What can we learn from half a century of surface-enhanced Raman spectroscopy: from nano- to AI-driven SERS?

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The field of surface-enhanced Raman spectroscopy (SERS) was initiated in the mid-1970s and revived in the mid-1990s. In 1974, the first surface Raman spectra that were dependent on the electrochemical potential were surprisingly observed from pyridine molecules adsorbed on a roughened silver electrode by Fleischmann, Hendra, and McQuillan [1]. This achievement stemmed from their pioneering work on the application of Raman spectroscopy to electrochemistry. In fact, this was the first SERS measurement, although it was not recognized as such at that time. Van Duyne and Jeanmaire soon carefully devised a procedure for measuring the surface enhancement factor thus discovered that the enhancement factor was of the order of 10^5-10^6. After a protracted review process, which was presumably due to the reluctance of reviewers to believe an unorthodox concept of surface enhancement, their paper was eventually published in 1977 [2]. Independently, Creighton and Albrecht published a paper on SERS in the same year [3]. In 1978, Moskovits first explained the effect of surface plasmons on roughened silver electrodes on SERS enhancement and predicted that the same effect may occur with Ag and Cu colloids covered with an adsorbate [4]. This prediction was verified experimentally using Ag and Au colloids by Creighton et al., and the effect was named as surface-enhanced Raman scattering (SERS) by Van Duyne in 1979 [5].

In the past 50 years SERS has gone through a tortuous pathway to develop into a powerful diagnostic technique [5,6]. My talk will provide the historical but forward-looking theme mainly through discussing the following questions.

What can we learn from the great pioneers and story of discovering SERS in 1970s?

Why is it essential to challenge the textbooks for opening a new scientific field?

How was SERS research boosted by nanoscience (Nano-driven SERS) in 1990s?

Will does AI usher in a new era in the study and application of SERS and break through the development bottlenecks of SERS (AI-driven SERS) in 2020s [7]?

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Nanoscale Study of Electrochemical Interfaces by Tip-enhanced Raman Spectroscopy

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Surface and interface are key to electrochemistry and determine the reaction mechanism, electrode process and performance of electrochemical devices. They are a region where nanoscale active sites play the key role and the multiple species undergo dynamic change over time and spaces. It requires characterization techniques with high sensitivity, high spatial resolution and at the same time molecular fingerprint information.

Electrochemical tip-enhanced Raman spectroscopy (EC-TERS), which combines electrochemical scanning probe microscopy (EC-SPM) and plasmon-enhanced Raman spectroscopy (PERS), is a powerful technique for the in-situ characterization of the electrochemical interface at the nanoscale and molecular level [1]. It is challenging to realize this technique since both a high sensitivity and a high stability are required. In the past 10 years, we have been developing EC-TERS so that it becomes possible to study the electrochemical interface [2-4]. We introduced water-immersion objectives to EC-TERS to avoid the optical path distortion between air and water, which allowed us to achieve a higher sensitivity. Both home-designed rigid EC-TERS cells and SPM heads were adopted to achieve a high stability. TERS tips with high TERS activity were fabricated by electrochemical method or nanofabrication (focused ion beam, FIB) method. These tips were then carefully insulated or protected for EC-TERS to give a long-time TERS enhancement. Benefited from the above improvements, we achieved EC-TERS imaging with a spatial resolution around 4 nm. With this powerful tool, we successfully probed the spatial distribution of plasmonically excited hot carriers [5] and visualized the structural evolution of individual active sites of MoS2 during hydrogen evolution reaction (HER)[6]. These discoveries offer new insights into our understanding of the electrochemical interface and may help the design of more active catalysts.

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Plasmon-Determined Selectivity in Photocatalytic Transformations on Gold and Gold-Palladium Nanostructures

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Noble metal nanostructures absorb light producing exciting coherent oscillations of the metal's electrons, so-called localized surface plasmon resonances (LSPRs). LSPRs can decay generating hot carriers, highly energetic species that trigger chemical transformations in the molecules located on the metal surfaces. The number of chemical reactions can be expanded by coupling noble and catalytically active metals. However, it remains unclear whether such mono and bimetallic reactions possess any sensitivity towards one or another chemical reaction if both of them can take place in one molecular analyte. In my talk, I will demonstrate the potential of tip-enhanced Raman spectroscopy (TERS), an emerging analytical technique that has single-molecule sensitivity and sub-nanometer spatial resolution, in the analysis plasmon-driven reactivity of 2-nitro-5-thiolobenzoic acid (2-N-5TBA) on gold and gold@palladium nanoplates (AuNPs and Au@PdNPs), Figure 1. This molecular analyte possesses both nitro and carboxyl groups, which can be reduced or removed by hot-carriers. We found that on AuNPs, 2-N-5TBA dimerized forming 4,4'-dimethylazobenzene (DMAB), the bicarbonyl derivative of DMAB, as well as 4-nitrobenzenehtiol (4-NBT), Figure 1. Our accompanying theoretical investigation based on density functional (DFT) and time-dependent density theory (TDDFT) confirmed these findings. The theoretical analysis shows that 2-N-5TBA first dimerized forming the bicarbonyl derivative of DMAB, which then decarboxylated forming DMAB. Finally, DMAB can be further reduced leading to 4-NBT. This reaction mechanism is supported by TERS-determined yields on these three molecules on AuNPs. We also found that on Au@PdNPs, 2-N-5TBA first formed the bicarbonyl derivative of DMAB, which is then reduced to both bihydroxyl-DMAB and 4-amino-3-mercaptobenzoic acid. The yield of these reaction products on Au@PdNPs strictly follows the free-energy potential of these molecules on the metallic surfaces.

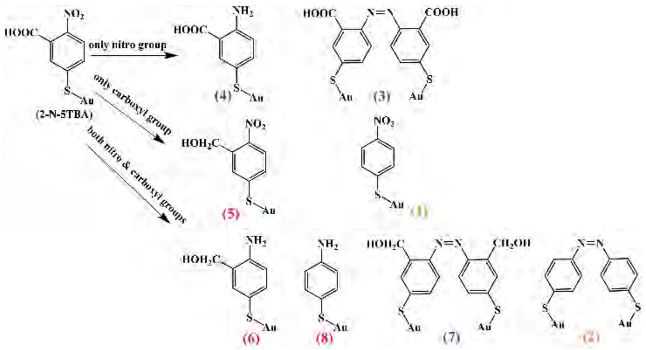

Figure 1: Possible reaction products that may be observed upon plasmon-driven reduction of 2-N-5TBA. The molecules labelled in blue and orange were observed in the presence of Au@PdNPs and AuNPs, respectively. The green-labelled molecule was produced on both Au@PdNPs and AuNPs. The red-labelled molecules were not observed in the experiment.

Vibrational and electronic surface-enhanced Raman spectroscopy at electrochemical interfaces

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Electrochemical interfaces are ubiquitous in both nature and technology including energy conversion, catalysis, and biological systems. To understand their dynamic nature, surface-enhanced Raman scattering (SERS) is often used as a vibrational spectroscopic method with high surface sensitivity. However, SERS can amplify not only vibrational Raman signals scattered by molecules, but alto electronic Raman signals scattered by free electrons in a substrate metal [1,2], which has long been neglected. When SERS is applied to measure an electrode/electrolyte interface, therefore, both electronic and vibrational information at the interface can be obtained simultaneously [3,4]. Moreover, we have developed an analytical method of SERS spectra in the terahertz frequency range, which can provide the dynamic information about hydrogen-bonded networks of liquid water or anion-cation interactions at electrochemical interfaces [5].

In this presentation, vibrational and electronic SERS observation is demonstrated at an electrochemical interface of ionic liquid (1-butyl-3-methylimidazolium hexafluoro-phosphate: $BMI^+PF_6^-$) and Au under electrochemical potential applications. Potential-induced changes of surface adsorbates, anion-cation interaction, and surface charge of Au electrode will be discussed, which can help to build a complete picture of electrochemical processes at interface.

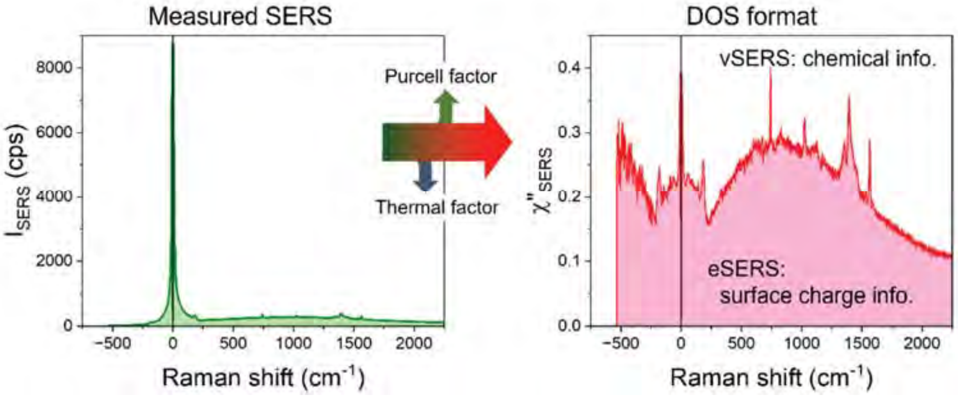

Figure 1: Measured SERS spectrum of $BMI^+PF_6^-$/Au interface and its DOS format.

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Overcoming electron transfer rate limitations in cyclic voltammetry using Surface Enhanced Raman Spectroelectrochemistry – seeing at and on the electrode

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Recently, we reported on the potential of surface enhanced Raman scattering (SERS) microspectroscopy to study redox processes occurring at the electrode solution interface.[1] In this presentation, we will develop this approach further using time-resolved SERS spectroelectrochemistry on a roughened gold surfaces to study the redox chemistry of non-heme iron catalysts. This approach allows for real time readout of the state of species at the electrode during cyclic voltammetry. We show that it is especially suited for redox processes where electron transfer kinetics are too slow for observation directly by cyclic voltammetry. We take as an example insight into the redox potentials and the important role of adsorption in the voltammetry of biomimetic non-heme iron complexes, such as (N4Py)Fe(II) (where N4Py is 1,1-bis(pyridin-2-yl)-N,N-bis(pyridin-2-ylmethyl)methanamine).[2] These species form Fe(IV)=O intermediates using chemical oxidants but these should be generated electrochemically also. The main challenges in doing so is to be certain of the Fe(III)-OH/Fe(IV)=O redox potential.[3,4] Characterising the Fe(IV)=O species electrochemically,[5-7] is challenging despite many efforts with a wide range of values proposed.[5-7] The approach taken in the present study overcomes many of the issues faced earlier and opens up a new approach to study the electrochemical activation of redox catalysts.

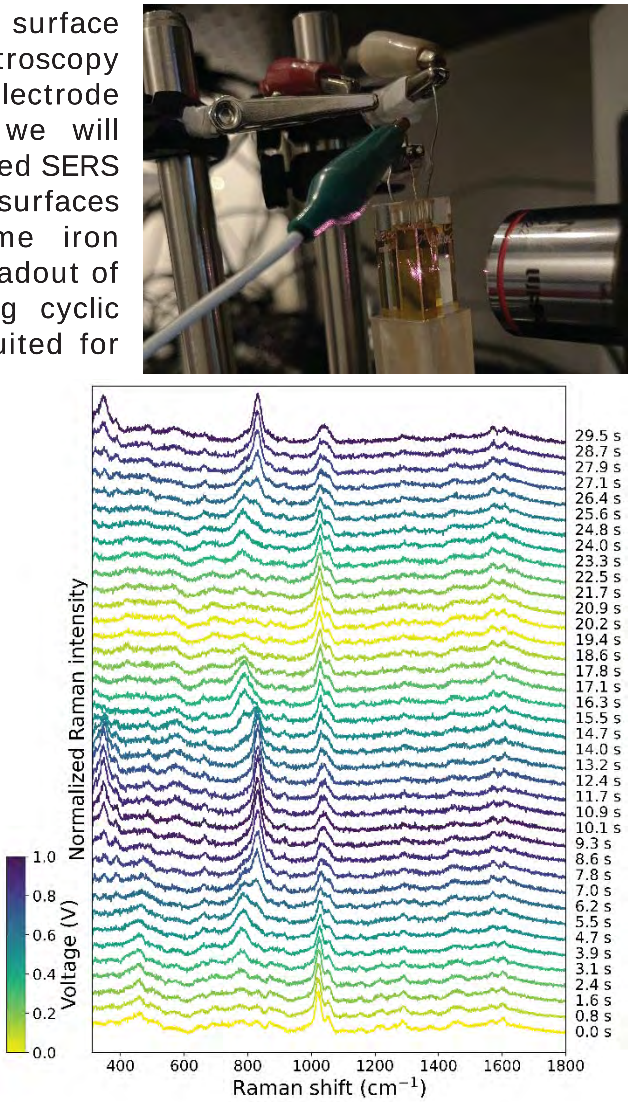

Figure 1: (top) Picture of the experimental setup. (bottom) SERS spectra recorded during the cyclic voltammetry experiment. Conditions: 2 mM $[Fe(II)(N4Py)(CH_3CN)](OTf)_2$, 0.1 M KNO_3 in H_2O, WE: gold bead, RE: Ag/AgCl, CE: Pt, scanrate: 0.1 V.s^{-1}. SERS spectra recorded with 0.5s acquisition time at a 785 nm confocal Raman microscope.

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Development of Tip-Enhanced Raman Spectroscopy in Controlled Electrolytes

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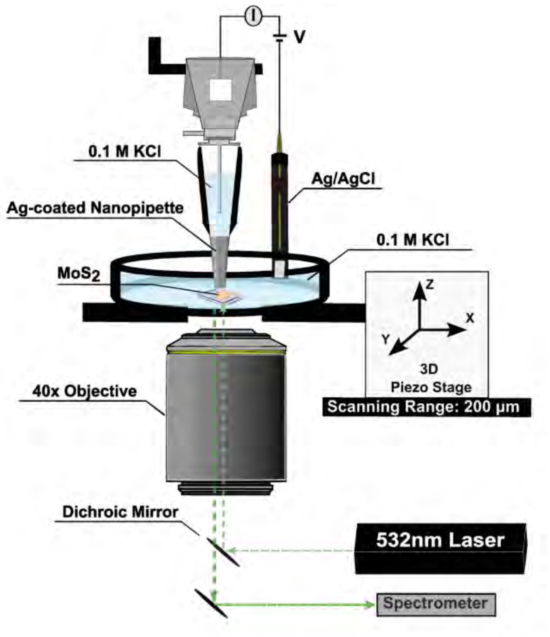
Abstract:

Tip-enhanced Raman spectroscopy (TERS) combines the nanoscale spatial resolution of scanning probe microscopy with the single-molecule chemical sensitivities of surface-enhanced Raman spectroscopy. TERS has achieved Ångström-scale spatial resolution in chemical imaging under ultrahigh vacuum conditions. We are developing custom-built laser-coupled scanning probe microscopes (SPM) for TERS operating in electrolytes, which enables the potential translation of this unprecedented spatial resolution in vibrational spectroscopy to liquid environments. Scanning ion-conductance microscopy (SICM) is an SPM technique that is particularly suitable for topological imaging of surfaces in controlled liquid environments. One technical challenge for implementing TERS in solution is the plasmonic probe fabrication. Surface-enhanced Raman spectroscopy (SERS) from the apex of the probes is used to optimize the fabrication procedures. Additionally, proof-of-concept experiments on two-dimensional transition metal dichalcogenides have already demonstrated the potential of the custom-built SICM-TERS setup. This new chemical imaging method opens a new avenue for researchers to obtain more detailed chemical information of soft surfaces in electrolyte solutions.



Modeling Atomistic Near-Field Spectroscopy

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Surface-enhanced Raman scattering (SERS) can be used to detect and image individual molecules. Experimentally and theoretically, it has been shown that it is possible to resolve molecule vibrations using the highly confined electric near-field at the surface of plasmonic metal nanoparticles. Understanding the specific interactions between the molecules and the plasmonic nanoparticles becomes important for correctly interpreting the images. However, atomistic simulations of surface-enhanced spectroscopes remain a challenge due the necessity of bridging the different length scale of the molecule and the metal nanoparticle. Here we present our recent progress on developing a reliable and efficient hybrid computational method that bridges classical electrodynamics and electronic structure theory. Focus will be on understanding chemical, resonance, and inhomogeneous electric fields in surface-enhanced spectroscopies. Specifically, we will discuss how a first-principles Raman bond model can be used to gain insights into the chemical enhancements in SERS. Finally, we will discuss our latest results focusing on understanding the effects of near-field localizations, field gradient, substrate effects on the resolution in Raman scattering images.

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Fully Atomistic Multiscale Modeling of Raman, SERS and Raman Optical Activity

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In modeling complex systems in the condensed phase, fully atomistic multiscale approaches have demonstrated considerable success. A particularly effective strategy for solvated systems involves focusing on a specific portion described at the Quantum Mechanical (QM) level, while treating the remaining portion classically using tailored Molecular Mechanics (MM) force-fields [1-3]. The extension of such methodologies to spectroscopy, and especially Raman, SERS and Raman Optical Activity, poses additional challenges [4-8].

In this lecture, I will present my recent contributions to the development of fully atomistic multiscale approaches for Raman, SERS and ROA of systems in complex environments, showcasing pilot applications that underscore the potential of these methods.

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Theory of Momentum-Based Nano-optics

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Owing to the negligible momentum carried by visible light in a vacuum, in conventional optical spectroscopy theory, the effects of the momentum are often overlooked, leading to the diffraction condition rules in inelastic scattering processes within periodic systems. In other words, visible light can only excite phonon vibrations near the Gamma point. In this presentation, we introduce a general theoretical framework at the quantized field theory level that can rigorously address the spatial distribution of optical fields.[1,2] Experimentally, it has been demonstrated using plasmonic optical cavities to confine light fields down to sub-nanometer scales in real space.[3] Leveraging the optical localization and the fundamental Heisenberg's momentum-position uncertainty principle in quantum mechanics (Figure 1a), the new theoretical framework predicts an innovative Raman scattering imaging technique based on the momentum distribution of the light field.[4] This technology adaptively satisfies both the energy and momentum conservation laws during the Raman scattering process, enabling quantitative measurement of phonon scattering spectra in low-dimensional hydrogen-rich systems, an inaccessible task with other methods. By utilizing the momentum distribution within localized optical fields, we further demonstrate how Raman scattering imaging can validate the convergence of vibrational and thermodynamic properties predicted by commonly employed cluster models and periodic boundary conditions. Lastly, we discuss the effects of increased momentum in surface plasmon polaritons on optical processes within the new theoretical framework (Figure 1b).[5] Through this presentation, we aim to stimulate broader discussions about the momentum characteristics in nano-optics and pave the way for a new dimension in this intriguing subject.

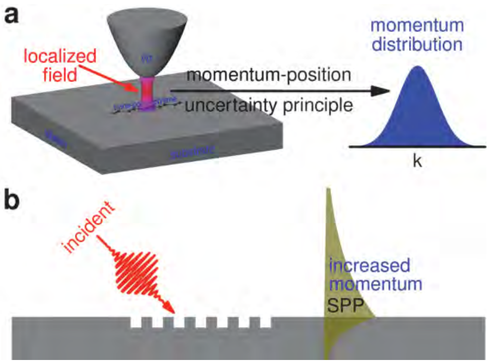

Figure 1: The momentum effects in (a) standing and (b) traveling nano-optics.

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Hybrid Quantum/Classical Multiscale Modeling of Surface-Enhanced Raman Scattering

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Surface enhanced Raman scattering (SERS) is a powerful analytical tool based on the huge enhancement of the Raman signal of a target molecule in the immediate surroundings of a metallic nanostructure [1]. The measured enhancement factors can reach values of 10^{14}-10^{15}, allowing even for single-molecule detection [1]. In this contribution, we present a multiscale model suitable for surface-enhanced spectroscopies, based on the coupling between a Quantum Mechanical (QM) description of the target molecule with a family of atomistic, classical, electromagnetic models which have been recently proposed by some of the authors [2-4]. These classical approaches, namely ωFQ and ωFQFμ, are able to describe the plasmonic properties of metal nanoparticles [2,4] and graphene-based materials [3,5] in a unified theoretical framework with more than 1 million atoms [6], also for systems in which quantum effects dominate the optical properties, such as in presence of geometrical defects [3,6] and subnanometer junctions [2,4]. We present the resulting QM/ωFQ(Fμ) model extended to the prediction of SERS signals [7], showcasing the robustness and reliability of the developed method for selected test cases and comparing with available experimental spectra.

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Stimulated Raman Photothermal Microscopy: Bond-selective imaging with ultrahigh sensitivity and ease of operation

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Stimulated Raman scattering (SRS) microscopy has shown enormous potential in revealing molecular structures, dynamics, and couplings in a complex system. Yet, the sensitivity of SRS is fundamentally limited to milli-molar level due to the shot noise and the small modulation depth. Additionally, the operation of SRS imaging is complicated by cross phase modulation and laser noise. We recently revisited SRS from the perspective of energy deposition (Figure 1). The stimulated Raman process pumps molecules to their vibrationally excited states. The thereafter relaxation heats up the surrounding and induces refractive index changes. By probing the refractive index changes with a laser beam, stimulated Raman photothermal (SRP) microscopy is developed [1], where a >500-fold boost of modulation depth is achieved. Moreover, SRP imaging can be operated with a relatively noisy fiber laser for excitation and a long working distance air condenser for signal collection, allowing system design to be much more flexible. In summary, SRP microscopy opens a way to perform bond-selective chemical imaging with ultrahigh sensitivity and ease of operation.

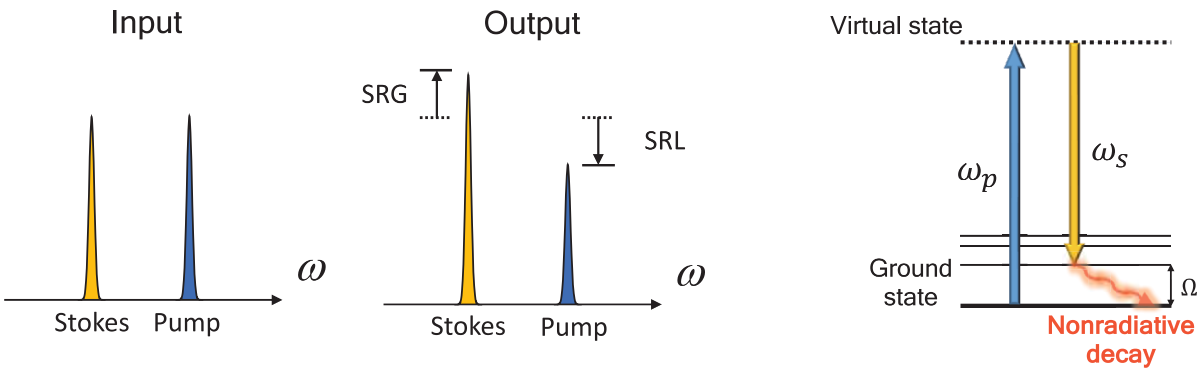

Figure 1: As a non-parametric process, stimulated Raman gain (SRG) and loss (SRL) collectively excite the target molecule to its vibrational excited states, which deposits energy onto the sample and generates heat via nonradiative decay on the picosecond time scale. This physics builds the foundation for highly sensitive photothermal detection of a Raman signal.

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Fundamental insights into the human gut microbiome obtained by correlative SRS imaging and fluorescence microscopy

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Microbiomes are the foundation of planetary health as they underpin almost all ecosystem services that humans depend on. In particular in the gut, microbes essentially aid their host in food digestion, delivery of nutrients, training of the immune system as well as the uptake and distribution of pharmaceuticals. However, deciphering the complex interplay between hundreds of individual members comprising the microbial consortium necessitates simultaneous interrogation of (sub)micron sized single cells with respect to their phylogenetic identity and metabolic function. For identification, *in situ* hybridization of fluorescently labelled oligonucleotide probes to 16S-rRNA (FISH) has become the workhorse. For evaluation of metabolic activity, stable isotope incubations can be conducted revealing single cells as active via isotopic enrichment. Chemical imaging techniques capable of isotope-specific analysis have been established, but widely suffer from limited through-put, which is a constraining factor in obtaining statistically robust data. Recently, we developed an imaging platform combining fluorescence microscopy with stimulated Raman scattering spectroscopy (SRS-FISH) offering an imaging speed of 10 to 100 ms per cell, which is two to three orders of magnitude faster than achievable by state-of-the-art methods [1].

Utilizing SRS-FISH together with heavy water (D_2O) incubations, we analysed the metabolic response of 30.000 individual cells from two major phylogenetic groups of microbes in the human gut (Bacteroidales and Clostridia spp.) to various mucosal sugars which represent host-derived nutrients. We found a significant interindividual variability of responses and, unexpectedly, that Clostridia can outperform Bacteroidales at foraging fucose.

In a follow-up study, we investigated the impact of the nervous system-targeted drugs entacapone and loxapine succinate on the gut microbiome [2]. *Ex vivo* supplementation of physiologically relevant concentrations of these pharmaceutic compounds significantly impacted the abundance of up to one third of the microbial species present. Importantly, we observed that the effect on microbial activity is much more pronounced than the impact on cellular abundances. Entacapone causes depletion of microbiota-accessible iron, resulting in restricted microbial growth, which could be reversed by iron replenishment. Remarkably, the entacapone-induced iron starvation selected for iron-scavenging organisms carrying antimicrobial resistance and virulence genes.

Collectively, these results demonstrate that the combination of SRS with FISH yields a powerful tool for microbiome characterization.

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Identifying Tumor-Associated Macrophage Polarization with Label-free Multimodal Nonlinear Optical Microscopy

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Tumor-associated macrophages (TAMs) are immune effectors largely diffused in the tumor microenvironment and play a crucial role in various processes like angiogenesis, metastasis, and immunosuppression. Depending on their polarization state, TAMs can either drive tumor progression or exhibit anti-tumor activity, making them promising targets for cancer therapy [1]. This study delves into TAMs activation using multimodal nonlinear optical imaging, combining Stimulated Raman Scattering (SRS) microscopy and Two-Photon Excited Fluorescence (TPEF) microscopy [2,3]. This noninvasive, label-free approach yields relevant chemical and metabolic information, which is used to discern distinct polarization states of murine TAMs (M1 and M2) in response to specific stimuli, such as lipopolysaccharide and interleukin 4, respectively.

We employ a state-of-the-art dual output tunable fiber OPO laser source (Picus Duo, Refined Laser Systems, Germany), which generates synchronized tunable pump and Stokes pulses of 7 ps duration at 40.5 MHz repetition rate, which can be tuned over a wide Raman shifts range (650 cm-1 to 3200 cm-1). A fraction of the Stokes output is extracted from a back port and used for balanced-SRS microscopy. The pump beam is modulated at 20MHz. The beams are overlapped in space and time and enter a lab-built microscopy unit. We employ a water-immersion 100X 1.25NA illumination objective and an oil-immersion 40X 1.30NA collection objective. A 3D motorized sample stage is used to perform raster scanning. For TPEF imaging, we collect the endogenous fluorescence of NAD(P)H and FAD in forward and epi-detection, respectively, using two photomultipliers. Spectral separation and isolation of biological signals is achieved using appropriate sets of optical filters.

Spectral analysis from the fingerprint region shows that M1 macrophages possess significantly reduced nucleic acids content. Furthermore, spectral information in the CH-region enables the differentiation of fatty acid metabolism between TAMs polarization states. Finally, the insights gained from the signals of NAD(P)H and FAD, which are important coenzymes involved in several metabolic processes, allow us to highlight polarization-dependent changes in cellular metabolism.

Our results illuminate TAM-mediated immune responses, paving the way for novel macrophage-centred therapies. These innovative strategies promise to complement existing cancer treatments, offering new avenues for improving patient outcomes.

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Identification of molecular signatures for diseases by vibrational spectroscopic imaging

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Accurate disease classification is crucial for clinical decision-making. Traditional histopathological evaluation often relies on morphology, extensive labeling, and labor-intensive analysis. Molecular-level information provides a more precise understanding of cellular physiology. Yet, ensemble-averaged measurements from complex tissues suffer from high variability, impacting disease classification. To address these challenges, spectroscopic imaging modalities were developed, directly measuring molecular concentrations and properties through chemical bond vibrations. In this talk, the development of submicron-resolution molecular imaging approaches based on Raman [1] and infrared spectroscopic modalities [2], along with their application in tissue imaging [3] and in tracking subcellular lipid dynamics in embryos will be discussed. These advancements facilitate the identification of spatio-spectral signatures of biomolecules toward disease classification.

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Spontaneous and coherent Raman microscopy-based evaluation of erythroid differentiation and heme biosynthesis

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An adequate supply and functioning of red blood cells (RBCs) are critical for tissue oxygenation under normal and stressful conditions and for preventing anaemia, etc. Mature red blood cells originate from the bone marrow in a tightly regulated, multistep process called erythropoiesis. As the isolation of erythroblast cells and precursors is an invasive method, *in vitro* studies of key players regulating this process are performed with human leukemic K562 cells treated with various pharmacologic agents [1].

Erythroid differentiation is associated with changes in morphology and phenotype even at the individual organelle level, especially mitochondria involved in iron metabolism and heme biosynthesis [2]. Therefore, the non-destructive identification of cellular composition with good spatial resolution performed with Raman microscopy [3] is a method of choice. We proved the utility of Raman spectroscopy in following erythroid differentiation and identified crucial changes in heme, nucleic acids, and lipid content. Additionally, to study the biochemical state of organelles, we introduced molecular Raman probes (RP). The presence of triple bonds or deuterium in RPs is followed in the 2000-2400 cm^{-1} spectral region, while the selective targeting group interacts with the subcellular compartment of interest. We applied MitoBADY, which accumulates according to the mitochondrial membrane potential, and EdU, a thymine analogue incorporating into DNA during *de novo* synthesis [3], proving that PR improves the sensitivity and specificity of classification. Moreover, the Raman markers of differentiation were selected and verified with a non-linear technique, Stimulated Raman Scattering, allowing for rapid classification of erythroblast and RBCs.

This work was founded by National Science Centre (Poland), project PRELUDIUM—No. UMO-2021/41/N/ST4/03069 and NAWA BEKKER program No.BPN/BEK/2023/1/00221

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Molecular Optomechanics with Plasmonic Nanocavities and Quantum Effects in Spontaneous Raman scattering

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Spontaneous Raman scattering is most naturally described within a quantum theory [1]. When it occurs inside a plasmonic nanocavity (as in SERS and TERS), it is also necessary to account for the backaction of the cavity field onto the molecular vibration. Molecular optomechanics is a theoretical framework for Raman scattering that is rooted is a complete quantum description of the molecular vibration and electromagnetic field [2,3]. I will discuss here two general experimental situations where using this framework is beneficial. First, I will review results of photon counting measurements in the regime of spontaneous Raman scattering in crystals and molecular liquids, evidencing the intrinsic quantum coherence that exists among the Raman scattered photons and a macroscopic vibrational state (Fig. 1, left). Second, I will discuss the theory of molecular cavity optomechanics and what it practically means in the context of SERS from plasmonic nanocavities (Fig. 1, right). My aim is to clarify how to use the theory in specific experiments and to highlight open questions and challenges in this field [6].

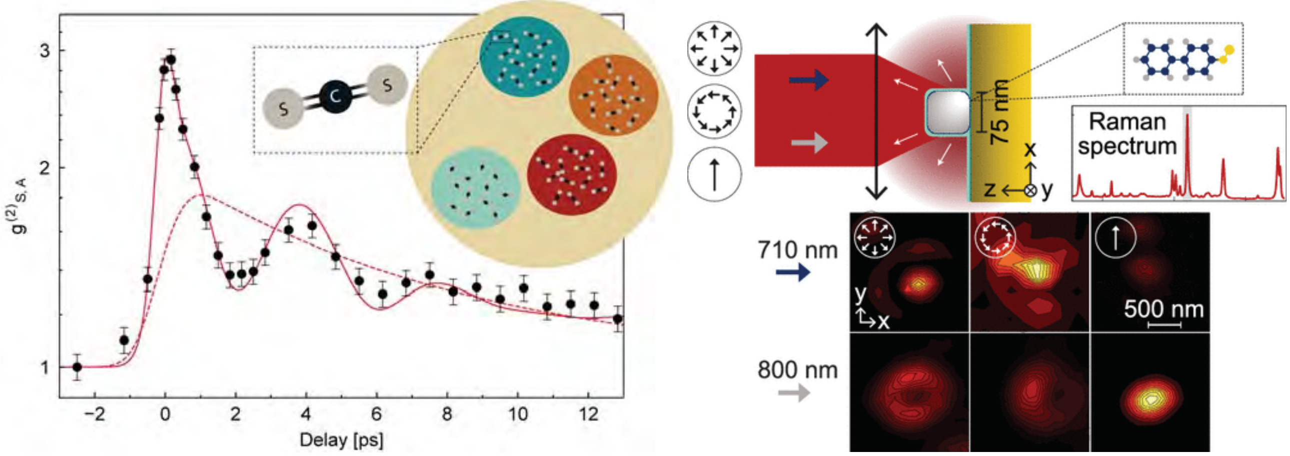

Figure 1: (left) Quantum beats in the normalized second-order correlation functions between Stokes and anti-Stokes photons scattered from liquid CS_2 are the signature of collective quantum coherence in spontaneous Raman scattering [4]. (right) Experimental evidence for mode-selective in-coupling rate of laser light to a nanocavity, depending on wavelength and polarisation [5].

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Sum-frequency vibrational spectroscopy with tip-enhanced nanocavities

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In only a few years, scattering-type scanning near-field optical microscopy (s-SNOM) has transformed into a key actor for the nanoscale imaging and spectroscopy of thin layers materials across the IR and THz spectral ranges. Extension of its abilities to other spectral regions and to properties of vibrational and phononic modes different from the mere absorption would be interesting for researchers in nanooptics but also for a number of technological developments in surface science and chemistry.

Here, we use s-SNOM to study vibrational sum-frequency generation (v-SFG), in which the sample's SFG cross-section is enhanced by many orders of magnitude by the s-SNOM microscope's tip [1]. By combining simultaneously mid-IR and VIS illuminations on gold nanostructures, we show the "on-demand" emergence of v-SFG signals controlled by the tip's nanomechanical positioning. Studying several vibrational modes, we additionally evidence and distinguish the tip contribution on both second-order nonlinear and Raman optical processes.

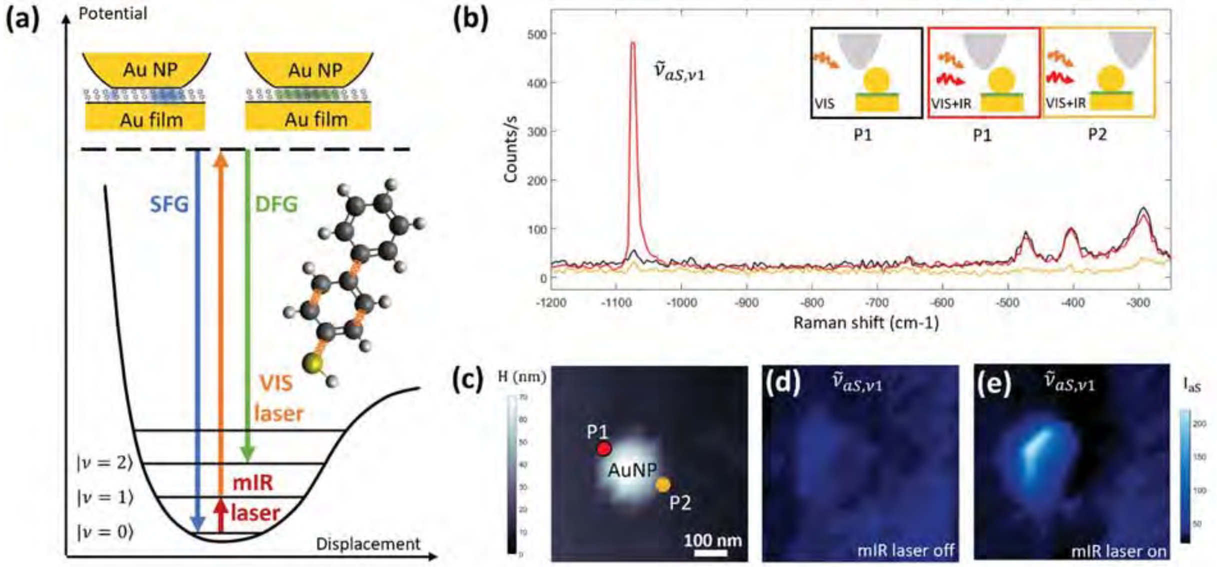

Figure 1: (a) Energy diagram of vibrational nonlinear optical processes. Above the diagram: Field distributions inside the plasmonic gap at the frequencies where SFG (blue) and DFG (green) occur. (b) Molecular spectra for different tip positions and illumination conditions (VIS or VIS+IR) (c) Topography image and intensity maps of the anti-Stokes sideband without (d) and with (e) IR illumination.

Our experiment demonstrates for the first time CW v-SFG with a near-field microscope, simplifying greatly the requirements on samples that can be addressed via upconversion spectroscopy [2]. Conversely, our work also leads to a novel near-field microscopy modality where the hyperpolarizability of thin molecular films and 2D materials could be probed at the nanoscale. More generally, by combining the coupling strength uniquely accessible to molecular vibrations in the mid-IR and the single-photon detection abilities available in the visible, our study suggests new strategies for the observation of cavity physics [3] and chemistry in the few-molecules limit.

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Convergence of Optical and Electron Microscopy: TERS in the atomistic near-field

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The atomic limit in optical microscopy was demonstrated through tip-enhanced Raman scattering (TERS) carried out in the atomistic near-field (ANF), by recording atom-resolved images of a surface [1], and by visualizing vibrational normal modes inside a molecule [2,3]. The demonstrated image resolution of **~** 1.6 Å is traditionally associated with electron microscopy, where the ~ 2 Å limit was reached two decades earlier through aberration corrected scanning transmission electron microscopy (STEM) [4]. The coincidence is not accidental. The ANF is accessed at plasmonic tunnel junctions, where Raman scattering can be demonstrated to be driven by photo-tunneling current rather than a locally enhanced external field, central to the orthodox SERS/TERS paradigm. At the tunnel junction, photon and electron become indistinguishable, TERS can be equally well regarded as near-field scanning optical or electron microscopy. In either case it derives its superior spatial resolution in part by the second order nature of Raman scattering, and in greater part because it measures vibronic intramolecular polarization currents, as opposed to electronic currents imaged in STM. We demonstrate these principles through bond-resolved TERS images of chemisorbed molecules, explain that the interpretations are a consequence of accepting the photon in matter as the quantized electromagnetic displacement, offer the image of the apex mode of the tip plasmon (Fig. 1) as that of an atomically confined photon, and discuss some implications of rethinking TERS/SERS: as it relates to outstanding anomalies, quantitative spectro-microscopy, direct wiring of photons into molecular electronic circuits [5], and more generally, as a direct probe of the merger of light and matter states.

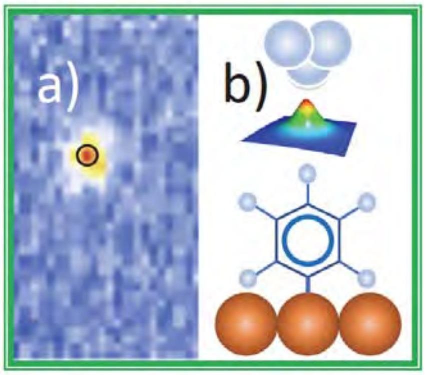

Figure 1: a) Image of atomically confined photon (diameter of enclosing circle is 2 Å), b) The apex mode of the tip plasmon is imaged with the para C-H bond terminal of chemisorbed phenyl on copper.

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Raman Spectroscopy for Forensic Purposes: The First Universal Method for the Identification of All Main Body Fluids

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Raman spectroscopy has been known for decades as the most selective spectroscopic technique. The development of inexpensive and reliable lasers and digital cameras opened the opportunity for numerous practical applications of the technique including that in forensic science. The technique is non-destructive, rapid and requires little or no sample preparation. Furthermore, portable Raman instruments are readily available allowing for crime scene accessibility. We have developed and are commercializing now (www.supremetric.com) the first universal method for the confirmatory identification of all main body fluids based on Raman spectroscopic analysis of biological stains. In addition, peripheral and menstrual blood as well as human and animal blood can be differentiated. The time since deposition of bloodstain can be estimated up to two years. Most recently, we demonstrated the proof-of-concept for phenotype profiling based on Raman spectroscopy of dry traces of body fluids and machine learning including determining sex, race, and age group of the donor.

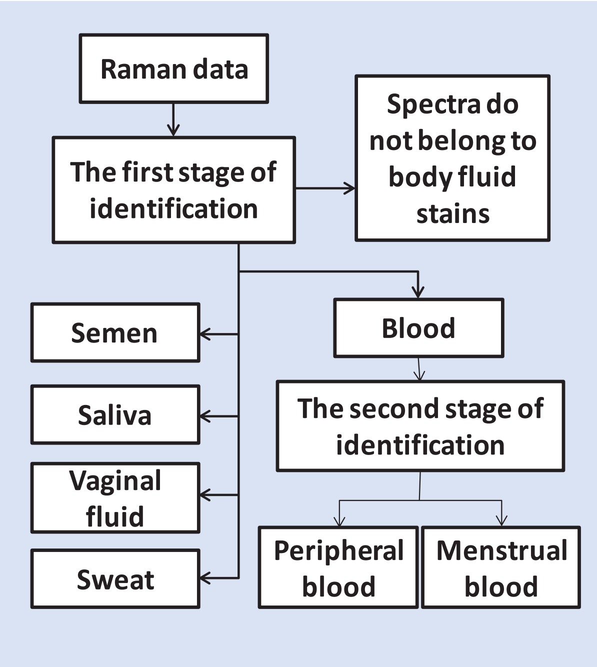

Figure 1: A universal method for the confirmatory identification of all main body fluids

"Revolutionizing Anti-Counterfeiting Strategies: 3D Printed Raman Ink Labels for Enhanced Security and Traceability"

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Abstract：

In the evolving landscape of anti-counterfeiting technologies, optical materials for encryption have emerged as a promising solution, offering unparalleled capabilities in information hiding and diversified response. However, the industry has grappled with the challenges of achieving programmable mass-manufacturing that combines high encoding capacity, unforgeability, and robust information traceability. Addressing these challenges, our research introduces an innovative direct Raman ink writing technique through 3D printing, which simplifies the fabrication of advanced encrypted codes. Utilizing a carefully formulated blend of triple-bonded Raman polymers, water-based resin, and light crosslinking agents, our approach eliminates the need for complex procedures and toxic organic additives, presenting a more straightforward and environmentally friendly method. The triple-bonded Raman inks stand out for their ability to encode information, thanks to their emission in the silent spectral region and exceptional multiplexing capabilities. By leveraging these inks, we achieve sophisticated information encryption and decryption, ranging from invisible Raman codes in 2D to 3D pixels. This technique facilitates spatial modulation of

Raman outputs, adherence to the 6-bit ASCII standard, and implementation of a "Codes in Code" strategy, allowing for unique identification of each item throughout the entire supply chain. This study not only demonstrates a viable mass-production method for light-curing triple-bonded Raman inks but also underscores the potential of 3D printing in crafting anti-counterfeiting labels that ensure secure information storage and retrieval, marking a significant advance in the field of anti-counterfeiting technology.

Relative quantification of titanium dioxide polymorphs in binary mixtures by Raman spectroscopy: interlaboratory comparison and foodstuff applications

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Titanium dioxide (TiO_2) is a semiconductor used in industry and research for a variety of applications. It is the most widespread white pigment in dyes and paints in the world, and it is employed for its photocatalytic activity, in cosmetics, and as a food additive (E171) — although it was banned for this purpose in 2022 in the EU. Its most utilised polymorphs are rutile and anatase. They differ in colour and ultraviolet absorption, band gap, and market price: from this, the industrial necessity arises of analytical methods for quantifying them. ASTM D3720−90 describes X-ray diffraction (XRD) as the current standard method for their relative quantification in TiO_2 mixtures. However, the method focuses on samples approaching purity of one or the other [1]. Raman spectroscopy is very suitable for the detection of anatase and rutile and their differentiation, because of their high cross section and distinct bands, and its applicability to complex and aqueous matrices. However, Raman is not normatively accepted yet for any kind of quantification: one of the main causes of this is the lack of standardization in terms of reference materials, operating procedures and data analysis, and even more so recently, due to the commonplace use of multivariate analysis on Raman data, often with no reproducibility effort by the users by a lack of disclosure of key information on models, procedures and parameters [2].

For these reasons, the prenormative interlaboratory comparison (ILC) "Raman spectroscopy for TiO_2 nanoparticles mixtures" was conducted under the framework of the Versailles Project on Advanced Materials and Standards (VAMAS), and its outcomes are here presented. By this ILC, including 11 experimental setups measuring TiO_2 films sourced from commercial powders, the performance and metrological uncertainty of Raman microspectroscopy for relative quantification of anatase-rutile binary mixtures was evaluated. Operating procedures were devised for the preparation of stable and uniform samples, for accurate and reproducible spectral acquisition by Raman microscopy, and for universally applicable data processing and multivariate data analysis by partial least squares (PLS). All models successfully quantified the samples with accuracies and precisions of less than 3% in the 5% – 95% range, with the majority of the datasets below 2%, indicating likely margins of improvement by refinement of the procedures on the basis of the ILC data. The ILC demonstrated the potential of Raman spectroscopy as a method for relative quantification in a field of considerable industrial and research interest. This approach could also be extended to a variety of samples containing TiO_2, such as foodstuff, with appropriate preparation. Strategies for this purpose are here illustrated, and a Raman study on anatase and rutile sub-micron particles relative quantification in liquid is presented, with direct applications in food and environmental analysis.

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Data analysis of low signal to noise Raman spectra for detecting traces of explosives on surfaces

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Remote, real-time threat detection is of great interest for security applications. For the detection of hazardous substances, techniques that are chemically selective and highly sensitive need to be available. Among various methods, UV Raman spectroscopy is especially promising [1-3], as it is far more sensitive than conventional Raman spectroscopy using visible or near-infrared light sources.

When scanning for explosives at security check points an inherently low detection time is accompanied by the need for sensitive trace detection. These prerequisites generally result in very low signal-to-noise spectra and difficult data analysis. Averaging multiple spectra in order to improve the signal-to-noise ratio, however, entails the risk of clouding important spectral signatures present only in a few of the spectra. Therefore, we investigate the use of secondary statistical analysis, such as variance or skewness, to improve the data quality and increase the probability of a successful threat detection.

For a first test, measurements of the explosive pentaerythritol tetranitrate (PETN) on a limited number of fabrics as well as the bare materials are performed. This dataset is used to test and compare different methods of statistical analysis and maximise the detection accuracy of an explosive. One example, where mean and variance spectra are compared, is shown in Figure 1.

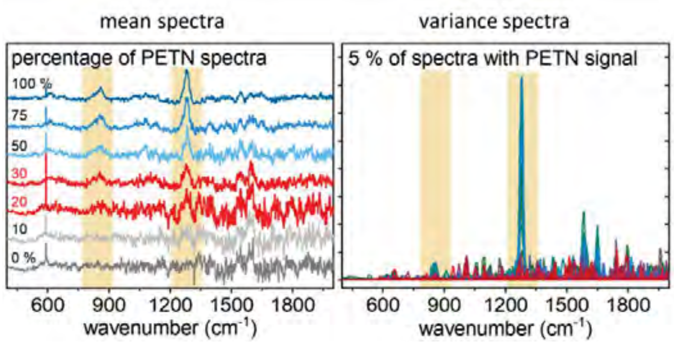

Figure 1: mean (left) and variance (right) UV Raman spectra of PETN on a red fabric substrate measured with a 266 nm laser. The fraction of PETN spectra in the dataset were varied. The variance spectra shown here were calculated from a dataset containing 5% of PETN spectra and 95% of substrate spectra [4].

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Detection of Exosomes for Cancer Diagnosis and Monitoring

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There has been a growing interest in using exosomes for early cancer diagnosis and anticancer therapy monitoring in recent years. Exosomes are small membrane-bound vesicles found in various body fluids that contain a variety of cellular components, including proteins, nucleic acids, and lipids, and reflect the physiological status of the cells. The use of exosomes for cancer diagnosis has the potential to be a sensitive and non-invasive approach that can reduce the pain and risks associated with traditional biopsy methods.

Raman spectroscopy is a powerful analytical tool used extensively in cancer diagnosis because it provides material-specific information about the samples in the fingerprint region. However, spectral analysis of biomaterials is complicated by the strong autofluorescence emitted by biological samples, particularly in the visible region, which interferes with Raman signal detection. Therefore, alternative excitation source is need to overcome the autofluorescence background in biological samples. The deep-UV Raman spectroscopy can increase the Raman intensity while minimizing the autofluorescence background.

In addition, surface-enhanced Raman scattering (SERS) is a promising and compelling analytical technique that enables ultra-sensitive analysis of chemicals and biomolecules. SERS has been successfully used in a variety of sensing applications, including the detection of heavy metals, disease biomarkers, and biological molecules. In particular, SERS-based biosensors have received much attention in the field of disease diagnosis.

In this study, SERS method was used to detect exosomes isolated from various cell lines with high sensitivity and selectivity. A deep-UV laser excitation of 244 nm to obtain Raman spectra of exosomes isolated from various cell lines was also used. In this presentation, potential of SERS-based assay and deep-UV Raman spectroscopy as powerful tools for exosome analysis and cancer diagnosis will be presented.

Real-time In Vivo Monitoring of Multiple Signaling Molecules in Plants by SERS

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Since plants cannot move against biotic- and abiotic stress, they communicate with chemical information with various signaling molecules. Even though there have been many trials to understand the biological and chemical dynamics in plants, molecular level detection is only successful for peroxide molecules in leaves, which does not give information on the origin of stress [1]. Surface-enhanced Raman spectroscopy (SERS), claiming single molecule level detection sensitivity, is a promising tool for monit oring signaling molecules in plants while it still faces challenges of autofluorescence in plant, competing abundant chemicals in plant, and most seriously low affinity of target molecules on SERS-active surface. For real-time sensing the plant signaling molecules, we developed SERS nanosensor particles that overcome these problems [2]. We fabricated PDDA-capped Ag nanoshell (AgNS@PDDA) and introduced it into plants through stoma. PDDA polymer effectively attracted signalling molecules by coulombic interaction. When plants leaf was under wound stress or cold stress, nasturlexin B, extracellular ATP and glutathione were successfully detected in vivo environment. Meanwhile, biotic stress was studied for the plants infected with fungal disease. F. Graminearum was injected into the plant, and signaling molecules was monitored with nanosensors according to the elapsed days after injection. Even only 2 hours after the fungi injection, when no lesion was detectable, SERS signals of eATP and salicylic acid related to systemic acquired resistance were successfully detected. This study shows the possibility of real-time monitoring plant signaling molecules with vibrational spectroscopy and will serve as an important tool of communicationg with plants.

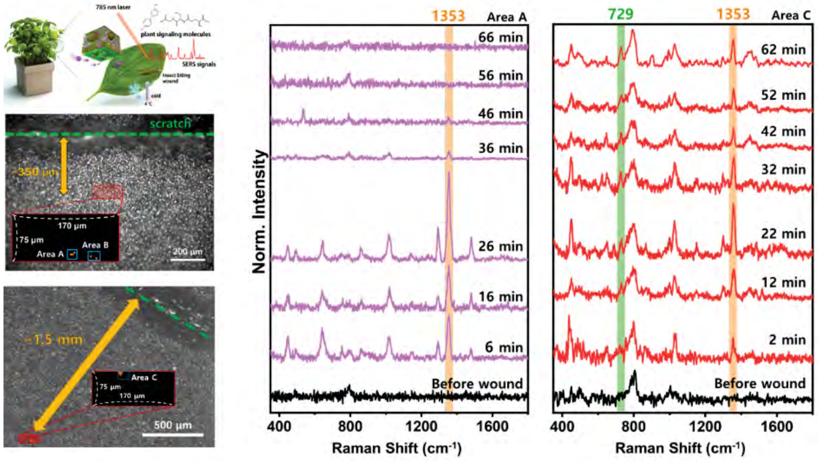

Figure 1: Detection of plant signalling molecules from living plants under abiotic stresses [2]

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Multimodal Raman biosensors for in vivo and liquid biopsy applications

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In this talk, we will describe our recent advancements in fiber optic sensing of tissue chemistry related to pathologies or functional perturbations. We will provide examples, including brain tissue and skin. A multimodal approach, incorporating other types of spectroscopies coupled with Raman, will be presented to enhance sensitivity and accuracy in tissue physiology determination. This approach will also be applied to liquid biopsy on various body fluids, demonstrating good sensitivity and accuracy in tissue or systemic pathology assessment. Additionally, we will discuss further improvements in sensitivity and specificity using SERS methodology, utilizing both fiber technology and a new sensor based on evanescent waves. In the latter case, we will showcase ultrahigh sensitivities, paving the way for early-stage detection of pathologies in body fluids.

Detecting A New Quasi-Particle With Raman Spectroscopy

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In materials, new quasi-particles can emerge as collective excitations of ordered states. Detecting these modes and their associated properties is an important step to understanding how the properties of materials combine to create these new phases. Here I will describe our recent efforts to detect a new Higgs Boson from a charge density wave (ie. When electrons form their own lattice). These modes were first observed by Raman almost four decades ago, but were always scalar, or just massive particles. I will discuss our discovery an unconventional axial Higgs mode of the charge density wave in the GdTe3.[1] The Axial Higgs mode is revealed using the interference of excitation quantum pathways in Raman scattering. I will discuss how Raman reveals the specific broken symmetries of the quasi-particle and system leading to its emergence. Providing evidence for a hidden orbital as well as charge density wave.

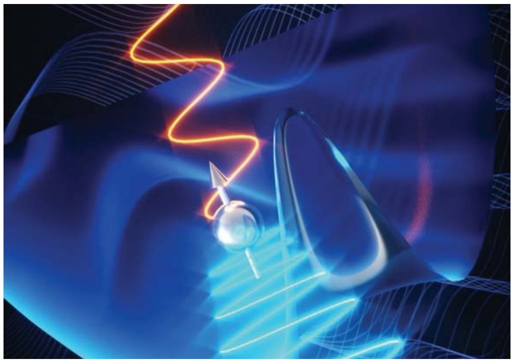

Figure 1: Artist rendition of the Axial Higgs Mode

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Spin rotational excitations in hexagonal *R*MnO$_3$ (*R*=Lu, Y)

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We have observed spin rotational excitations in *R*MnO$_3$ (*R*=Lu, Y) below the Neel ordering temperatures through resonant Raman scattering. We could assign all the spin excitation peaks in terms of Heisenberg spin interaction, and found that the spin rotational angles are predominantly 60, 120, 180 degrees commensurate with the triangular lattice of the Mn-ion spins. Unlike usual spin-wave excitations, this spin rotational excitations are limited to one triangular unit cell, thus costing relatively high excitation energy (~ 0.1 eV). Optically pumped and optically detected spin rotational excitations confined in a triangular cell can be a good candidate for future spin logic/memory devices.

Helium-closed-cycle cryostat was used to control the temperature of the sample from 15 to 120 K in vacuum chamber. Raman scattering spectra were obtained by Horiba LabRam spectrometer coupled with a liquid-nitrogen-cooled CCD under $z(yx)\bar{z}$ cross configuration. Excitation light source was visible red laser which has continuous 671 nm (~1.85 eV) wavelength, with the power of 40 mW on the sample chamber window. Figure 1 shows temperature-dependent Raman spectra of the hexagonal LuMnO$_3$ single crystal in cross polarization scattering geometry. The broad Raman peaks near 197, 580, and 805 cm^{-1} are non-phononic in origin, get stronger at lower temperatures below the Neel ordering temperature. Similar peaks, but at different wavenumbers were observed in singlecrystal YMnO$_3$. These broad peaks below T$_N$ are found to be excited through the resonance with the Mn *d*-*d* transition by the incident red laser (~1.85 eV).

Along with theoretical analyses of the spin-spin interaction Hamiltonian and the Raman selection rules, we could conclude that the spin excitation peaks are due to simultaneous rotation of all three Mn spins in one Mn-trimer in hexagonal *R*MnO$_3$ at the antiferromagnetic state. Only special-angle rotations of the three Mn spins are allowed to preserve the triangular symmetry with the antiferromagnetic ordering, thus the spin rotation angles are quantized due to the triangular symmetry of the hexagonal *R*MnO$_3$.[1]

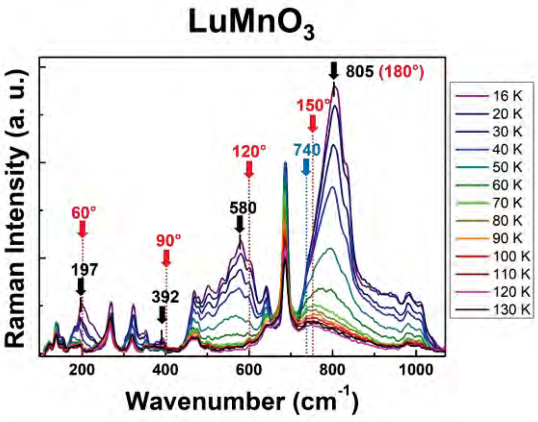

Figure 1 Spin rotational excitation peaks are observed in hexagonal LuMnO$_3$ below T$_N$. The angles denoted are for the spin rotations of all three Mn-ion spins in one trimer with respect to the neighboring spins.

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Polarization-sensitive B-CARS hyperspectral imaging of ferroelectric domain walls

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Raman spectroscopy in terms of both spontaneous Raman scattering (SR) as well as broadband coherent anti-Stokes Raman spectroscopy (B-CARS) enables imaging of local lattice distortions[1,2] and, accordingly, identifying ferroelectric domain walls (DWs).[3] For SR, DW imaging is based on small intensity changes of the bulk lattice peaks at the DW boundary, with the contrast mechanism being well understood to date.[3] B-CARS bears the advantage of an up to 1000x increased signal-to-noise ratio as compared to SR, hence allowing for the much faster data acquisition. Moreover, B-CARS detects additional Raman peaks in lithium niobate (LNO), which further enhances its contrast and allows tracking DWs[4].

In this work, we apply both polarization-sensitive SR and B-CARS for DW imaging to a broad range of ferroelectrics (FEs) such as LNO, lithium tantalate (LTO), and potassium titanyl phosphate (KTP). We find firstly, that B-CARS shows strongly enhanced signals as compared to SR for all materials. Secondly, in comparison to SR, additional Raman peaks occur for both bulk FEs and at DWs (see Figs. 1a,b), resulting in a strong DW-to-bulk contrast that enables *in-situ* identification and localization of the DWs in two-dimensional maps (Figs. 1c). Furthermore, combining the *in-situ* DW localization via B-CARS with depth-dependent SR allows imaging of the DW distribution within the bulk crystal. Hence, a comprehensive DW analysis becomes possible, which reveals exciting information about the structure of domain walls in FE crystals with much faster acquisition times[4], leading to a fundamental understanding of the B-CARS contrast mechanism at two-dimensional interfaces.

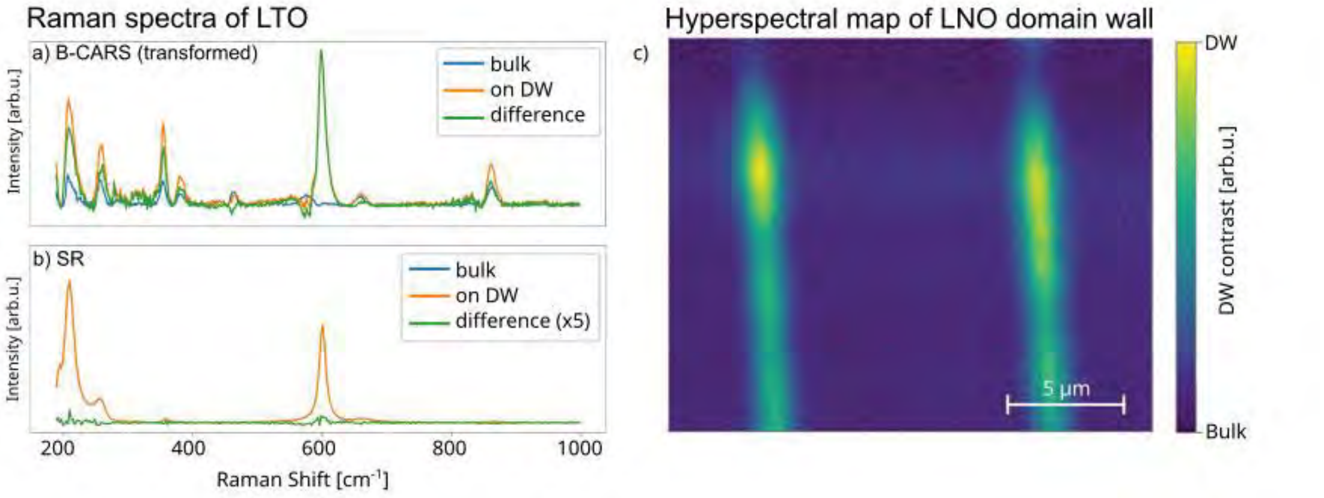

Figure 1: Polarization-sensitive Raman spectra on lithium tantalate (LTO) showing additional peaks for B-CARS (a) in contrast to SR (b). Comparing peaks between bulk and domain walls (DW) reveals a characteristic signature (shown in green) due to both additional peaks as well as intensity changes. (c) *In-situ* DW imaging in lithium niobate (LNO) utilizing B-CARS. DWs are the bright regions.

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Pressure-Induced Topological and Structural Phase Transitions in 3D Topological Insulator $TlBiTe_2$

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In strong spin-orbit coupling systems, pressure (strain) offers a foundation for the fundamental understanding of topological quantum phase transitions (TQPT) and for optimizing material features, such as thermoelectric performance, for technological applications [1]. In this context, materials belonging to the $TlBiX_2$ (X = S, Se, Te) family of ternary chalcogenides have garnered significant attention because of their numerous TQPTs. The compounds $TlBiS_2$, $TlBiSe_2$, and $TlBiTe_2$, with E_g = 0.42, 0.28, and 0.11 eV, respectively, crystallize in a rhombohedral structure (SG: $R\bar{3}m$) with strong interlayer coupling between the layers, resulting in a three-dimensional structure [2]. Recent high-pressure Raman spectroscopy investigations on $TlBiS_2$ combined with X-ray diffraction and first-principles calculations have shown its evolution into topological insulating and topological crystalline phases at 0.5 GPa and 1.8 GPa, respectively [3]. $TlBiS_2$ displays TQPT when Se is chemically substituted at the S site as well. [4]. Here we present comprehensive studies of the 3D topological insulator $TlBiTe_2$ using Synchrotron X-ray diffraction (XPRESS, Elettra) and high-pressure Raman spectroscopy, examining the pressure-induced topological, electronic, and structural phase changes. We comprehend the anomalous electron-phonon interaction associated with the TQPT from the phonon anomalies of A_{1g} and E_g modes under hydrostatic pressure. The phonon anomalies correlate to a transition to a topological crystalline insulator phase at 3 GPa, according to our density functional theory calculations. The bulk modulus of the system and the linear compressibility of the axes were determined by fitting and analyzing the pressure dependence of the unit cell parameters using the Murnaghan equation of state. The X-ray diffraction and Rietveld refinement results show the structural transition from rhombohedral to monoclinic phase starting at 7.5 GPa with the coexistence of phases over some pressure range and another phase transition to a high symmetry phase at 10.7 GPa. Both synchrotron X-ray diffraction and Raman scattering experiments show that the structural transformation is reversible upon pressure release. We have also attempted to unify our understanding of the pressure-induced phenomena of the $TlBiX_2$ (X = S, Se, Te) family of materials by conducting a comparative investigation of their phonon and structural behaviour in this study.

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Planetary Mineralogical Investigation by Raman Spectroscopy

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Raman spectroscopy is a relatively new analytical method for exploring the mineralogical composition of planetary surfaces from rovers and landers. The NASA Perseverance rover includes the first two Raman spectrometers sent to Mars as part of the SuperCam [1, 2] and the SHERLOC (Scanning Habitable Environments with Raman & Luminescence for Organics & Chemicals) [3] instruments. The SuperCam instrument is capable of remote 532 nm Raman spectroscopy along with Laser-Induced Breakdown Spectroscopy (LIBS), Passive Visible and Near Infrared Spectroscopy (VISIR), and Time-Resolved Luminescence Spectroscopy. SuperCam also collects color context images of the targets analysed with an integrated high resolution Remote Micro-Imager (RMI). The integration of multiple analytical modalities enables a more complete description of the samples probed. The SuperCam suite of analytical techniques enables both LIBS elemental quantitative analysis with complementary mineralogical analysis by Raman and VISIR in a co-borsighted configuration.

This presentation will highlight some of the SuperCam Raman observations from the Perseverance rover's exploration of Jezero crater as the foundation for future planetary missions. An integrated Raman and LIBS instrument similar to SuperCam has been proposed to investigate the Venus surface from within the safety of a lander [4]. The Venus surface consists of ~92 atm of supercritical CO_2 at ~465°C. These extreme surface conditions severely limit the lander's operational lifetime and rapid remote analytical techniques like Raman and LIBS are required [4-6].

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Surface Enhanced Raman Spectroscopy for the Detection of Biosignatures on Icy Worlds and Martian Polar Caps

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Surface Enhanced Raman Spectroscopy (SERS) is a powerful technique allowing characterisation of molecules at extremely low concentrations, which is widely used in research and life science. However, the use of SERS in planetary sciences has been limited due to issues associated with SERS substrate degradation and spectra reproducibility. Here we present ongoing work on the Compact Integrated Raman Spectrometer (CIRS) instrument proposed for the Europa Lander Mission Concept [1], which incorporates SERS capability into the green-wavelength Raman system in order to allow detection of biosignatures at extreme low concentrations down to 10nM.

In this work we optimise a SERS method based on chemically stable AgCl precursor coating [2], which is reduced to SERS active AgNPs in-situ, thus eliminating any potential degradation of the SERS active AgNPs during transit. The AgCl coated SERS substrates are irradiated with the CIRS's green laser light in-situ. We optimise the coating fabrication, deposition and activation method for the detection of biosignatures on Icy Worlds and Martian Polar caps and for use in combination with the CIRS' Serially Shifted Excitation (SSE) fluorescence mitigation mechanism, which requires multiple stable Raman spectra taken over a short period of time. We explore the AgCl coating fabrication methods including solution concentration, method of deposition and activation methodology in order to produce reproducible and stable spectra of biosignatures relevant for the search for life on Icy Worlds and Martian Polar regions.

This method greatly enhances the scientific capabilities of the CIRS instrument as it allows the detection of organics and biosignatues at extreme low concentrations, which is critical for the search for life on Icy Worlds and Martian Polar Caps. This enhancement does not require any additional sample preparation or mechanisms, the SERS active coating is applied to the sample cup at little additional cost or complexity. This makes this optimised SERS capability for CIRS a simple and cost effective solution for the search for biosignatures on icy bodies in our solar system and beyond.

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Coherent Phonon Spectroscopy: an emerging alternative to Raman Spectroscopy in Space Exploration?

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Robotic missions to extraterrestrial objects in our solar system are nowadays often equipped with instruments allowing to explore the geochemistry of the surfaces by e.g. identification of their characteristic vibrational fingerprints. Femtosecond lasers have in recent years been shown to be in principle space qualified, opening up the opportunity to explore the potential of different time-domain techniques as compact, robust alternatives to e.g. Raman spectroscopy [1,2]. In this contribution the potential of coherent phonon spectroscopy (CPS) as an emerging in-situ spectroscopic technique to identify solids by their characteristic phonon spectra is discussed based on exemplarily measurements of different (planetary) materials. It is shown that CPS is not hampered by fluorescence backgrounds and allows to avoid bulky opto-mechanics. It is outlined how the bandwidth of the technique is related to the available laser pulse duration. CPS Measurements with a bandwidth of beyond 1000 cm^{-1} (30 THz) and a resolution of better than 4 cm^{-1} (100 GHz) are demonstrated and compared to complementary Raman measurements.

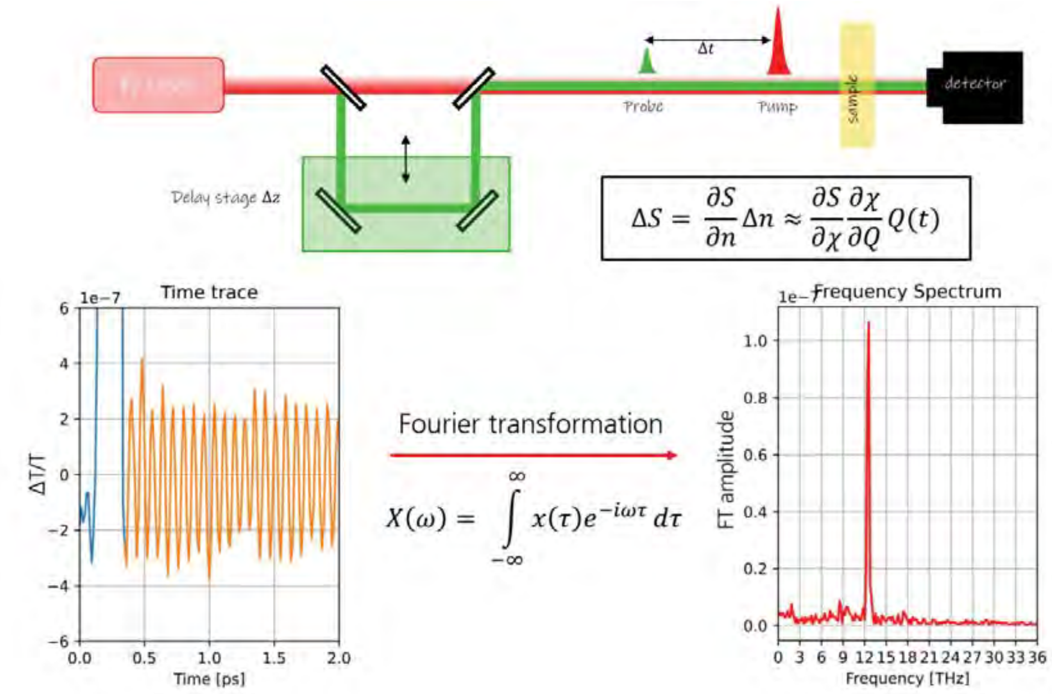

Figure 1: Fundamental principle of CPS. Raman-active phonon modes can be observed by the differential changes ΔS of optical properties in the time-domain which can in principle be probed in transmission, reflection or scattering geometries.

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Analysis of space weathering using correlation of luminescence and Raman signatures for silicate mineral mixes

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Analytical capability of Raman scattering spectroscopy to provide comprehensive clues into geochemical, thermal, or radiation processes that led to the formation and alteration of rock-forming minerals, occurrent at surfaces of atmosphere-less celestial bodies. This causes the intensified development of space research instrumentation (such as the dedicated to in-situ exploration of the Martian Moon Phobos [1]) and new studies related to space weathering (SW) of rock-forming minerals, mainly by the simulated micrometeoritic bombardement [2]. Photoluminescence (PL) contributions to the Raman spectra have been observed for the samples returned from space missions to asteroids [4] and are generally expected to be presented for atmosphere-less bodies. We discuss the use of the correlations between PL and Raman signals (RS) in the spectra of silicate mix samples under simulated SW using pulsed laser ablation (Fig. 1); the relationship of RS/PL contrast to the alteration extent (due individual enthalpy of fusion, melting temperature, irradiation laser absorption) and to the relevant Raman studies on extraterrestrial samples.

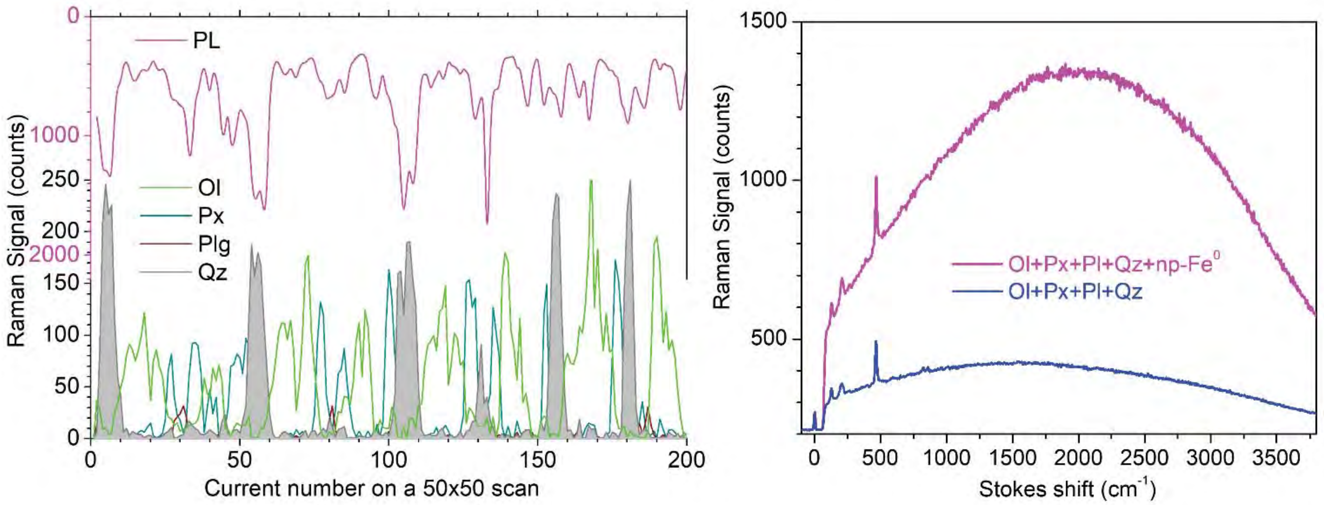

Figure 1: (Left) Correlation of PL signal with particular mineral phases (Qz, Px) in the silicate mix sample; (Right) Typical averaged spectra of Raman spectra scan over original (blue) and irradiated (magenta) mineral mix samples (example: Olivine, Pyroxenes, Plagioclase, Quartz).

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Beyond Single-Molecule Tip-Enhanced Raman Spectroscopy

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Aspirations for reaching atomic resolution with light have been a major force in shaping nano-optics, whereby a central challenge is to achieve highly localized optical fields. The plasmonic picocavity defined by the coinage-metal tip and substrate in a scanning tunneling microscope (STM) can provide highly confined and dramatically enhanced electromagnetic fields, which can modify both the excitation and emission of a single molecule inside the nanocavity and improve the spatial resolution up to a new microscopic limit [1]. In this talk, I shall demonstrate that the spatial resolution of tip-enhanced Raman spectroscopy (TERS) has gone beyond the single-molecule level [2], driven down to the sub-molecular angstrom scale at the single-bond limit [3,4]. Such a capability not only yields a new methodology called scanning Raman picoscopy for structural reconstruction [4], but also enables to track bond breaking and forming of surface reactions [5,6] as well as tip-induced bond weakening [7], all at the single-bond level. I shall also show three recent examples of its applications. The first one is to clarify the chemical enhancement mechanism in TERS through well-controlled local contact environments [8]; The second is to provide a noninvasive Raman thermometry for measuring the local thermal effect of a single molecule under non-equilibrium [9]; The third is to spectrally resolve in real space single nucleobases in single-stranded DNA molecules artificially designed. Our results provide new routes to studies on ultrahigh-resolution molecular recognition, surface reactions, and optical spectromicroscopy at the sub-nanometer scale.

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Mechanistic Understanding of Catalytic Reactions using Tip-Enhanced Raman Spectroscopy

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Over the past two decades, Tip-Enhanced Raman Spectroscopy (TERS) has emerged as a powerful tool for nanoscale surface chemical analysis [1]. By combining the high spatial resolution of scanning probe microscopy with the chemical sensitivity and specificity of surface-enhanced Raman spectroscopy, TERS allows visualization of surface chemical transformations beyond the diffraction limit of visible light [2].

In my talk, I will first provide a brief overview of the fundamental principles of TERS, with a particular focus on the strengths and limitations of AFM- and STM-based TERS techniques. In the second part of my talk, I will demonstrate the practical applications of TERS in the field of heterogeneous catalysis by sharing findings from two different studies conducted in our laboratory. The first study showcases how hyperspectral TERS imaging in combination with molecular-resolution STM imaging and DFT modeling can provide molecular-level insights into the reactive arrangements involved in on-surface photocatalytic coupling reactions [3]. In the second study, I will discuss how TERS can elucidate mechanistic understanding of oxygen activation on bulk Au(111) surfaces [4].

Overall, this talk will highlight the potential of TERS in the nanoscale investigation of surface catalytic processes. Through high sensitivity and nanoscale hyperspectral imaging, TERS can offer valuable insights to advance our mechanistic understanding of surface chemistry.

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Different Enhancement of Individual Modes in TERS depending on the tip and tip-sample distance

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To increase the intensity of the Raman signal, tip-enhanced Raman spectroscopy (TERS) can be used. In TERS a noble metal tip is brought in close proximity to the irradiated molecule which is then excited by the near field of the tip. The processes that lead to the enhancement are numerous and contribute to the total enhancement to varying amounts [1, 2]. A selective and well-aimed variation of measurement parameters may help to elucidate the role of individual enhancement processes in TERS.

To gain insight into the role of individual parameters we used a well-defined substrate and molecule system to limit the number of variations between measurements. We used a thermally evaporated few-layer coverage of Hexadecafluoro-cobaltphtalocyanine (CoPc-F_{16}), which aimed to limit the variation in molecular orientation. We employed a template stripped gold film as the substrate to ensure an ultra-smooth surface avoiding the SERS effect. The systematic tip-sample distance change was controlled using a shear force feedback.

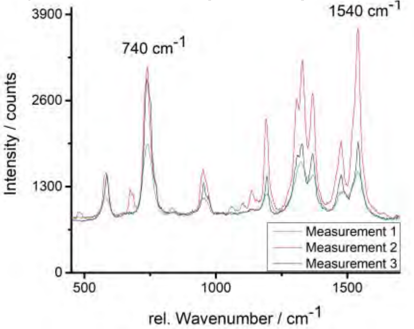

Figure 1: Influence of different tips on the intensity of individual modes

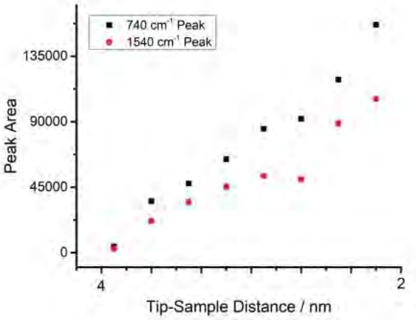

Figure 2: Peak area increase for different tip sample distances using the same tip

We observed that for the same kind of sample, different tips produced TERS of different relative peak ratios. In figure 1, TERS measurements with three different tips were shown. The peak at 740 cm^{-1} shows greater maxima for tips 1 and 3. For tip 2 the peak at 1540 cm^{-1} is most intense. Furthermore, even with the same tip, the TERS enhancements of different vibrational modes showed different dependences on the tip-sample distances. In figure 2, when the tip-sample distance is reduced, the intensity for the 740 cm^{-1} peak increases faster than the 1540 cm^{-1} one. Possible reasons for these varied spectral features were discussed considering 1) the charge transfer processes for variations in the molecular orientation [2]; 2) the symmetry of Raman modes vs. the electromagnetic field polarization; 3) the exact positions of few-atoms protrusions in either the tip or the substrate [1]. Further variation of measurement parameters, such as the substrate used, will offer additional insights into the observed spectral variations and their possible sources.

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Quantum Mechanical Modelling of Plasmon-enhanced Raman Spectroscopy

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Recent TERS experiments suggest unexpectedly high lateral resolutions in the molecular or even sub-molecular regime, despite the size of the plasmonic particle.[1,2] To elucidate the interaction between the plasmonic nanoparticle and the immobilized substrate, i.e., in the scope of TERS, a quantum mechanical description is indispensable – addressing the so-called “chemical effect” as well as the “electromagnetic effect”. In this contribution, we go beyond the computational description of the non-resonant chemical effect, as already introduced successfully by our group based on 3-dimensional grid calculations at the density functional level of theory (DFT),[3] while the immobilized molecule is mapped by the plasmonic “tip” mimicked by a single silver atom. Firstly, resonant effects as well as charge transfer states are incorporated by time-dependent DFT (TDDFT) simulations to cover the complete “chemical effect”, exemplarily for the surface-immobilized tin phthalocyanine (SnPc).[4] Secondly, complementary electrodynamic calculations based on the finite element method are performed to describe the electric field confinement in the near-field. The static electric field, obtained in the vicinity of an atomistic sized feature – the “electromagnetic effect”, is then incorporated in the quantum chemical simulations. This way, TERS spectra and intensity maps of SnPc are computationally evaluated under non-resonant and resonant conditions, see Figure 1.[5] Finally, our computational approach is applied exemplarily to elucidate the thermodynamics of plasmon-induced reactions.[6]

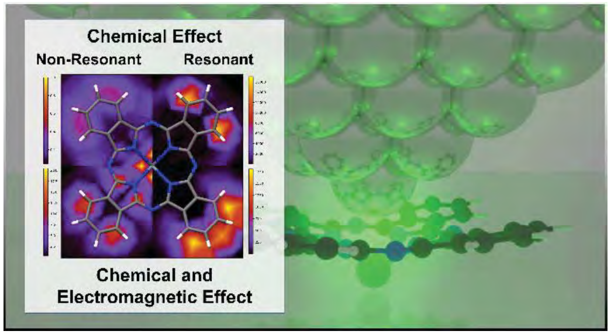

Figure 1: Simulated TERS maps of SnPc under non-resonant (DFT) and resonant (TDDFT).

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Real-time, real-space TDDFT for near-field Raman spectroscopy with a multipolar Hamiltonian

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Near-field Raman spectroscopy with multipolar Hamiltonians offers a powerful theoretical tool for analysing molecules at the nanoscale, overcoming limitations of conventional methods. While traditional ab initio codes struggle with non-dipolar excitations typical of near-field interactions, our approach leverages a multipolar Hamiltonian within real-time, real-space time-dependent density functional theory (RT-RS-TDDFT) [1-2]. This framework enables computation of both on- and off-resonance Raman spectra, providing a comprehensive theoretical foundation for near-field Raman spectroscopy.[3]

In the multipolar Hamiltonian, the interaction between the near field and molecule is described by the spatial integration of the inner product of the molecular polarization field and electric field, both of which can depend on the position as follows:

$$H_{\mathrm{int}}(\boldsymbol{r},t) = -\int \widehat{\boldsymbol{P}}(\boldsymbol{r}',t)\cdot\boldsymbol{E}(\boldsymbol{r}',t)d\boldsymbol{r}'$$

where $\widehat{\boldsymbol{P}}$ is the electric polarization field operator, whose explicit form is

$$\widehat{\boldsymbol{P}}(\boldsymbol{r}) = \sum_{\alpha} e_{\alpha}(\widehat{\boldsymbol{q}}_{\alpha}-\boldsymbol{R})\int_{0}^{1}\delta[\boldsymbol{r}-\boldsymbol{R}-\lambda(\widehat{\boldsymbol{q}}_{\alpha}-\boldsymbol{R})]d\lambda$$

where e_{α} and $\widehat{\boldsymbol{q}}_{\alpha}$ are the charge and position operator of the αth electron in the molecule, respectively. While the Taylor expansion of this electric polarization field gives multipoles such as dipoles, quadrupoles, and octupoles, our method uses the original form of the polarization field without any approximation.

Using this interaction Hamiltonian, near-field-induced electronic excitations were calculated with the RT-RS-TDDFT program Octopus. For the Raman spectra, the near-field-induced dipole moments of a target molecule are calculated for slightly distorted geometries from its optimized geometry and then the normal coordinate derivatives of the induced dipole moment of the molecule are obtained.

We calculate near-field Raman spectra of benzene using a model near-field that mimics tip-enhanced Raman spectroscopy (TERS) in scanning tunnelling microscopy (STM) setups [4, 5]. To avoid chemical interactions, longer tip-molecule distances are employed. Off-resonance selection rules are well-understood by considering both the near-field's spatial structure and molecular vibrations. Under on-resonance conditions, the spectra are strongly influenced by the excited state's nature. Notably, even when the near-field forbids the equilibrium π-π* transition, vibronic couplings from structural distortions can activate on-resonance Raman spectroscopy.

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Experimental and theoretical characterization of 1,3-Diphenyl-3-tosylpropan-1-one

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ABSTRACT

1,3-Diphenyl-3-tosylpropan-1-one (DTP) was studied by recording its FT-IR (4000-400 cm-1), FT Raman (4000-50 cm-1) and UV-Visible spectra (200-400 nm). Barrier heights around flexible bonds were estimated by evaluating torsional potentials using optimized structure. Density functional theory, utilizing B3LYP exchange-correlation functional along with 6-311++G(d,p) basis set, was used to determine the ground state structure parameters, general valence force field, harmonic vibrational frequencies, potential energy distribution, intensities of infrared and Raman bands, oscillator strengths and absorption maxima for electronic transitions in a solution of DMSO-d6 with TD-DFT, frontier molecular orbital characteristics, NLO behaviour, NBO parameters, Fukui functions, molecular topology and MESP analysis. The observed and estimated quantities for structural parameters, IR, Raman and UV-Vis transitions showed good agreement. All the frequencies of the molecule were assigned unambiguously for the first time using PED and eigenvectors. The NBO study confirmed that the chemical was suitable for NLO applications. The drug characteristics of the molecules were evaluated by using drug-likeness. The target protein and the ligand reactions were investigated using molecular docking.

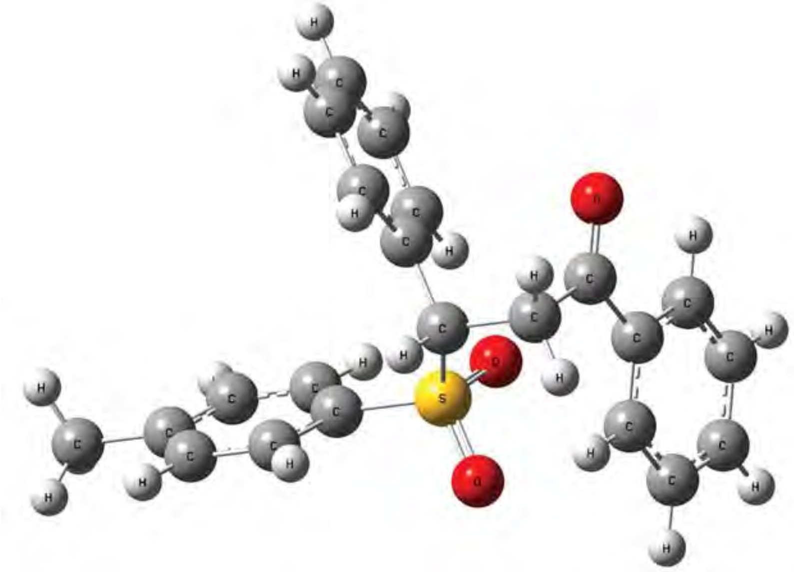

Figure: Optimized molecular structure of DTP

Automated, turnkey infrared and Raman spectra from first-principles

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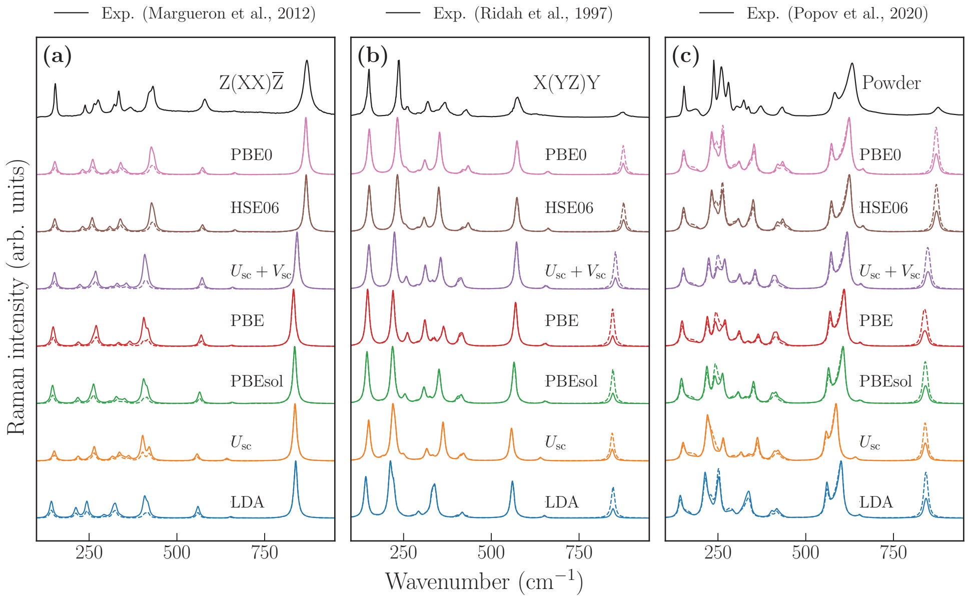
We present an automated, open-source, user-friendly approach based on density-functional theory to allow seamless calculation of first-principles infrared absorption and reflectivity, Raman spectra, complex dielectric tensors and non-linear optical susceptibilities. By employing finite displacements and finite fields we allow for the use of any exchange-correlation functional or pseudopotential formalism, as well as an efficient treatment of large and low-symmetry structures. To demonstrate the capabilities of the approach, we use different semi-local, Hubbard corrected, and hybrid functionals to study ferroelectric LiNbO3, finding the best agreement with experiments when using extended Hubbard or hybrid functionals. The need for advanced funcionals is seen even more pronouncedly when looking at the spectra of Li-ion phospo-olivine cathodes, that show a close agreement with experiments when using self-consistent extended Hubbard corrections (DFT+U+V). The entire framework (aiida-vibroscopy)[1] is fully and openly available, and exploits the back-end capabilities of AiiDA and the Quantum ESPRESSO distribution, together with a front-end AiiDAlab GUI.



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Lipid metabolic profiling via quantitative stimulated Raman scattering imaging opens up new avenues for precision medicine

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Lipid droplet (LD) is a dynamic organelle closely associated with cellular functions and energy homeostasis. Dysregulated LD biology underlies an increasing number of human diseases. Commonly used lipid staining and analytical tools are difficult to provide the information regarding LD distribution and composition at the same time. To address this problem, stimulated Raman scattering (SRS) microscopy uses the intrinsic chemical contrast of biomolecules to achieve both direct visualization of LD dynamics and quantitative analysis of LD composition with high molecular selectivity at the subcellular level. Recent developments of Raman tags have further enhanced sensitivity and specificity of SRS imaging without perturbing molecular activity. Taking these advantages, SRS microscopy has offered great promise for deciphering LD metabolism in single live cells. Our work showcases the applications of SRS microscopy as an emerging platform to dissect LD biology in cancer development and progression. For instance, our study developed advanced SRS imaging methods to quantitatively map lipid molecules in LDs in liver fibrosis, cirrhosis and hepatocellular carcinoma. Our data revealed that the lipid homeostasis was remarkably dysregulated in the late-stage compared to the early-stage fibrosis, including increased unsaturated triglycerides with decreased lipid unsaturation degree and decreased membrane fluidity. Such alterations were likely due to up-regulated lipogenesis, desaturation, and peroxidation, which consequently led to endoplasmic reticulum stress and cell death. Inspiringly, injured hepatocyte could be rescued by remodeling lipid homeostasis via either supply of unsaturated fatty acids or enhancement of membrane fluidity. Moreover, in contrast to the conventional wisdom that unsaturated lipids enhance tumor proliferation, we unexpectedly found substantial amount of saturated fat accumulated in hepatocellular carcinoma, whereas considerable lipid peroxidation products seen in their adjacent cirrhosis tissues. Such lipid accumulation was shown to be closely correlated with patient prognosis. Collectively, our work improves current understanding of the role of LD in liver disease development and progression.

Exploring Skin Physiology and Pharmacology through CARS Microscopy

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The application of Coherent anti-Stokes Raman Scattering (CARS) microscopy in biomedical research represents an extension in our ability to study complex biological systems with unique resolution and specificity. This presentation highlights the use of CARS microscopy to explore various aspects of skin physiology and pharmacology, demonstrating the technique's versatility and its potential to extend our understanding of biological tissues and treatment efficacy.

Firstly, utilizing CARS microscopy enabled the direct observation of time-dependent, spatially resolved diffusion of water (D2O) within human skin tissue, unveiling significant variations in diffusion coefficients across different strata of the Stratum Corneum (SC). This heterogeneity challenges the prevailing notion of the SC as a monolithic barrier, instead positing it as a complex, layered defense mechanism. This study show CARS microscopy's ability to quantitatively measure diffusion coefficients at varying tissue depths and locations.

Further, the examination of dissolvable microneedles for transdermal drug delivery underlines CARS microscopy's unique ability to visualize penetration of the microneedles and follow their morphology within the skin. By providing detailed images of microneedle degradation and drug dispersion, CARS microscopy offers valuable insights into the mechanisms of drug release and absorption, supporting the development of more effective transdermal therapeutics.

Additionally, employing CARS microscopy in conjunction with a perspiring skin simulator to assess sunscreen substantivity offers a novel approach to studying the interaction between topical formulations and physiological processes like sweating. This application highlights the technique's potential to dynamically follow the redistribution of specific active ingredients in the sunscreen during perspiration. Thus, evaluate product performance under realistic conditions, informing the design of more resilient and effective sunscreens.

Overall, these studies exemplify the power of CARS microscopy to provide detailed, molecule-specific insights into complex molecular interactions in tissue.

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Pre-resonance Raman and SRS for detecting carotenoids; applicability to Alzheimer Disease brain tissue samples

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Alzheimer's disease (AD) is considered the main cause of dementia. It is characterized by extracellular deposits (plaques) of aggregated amyloid peptides. We developed a workflow of label-free and non-invasive techniques to study post mortem AD human brain tissue. Working with snap-frozen, unstained tissue slices allows us to do so as much as possible in their native form [1]. Suspected amyloid plaques are first localized by means of green autofluorescence; that area is then Raman mapped at 532 nm. Surprisingly, we observed that (cored) plaques consistently show strong Raman bands around 1150 and 1514 cm^{-1}, indicating the presence of carotenoids (see Figure). In absence of pre-resonance enhancement (785 nm exc.) these compounds were not detected. The association of carotenoids (known as powerful anti-oxidants) with plaques had not been reported before, and their role in the development of AD requires further study.

Since spontaneous Raman mapping is rather slow and therefore unpractical for larger tissue areas or numbers of samples, stimulated Raman scattering (SRS) was also tested on brain tissue samples, using two synchronized ps NIR laser beams. We observed the expected blueshift of the Amide I peak (typical for proteins in a beta-sheet conformation), but with our NIR-SRS system (off-resonance) carotenoids could not be detected [2]. Most recently, in order to enhance the SRS sensitivity and selectivity for carotenoids, a frequency doubling unit was added to the OPO signal and idler beams. In combination with the 532-nm beam, this resulted in a tuneable blue output for resonance SRS, or in a tuneable yellow output for pre-resonance SRS. Preliminary findings with these visible-SRS configurations in comparison with NIR-SRS will also be discussed.

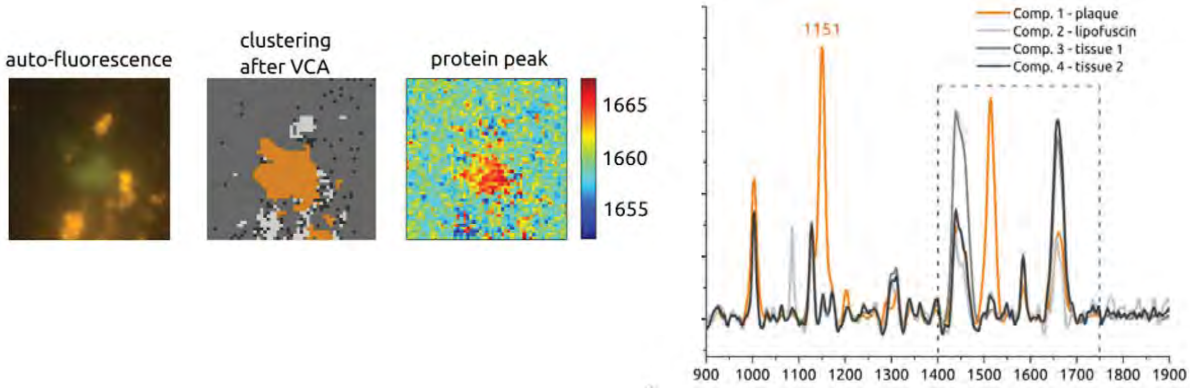

Figure 1: Fluorescence and Raman imaging of AD plaque area; the orange spectra recoded at the plaque location show typical carotenoid bands at 1150 and 1514 cm^{-1}; exc. 532 nm [2]

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AI-driven endomicroscopic morphochemical imaging for tumor identification and fs-laser ablation for selective tissue removal

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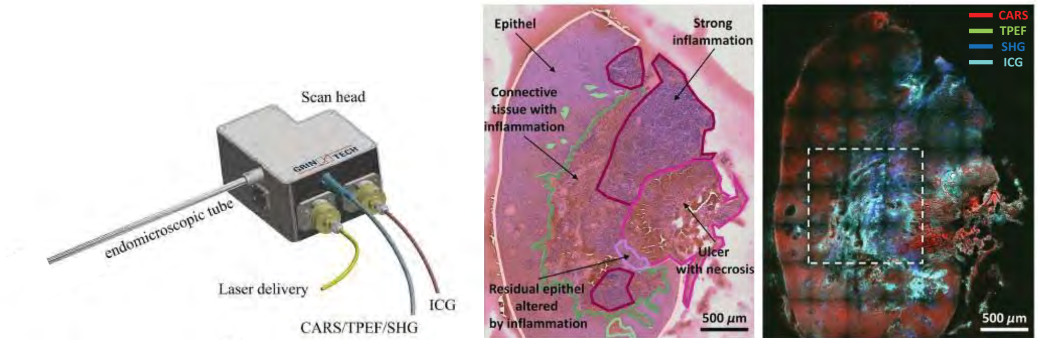
A major challenge in modern medicine is the effective prevention, diagnosis and treatment of cancer. However, diagnostic and surgical tools for early detection and minimally invasive treatment are still lacking. Laser-based endoscopy is among the most promising technologies to improve cancer diagnostics. Endoscopic probes allow the implementation of non-invasive imaging modalities and laser systems for tissue ablation into compact platforms.

To assess both chemical composition and morphology of tissue, we combine the nonlinear imaging techniques coherent anti-Stokes Raman scattering (CARS), second harmonic generation (SHG) and two-photon excited fluorescence (TPEF) in a single rigid endomicroscopic system for label-free head and neck cancer diagnosis. The use of multiple imaging modalities allows the detection of molecular changes at early stage of disease progression and enables distinguishing cancer from healthy tissue. Alongside the imaging capability, we integrate the system with a high-power femtosecond laser to implement fs-laser ablation and remove selected parts of tissue.

A complete endomicroscopic system with a field of view (FOV) of 650 µm and a lateral resolution of 1 µm has been developed and assessed[1]. The device has been tested in a preclinincal study, where a cohort of 15 patients was examined *ex-vivo*, using multimodal nonlinear microscopy to diagnose cancer. The measurements have demonstrated high signal quality and the ability to analyze the tissue composition. Deep learning-based semantic segmentation was used to automatically differentiate malignant from healthy tissue, achieving a sensitivity and specificity of 88% and 96% respectively. To combine diagnostics with treatment, machine learning driven image-guided selective tissue removal was demonstrated, integrating femtosecond laser ablation into the endomicroscope. This enables a completely novel intraoperative seek-and-treat approach, paving the way for a new surgical treatment.



(a) (b) (c)

Figure 1: (a) Endomicroscopic system for multimodal imaging and laser ablation. (b) H&E stained image of a head and neck tissue slice with annotations from a pathologist. (c) Multimodal image of the same tissue slice combining 3 nonlinear imaging modalities (CARS, TPEF and SHG) with linear fluorescence from indocyanine green (ICG).

Acknowledgments

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Machine learning tools for SERS sensing and synthesis of nanoparticles

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SERS and Raman spectroscopy yields large data sets with information-rich spectra. Classical linear methods have limitations, especially for SERS spectra of single molecules, where the spectra are highly dependent on the orientation of molecules on surfaces, and for large data sets [1]. Methods from data sciences are increasingly used to classify spectra into categories and predict SERS spectra for new data based on trained algorithms [2]. In one example, classification of single molecule spectra will be shown for neurotransmitters and other biological metabolites were identified with a barcoding data processing method, processed with TensorFlow using a convolutional neural network architecture [3]. This machine-learning driven data processing significantly improved the positive assignment rates for a series of metabolites and allows for complex measurements of the cell's biochemistry. In another example, efforts for the control and optimization of nanoparticle synthesis using a continuous flow chemistry approach that can be controlled with machine learning will be presented. Finally, efforts in the classification and concentration prediction will be presented with different sensing schemes.

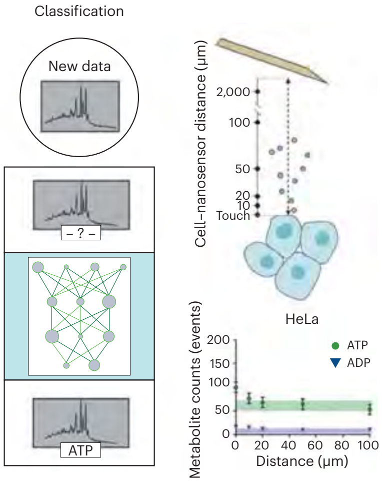

Figure 1: Convolutional neural network processing of SERS spectra allows for multiplexed detection of metabolite gradients

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SERS Sensing Applied to Drug Discovery

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Monitoring dynamic processes in complex cellular environments requires the integration of uniformly distributed detectors within such three-dimensional (3D) networks, to an extent that the sensor could provide real-time information of nearby perturbations in a non-invasive manner. In this context, the development of 3D-printed structures that can function as both sensors and cell culture platforms, emerges as a promising strategy, not only for mimicking a specific cell niche but also toward identifying its characteristic physico-chemical conditions, such as concentration gradients [1,2]. This talk will provide an overview of the fabrication of 3D cancer models that incorporate hydrogel-based scaffolds containing gold nanoparticles [3,4]. In addition to sustaining cell growth, the printed nanocomposite inks display the ability to identify cancer-relevant metabolites, as well as to uncover drug diffusion profiles by surface-enhanced Raman scattering, with high spatio-temporal resolution [5,6]. We propose that the acquired information could pave the way to designing novel strategies for drug discovery in cancer therapy, through correlation of drug diffusion with cell death.

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Deep learning assisted real time field of view optimization in stimulated Raman histology for brain tumor detection.

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An alternative technique to conventional histology is Stimulated Raman Histology (SRH). It has been reported to provide images comparable to H&E staining in a significantly shorter time [1]. SRH tiles are acquired using Stimulated Raman Scattering (SRS) for two Raman shifts 2845 cm^{-1} and 2930 cm^{-1} corresponding to CH_2 and CH_3 chemical bonds. After acquisition, tiles are processed, stitched and mapped to an H&E-like colour code to generate an image suitable for diagnosis [2]. However, the information provided by the scanned tiles may not always be relevant leading to a waste of valuable time. Here we propose a path finding algorithm coupled with a convolutional neural network (CNN) to optimize the mosaicking task performed by an SRS point scanning microscope on brain tumour samples. The proposed CNN architecture automatically detects regions of interest for diagnosis, *i.e.* zones where cell nuclei are sufficiently contrasted with respected to the background. Starting at a random position, our strategy aims to find a path that maximizes the presence of relevant areas.

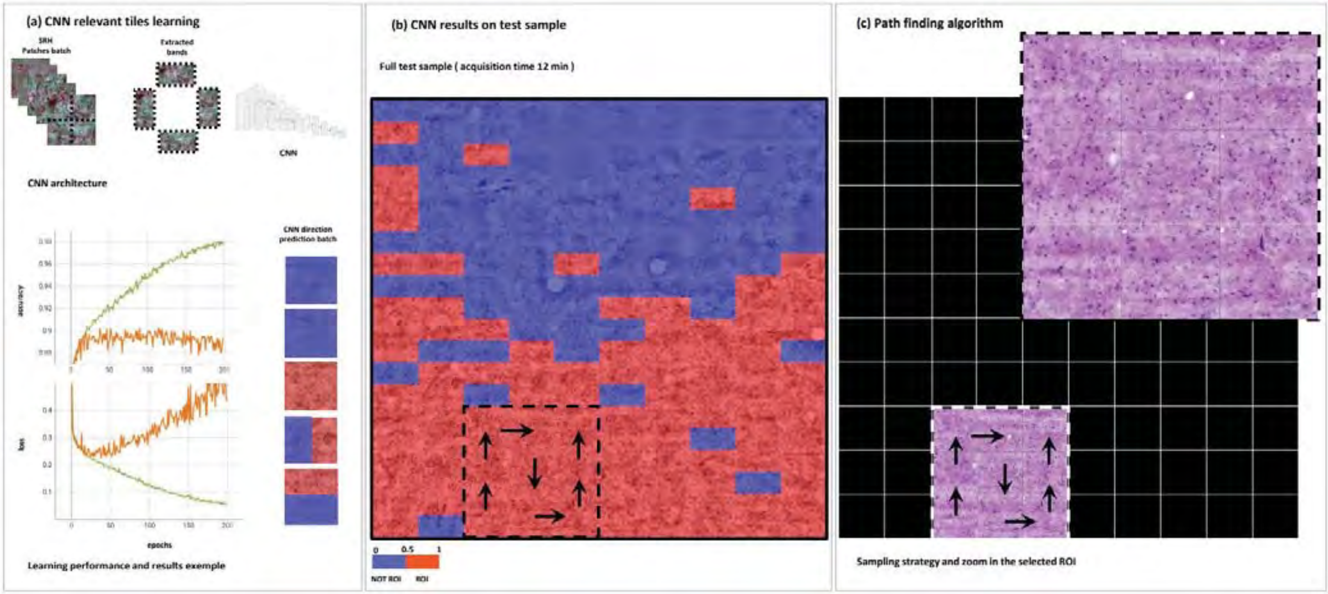

Figure 1: Design and performance of the mosaicking approach. A CNN **(a)** is trained to discriminate relevant tiles (i.e. containing histopathological useful features for diagnosis) from empty or useless tiles. Probability heatmap **(b)** illustrates our classification performances on a test sample. The arrows indicate the direction given by the border with the higher ROI probability **(c)** Starting from an empty map, our path finding algorithm designs an acquisition plan maximising relevant content while scanning a reduced number of tiles.

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Transfer Learning Techniques for Raman Spectra Classification Using Artificial Training Data

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Raman spectroscopy, a non-invasive vibrational spectroscopy technique providing specific molecular fingerprints, features significant potential in biomedical applications [1], particularly for rapid diagnosis. However, the method faces challenges due to the scarcity of substantial spectral datasets needed to train deep learning models owing to the limited availability of samples and the inherent weakness of the Raman effect requiring long measurements times to record the training data. This study addresses these issues by generating an artificial spectral dataset using GFN2-xTB [2], a semi-empirical quantum chemistry method, for the small molecule set in the Worldwide Protein Database (wwPDB) [3]. The dataset is used to pretrain deep learning models which can then be applied to real data and improve the models' performance on experimental dataset of bacterial Raman spectra using transfer learning techniques. An overview of the study is shown in Figure 1. The artificial dataset includes variations such as broadening, noise, and artefacts, mimicking real-world conditions to improve model robustness. By adjusting parameters, the inherent flexibility of this approach enables the generation of synthetic data which can be tailored to specific scenarios, thereby enhancing model applicability and precision. The study evaluates the effectiveness of transfer learning with different deep learning models using cross validation schemes. Additionally, Explainable AI (XAI) methods have been explored to gain deeper insights into the models' decision-making processes. These efforts are aimed at improving the interpretability of deep learning applications in Raman spectroscopy, ensuring more reliable and insightful analyses in biomedical applications.

Figure 1: An overview of the workflow: Synthetic dataset generation for Transfer Learning

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Raman spectroscopy for the study of ancient materials and objects: from the laboratory to sites

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Raman spectroscopy has been used to study cultural heritage materials and objects for over 40 years now, although the explosion in the number of studies has occurred more recently in conjunction with instrumental revolutions that have increased analytical performances [1]. The study of these heritage samples and objects raises a number of analytical challenges due to their complex chemistry and the constraints imposed by their specific characteristics (heterogeneity, diverse nature, need for non-invasive approaches, capability for portable measurements, etc.). After a brief general history of the studies in the field, this presentation will give an overview of the recent contributions of Raman spectroscopy to various issues related to cultural heritage: from material identification and knowledge of the history of technologies to issues related to the conservation of objects.

Special emphasis will be given to the original contributions of Raman spectroscopy to these issues. On the one hand, questions of data acquisition, whether in the laboratory or in the field with portable instruments, and instrumental parameters for optimising the results will be addressed. On the other hand, we will present strategies for the extraction of meaningful information from spectra that require both spectroscopic know-how and the ability to take into account the specific characteristics of heritage materials. The examples presented will illustrate the diversity of materials for which Raman spectroscopy is relevant, including natural materials, whether inorganic, organic or composite, as well as materials that have been transformed or synthesised by man. These studies will therefore cover very different time periods and cultures.

The topics addressed will cover the following areas. How the mineralogical characterisation of archaeological artefacts allows us to study the circulation of prehistoric raw materials. What the identification of natural organic substances used in "paint materials/colours" tells us about the variety of substances used and technical developments from Prehistory to Modern Art. The way in which the study of ceramic pigments and opacifiers illustrates the complex chemistry of these Arts of Fire and thus enables the technological innovations of enamelling and their transfer to be traced. And finally, how the characterisation of metal corrosion products provides tools for diagnosing their state of conservation.

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Corrosion, the most worrying issue for curators: Raman spectroscopy applied to the study of metals

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Metal corrosion processes are among the most aggressive degradation factors for archaeological and modern cultural heritage artefacts. In the case of archaeological tools, the characterisation of both the original composition, the corrosion layer and degradation compounds is crucial to obtain data on the manufacturing processes. In addition, a well-defined multi-analytical approach is key to an in-depth characterisation of the degradation patterns experienced by the artefacts due to aggressive exposure to the atmosphere after centuries of burial.

On the other hand, the development of corrosion-resistant materials has given rise to a number of innovative alloys, which offer new creative opportunities for artists. Corten steel, for example, provided an alive material for artists and architects because, depending on the environment to which it was exposed, its surface took on different shades and textures. These specific characteristics attracted professionals because of the opportunities the material offered from a creative point of view.

However, the surrounding environment could alter the normal corrosion processes, leading to the degradation and destruction of the material and the pieces. A correct multi-analytical study of these degradation patterns and the identification of the physical-chemical stressors is the basis for developing and applying the most appropriate conservation and restoration treatments, ensuring the preservation of metal pieces and materials.

In this way, Raman spectroscopy has proven to be a very versatile analytical technique for a complete evaluation of the structure of a metal and a better understanding of its evolution in specific environments.

In this work, we present different case studies in which Raman spectroscopy was key to elucidate conservation problems (Figure 1). In addition, its potential when combined with other analytical techniques will also be explored.

Figure 1. Portable Raman spectroscopy in an in-situ steel sculpture study.

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Direct Point Analysis and 2D-Mapping on Easel Paintings from the National Gallery-Alexandros Soutsos Museum (ATH, GR)

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Raman spectroscopy has proven to be a powerful approach for the analysis of cultural heritage objects. Moreover, when the technique is applied with mobile systems the results are very promising, thus working towards the non-invasive analysis of the artefact. Compared to laboratory studies, when bringing mobile Raman spectroscopy instruments on the field, challenges can arise from many factors including environmental conditions, ambient light, the measurement of the unknown etc. but also from the characteristics of the systems themselves.

During 2023, a multi analytical campaign was realized that took place in the National Gallery-Alexandros Soutsos Museum in Athens, Greece. The main question was to identify and document the palette of selected painters (Nikiforos Lytras, Andreas Kriezis, Francesco Pige and Symeon Savvidis) of the early 20th century belonging to the permanent collection of the museum.

Mobile Raman spectroscopy acted as the cornerstone of the research campaign, identifying the pigments of the artists. Together with point analysis, 2D direct Raman spectroscopy mappings were performed revealing the distribution of the pigments used in particular details of the paintings; some are heavily varnished. Handheld X-ray fluoresence (hXRF) and hyperspectral imaging (UV-vis-NIR) were used as auxiliary techniques to document more the materials and their mixtures, thus shedding light on the production technique of these unique masterpieces.

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Photonic Data Science: Challenges and Opportunities in Raman Spectral Analysis

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Photonic measurement techniques, including vibrational spectroscopy such as Raman-spectroscopy, are increasingly used in various disciplines, such as life science and medicine. This increased utilization is linked to improvements in measurement methods and setups, as well as the development of data science methods and computational infrastructures. With these photonic data science methods, it is feasible to detect and extract high-level information from subtle differences in (bio-medical) Raman spectra. The high-level information in this case depends on the task and the sample. For instance, it may involve predicting tissue types, disease states, or properties of the samples, such as concentrations of constituents.

Vibrational spectroscopic techniques, such as Raman spectroscopy and nonlinear variants of it like coherent anti-Stokes Raman spectroscopy, offer several advantages. They can be used as non-destructive fingerprinting techniques. To fully utilize the potential of vibrational fingerprints, it is important to study the entire data life cycle of spectroscopic data, from its generation to archiving, in a holistic manner. This includes careful consideration of experimental design, sample size planning, data pre-treatment, data pre-processing, chemometric and machine learning-based data modeling, and model transfer methods. Transfer learning is also an important aspect to consider. All aspects and methods mentioned above need to be combined in a data pipeline to standardize vibrational data and extract reliable high-level information.

Herin, recent studies aimed at constructing a standardized data analysis pipeline for biomedical Raman spectra and image data [1,2,3] will be presented and pitfall, challenges and opportunities in this pipeline will be highlighted. Additionally, follow up studies to a European ring trial [4] for Raman spectra aiming at the comparability of Raman spectra between instruments and labs will be presented.

Acknowledgements

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On-the-Fly Raman Microscopy Guaranteeing the Accuracy of Discrimination

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Accelerating the measurement for discrimination of samples, such as classification of cell phenotype, is crucial when faced with significant time and cost constraints. Spontaneous Raman microscopy offers label-free, rich chemical information but suffers from long acquisition time due to extremely small scattering cross-sections. One possible approach to accelerate the measurement is by measuring necessary parts with a suitable number of illumination points. However, how to design these points during measurement remains a challenge. To address this, we developed an imaging technique based on a reinforcement learning in machine learning (ML). This ML approach adaptively feeds back "optimal" illumination pattern during the measurement to detect the existence of specific characteristics of interest, allowing faster measurements while guaranteeing discrimination accuracy. Using a set of Raman images of human follicular thyroid and follicular thyroid carcinoma cells, we showed that our technique requires 3333-31683 times smaller number of illuminations for discriminating the phenotypes than raster scanning. To quantitatively evaluate the number of illuminations depending on the requisite discrimination accuracy, we prepared a set of polymer bead mixture samples to model anomalous and normal tissues. We then applied a home-built programmable-illumination microscope equipped with our algorithm, and confirmed that the system can discriminate the sample conditions with 104-4350 times smaller number of illuminations compared to standard point illumination Raman microscopy [1]. The proposed algorithm can be applied to other types of microscopy that can control measurement condition on the fly, offering a new approach for the acceleration of accurate measurements in various applications including medical diagnosis. We will also present our bar-code-type on-the-fly Raman microscopy if time allows.

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A Deep Learning Assisted Identification of Single-Molecule Peptide Post Translational Modification using Plasmonic Nanopore Surface-Enhanced Raman Spectroscopy (SERS)

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Nanopore resistive pulse sensors are emerging technologies for single-molecule protein sequencing [1]. But they can hardly detect small post-translational modifications (PTMs) such as hydroxylation in single-molecule level. While a combination of surface enhanced Raman spectroscopy (SERS) with plasmonic nanopores can detect the small PTMs, the blinking Raman peaks in the single-molecule SERS spectra leads to a big challenge in data analysis and PTM identification. Herein, we developed and validated a one-dimensional convolutional neural network (1D-CNN) for amino acids and peptides identification from their PTMs including hydroxylation and phosphorylation using their SERS spectra, named Single Amino acid and Peptide Network (SAPNet) [2]. Our work combines cutting-edge plasmonic nanopore technology for SERS signal acquisition and deep learning for fully automated extraction of information from the SERS signals. The SAPNet model achieved an overall accuracy of 99.06% for the identification of amino acids from their modification, and 96.85% for the identification of peptides from their PTM translation. We also evaluated the model with out-of-sample examples with good performance. Our work can be beneficial for early detection of diseases such as cancers and Alzheimer's disease.

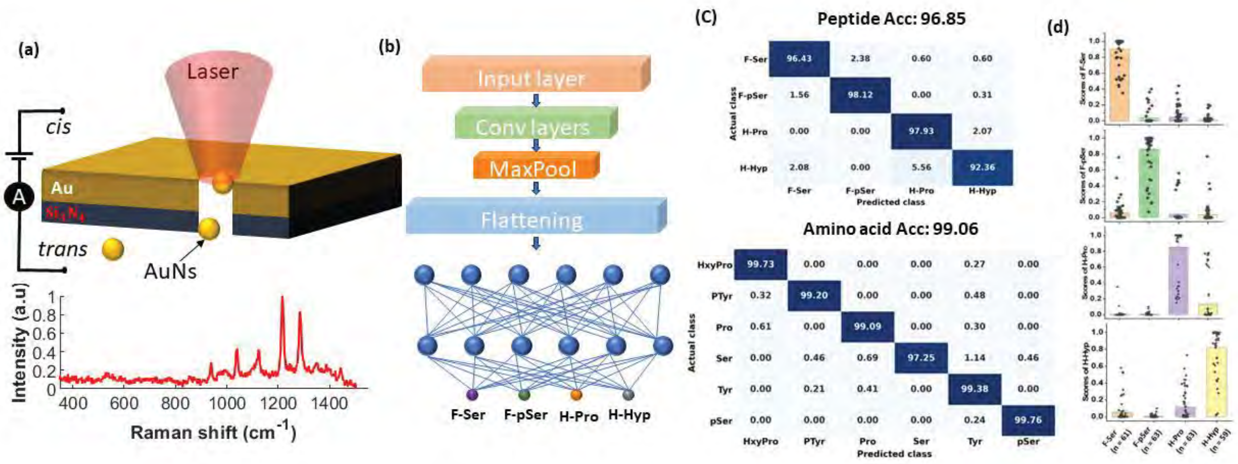

Figure 1: (a) Plasmonic particle-in-pore single -molecule SERS signals acquisition sensor, (b) the highlights of SAPNet model, (c) the overall testing set accuracy of SAPNet model, and (d) the post-evaluation probability scores of peptides.

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Towards a combined Surface-enhanced Raman scattering, Seed amplification assay and Machine Learning approach facilitating Alzheimer's disease diagnosis

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Alzheimer's disease (AD) is the most common neurodegenerative disorder in the elderly with an incidence that progressively increases worldwide. One of the main neuropathological hallmarks of AD is the presence of amyloid-b protein (Aß) aggregates which forms extracellular amyloid plaques. Current diagnostic protocols for AD rely on a comprehensive analysis involving clinical, instrumental, and laboratory assessments. However, signs, symptoms and biomarker alterations observed in AD might overlap with other form of dementias, resulting in misdiagnosis [1].

In this work we present a new diagnostic approach for AD which takes advantage of the boosted sensitivity in biomolecular detection, as allowed by seed amplification assay (SAA), combined with the unique specificity in biomolecular recognition, as provided by surface-enhanced Raman spectroscopy (SERS) [2].

The SAA-SERS approach supported by machine learning data analysis [3] allowed efficient identification of pathological Aβ oligomers in the cerebrospinal fluid of patients with a clinical diagnosis of AD or mild cognitive impairment due to AD [4].

Such analytical approach can be used to recognize disease features, thus allowing early stratification and selection of patients, which is fundamental in clinical treatments and pharmacological trials.

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From copper surface synchrotron studies to deep UV-SERS applications

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The UV range, in particular, is very promising for proteins because they specifically absorb light below 400 nm, thereby resonantly amplifying vibrational modes characteristic of the peptide backbone and some amino acid residues. A large number of different fabrication strategies are available for the fabrication of a powerful SERS substrate. However, despite its enormous potential, SERS has not yet been applied in routine bioanalytical applications, often due to the challenges of preparing low-cost/efficient SERS surfaces with high reproducibility and signal amplification properties. The use of copper as a plasmonic material could play a crucial role in the field of SERS research, as this issue is very poorly understood and studied. According to existing publications, the SERS activity of copper (Cu) nanostructures is associated only with the near-infrared spectral range (600-800 nm), where copper exhibits weaker SERS activity compared to gold or silver nanostructures.

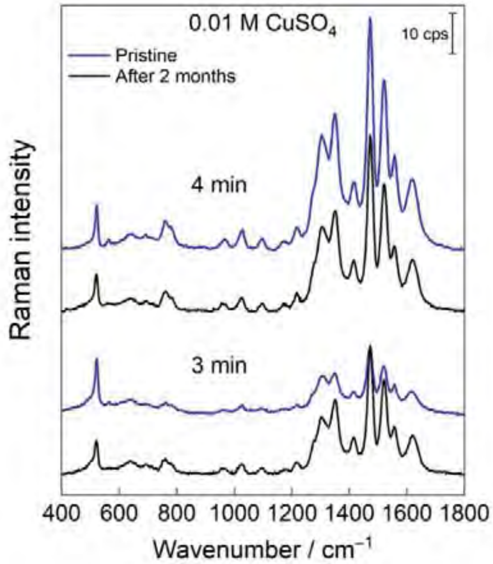

Figure 1: SERS spectra of 10^{-5} M adenine under 325 nm excitation (3 mW; 1 s) on 3 min and 4 min grown copper-based surfaces measured at initial (pristine) stage and after surface storage at ambient conditions for 2 months (after 2 months).

In the present work, for the first time on the basis of Cu/CuO_x nanostructures deposited on silicon surface using galvanic displacement effect, we found significant enhancement of Raman signal of biomolecules (adenine, guanine, riboflavin, KSCN) by copper nanostructures at excitation wavelengths of 229, 244, 257, 325 nm. The obtained copper surfaces show significant chemical resistance to oxidation, which may be related to the binding of biomolecules to the surface. In addition, to study the interaction of biomolecules with the copper surface and possible energy transfer, the copper surface was coated with a layer of silica deposited by ALD. The surface peculiarities were studied by X-ray photoelectron and absorption near edge structure spectroscopies at the Solaris synchrotron (PHELIX). A more detailed discussion of the discovery will be presented in the paper. From this point of view, our discovery may provide a new stimulus for the development of materials for UV-SERS applications.

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Phonon anharmonicity in confined carbyne probed by Raman spectroscopy

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Confined carbyne are long linear carbon chains (>100 atoms) encapsulated in carbon nanotubes and represents the closest realization of carbyne, the truly 1D carbon allotrope [1]. The interest in confined carbyne is driven by the outstanding predicted properties of carbyne, including exceptional optical activity and intriguing metal-to-semiconductor phase transition [2]. Recently, DFT studies predicted that (confined) carbyne shows this phase transition only at unphysically large temperatures, because of quantum anharmonicity [3]. The properties of confined carbyne are independent of the chain length but are modulated by the hosting nanotube due to strong chain-nanotube interactions [4].

In this work, we experimentally investigated the phonon anharmonicity of confined carbyne by studying the higher-order modes of the Raman-active C-mode (Fig. 1a) of carbyne chains by confocal and tip-enhanced Raman spectroscopy. We observed large frequency shifts in C-mode overtones compared to the expected harmonic frequencies (*i.e., n*-times the C-mode frequency, Fig. 1b), implying strongly anharmonic properties. We noticed that the relative frequency spacings between experimental C-mode overtones and harmonic approximated spacings increase for decreasing chains' C-mode frequency. We interpreted our results with a simple analytical model, showing that the nanotube host diameter parametrically modulates the C-mode anharmonic contribution.

Our findings enable further insight into the properties of confined carbyne, guiding us to a comprehensive description of this material as a necessary step toward its application in real devices.

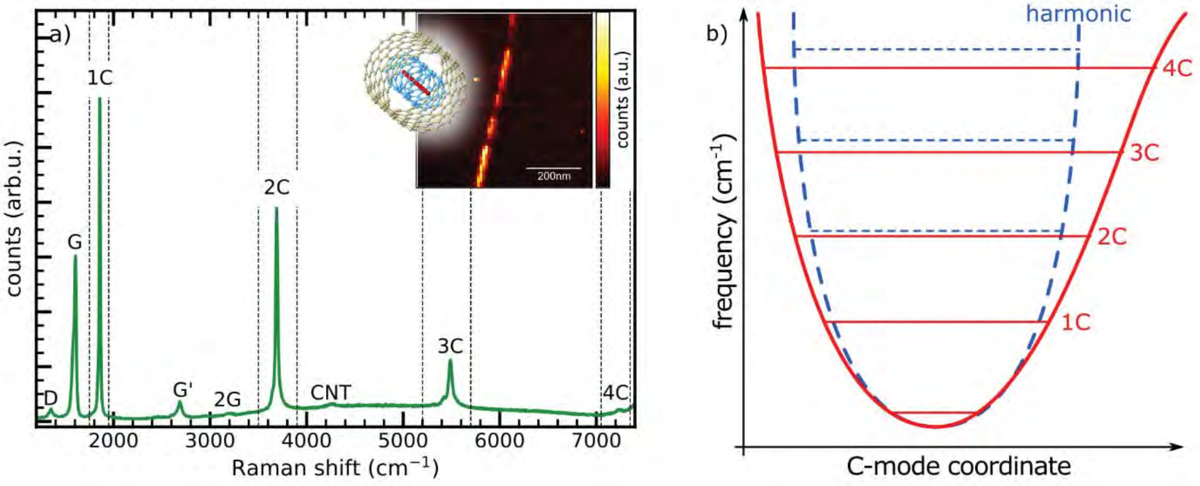

Figure 1: a) Raman spectrum of confined carbyne collected at 532 nm. In the inset, a representation of a confined carbyne and a tip-enhanced Raman image of a single confined carbyne chain. b) Experimental vibrational frequencies of a carbyne chain's C-mode (red lines) compared to the expected harmonic frequencies (blue dashed lines). The potential energy surfaces are schematically shown.

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High pressure Raman study of dipeptide glycyl-L-phenylalanine

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Peptides have garnered considerable attention owing to their substantial potential for multifaceted applications. These molecules comprise organic compounds formed through the linkage of amino acids by covalent bonds, commonly referred to as peptide bonds. In this study, we investigate the vibrational properties of the dipeptide glycyl-phenylalanine (Gly-L-Phe) utilizing Raman and infrared spectroscopic techniques. Employing a diamond anvil cell, a small sample of Gly-L-Phe underwent investigation via Raman spectroscopy, extending up to 7 gigapascals (GPa). The Raman-active modes within the spectral range of 100 to 3200 cm^{-1} were scrutinized, revealing alterations across the entire spectrum at pressures nearing 1.0 GPa. In summary, several effects were observed: the disappearance and emergence of modes in the low wavenumber region of the spectrum; a shift in the wavenumber of the deformation modes involving carbons of the molecule's skeleton; a shift in the wavenumber of the deformation modes involving carbons belonging to the ring and the carboxyl group; changes in wavenumber and intensity of bands associated with ring torsion mode; the splitting of a band associated with the NH_3^+ group. This investigation provides novel insights into a critical system wherein hydrogen bonding plays a fundamental role.

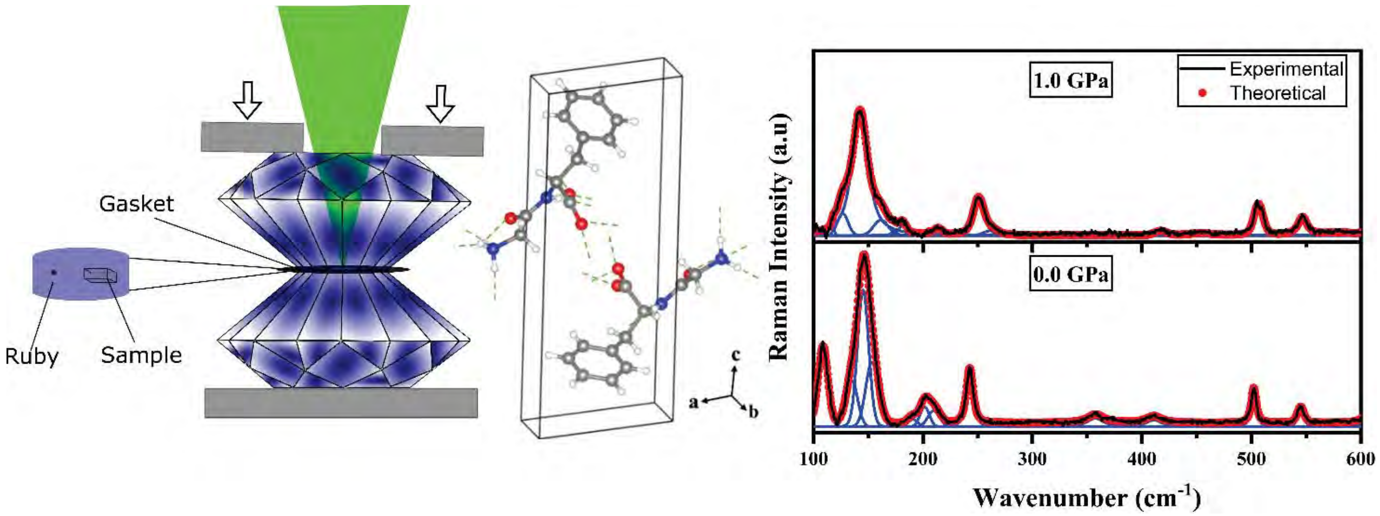

Figure 1 : Raman spectra of dipeptide Gly-L-Phe at selected pressure.

Plant nanobionic sensor based trace VOC detection

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Demands on efficient VOC sensors have been uprising from industrial, medical, and environmental monitoring field. VOC sensors for those usages need to be capable of detecting low-concentration VOC with high conveniency, but lack of sensitivity and complex instrumental structure limit their usage on practical VOC sensing. Moreover, those sensors possess fundamental limitation in that essentially accompany separate configurations for VOC collection and concentration. Herein, to overcome above problems and build effective VOC sensor to efficiently detect trace analytes, we suggest plant-based nanobionic VOC sensor which can simultaneously act as a concentrator and SERS substrate for VOC compounds. After surface modifications with polymer which stabilize nanoparticle and often specifically capture targeted molecule, plasmonic NIR-nanoprobe was integrated with plant leaf. Our plant-based nanobionic VOC sensor enables sensitive and rapid for VOC analyte detection within few minutes. Such sensor was capable of multiplexed detection of VOC analytes and integrated with customized portable Raman device to demonstrate feasibility for on-field VOC sensing. By varying the surface modification strategy, we proved practical usage of such sensor on detecting various VOC analytes.

Raman Instrumentation for Planetary Exploration

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Planetary applications of Raman systems require low mass, volume, and power consumption. A number of scientific groups have developed advance Raman hardware for planetary applications (e.g., [1-3]). For the first time, two complementary Raman spectrometers (SuperCam and SHERLOC) on board NASA's Mars 2020 Perseverance Rover are exploring Jezero Crater since the rover landed on Mars on February 18, 2021. SHERLOC is a Deep UV micro-Raman and luminescence system on the rover arm, useful for high spatial resolution proximity measurements [4], while SuperCam has a 532-nm pulsed laser-based system and allows long-range time-resolved Raman and luminescence measurements with lower spatial resolution [5, 6]. These Raman Instruments are providing unambiguous information about the types of minerals, hydrated and anhydrous inorganic salts, and their distributions on the Martian surface.

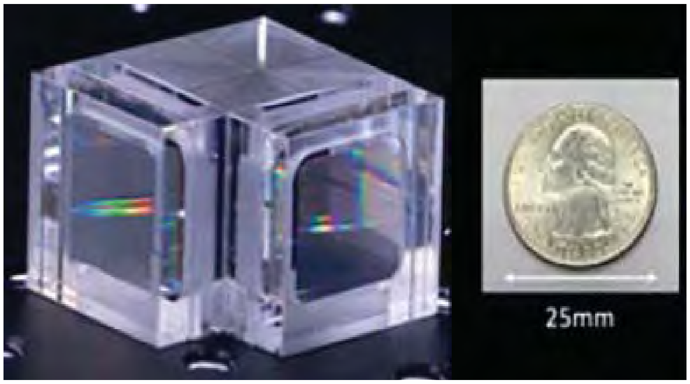

Figure 1: Two-grating monolithic SHRS (2g-mSHRS) compared to the size of a US quarter [7]

This talk will briefly discuss existing planetary Raman systems and present one of the emerging Raman technologies, namely, a miniature monolithic Spatial Heterodyne Raman Spectrometers (SHRSs) that provides significantly low mass (80 g) and volume (3.5 x 3.5 x 2.5 cm^3) with higher spectral range and resolution (Fig. 1). The SHRSs can provide capability for use with visible and UV laser excitations for future space exploration.

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Raman Laser Spectroscopy in Planetary Exploration: A historical perspective.

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For decades Laser Raman Spectroscopy has been recognized as a powerful analytical technique for the chemical and structural study of materials in the solid, liquid or gaseous state. Given this, its application to planetary exploration was quite natural and was proposed from the beginning of the rover-based robotic exploration of Mars surface [1].

Applications have extended over the following years with the proposal of Raman or combined Raman-LIBS instruments for the exploration of Mars [2, 3, 4] and Phobos [5], Venus [6], Europa [7] and our Moon [8]. Part of these instruments are successfully operating on the red planet (SuperCam, Sherloc) others are on track for the launch (RAX, RLS) and others are under development or study for new planetary or lunar missions.

This work presents a summary of these developments with particular interest in the Raman instrument for the Exomars “Rosalind Franklin” Rover 2028 mission to Mars, the Raman-Laser Spectrometer (RLS). The work will be presented from a historical perspective emphasizing the key role of RLS capabilities to fulfil the mission scientific objectives. Among those stand out the possibility to detect traces of past or present life on Mars through the combined analysis of subsurface samples at the mineral grain scale.

The work will also include a discussion on innovative specific Raman concepts with potential applications to future planetary exploration missions.

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1000 Sols of Supercam Raman Activities On Mars – Review Of Data Processing And Mineral Detections At The Jezero Crater

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The Perseverance rover landed in Jezero Crater, Mars, in February 2021, carrying the SuperCam instrument [1-3], a multi-analytical instrument suite featuring LIBS, time-resolved Raman and luminescence spectroscopy (TRR-LS), visible and near-infrared (VISIR) reflectance spectroscopy, imaging and a microphone. When operating in Raman mode, the SuperCam instrument uses a frequency-doubled 1064 nm pulsed excitation laser source with a 4 ns pulse, typically acquiring 100 to 400 shots per analyzed spot [2].

Conducting Raman spectroscopy on a different planetary surface is challenging and has required great efforts to optimize the Raman acquisition and post-processing of the Raman data acquired on the surface of Mars. During the mission SuperCam Raman has been key for the robust identification of olivines, perchlorates, carbonates and sulfates including the remarkable identification of anhydrite veins present on the sedimentary deposits at the delta front of the Jezero crater paleolake. Regular acquisition of Raman data on several SuperCam Calibration targets [3] enabled to monitor the degradation suffered by organic samples [4] and minerals [5] under the Martian environment. A summary of these results will be discussed as part of this work.

Raman data was acquired on 500+ points during the first 1000 sols of operations on Mars, providing key insights into the Martian mineralogy, confirming the significant potential and utility that Raman spectroscopy will hold in planetary exploration missions.

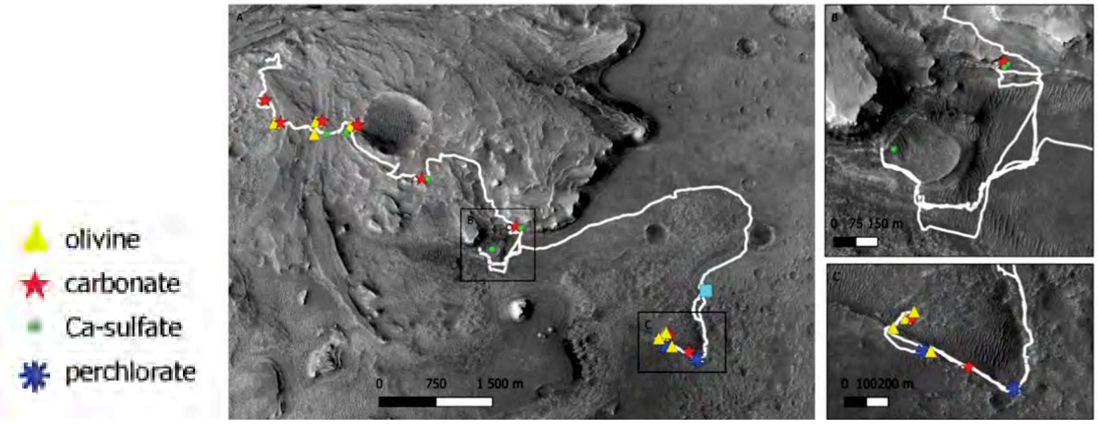

Figure 1: Summary map of detections by SuperCam Raman on the Jezero crater during the first 1000 sols

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Molecular optomechanical approach to Surface-Enhanced Raman Scattering

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Molecular vibrations constitute one of the smallest mechanical oscillators available for nanoengineering. The energy and strength of molecular oscillations depend on the attached specific functional groups as well as on the chemical and physical environments. By exploiting the inelastic interaction of molecules with optical photons, Raman scattering can access the information contained in molecular vibrations. However, the low efficiency of the Raman process typically allows only for characterizing large numbers of molecules. To circumvent this limitation, plasmonic resonances supported by metallic nanostructures and nanocavities can be used to localize and enhance light at optical frequencies, enabling surface-enhanced Raman scattering (SERS), where the Raman signal can be dramatically increased. This enhancement enables few- or even single-molecule characterization. The coupling between a single molecular vibration and a plasmonic mode constitutes an example of an optomechanical interaction, analogous to that existing between cavity photons and mechanical vibrations [1].

Optomechanical systems have been intensely studied because of their fundamental interest as well as their application in practical implementations of quantum technology and sensing. In this context, SERS brings cavity optomechanics down to the molecular scale and gives access to larger vibrational frequencies associated with molecular motion, offering new possibilities for novel optomechanical nanodevices. The molecular optomechanics description of SERS is recent and its implications have only started to be explored. The quantum description of molecular optomechanics is fully consistent with standard classical and semiclassical models of SERS, and it provides a framework particularly suited to address novel regimes of fundamental and practical interest in SERS, giving rise to the emergence of non-linear effects, collective phenomena involving many molecules, or the modification of the effective losses and energy of molecular vibrations due to plasmon–vibration interaction.

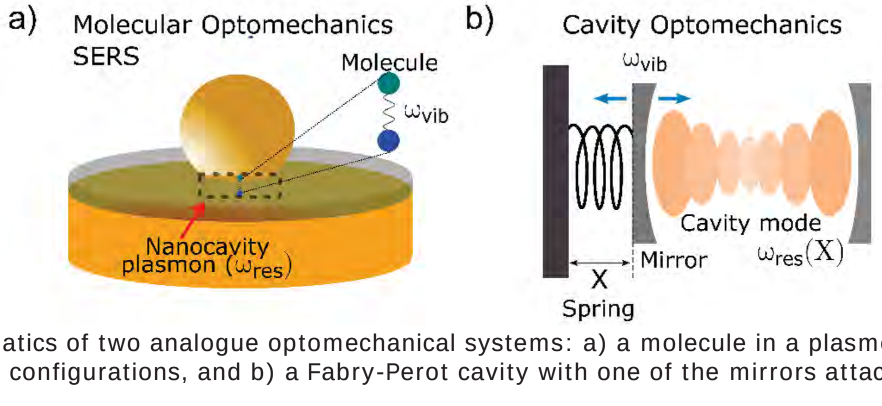

Figure 1: Schematics of two analogue optomechanical systems: a) a molecule in a plasmonic nanocavity as in typical SERS configurations, and b) a Fabry-Perot cavity with one of the mirrors attached to a spring.

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Probing Chemistry at the Ångström-Scale via Tip-Enhanced Raman Spectroscopy

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My research group is interested in investigating how local chemical environments affect nanostructure properties with Ångström scale resolution. This talk will start with Tip-Enhanced Raman Spectroscopy (TERS), which affords the spatial resolution of traditional Scanning Tunneling Microscopy (STM) while collecting the chemical information provided by Raman spectroscopy. By using a plasmonically active material for our scanning probe, the Raman signal at the tip-sample junction is incredibly enhanced, allowing for single-molecule probing. This method, further aided by the benefits of ultrahigh vacuum, is uniquely capable of controlling localized plasmons via an atomistic approach. We are able to obtain (1) single-molecule chemical identification; (2) structural identification of new synthetic 2D materials; (3) atomic-scale insights into the oxygen reactivity of 2D materials; (4) local strain effects in an organic/2D materials heterostructure. By investigating substrate structures, superstructures, 2D materials lattices, and the adsorption orientations obtained from vibrational modes, we extract novel surface-chemistry information at an unprecedented spatial (< 1 nm) and energy (< 10 wavenumbers) resolution. Another application of localized surface plasmons is to achieve site-selective chemical reactions at a sub-molecular scale. We recently selectively and precisely activated multiple chemically equivalent reactive sites one by one within the structure of a single molecule by STM tip-controlled plasmonic resonance. Our method can interrogate the mechanisms of forming and breaking chemical bonds at the Ångström scale in various chemical environments, which is critical in designing new atom- and energy-efficient materials and molecular assemblies with tailored physical and chemical properties.

Imaging Electronic and Atomic Motion in Molecules

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Abstract

Motion of electrons is at the heart of any chemical transformation, photo-induced charge or energy transfer process in molecules. Contemporary techniques in ultrafast science have the capability to generate and trace the consequences of this motion in real-time, but not in real-space. Scanning tunnelling microscopy (STM), on the other hand, can locally probe the valence electron density in molecules, but cannot provide by itself dynamical information at this ultrafast time scale. In my talk, I will show you how dynamics of coherent superposition of valence electron states generated by < 5 femtosecond long carrier-envelope-phase (CEP) stable laser pulses, can be locally probed with angstrom-scale spatial resolution and 300 attosecond temporal resolution simultaneously, at the single orbital-level with the help of an STM, defying the previously established fundamental space-time limits [1-4]. Electronic motion in molecules is usually coupled with atomic motion, especially in molecules undergoing photo-induced charge/energy transfer, structural or chemical transformation. In order to understand this coupling, we have recently realized femtosecond broadband coherent anti-Stokes Raman spectroscopy (CARS) in an STM and it has enabled tracking of coherent atomic motions in a single graphene nanoribbon with sub-angstrom scale spatial, meV energy and ~20 fs temporal resolution, simultaneously [5-6]. Time-resolved CARS implemented in an STM is the key to probing both electronic and atomic motion at the same time. These recent developments pave the way towards direct real space-time imaging of chemical reactions and phase transformations in two-dimensional materials.

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Atomically Resolved Tip-Enhanced Raman Scattering Imaging of Chemisorbed Molecules

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While TERS images of physisorbed molecules are relatively straightforward to interpret due to their minimal interactions with the substrate and flat adsorption geometry [1], chemisorbed molecules require more stringent analysis because the adsorption geometry becomes often 3-dimensional through chemical bonds. Therefore, the complexity in the case of chemisorption involves the inhomogeneous polarization of the interior of the adsorbate through multiple imaging planes and altered polarizability stemming from the chemical interaction with the substrate. Furthermore, as the tip approaches the molecule within a subatomic gap distance, electron tunnelling takes over the field excitation, requiring accurate description of the electronic structure of the molecule-substrate complex.

In this talk, the theoretical modelling addressing the abovementioned complexity to reproduce experimental images will be discussed.

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Towards Single-Molecule Sequencing: Resolving Single Nucleobases in a Single-Stranded DNA by using TERS

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DNA molecules are made up of four types of nucleotide subunits linked with a sugar-phosphate backbone, and the sequence of these nucleotides are crucial for understanding the enormous diversities in living organisms and also for applications in biological and medical fields. Although some single-molecule DNA sequencing techniques (e.g., the nanopore technique) have attracted extensive attentions because of their potential advantages to specify single nucleobases without any amplification or labelling, their heavy reliance on the electric current measurements alone suffers from insufficient chemical information for sequencing, limiting their accuracy in nucleobase identification. Recently developed tip-enhanced Raman spectroscopy (TERS) technique has emerged as a promising tool for visualizing the chemical structure of a single molecule with both sensitive chemical specificity and high spatial resolution at the sub-nanometer scale [1−3], which has been applied to spatially resolve adjacent DNA-base molecules adsorbed on surfaces [4]. Here, we carry out a proof-of-principle study to demonstrate in real space the spectral identification of individual nucleobases within a short single-stranded DNA (ssDNA) molecule. By delicately combining three strategies including tip-apex engineering, cryogenic cooling and pulse-injection deposition, sub-nanometer resolved TERS imaging for the well-defined ssDNA molecules on atomically clean metal surfaces can be achieved. Such a capability of single-nucleobase resolution can be better revealed by panoramic TERS mapping, which not only resolves adjacent nucleobases in a single ssDNA, but also provides important information on the adsorption configuration of the molecule including the spatial arrangements of bases and sugar-phosphate backbone. The further combination with the matching algorithm and machine learning enabling us to identify the single nucleobases and even their orientations in a long ssDNA chain. Our technique also provides a way to track the modification or activity sites in other bio-molecules including glyco-peptide complexes, which could be crucial for understanding their biological functions and might be instructive for the design of drug molecules, DNA damaging/repairing, and even gene-editing treatment.

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Nonlinear and collective effects in Surface Enhanced Raman Spectroscopy

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A quantum mechanical theory of Surface Enhanced Raman Spectroscopy (SERS) based on molecular optomechanics predicts non-linear effects with increasing laser intensity I_{las} that go beyond the classical description [1,4]. At low I_{las}, this framework recovers the classical enhancement of the signal with the fourth power of the field enhancement induced by an optical cavity. At large I_{las}, it describes more complex behaviours including a quadratic scaling of the anti-Stokes signal with I_{las} due to vibrational pumping [5], a divergence called parametric instability and changes on the energy of the molecular vibrational modes.

In this talk, we discuss the molecular optomechanical theory and describe the variety of nonlinear effects captured by this framework. We highlight that this formalism indicates that, for large enough I_{las}, the molecules cannot be treated as independent, contrary to the typical assumption. In contrast, the cavity-mediated molecule-molecule interactions lead to the emergence of novel collective effects that strongly increase the Raman signal (Figure 1) [6,7]. Last, we compare the theoretical predictions with experimental measurements [7]. This work thus shows that the molecular optomechanical framework can open a new landscape of intriguing phenomena in SERS [8].

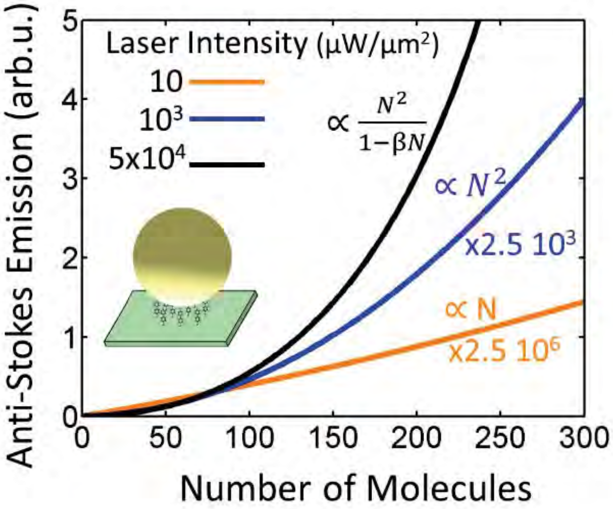

Figure 1: Scaling of the anti-Stokes emission with number of molecules for different laser intensities, illustrating the collective SERS response in the non-linear regime.

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Chemical and orientation contributions to the SERS enhancement factor of resonant molecules

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Surface Enhanced Raman Spectroscopy (SERS) enhancement factors (EFs) are central to SERS [1]. They are crucial to our fundamental understanding of the mechanisms, electromagnetic and chemical, underlying SERS. They are also one of the most important considerations for many applications: How large is the EF? Does it apply equally to all analytes? Is it sensitive enough for trace or even single molecule detection?

One of the main difficulties in pinning down a general definition and method for measuring SERS EFs is that for a given SERS substrate, the SERS EF is strongly molecule-dependent [2]. Two important factors for this dependence are chemical enhancements and orientation effects.

In this talk we will discuss the importance of these effects for SERS in resonance Raman conditions (SERRS), typically when using dyes. We will argue that dyes are particularly susceptible to chemical enhancements effects due to adsorption-induced shifts in their resonance conditions [3] and discuss examples where orientation further affects SERS EFs [4]. These results will be discussed in the context of improving the reliability of SERS EFs determination and comparison.

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Plasmon-Enhanced Ultrafast and CW Raman Spectroscopies: A Unified Theoretical Description and Comparison with Experiments

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Spontaneous and stimulated Raman spectroscopies provide label-free, diffraction and sub-diffraction limited imaging capabilities, particularly valuable for biomedical applications. Plasmonically enhanced (PE) nonlinear versions of these spectroscopies can potentially provide even higher sensitivities enabling more rapid chemical imaging of a wider range of analytes for "real time" applications. A unifying density matrix framework for treating all plasmon-enhanced molecular spectroscopies is developed.[1] The temporal description of PE optical electric fields of any pulse duration is an essential first step for this approach. The effects of the complex plasmonic enhancement factor on ultrafast, picosecond and cw pulses based on an idealized Lorentz oscillator model and observed dielectric properties of metal nanoparticle structures is shown. In particular, plasmonic enhancement effects on the optical phase, carrier frequency and pulse duration of incident ultrafast pulses are demonstrated. Unlike spontaneous PE Raman (SERS), the locally generated signal field of plasmon-enhanced stimulated Raman spectroscopies is also itself enhanced by a plasmonic response. A density matrix framework formulism is used to describe plasmonically enhanced femtosecond stimulated Raman scattering (FSRS), stimulated Raman gain/loss (SRG/L), impulsive stimulated Raman, CARS and spontaneous Raman. Plasmonic enhanced ultrafast pulses result in Raman spectroscopies that display dispersive vibrational line shapes (FSRS) [2] as shown in Figure 1, or mixed dichroic and birefringent nuclear coherences (pump-probe) in agreement with experimental observations.

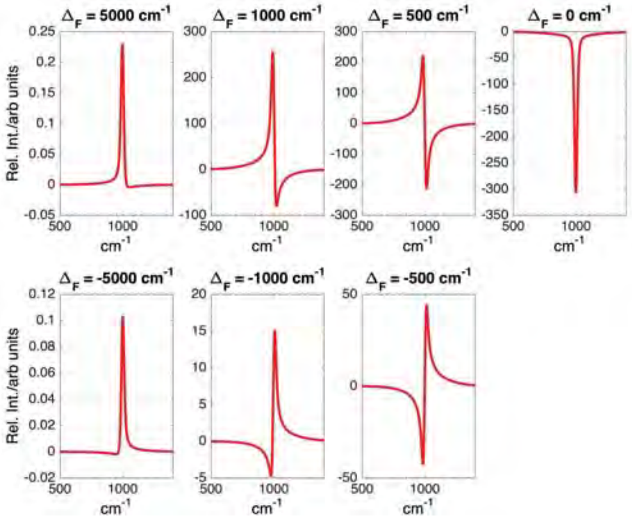

Figure 1: Calculated PE-femtosecond stimulated Raman spectra (PE-FSRS) for a 1000 cm^{-1} Raman active mode as a function of detuning between the femtosecond probe and the plasmon resonance frequency (Δ_F).

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Molecular Modeling of (Resonance)-Raman spectroscopy of Large Systems

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Resonance Raman (RR) spectroscopy -and especially UVRR- has demonstrated potentialities to unveil the structural and electronic properties of systems providing information about local environments.[1] The tunability of the incident wavelength to match a specific electronic transition of the system allows for the enhancement of particular vibrations associated with specific chromophores and gives RR selectivity and sensitivity.[2] The technique is employed to study many biological systems experimentally. Its molecular modeling can serve as a tool to interpret experimental spectra and predict UVRR profiles when measurements are unavailable. However, computing the property is far from trivial[3,4] and all the effects arising from electronic and vibrational parts (electronic transitions, normal modes, and polarizabilities) must be coherently inserted into the model. Besides, the computational complexity increases when modeling RR of large systems due to two main factors: (i) treating large systems implies including hundreds of vibrations in the calculation, and (ii) an adequate sampling of the configurational phase space of the target-environment system is needed to obtain reliable spectroscopic signals.

Our atomistic multiscale computational protocol, proven to be successful in modeling spectral signals of molecules in aqueous solutions[5,6,7], has been extended to more complex systems, such as chromophores embedded in biological matrices. In such an extension, a series of strategies to compute the normal modes at different levels of sophistication is proposed. We use Doxorubicin, a widely used chemotherapy agent, intercalated into DNA[8], to test the computational protocol. The good agreement between computed and experimental data allows us to easily identify the main features of RR spectra in both aqueous and DNA solutions, confirming the reliability of our methods. This methodology is very versatile and thus suitable for promising applications to complex biosystems as peptides and proteins.

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Acknowledgements

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Ultrabroadband Coherent anti-Stokes Raman Scattering Microscopy for Biological Applications and Material Science

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Coherent anti-Stokes Raman Scattering (CARS) microscopy is a label-free vibrational imaging technique that delivers chemical maps of cells and tissues. CARS employs two narrowband picosecond pulses (pump and Stokes) that are spatiotemporally superimposed at the sample plane to probe a single vibrational mode. Broadband CARS (BCARS) combines narrowband pump pulses with broadband Stokes pulses to record broad vibrational spectra. Despite many technological advancements, BCARS microscopes still struggle to image biological samples spanning the entire Raman active region of biological samples (400-3100 cm^{-1}).

Here, we demonstrate a novel BCARS method to answer this need [1-2]. Our experimental setup is based on a femtosecond fiber laser at 1035 nm and 2 MHz repetition rate, thus delivering high energy pulses used for generating sub-20-fs broadband Stokes pulses by white-light continuum in a bulk YAG crystal, a compact and alignment-insensitive technique. Combining them with narrowband picosecond pulses, we can generate a CARS signal with high (< 10 cm^{-1}) spectral resolution in the entire Raman window exploiting both two-color and three-color excitation mechanisms. The system is equipped with a home-made transmission microscope to image cells and tissue at high-speed (<3 ms) and large field of views. Using a post-processing pipeline, we deliver high-quality chemical maps, identifying the main chemical compounds in cancer cells and discriminating tumorous from healthy regions in liver slices of mouse models, unveiling the path for applications in histopathological settings.

We also applied our BCARS microscope for the study of the so-far largely neglected group of crystalline materials with varying structural complexity, ideal model systems due to their homogeneity and defined orientations. We could image domain walls, acting as 2D quasi-structures.

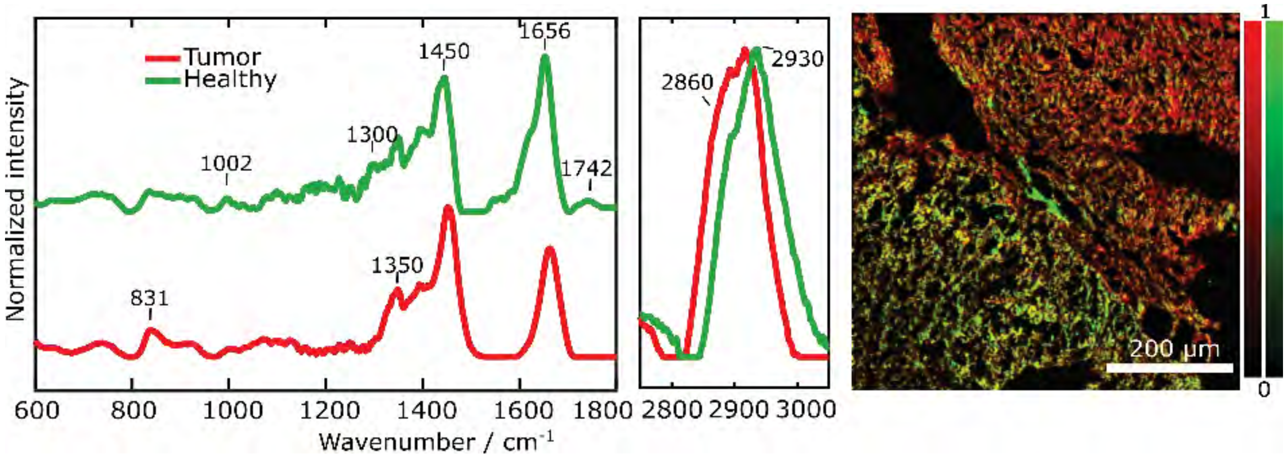

Figure 1: 600 x 600 μm^2 BCARS image on a liver slice of a mouse model with hepatocellular carcinoma tumor cells. Imaging settings: 3ms pixel dwell time; 2 μm pixel size.

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Stimulated Raman Scattering Imaging: the Next Frontier of Light Microscopy

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All molecules consist of chemical bonds, and much can be learned from mapping the spatiotemporal dynamics of these bonds inside cells, tissue and animals. Since its invention in 2008, stimulated Raman scattering (SRS) microscopy has become a powerful modality for imaging chemical bonds with high sensitivity, resolution, speed and specificity. The past dozen years have witnessed the blossoming of SRS microscopy, where advances in both optical instruments and imaging probes have found broad applications in life sciences.

In this talk I will first discuss the recent advances in theoretical understanding and technical innovations of SRS imaging. Then I will highlight new research areas and applications, including (1) single-molecule vibrational spectroscopy and nanoscopy, (2) bioorthogonal chemical imaging, (3) super-multiplexed optical imaging, (4) single-particle nanomedicine, (5) VIBRANT for drug discovery, and (6) vibrational spatial omics.

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Superresolved CARS imaging by coherent image scanning

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Superresolution microscopy has transformed many life science imaging applications. Yet, a similar advance towards breaking the resolution barrier in coherent nonlinear microscopy is yet to occur. Here we present the implementation of image scanning microscopy (ISM) to coherent anti-Stokes Raman imaging, showing a significant resolution enhancement. In ISM, a pixelated detector serves as the pinhole in confocal microscopy, whereby the excitation spot is magnified such that every pixel acts as a small pinhole. Signals collected from multiple pixels are then analysed to generate a higher resolution image. The difficulty in applying ISM to coherent imaging is that the analysis process requires exact knowledge not only of the amplitude of the signal but also its phase [1,2]. We implement CARS-ISM using nearly inline interferometry, whereby a reference signal is generated in a glass slide and is directed towards the sample along with the pump and stokes beams. Dispersion compensation and phase stepping of the reference beam is done in a 4f pulse shaper inserted between the generation of the reference and the sample.

Using this system, we obtain superresolved CARS images in the lipid C-H stretch band when using pump and Stokes beams generated by a Ti:Sapphire laser and a synchronously pumped OPO. As can be seen in Figure 1, images show both lipid droplets and other cellular organelles at a resolution improved relative to the 'bare' CARS image. Importantly, the phase of the CARS signal is spatially variant due to the different rations of resonant and nonresonant contributions. To further characterize the resolution enhancement we use polymer air-force like grating targets which show that the use of ISM can lead to a ~1.5 times better resolution even without advanced image processing, and holds the potential for further resolution increase. The potential of this technique as a general-purpose booster of resolution in coherent nonlinear microscopy and possible implementations in epi-detection mode will also be discussed.

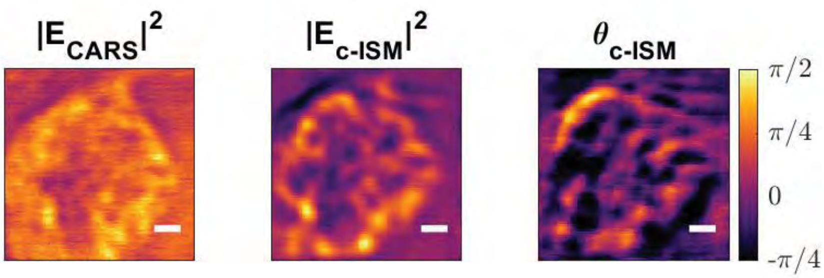

Figure 1: Comparison of conventional CARS imaging with CARS-ISM: Image of a cell in the lipid band by conventional CARS imaging (left) ; Extracted CARS intensity from the ISM image (center) ; extracted phase from the ISM image (right). A clear improvement in resolution is evident going from the conventional to the CARS-ISM images.

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A Versatile Laser System for Wide-field Nonlinear Optical Microscopy

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In the last decades, nonlinear optical microscopy techniques, such as Two-Photon Excited Fluorescence (TPEF), Sum Frequency Generation (SFG), and Hyperspectral Coherent Anti-Stokes Raman Scattering (H-CARS) microscopy, emerged as powerful tools to image biological samples in a label-free, fast, and non-destructive way. Traditionally, these techniques are performed with single point-scanning acquisition schemes that feature a limited field of view and often lead to sample damage when high laser power is used to increase the acquisition speed. Wide-field illumination and camera-based detection schemes offer a solution to these issues enabling fast imaging over a large field view. However, current laser sources are not able to induce nonlinear effects in non-linear wide-field imaging configurations over areas larger than 100x100 μm^2.
Here, we propose an innovative and powerful laser source (Figure 1.a) that addresses this issue enabling video rate (>60 frame/s) TPEF, SFG, and H-CARS wide-field imaging over >1x1 mm^2. The laser source starts with a femtosecond laser system (TANGOR 100) centred at 1030 nm with 100 W average power, operating at 200KHz or 1 MHz repetition rate. When set at 200 KHz, the laser source is used to feed two lines of optical parametric amplifiers (OPAs) with two optical amplification stages, respectively. The two OPAs output 800-fs laser pulses with 1.6-2.5 μJ pulse energy in the entire range of 700-900 nm and can be quickly tuned (max. speed 100KHz) within 20 nm bandwidth through two independent acousto-optic programmable dispersive filters (AOPDF). We will show CARS spectroscopy spanning from 1400 to 3200 cm^{-1} and unprecedently fast (10-ms/frame) and large wide-field SFG, TPEF, and H-CARS images of test samples (Figure 1.b). We foresee that this type of laser system, coupled with state-of-the art microscopy techniques, will bring multimodal nonlinear microscopy to a novel wide-field era.

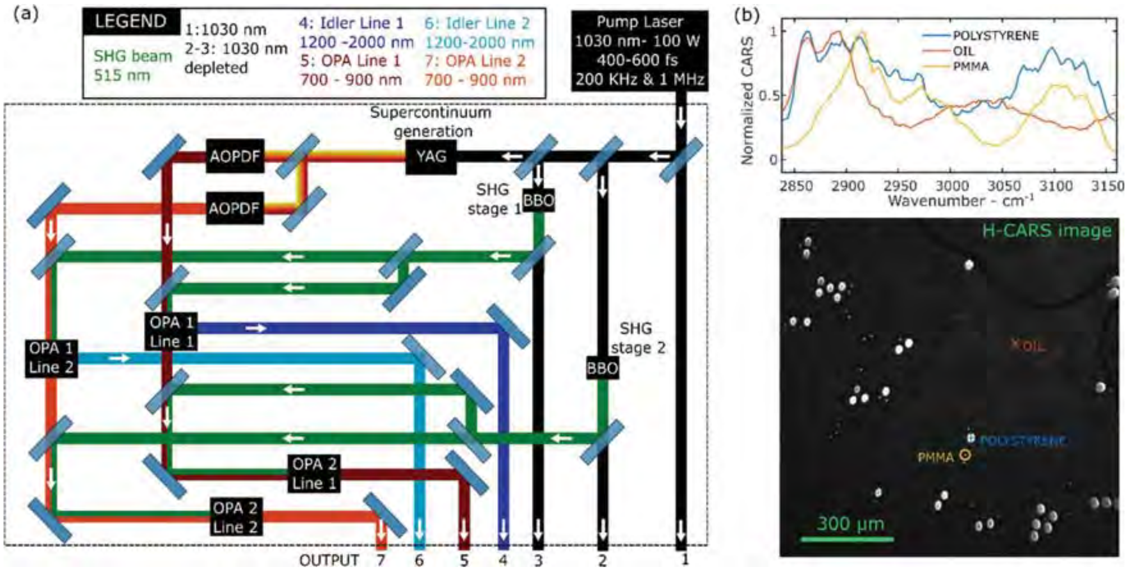

Figure 1 (a) Laser system configuration at 200 KHz with 7 laser outputs. 5-W of the fundamental laser beam generates the narrowband Stokes beam at 1030 nm (output 1), 0.5-W is used for supercontinuum generation into a 10mm-YAG crystal (700-900 nm), 18.5-W and 76-W are used for SHG in BBO crystal that serve, respectively, as pump beams for the first and second stage of amplification of the two OPAs. (b) 1.1x1.1 mm^2 wide-field hyperspectral CARS images of polystyrene (30 μm) and polymethacrylate (20 μm) beads in oil.

Impact of Artificial Intelligence and Resonance Raman Spectroscopy in Biomedicine

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Raman spectroscopy is a valuable tool for biomedical investigations due to its ability to provide detailed molecular information in a non-invasive and rapid manner. Biological samples like cells, tissues and biofluids can be probed using laser light of a suitable wavelength to study disease states and cellular processes. However, conventional Raman spectroscopy can be limited by weak signal intensities, especially in complex biological environments. This is where resonance Raman spectroscopy proves useful. Prosthetic groups like tetrapyrrole rings, polyene chains and protein bound metal ions are some examples of protein bound chromophores studied by Resonance Raman spectroscopy.

In this talk, various applications of Resonance Raman spectroscopy for the study of bacteria and disease markers will be discussed. The enhanced carotenoid peaks observed in the Resonance Raman spectra collected using a 514 nm laser were used to differentiate between two Mycobacterial species co-occurring in immunocompromised individuals, a task difficult to achieve with conventional methods[1]. In another study, we identified a novel Resonance Raman peak at 740 cm^{-1} for Escherichia coli during the log phase of bacterial growth, using a 633 nm laser. Through experimentation with various mutant strains, we demonstrated the involvement of cytochrome bd subunit II in generating this peak [2]. Monitoring this peak allowed us to investigate the development of resistance in Escherichia coli on exposure to herbicides[3]. We further used Resonance Raman spectroscopy to identify spectral markers of sepsis in mice models. Sepsis, a critical condition triggered by exaggerated immune responses lacks specific and rapid diagnostic methods. The spectra collected using a 442 nm laser from blood sera demonstrated an increase in the resonant Raman bands at 670 and 1350 cm^{-1} from the pyrrole ring breathing vibrations of the porphyrin molecule indicating sepsis induced hemolysis[4]. These findings in combination with the application of artificial intelligence is likely to offer promising avenues for precise and timely disease detection across various medical contexts.

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Advances in the biological Raman spectroscopy field show its remarkable promise as a rapid diagnostic for infection, cancer, and even metabolic conditions with high sensitivity and specificity. However, clinical translation of Raman spectroscopy requires 1) efficient and reliable pipelines that can enable seamless processing of large numbers of samples and 2) equally reliable rapid data processing pipelines that provide conclusive results interpreted from the spectra in clinical terminology. In this talk I will present our work on 1) liquid sample processing pipelines using acoustic inkjet printing for generating single-cell droplets, 2) robotic pipelines for end-to-end automated sample handling including spectral collection and waste removal, and 3) highly-reliable custom machine learning pipelines with computational and energy needs fit for point-of-care systems. We believe our work enables adoption of Raman spectroscopy for clinical and large scale fundamental biological studies.

Machine Learning of Raman Spectroscopic Data: A Systematic Comparison of Validation Strategies

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Machine learning (ML) methods provide powerful tools for analyzing complex biological SERS spectra enabling the detection of minute differences in cell composition [1].

Besides choosing the best classifier, there are many challenges in data analysis, starting from preprocessing, where many different methods are available and the choice has to be made carefully, to different validation strategies that could be applied to avoid overfitting and obtain an accurate estimate of the error [2], [3]. In this work we focused on the application and systematic comparison of different data and training validation strategies and their impact on different ML classifiers.

For a fast and reliable data analysis we established a machine learning workflow [4] which, after quality control and preprocessing, uses more than 10 different classifiers. This workflow was successfully tested with different datasets using a computationally intense nested cross validation (CV) for hyperparameter tuning and performance estimation [5]. To further optimize and develop this workflow, we systematically compared 5 different CV strategies with 3 different data sets with increasing biological complexity:

(1) Prokaryotic dataset: differentiation between different prokaryote species [6]
(2) Cyanobacteria dataset: detection of poly-hydroxybutyrate (PHB), which is of interest for the biopolymer industry as biodegradable plastic [7]
(3) HL (Hodgkin lymphoma) dataset: determining the effect of cytostatic treatment compared to an untreated control [5]

For validation we compared Leave-One-Group-Out, Repeated K-Fold, Stratified K-Fold, (Unstratified) K-Fold and Leave-P-Out (more precisely P=1 Leave-One-Out) from scikit-learn with the already established gold standard Nested CV in data analysis workflow mentioned above. To determine the performance, we used generalization accuracy, p-values of McNemar tests, confusion matrices, and ROC curves.

The here discussed validation methods generally deliver results that are almost of the same quality as those of Nested CV for the investigated datasets, and in less time. Of the four strategies, Unstratified K-Fold often delivers recognizably worse results than the other three methods. Leave-One-Group-Out generally delivers lower probability estimates compared to other methods.

These results clearly indicate that the computationally expensive "Nested CV" could in many cases be replaced by simpler methods, especially when time or computational resources are limited, while maintaining the same performance in terms of accuracy and efficiency. However, the possibility of overfitting must be considered more carefully when using these simpler methods.

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Deep Learning Transformations in Raman Spectroscopy: From Fundamental Principles to Innovative Applications

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Remarkable strides have been made in the field of Deep Learning (DL), greatly due to the rise of NLP-focused foundation models demonstrating stunning capabilities. Such an explosion in interest dramatically changed the overall paradigm of DL implementations, giving way to the development of Large Foundation Models. Deep Learning has been explored as a robust tool for Raman Spectra identification [1][2]. Despite this, recent works in the field still struggle to keep up with modern Deep Learning practices. In this work, we explore the possibility of building the first Foundation model [3] for Raman Spectroscopy, benchmarking modern architectures, such as ViTs [4] and SSMs [5], over contrastive pre-training of Large embedding models capable of surpassing current techniques in spectrum representation and downstream tasks, such as classification, denoising, relative abundance regression, semantic segmentation. We explore the pretrained model's capabilities with spectra of organic compounds of different types, both in Supervised and Zero-Shot regimes.

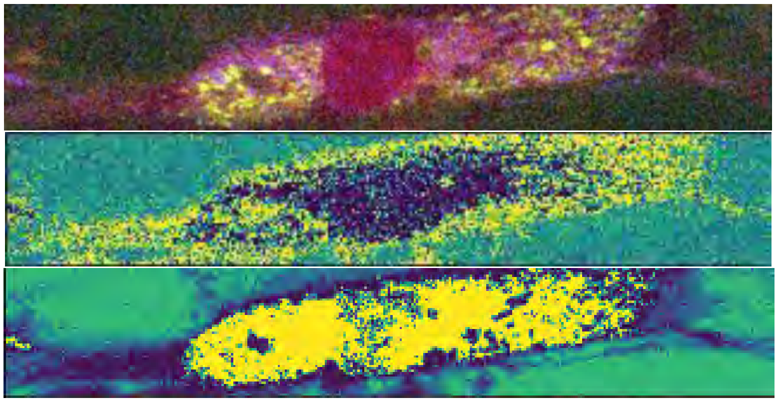

Figure 1: Image 1 is a manually segmented Raman Map of a human fibroblast during apoptosis. Image 2 is a simple k=4 k-means semantic segmentation of the same map.. Image 3 is the Deep-Learning-based k=4 k-means segmentation.

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Revisiting silver nanoisland films (AgNIFs) as substrates for SERS analysis of organic colorants in works of art

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Over the last twenty years SERS has found consistent use in the microanalytical identification of organic colorants in works of art [1]. Essentially all this work has been carried out using silver colloids as the plasmonic substrate: in fact, we reviewed the performance of silver colloids, chemically deposited silver films, and evaporated nanoisland films several years ago, finding colloids preferable as slightly more sensitive, and easier to prepare reproducibly [2], particularly when using a modified Lee-Meisel type reaction [3]. Recently, we reevaluated the use of high vacuum evaporated silver nanoisland films (AgNIFs) developing new approaches to extract the dyes of interest from the microscopic samples obtained from the work of art and to transfer them to the active surface. Notably, we were able to obtain high quality spectra of natural and synthetic dyes from works of art in cases where Ag colloids failed to deliver any results. Among others we will discuss the example of a Japanese painting long thought to be a late 19th century forgery and reattributed to the studio of Hokusai based on the results of Raman analysis.

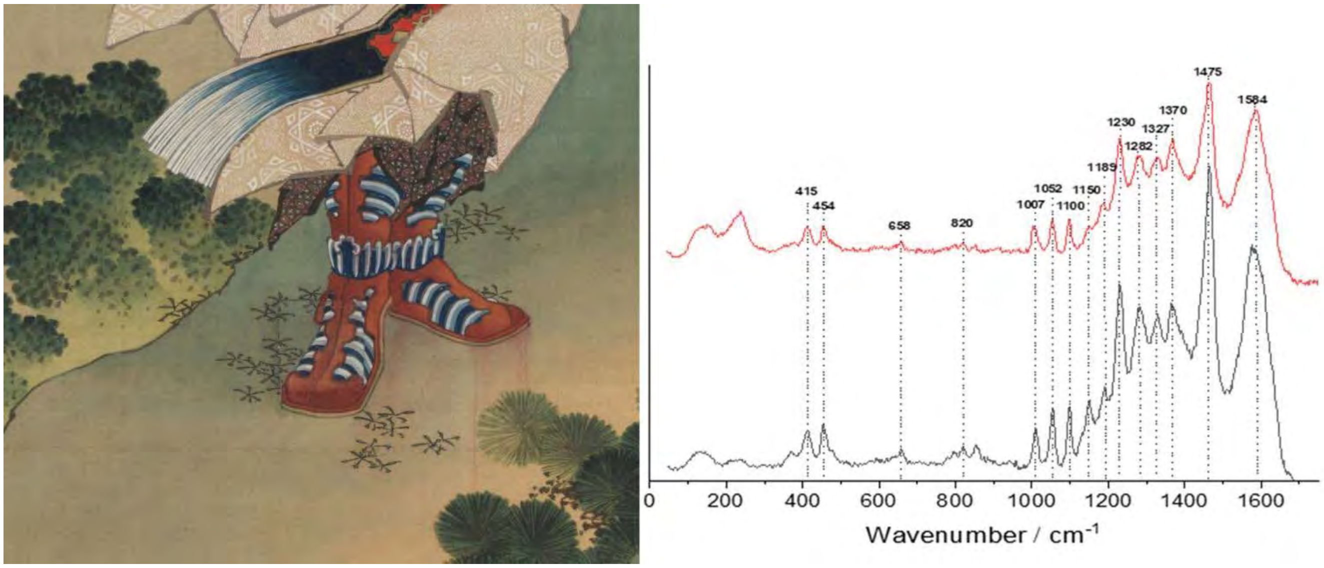

Figure 1: Left. The Legendary Empress Jingū. (Detail). Hanging scroll on silk. Studio of Katsushika Hokusai, Japanese, dated 1847. The Metropolitan Museum of Art (14.36.76). **Right**. Red trace: spectrum of a sample from the dye bleed area under the figure proper left shoe (AgNIF substrate). Black trace: lac dye reference (Ag colloid).

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New integrated approaches in Heritage Science: correlative Brillouin and Raman microspectroscopy - BRaMS

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Heritage Science is boosted by a new tool, correlative Brillouin, and Raman microspectroscopy (BRaMS) enabling to simultaneously probe the compositional and viscoelastic properties of materials. This integrated approach, which has already been proven effective in studying biological materials [1], it has only recently been applied to study complex and heterogenous materials of our Cultural Heritage [2].

The possibility of investigating the mechanochemical properties at a time scale of hundreds of picoseconds -hardly reachable by other methods- makes BRaMS a valuable tool for studying degradation processes in which chemical and mechanical changes are intimately connected. Recent applications of BRaMS in Heritage Science, combined with state-of-the-art noninvasive portable spectroscopies, are presented.

First and foremost, BRaMS was employed to understand the interplay between molecular and structural changes occurring during oil paint polymerization and curing. As a real case application, the feasibility of the BRaMS approach was then assessed by investigating a precious micro-fragment from the iconic painting *Alchemy*, Jackson Pollock's masterpiece of American abstract expressionism.

As a further example of BRaMS application in Heritage Science, the mechanochemical monitoring of acrylonitrile butadiene styrene-ABS plastic photoaging is discussed. A multimodal approach based on portable broad-range infrared reflection spectroscopy and NMR-relaxometry, combined with benchtop BRaMS, enabled the unveiling of the state of conservation of historical plastic objects of the Italian design.

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Deep Raman for Heritage Science: state of the art

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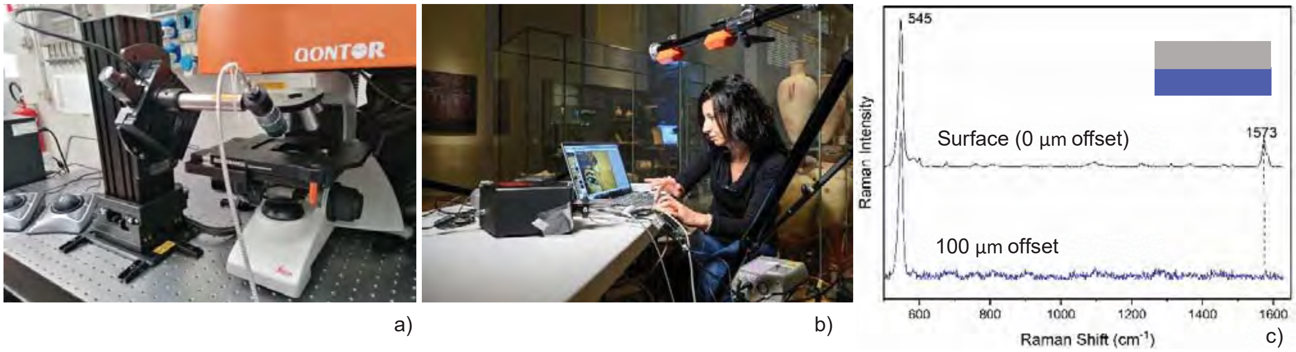
The analysis of diffusely scattering compounds at depths is a topical and fast evolving area in Heritage Science. The arrival of the micro-scale variant of SORS, micro-Spatially Offset Raman Spectroscopy (micro-SORS), in this field enabled the non-invasive probing up to several tens of micrometres inside painted layer sequences and artistic objects [1].

This presentation will focus on recent technological and methodological developments of micro-SORS at the Raman Spectroscopy Laboratory of ISPC-CNR for applications in Cultural Heritage. Two micro-SORS prototypes, benchtop and portable, with laser excitation wavelength at 785 nm have been recently optimized and tested with artistic objects for identifying non-invasively the molecular composition of the compounds and their mutual position within the materials. The benchtop prototype has high spectral and lateral resolutions and high micro-SORS surface/subsurface contrast [2]; moreover, the coupling of micro-SORS with imaging/mapping modalities paves the way for studies of high-resolution molecular distribution of compounds hidden by external opaque layers in art objects. The in-house portable prototype represents a technological evolution of existing commercial portable Raman spectroscopy since the detection of Raman signals of the surface and subsurface is achieved by using a micrometric linear fibre bundle to preserve the offset information on the detector; in this way, a simultaneous acquisition of Raman photons emerging from the surface and subsurface in separate spectra is achieved. Preliminary outcomes of a study focusing on the comparison of micro-SORS and traditional macro-scale SORS will also be presented. The extension of the application areas to biomedical and energy fields will also be discussed.



a) b) c)

Figure 1: Benchtop prototype (a), in-situ measurements with portable prototype (b, © Nicola Dell'Aquila – Museo Egizio), micro-SORS spectra (c).

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Characterization of carbonate high-pressure phases with Raman spectroscopy

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Raman spectroscopy is an important analytical tool for the characterization of carbonates and allows *in situ* investigations at extreme pressure conditions in diamond anvil cells. Here, we demonstrate the application of Raman spectroscopy for the identification of high-pressure phases of carbonates [1-4].

Typical “conventional” carbonates such as $Ca[CO_3]$ or $Al_2[CO_3]_3$ are characterized by the presence of isolated planar trigonal $[CO_3]^{2-}$-groups with sp^2-hybridized carbon orbitals [1,3]. These building blocks are characterized by symmetric stretching modes at around 1087 cm^{-1} (Fig. 1b,c). In the last 20 years, there has been a significant expansion of our understanding of the crystal chemistry and the structures of carbonates, mainly triggered by structure determinations from X-ray experiments. Recent studies demonstrated that new carbonates containing different building blocks, such as $[C_2O_5]^{2-}$ groups, mixture of $[C_2O_5]^{2-}$-groups with $[CO_3]^{2-}$-groups and carbonates with polymerized $[CO_4]^{4-}$-groups can be synthesized at extreme conditions [2-4]. The characteristic vibrational signatures of these building blocks can be easily identified by Raman spectroscopy.

The distinguishing feature of the $[CO_4]^{4-}$-groups in $Sr_2[CO_4]$ structure is the stretching modes at 885 cm^{-1} at ambient conditions (Fig. 1a). In the case of $Al_2[C_2O_5][CO_3]_2$, the characteristic Raman modes of the $[C_2O_5]^{2-}$-groups are located at approximately 979 cm^{-1} at ambient conditions (Fig. 1c). This mode can be assigned to the in-plane bending mode of the $[C_2O_5]^{2-}$-groups. DFT calculations were used to benchmark the experimental Raman spectra and to derive the assignment of the Raman modes. The agreement between the experimental and calculated Raman spectra in $Sr_2[CO_4]$, $Al_2[CO_3]_3$ and $Al_2[C_2O_5][CO_3]_2$ is very convincing (Fig. 1) and confirms the presence of the different building blocks.

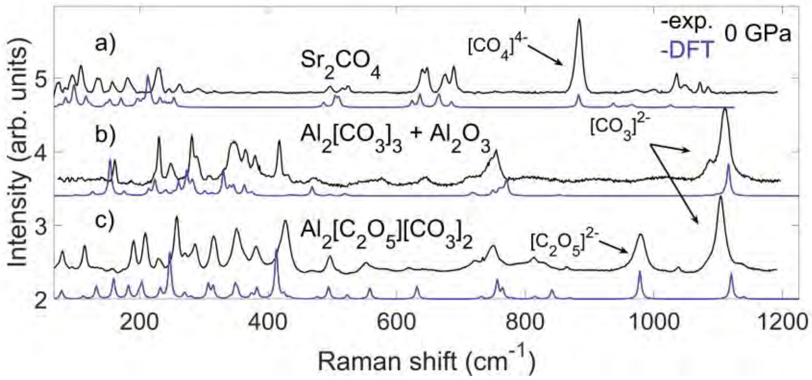

Figure 1: Raman spectra of (a) $Sr_2[CO_4]$ (b) $Al_2[CO_3]_3$ and (c) $Al_2[C_2O_5][CO_3]_2$ at ambient conditions. Arrows indicate the positions of the characteristic Raman modes.

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Predictive analytics with Raman spectroscopy – progress towards development of assays for oncological applications

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Vibrational spectroscopy, encompassing techniques such as Raman spectroscopy and Fourier-transform infrared (FTIR) spectroscopy, has emerged as a powerful tool in biomedical research for its potential in non-invasive diagnosis and therapeutic monitoring of cancer.

As the acquisition of Raman spectra from biological systems requires minimal sample preparation and the ability to analyse samples in a label-free manner, preserving the integrity of biological specimens. This aspect is crucial for longitudinal studies where repeated measurements are necessary to monitor the therapeutic response over time. This paper will review research at TU Dublin to employ Raman spectroscopy for the development of predictive models for treatment outcomes. These studies have Raman spectroscopy in identifying radiation sensitivity and radiation resistance in model cellular systems *in-vitro* and *ex-vivo*[1], [2] and have also developed modelling approaches to identify patients at risk of the development of future adverse radiotherapeutic treatment responses within clinical trial studies,[3] including using liquid biopsies[4]. More recent work is exploring the potential of the technique for the prediction of chemotherapeutic response.

Raman spectroscopy holds promise for monitoring and predicting cancer therapeutic response to a range of therapeutic pipelines using a range of tissue sample types, offering a non-invasive, rapid, and detailed molecular understanding of treatment efficacy. We review current challenges to furthering research in this field, to maximise the contribution Raman spectroscopy can make to personalized medicine.

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Accelerating confocal Raman microscopy by capturing essential spectral information

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Point-by-point Raman confocal hyperspectral imaging is a widespread technique through which both spectral and spatial information can readily be extracted, allowing one to have a global understanding of the samples under study. However, when the surface to analyze is large, its characterization may require from few hours to days, which hampers specific analytical applications requiring fast identification or inducing photodamage. To circumvent these issues, we propose an approach based on the assessment of Essential spectral Information in the Fourier Domain (EIFD) that permits to accelerate confocal Raman imaging by 50-fold compared to classical point scanning [1]. The set of essential spectral profiles selected encompass the most linearly independent (dissimilar) spectra measured and the sole use of these spectra enables the reproduction of the collected data in a convex linear way [2-3]. The selection of EI has proven to be a very useful and reliable data reduction tool for linear spectral unmixing, brings advantages in terms of speed and compression and facilitates challenging analytical tasks such as the detection of minor species [4]. One of the major assets of EIFD is that essentiality can be assessed independently for each spectral pixel and potentially as the data acquisition is ongoing. This paves the way to acquisition processes that would be based on the EI content of a spectral pixel and that would quicken the collection at those locations where information is not essential, considerably accelerating the whole imaging procedure. To illustrate our approach, we show outcomes obtained for several Raman hyperspectral images resulting from the analysis of samples of varying complexity (up to pharmaceutical commercial tablets) and outline a new data acquisition scheme relying on targeted sampling based on EIFD that could foster a real paradigm shift in the imaging domain.

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Robust Spectroscopic Analysis through Image-Based Spectral Representation and Deep Learning Techniques

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Due to its wide range of practical applications, Raman spectroscopy has become an important tool in a variety of fields, including biomedical diagnostics. However, maintaining device-to-device and long-term stability in wavenumber calibration is a significant challenge in its application, as this stability is critical for accurate spectrum classification. In our study, we present a new method to address this problem by transforming the spectral data into spider plot images in which the inner sections are color-coded based on their intensities, thus adding a new dimension to the data. This transformation effectively adapts image-focused pre-trained networks originally developed for traditional image datasets to Raman spectral analysis. The dataset we used in our research contained spectra from six classes of bacteria, and we systematically applied wavenumber shifts to the dataset and evaluated the adaptability of our classification model. While the traditional PCA-LDA classification method showed a marked accuracy decline from 0.90 to an average of 0.77 with spectral shifts, our approach using spider plot images and the EfficientNetB7 network demonstrated robustness against these shifts. The accuracy remained high at 0.87 without shifts and only slightly decreased to 0.83 with shifts. This stability is attributed to the structural consistency of spider plot images, where wavenumber shifts result in image rotations, a variation well-handled by the pre-trained network. Our results highlight the efficacy of image-based representations in spectroscopy, showcasing the adaptability of the proposed method to spectroscopic data and their robustness against spectral shifts.

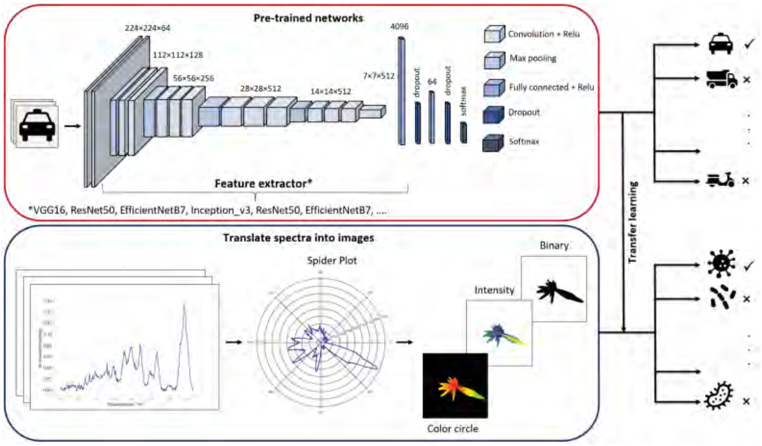

Figure 1: Adapting pre-trained networks for spectra classification. In a basic analogy, consider various vehicles being classified using pre-trained networks: Given an image, the network can determine, for instance, that the image depicts a car. Using a similar rationale, to classify bacterial Raman data, we first transform these data into an image representation. Then, leveraging the pre-trained networks, we can identify the specific bacterial type represented by the input.

Acknowledgement
This work is supported by the BMBF, funding program Photonics Research Germany (13N15466 (LPI-BT1)) and is integrated into the Leibniz Center for Photonics in Infection Research (LPI). The LPI initiated by Leibniz-IPHT, Leibniz-HKI, Friedrich Schiller University Jena and Jena University Hospital is part of the BMBF national roadmap for research infrastructures.

IRRSmetrics4stem: a new tool for prediction of consecutive stages of Adipose-Derived Mesenchymal Stem Cells differentiation

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Dynamic processes such as cell differentiation in regenerative medicine are orchestrated by several temporal and spatial molecular transformations inferred by alternations of cell morphology, metabolic activity, and molecular composition. Therefore, the standardization of reproducible differentiation of stem cells requires several biochemical assays [1,2].

In this study, combining label-free FTIR and Raman hyperspectral imaging, detecting the molecular composition, with data mining enables non-invasive monitoring of the stem cell differentiation and identification of biochemical heterogeneity accompanying this process [1]. We previously developed the employed methodology for tracking intermolecular interactions of the progenitor cells [2].

Primary adipose-derived mesenchymal stem cells (AD-MSCs) isolated from C57BL6 mice were cultured. Their differentiation into adipocytes and osteoblasts was followed within a few weeks by Raman, FT-IR, and O-PTIR imaging guided by histochemical and fluorescence staining. A workflow of the data analysis (IRRSmetrics4stem) was designed to identify spectral predictors of the adipogenesis and osteogenesis stages and test machine-learning (ML) methods (hierarchical clustering, PCA, PLSR) for the rapid control of the AD-MSC differentiation degree.

IRRSmetrics4stem delivered critical findings that provide insights into the chemism of differentiation, particularly at the early stages. With single-cell tracking of AD-MSCs as an *in vitro* model cultured in the differentiation medium, we established IRRS metrics of lipids, proteins, and DNA specific for the key stages of cellular transformation. The over 90% predictive efficiency of the selected ML methods proved the high sensitivity of the IRRS metrics for recognizing the process.

The proposed IRRS metrics unequivocally recognize a switch from proliferation to adipogenic/osteogenic lineage. This study introduces a new bioassay to identify molecular markers indicating metabolic and molecular transformations in the differentiating cells and to achieve rapid and machine learning-based monitoring of MSC growth that can be also relevant to other differentiation processes.

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Raman spectroscopy and self-supervised learning: an approach to a general analysis method

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Analysis of Raman spectra from biological samples is a persistent challenge in the application of Raman spectroscopy [1]. This is further compounded by variations in the properties of the spectra produced by different measurement systems and procedures, making direct comparisons between the works of different laboratories challenging. To meet this challenge, we propose an approach using a novel self-supervised deep learning algorithm with the goal of extracting chemically significant information from Raman spectra to differentiate extracellular vesicles in the presence of different levels of noise and distortions. By having the algorithm explicitly consider both the intensities and wavelength of each point in the spectrum, it becomes able to adapt to both distortions and differences in spectral range from different instruments, as shown in figure 1.

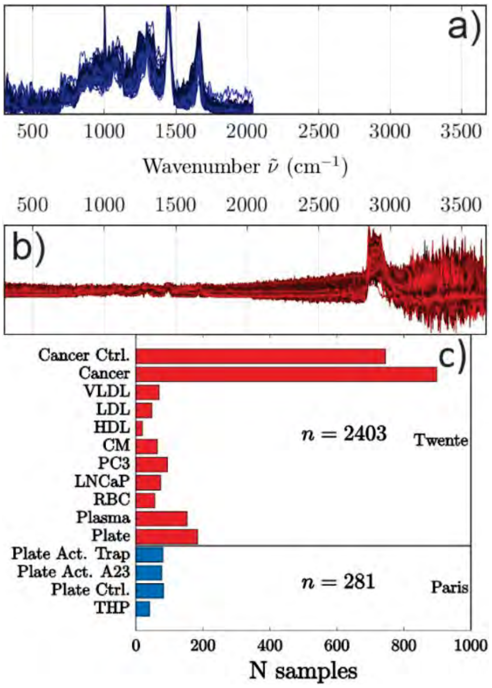

Figure 1 a) Spectra from Paris-lab; b) Spectra from Twente-lab; c) Distribution of particles by lab and biological origin

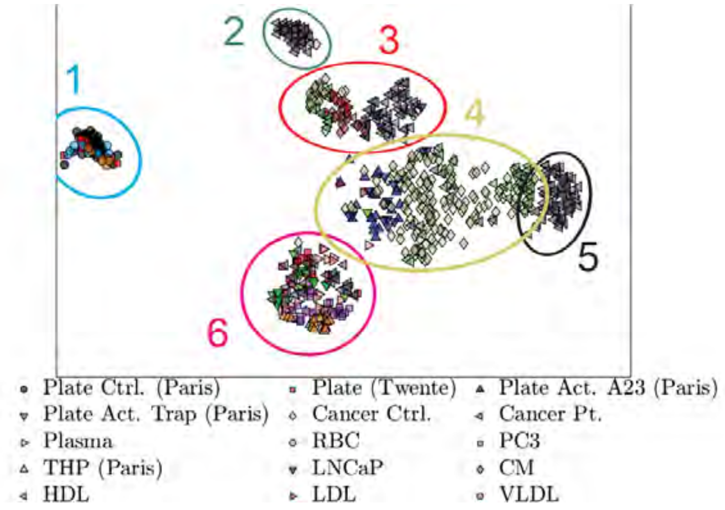

Figure 2 t-SNE projection of the latent information from 2684 spectra of 15 biological origins

The algorithm is trained using un-labeled spectra, which it decomposes into 100 latent variables containing the information in the spectra. The spectra are also subject to randomly generated gaussian noise as random distortion and truncation of its wavelength-axis, emulating differences between measurement systems. Once trained, the algorithm can differentiate extracellular vesicles and liposomes by the latent representations of their spectra, as shown in figure 2. The clustering of particles by their biological origin is here produced by how similar the algorithm sees them, as the true biological origin of the particles is not made known to the algorithm.

Using the latent variables, the particles from 15 biological origins can then be classified by an auxiliary model, demonstrating a sensitivity of 92.2% and a selectivity of 92.3% over a test set of 769 particles. The robustness of the model can be further demonstrated by maintaining an accuracy of at least 81.5% over the same set when the spectra are subject to artificial noise, distortion, and truncation. This demonstrates the flexibility and potential of deep learning in analysis of Raman spectra of biological particles, both for research and possible diagnostic purposes.

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SERS and DFT study of rare earth-citrate complexes

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Rare earth (RE) elements are difficult to be classified due to their similar chemical properties[1]. In this study, the surface-enhanced Raman scattering (SERS) spectra of two non-fluorescent RE^{3+} ion-citrate complexes (RE: La, Gd) were used for the qualitative classification of La^{3+} and Gd^{3+} ions due to the spin effect in their SERS spectra[2]. Through evaluating the spectral relationship of sample solutions with and without citrate-capped silver nanoparticles(citrate@AgNPs), we confirmed that the enhanced Raman signals of Ln-citrate complexes could be obtained by adding a small amount of $RE(NO_3)_3$ solution to citrate@AgNP solution. Combined with our density functional theory (DFT) simulations employed with the new SERS simulation method for RE complexes, the experimental characteristic SERS peaks near 1065 cm^{-1} and 1315 cm^{-1} were assigned to spin-related bands (C-O···RE) and (COO^{-}), respectively. By evaluating the spin population and vibration mode of RE-citrate complexes, their relative intensity changes were related to the electron density in the region coordinated by Ln^{3+} ions. The relative SERS intensity ratios of these two peaks (I_{1065}/I_{1315}) for La-citrate and Gd-citrate were in the range of approximately 1 and 0.55, respectively. Thus, this relative intensity relationship of these SERS peaks can be used to classify La^{3+} and Gd^{3+} ions.

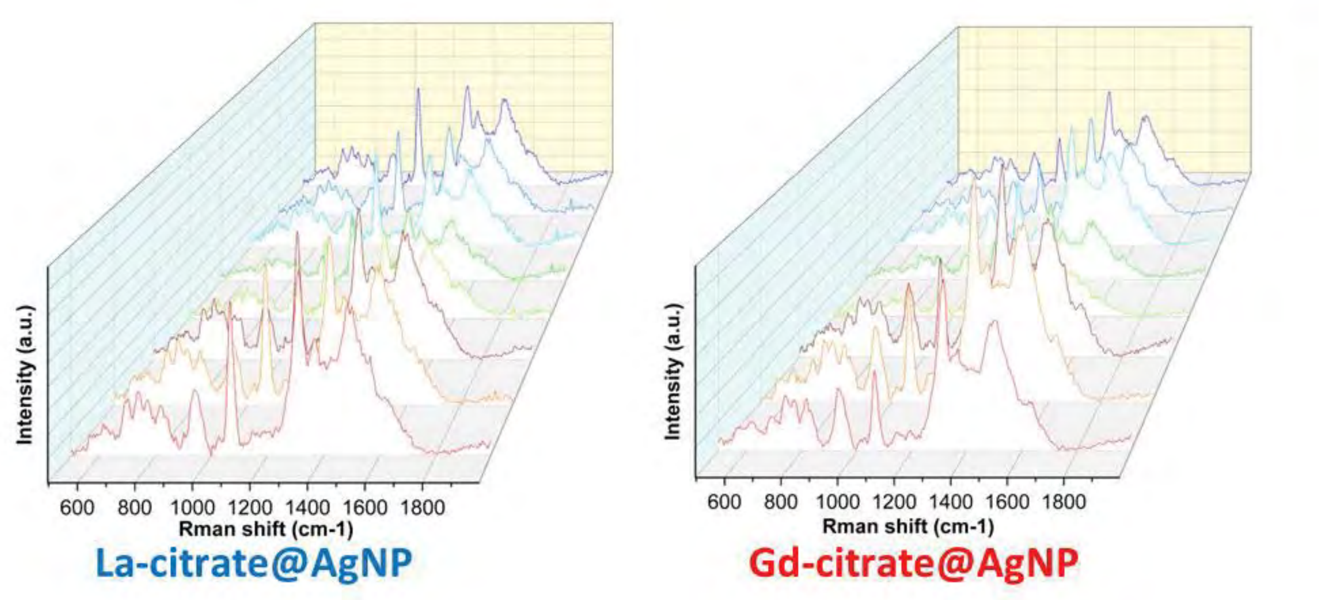

Figure 1: SERS spectra of La-citrate and Gd-citrate excited at 532nm

Keywords: rare earth, surface-enhanced Raman scattering, DFT, SERS simulation

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Temperature dependent structural evolution of PCE11

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In the last few years, a significant evolution in the field of organic photovoltaics (OPVs) has been achieved thanks to the combination of low-bandgap donor-acceptor polymers with non-fullerene acceptors, with reported power conversion efficiencies (PCE) surpassing 19%. This study focuses on a donor-acceptor polymer known as PCE11 (poly[(5,6-difluoro-2,1,3-benzothiadiazol-4,7-diyl)-alt-(3,3´´´-di(2-octyldodecyl) 2,2´;5´,2´´;5´´,2´´´-quaterthiophen-5,5´´´-diyl)]). PCE11 is a polymer that shows highly tunable optical properties under various processing conditions and has a reported PCE of 11.5%.[1] Temperature-dependent absorption measurements of thin films of PCE11 showed a similar trend as other donor acceptor polymers,[2] with a slight red shift and a clear vibronic structure as the temperature is decreased, usually interpreted as increase in polymer planarity and consequently conjugation length. However, structural data does not usually accompany such measurements. Here, we employ temperature-dependent Resonance Raman spectroscopy (RR) to develop a basic understanding on how temperature affects the polymer conformation and thus its optoelectronic properties. We find, contrary to the interpretation of temperature-dependent absorption spectra, increase in the polymer backbone torsional disorder when cooling, attested by upshifts in the thiophene C-C symmetric stretch and increase in the ratio between the acceptor and donor modes. These spectral observations were confirmed by DFT calculations that scanned the dihedral angle along the polymer chain. These results suggest that interpretation of the optical spectra is not as simple as described in the literature so far, but a combination of the structural information obtained by RR and excitonic models for photophysical aggregates are necessary to understand the optical properties. Interestingly, following the frequency shift and the relative intensities as a function of temperature we observe a transition around 200 K that could be interpreted as the structural change that leads to modification of the excitonic coupling from inter- to intra-molecular, explaining thus the increase in the 0-0 band with cooling, as expected for J-aggregates.

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Visible and UV-Resonance Raman spectroscopy to probe peptide supramolecular assemblies in sol, crystals, and gels

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Minimalistic peptides composed of just a few amino acids have attracted great interest as building blocks of functional supramolecular structures, for their ease of preparation, low cost, biodegradability and possibility to encode biological messages to cells for therapy. In particular, those containing also D-amino acids at specific positions offer further advantages, such as increased resistance against enzymatic degradation, and possibility to attain different supramolecular structures with enhanced cytocompatibility and bioactivity [1]. Possible applications include the stabilization of protein biotherapeutics, drug delivery, nanoparticle templating, antimicrobial agents and gels, and catalysis [2-5]. However, understanding their supramolecular structures, which often bear the more interesting bioactivity, is far more trivial. This lecture will detail how the use of visible Raman complemented with UV-Resonance Raman proved instrumental to unveil supramolecular interactions and their differences across phases, such as sol, gels, and crystals, thus proving to be an extremely versatile tool to probe peptide assemblies [6-7].

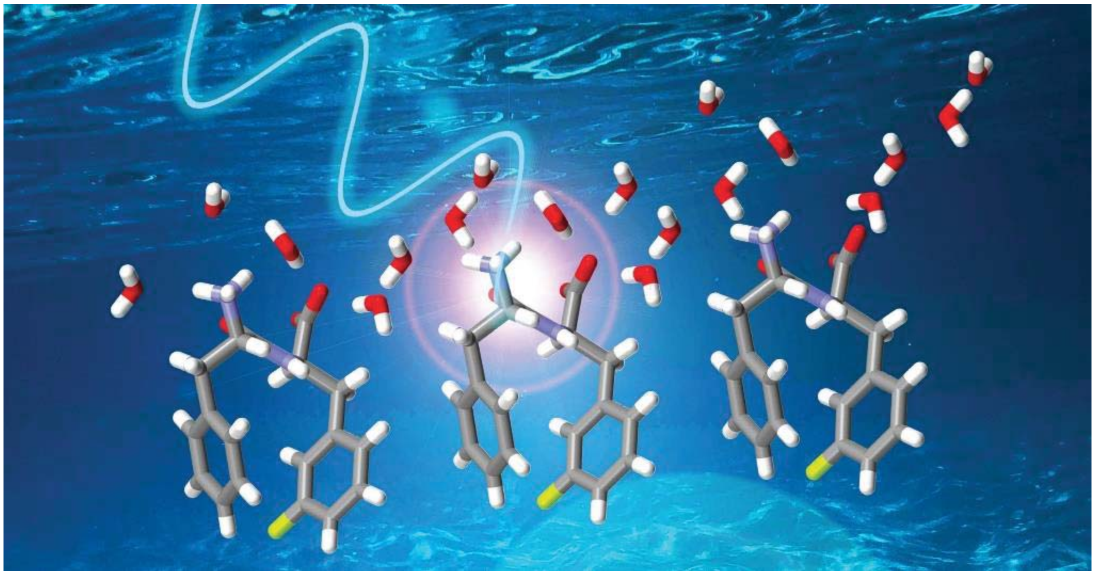

Figure 1: Raman spectroscopy to probe minimalistic peptide assemblies in water [6].

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Charge-transfer / polarization effects in the IR and Raman spectra of advanced π-conjugated materials

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Since the early synthesis of semiconducting polymers and oligomers, Raman and Infrared (IR) spectroscopies have been extensively used for their structural characterization. In the case of molecular materials characterized by delocalized π electrons, Raman spectroscopy allows to elucidate the fine-tuning of the electronic structure caused by changes of the molecular conformation, size confinement and inter-molecular interactions [1]. Conversely, the strong interdependence between the molecular geometry and the electron density explains the remarkable geometry relaxation associated with relevant electronic processes, like chemical functionalization and doping.

We present here a theoretical investigation of phenomena that are relevant in the application of advanced π-conjugated molecular materials, like the charge transfer by chemical doping and the polarization of the π-electrons cloud (*e.g.*, in polyenes or polyynes with asymmetric/push-pull ends). Density functional theory calculations applied on suitable molecular models are used to describe how these phenomena affect the Raman and IR spectra. This highlights remarkable physical effects, like the relaxation of the molecular geometry and the softening of the vibrational frequencies of specific normal modes. In the case of doped conducting polymers (polyacetylene, polythiophene) the analysis of the calculated spectroscopic observables and the comparison with their experimental counterparts give insights about the origin of the strong doping-induced Infrared Active Vibrational transitions (IRAVs), closely related to the major Raman bands of the undoped polymers [2,3]. IR and Raman intensities can be analysed through local contributions based on electro-optical parameters and reveal that intramolecular charge hopping is promoted by collective nuclear displacements along the Effective Conjugation Coordinate (ECC). The same mechanism is responsible for the remarkable vibrational contribution to first hyperpolarizability observed in push-pull species [1].

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Exploring the formation of Poly(3-heylthiophene) aggregates and interchain interactions using Raman spectroscopy

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Conjugated polymers boast significant applicability potential due to their optimal electronic properties and their ability to form ordered structures (aggregates) [1,2], which manifest distinct properties compared to isolated polymer chains. The formation of these aggregates can be accomplished through solvent mixing, temperature variation, photoaggregation, etc. [1,2]. Here, we induced aggregate formation of regioregular poly(3-heylthiophene) (P3HT) by adding different aliquots of methanol in P3HT chloroform solution as well as, subjecting the P3HT chloroform solution to irradiation with a nanosecond pulsed laser (6ns, 532 nm). We employed UV-Vis absorption spectroscopy along with Raman spectroscopy, to analyse the changes in the electronic and vibrational properties of P3HT resulting from aggregate formation. In both experiments, the pristine solution exhibited the distinct absorption band centered at 450 nm. The solution with P3HT aggregates displayed additional absorption bands, located at approximately 560nm, and 610nm. The lower energy electronic band arose from interchain interactions within the aggregated structures, while the higher energy band corresponds to the vibronic progression [2]. The Raman spectroscopy results unveil that the formation of aggregates enhances the Raman signals of the P3HT isolated chain bands, at approximately 1380cm^{-1} and 1440cm^{-1}, corresponding to C-C and C=C stretches in the polymer backbone (**Figure 1**).

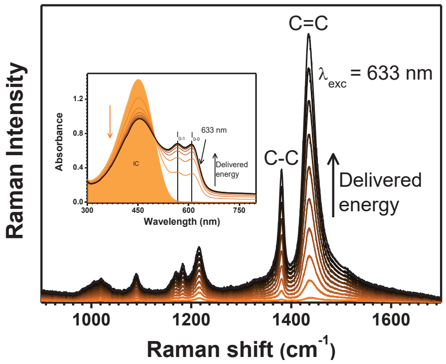

Figure 1: Raman spectra acquired for pristine(orange) and photoaggregated(black) P3HT in chloroform.

The observed effect is attributed to the resonance between the excitation (633 nm) and the new absorption band of the aggregates. Our findings suggest that the interchain interaction within the formed aggregate is weak. Furthermore, since the formed aggregates lacks fluorescence, they offer an opportunity for detailed investigation into the vibrational properties of P3HT aggregates in solution. This can be accomplished while tracking the formation of these aggregates using resonance Raman spectroscopy.

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Vibrational imaging of protein backbone structure with sum-frequency generation microscopy

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Vibrational imaging offers a label-free approach to visualizing biological materials with spectroscopic contrast. Besides the ability to identify selected molecular targets based on their vibrational fingerprints, vibrational imaging also provides information about the structure and local environment of biochemical compounds. For structures that exhibit non-centrosymmetry, sum-frequency generation (SFG) microscopy represents an attractive technique for linking spatially resolved maps to molecular structural information. [1,2] We have previously used SFG microscopy to study the structure and orientation of collagen type I fibrils in tendon tissue, employing the intrinsic carbon-hydrogen stretching vibrations as a structural probe for the organization of selected amino acid residues in the polypeptide chain. [3] In the current work, we focus more directly on protein structure by mapping structural diversity in the protein's fibrillar backbone. We achieve this by tuning the mid-infrared incident beam to the Amide I stretching vibration of collagen. In this contribution, we present our experimental approach, reveal high-resolution polarization-resolved SFG images of fibrillar collagen in the Amide I stretching range, and discuss our findings in the context of collagen's molecular structure.

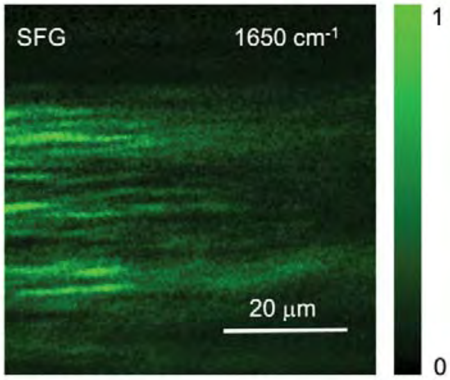

Figure 1: Vibrationally resonant SFG image of collagen, with contrast derived from the Amide I vibration.

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Deep UV Raman Spectroscopy, from Earth to Mars and Back Again

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Deep UV Raman spectroscopy has made significant strides from large lab-based systems to compact, highly ruggedized instruments being used for planetary exploration such the SHERLOC instrument on the Mars Perseverance rover [1]. This presentation will present benefits of fusing deep UV fluorescence and Raman spectroscopy, how it was implemented as in planetary instrument, and a summary of key observations the SHERLOC team has made over the last 1000 sols on Mars [1-6]. These capabilities not only provide support for samples to return to earth, the engineering development and science efforts advance instrumentation for future planetary instruments, including support of human spaceflight, as well as for use on Earth. In addition to developing a series of analog instruments for SHERLOC and future planetary science efforts, compact, portable deep UV Raman/fluorescence instruments are now opening new research opportunities and industrial applications in a wide range terrestrial applications that will be presented here.

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Camera System Development for Planetary Raman Spectrometers

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Several Raman spectrometers have been developed for planetary exploration missions in recent years [1,2,3,4]. In-situ instruments, such as the Raman Laser Spectrometer (RLS) in the Analytical Laboratory Drawer (ALD)[1] of the ESA ExoMars rover and the Compact Integrated Raman Spectrometer (CIRS)[2], initially developed for Mars but also considered for destinations such as Europa and the moon, typically utilise 532 nm lasers as excitation sources in order to perform mineralogical analyses, and, in some cases, to search for signs of extinct or extant life. One of the key aspects of instrument design that has a significant effect on the scientific performance of such spectrometers is the camera system. Most of the Raman instruments developed for space applications utilise CCD detectors due to their high detection efficiencies, low noise performance and significant levels of space heritage. However, in recent years, the performance of CMOS detectors has increased significantly and are now considered to be competitive alternatives to CCDs due to their greater flexibility and inherent radiation hardness.

In recent years, the University of Leicester have worked with teams at the Jet Propulsion Laboratory and Washington University on the development of two separate camera systems for the CIRS instrument, one for the Raman spectrometer channel and one for a Context Imaging channel. CCD and CMOS detectors were considered for both channels and radiation qualification tests were performed in order to verify the optimum choice and design for a given mission's science goals.

In this presentation, we provide overviews of the camera system development and testing programmes for the Raman spectrometer and the Context Imager, and present results from end-to-end instrument performance testing using terrestrial analogues of planetary materials. We also discuss the suitability of various camera designs for deployment on the Moon, Mars, and Europa, and describe the radiation testing campaigns performed to verify the performance of the camera systems at end of mission dose levels.

Figure 1: Inside the Compact Integrated Raman Spectrometer [2].

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Raman Spectroscopy for Future Lunar Missions

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Missions to the Moon enable us to study formation processes and materials that were present in the early history of rocky bodies in the inner Solar System. Lunar petrology provides the opportunity to study the processes that took place during the first stages of planetary formation, processes that result in a diversity of rocks that will be thoroughly characterised by in-situ analytical payloads onboard planned Space Resource Utilisation (SRU) and astronaut exploration missions. The Lunar Exploration Analysis Group has recently identified eight scientific goals for future lunar exploration [1]; of these goals, three of them would significantly benefit from in-situ studies performed with a Raman spectrometer: (i) key planetary processes are manifested in the diversity of lunar crustal rocks; (ii) lunar volcanism provides a window into the thermal and compositional evolution of the Moon; and (iii) the Moon is a natural laboratory for regolith processes and weathering on anhydrous airless bodies.

The commercial company, ispace, is developing a series of small commercial lander and rover missions (HAKUTO-R) to explore the lunar surface. The goals include the development of the cis-lunar economy and the establishment of SRU infrastructure on the Moon by breaking down the barriers of access to the lunar surface. The University of Leicester is working alongside INTA/University of Valladolid and RAL Space to deliver a Raman spectrometer instrument (based on the Raman Laser Spectrometer, RLS, delivered to the ESA Rosalind Franklin rover mission [2]) for one of the ispace HAKUTO-R lunar missions, due for launch in late 2026.

In this presentation, we provide an overview of the mission and the instrument development, qualification, and surface operations plan. We also describe some of the early Raman analysis performed with a flight representative instrument on terrestrial analogue samples relevant to the lunar mission.

Figure 1: An artist's impression of an ispace HAKUTO-R lander orbiting the Moon [3].

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Probing The Effect of Proton Irradiation on Liquid Phase Exfoliated Graphene Layers

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Graphene and other 2D materials are being extensively studied around the world due to their unique physical properties [1,2]. These materials can be used in various technological fields and environments. For example, they can be used in environments where there is a proton radiation background (Nuclear plants, space, etc.). Therefore, it is very important to understand the effect of proton irradiation on the properties of these materials.

In turn, graphene layers synthesized by liquid-phase exfoliation (LPE) exhibit interesting optical properties, especially when doped simultaneously in solution [2,3]. In our research, we investigate the influence of proton irradiation on liquid phase exfoliated graphene (LPE graphene) through the utilization of Raman spectroscopy and imaging, as well as infrared spectroscopy. By combining Raman and FTIR spectra, it was possible to analyse the effect of irradiation on the Fermi level and the optical properties of the layers [4].

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Raman inputs on the pressure-induced structural modifications in Remeika phase quasi skutterudite stannides

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The intermetallic stannides with formula $R_3T_4Sn_{13}$ (3-4-13) and $R_5T_6Sn_{18}$ (5-6-18) (where R= rare-earth/alkali metal; T = transition-metal) are attracting much attention due to their peculiar cage-like structure as well as interesting physical properties, particularly superconductivity. We have recently reported the Raman spectrum of some of these systems [1-3]. Coupled with the high-pressure (HP) diffraction studies, our systematic HP Raman studies provide the phonon mode dependence on the pressure-dependent structural evolution. We are currently in the process of extending these studies to correlate the same with pressure induced structural phase transitions [4] as well as the pressure dependent superconducting properties. Details on these studies will be presented.

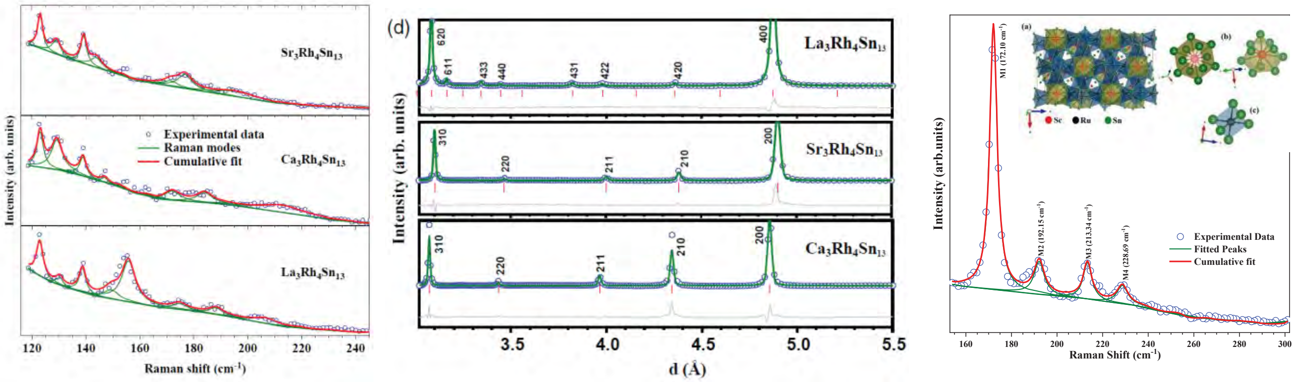

Figure 1: Left panel: Raman spectra of $R_3Rh_4Sn_{13}$ (R=Sr, Ca and La) with excitation wavelength 532 nm together with spectral deconvolution using a set of Lorentzian line shapes (solid lines). The Raman mode intensities are found to be very weak in these systems. Middle panel presents a zoom over the low-2theta part of the diffraction pattern together with the Rietveld refinement results with x-axis in d-spacing (Å). $Sr_3Rh_4Sn_{13}$ and $Ca_3Rh_4Sn_{13}$ have identical Bragg reflections, whereas $La_3Rh_4Sn_{13}$ presents several additional weak reflections in-agreement with the lattice doubling in this system. Left panel: Raman spectra of $Sc_5Ru_6Sn_{18}$ with excitation wavelength 532 nm together with spectral deconvolution using a set of Lorentzian line shapes (solid lines). Inset (a) Unit cell of $Sc_5Ru_6Sn_{18}$ showing an array of corner-sharing trigonal prisms $[RuSn_6]$ with intercalated Sn_{12} cuboctahedra around Sc_1. (b) Local environment of Sc_1 and Sc_2 with Sn atoms. (c) Trigonal prisms formed by $[RuSn_6]$.

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Towards high-throughput SERS imaging and sensing

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Surface-enhanced Raman scattering (SERS) offers unprecedent potential for ultrasensitive chemical analysis and imaging of two- and three-dimensional samples. However, albeit record-sensitivities the often excessively long spectral acquisition-times make it difficult to exploit the full analytical potential of SERS. Multiplexed readout modalities promise to overcome this limitation by eliminating one major bottlenecks: single-point, confocal, read-out schemes. Albeit their great promise, very few attempts to implement such schemes have been reported.

I will present our recent progress in combining highly-multiplexed SERS imaging and sensing schemes with tailored SERS codes. To this end, I will discuss high-speed approaches that enable widefield SERS spectroscopy[1], and even holographic 3D imaging[2]. These straight-forward to implement imaging schemes allow increasing SERS-code readout rates >100-fold, thus promising to act as enabling technologies for future SERS-based analytics.

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Surface-enhanced Raman spectroscopy on non-plasmonic vanadium oxide nanoparticles

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The metal-oxides (MeO) are promising non-plasmonic platforms for surface-enhanced Raman scattering (SERS) spectroscopy. Since their main enhancing mechanism is a charge transfer, they provide lower signal enhancement as compared to plasmonic metallic platforms. On the other hand, MeO-based SERS substrates offer good signal uniformity, stability, spectral reproducibility, and low local heating upon laser irradiation [1].

We developed a new approach for the synthesis of vanadium pentoxide (V_2O_5) nanoparticles (NPs) using magnetron-based gas aggregation sources of vanadium NPs (Fig. 1a) and thermally induced transformation of deposited vanadium NP films into V_2O_5 ones (Fig.1b) [2]. The solution of the analyte was then dropped onto the surface and dried in the air (Fig. 1c). Finally, SERS spectra were measured (Fig. 1d). Spectral detection limits of various model molecules (using 632.8 nm excitation) were determined as 5×10^{-8} M, 1×10^{-6} M, and 2×10^{-5} M for methylene blue, crystal violet, and rhodamine 6G, respectively. Spectral mapping over the surface proved excellent spectral reproducibility (RSD about 10%) [2]. Moreover, a combination of V_2O_5 NPs with nanostructures of various noble metals (Au, Ag) has shown to be promising for further enhancing the Raman signal while retaining previously mentioned advantages.

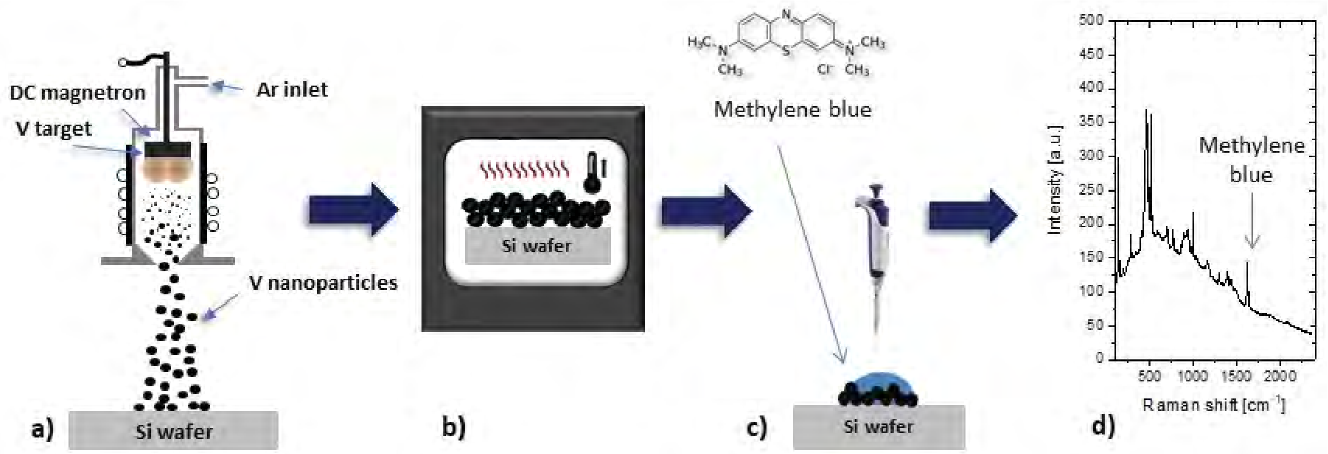

Figure 1: a) Deposition of vanadium (V) NP films, b) heat-induced formation of V_2O_5 NPs, c) drop deposition of an analyte, and d) SERS detection.

The financial support by the Czech Science Foundation (22-16667S) is acknowledged.

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Spatially resolved polarization dependent SERS using single gold nanoparticle on ultrasmooth mirror configurations

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Surface enhanced Raman scattering (SERS) has proven to be a powerful technique to strongly improve the optical response that can be acquired of a wide range of samples. Enhancing the Raman signal is indispensable when trying to gain deeper insights into light-matter interaction for samples with low Raman scattering cross-section that would otherwise not be visible due to low signal yield. The two most popular ways of achieving strong SERS enhancement factors are using either rough plasmonic substrates covered with molecules or relying on plasmonic nanoparticles deposited on top of the sample, frequently combined with a metallic mirror beneath. For the latter, it has been shown that each hotspot must be treated individually due to inhomogeneous geometries within a nanocavity, which seems to be a driving factor regarding their behaviour [1].

In this work, we present our new insights into spatial distribution of Raman signal emerging from the sample using energy-momentum spectroscopy combined with the nanoparticle-on-mirror SERS. We used a system that consists of individual nanospheres deposited directly on organic molecules of about monolayer coverage that were thermally evaporated on an ultrasmooth gold mirror. Investigating the spatial distribution of Raman signal is done by implementing a spatial filter that physically blocks the majority of the collected radiation, allowing for an investigation of separate spatial fractions of the entire beam. This requires a strong signal enhancement to compensate for the majority of the signal being blocked by the spatial filter. We supplemented the spatially resolved experiments with an in-depth analysis of excitation-polarization dependent SERS enhancement. We demonstrate that individual nanoparticle-on-mirror hotspots show unique optical responses to different laser polarizations, deviating from the general expectation of a preferred excitation with an out-of-plane electric field [2]. While on average radial polarization (dominant out-of-plane electric field) results in the strongest enhancement factor, the enhancement for azimuthal polarization (exclusively in-plane electric field) is not only much stronger than anticipated, but also overcomes that of radial polarization occasionally. Furthermore, we observe polarization dependent enhancement factors for different Raman peaks, which opened the way to discuss molecular orientation within the nanocavities. The project is concluded by combining this with energy-momentum spectroscopy to show that the angular differences in Raman scattering from individual hotspots can be achieved.

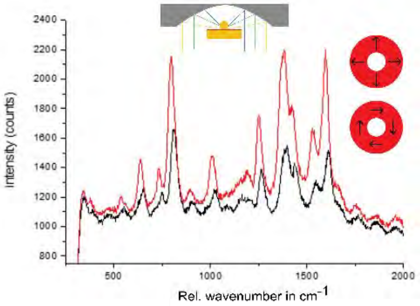

Figure 1: Difference of SERS using radial (red) and azimuthal (black) polarisation

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Polarization dependent plasmon driven reactions on chiral SERS substrate

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Chiral plasmonics has garnered significant attention within the nanomaterial scientific community due to its profound potential in light manipulation, optical activity, chiral sensing, and metamaterials [1,2]. Here, we present a plasmonic catalysis on 3-D chiral gold helicoids synthesized through chirality transfer from chiral molecules (amino acids and peptides) to metal nanoparticles [3]. The helicoids gold nanocubes (AuNC) exhibit substantial optical activity with an optical dissymmetric factor (g-factor) of 0.2. The dehalogenation of 8-Bromoadenine (BrA), adsorbed on chiral AuNC, has been employed as a model reaction to understand the effect of polarized light on chiral plasmonic reaction. The reaction pathway primarily involves hot electron transfer to the molecule adsorbed on the AuNC upon illumination with laser light, leading to cleavage of C-Br bond which is real time monitored in the Raman spectra [4]. The excitation wavelength (633nm) is chosen to maximize the optical circular dichroism of AuNC. Our observations reveal that Left-handed cubes (L-AuNC) display a higher reaction rate when exposed to right-circularly polarized (RCP) light compared to left-circularly polarized (LCP) and unpolarized light (UP). The photochemical g-factor surpasses the optical g-factor by a factor of 2.

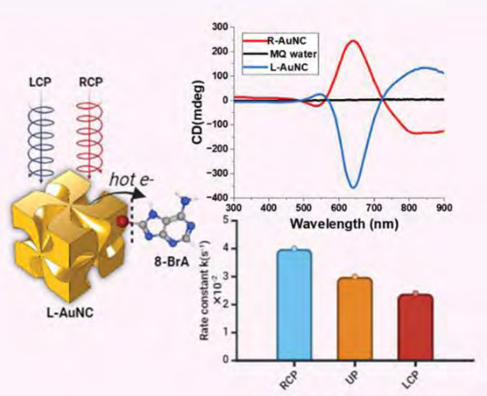

Figure: The illustration of chiral plasmonic nanostructures AuNC illuminated by LCP and RCP. Circular dichroism (CD) spectra of L-/R-AuNC in water. Depiction of rate constants with different polarized light.

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Optimised fabrication of gold nanoparticle monolayer SERS substrates at the liquid-liquid interface

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Bottom-up approaches to SERS substrate fabrication using self-assembly of nanoparticles are typically more accessible and less technically complex than top-down fabrication methods[1]. Self-assembled monolayers of colloidal citrate-capped gold nanoparticles formed at the oil-water liquid-liquid interface[1] have been used previously for SERS but are typically limited by poor reproducibility and uniformity[2,3].

Here the aim is to develop and optimise the method of self-assembly of gold nanoparticles at the liquid-liquid interface to fabricate highly reproducible, uniform, and low-cost SERS sensors. Three substrate materials (glass, polyvinyl chloride-PVC, and polycarbonate-PC) for gold nanoparticle monolayers (GML) were trialled and both the oil-phase and the aqueous phase were varied. The SERS sensors were characterised using 532 nm correlative SEM-Raman mapping and 785 nm Raman. The performance of the GML sensors was evaluated using 4-mercaptobenzoic acid (4-MBA) as the SERS probe.

We measured the uniformity and reproducibility of GMLs on different substrates (glass, PVC, PC), as well as the effect of varying the ionic strength of the aqueous phase and the solvent in the oil phase. We further explored the use of mixed solvents in the oil phase to optimise uniformity and reproducibility. GMLs fabricated by our optimised method improved the Relative Standard Deviation (RSD) of SERS signals from >70% to <10%. Thus, indicating high SERS sensor uniformity (RSD <20% are considered uniform[2]) with the improved method. Such SERS substrates have immediate applications in biosensing and rapid identification of biological targets[4,5].

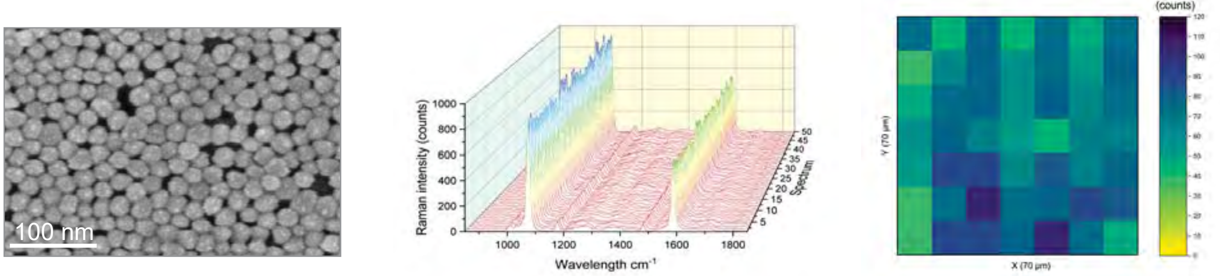

Figure 1 (a) SEM image (500,000x magnification) of a close-packed nanoparticle monolayer fabricated using optimised self-assembly and transferred to a PVC substrate. (b) SERS spectra of 4-MBA from nanoparticle monolayer on a PVC substrate. (c) Heatmap of SERS signal at 1582 cm^{-1} shows minimal variation across a GML sensor.

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Impact of metal nanoparticle size on plasmon-induced reactions

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Hot charge carriers are generated through the non-radiative decay of localised surface plasmon resonances in noble metal nanoparticles. They can induce chemical reactions of molecules adsorbed to the plasmonic nanoparticles.[1] To understand the influence of nanoparticle size on the hot electrons-induced reactions, we studied the decomposition of 8-Bromoadenine (BrAde) forming Adenine (Ade) adsorbed on different sized Au- and Ag nanoparticle aggregates by surface enhanced Raman spectroscopy (SERS). By using the decay curve of the ring breathing mode of BrAde the reaction rate was calculated and compared for each size. The results show a clear increase in the decomposition reaction rate as the Au- and Ag nanoparticle diameter increased in diameter. The main reason for that increase is the larger absorption cross section of larger diameter nanoparticles.[2] Further insight is gained through the analysis of the rate in function of the laser wavelength, where the reaction at 532 nm is faster than at 633 nm and 785 nm. Given that this investigation focused on aggregated structures to deepen insights into a smaller component within a plasmonic system, we employed a DNA origami nanofork plasmonic nanoantenna for the examination of plasmon-induced reactions.

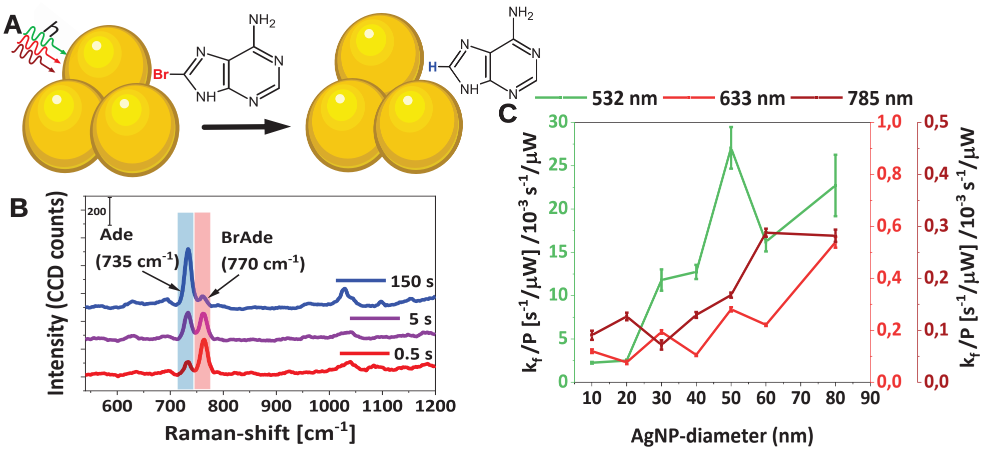

Figure 1: A) schematic illustration of Decomposition of BrAde on plasmonic metal nanoparticles; B) Average SERS spectra of BrAde adsorbed on 60 nm AgNPs, measured with 633 nm (300 µW); C) Plot of calculated reaction rate (k_f) of the Decomposition of BrAde normalized by the used laser power for different AgNP sizes and different wavelengths.

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Metasurfaces for enhanced Raman scattering and photocatalysis

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Dielectrics are a new frontier for Surface Enhanced Raman Scattering. They can serve either as a complement or an alternative to conventional, metal-based SERS, offering key advantages in terms of low invasiveness, reproducibility, versatility and recyclability. In comparison to metals, dielectric systems are characterized by a much greater variety of parameters and properties that can be tailored to achieve enhanced Raman scattering or related effects. Light trapping and sub-wavelength focusing capabilities, morphology and size-dependent resonances, control of band gap and stoichiometry, charge transfer between substrate and molecules and vice-versa are a few examples of the manifold opportunities associated to use of dielectrics as SERS-active materials [1,2]. In this context, dielectric metasurfaces, which can be designed to manipulate light with unprecedent control, represent an exciting, yet still quite unexplored field for Raman spectroscopy and photocatalysis.

Here we will show how metasurfaces based on TiO_2 optical antennas can be exploited to enhance Raman scattering, providing a level of reproducibility and reliability that overcomes conventional plasmonic SERS [3]. This approach can be extended to the study of light-driven surface processes [4], allowing to finely control selectivity and efficiency of photocatalytic reactions.

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Impulsive stimulated Raman spectroscopy with frequency-correlated entangled photons and with X-ray pulses

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Multidimensional spectroscopy has been instrumental for probing dynamical processes in a wide variety of material systems ranging from atoms, molecules to biological complexes. These techniques traditionally rely on sequences of coherent laser pulses with electric fields with well-defined envelopes and phases. Quantum light sources have been developed for broad applications such as quantum information processing, secure communication, and lithography. Employing quantum light in multidimensional spectroscopy is opening up many exciting opportunities to enhance the signal-to-noise ratio, improve the combined temporal, spatial, and spectral resolutions, and simplify nonlinear optical signals by selecting desired transition pathways in second and third order signals. We show that the spectro-temporal resolution of a stimulated Raman process with frequency-correlated entangled photons is superior to the conventional method which uses a broad- and a narrowband classical field. The entangled photon signal follows the instantaneous Raman frequency more closely and a sufficient temporal resolution is achieved at longer pulses compared to the broadband pulses needed in the classical case. The entangled photon signals further makes use of the entire frequency bandwidth of the photons while the classical method only utilizes the regime where the two fields are temporally overlapping, causing a reduction in signal intensity.

Novel X-ray pulse sources from free-electron lasers and high-harmonic generation setups enable the monitoring of molecular events on unprecedented temporal, spatial and energetic scales. The attosecond duration of X-ray pulses, their large bandwidth, tunable energy range, and the atomic selectivity of core X-ray excitations offer a uniquely high spatial and temporal selectivity for non-linear Raman spectroscopies. X-ray signals reveal detailed information about the ultrafast passage through conical intersections. We show how the orbital angular momentum of twisted X-ray light can be leveraged to detect electronic and vibrational coherences and time evolving chirality emerging at conical intersections due to the bifurcation of molecular wavepackets.

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Impulsive Stimulated Raman Spectroscopy on Large-Amplitude Wave Packet Dynamics in Gas-Phase Molecules

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Low-frequency large-amplitude motion (LAM) induces substantial structural change of molecules and molecular aggregates. It may cause modification of electronic properties of molecular systems and give rise to chemical reaction, *e.g.* isomerization or twisted intramolecular charge transfer. Impulsive stimulated Raman (ISR) spectroscopy is suitable for observation of LAMs, since it can securely locate transitions well below 100 cm^{-1} without interference by strong Rayleigh scattering. However, when applying ISR spectroscopy to gas-phase dilute molecular samples, highly sensitive detection is needed. In addition, incorporation of species selectivity will be required.

This presentation will introduce two approaches for low-frequency ISR studies of gas-phase isolated molecular systems. The first is based on pump-probe scheme, where the pump and probe time regimes are quite different. As a pump, a pair of two ultrashort pulses (~800 nm, < 100 fs duration) is irradiated to induce vibrational coherence via non-resonant ISR excitation in the electronic ground state of molecular systems. The vibrational wave packet created by the first pulse is interfered by the second pulse, and the population of each vibrational eigenstate is modulated when the mutual delay between the two pump pulses is changed. Then, such a delay dependent change in vibrational-state population is monitored via resonant two-photon ionization with time-of-flight mass spectrometry, by using a wavelength-tuneable UV probe pulse (~10 ns duration, frequency resolution of < 0.1 cm^{-1}). Such state- and mass-selective measurements afford us definite molecular identification even when the gas sample is a mixture of various molecular species. This approach was successfully applied to the studies on LAM dynamics in substituted biphenyls [1] and molecular clusters, *e.g.* benzene–CH_4.

The second approach adopts fs time-resolved Coulomb explosion imaging, which has been applied to experimentally visualize rotational wave functions of molecules [2]. Here, an ultrashort pump pulse (~800 nm) is again used to induce non-resonant ISR excitation. The resultant spatiotemporal evolution of probability distribution of the intermolecular axis is captured by monitoring images of fragments promptly ejected from multiply charged ions created by another time-delayed more intense ultrashort probe pulse. The fragments from molecular species to be studied fly with substantial recoil velocity and can be clearly discriminated against signals of unfragmented ions. The time-dependent alignment thus measured shows oscillatory dynamics of overall rotation as well as much faster change due to intermolecular vibration in molecular clusters, *e.g.* Ar_2 and N_2–Ar.

In the presentation, experimental setups of the two approaches will be described in some details. Results on some of molecular systems and future perspective will also be discussed.

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Time-Resolved Resonance Raman Spectroscopic Observation of Selected Arylnitrenium Ions

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Arylitrenium ions are highly reactive intermediates that are important in some synthetic chemistry reactions and in biological processes as suspected carcinogens that can induce DNA damage and modification. We reported the first time-resolved resonance Raman (TR^3) direct observation of the 2-fluorenylnitrenium ion (**2FN**) in aqueous solutions.[1] Later, we used TR^3 to study the reaction of **2FN** with guanosine to generate the C8-intermediate (dG-C8-NH) [2] for the first time-resolved vibrational spectroscopic investigation of an arylnitrenium ion reaction with a guanine derivative and also the first vibrational spectroscopic characterization of a C8 intermediate species. We then extended TR^3 study for reactions between the **2FN** and DNA oligomers which found that the observed C8 intermediates were similar to those formed from the monomeric guanine derivatives, consistent with product analysis and biological experimental observation in vivo.[3] The oligomeric structure does not greatly influence the reaction intermediate structure but does accelerate the reaction rate of the guanine units towards the arylnitrenium ion and may modestly affect the structure of the intermediate.[3] All of the arylnitrenium ions examined in these previous studies are singlet arylnitrenium ions and the triplet states of arylnitrenium ions and their reactions have not yet been similarly directly detected with time-resolved spectroscopic methods. We have recently detailed the development of a new photoprecursor compound whose excited state can undergo heterolysis to form the singlet arylnitrenium ion and also concurrently undergo intersystem crossing (ISC) with a subsequent spontaneous heterolysis to produce the triplet p-iodophenylnitrenium ion (np).[4] This triplet arylnitrenium ion then proceeds by ISC to produce the ground singlet state that eventually undergoes proton and electron transfer to form a long-lived radical cation that goes on to form the reduced p-iodoaniline final product.[4] We have also recently discovered another new photoprecursor compound that can form a ground state triplet arylnitrenium species.[5] These new results provide an improved understanding of the photophysical characteristics and reactivity of two different spin states of arylnitrenium ions (eg singlet and triplet) and also report the first direct time-resolved observations of both an excited state and ground state triplet arylnitrenium ion species.[4,5]

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Impulsive stimulated Raman spectroscopy of squaraine dye molecules encapsulated inside carbon nanotubes

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Carbon nanotubes (CNTs) represent an ideal system to confine molecules in a one-dimensional (1D) nanospace, due to their hollow structure, their smooth and impermeable sidewalls and the precise tunability of their diameter [1]. These unique characteristics can be exploited to study the behavior of atoms and molecules confined in 1D, or to realize new hybrid structures (mol@CNTs) with different and engineered optical properties, due to the interaction of the two species [2,3]. In this presentation, transient absorption and impulsive Raman spectroscopies are exploited to study the electronic, optical and vibrational properties of squaraine dye molecules encapsulated inside CNTs in the time domain. The electronic and vibrational relaxation dynamics of the squaraine molecules were tracked from the first tens of femtoseconds upon the photoexcitation to hundreds of picoseconds. Furthermore, by comparing the Raman and the electronic responses of the squaraine molecules encapsulated inside CNTs and solubilized in an organic solvent, we show how the encapsulation modifies the vibrational properties of squaraine.

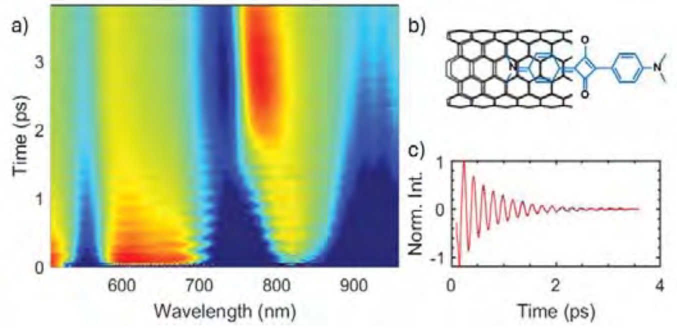

Figure 1: a) Coherent oscillations (ISRS response) and transient absorption of squaraine molecules encapsulated inside CNTs. b) Pictorial representation of a squaraine molecule inside a CNT. c) Coherent oscillations of squaraine at 750 nm.

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Nonlinear Raman spectroscopy of ChCl-EG Deep Eutectic Solvents

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Deep Eutectic Solvents are non-aqueous solvents obtained by mixing Lewis or Brønsted acid with bases. They are being considered for application as next-generation electrolytes in different battery chemistries, among which the Zinc-ion technology, where ChCl-EG, in particular, can ease anode shape-change control [1]. Generally poor ionic conductivity of pure DESs can be improved with addition of water below the salt-in-water threshold and molecular-level understanding of Zn^{2+} coordination is crucial to jointly optimize charge-transfer kinetics, electrodic chemistry and mass transport. Raman spectroscopy has the potential to elucidate the structural changes incurred with the addition of water in ChCl-EG DES but, in its spontaneous version, it is challenging due to fluorescence background and low signal strength. Here we circumvent these limitations with the use of Stimulated Raman Scattering (SRS) and Impulsive Vibrational Spectroscopy (IVS) in the frequency and time domain, respectively [2,3].

We observe that addition of water causes changes to the vibrational modes at 280 cm^{-1} and 3470 cm^{-1}, namely spectral peak shift and change in dephasing time, which give information about the zinc ligand mode (Fig. 1e) and zinc aqueous complex respectively. Results on this particular system, in addition to specific interest for Zinc-ion batteries, also prove the potential of nonlinear Raman spectroscopy for the investigation of battery electrolytes and other chemistries and *in operando* measurements.

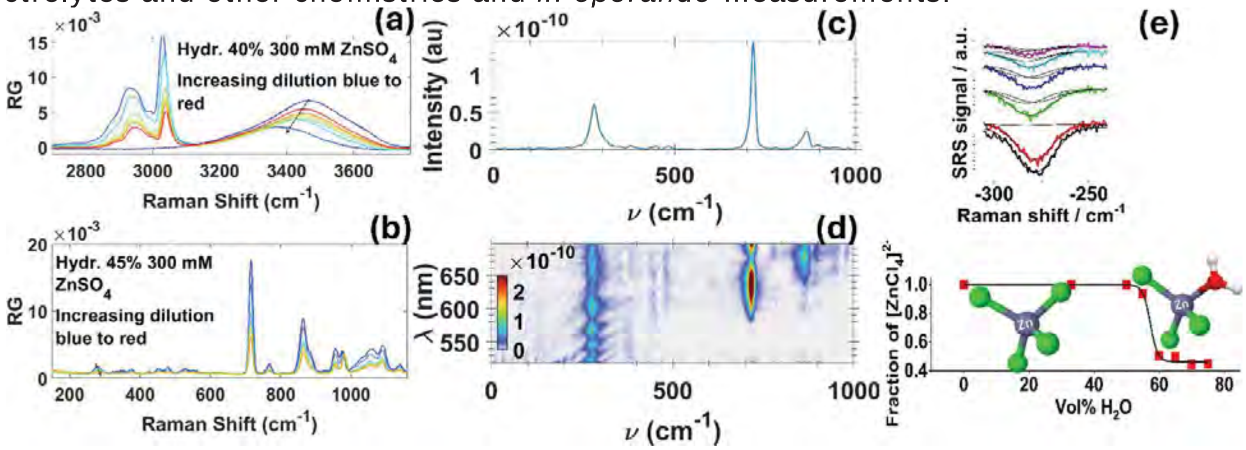

Figure 1: (a)SRS spectrum of high frequency region and (b)low frequency region. (c) Typical IVS power spectrum and (d) colormap of anhydrous ChCl-EG DES sample. (e) Fitting of the ν(Zn^{2+}-Cl^{-}) mode with $[ZnCl_4]^{2-}$ and $[ZnCl_3H_2O]^{-}$ components and the thereby estimated fraction.

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Single-pixel field-resolved coherent anti-Stokes Raman scattering with three-color excitation

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Coherent Anti-Stokes Raman Scattering (CARS) spectroscopy is an advanced optical technique that offers a significant improvement in the detection and analysis of molecular vibrations over spontaneous Raman methods. Two-color Broadband CARS (BCARS) spectroscopy employs a broadband Stokes frequencies to simultaneously excite multiple vibrational modes, giving a comprehensive Raman response in a single shot manner [1]. However, despite the advantages of BCARS approach, its detection efficiency is limited by the inefficient stimulation of Raman transitions. Three-colour- excitation has been recently proposed to address this issue [2]. However, similar to the shortcomings of the single-pulse CARS approach [3], the three-colour scheme is primarily applicable only to the fingerprint region, indicating that a more efficient BCARS approach within the CH/OH stretch region still remains to be developed. Furthermore, these approaches are based on cameras, which have limited imaging speed.

In this presentation, we revisit the three-color excitation strategy however applicable to the CH/OH stretch region, using a broadband pump/probe and narrowband Stokes beam. This technique leverages a programmable pulse shaper [4], enabling multiple modulations of the pump/probe beam's spectrum. Detection of the CARS signals excited by each modulation are captured by a single-pixel detector, an approach significantly faster than exploiting cameras. We develop analytical and computational methods to demonstrate exact retrieval of the third-order susceptibility $\chi^3(\omega)$.

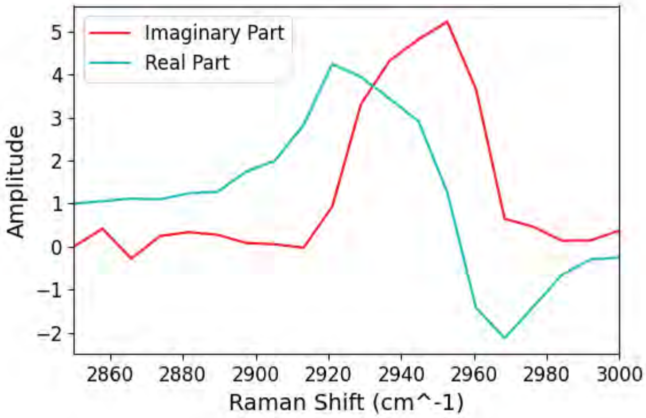

Figure 1: Measured complex $\chi^3(\omega)$ of Acetonitrile using proposed method.

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Stimulated Raman Imaging of Lysosomotropic Drug Analogs and Lipid Dynamics in Live Cancer Cells

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In the realm of cancer therapy, understanding the cellular mechanisms affected by drug treatments is crucial for enhancing efficacy and overcoming resistance. This study employs stimulated Raman scattering (SRS) imaging[1] to elucidate the interactions between lysosomotropic drug analogs and the lipid metabolism in live cancer cells. By integrating these drug analogs with a specifically designed Raman-active probe, our research offers unprecedented visualization of their localization within lysosomes. Our findings reveal significant alterations in lipid dynamics triggered by these drugs, suggesting a novel mechanism through which they may exert their therapeutic effects or contribute to the development of resistance. The implications of these results extend beyond cancer therapy, highlighting the versatility of SRS imaging as a tool for real-time observation of cellular processes and drug action. Through this study, we not only deepen our understanding of lysosomotropic drug behavior but also underscore the potential of SRS imaging in advancing the precision of cancer treatments and possibly identifying new targets for combating drug resistance[2]. Current and future work aim to image antibody-drug conjugates (ADCs) trafficking into lysosomes in live cancer cells using SRS imaging with Raman tags.

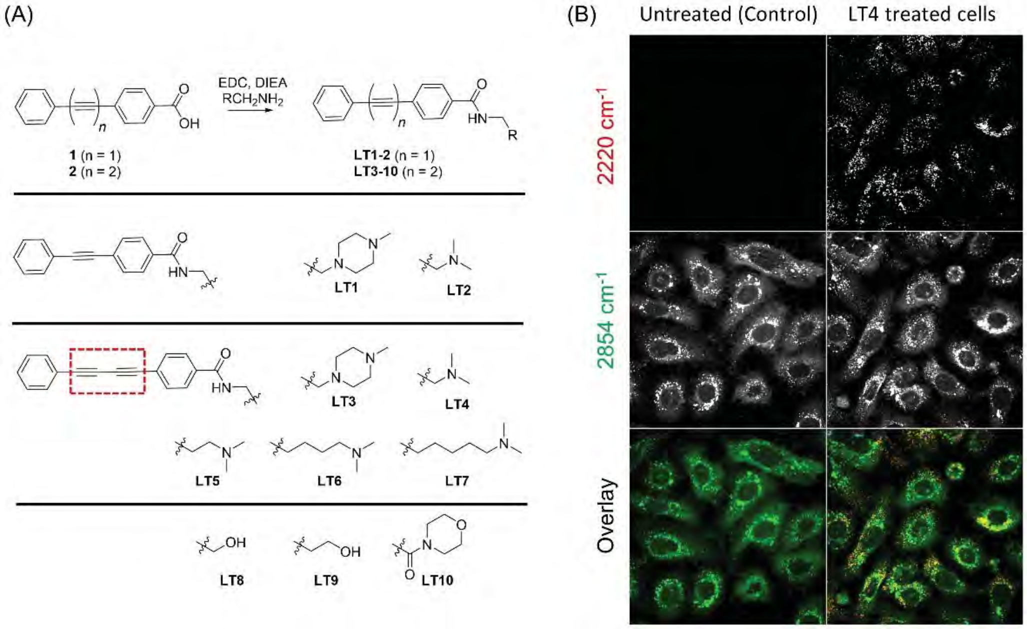

Figure 1: (A) synthesized lysosomotropic (LT) drugs containing alkyne groups, which exhibit a Raman peak at around 2200 cm^{-1}. (B) SRS imaging of live cancer cells treated with the LT4 compound at 2200 cm^{-1} (C≡C) and 2854 cm^{-1}(lipids, CH_2), respectively[2].

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Silicon-enhanced alkyne tags for Raman and infrared-based imaging

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Vibrational tags have unique vibrational modes in the silent regions; they are essential tools for bio-imaging studies. Alkyne-based vibrational tags have proven efficient and successful because of their narrow Raman bandwidth, strong response [1], spectral tunability, and inert chemical character in the cellular environment. Whereas Raman tags have seen significant development, IR-based tags have yet to benefit from systematic design optimization in a similar way. In this contribution, we present the design of silicon-enhanced alkyne tags that feature substantial IR activity while also retaining a strong Raman response [2]. For this purpose, we optimize the chemical motifs with density functional theory (DFT). The synthesized motifs are tagged to lipids for exemplary cellular imaging. The cell samples are evaluated using stimulated Raman scattering, Fourier transform infrared (FTIR) absorption microscopy, and photothermal microspectroscopy.

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Electronic Pre-Resonance Coherent Anti-Stokes Raman Scattering Microscopy

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[a]*University of Konstanz*

Coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) microscopy are important methods for label-free imaging with vibrational contrast. Their broader application, however, is often limited by their relatively poor sensitivities. Recently, it has been shown that similar to spontaneous resonance Raman spectroscopy, the sensitivity of SRS microscopy can be boosted significantly by exploiting electronic pre-resonances [1,2]. Electronic enhancement of CARS signals in spectroscopy has been reported in the seventies [3]. Here, we present experimental results on the electronic enhancement in CARS microscopy. We demonstrate that by choosing one of the excitation wavelengths close to an electronic transition in the sample molecules, significant sensitivity enhancements of CARS microscopy down to the low μM detection level can be achieved. As is the case for pre-resonance SRS microscopy, however, also background not present in the electronically non-resonant counterparts is generated. We will discuss the origins of these background signals and show how they can be removed.

Our findings not only contribute to the understanding of electronic pre-resonance effects in CARS, but also offer a practical solution to enhance sensitivity, opening new avenues for applications in chemical and biological imaging at lower concentrations.

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Broadband background-free stimulated Raman microscopy with a new frequency-modulation scheme

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Stimulated Raman scattering (SRS) microscopy is usually considered a backgroundfree technique due to the absence of the so-called non-resonant background present in coherent anti-Stokes Raman scattering. However, there are other optical processes – such as two-color-two-photon absorption, thermal lensing, and cross-phase modulation – that spectrally overlay with the resonant Raman signal and introduce a background signal that can limit the contrast and sensitivity of SRS measurements [1].

We propose a new broadband background-free SRS microscopy scheme based on the frequency modulation approach (FM-SRS). A 1030nm femtosecond laser is frequency doubled using a high-efficiency spectral compression method to pump a picosecond optical parametric oscillator, whose output is used as the pump beam for the SRS setup. The broadband Stokes beam is split into two intensity-modulated arms with orthogonal polarizations using an electro-optic modulator and a polarizing beam splitter. The beam in one of the arms is spectrally filtered by a high-speed, narrowband acousto-optic tunable filter [2], while, in the other arm, spectral selection is achieved using a narrowband etalon. After matching their optical paths, the two Stokes sub-beams are recombined with another PBS. A half-waveplate is used to rotate the polarizations of the Stokes sub-beams by an arbitrary degree, and a polarizer is used to select their projections over the polarization of the pump beam for efficient SRS generation. The pump and the Stokes beams are then recombined by a dichroic mirror and focused on the specimen (Fig. 1a).

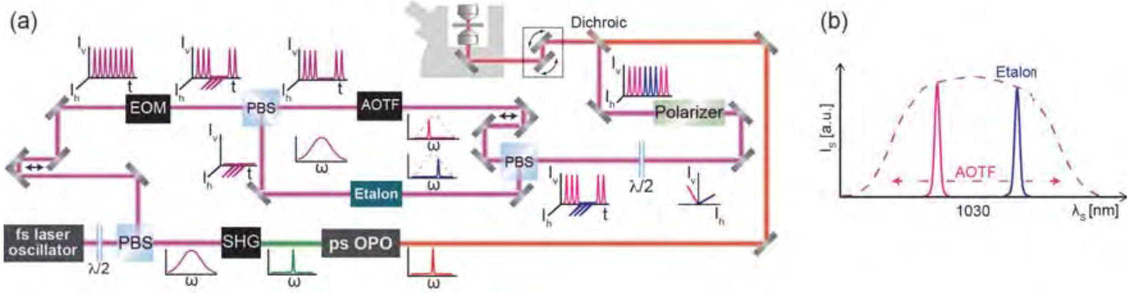

Fig. 1, a) Our FM-SRS setup. PBS: polarizing beam splitter; SHG: second harmonic generation; OPO: optical parametric oscillator; EOM: electro-optic modulator; AOTF: acousto-optic tunable filter; b) wavenember-pair spectral selection

The broad bandwidth (~300 cm^{-1}) of the Stokes beam, combined with the fast and automated tunability of the AOTF, gives high flexibility and ease in selecting the pair of probed wavenumbers in FM-SRS (Fig. 1b). This new FM-SRS architecture allows background-free acquisitions from the fingerprint to the CH-stretch region of the Raman spectrum. We present application of our FM-SRS microscope for imaging crystals within microalgae in the fingerprint region.

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Chirp modulation stimulated Raman scattering microscopy: completely background free vibrational imaging

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Stimulated Raman scattering (SRS) microscopy has emerged as a powerful tool for label-free chemical-specific imaging [1]. In SRS, molecules are coherently excited by two laser fields (pump and Stokes beams) frequency detuned by the molecular vibrational frequency. Typically, a modulation transfer / lock-in detection scheme is implemented. In spectral focusing [2], the input femtosecond pulses are co-chirped (same sign and magnitude of chirp) to picosecond durations. Vibrational spectroscopy obtains from scanning the time delay between the chirped pulses. The SRS signal is linear in molecular concentration and unimpacted by non-resonant Four Wave Mixing background, offering a useful tool for quantitative chemical imaging. However, non-Raman background processes can also couple the two fields in a sample, appearing as modulated signals which limit the sensitivity and contrast of SRS. These non-Raman processes, where the presence of one laser field affects the propagation of the other, can be broadly classified into two categories: 1) Nonlinear (two-photon two-colour) absorption; 2) Nonlinear scattering (transient lensing) where the finite solid angle of the detector does not collect the full scattered field.

To date, several SRS background reduction schemes were demonstrated. These discriminate Raman from certain non-Raman responses based on, for example, their frequency [3] or polarization responses [4], or their contribution to the pump and the Stokes intensities [5]. Here we present a novel scheme: Chirp modulation (CM) SRS. For spectrally integrated detection in a spectral focusing configuration, the non-Raman terms are insensitive to the relative sign of chirp between the input pulses. By modulating between co-chirped and contra-chirped (opposite sign but same magnitude of chirp) arrangements, we show that all non-Raman background signals are removed, yielding a completely background free Raman spectrum. CM SRS opens the door to very low-concentration chemical imaging relevant to broad applications in biology, pharmacology, mineralogy, and material sciences.

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Raman Optical Activity: Biological and Biopharmaceutical Applications

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Raman optical activity (ROA) and its related infrared technique vibrational circular dichroism (VCD) comprise the field of vibrational optical activity (VOA)[1,2]. VOA is defined broadly as the difference in intensity in right versus left circularly polarized (RCP vs. LCP) radiation for a chiral molecule or molecular entity undergoing a vibrational transition. There is only one form of VCD, the difference in absorbance for LCP minus RCP infrared (IR) radiation. ROA, on the other hand, has four forms, incident circular polarization (ICP), scattered circular polarization (SCP) and two forms of dual circular polarization (DCP_I and DCP_{II}), referring to whether the CP difference is in the incident laser radiation, the scattered radiation, or both, either in-phase or out-of-phase, respectively. VOA intensities are roughly three to five orders of magnitude smaller that their parent IR and Raman intensities.

Both ROA and VCD were discovered in the 1970s and have evolved instrumentally and theoretically over the past five decades so that today VOA instruments and computational software are available commercially for a wide variety of impressive applications. Instrumentation includes Fourier-transform (FT) IR for VCD and multichannel charge coupled device (CCD) detection for ROA providing simultaneous measurement of VOA over hundreds of wavenumbers with no need to scan the spectrum.

To date most applications of VCD involve small chiral molecules dissolved in non-aqueous media whereas most ROA applications have involved biological molecules in aqueous media where ROA has the advantage of weak scattering from water. Another advantage of ROA derives from the variety of instrumental configurations, such as scattering geometry (right-angle, back, and forward scattering) and laser radiation wavelength (UV, visible and near IR) which expands the range of applications that can be explored. Unique applications of ROA to biological molecules have also included resonance enhancement as RROA with laser excitation in the visible and in the near-IR, and the sensitivity of ROA to supramolecular chirality in protein fibrils.

The focus of this talk will be on bio-related applications of ROA, starting with biological molecules including amino acids, peptides proteins, carbohydrates and glycoproteins and moving to a variety of biologic and biosimilar pharmaceutical molecules. Here there is growing list of modalities that includes immunoglobin antibodies (mAbs), recombinant DNAs, messenger RNA vaccines (mRNAs), fusion proteins and various types of small molecule protein combinations.

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Application of Raman optical activity spectroscopy to the study of microbial rhodopsins

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Microbial rhodopsins are the seven-transmembrane photoreceptor proteins that are widespread over microrganisms (Figure 1A). They contain the retinal chromophore to catch the photon energy, and their representative functions include the light-driven ion pump, light-gated ion channel and photosensor. These biological functions are mediated by the photoreactions of the retinal chromophore (Figure 1B) in the active site, where the chromophore fits with the specific three dimensional structures. The conformation of chromophore inside the active site is important because it is closely related to which color of light the chromophore is sensitive to and how the chromophore photoreacts. In order to capture this conformation of the retinal chromophore in microbial rhodopsins, Raman optical activity (ROA) spectroscopy can be powerful [1-4], due to its capability of reporting the three dimensional molecular structures most sensitively.

ROA spectroscopy detects the small difference of Raman intensities obtained with right- and left-circularly polarized incident lights (Figure 1C). In this study, we applied ROA spectroscopy to the investigation of microbial rhodopsins. We show that the ROA spectra provide the three-dimensional structural information of the retinal chromophore, which is not obtained by X-ray crystallography or conventional vibrational spectroscopies.

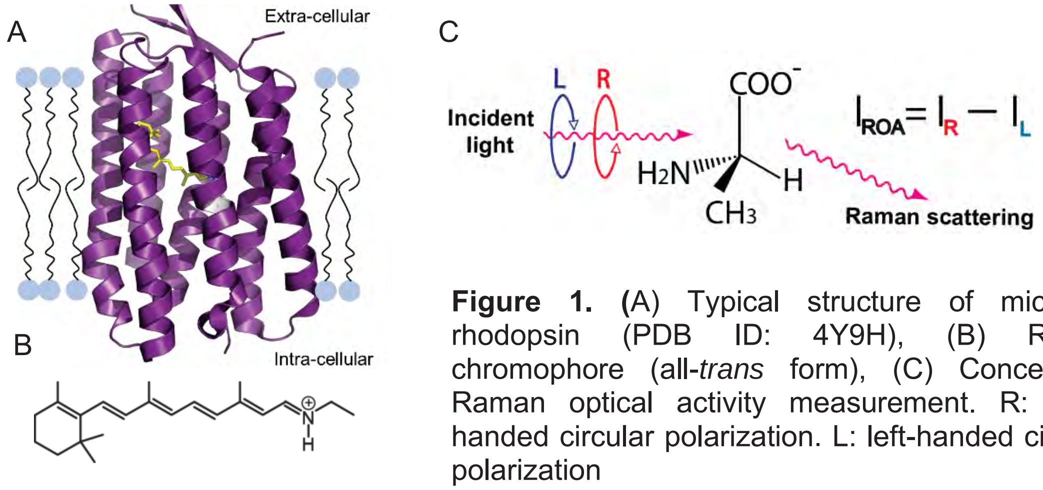

Figure 1. (A) Typical structure of microbial rhodopsin (PDB ID: 4Y9H), (B) Retinal chromophore (all-*trans* form), (C) Concept of Raman optical activity measurement. R: right-handed circular polarization. L: left-handed circular polarization

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Probing stereochemistry and Structure Using Raman optical activity (ROA)

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RMIT University

Raman optical activity (ROA) measures a small difference in the intensity of Raman scattering, when using circularly polarized photons, from chiral molecules. As ROA band shapes and band intensities are highly sensitive to the vibrational dynamics of the stereochemistry of the atoms and bonds involved, ROA spectra are also highly sensitive to successive hierarchies of biopolymer structure: primary (subunit identity), secondary (local interactions) and tertiary (folding). This presentation will explore how the details of ROA spectra can provide useful new perspectives on the structures of proteins, polysaccharides and viruses.

Raman Optical Activity of Chiral Biosupramolecular Systems

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Raman Optical Activity (ROA), a chiral variant of Raman spectroscopy, is still a highly unexplored technique, mostly due to its low analytical sensitivity. Our recent research concentrates on supramolecular biogenic systems that provide enhanced ROA due to either resonance of an incident radiation with an electronic transition[1-3] or exciton coupling[4]. In particular, we focus on carotenoid aggregates that show intense resonance ROA at 532 nm excitation and amyloid fibrils that provide excitonically enhanced signal. Our results demonstrate that ROA can be an unique tool for studying aggregation processes and organization of carotenoids in membranes[1], micelles[2] or complexes with proteins[3]. Amyloid fibrils generate exceptionally intense and distinct ROA already for prefibrillar states, making ROA an ultrasensitive tool to characterize fibril formation and structures (Fig. 1)[4]. Overall, we show that employing signal enhancement could make more realistic applications of ROA for analysis of complex biological systems, which predominantly consist of supramolecular assemblies regulated by non-covalent interactions and chiral-recognition mechanisms.

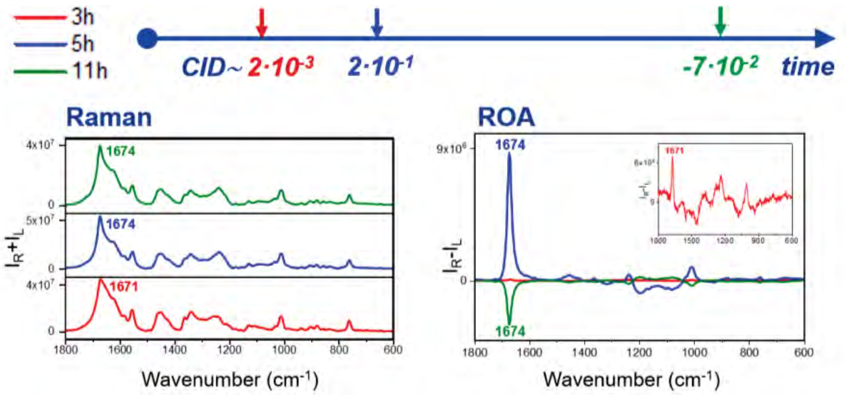

Figure 1: Raman and ROA spectra of prefibrillar states (—), early-stage (—) and mature (—) amyloid fibrils of hen egg white lysozyme obtained at different incubation times (3, 5 and 11 h, respectively). CID is the ROA to Raman intensity ratio. Based on [4].

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New horizons for Raman optical activity

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Raman optical activity (ROA) is based on the measurement of the small difference between right- and left-handed circularly polarized Raman scattering [1]. High quality spectra are of paramount importance and the work of W. Hug [2] represents undoubtedly a major milestone, allowing a wider use of the technique.

ROA has proved to be a very sensitive analytical tool for the study of absolute configuration [3], the determination of enantiomeric excess with unprecedented accuracy [4], conformation of molecules in solution [5], often revealing anharmonic spectral features [6], intermolecular interactions [7] and the details of large molecular systems such as globular proteins [8].

Much effort is currently being devoted to finding ways to increase the sensitivity of ROA, one of which is resonant Raman optical activity (RROA). It has been recognized that RROA is often obscured by the stronger circularly polarized Raman scattering in samples exhibiting electron circular dichroism [9]. This effect can be exploited for investigation of chiral samples, but must be subtracted to obtain the RROA. For this purpose, an efficient method has been found and RROA spectra have been confirmed for a number of molecules [10,11]. High quality spectra measurable for various polarization modulation schemes, interpreted with state-of-the-art *ab initio* calculations, thus reveal details of the molecular structure in the resonant state, while fulfilling the dream of signal enhancement [11].

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Resonance Raman Optical Activity in the study of the iron oxidation state in heme proteins

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Heme proteins are biologically important molecules that are widespread in human organisms. Their characteristic feature is possessing an iron ion in the center of the porphyrin ring, whose oxidation state, Fe^{2+} or Fe^{3+}, is determined by the reductive or oxidative environment, respectively. These proteins perform many important biological functions such as the transport of oxygen and its storage (hemoglobin and myoglobin, respectively), electron transfer (e.g. cytochrome c and cytochrome b_5), and redox-regulated signaling (e.g. cytoglobin).[1,2] Structures of selected heme proteins are presented in **Figure 1**.

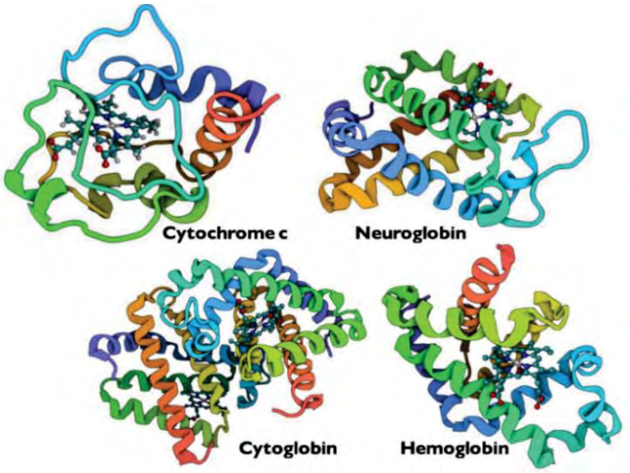

Figure 1: Structures of selected heme proteins (created in BioRender.com from RCSB Protein Data Bank).

To study various heme proteins we have applied Raman Optical Activity (ROA), a dynamically developing chiroptical spectroscopy method. It is defined as a difference of right and left circularly polarized light which is detected in Raman scattered light ($\Delta I = I_R - I_L$). ROA is a useful sensitive analytical tool for the investigation of biological compounds in their native aqueous environment. In resonance conditions and while multiple electronic states are involved in the excitation, resonance ROA (RROA) spectra are bisignate. Multiple and bisignate RROA bands provide information about optical activity, hindered for resonance Raman spectroscopy (RR). Moreover, it provides more specific information about the molecular structure of chiral systems compared to RR.[3]

Herein, we report the new application of RROA to study the oxidation state of the iron ion in different heme proteins, e.g. cytochrome c. The obtained bisignate RROA spectra of reduced and oxidized forms of this protein exhibited significant differences which enabled us to distinguish them and delivered additional structural information.

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A multi-analytical approach to investigate the altars' stuccoes in the Sant'Angelo complex in Barbarano Romano

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In this last year, an intensive restoration campaign has been carried out on the stucco altar of Saint Michael the Archangel in the Sant'Angelo complex at Barbarano Romano. As comparison, the Major altar (XVII century) was also investigated. An integrated analytical procedure, based on the use of non-invasive and micro-invasive techniques, has been performed aiming at defining the painting materials, the execution technique, the state of conservation and the degradation products as well as previous conservation treatments. Preliminary *in situ* investigations have been carried out by X-Ray Fluorescence (XRF) spectroscopy and UV imaging. Further analyses on selected fragments have been performed by using μ-Raman spectroscopy, Fourier Transform Infrared Spectroscopy (FT-IR), and Scanning Electron Microscopy equipped with an Energy Dispersive X-ray detector (SEM-EDS). Data analyses have revealed the unexpected presence of a mixture of gypsum, calcite, and lead white. Lapis lazuli, red ochre, yellow ochre, and some alteration products, such as (para)coquimbite, have been detected. Noteworthy, oxalates and carotenoids, likely related to a microbiological attack [1], and basic lead chloride (Figure 1), interpreted as a possible alteration product due to previous restoration interventions, have been identified [2]. FT-IR analysis confirmed the presence of gypsum, calcite, oxalates and lead white and showed signals attributable to organics, probably proteins. The proposed multi-analytical approach allowed a complete characterization of the investigated works of art, addressing a much more focused and adequate restoration treatment.

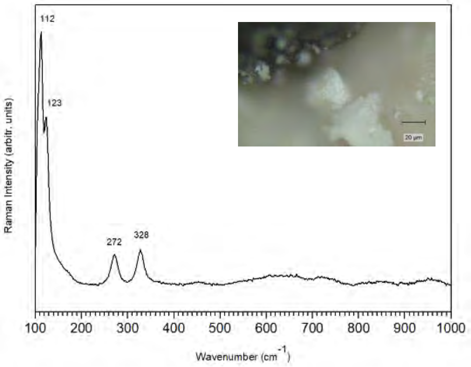

Figure1 Raman spectrum of lead basic chloride and its related micrograph

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Industrial coatings under the spotlight: Composition of paint layers of coated scrap metals

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The conservation of movable industrial heritage primarily focuses on preserving machinery, equipment, and tools associated with various industries. However, the presence of industrial scrap metals as art objects underscores the necessity for art conservation to also give due consideration to the components of industrial artifacts. This necessity is made apparent by Gonçalo Mabunda's sculptures (Fig. 1) which are made up of coated scrap metals and are undergoing paint loss due to the corrosion of metallic substrates. This research aims to analyze the coatings present in Mabunda's sculptures, contributing to an enhanced understanding of the materials and their degradation. The ultimate goal is to facilitate a development of an appropriate conservation approach for industrial scrap metals utilized as artworks.

To achieve this goal, the paint coatings were sampled, and cross-sections were observed under optical microscopy. The key elements of the pigments were analyzed by XRF spectroscopy, and the composition of compounds was identified through RAMAN spectroscopy. Binder identification was done using ATR-FTIR spectroscopy.

Preliminary results showed that the pigments present in Mabunda's sculptures, namely blue and green phthalocyanine, chrome yellow and yellow-orange, titanium white, and azo red pigments, are those which have been reported in previous studies[1] [2] [3] that analyzed the coatings applied to industrial heritage objects and machines from 19th to 20th Centuries. Initial findings for the binders identified alkyd as the binding agent for most samples except for one that had an acrylic binder. The most interesting discovery, however, was the unique composition of the sampled retroreflective sheet (Fig. 2), which contains silica beads. As far as we are aware, material analysis of retroreflective sheets with silica beads in the heritage conservation context is something novel.

Figure 1. "*Dá ao Povo o que é de Cesar*." (2013) Sculpture by G. Mabunda. Wood, metal, and painted metal. 165 cm x 90 cm.

Figure 1. Cross-section of a retroreflective sheet sample taken from the sculpture in Fig. 1, comprising of four layers: 1) silica beads, 2) adhesive, 3) red color, 4) yellow color.

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New Insights into Iron Gall Inks: Exploring Variations in Raman spectra through a Chemometric Approach

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To develop effective conservation treatments, it is crucial to understand the molecular structure of Iron Gall Inks (IGI) and the degradation processes that occur in these valuable manuscripts. However, the composition of the IGI complex is not well understood. Interdisciplinary studies have made progress in bridging this knowledge gap through comprehensive molecular characterization of phenolic extracts obtained from historically accurate reconstructions. Raman and infrared spectroscopies have shown that medieval writing inks are not represented solely by iron complexes with gallic acid [1]. Raman bands used to identify iron-gall inks in historical documents are around 1470 cm^{-1}, between 1315 and 1350 cm^{-1}, 490–640 cm^{-1} (broad), and 400 cm^{-1}[2]. For iron-gallate, Ponce et al [3], proposed the following assignments: C–C ring vibrations at 1470 cm^{-1} and COO− and C–O stretching modes between 1315 and 1350 cm^{-1}. The two medium–low intensity bands, at circa 1430 cm^{-1} and 1579 cm^{-1}, were attributed to the symmetrical and asymmetrical vibrations of a coordinated –COO−COO−, to a metallic ion in their iron-gallate precipitate. More research from the iron-gallate Raman spectra is needed to fully understand the bonds between carboxylate and iron-gallate, as some studies [3] have not detected these types of bindings in their Raman spectra.

In this study, we propose an in-depth analysis of Raman spectra (using laser 785 nm) of reconstructed iron gall ink recipes using period-appropriate ingredients. These recipes were selected based on historical sources, including Iberian written sources of medieval techniques (15-17th c.) [1], as well as Islamic technical sources attributed to Ḥubayš Tiflīsī (12th c.) and al-Qalalūsī (13th c.) [4]. The variations observed in Raman spectra reflect differences in the chemical composition of the inks resulting from these recipe variations. By utilizing the distinct spectral fingerprints and a robust dataset of Raman spectra, we will apply chemometric analysis to identify patterns and correlations.

This combined spectroscopic and chemometric approach will enable us to classify and discriminate different IGI samples. We aim to create a model that will advance their rapid and in situ identification in historical manuscripts and ultimately contribute to their preservation.

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Non-Invasive Molecular Diagnostics of the Allegory and Effects of Good and Bad Government by Ambrogio Lorenzetti

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Here, we report the results of a thorough compositional diagnostic study of the celebrated masterpiece by Ambrogio Lorenzetti (1290-1348) known as the Allegory and Effects of Good and Bad Govern (1338-1339). The study was conducted as part of a conservation project involving multiple research groups. Important material evidences were collected on previous restoration interventions and on the overall status of preservation of the present unique masterpiece. Furthermore, the study also provided the precious opportunity to collect additional material data on the pictorial techniques of the greatest painter of the Sienese School and then on the Renascence of painting between Late Middle Ages and Early Renaissance.

Figure 1: Allegory of the Good Government.

Portable Raman instruments, including the iRaman B&W Tek (exc. 785 nm) and a homemade prototype (exc. 1064 nm), were used in combination with portable FTIR and XRF. Moreover, μRaman and petrographic analyses were carried out on a few material samples to obtain a comprehensive compositional and microstructural analysis of the conservation issues. The present work also allowed extending the results of previous studies on the photothermal control during Raman spectroscopy [1-2], by comparing the photothermal behaviours of different excitation wavelengths using a novel accessory that can easily be adapted to several commercial Raman probes [3].

Acknowledgements

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Flexible hydrogels for the non-invasive analysis of heritage dyes: introducing a new single-step formulated silver SERS substrate

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Surface-Enhanced Raman Spectroscopy (SERS) is a highly sensitive technique, ideally suited to the analysis of heritage objects, and invaluable in identifying traces of dyes in processed organic materials such as hides, feathers or fabrics. In some investigations, sampling is not allowed, which confirms the need to develop non-invasive SERS substrates. Among these, a wide variety of mild polymer-based devices have been reported to date [1]. Dyes are a relevant technological marker of the know-how of ancient societies and the resources they used. Identifying their nature makes it possible to reconstruct the processes involved in obtaining, transforming and using dyes.

To access the dyes' spectral signatures, we present a new single-step formulated SERS substrate based on an aqueous hydrogel. Agar-agar was chosen here for its widely use in Art restoration, environmental sustainability, ease of processing, but also for its capacity to reduce silver nanoparticle (NPs) precursors *in situ* without further reagents. The gel and silver nanoparticles are prepared in one same step, providing a SERS substrate stable over time (with storage before use), with a homogeneous spatial distribution of NPs, and a high level of compliance with the analytes. This substrate has the advantage of through-gel Raman analysis and can be removed after measurement without sample contamination, unlike the colloidal suspensions commonly used [2].

First this new hydrogel-based SERS substrate proved to be effective for dyes detection on textiles and porcupine quills mock-ups (anthraquinones, flavonoids, tannins...), while limiting invasiveness, enabling *in situ* analysis and offering good measurement repeatability. After this proof of concept on model samples, we applied this strategy to the challenging analysis of coloured ancient samples constituted of the same materials. On these complex objects, which are sometimes contaminated by the burial environment, but also degraded, the analytical strategy developed turned out to be especially effective in identifying various organic dyes.

Keywords: Surface-Enhanced Raman Spectroscopy (SERS), historical dyes, non-invasive methodology, silver nanoparticle-embedded hydrogel

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Challenges in translating Raman spectroscopy for clinical applications

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My lab has been focused on developing Raman spectroscopy for in vivo detection of various clinical conditions including preterm birth, hydration status, otitis media, inflammatory bowel disease, oropharyngeal cancer etc. Regardless of the application, various challenges need to be addressed when developing Raman spectroscopy for in vivo use. These include study design, system calibration, probe design, system design, data processing and data analysis. In this presentation, I will provide an overview of two applications, where these challenges have been addressed and discuss future prospects of this technology.

Depth-coded Stimulated Raman Scattering Tomography Enables Label-free Biomolecular and Functional 3D Imaging of Live Cells and Tissue

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Abstract

Stimulated Raman Scattering (SRS) microscopy is an attractive label-free imaging technique in tissue and cells with high biochemical specificity. The conventional SRS microscopy uses the tightly focused Gaussian beams to excite the tissue sample, which suffer from the strong light scattering in turbid media (e.g., tissue) resulting in a very limited penetration depth that unsuits for acquiring volumetric deep tissue image. Here, we present novel z-scanning-free SRS tomography (SRST) techniques with depth-encoding methods for rapid functional and biomolecular 3D imaging in tissue and cells. In SRST, the Bessel excitation beams are generated to enhance the imaging penetration depth, while time- and frequency-domain encoding approaches enable us to retrieve the depth information without the need of mechanical z-scanning for SRST 3D imaging. SRST provides over 2-fold improvement in penetration depth in highly scattering media as compared to the conventional point-scan SRS microscopy. Real-time 3D dynamic and metabolic functional imaging of live cells and tissues can be achieved using rapid SRST technique. The z-scanning-free optical sectioning ability of spatial-encoding techniques in SRST is universal, which can also be readily extended to any other optical imaging modalities for advancing 3D tissue and cells imaging in biological and biomedical systems.

Quantification of Liver Fat: Size and Content Assessment via Combination of Raman Scattering and Reflectance in Human Specimens

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Fat accumulation in livers, hepatic steatosis (HS), negatively influences transplantation outcomes. As a result, precise quantification of liver fat content (HS) during donor liver evaluation is of great import. Lipid droplet size is a further critical indicator of transplant viability. Macrodroplet (typically > 1 micron) lipid accumulation in hepatocytes, known as macrovesicular steatosis (MaS), lead to poor outcomes but is challenging to quantify in the absence of time-consuming histological exams. Our study introduces a novel, non-imaging, Raman/reflectance approach using a portable optical device to quantify **both** overall HS **and** MaS. Snap-frozen human liver samples were assessed using biochemical and histopathological methods and correlated with coincident Raman/Reflectance measurements. Analysis of 16 samples (phase I) demonstrated significant correlation between fat levels measured by Raman/reflectance and triglyceride content. Most importantly, convoluting Raman and Reflectance data allowed us to **distinguish various degrees of HS and MaS** in 66 samples (phase II). Oil-in-water emulsion experiments and Monte Carlo simulations confirm the trends. This dual-channel method overcomes the limitations of a Raman-only approach, creating a promising tool to decrease liver discards, offering hope for those awaiting life-saving transplants.

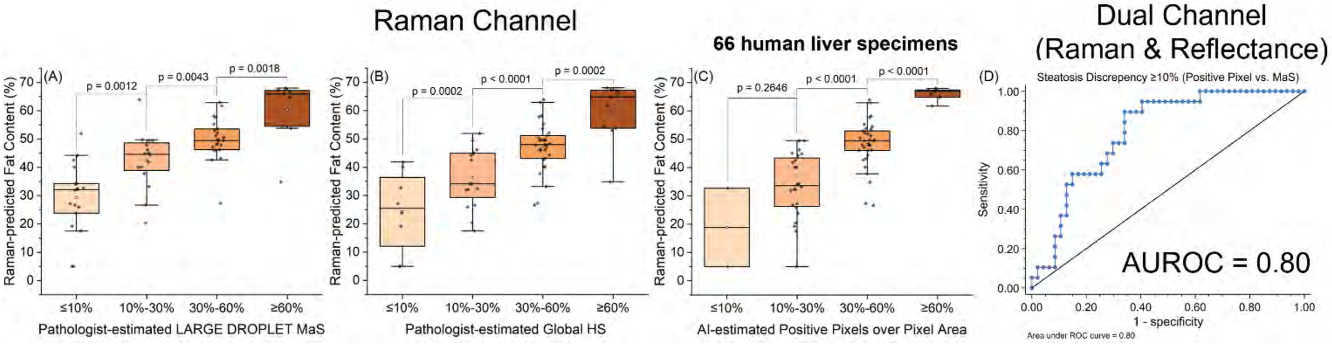

Figure 1: Boxplots of Raman-estimated fat content and receiver operating characteristic of dual-variable binary predictions on ≥10% steatosis discrepancy between overall HS and MaS.

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Insights into osteopetrosis: Raman and Brillouin microscopies for studying a rare genetic disorder

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Osteopetrosis (OP) is characterized by osteoclast dysfunction, resulting in increased bone density leading to fatal complications in the first decade of life if not treated. Current diagnostic and research techniques lack to provide direct insight into bone quality and composition thus preventing deep understanding of the pathogenesis in both humans and animal models. Instead, Raman and Brillouin spectroscopies could be optimal tools for the study of bones, potentially leading to the development of non-invasive diagnostic and therapy monitoring tools.

Fixed, bone samples (femur, tibia, vertebrae and skull) from oc/oc mice ($n = 6$), as a model of severe OP, Rankl knock-out (Rankl KO) mice ($n = 4$), as a model of mild osteopetrosis, and healthy wild type (WT) mice at different ages ($n = 16$) were analysed using a home-built Raman-Brillouin microscope equipped with a 660 nm laser and coupled with a standard CCD-spectrometer and a BIPD-assisted single-stage Virtually Imaged Phased Array (VIPA) spectrometer for the detection of Raman and Brillouin signals, respectively.

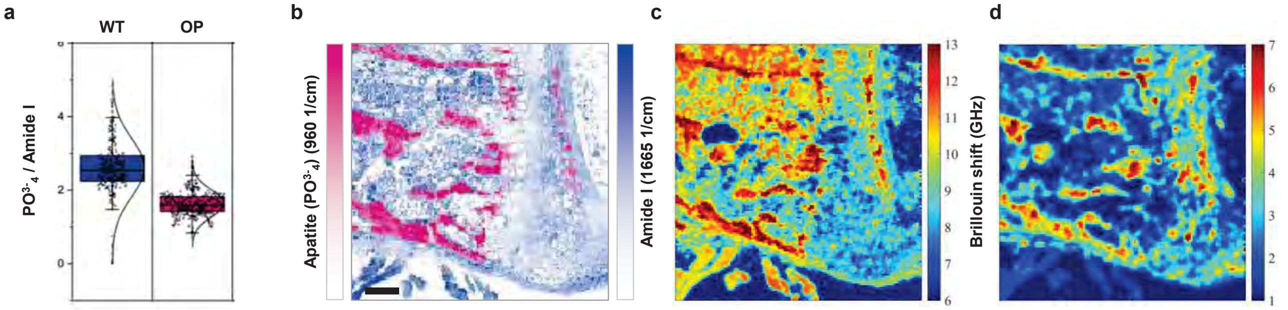

Figure 1: box plot of Raman data (mineral / organic matrix) collected from bone of WT and OP (oc/oc) mice (a); Raman (a) and Brillouin maps (c, d) of a WT murine vertebra. Scale bar 100 µm.

Single-point Raman spectra collected from the two OP mice models demonstrated lower mineral-to-matrix ratio ($p < 0.001$) if compared with WT mice (Fig.1a). Crystallinity was significantly lower in oc/oc vs WT ($p < 0.001$) but higher in the Rankl KO vs WT mice ($p = 10^{-4}$). Brillouin data revealed significantly higher ($p < 0.001$) frequency shift and linewidth in OP mice, compared to the WT. Raman and Brillouin maps (Fig.1b-d) of vertebra slices also showed difference in both mineral and biomechanical features in OP and WT models.

For the first time, Raman and Brillouin data reveal alterations in bone matrix composition and mineralization in osteopetrosis, as well as its crystalline and biomechanical properties, not detectable with standard techniques. The approaches used here can serve as a novel, direct, and non-invasive solution for studying osteopetrosis and as a proof of concept of their application to other relevant bone diseases, toward patient-friendly diagnostic and monitoring tools.

Investigation of cell oxidative stress and scavenging strategies by Raman Spectroscopy in cytological thyroid samples

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Thyroid gland plays a key role in the metabolic regulations through hormones synthesis, thus inducing a physiological production of reactive oxygen species (ROS) in thyrocytes [1,2]. Therefore, the detoxification system plays a crucial role in protecting thyrocytes from an ROS excess. The imbalance between ROS production and the defence system results in the cell oxidative stress (OS). The increased OS levels are a risk factor for carcinogenesis [3]. In recent years, Raman spectroscopy (RS) has been successfully used for thyroid cancer diagnosis [4,5]. Here, RS is proposed as a tool to investigate the thyroid disease development through a cell OS study. We enrolled 28 patients, submitted to a first and second thyroid fine needle aspiration (FNA) during follow up. The cytological samples were investigated by RS (Fig. 1a) and morphological examination. On the basis of the evolution of the Raman features over the two FNAs, the 28 patients have been classified into 4 categories, evidencing a different cell OS. In particular, RS shows the presence of carotenoids bands whose uptake may be activated to mitigate the ROS increase, as suggested by the intensity ratio of the peaks assigned to oxidized/reduced cytochrome *c* [6]. Moreover, the presence of fatty acids droplets in papillary thyroid carcinoma samples is evidenced, as an additional pathway against oxidative stress (Fig. 1b). Overall, our results suggest a correlation among changes in oxidative stress, carotenoids uptake, fatty acid droplet and thyroid diseases, which could inspire new fundamental research on biomarkers and signalling pathways involved in thyroid OS.

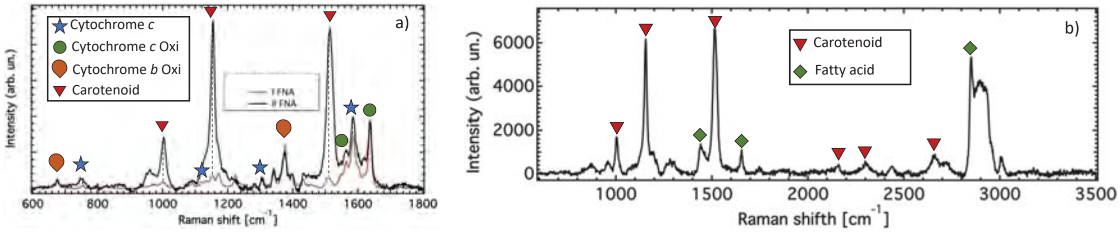

Figure 1: a) An example of comparison between the spectra collected on two FNAs of the same patient. b) Raman spectrum of a cytological PTC sample.

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The potential of Raman Spectroscopy for Accurate Identification of Hemoglobin Variants in Diabetes Diagnostics

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Approximately 500 million people globally suffer from diabetes mellitus, commonly known as diabetes. Diabetes, regardless of type, can lead to complications in various body parts and increase the risk of premature mortality. Consequently, early and timely diagnosis is vital for diabetes management. Accessible and affordable basic diagnostics, such as blood glucose testing, should be available in primary healthcare settings. Glycated hemoglobin (HbA1c) levels are considered the gold standard for assessing long-term glycemic control. Many Point-Of-Care (POC) devices currently available diagnose diabetes through HbA1c sensing [1]. While these POC tests offer quick results, their detection accuracy and performance require further improvements. Additionally, for patients with inherited hemoglobin disorders, HbA1c tests may underestimate diabetes prevalence due to abnormal hemoglobin affecting red blood cell lifespan and glucose levels [2]. Hence, HbA1c measurement techniques that accurately detect and quantify hemoglobin (Hb) variants are critical for precise HbA1c results. Existing laboratory methods and POC tests cannot simultaneously provide high-quality HbA1c analysis and detect Hb variants in a single run. This study demonstrates the potential of Raman spectroscopy in analyzing the fingerprint spectra of hemoglobin variants, enabling the identification of different variants. We evaluated this potential using commercially available hemoglobin fractions, including fetal hemoglobin. The classification process was automated with machine learning techniques such as support vector machines (SVM) and fully connected neural networks (NN), finely tuned and optimized to classify these fractions. The models achieved test accuracies of 98.2% and 98.5%, respectively [3]. Our research underscores the potential of combining Raman spectroscopy with high-performance liquid chromatography (HPLC) as a powerful identification tool. Additionally, this detection approach is amenable to miniaturization and integration with microfluidics, making it a promising solution for lab-on-chip applications.

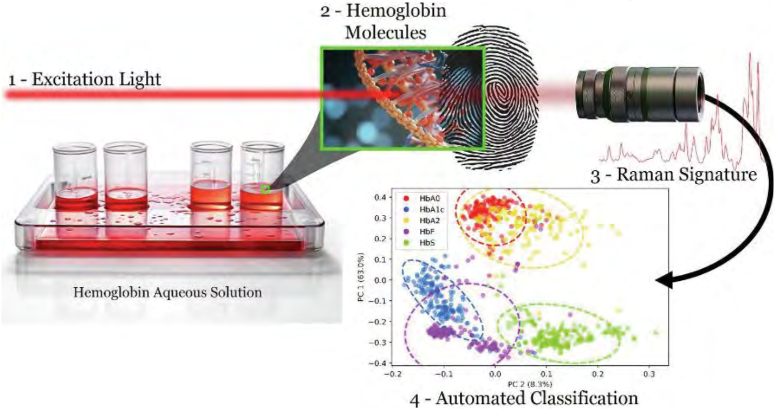

Figure 1: The protocol for the identification of Hb variants: sample preparation, Raman measurements, and automated classification with machine learning approaches.

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Lattice reconstructions and strain in moiré superlattices

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In moiré crystals formed by stacking van der Waals (vdW) materials, surprisingly diverse correlated electronic phases and highly tunable optical properties can be realized. It has been recognized that lattice reconstruction can significantly influence both electronic and optical properties. We find that over a range of small twist angles in twisted MoS_2 bilayers, the phonon spectra evolve rapidly due to ultra-strong coupling between different phonon modes and atomic reconstructions of the moiré pattern [1]. A new low-energy continuum model is developed to explain this evolution of phonon modes with twist angle. This continuum model overcomes the outstanding challenge of calculating properties of large moiré supercells and successfully captures essential experimental observations.

Lattice reconstruction occurs due to a competition between the interlayer coupling and strain in the twisted bilayers. Intrinsic strain associated with the moiré superlattice and unintentionally introduced uniaxial strain may be present simultaneously. Both strains are able to lift the degeneracy of E_{2g} phonon modes in Raman spectra. Because of the different rotation symmetry of the two types of strain, the corresponding Raman intensity exhibits a distinct polarization dependence. We compare a 2.5° twisted MoS_2 bilayer, in which the maximal intrinsic moiré strain is anticipated, and a natural MoS2 bilayer with an intentionally introduced uniaxial strain. By analysing the frequency shift of the E_{2g} doublet and their polarization dependence, we can not only determine the direction of unintentional uniaxial strain in the twisted bilayer but also quantify both strain components [2]. This simple strain characterization method based on far-field Raman spectra will facilitate the studies of electronic properties of moiré superlattices under the influence of combined intrinsic and external strains.

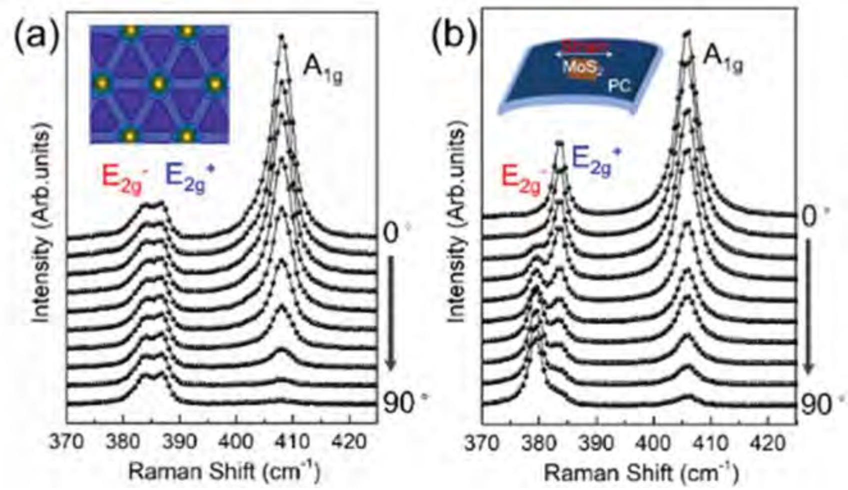

Figure 1: Comparison between Raman spectra taken from (a) 2.5° twisted MoS_2 bilayer and (b) a natural MoS_2 bilayer

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Temperature- and pressure-dependent phonon dynamics properties of GaSeTe

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In this work, we present a temperature- and pressure-dependent Raman study of bulk GaSe0.5Te0.5 in order to access its thermal and mechanical properties. By combining density functional theory (DFT) calculations and temperature-dependent Raman measurements, we calculate the anharmonic constants relative to three- and four-phonon decay processes, the mode-Grüneisen parameters, Debye temperature, and the thermal expansion coefficient. From the high-pressure experiments and DFT calculations, we show that A1g and E2g Raman modes present a nonlinear pressure dependence, with A1g modes being more compressible higher $\partial\omega/\partial P$ values than E2g mode. Furthermore, a comparison with the GaSe system shows that the alloy is more compressible, thus presenting higher pressure coefficients and smaller bulk modulus. [1].

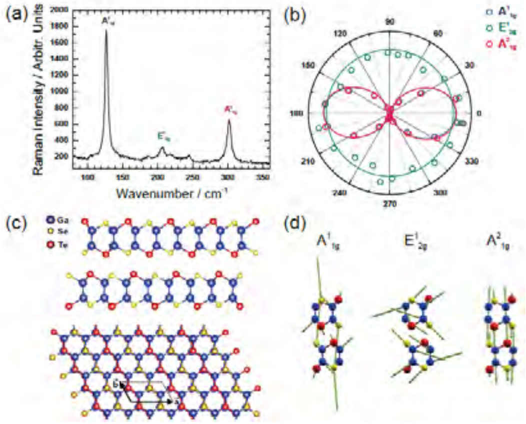

Figure 1: a) Non-polarized Raman spectrum and (b) polar plots of A11g(blue circles), E12g(green circles), and A21g(red circles) phonon modes for GaSe0.5Te0.5. The solid colorful curves are from fitting the experimental data by using the Raman tensors of A1g and E2g irreducible representation of GaSe. (c) Side-view and top-view schematics of AA stacking geometry for bulk GaSe0.5Te0.5. The primitive cell is also indicated by the in-plane a and b primitive vectors. (d) Eigenvectors of A1g and E2g vibrational modes.

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Raman spectroscopic studies of commercial graphite fibres modified with copper (II) chloride

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Carbon fibres are a class of materials with very diverse properties [1]. A particular group of carbon fibres are graphite fibres, which are produced from the mesophase pitch (aromatic hydrocarbons) precursors by high-temperature treatment up to 2900 °C. Such fibres are characterized by high structural ordering, similar to the structure of natural graphite. They have a very high elastic modulus (910 GPa), thermal conductivity (1100 W/mK) and low electrical resistivity (1.13 μΩm). Commercially available K-1100 graphite fibres (Amoco Performance Product) were the subject of research as potential light and strong electrical conductors. Their electrical conductivity can significantly be increased by intercalation, i.e., by introducing between the basal graphite layers (002) anhydrous copper chloride (CuCl2). Raman microspectroscopy was used to analyze such graphite fibres after intercalation, using laser lines with wavelengths of 413.2 nm, 442 nm, 514.5 nm, 532 nm and 785 nm. In this way, the impact of modifications of graphite structure was determined. G-band dispersion, defined as the change in the position of the G-band as a function of the excitation wavelength, occurs when disorder is present in the material. The nature of these changes is shown in Fig. 1. In the case of CF-$CuCl_2$ graphite fibres, a slight dispersion lack of the G-band is observed. Such a result may confirm the mechanism of separation of basic graphite layers by the presence of CuCl2 and the formation of single graphene layers. The D band is the main feature indicating the presence of a disorder in the tested systems. The D-peak dispersion is clearly marked for all of the studied materials. The intensity of the D peak decreases significantly at higher excitation energy, which may indicate nanographite-type ordering in the tested systems [2]. The possibility of modifying fibres opens up a wide range of applications, and research using Raman spectroscopy can indicate their properties on the microscale.

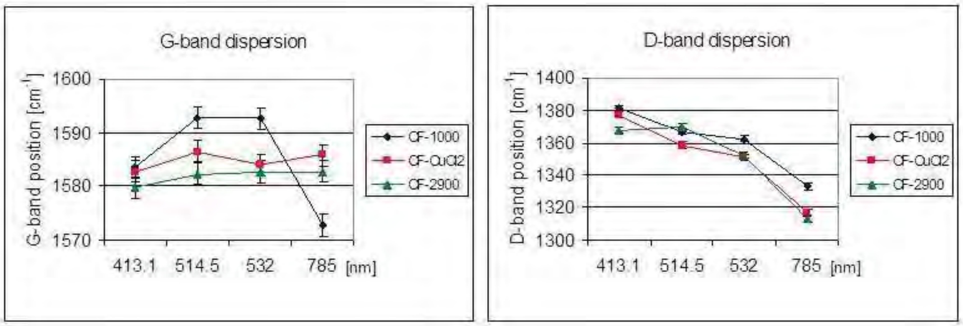

Figure 1: G and D band dispersion for the tested carbon fibre materials.

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Photoluminescence and Raman Map Analysis of Methylammonium Chloride Treated Formamidinium Lead Iodide ($FAPbI_3$) Microstructural Phases

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Formamidinium lead iodide ($FAPbI_3$), one of the organic-inorganic lead halide perovskites, has shown remarkable power conversion efficiency. However, the photoactive α-$FAPbI_3$ phase is thermodynamically unstable in ambient conditions, which tends to transform into the useless photoinactive δ-phase. The addition of methylammonium chloride (MACl) is beneficial to reduce the δ-phase of $FAPbI_3$. To understand the role of MACl, we studied the effects of different concentrations of MACl (10, 20, and 40 mol %) on $FAPbI_3$ perovskite films using photoluminescence (PL) and Raman map (25 µm × 25 µm = 625 μm^2) spectroscopies. **Figure 1** shows the PL intensity and Raman shift heat maps of $FAPbI_3$ thin-films treated with the different MACl concentrations. The PL map reveals that $FAPbI_3$ with 20 mol % MACl exhibits the highest PL intensity distribution followed by $FAPbI_3$ with 10 and 40 mol % concentrations. The Raman studies show a Raman peak of α- and δ- $FAPbI_3$ at 111 and 122 cm^{-1}, respectively [1-2]. In addition, Raman map results show that the higher proportion of α-$FAPbI_3$ is spatio-correlated to the higher distribution of the PL intensity. The α-$FAPbI_3$ phase increases around 75.20 % as the MACl concentration rises to 20 mol %, indicating that the MACl addition is effective for the formation of α-$FAPbI_3$. However, the excessive MACl concentration (40 mol %) results in decreased stability (more δ-formation) and lower PL intensity. We also investigate the recovery process of δ-$FAPbI_3$ phase using temperature-controlled ultra-low frequency Raman spectroscopy. We found that the δ-$FAPbI_3$ phase can be recovered to α-$FAPbI_3$ phase by heating at 430 K. Overall, this study elucidates the intricate relationship between MACl concentration, quantitative phase composition, and optical properties in large $FAPbI_3$ perovskite films, offering suggestions for optimizing their performance in large-area photovoltaic devices.

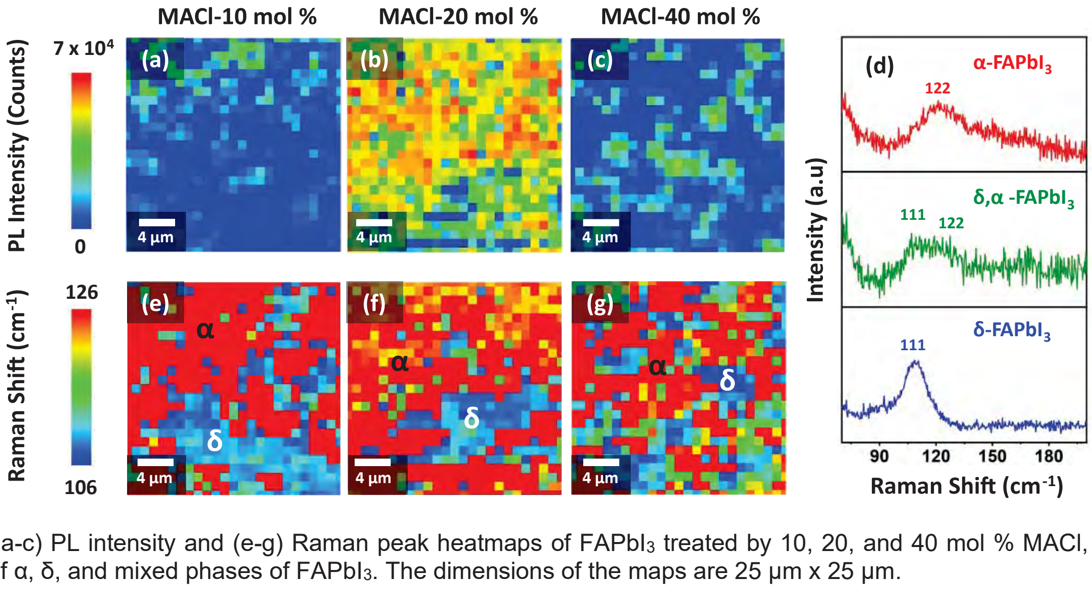

Figure 1: (a-c) PL intensity and (e-g) Raman peak heatmaps of $FAPbI_3$ treated by 10, 20, and 40 mol % MACl, (d) Raman spectrum of α, δ, and mixed phases of $FAPbI_3$. The dimensions of the maps are 25 µm x 25 µm.

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Vibrational spectroscopy inside silicon by stimulated Raman scattering at telecom wavelength

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Stimulated Raman scattering (SRS) microscopy, initially popular in biological studies, now garners attention for its potential in material science [1]. Among these applications, semiconductor measurements hold promise for designing complex geometries like three-dimensional integrated circuits and demonstrating their reliability. Nevertheless, there has been a notable absence of studies observing SRS of silicon (Si), the fundamental material in integrated circuits. This gap primarily exists because the wavelengths typically used in SRS spectroscopy range from visible to 1 μm, where Si's absorption severely limits SRS acquisition. While spontaneous Raman scattering provides valuable information about its strain and crystal quality on Si's surface[2-4], its limited sensitivity and penetration length hinder comprehensive imaging. If SRS imaging of Si is realized, it has the potential to significantly assist in characterizing and optimizing the design of complex integrated circuits.

In this report, we demonstrate SRS spectroscopy inside a Si substrate by using the pump beam at the wavelength of 1.56 μm and the Stokes beam at the wavelength of 1.7 μm, where Si's absorption is very small compared to the traditional SRS spectroscopy.

In our experiment, we used a mode-locked Er-doped fiber oscillator with a repetition rate of 44.5 MHz and a center wavelength of 1.56 μm as our primary laser source. Optical pulses from the oscillator were amplified by an Er-doped fiber amplifier (EDFA) and divided into two beams: one serving as the pump beam and the other as the Stokes beam. The Stokes beam was intensity-modulated by an electro-optic modulator, halving its repetition rate. Subsequently, the Stokes beam was amplified by EDFA and redshifted to 1.7 μm by the soliton self-frequency shift [5-6]. Both the pump beam and the Stokes beam were negatively chirped by transmission grating pairs for wavenumber scanning by the spectral focusing method [6-7]. They were recombined at a bandpass filter and focused onto the sample with a 50x objective lens (Olympus). After the sample, only the pump light was extracted and detected by an InGaAs photodiode. SRS signal was then extracted using a lock-in amplifier (LIA) with time constant 30 ms. The SRS spectra were acquired by scanning the delay stage of the pump beam by a servo motor.

We measured the SRS spectrum inside a non-doped (111) Si substrate (10×10×0.525 mm). The optical power was 8.1 mW for the pump beam and 4.3 mW for the Stokes beam. Fig. 1 illustrates the resulting SRS spectrum. The wavenumber was calibrated using a GaN substrate, which shows E_2 (high) peak at 568 cm^{-1}[8]. The signal-to-noise ratio was calculated as 307. The full width at half maximum was 4.58 cm^{-1}. Assuming that the spectral width of Si is 3.10 cm^{-1}[9], the spectral resolution is 3.37 cm^{-1}, which is sufficient for detecting tiny vibrational shift of ~0.01 cm^{-1}.

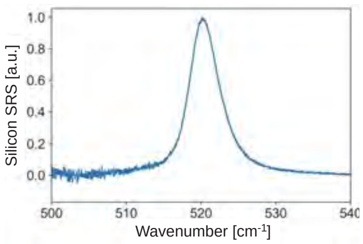

Figure 1. Obtained SRS spectrum of Si.

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Investigation of the Marangoni bursting effect on a non-linear optical dye with Raman microspectroscopy.

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The control over phase and aggregation of an organic chromophore is a determinant of its optical properties. The Marangoni bursting effect (MBE) is a bottom-up strategy to obtain fiber-like structures, taking advantage of the thermocapillary effect and the difference in solubility of the molecule of interest in two solvents, *e.g.*, water and isopropanol (IPA).[1] As shown recently by Ślemp *et al.* [2], the (3-(2,2-dicyanoethenyl)-1-phenyl-4,5-dihydro-1H-pyrazole) (DCNP) crystal can be used to generate fibrous microstructures using MBE with different aggregation which manifest in different luminescent properties: either showing red luminescence (common for DCNP) or blue luminescence (not observed until now). In this work, we study the Raman spectroscopy and non-linear optical properties of a novel dye here named A2EN (N,N-Diethyl[(2-methoxy-4-nitro)phenylazo]anile), for which its structure is shown in Figure 1A. As a bulk crystal, A2EN shows Second Harmonic Generation with an effective $\chi^{(2)}$ *ca.* ten times larger than potassium dihydrogen phosphate. Using the MBE, we can obtain wire-like structures with diameters ranging from 0.5-2 μm (shown in Figure 1B as brightfield and cross-polarized images), but also plates (with 0.5-1 μm in height) and spherical droplets (which are amorphous and have diameters smaller than 10 μm). Raman microspectroscopy (shown in Fig. 1C) reveals that the microstructures generated with MBE differ from their bulk forms, as evidenced in changes of modes associated with different aromatic-ring deformations (shown in Fig. 1D, obtained from quantum chemistry calculations using density functional theory). We argue that these changes in aggregation may alter the non-linear optical properties of A2EN.

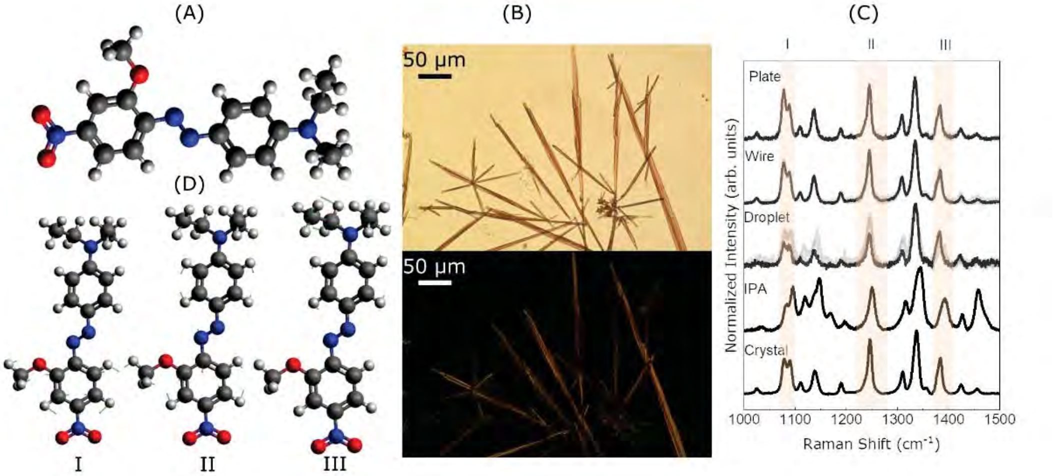

Figure 1: (a) Structure of the A2EN. (b) Optical image of MBE generated structure (normal on top and cross-polarized on bottom). (c) Raman spectra of different forms of A2EN (from top to bottom): crystal, solution in IPA, droplets, wires, and plates. The average spectra of the MBE structures are shown black and individual samples are shown in gray (d) Normal modes related to ring deformations of A2EN.

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The role of Mobile Raman Instrumentation in Geosciences – *Sensu Lato*

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Thanks to its favourable properties, Raman spectroscopy is well-appreciated as an analytical approach in geosciences. Laboratory spectrometers retrieve molecular and crystalline information on particles or inclusions down to 1 µm. Moreover, in general the measurements are relatively fast and allow for the identification of inorganic as well as organic species. Consequently, the range of applications includes mineral species identification, the study of inclusions, as well as applications in astrobiology and the identification of minerals in a cultural heritage context. Miniaturisation of the instrumentation and the use of fibre optics have paved the way for *in situ* applications. Mobile instruments were adapted to be used for the study of minerals, as well as biomarkers at outcrops, as well as for the non-destructive examination of minerals and gemstones in precious artefacts, for instance in a museum context. However, when using mobile instrumentation, precautions have to be taken to avoid environmental interferences, for instance from ambient light. Whereas benchtop instruments are excellently suited to perform a Raman mapping to study the spatial distribution of species in a small sample, the sampling volume of mobile instruments is typically significantly larger. Nevertheless, it is possible to construct a macro-Raman mapping set-up that account for Raman mapping of larger areas – spanning several cm².

Figure 1: Implementation of mobile Raman instrumentation for in situ analysis (left) and using similar instruments for macro-Raman mapping of an artefact (right)

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Raman Spectroscopic and X-ray diffraction studies to characterise Burugubanda Graphite Deposits in south-eastern parts of Eastern Ghats Mobile Belt, India

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The Eastern Ghats Mobile Belt (EGMB) is a poly phase deformed and regional granulite faciesterrain, mainly consistsof metamorphosed assemblages of both metasedimentary and metaigenous granulite facies rocks, which were subsequently intruded by the Proterozoic anorthosite and alkaline and granitoids rocks. The metasedimentary rocks mainly include garnet-silimanite gneiss (Khondolites), Quartzites, and Calc-silicates. The metaigneous rocks range from basic to felsic in composition and are essentially hypersthene-bearing (Charnockites)[1].

The Burugubanda Graphite deposit fallsin south-eastern part of EGMBand comprises of khondalites suite rocks of garnet-sillimanite gneiss, magnetite-manganiferous quartz, quartzite, calc-silicates. The Graphite ore confined within the granet-silimianite bands and also associated with the quartz and quartzofeldspatic veins and occurs as a crystalline variety of mostly flaky nature type and fine grained flaky ground mass and also small veins, which are intruded within the fine groundmass.

The powder X-ray diffraction (XRD) studies shows the graphite mineral phase 2Ө positionat 26.39 (D-space 3.39) and other associated mineral phase such as quartz phase 2Ө position at 26.54 (D-space 3.34), orthoclase phase 2Ө position at 27.38 (D-space 3.24), antigorite 2Ө positionaT 12.19 (D-space 7.25) and mica group mineral annite 2Ө position at 8.67 (D-space 10.19). The %crystallinity calculated by equation (area under the crystalline peaks)/(area under all peaks) *100). The%Crystallinity of graphitemineral is about 40% and remaining 60% amorphous.

The Raman spectroscopic studies carryout out in different types of graphite'soccurrence in the study area. The most common type i.e. flaky nature graphite and shows the prominent G band at 1581 cm^{-1} and 1581 cm^{-1} and without D1 and D2 bands, suggesting variable degrees of structural disorder and the second order spectra at 2729cm^{-1} and 2727cm^{-1}.The fine grained graphite are characterized by G band at 1580 cm^{-1} and 1580 cm^{-1} and with D1 band at 1354 cm^{-1} and 1350 cm^{-1} and without D2 band with the second order spectra at 2729cm^{-1} and 2723cm^{-1}. The Raman spectra of small veins shows G band at 1581 cm^{-1} and 1581 cm^{-1} and with D1 band at 1361 cm^{-1} and 1352 cm^{-1} and also with D2 band at 1618 cm^{-1} and 1624 cm^{-1}indicating structurally ordered and the second order spectra at 2731cm^{-1} and 2730 cm^{-1}.

The temperature and degree of graphite crystallization determined based on the parameters i.e.ratio R1= (D1/G)H (maximum intensity of peak) and ratio R2=(D1/G+D1+D2)A (area of the peak)[2]and thermometer formulaTGr(°C)= - 445 x R2 + 641. The flaky graphite ratio is zero and it suggests a corresponding temperature above the working limit >640ºC and the fine grained graphite ratio is 0.144 and corresponding temperature is 576.9 ºC and the small vein type graphite ratio is 0.360 which corresponding temperature 480ºC. The low values of R1 and R2 ratios ranges from 0.070 to 0.130 and 0.144 to 0.363 respectively indicate the low degree of graphitization.

The Burugubanda Graphite deposit falls in the south-eastern part of Eastern Ghats Mobile Belt and graphite confined within the granet-silimianite bands, quartz and quartzfeldspathic veins. The graphite mineral phaseof XRD at 26.39 (2Ө position) with dspace 3.39 and crystallizing in rhombohedral (hkl:002) and the %crystallinity of graphite mineral is about 40 percent. The Raman study confirms the different types of graphite with G band (1581cm^{-1}, 1580 cm^{-1}), D1 band (1354 cm^{-1}, 1350 cm^{-1}) and D2 band (1618 cm^{-1}, 1624 cm^{-1}). The low values of R1 and R2 rations indicate low degree of crystallisation and the temperature ranges from 480ºC to >640ºC, which is the graphite mineral formation and deposition condition in Burugubanda area, Eastern Ghats Mobile Belt (EGMB).

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Development of a Raman Spectrometer Autofocus System

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In recent years, Raman spectrometers have been proposed for a number of planetary exploration mission payloads (including the ESA Rosalind Franklin, NASA Perseverance, and JAXA MMX missions [1,2,3]) due to their sensitivity to organic and inorganic compounds and their ability to unambiguously identify biosignatures [4]. They are a particularly strong analytical instrument technique for search for life missions or for helping to assess the habitability of a particular landing site. However, in a recent study [5], it was demonstrated that the specific surface topology and morphology of sample material can significantly degrade the spectral performance of an instrument and in some cases can degrade the SNR achieved during autonomous mission surface operations to a level where the mission science goals can no longer be met.

One proposed method to address this issue is to incorporate an autofocus mechanism in the instrument’s optical head. Indeed, this approach has been adopted for the Raman Laser Spectrometer [1] (RLS) onboard the ESA Rosalind Franklin rover. The RLS instrument utilises a mechanism that can autonomously adjust the position of the spectrometer’s optical head in order to optimise the measurement being made.

In this presentation, we consider the implementation and performance of several different types of auto-focus system in order to identify the optimum system to use for particular material types and for specific sample extraction and distribution mechanisms. Results from software based instrument modelling and laboratory prototype testing with planetary analogue materials are discussed.

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Using Raman spectroscopy to help solve the Stonehenge enigma

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Located in Wiltshire, UK, Stonehenge is one of the most iconic historic monuments in Europe, dating to the late Neolithic period. At its centre lies the Altar stone. This grey-green, micaceous sandstone is anomalous in its composition and dimensions when compared to the other bluestones that form the inner circle of Stonehenge. Earlier suggestions stated that the Altar stone originated from West Wales, close to the source of the other bluestones, *ca.* 225 km from Stonehenge. However, a recent study by Bevins *et al*. identified significantly different mineralogical properties in the Altar stone compared to those found in West Wales, notably the unusually high Ba content.[1] This was supported by portable XRF analysis (performed *in situ*), complemented by SEM-EDS, optical petrography, and Raman spectroscopy on debitage fragments, presumably derived from the Altar stone. The team has therefore proposed that the stone be declassified as a bluestone. Yet, its source cannot be confirmed.

Raman microscopy is ideal for this analysis as it is sensitive to the chemical and mineralogical composition of geological samples, which can provide strong clues to the Altar stone provenance. Contrary to handheld Raman analysers, a Raman microscope can provide structural and chemical information with wavenumber spectral resolution and microscopic spatial resolution. However, the application of a Raman microscope can be impractical in remote locations and with gigantic samples, such as the Altar Stone, which is partly buried and weighs an estimated six tonnes.

In this study, we show that geochemical research can be performed *in situ* at Stonehenge using a transportable Raman microscope (Renishaw Virsa™ equipped with a 532 nm laser, ×20 and ×50 LWD objectives) for the identification of the compositional characteristics of the Altar stone.

Results of *in situ* and *ex situ* single point spectra and high-resolution Raman images confirmed the presence of a suite of different heavy mineral grains, previously identified by the petrographic analysis of fragments, matching the mineralogic fingerprint of the stone. From it, baryte was also detected, which is consistent with the previous XRF findings. By correlating Raman data acquired at Stonehenge with previous results on the Altar stone debitage, this methodology will aid the ongoing investigations not only in confirming the authenticity of such fragments but ultimately in confirming the unique origin of the Altar stone.

Figure 1. Using a Raman microscope on the Altar Stone at Stonehenge

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Unlocking Qualitative and Quantitative Soil Inspection by Means of Shifted Excitation Raman Difference Spectroscopy

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In-depth information about soil composition is an important requirement in precision agriculture to enable site-specific farming. Established soil laboratory analyses are often complex, expensive and time-consuming but they could be complemented by fast and non-destructive optical methods. With the ability to retrieve molecular information about soil constituents, Raman spectroscopy is a promising technique for this task.

Interferences by fluorescence and ambient lights can effectively be addressed by shifted excitation Raman difference spectroscopy (SERDS) based on a physical approach. Here, we present an overview of laboratory and on-site SERDS investigations applying in-house developed dual-wavelength diode lasers with two emission lines around 785 nm as excitation light sources for SERDS.

Initial SERDS laboratory investigations were conducted for a better understanding of soil as the target sample and for reference to subsequent field investigations. A raster scan approach probing 100 individual measurement positions with a spot size of 100 µm within an area of 1 cm^2 was used to account for the intrinsic heterogeneity of soil constituents. Using a set of 150 soil samples collected from an agricultural field in Germany, it was shown that 13 soil minerals such as silicates, titanium dioxides carbonates and phosphate as well as amorphous carbon could be detected and identified. Quantitative analysis using partial least squares regression of the SERDS spectra against laboratory elemental analysis or X-ray fluorescence as reference values is exemplarily demonstrated for the prediction of the soil parameters organic matter content [1] and carbonate content [2].

Subsequently, a portable SERDS instrument [3] was developed and applied on-site on the above-mentioned agricultural field to validate our laboratory investigations. The field study was conducted at 25 measurement positions distributed along a length of 384 m. Each position was probed at 10 different points to account for soil heterogeneity at the millimetre scale. Qualitative analysis shows that 10 soil constituents (amorphous carbon and minerals) could be detected even in the presence of strong background signals. Moreover, quantitative soil inspection is demonstrated showing a prediction performance comparable to the SERDS laboratory investigations for the contents of e.g. organic carbon and inorganic carbonates.

The findings of our investigations demonstrate the ability of SERDS for qualitative and quantitative on-site soil inspection, e.g. for site-specific farming.

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The Compact Integrated Raman Spectrometer (CIRS)

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The Compact Integrated Raman Spectrometer (CIRS) is specifically engineered for planetary exploration, designed for use across a diverse array of environments including the Moon, Mars, Venus, and the icy moons such as Europa, Enceladus, and Ceres[1]. CIRS is configurable as an integrated instrument or as a spectrometer with a fiber-coupled probehead, depending on the specific accommodation requirements of the mission.

CIRS employs continuous-wave green laser excitation (532nm), an optimal wavelength for mineral and organic identification, offering spectral coverage ranging from 150-4000 cm^{-1} with a resolution of 7-8 cm^{-1}. The use of continuous-wave laser excitation is advantageous as it minimizes potential photochemical or thermal degradation of the sample. However, it may induce confounding fluorescence, which CIRS addresses using serially shifted excitation (SSE). SSE involves collecting a series of raw spectra at slightly shifted laser excitation wavelengths, followed by employing maximum likelihood estimation to solve for the fluorescence and Raman spectral components[2].

The detection of low levels of organics (e.g. nanomolar, 10^{-10} wt/wt, etc.) has been identified as a crucial requirement for *in situ* life detection instruments. Resonance enhancement enables lower detection limits for larger conjugated or aromatic organic molecules, (e.g., carotenoids) with green laser excitation. To address the sensitivity requirements for smaller organic molecules, CIRS incorporates photoactivated surface-enhanced Raman spectroscopy (SERS)[3]. Integrated with CIRS's sample handling system, the CIRS SERS substrate remains inactive indefinitely. Once exposed to light, silver nanoparticles are formed, conferring SERS enhancement to nanomolar levels.

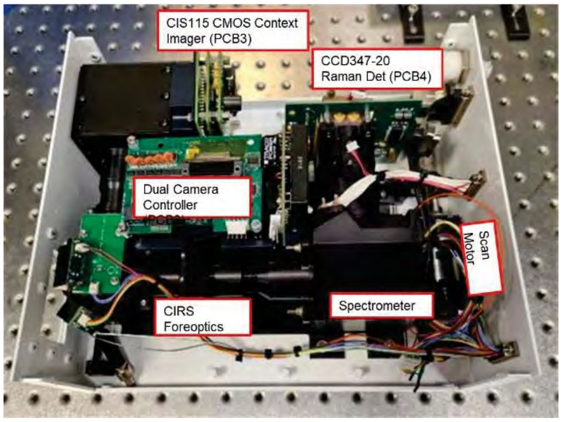

Figure 1: The Compact Integrated Raman Spectrometer (CIRS)

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Surface-enhanced Raman scattering of biological samples

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Surface-enhanced Raman scattering (SERS) is a remarkably strong effect that has extended the use of Raman spectroscopy to many applications, ranging from biodiagnostics to materials analysis or photocatalysis. SERS probing unavoidably relies on the nanometer-proximity, often a direct physical or chemical interaction, of the molecules with a SERS substrate, usually a plasmonic nanostructure, as the enhancement takes place in extremely localized, high optical fields. Therefore, SERS spectra give us selective information on the molecular structure and composition at surfaces of nanostructures and are ideally suited to characterize such interactions also in complex environments [1].

In this talk, the results of SERS experiments with biological samples, specifically living cells and biofluids will be shown. As an important aspect, the availability of the surfaces will be discussed for different types of optical probes that can be used in bioanalytical SERS. Using SERS to characterize the processes that occur in different organelles of living cells relies on the transport of optical probes to specific targets inside the cells. SERS spectra can reveal many aspects of these pathways that begin at the extracellular membrane and lead through the endolysosomal compartment to their final targets, such as the nucleus [2]. They involve different types of molecular species, including membrane lipids, proteins, and important actors in cellular metabolism, such as enzymes along with their substrates and inhibitors. The results demonstrate the nature of the data that are obtained, and ways to harness the spectral information to better understand molecular composition and molecule-molecule interactions.

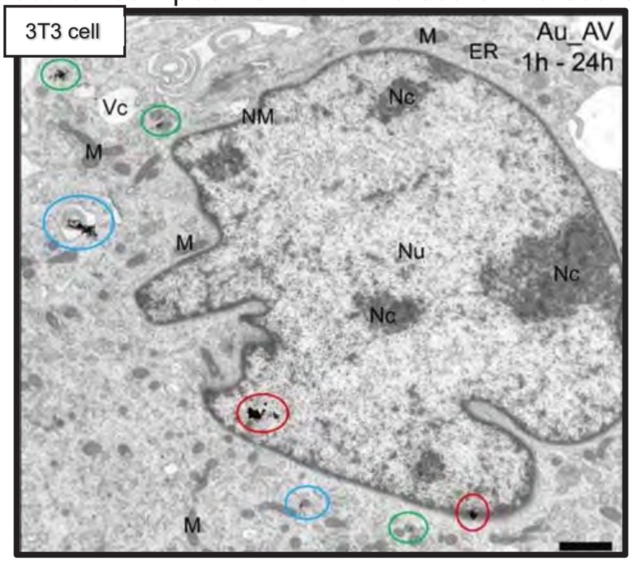

Figure 1: Transmission electron micrograph of SERS probes on their way to the nucleus of a mouse fibroblast cell. Scale bar: 1 µm. The colored markings indicate particles in endosomes (green), escaping from the vesicles (blue), and entering the cell nucleus (red). Reproduced with permission from [2]

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European Research Council info session

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The European Research Council (ERC) is Europe's leading funding agency supporting excellent and investigator-driven frontier research across all fields of science.

ERC calls are open to applicants from all over the world and provide several outstanding funding opportunities with grant-budgets ranging from 1.5 to 3.5 million euro. All nationalities are welcome, provided that the projects are carried out at a host institution located in Europe (i.e. European Union Member States or the countries which have associated to the EU research funding programmes).

Since 2007, the ERC has financed ca 550 individual research projects in the field of Physical and Analytical Chemical Sciences[1], for an overall budget of €1026M.

In this informative session, the speaker will present the main features of the ERC individual grants (i.e. Starting, Consolidator, Advanced), together with general information on the application and evaluation process. Advice will be provided on relevant aspects such as identifying a solid idea and writing a successful proposal.
Finally, anybody from the audience will have the chance to ask questions.

This ERC session is open to all the attendees of the ICORS congress and will be particularly helpful to early-career and mid-career scientists who think about applying for funding to conduct breakthrough-research in Europe.

European Research Council
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References

[1] https://erc.europa.eu/projects-statistics

Raman-based subcellular pharmaco-metabolomics

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The use of molecular probes in Raman imaging is a relatively new technique in subcellular research, however, very fast and dynamically developing. Compared to the label-free method, it allows for a more selective visualization of organelles within a single cell. Directly visualizing biological structures and activities at the cellular and subcellular levels remains by far one of the most intuitive and powerful ways to study biological problems.

For hyperspectral detection and imaging of living cells, it is very desirable to use probes with strong and unique Raman vibrations in the biological silent region (1800 – 2800 cm-1). Here it is shown a biorthogonal chemical imaging of cells to track biochemical changes associated with nucleus and mitochondrial function at the cellular level in the in vitro models. Both commercially available and newly synthesized highly sensitive Raman probes for selective imaging of mitochondria and nucleus in live cells are presented. In addition to spontaneous Raman microscopy, nonlinear techniques were used for rapid cell imaging.

This research was funded in whole or in part by the National Science Center Poland (NCN), Maestro 2022/46/A/ST4/00054 to Malgorzata Baranska.

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Title:

Enhanced AFM with chemical signature for comprehensive material characterization

Abstract:

The hyphenization of Atomic Force Microscopy (AFM) and Raman spectroscopy provides extensive understanding into the complex dynamics of various materials. Recognizing the crucial role of a comprehensive characterization of the studied specimen and it intrinsic properties, this colocalized method serves as an ease method, enabling the structural and chemical characterization of the specimen, including the analysis of contamination and trace materials.

SignatureSPM, the first multimodal characterization system built on an automated AFM platform and integrating a Raman/Photoluminescence spectrometer, enabling true colocalized measurements of physical and chemical properties. Raman spectroscopy, within this colocalized approach, facilitates the identification and characterization of various compounds, providing essential data on their molecular structures and compositions, including potential contaminants at microscale. Simultaneously, AFM contributes vital information on the topographical and mechanical properties of these samples, offering detailed data into their surface textures, surface adhesion, roughness, and stiffness at nanoscale.

HORIBA, has designed SignatureSPM, the first multimodal characterization system built on an automated AFM platform and integrating a Raman/Photoluminescence spectrometer, enabling true colocalized measurements of physical and chemical properties. SignatureSPM enables fast acquisition, ensuring precise pixel-to-pixel correspondence in the images.

While users are obliged to use several instruments, trying to extract a comprehensive information about their sample, our distinctive colocalized AFM-Raman approach distinguishes itself as a uniquely multimodal system, providing clear and correlated understanding of skin or hair composition through rapid scanning.

Keywords:

SignatureSPM, colocalized AFM-Raman, spectroscopy, chemical correlation

Applications of THz-Raman in pharmaceutical drug discovery, formulation, and manufacturing

Anjan Roy, PhD
Coherent Corp.

THz-Raman spectroscopy extends traditional Raman spectroscopy into the low-frequency or “Terahertz regime,” capturing both chemical and structural spectral signatures. This technique enhances sensitivity and improves identification and analysis of a wide variety of materials, including pharmaceuticals, explosives, and biologicals, offering a unique solution for comprehensive material characterization.

In the pharmaceutical industry, THz-Raman provides rapid and non-destructive analysis of pharmaceutical samples. THz-Raman enables **rapid polymorph identification** during drug development, manufacturing, and quality control. Different polymorphs can impact bioavailability and efficacy, making their identification critical and a requirement by regulatory agencies. THz-Raman provides a superior method to detect **pseudo-polymorphism** and distinguish between solvate crystals and drug crystals**.** Ease of sampling and the fact that low frequency Raman modes are often an order of magnitude larger in intensity makes THz-Raman ideal for **High-Throughput Screening (HTS)**. THz-Raman systems integrated with well plate readers accelerates research and development processes. These HTS systems are extensible to other optical imaging modalities, such as **polarized light microscopy (PLM),** amongst other methods. Finally, THz-Raman is an ideal method to detect residual crystallinity in rapidly expanding area of **Amorphous Solid Dispersions (ASD)** manufacturing**.**

THz-Raman spectroscopy reveals “structural fingerprints” that differentiate polymorphs, isomers, co-crystals, and other structural variations, enhancing pharmaceutical analysis and quality control.

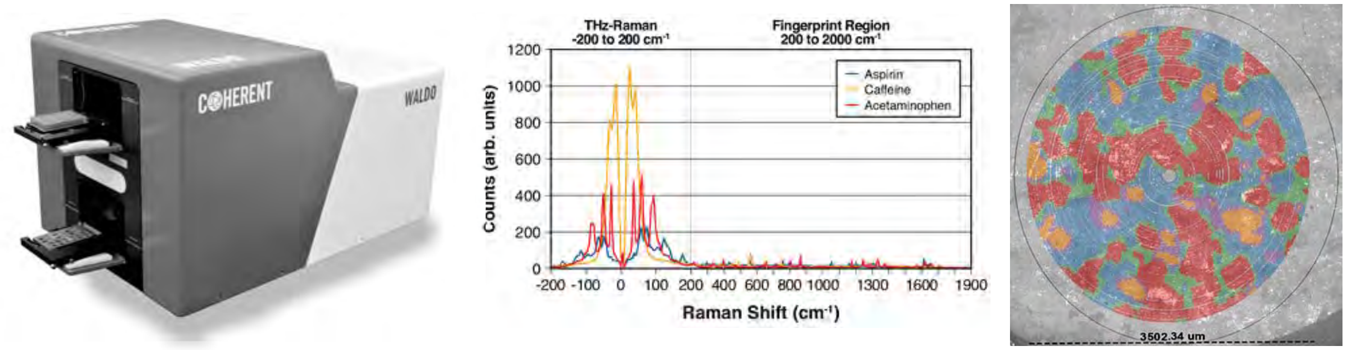

Figure 1: (Left) WALDO, THz-Raman High Throughput System. (Centre) Representative THz-Raman spectra of individual components of a multicomponent pharmaceutical tablet. (Right) THz-Raman imaging of components in the tablet cross section.

Simultaneous and colocalised Raman and SEM imaging for correlated multimodal analysis

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Here, we introduce the inLux SEM Raman interface, a novel interface that can be attached to new or existing SEM microscopes to enable colocalised and simultaneous SEM and Raman imaging. The analysis is made simultaneously from the same sample region (without moving the sample), thus avoiding correlation errors and the challenging and labour-intensive approach of transferring samples between instruments. The inLux interface enables you to combine Raman, optical and cathodoluminescence methods with standard SEM techniques such as SE, BSE and EDX.

We will discuss the application potential of correlative SEM and Raman imaging using the inLux interface on batteries, polymers, pharmaceuticals, and biologic samples. With these examples, we illustrate how the multimodal correlative analysis can increase understanding of materials, and the power and ease of use when these techniques are effortlessly combined inside a SEM using this innovative technology. We also highlight in Figure 1 the benefits of the multimodal approach on a mineral section which demonstrates the clear and accurate overlay between minerals found in the Raman, BSE and EDX images. The complementary potential of the analysis showed species detected only with Raman (anatase) or EDS (Zn, sphalerite), thus contributing to the increased understanding of the sample's properties.

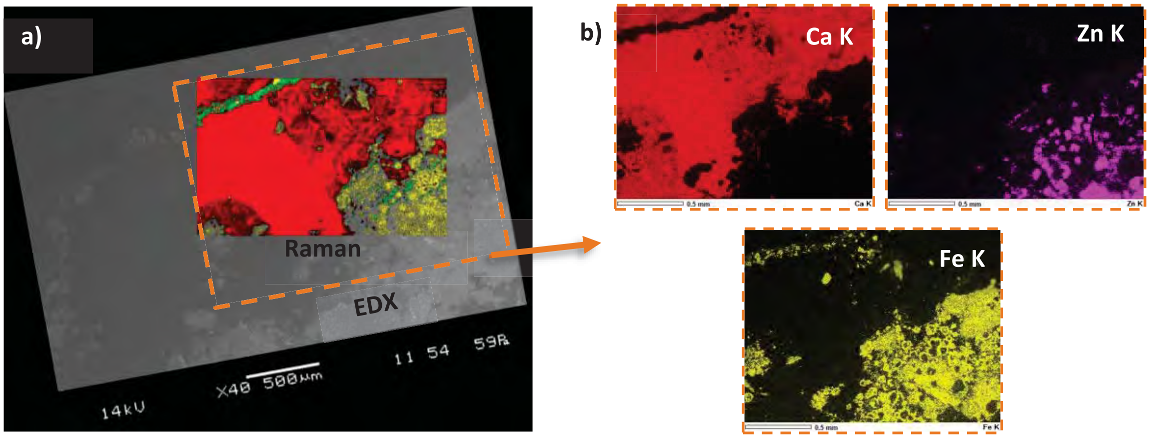

Figure 1: a) BSE image of a mineral section with the overlayed Raman image illustrating the distribution of calcite (red), pyrite (yellow), and anatase (green); b) complementary EDX information correlating with the Raman data, with the additional detection of Zn due to the presence of sphalerite. The presence of anatase also shows that Raman spectroscopy is sensitive to the many polymorphs of TiO$_2$.

Miniaturized high-end Raman spectrometers and microscopes without performance compromise

Oleksii Ilchenko

Lightnovo ApS, Birkerød 3460, Denmark

Lightnovo was established in 2019 and specializes in the development, production, and sale of Raman spectroscopy-based instruments.

It is our ambition to democratize the power of high-end Raman spectroscopy for the benefit of mankind.

The miniRaman Spectrometer, is an innovative, compact, handheld Raman device, utilizes a patented internal reference channel for constant recalibration. This spectrometer ensures unparalleled accuracy and operational stability with each acquisition. The spectrometer's ground-breaking approach to optical optimization, including the novel use of real-time spectral deblurring, effectively addresses laser wavelength drifts, enhancing resolution without the need for energy-intensive cooling systems.

To expand the possibilities of research, Lightnovo company has developed a world’s smallest confocal miniRaman microscope with modular design for chemical and structural analysis. The heart of the microscope is the miniRaman spectrometer.

This instrumental setup allows for both sample viewing using the optical microscopy capabilities and performing measurements by Raman spectroscopy simultaneously.

The visual observation reveals morphological details of a sample (e.g. color, size, shape), whereas the spectroscopic measurement reveals information about the molecular structure and chemical composition of a sample.

For applications that require high spectral and spatial resolution, large mapping area, extremely stable laser power, high sensitivity and broad spectral range (from low frequency to high frequency Raman shift), we suggest RG Raman spectrometers and microscopes.

Another direction of our company is the development of advanced microscope THOR. The system features up to four lasers with motorized switching, simultaneous Raman spectroscopy, and ambient light-blocking sliding doors for secure measurements.

THOR, based on mirrorless Raman beam delivery technology, are ideally suited for sophisticated applications in Raman microscopy: fast 3D Raman imaging, SERS imaging, polarized Raman imaging, low frequency Raman measurements, Stokes/Antistokes Raman measurements, and fast optical shutter technology (<1ms).

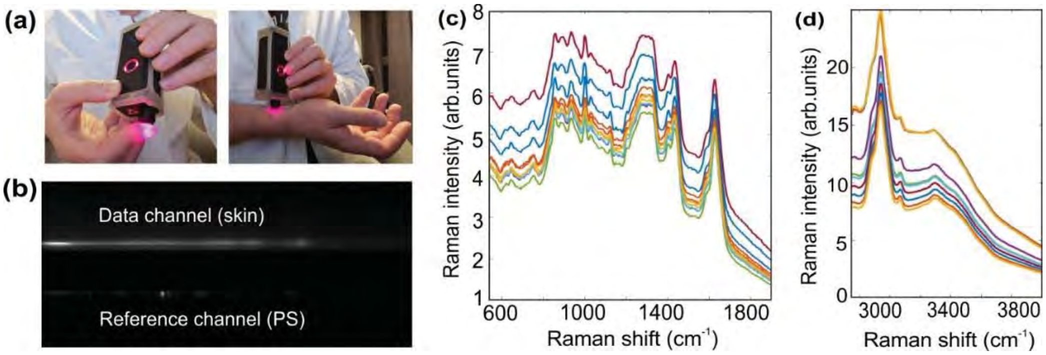

Figure 1: (a) Photograph of compact Raman device, (b) sensor image with data and reference channels, (c,d) typical Raman spectra of human skin obtained by compact Raman device.

Ultrafast Lasers for Raman Spectroscopy and Microscopy

Jonas Berzinš, Vaidotas Stalilionis, Dominykas Gudavičius,
Lukas Kontenis, Mantvydas Mikulis, Marco Arrigoni
Light Conversion, Vilnius, Lithuania

Ultrafast lasers have revolutionized multiple scientific and industrial applications, Raman being one of them. Coupled with wavelength-tunable extensions, the ultrafast lasers provide access to a wide spectral range of tunability, which plays a vital role in stimulated Raman scattering (SRS) and coherent anti-Stokes Raman scattering (CARS).

SRS is a powerful technique that enables label-free detection of chemical bonds with high specificity. However, typically the SRS is prone to low sensitivity; thus, limiting its application to bioimaging. To address this bottleneck, the electronic pre-resonance SRS (EPR-SRS) technique has been developed to enhance the Raman signals by shifting the excitation frequency toward the molecular absorption. This has been showcased with both amplifier system using multiple-plate continuum to obtain a broad spectrum [1] and using CRONUS-2P OPO-based laser source with two independently tunable channels [2]. More so, the latter laser source with its two independent channels was used for dual CARS microscopy across the vibrational spectrum [3].

Another development is towards widefield Raman techniques, e.g., a widefield CARS based on random illumination microscopy (RIM) [4]. CARS-RIM provides super-resolved reconstructions and optical sectioning of the sample from the second-order statistics of multiple images obtained under different speckled illuminations. The advent of high-power laser oscillators and OPAs makes it a promising technique for imaging large samples with molecular specificity at high acquisition rates.

Lastly, ultrafast laser direct writing can be used to obtain differently shaped periodic gold nanostructures on the surface of a thin metal film [5]. The arrays excite hybridized plasmonic modes and hold potential as simple and reproducible substrates for surface-enhanced Raman spectroscopy (SERS).

In this work, we will provide an overview of Light Conversion's ultrafast technology, including femtosecond amplifiers and oscillators, OPAs, and OPOs – tools that are indispensable for advancing Raman spectroscopy and microscopy techniques.

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Multimodal submicron Widefield IR, Simultaneous Raman and Fluorescence microscopy:

When Autofluorescence becomes your new friend

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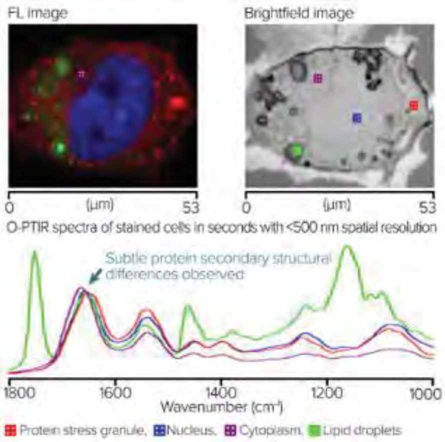
[1]Photothermal Spectroscopy Corp., Santa Barbara CA USA

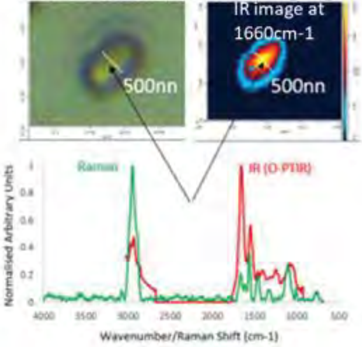
Using Optical Photothermal IR (O-PTIR) spectroscopy, the accepted limitations of traditional IR microcopy are broken, providing for true submicron spatial resolution operating in contact-less reflection mode (no ATR), whilst providing for FTIR transmission/ATR-like data quality. Furthermore, it bridges the gap between conventional IR microspectroscopy and nanoscale IR spectroscopy. Now, in a world first, it also provides **IR**+**Raman** - simultaneous IR and Raman measurements at the same time, from the same spot and the same resolution to take full advantage of well known complementarity of Raman and Infrared spectroscopy, whilst also providing submicron spatial resolution vibrational spectroscopy on challenging samples, such as highly fluorescent and/or easily damaged samples.

In recent developments, a new modality, “counter-propagating” has been engineered to provide for enhanced IR spatial resolution and sensitivity, with the use of high NA refractive objectives. This improves spatial resolution to ~300nm for both IR and Raman, whilst also improving sensitivity, image quality and facilitating immersion objective studies in fluid. Further, we demonstrate widefield epifluorescence to facilitate a novel concept – fluorescence guided (or fluorescence co-located) O-PTIR microspectroscopy with very recent advances now using the (auto) fluorescence signal as means to deliver true widefield, snapshot IR imaging, with super-resolution IR with 50x increase in speed of measurement.

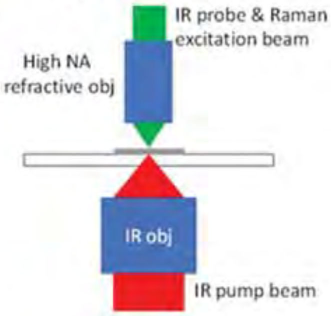
Several examples from published literature in the life sciences to materials will be reviewed, such as live cell lipid metabolism in water, hydrated tissues analysis for protein mis-folding to various bacterial studies with stable istopic labelling and the characterization, simultaneous SERS/SEIRA and the synergist application of IR+Raman for more thorough microplastics characterization.



Ultra high resolution single cell bacterial IR+Raman imaging



Counter-Propagating Mode O-PTIR



Multiplexing stimulated Raman microscopy for biomedical imaging and chemometric histology

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So far, multiplex stimulated Raman scattering (SRS) microscopy has been implemented only in few scientific publications due to the intrinsic technical challenges [1,2] in terms of both the laser source and the detector. The potential of a parallelized acquisition of the whole coherent Raman spectrum in a single-shot is evident and would represent the most advantageous coherent translation of spontaneous Raman.

Cambridge Raman Imaging (CRI) has developed multiplex SRS systems, starting from the laser source (STRALE) and the multi-channel detector (CHAMP) to the complete integrated imaging system (CORAL). STRALE is a passively synchronized dual-output fiber laser, based on a CRI patented technology, which generates a narrowband/picosecond pump and a broadband/femtosecond Stokes, whose bandwidth covers the entire CH-stretching region (2800 - 3100 cm^{-1}) in a single shot. CHAMP is a unique-on-market multi-channel lock-in amplifier spectrometer, able to retrieve the stimulated Raman spectrum in few microseconds with a sensitivity down to 10^{-6}. CORAL is an integrated imaging research platform incorporating STRALE and CHAMP in a ready-to-use inverted laser scanning microscopy system, ensuring day-to-day reproducible results in bioimaging. The unique multiplexing capabilities of CRI technology enhance data-driven image analysis for fast and accurate chemometric classification, pushing the boundaries of label-free image-based diagnostics.

Figure 1: CRI's product portfolio: the STRALE laser (left), the CHAMP detector (center) and the imaging platform CORAL (right).

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Gap-mode TERS of TMDs on metallic substrates: unexpected peculiarities and applications for Janus TMDs nanoscale structural characterization.

Andrey Krayev, HORIBA Scientific

In my talk I'll discuss recent results on the excitation profile of the gap-mode TERS response from transition metal dichalcogenides (TMDs) such as WS_2 and MoS_2 on silver and application of TERS imaging, cross-correlated with other scanning probe microscopy channels such as the topography, surface potential or photocurrent for the nanoscale spectroscopic and consequently, structural characterization of various growth- and conversion-related defects in Mo-based Janus TMDs (MoSeS and MoSSe).

In the first part of the talk I'll discuss the evolution of the gap-mode TERS spectra of the mono- and bi-layer WS_2 and MoS_2 on silver as a function of the excitation laser wavelength. In the course of this study we collected consecutive TERS maps of the same area in the sample containing mono- and bi-layer regions with same TERS probe with 6 different excitation lasers. To decrease the number of collected TERS maps, we used concurrent excitation and collection with two lasers simultaneously. We found that the E_{2g}/A_{1g}.peak intensity ratio for the bilayer WS_2@Ag and the ratio of the A'/A_{1g} peak intensity of the out-of-plane mode for the mono- and bi-layer, change in a significantly non-monotonous way as the excitation laser energy is swept from 1.58 eV to 2.62 eV. The former ratio increases at energies corresponding to A and B excitons in bilayer WS_2. Absolute intensity of the A' peak in the monolayer and, correspondingly- the A'/A_{1g} ratio is surprisingly high at lower excitation energies, but dips dramatically at the energy corresponding to A exciton, being restored partially in between A and B excitons, but still showing the descending trend as the excitation laser energy increases.

Somewhat similar picture was observed in mono- and bi-layers of MoS_2@Ag, though the existing set of the excitation lasers did not match excitonic profile of this material as nicely as for the case of WS_2.

We can draw a rather *counterintuitive conclusion* that for successful observation of the gap-mode TERS signal from TMDs in intimate contact with a metallic substrate, the energy of the excitation laser should be below A exciton, the use of Raman laser which is in resonance with A or B excitons is actually counterproductive. Based on this observation and consistently strong TERS response from the WS_2 and MoS_2 bilayers, an "ideal" substrate for efficient TERS and tip enhanced photoluminescence (TEPL) measurements will be discussed.

In the second part of my talk I'll show how the TERS imaging of Janus MoSSe and MoSeS TMDs on gold and silver performed with 785 nm (1.58 eV) excitation laser, which is below the A exciton in these materials (~1.7 eV) can identify various growth-related nanoscale defects of MoS_2/$MoSe_2$ precursors such as occasional bi-/multi-layer islands in predominantly monolayer crystals, as well as the conversion-related defects in these Janus TMDs (Fig.1) :

- appearance of the physical cracks or the submicron-scale domains with the over-converted borders in MoSeS Janus monolayers converted from $MoSe_2$ precursor, related to significant tensile strain occurring after the replacement of selenium atoms by sulfur;

- appearance of somewhat similar domains with wrinkled borders in MoSSe Janus crystals converted from MoS$_2$ due to the compressive strain appearing after the replacement of sulfur with selenium;
- Spatial variations at the scale of only a few tens of nanometers of the intensities and the spectral position of A$_1^1$ and A$_1^2$ TERS peaks that can be interpreted as the local variation of the perfection of Janus conversion;
- Nanoscale domains of non-converted material.

We'll discuss how the understanding of the nanoscale structure of the Janus monolayers can help avoiding misinterpretation of the Raman data and pave the way to sophisticated improvements of the synthetic and conversion steps for the production of perfect Janus crystals or Janus crystals with desired and well-controlled defects.

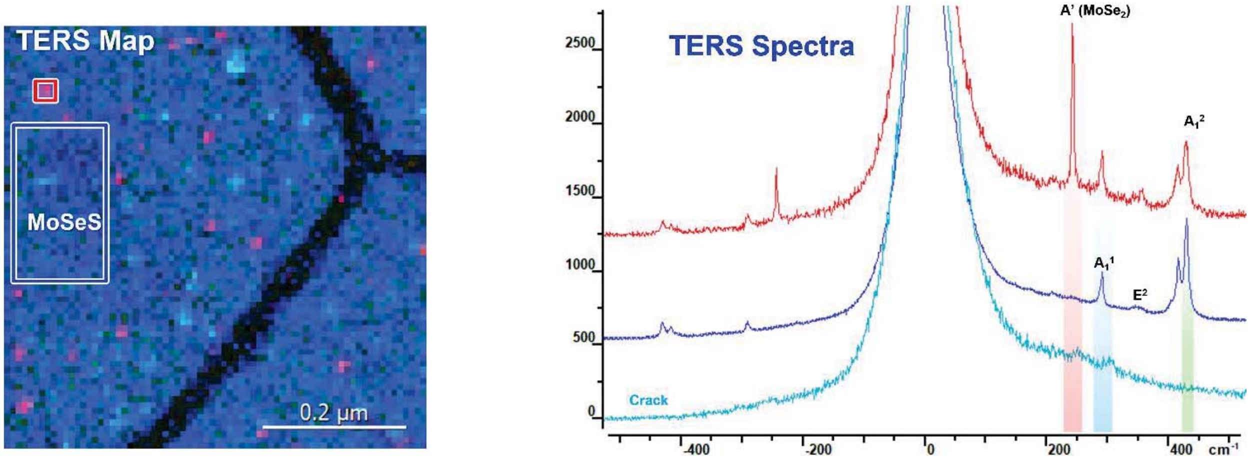

Figure 1. Combined TERS map showing the distribution of the intensity of correspondingly highlighted Raman peaks: A'(MoSe$_2$)-red, A$_1^1$(MoSeS)-blue and A$_1^2$(MoSeS)-green. TERS spectra averaged over MoSeS (blue), MoSe$_2$-MoSeS vertical heterostructure (red) and over the cracks (cyan). Absence of the Raman signal of both MoSeS or MoSe2 over the cracks confirms that the domains in MoSeS crystals are physically separated by gaps a few tens of nanometers wide. Presence of the Raman bands of MoSe2 coming from tiny, 20-30 nm across, nano-islands may be misinterpreted in the far-field measurements as incomplete conversion.

Tip-Enhanced Raman Spectroscopy for Chemical and Structural Characterization of Tau Amyloid Fibrils

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Tip-enhanced Raman spectroscopy (TERS) has emerged as a powerful technique for chemical and structural characterization of biomolecules with nanoscale spatial resolution [1]. Due to their nanoscale composition and structural heterogeneity, amyloid fibrils are in particular interesting samples for TERS studies. Amyloid fibrils are important markers of neurodegenerative diseases. They are formed by aggregation of normally soluble proteins and peptides, and can lead to the formation of deleterious protein deposits on neurons. Among them, tau amyloid fibrils implicated in tauopathies (e.g. Alzheimer's disease) can be stabilized by a variety of cofactors such as heparin sodium (HS), phospholipids and RNA. The latter two are especially relevant in the frame of human neurodegenerative disorders, but it remains unclear whether these cofactor molecules are inserted into fibril structures and, if so, how they are spatially distributed in fibrils.

Here, we present the TERS characterization of tau amyloid fibrils using bottom-illumination TERS systems [2-5], as well as technical developments performed to achieve this goal [2,3]. Three types of tau fibrils are investigated. Well-documented tau fibrils formed in the presence of HS as cofactor [3,4] are considered as reference fibrils for comparison with the two other types, namely tau-PIP2/POPC fibrils formed from a mixture of phosphatidylinositol (PIP2) and phosphatidylcholine (POPC) [4], and tau-polyA fibrils formed in the presence of poly-adenosine (polyA) cofactor [5]. Contrary to HS, PIP2 is present inside the inner cytoplasmic membrane and RNA strands are expected in neurons too. PIP2 and polyA are thus biologically more relevant than HS as prototypical cofactors implicated in neurodegenerative diseases. TERS studies reveal different secondary structures as a function of the cofactor, with distinct β-sheet and random coiled compositions [4,5]. They prove that PIP2 is inserted inside the fibril structure [4], and polyA is superficial to the β-sheet fibril core, but nonetheless enveloped within the fuzzy coat [5]. TERS imaging also show the colocalization of adenine with positively charged histidine and arginine moieties implicated in C-terminal core binding, thus confirming specific tau-polyA interactions and providing valuable clues to understand the efficiency of polyA as aggregation cofactor [5]. These first studies are promising to better characterize tau fibrils formed from various cofactors and better understand molecular mechanisms involved in amyloid aggregation processes.

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Selective Accumulation of SERS Signal by Density-Based Clustering Analysis

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We developed a new method for obtaining surface-enhanced Raman scattering (SERS) spectra with extremely high sensitivity and spectral resolution[1]. In this method, thousands of SERS spectra are acquired, followed by a data selection procedure based on density-based spatial clustering of applications with noise (DBSCAN)[2]. Each spectrum is recorded by exposure with a single nanosecond laser pulse to avoid the effect of time averaging. The reconstructed spectrum consists of the data which belong to the clusters. The method was applied to a crystal violet (CV) aqueous solution with a concentration of 10^{-7} mol/L. The results suggest that several minor Raman peaks were successfully recovered which cannot be detected in conventional SERS measurements.

Methods

The concept of the data selection using DBSCAN is explained as follows. Firstly, thousands of SERS spectra were acquired, with each spectrum measured by a single-shot nanosecond laser pulse to avoid time averaging. Secondly, an intensity–wavelength plot (I–W plot) was constructed by extracting the data sets of the highest intensity and its pixel number from each SERS spectrum. Thirdly, we applied DBSCAN to the I–W plot to automatically separate the signal (cluster) from the noise (non cluster). DBSCAN process classify the high density of data points as a cluster, and the other part as a noise. Since each data point on the I–W plot originates from the maximum value of the SERS spectrum, it is highly probable that the SERS signal involves in the cluster. Therefore, only the data within clusters are selected to form a new spectrum.

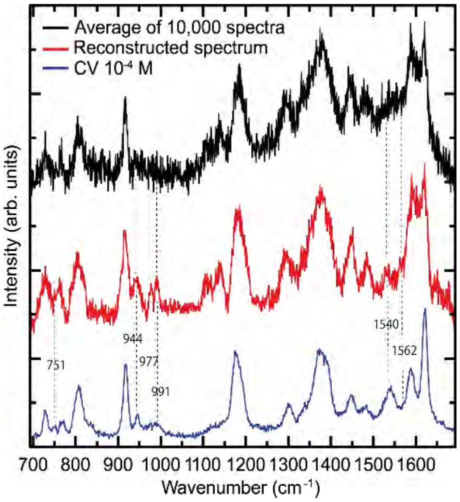

Figure 1. Reconstructed single-pulsed SERS spectra of 10^{-7} mol/L CV (532 nm excitation). Red line: DBSCAN reconstruction, Black line: Average of all (10,000) spectra, Blue line: thick solution.

Results

Figure 1 shows the Raman spectra of CV, reconstructed by single-pulsed SERS with DBSCAN. The red line indicates the reconstructed spectrum obtained by DBSCAN with MinPts = 400 and Eps = 5.4, while the black line indicates the average of 10,000 single-pulsed spectra. For comparison, a spectrum of high-concentration CV solution (10^{-4} mol/L) is shown by the blue line. According to Figure 1, it can be observed that the reconstructed spectrum improved signal-to-noise ratio in comparison with the averaged spectrum. In addition, fluorescence background appeared at around 1300-1600 cm^{-1} is reduced. Several peaks are clearly recovered in the reconstructed spectrum that are not visible in the averaged spectrum. The peaks 751, 944, 977, 991 cm^{-1} are clearly recognized in the red spectra, while in the black spectra, these peaks are difficult to see. Similarly, the peak at 1540 and 1562 cm^{-1} is more easily observed in the red spectrum than in the black spectra. These results suggest that we successfully recovered all the Raman peaks found in the spectrum under high concentration.

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Simulation of IR and Raman signals in liquid water from molecular dynamics: Effect of the hydrogen bond network

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Water is an ubiquitous liquid that has several exotic and anomalous properties. Despite its apparent simple chemical formula, its capability of forming a dynamic network of hydrogen bonds leads to a rich variety of physics. Here we study the vibrations of water using molecular dynamics simulations, mainly concentrating on the Raman and infra-red spectroscopic signatures, and compare our simulated results with the measured ones [1]. We investigate the consequences of the temperature on the vibrational frequencies, and we enter the details of the hydrogen bonding coordination by using restrained simulations in order to gain quantitative insight on the dependence of the frequencies on the neighbouring molecules. Further we consider the differences due to the different methods of solving the electronic structure to evaluate the forces on the ions, and report results on the angular correlations, isotopic mixtures HOD in H_2O/D_2O and and the dielectric constants in water [2]. We also explore the collective vibrational excitation modes in the liquid [3].

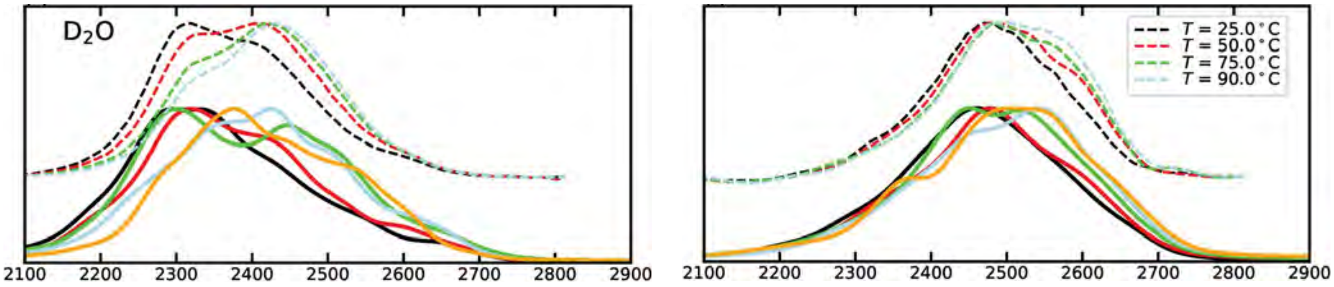

Figure 1: The measured [1] – dashed lines – and simulated [2] – solid lines – Raman spectra in the isotropic – left – and anisotropic – right – mode in liquid D_2O

The results from the vibrational spectroscopies allow us to better understand the structure and dynamics of the hydrogen bond network in liquid water. Also the comparison between the measured and simulated spectra provide interesting insight into the structure and other properties of water.

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Raman spectroscopy of heavily doped graphenes

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The temperature-dependent (T-dependent) linewidth and frequency shift of the G mode provide valuable information on the phonon anharmonicity of graphene-based materials. We probed the intrinsic phonon anharmonicity of heavily-doped graphene by T-dependent Raman spectra based on FeCl3-based stage-1 graphite intercalation compound (GIC), in which the EPC contribution is negligible due to the large Fermi level shift. The linewidth and frequency shift of the G mode exhibit a nonlinear decrease and noticeable broadening with increasing temperature, respectively, which are both dominated by phonon anharmonicity processes. The contribution of phonon anharmonicity to frequency shift of heavily-doped graphene decreases as the EF approaches to the Dirac point. However, the T dependence of frequency shift is almost independent on EF and qualitatively agrees with the theoretical result of pristine graphene. These results provide a deeper understanding of the role of phonon anharmonicity on the Raman spectra of heavily doped graphene. Furthermore, Graphene is an ideal platform to study the coherence of quantum interference pathways by tuning doping or laser excitation energy. The latter produces a Raman excitation profile that provides direct insight into the lifetimes of intermediate electronic excitations and, therefore, on quantum interference, which has so far remained elusive. We control the Raman scattering pathways by tuning the laser excitation energy in graphene doped up to 1.05 eV. The Raman excitation profile of the G mode indicates its position and full width at half-maximum are linearly dependent on doping. Doping-enhanced electron−electron interactions dominate the lifetimes of Raman scattering pathways and reduce Raman interference. This will provide guidance for engineering quantum pathways for doped graphene, nanotubes, and topological insulators.

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Optical transition energies and symmetry-dependent electron-phonon enhancement in graphene nanoribbons obtained by resonance Raman scattering

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We report in this work a multi-excitation resonance Raman study of atomically precise graphene nanoribbons (GNRs) produced by surface-assisted coupling of molecular monomers designed to synthesize armchair graphene nanoribbons with 7 and 9 atoms width (7-AGNR and 9-AGNR). Resonant Raman excitation profiles (REP) for both low and high energy phonon modes were measured using several laser excitation energies in the range from 1.37 eV (904 nm) to 2.81 eV (441 nm), and the optical transition energies were obtained from the analysis of the REPs. Resonances with direct E_{ii} optical transitions are observed for all the phonon modes of 7-AGNR, showing that all Raman peaks are intrinsic features of the GNR, including the peaks in the range 1200-1400 cm^{-1} , which comes from the TO and LO phonon branches of graphene near the K point. On the other hand, results for the 9-AGNR show resonances with crossed $E_{i,i\pm1}$ optical transitions observed for some modes, revealing a symmetry-dependent electron phonon coupling effect in this material.

Progress of biomedical Raman microspectroscopic imaging

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Raman microspectroscopic imaging combines a Raman spectrometer, a microscope and a motorized stage or scanning mirrors. Hyperspectral images are registered by sequential acquisition of Raman spectra at each position. Key experimental parameters include lateral resolution that is determined by the step size and numerical aperture of the objective lens, time per spectrum that determines the signal to noise ratio, and image size that determines the number of spectra. The total collection time is the product of number of spectra and time per spectrum which can become long resulting in slow throughput. Early imaging work of my lab is presented for single cells, brain tumor tissue and sections, the complementary application of Raman and FTIR imaging, and chemometric data processing. Then, recent progress in Raman imaging will be reported.

Towards intraoperative assessment of bladder cancer, a workflow separated unwanted contributions from substrate, water and embedding medium from tissue, tumor, and necrosis by hyperspectral unmixing. In addition, in 13 out of 20 specimens particles were detected and identified using a spectral library[1].

Shifted excitation Raman difference spectroscopy (SERDS) for fluorescence suppression was implemented with a 20x20 fiber array, switching the excitation wavelength in the millisecond range and interleaved nod & shuffle. This approach enabled rapid and background-free Raman imaging which was demonstrated for tissue samples[2].

Collagen crosslinking was induced by glutaraldehyde, genipin and glycation and monitored by Raman spectroscopy, multiphoton SHG and TPEF microscopy and atomic force microscopy (AFM). SERDS was also applied here because the genipin crosslinker turned crosslinked collagen dark blue. Whereas AFM resolved single fibers, SHG and TPEF revealed detailed images. Raman spectra provided specific fingerprints of each crosslinking process[3].

A novel experimental approach combines Raman spectroscopy and optical photothermal infrared (OPTIR) spectroscopy. Both spectral information are complementary to some extent and can simultaneously be registered. Rapidly acquired OPTIR images enable selecting points of interest for full Raman and IR spectroscopy. Biomedical applications will be demonstrated.

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Optical Photothermal Infrared (O-PTIR) and Raman Spectroscopic Study of Tauopathy Signatures in a Mouse Model of Alzheimer's Disease

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Alzheimer's disease (AD) is the most common form of dementia. There are currently over 55 million people affected by dementia worldwide, with this number predicted to increase significantly in the coming decades due to ageing population [1]. However, there is no definitive diagnostic test or cure for AD so far and the effectiveness of medical interventions is limited. The two major hallmarks of AD are amyloid beta (Ab) plaques and neurofibrillary tangles (NFTs) of hyperphosphorylated tau protein in the brain. In our team, we have previously investigated Ab plaques in a mouse model of amyloidopathy (TASTPM) using a combination of label-free vibrational spectroscopic methods based on Raman scattering and IR absorption [2]. In this work we are interested in the study of tauopathy through another experimental model, the rTg4510 mouse. The emerging Optical Photothermal Infrared (O-PTIR) microscopy was applied alongside Raman and immunofluorescence imaging to study *ex vivo* brain tissue samples from 12-month-old rTg4510 mice and littermate controls [3]. The higher spatial resolution afforded by O-PTIR enables the subcellular investigation of tauopathy signatures in the brain hippocampus, showing putative NFTs which are smaller than the optical resolution limit. These observations provide valuable insights into the biological and chemical characteristics of tauopathy, thus contributing to a better understanding of the underlying mechanism of AD pathogenesis.

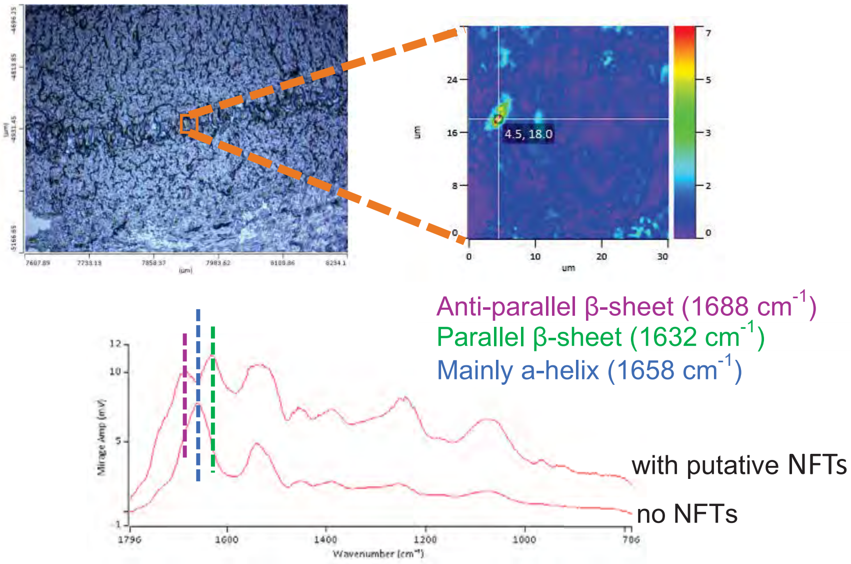

Figure 1: White light image and O-PTIR map at lipid ester (1732 cm^{-1}) of transgenic mouse hippocampus with representative spectra of putative NFTs and no NFTs

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Development of a unified Raman spectroscopy and mass spectrometry bioimaging instrument

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Lipid metabolism and signaling hold crucial importance in biological systems and disease progression. Despite their significance, the absence of an optical technique capable of directly performing lipidomics tissue analysis with high molecular specificity persists. In this study, we have developed the first integrated Raman spectroscopy and desorption electrospray ionization (DESI) - mass spectrometry imaging in a unified instrument (Figure 1)[1]. A computational framework for inter-modality regression analysis was devised to extract lipidomic abundances from optical vibrational spectra. We demonstrate correlative Raman and mass spectrometry imaging of various tissues, including cancer tissues and transient cerebral ischemia-reperfusion injury in a murine model of ischemic stroke. By utilizing mass spectrometry to infer the molecular composition in compounded tissue Raman spectra, we offer a new approach for lipid visualization and identification. The unified Raman and mass spectrometry imaging technique opens novel opportunities for performing lipidomic analysis using vibrational optical spectroscopy.

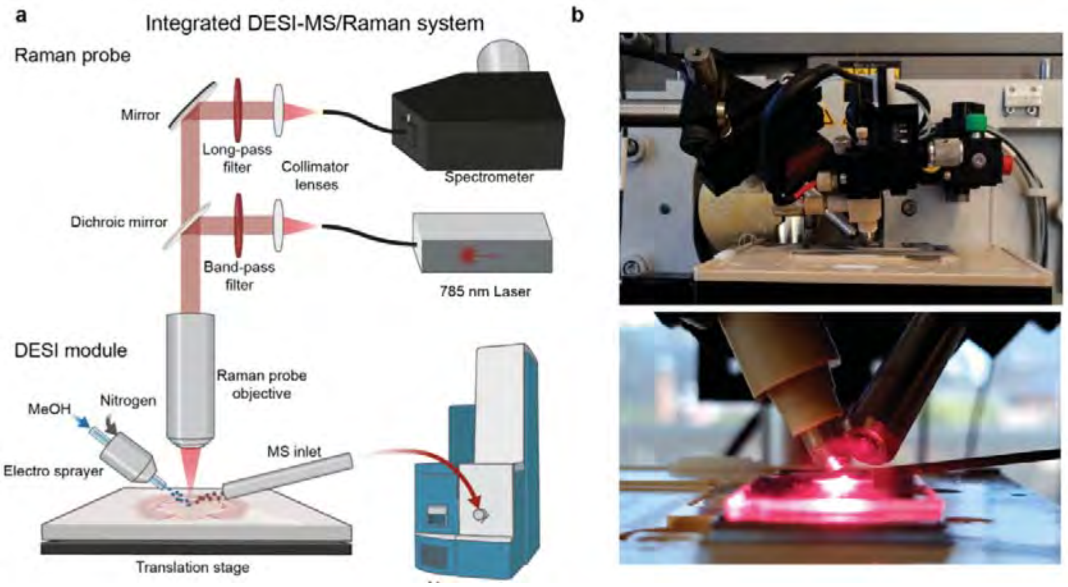

Figure 1 illustrates the integrated Raman and DESI-MS imaging system. Reproduced from [1]. (a) Schematics depict the setup where the electrosprayer focuses a jet of electrically charged MeOH solvent onto the tissue sample via pressurized nitrogen. Desorbed molecules then travel through ambient air into the mass spectrometer (MS) inlet. For Raman spectroscopy, a 785 nm laser light is directed through an objective lens, focusing onto the sample at a 45-degree angle. Raman scattered light is returned through the objective, separated from the laser path by a dichroic mirror and longpass filter, and finally fiber-coupled into a near infrared (NIR) spectrometer. (b) A photograph of the integrated Raman/DESI-MS imaging system employing a commercial Raman probe (InPhotonics).

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Differentiability of cell types enhanced by detrending non-homogeneous pattern in line-illumination Raman microscope.

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Line illumination Raman microscope allows to record Raman images a few hundred times faster than raster scanning [1]. This enables us to explore a wider range of applications including biological samples such as cells and tissues. However, a non-uniform intensity distribution of the laser line illumination may induce some artifacts in the data and lower the accuracy of machine learning models trained to predict sample class membership. In this contribution using cancerous and normal human thyroid follicular epithelial cell lines, FTC-133 and Nthy-ori 3-1, whose Raman spectral difference is not so large, we show that standard preprocessing of spectral data widely used for raster scanning microscope cannot fully correct spatial experimental bias. To address this issue, we proposed a spatial de-trending scheme based on random forest regression, a nonparametric model-free machine learning algorithm, combined with position-dependent wavenumber calibration scheme along illumination line. It was shown that the detrending scheme minimizes the artificial biases arising from non-uniform laser source and significantly enhances the differentiability of the sample states, i.e., cancerous, or normal epithelial cells, compared to the standard preprocessing scheme [2].

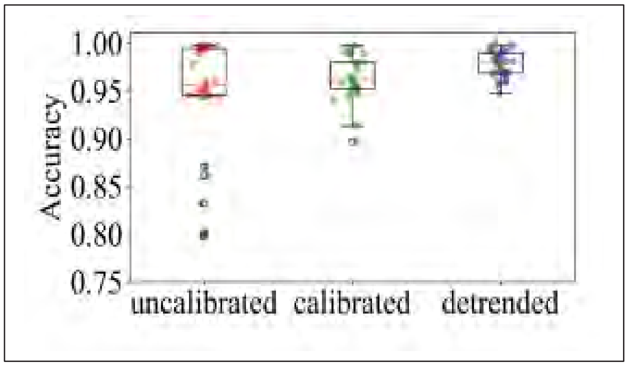

Figure 1: Comparison of machine learning performance for a classification task among the standard preprocessing pipeline and the detrending line preprocessing tailored for line-illumination Raman microscope.

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Raman imaging of Fabry disease-specific lipid accumulations in cardiac cells

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Fabry disease (FD) is a rare inherited lysosomal storage disorder due to alpha-galactosidase A (α-Gal A) deficiency causing progressive multiorgan damage [1]. The most common cause of death in FD patients is due to cardiac lipid manifestations, which are often not diagnosed until irreversible damage has already occurred. All this makes a fast and reliable diagnostic tool necessary and desired. Previously, we demonstrated the application of coherent anti-Stokes Raman spectroscopy (CARS) for differentiation between diseased and healthy mouse tissue samples with high sensitivity [2].

Here, Raman spectroscopy (RS) is applied to detect the disease-associated lipid biomarkers in different cardiac cell types along with their intracellular distribution. This complements preliminary results by a deeper understanding of the disease behaviour at early stages on a cellular level. About 50 fibroblasts and 30 adult cardiomyocytes were isolated from mice at the age of 20 weeks of both α-Gal A-knockout and wild-type mice, corresponding to the early disease stage. The fixed cell samples were imaged spectroscopically and pre-processed afterwards [3]. Finally, an in-house written lipid detection algorithm was applied to localize different lipid accumulations within the cells and classify them.

Indeed, lipid accumulations associated with FD biomarkers were found in both cell types. For cardiomyocytes these accumulations were found exclusively in diseased α-Gal A-knockout mice and the bands of reference spectra were identified in the corresponding mean spectrum of the specified clusters. In contrast, a far higher lipid proportion compared to cell size along with a higher amount of lipids was detected in fibroblasts (see Figure 1). This was detected for both knockout and wild-type genotype, making a distinction between two groups more complex. In conclusion, this work shows the potential of RS to detect and localize disease-associated lipid accumulations, and, importantly, allows their visualization intracellular already at the early disease stage.

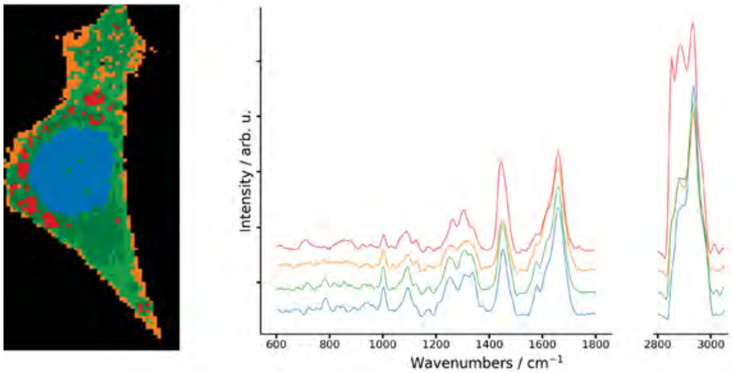

Figure 1. Left: colour map representation of isolated fibroblast from diseased mice. Right: corresponding to the image cluster representation with specific lipid peaks in high frequency and fingerprint region.

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Towards Asymmetric Reaction Monitoring in Organocatalysis by Coherent Raman Optical Activity (CARS-ROA) Spectroscopy

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Coherent Anti-Stokes Raman Scattering-Raman Optical Activity (CARS-ROA) spectroscopy is the coherent version of spontaneous ROA. Both spontaneous ROA and CARS-ROA are enantioselective techniques and, in principle, suited for monitoring asymmetric reactions. However, spontaneous ROA signals are very small, typically only ~10^{-4}-10^{-5} of the spontaneous Raman signals. This requires several hours of acquisition times to record a decent spectrum of a diluted solution of chiral molecules in an achiral solvent. On the other hand, CARS-ROA has been theoretically predicted to provide orders of magnitude enhanced chiral Raman signals compared to spontaneous ROA. The first experimental demonstration of CARS-ROA was achieved by the heterodyne-detected CARS-ROA spectra of both enantiomers of a chiral liquid and required just one minute of acquisition time [1]. The achiral CARS signals are typically ~10^{3}-10^{5} times larger than the spontaneous Raman signals, consequently, the CARS-ROA signals are sufficiently large to detect diluted solutions of chiral molecules in an achiral solvent in less than a minute. Recently, we have demonstrated heterodyne-detected CARS-ROA spectra of solutions of chiral MOM-BINOL molecules in the achiral organic solvent DCM acquired in one minute [2]. MOM-BINOLs are the precursor to a family of widely used organocatalysts in asymmetric synthesis. This paves the path for employing CARS-ROA in monitoring asymmetric organocatalysis that typically happens in an hour or more. In a closer step towards it, very recently we have acquired a CARS-ROA spectrum of a diluted solution of a real chiral catalyst shown in the Figure.

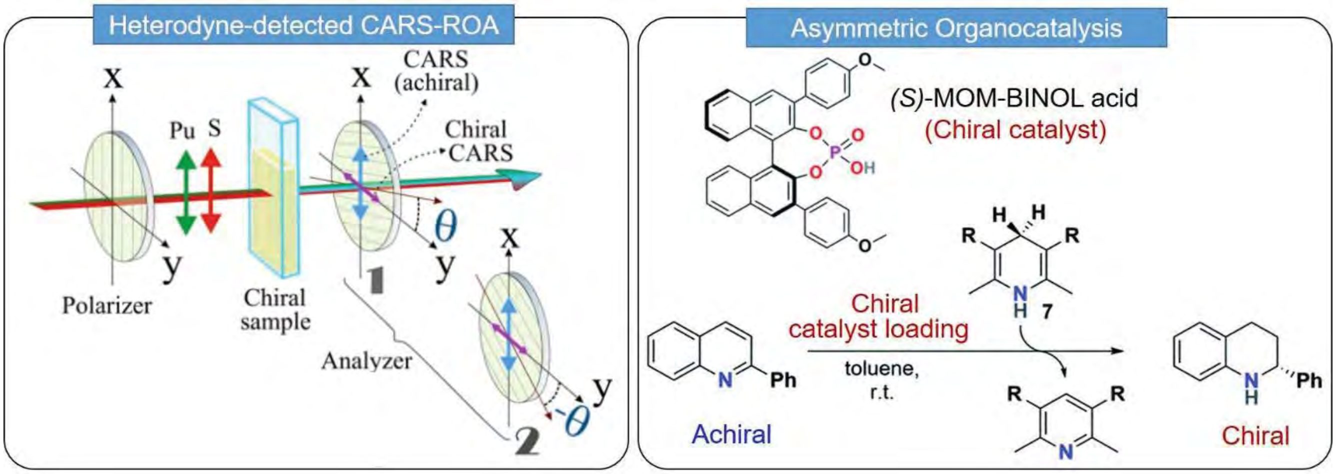

Figure: (Left) Schematic of the CARS-ROA setup. (Right) An example of asymmetric organocatalysis.

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Unraveling the Structural Polymorphism of Mononucleotide G-Quadruplexes *via* Raman Optical Activity

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Over the past two decades, mononucleotide G-quadruplexes (mG4), intricate nanostructures of guanine nucleotides, have garnered significant attention in genomics and bionanotechnology [1]. Yet, fundamental mysteries persist regarding their structure, function, and dynamics, crucial for understanding the details of mG4 formation and stability. Notably, the relationship between mG4 topology and stabilizing cations remains unclear, alongside ongoing debates on hydrogen bonding patterns and stacking configurations [2].

We introduce Raman optical activity (ROA) as a powerful tool to probe mG4 association and dynamics. ROA exhibits heightened sensitivity to mG4 formation compared to Raman scattering, revealing agglomeration patterns and higher-order organization in the presence of various cations. Particularly striking is the greatly enhanced terahertz ROA signal (< 200 cm^{-1}), offering unique insights into mG4 arrangements. Additionally, our study highlights the crucial role of hydrogen bonding in mG4 stability and uncovers a reversible transition between mG4 arrangements in Li-stabilized agglomerates — a novel observation. Complementing our experimental findings, molecular dynamics and quantum mechanics simulations provide deeper insights into these phenomena.

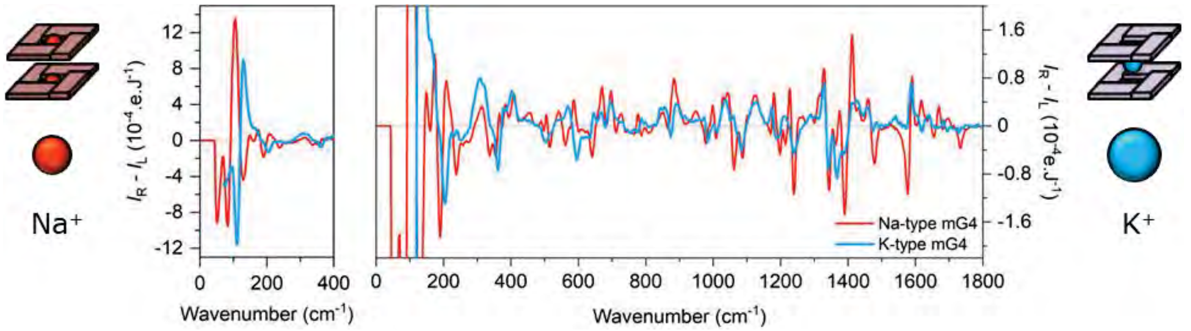

Figure 1: ROA spectra of Na- (red) and K-type (blue) of mG4. The low-wavenumber (THz) regions of ROA spectra are shown once more on the left due to their much higher intensity.

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Acknowledgment

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Plasmonic nanohelices for surface enhanced Raman spectroscopy with circularly polarized light

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Advanced surface enhanced Raman scattering (SERS) substrates have become important to enhance intrinsically weak Raman signals. However, these substrates are difficult to compare to one another. Here, we demonstrate a high-performance SERS substrate and, for meaningful comparison, we benchmark its properties using a commercially available product. Our substrate efficiently couples to circularly polarized light offering two novel ways to detect the chirality of molecules and nanostructures.

Raman spectroscopy is becoming a key tool to identify molecules associated with pollutants and environmental challenges [1]. To meet these challenges, SERS substrates must produce strong and reproducible signal enhancements over large areas between separate samples along with low fabrication costs. Here, we show that dense arrays of plasmonic nanohelices, which are of interest for many nanophotonics applications, also present excellent SERS properties [2]. We compare the performance of these nanohelices to commercially available conglomerates of Au nanoparticles.

Figure 1A shows the dimensions of left-handed Ag nanohelices; at a length of ≈100 nm these are the smallest helical structures used for SERS [3]. The SERS properties of the helices are demonstrated through two new methods for probing near-field enhancement generated with circular polarization at chiral metasurfaces (see Figure 1B). The first method is based on a single, element specific, achiral molecular vibrational mode (i.e., a single Raman peak; see Figure 1C). The second method is based on the Raman spectra of achiral molecules (crystal violet; see Figure 1D).

These advanced plasmonic materials are set to find broad applications in SERS spectroscopies for environmental science applications. One such application that could benefit is hyper Raman spectroscopy. Hyper Raman's very weak signal limits its use as a complimentary technique to standard Raman but use of these substrates will allow wider use of the technique.

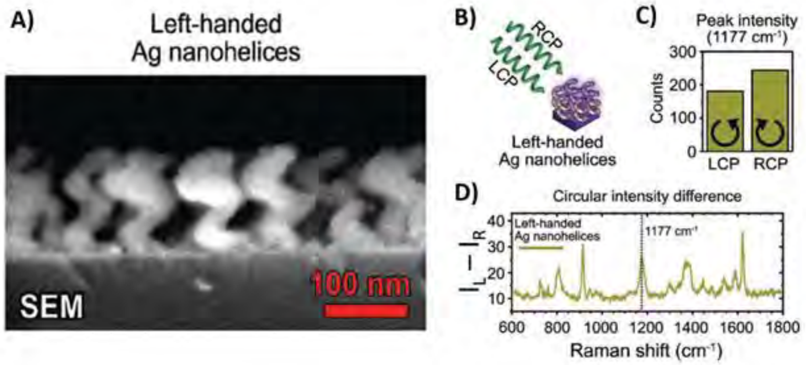

Figure 1: SEM image and data related to left-handed Ag nanohelices. A) Side view SEM of the nanohelices. B) Diagram of illuminating nanohelices with left- and right-handed circularly polarized light (LCP and RCP, respectively) at 532 nm. C) Peak intensity of the Raman vibrational mode at 1177 cm^{-1}, for LCP and RCP illumination. D) The circular intensity difference (CID, defined as IL – IR) spectrum of crystal violet for dense arrays of nanohelices; irradiance 1.7 kW cm^{-2}. [2]

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Giant Raman Optical Activity in chiral and polar Ni_3TeO_6

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Chiral phonons in chiral crystals have been a topic of great interest in the phonon and Raman scattering community for the past years. Their main method of identification in Raman scattering has been a splitting of phonon modes that can be observed through excitation with circularly polarised light, e.g. in α-Quartz [1], α-HgS [2] and Te [3].

In the chiral & polar magnet Ni_3TeO_6, we show that instead of an energy splitting, the chirality of the system leads to an intensity difference for cross-circularly polarised scattering geometries known as Raman Optical Activity. Originally developed for molecular systems [4], this effect can also be observed for phonons in chiral crystals [5], although the magnitude of the effect rarely exceeds magnitudes of 10^{-3}. Remarkably, in Ni_3TeO_6, this effect can be up to a hundred times larger.

The different scattering intensities when excited with right- (σ^-) or left (σ^+) circularly polarised light can be attributed to a non-negligible influence of the interaction of electric dipole moments with the magnetic field and electric field gradient of the incident light for optically active materials.

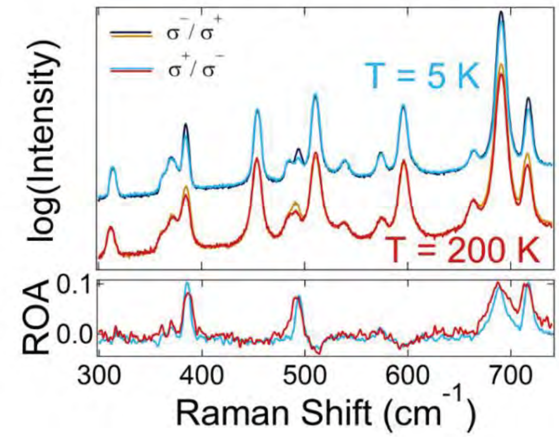

Figure 1: Raman spectra (upper panel) and Raman Optical Activity spectra (lower panel) of Ni_3TeO_6 in σ^+/σ^- (incident/analysed) and σ^-/σ^+ polarization configurations at T = 5 K and T = 200K. The phonons at 390 cm^{-1}, 490 cm^{-1}, 690 cm^{-1} and 720 cm^{-1} show intensity differences of up to 10% between the two configurations.

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Flame-fabricated SERS substrates for chemical and biological sensing

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Surface-enhanced Raman scattering (SERS) is a powerful technique for chemical and biological sensing, due to its advantages of label-free, fingerprint recognition, high sensitivity, rapid detection, and user-friendliness [1]. However, the commercialization of such SERS chemical and biological sensors is still limited by their sensing substrates due to high-cost and poor batch-to-batch reproducibility. To address this challenge, we examine the economic and massive manufacturing of SERS substrates using a highly scalable and reproducible flame aerosol technology, flame spray pyrolysis (FSP). The fabricated SERS sensing substrates have demonstrated high surface uniformity, high sensitivity, good batch-to-batch reproducibility and stability. The massive manufacturing of 150 samples can be achieved within 1.5 hours with a lab-scale flame reactor. The SERS substrates can rapidly detect pesticide residues both on apple surface and in orange juice, offering a proof-of-concept of their promising application in food safety diagnostics [2-3].

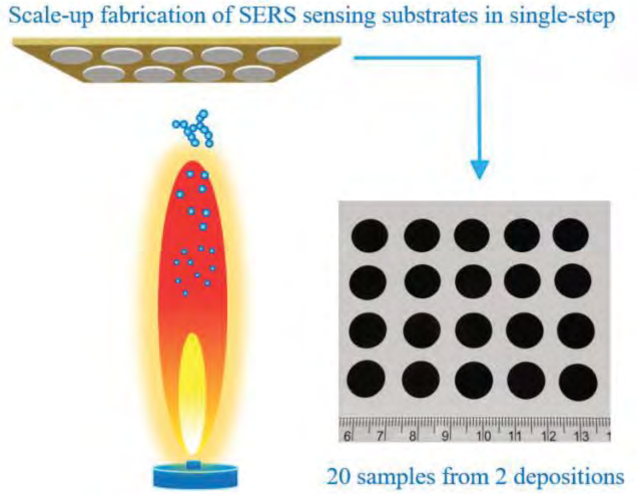

Figure 1: Scale-up fabrication of SERS sensing substrates in one-step using flame aerosol technology. 10 cover-glass based samples were manufactured from one deposition within 40s.

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Nanomotors: A dynamic approach for rapid capture and SERS detection of low-concentration emerging pollutants

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To effectively address the risks posed by emerging pollutants on ecological and human health, it is crucial to employ innovative and effective solutions [1, 2]. In this regard, a dynamic approach has been introduced, which can rapidly concentrate and detect these pollutants even at ultra-low concentrations. This approach utilizes responsive autonomous propulsion R-Fe_3O_4@Au@β-CD-EG-PF127 (RAP) nanomotors, which are capable of efficiently adsorbing pollutants from large-scale water sources using the "fishhook capture" concept (Figure 1). Moreover, the RAP nanomotors leverage external magnetic fields to accelerate the flow of pollutants, enabling more efficient and broader pollutant capture within three minutes. Furthermore, the method incorporates a temperature-sensitive self-cleaning process for the nanomotors, enhancing their practicality and reusability. The analysis and identification of captured pollutants are achieved in situ through surface-enhanced Raman spectroscopy (SERS), with a Principal Component Analysis algorithm applied to optimize spectral information extraction. This innovative approach highlights the potential of nanomotors as powerful and cost-effective tools for real-time monitoring of water quality and managing emerging pollutants.

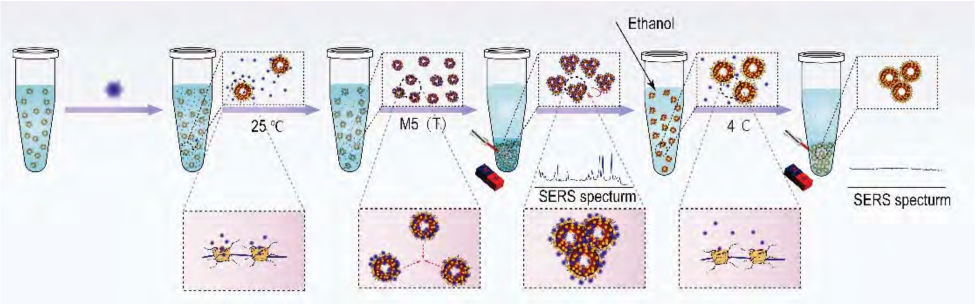

Figure 1. Schematic of dynamic enrichment and cyclic SERS detection.

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Silver Nanoparticles in the Langmuir-Blodgett Film matrix of Stearic Acid as SERS active sensor for Detecting Paraquat Herbicide

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Surface-enhanced Raman spectroscopy (SERS) has emerged as a potent on-site spectroscopic technique renowned for its swift, distinctive 'fingerprint' identification, and heightened sensitivity to molecules [1]. This study introduces a novel method for fabricating SERS-active substrates, known as SA-LB/Ag sensor, enabling ultra-trace paraquat detection in controlled environments and demonstrating the practical utility of these substrates in various field applications [2]. The fabricated substrates exhibit high sensitivity, uniformity, and reproducibility, making them potential substrates for herbicide and pesticide analytes, such as Paraquat (PQ). The sensitivity of the substrates was demonstrated by recording enhanced Raman signals at concentrations ranging from 200 ppm to 75 ppb. Quantitative assessments demonstrate a linear regression within the concentration range of same concentration, showcasing a remarkable detection limit of 14 ppb. Practical usability evaluations indicate that the developed SERS sensor is proficient in detecting residues in intricate water compositions, encompassing bodies of water affected by waterlogging, agricultural water such as rice field and vegetable field water, as well as extracts from soil, underscoring its potential applicability in the realms of food safety and environmental analysis.

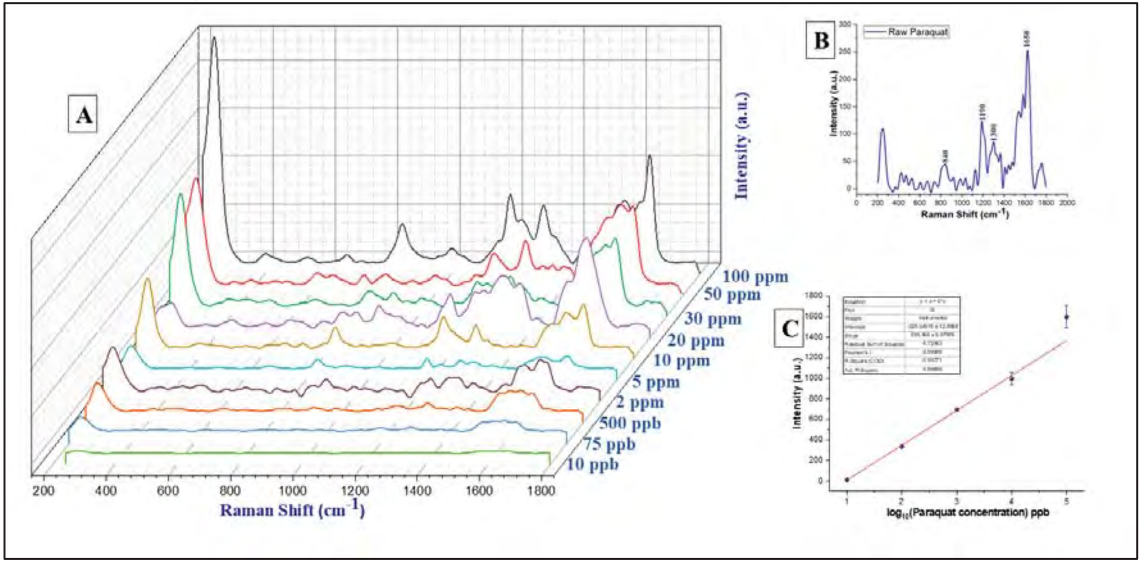

Figure 1: SERS spectra of PQ in the concentration range 10 ppb– 100 ppm.

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SERS detection of marine biotoxins

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Efficient and cost-effective detection of marine biotoxins poses a growing scientific and technological challenge, intensified by the proliferation of harmful algal blooms (HABs). These blooms, resulting from excessive algae accumulation in aquatic environments, can produce toxins harmful to human health. The escalating occurrences of HABs, amplified by ocean warming due to climate change, underscore the pressing need for action. These events not only pose threats to human health but also comprise significant economic impacts, particularly in sea-related economical activities like aquaculture. Hence, innovative approaches are imperative to effectively and selectively detect marine biotoxins, safeguarding both public health and economic stability in marine industries [1].

To address this challenge, our group has developed a microfluidic system for Surface-Enhanced Raman Scattering (SERS) detection of marine biotoxins. This innovative system features an organic–plasmonic hybrid composite engineered to selectively adsorb different biotoxin groups, enabling their detection at trace levels. By integrating gold nanoparticles (GNPs) with a covalent organic polymers (COPs) or covalent organic frameworks (COFs), we aim to achieve high affinity and preferential toxin adsorption alongside SERS detection [2]. The SERS signal generated by the composite-toxin system is validated through DFT calculations, ensuring specific toxin identification and quantification.

Preliminary assessment of these hybrid composites reveals promising results for trace toxin detection, paving the way for the development of an integrated detection system tailored for implementation in aquaculture farms situated in the open sea. This envisioned system leverages automated detection through chemometrics and machine learning algorithms, enhancing efficiency and accuracy in toxin analysis and monitoring processes.

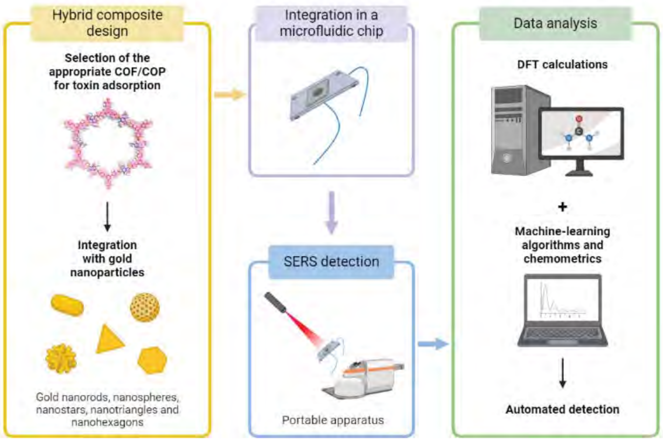

Figure 1: Graphic representation of the system designed for detecting trace levels of marine toxins by SERS.

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Detection of Vaginal Lactobacillus using Surface-Enhanced Raman Spectroscopy

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The vaginal microbiome plays a vital role in maintaining vaginal health and is dominated by *Lactobacillus* species – namely *Lactobacillus crispatus* (*L. crispatus*) and *Lactobacillus iners* (*L. iners*)[1]. Reductions in vaginal *Lactobacillus*, known as vaginal dysbiosis, can lead to numerous complications for women including development of bacterial vaginosis, increased risk of acquiring a sexually transmitted infection, preterm birth, and premature rupture of membranes during pregnancy[2,3]. Additionally, dominance of the microbiome by *L. iners* rather than *L. crispatus* has been linked to dysbiotic conditions, which routinely go undetected using standard methods[4–6]. Despite the importance of the vaginal microbiome, current methods for detecting and monitoring vaginal *Lactobacillus* are lacking. Standard techniques include visual, olfactory, and microscopic investigations, or microorganism culturing, but they all suffer from numerous limitations including subjectivity and variability[7]. Vibrational spectroscopy, such as surface-enhanced Raman spectroscopy (SERS), shows promise for filling the gap in timely detection of alterations in the vaginal microbiome because it is a rapid, label-free, and non-destructive technique[8]. In this study, we hypothesized that SERS can detect key biochemical differences between *L. crispatus* and *L. iners*, discriminate between co-cultures with varying ratios of *L. crispatus* and *L. iners*, and be able to detect *L. crispatus* and *L. iners* in complex mixtures.

L. crispatus and *L. iners* were grown from frozen stocks following standard procedures, washed, and diluted to the physiological concentration found in the vaginal microbiome. To collect SERS spectra of each species, the washed bacteria were mixed with citrate-stabilized gold nanoparticles (AuNPs) (45 nm diameter) at a 1:4 volumetric ratio (bacteria:AuNPs) and droplets of this solution were applied to aluminium foil and allowed to dry. Spectra were collected from the dried samples using a Renishaw inVia Raman microscope with 785 nm excitation. Following spectral collection from each species individually, to determine our ability to detect the bacteria in the co-cultures, AuNPs were added to mixtures of *L. crispatus* and *L. iners,* and SERS spectra were collected. The SERS spectra of *L. crispatus* and *L. iners* reveal key variations in protein, amino acid, and nucleic acid content. The intensity of the 734 cm^{-1} peak representing adenine and bacterial cell wall components was found to be stronger in *L. crispatus*. Furthermore, *L. iners* was found to have an increase in signal from phenylalanine, amide III, and nucleic acids. Using these key peaks for each species, we were able to determine the dominant species when mixed in co-culture at various *L. crispatus*: *L. iners* ratios. The ability to determine the dominant species in co-cultures has significant clinical relevance as the shift from *L. crispatus* to *L. iners* dominated microbiome is a potential indicator of the microbiome entering a dysbiotic state. Finally, the distinct spectral signatures of *L. crispatus* and *L. iners* allow for detection of these species in the presence of confounding factors such as vaginal fluid components or other bacteria. In summary, these results demonstrate that SERS can detect biochemical variation in two similar *Lactobacillus* species, and further determine the dominant species in co-cultures at various bacterial ratios.

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Applications of Multi-Excitation Raman Spectroscopy (MX-Raman) for Rapid Bacterial Identification

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Raman spectroscopy is extensively used in modern biosciences for its excellent optical fingerprinting capabilities, making use of intrinsic vibrational information from chemical bonds to distinguish between targets. However, identification of complex biological targets such as bacteria remains a significant challenge, especially for distinction at the species and strain level.

In previous work we demonstrated how we can exploit the wavelength dependence of the Raman scattering cross-section to distinguish between bacterial strains [1], and between targets in complex media [2,3].

In this presentation we will describe new developments in the use of multi-excitation Raman spectroscopy for rapid identification of wastewater bacteria. We will describe (1) the creation of a spectral library of key functional bacteria that are representative of microbial populations in European wastewater treatment plants, (2) our approaches to data processing and treatment of Raman spectral data to enable effective classification & segmentation to support bacterial identification, and (3) the identification of bacteria from mixed samples. We will discuss perspectives on how wastewater industry partners may feasibly use MX-Raman in their operations for rapid-turnaround bacterial identification, and its potential impact.

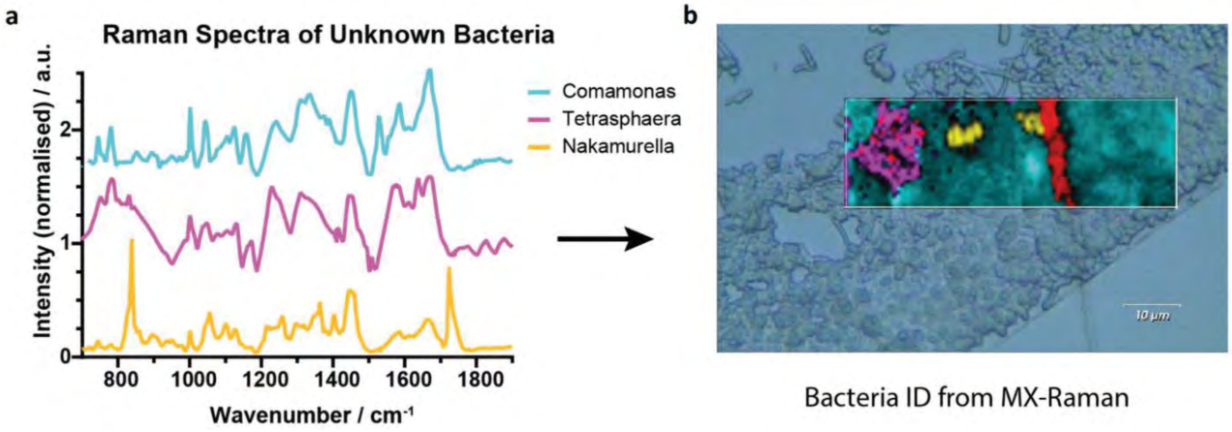

Figure 1 (a) Raman spectra of unknown bacteria are compared to a reference library for identification. (b) Bacteria in a mixed population can be identified using their spectral signature.

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Unlocking Bacterial Health with Raman Marker Analysis: Insights into Membrane Dynamics and Cellular Resilience

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Raman spectroscopy offers a promising avenue for assessing the health status of bacterial cells. Here, we present a protocol designed to harness its efficiency in this regard. Central to our approach is the Raman Marker (RM), derived from the intensity ratio of CH_3 (~2930 cm^{-1}) and CH_2 (~2850 cm^{-1}) symmetric stretching bands [1,2]. This marker proves sensitive to conformational changes in lipidic membranes [3]. Our investigation starts with model membranes, utilizing liposomes, artificial vesicles composed of phospholipid bilayers, to study how lipidic composition affects the phase-transition dynamics of cell membranes. Thus, we designed a temperature-dependent experiment spanning from 22 to 45°C to analyze the modifications of the physical state of different liposomes, where we systematically introduced specific lipid species to mimic complex compositions akin to real systems such as lung cell membranes. This analysis reveals how lipid composition influences phase-transition dynamics, with RM efficiently detecting these modifications, as corroborated by differential scanning calorimetry. Extending our study to real systems, we employ RM to monitor the physical state of four bacterial strains of *Acinetobacter baumannii* (an opportunistic nosocomial pathogen [4]) subjected to dehydration and temperature excursions. Our findings not only showcase RM's capability in detecting phase transitions under such stresses, irrespective of membrane composition, but also highlight the strong correlation of RM with the clonogenic ability of bacterial cells, following a power-law relationship [3]. This correlation underscores the intrinsic link between bacterial physiology and membrane condition, emphasizing the pivotal role that the RM analysis could play in assessing the health status of bacterial cells.

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Detection of Microbial Species using Raman Spectroscopy

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According to Inc42, the Indian agricultural sector is predicted to increase to US$ 24 billion by 2025[1], necessitating advancements in agricultural technologies to sustainably meet rising demands. Soil microbiomes, especially bacteria, play pivotal roles in augmenting crop productivity and quality[2]. Utilizing plant growth-promoting bacteria like Agrobacterium and Bacillus is established for soil fertility enhancement. However, comprehensive knowledge of soil microbiomes is imperative for targeted microbe enrichment and pathogen mitigation. A cost-effective, portable platform for identifying soil bacteria, particularly non-viable ones, is urgently required. Raman spectroscopy, which analyzes light scattering interactions with sample molecules[3], offers a promising solution. Each bacterial species exhibits a distinct Raman spectral signature, enabling species identification without sample extraction or purification. Our study successfully demonstrates characteristic Raman spectra for *B. subtilis, E. coli, and C. vulgaris.* Additionally, we detect and map *C. vulgaris* presence within consortia, even at 10X magnification, showcasing the potential for specific microbe localization. Multivariate statistical analysis further highlights the capability to differentiate Raman spectra based on characteristic peak features or variants among microbes. This research represents a significant advancement in agricultural practices and soil management strategies.

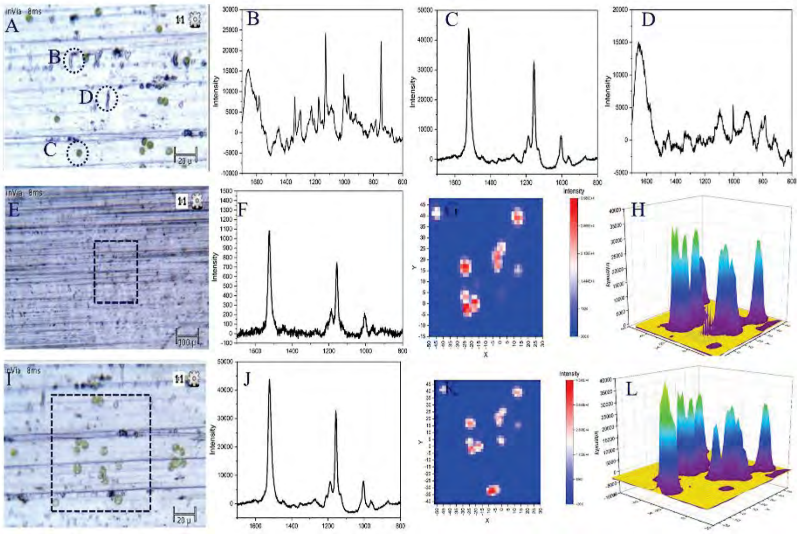

Figure 1: (A) Represents the optical image of consortia of microbes, (B, C, D) Raman spectroscopy at spot 1, 2 and 3 respectively. (E) Represents the 10X mapping view. (F) Raman spectra of C. vulgaris at 10X. (G,H) Mapping image of area. (I) Represents the 50X mapping view. (J) Raman spectra of C. vulgaris at 10x. (K,L) Mapping image of area.

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A Quantitative Chemometric Study of Pharmaceutical Tablet Formulations Using Multi-Spectroscopic Fiber Optic Probes

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Pharmaceutical products are typically composed of an active pharmaceutical ingredient (API) and an excipient which the role is help facilitate the API.[1] Different formulation approaches can impact solid-state properties, particle size, and geometry of pharmaceutical products. This leads to designing various solid dosage forms, suspensions, and controlled release formulations for different specific applications.[2] So, it is important that pharmaceutical manufacturers follow strict regulation and demonstrate compliance to ensure output of consistently fit-for-purpose products. Raman spectroscopy and near-infrared spectroscopy are rapid, non-destructive, and relatively inexpensive methods which can provide complementary information of complex mixtures and systems, with the potential for identification, quantification, classification, and process monitoring of pharmaceutical systems. Our long-term objective is to develop a versatile multi-spectroscopic fibre optic probe with a wide range of uses, including a point-of-care tool for diagnosis of gastrointestinal illnesses such as coeliac disease and cancer. This work is to build upon the proof-of-concept work by Fraser-Miller *et al*. where the capabilities of complementary sensors, namely Raman spectroscopy and near-infrared spectroscopy, were investigated as promising tools for diagnosing coeliac disease within patient biopsies.[3] In this study, the capabilities of custom-made multi-spectroscopic fibre optic probe prototypes were investigated with estimating the hydroxypropyl methylcellulose (HPMC), α-lactose monohydrate (LAC-MH), anatase (a-TD), and γ-indomethacin (γ-IND) content within tablet formulations and evaluated through data fusion approaches and multivariate analysis methods such as principal component analysis (PCA) and partial least squares regression (PLSR).

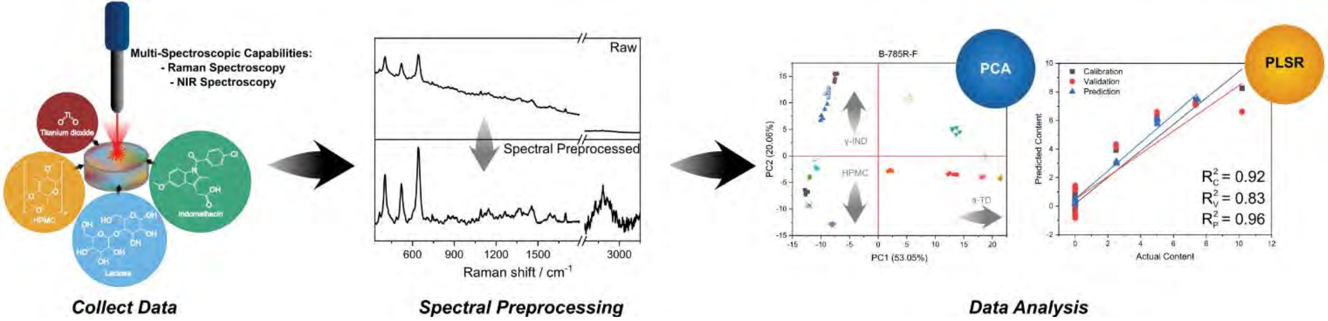

Figure 1: Graphical Abstract of Study

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Improved detection of therapeutic drugs through electrochemically assisted surface-enhanced Raman spectroscopy (EC-SERS): towards therapeutic drug monitoring

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The performance of therapeutic drug monitoring (TDM) to a wide range of drugs in clinical settings can improve the treatment and recovery of patients [1]. However, today drug routine measurement in clinics requires long time, highly trained personnel, and costly instrumentation. Therefore, the development and implementation of fast, robust and easy to operate methods might advance the applicability of TDM for almost all the drugs. Electrochemically assisted Surface-enhanced Raman spectroscopy (EC-SERS) is a powerful technique for improving the sensitive detection of small molecules, like therapeutic drugs [2]. Although, several physicochemical aspects should be understood and controlled in EC-SERS experiments, it provides robust advantages for high sensitivity detection of TDM compounds. In this work we present a modified EC-SERS assay to improve the sensitivity and label-free detection of TDM compounds, using methotrexate (MTX) and lamotrigine (LTG) as model drugs. Our approach consisted of an electrochemical surface modification of the SERS substrate, an ordered Au nanopillar (NP) chip, that helped to bring the therapeutic drugs closer to the surface and thus boosting the vibrational bonds. This approach allowed us to acquire robust SERS mapping of the analyte, reproducible measurements and improve the sensitivity detection (Figure 1). In this method MTX was possible to be detected lower than 0.25 µM, while LTG was detected as low as 0.5 µM in electrolytic media. In conclusion, this approach shows a promising strategy for improving the sensitivity detection of therapeutic drugs under a modified EC-SERS approach that might advance the implementation of TDM point-of-care in clinical use.

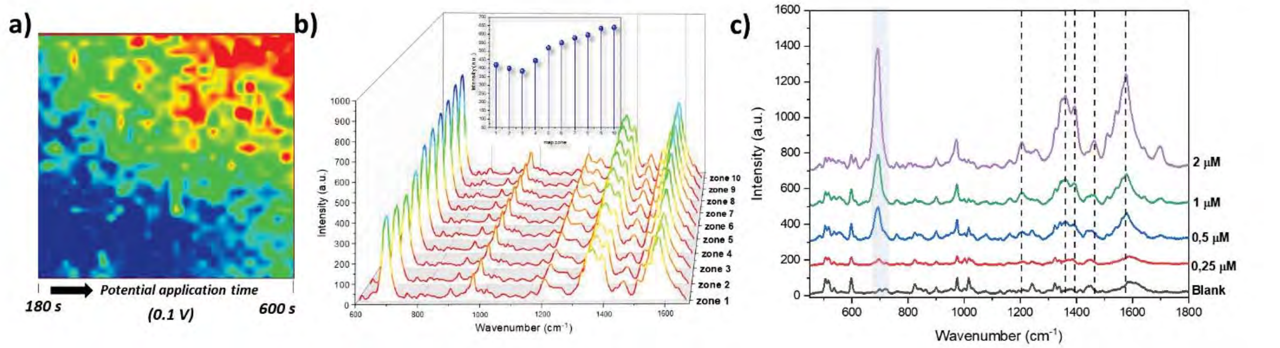

Figure 1: a) Heat map of MTX main band under EC-SERS measurement. b) Spectral distribution of MTX in the SERS map. c) SERS profile of MTX at different concentrations under the modified EC-SERS method.

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Operando Raman studies on functional materials: assessing structure-activity relationships on oxide-based and shaped catalysts

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The term "*operando*" was coined to describe the characterization of a catalyst's performance and relevant chemistry during catalytic operation, to establish structure-activity relationships. [1-3]. This approach is widely used now, and it has become a complex yet efficient junction where spectroscopy, materials science, catalysis, and engineering meet. The *operando* methodology involves using a specialized cell that is suitable for in situ spectroscopy. The *operando* methodology enables researchers to gain insights into the state of the catalyst at work and concomitantly connect how it changes during the reaction with changes in catalytic performance.

The presentation will summarize the study of supported oxides for selective and total oxidation and ammoxidation reactions, the role of additives, support, hydration and reaction conditions on the states of supported catalysts. A particular emphasis is given to the capacity of Raman spectroscopy to assess the state of molecularly dispersed oxides and their interaction with support-stabilized nanoscaled oxides (e.g., V and Sb on gamma-alumina). Furthermore, engineering ceria support from MOF precursors enables modulating the performance through fine-tuning the interaction between the supported active phase (Pt, Au, V) and the support (ceria) [4,5]. of This is applied to assess the molecular basis for activation/deactivation and the nature of the catalyst active site for oxide reduction, alkane oxidative dehydrogenation, ammoxidation and for environmental selective catalytic reduction of NOx and remediation of toluene. I will illustrate how *operando* methodology engages with complementary methodologies, such as computational chemistry, engineering, and other spectroscopies. The transversal nature of the *operando* approach places it at the junction between fundamental catalytic chemistry and applied chemical engineering. An approach to harmonizing Raman spectra is also a new pillar in a broader context under the CHARISMA h2020 project.

Acknowledgments: This work has received funding from the European Union’s Horizon 2020 Research and Innovation Program under Grant Agreement No. 952921 *CHARISMA* and by La CaixaFoundation Health Research project HR21-00813 *SafeAir*.

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Ultrasensitive plasmon-enhanced Raman spectroscopy: from single-molecule detection to *in situ* / *operando* characterizations

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The investigation of physical and chemical processes at the molecular/atomic level plays a crucial role in the development of material sciences. Based on plasmon resonance effect, surface-enhanced vibrational spectroscopy is featured at molecular chemical recognition of surface material and surface adsorbed species with exceptional detection sensitivity, and it has been widely used in the *in situ*/ *operando* spectroelectrochemistry[1].

By coupling plasmonic nanoantennae with metal film, a plasmonic nanocavity substrate with tuneable size was obtained, which allows us to mediate the plasmonic field distribution in the hot spot to achieve an ultrahigh Raman intensity enhancement. With this ultrasensitive plasmonic substrate, a distinct structural transformation and the subsequent photo-induced carbon-carbon bond cleavage of a single rhodamine B molecule were probed in real-time (Fig.1) [2]. Furthermore, using the *in situ*/ *operando* plasmon-enhanced Raman spectroscopy, molecular/atomic structures and physicochemical features of the electrode-electrolyte interface or interphase in the Li-ion electrolytes were revealed [3-4].

This highly sensitive spectroscopic method shows a significant potential for understanding the mechanisms of single-molecule reactions and is expected to have a broad appeal to various scientific fields including chemistry, physics, materials, and energy.

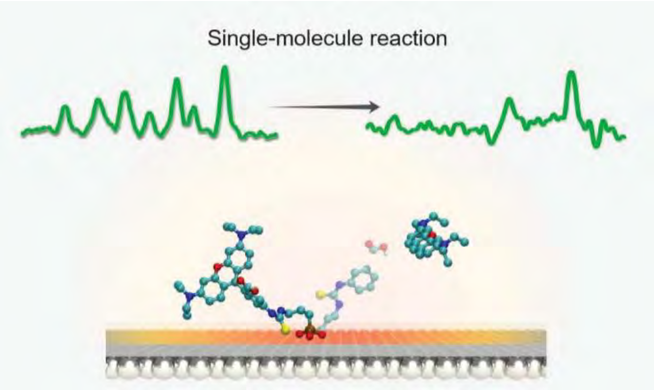

Figure 1: Schematic of ultrasensitive plasmon-enhanced spectroscopy in single-molecule detection

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SERS characterization of atomic level adsorption sites on Pd/Au catalysts during electrocatalytic CO_2 reduction

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Bimetallic catalysts with unique surface structure often show improved catalytic activity and selectivity. Identification of catalytically active sites and reaction intermediates are vital for rational catalyst design, yet difficult to achieve due to the complex atomic-level surface sites.

In this study, in situ surface-enhanced Raman scattering (SERS) was utilized to monitor electrocatalytic carbon dioxide reduction reaction (CO_2RR) on Au@Pd catalysts with atomically well-defined surface structures. SERS is a powerful tool to study the adsorption behavior of surface species in catalytic reactions, providing a highly sensitive chemical identification of trace species. SERS spectra with different adsorption configurations of *CO intermediate were observed. *CO exists in three adsorption configurations (top-site, bridge-site, and hollow-site) on different catalysts surface, including bare Au nanocube, and Pd single atom, Pd sub-monolayer, and Pd monolayer on Au nanocube surface. The signals also show different potential dependence that can be correlated with the electrocatalytic activity. The results provide atomic-level insights into the varied activity of the four kinds of catalysts and will be valuable for developing efficient CO_2RR systems.

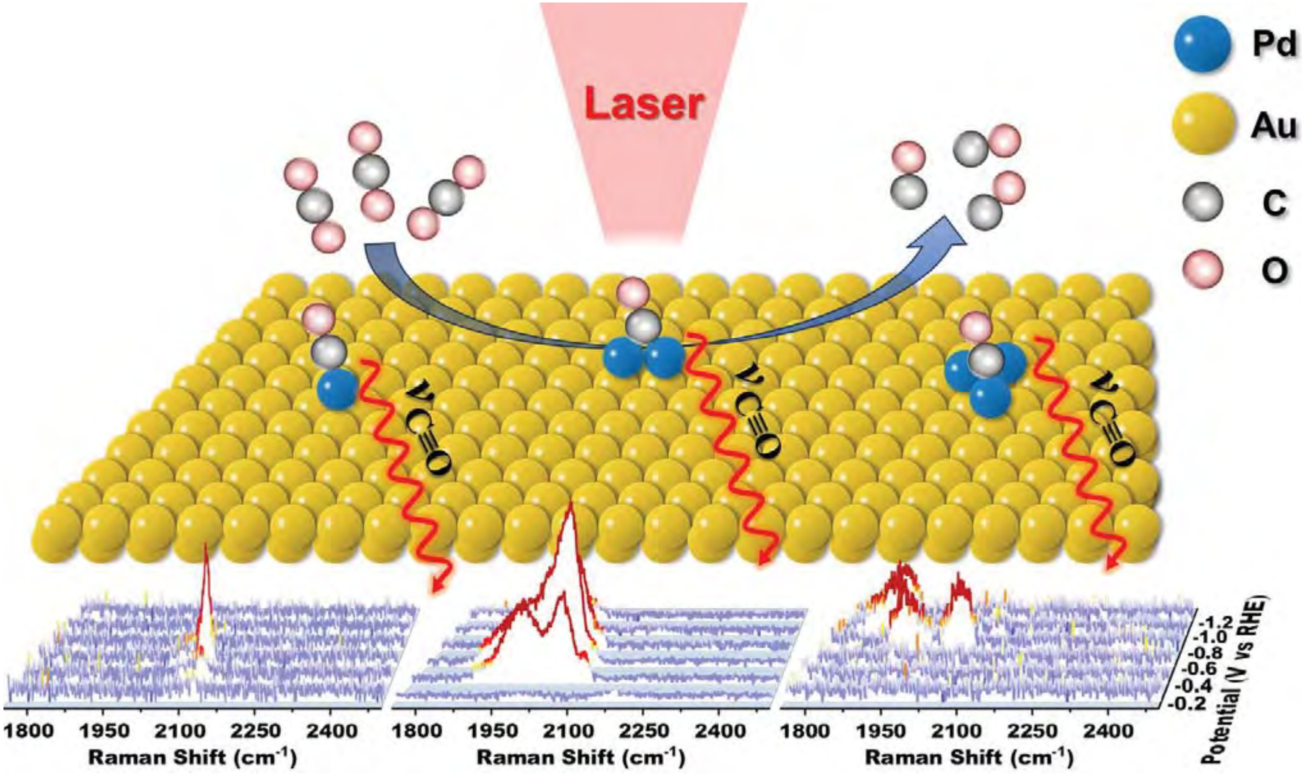

Figure 1: SERS characterization of atomic level adsorption site on Pd/Au during electrocatalytic CO_2RR.

Raman spectroelectrochemistry of IrO_2 and Ir nanoparticles in oxygen evolution reaction

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Oxygen evolution reaction (OER) is known to be the limiting water splitting process in proton exchange membrane water electrolyzers (PEMWE). Under acidic conditions, iridium is recognised as one of the most active and stable electrocatalyst for OER, which has stimulated the investigation of Ir-based materials using various techniques. Raman spectroscopy can be a powerful tool to gain additional insight into OER mechanism and the active side of iridium, which are still under debate [1,2].

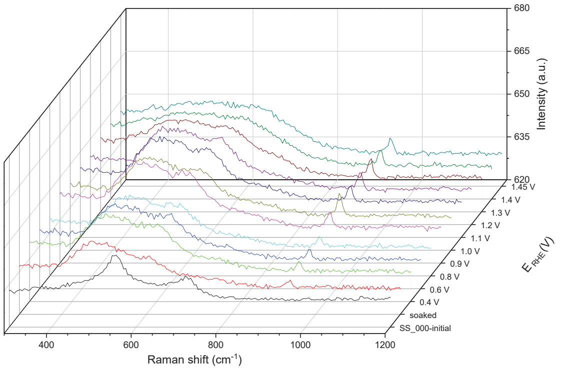

Figure 1: *In situ* Raman spectra of Ir nanoparticles drop-casted on GCE. Electrolyte was 0.1 M $HClO_4$ and measurements were recorded for initial and soaked states, and at potentials from 0.4 to 1.45 V_{RHE}.

In this study, rutile IrO_2 and Ir nanoparticles (Ir NPs) are examined using *ex situ* and *in situ* Raman spectroelectrochemistry, which offers interesting comparisons. Both compounds were deposited on glassy carbon electrode (GCE) and activated in 0.1 M $HClO_4$ electrolyte. Ir NPs show partial oxidation in the as-deposited state, which was observed by EDS. As a result, characteristic Raman bands of IrO_2 [3] were found for both compounds drop-casted on GCE: the E_g mode (552 cm^{-1} for Ir NPs) and the overlapping B_{2g}, A_{1g} modes (724 cm^{-1} for Ir NPs). IrO_2 and Ir NPs were then subjected to three different activation protocols: 0.05 to 1.45 V_{RHE} , 0.05 to 1.6 V_{RHE}, and 1.1 to 1.6 V_{RHE}. The different potential ranges lead to distinct oxidation states of iridium and thus different OER activity. Therefore, information about OER and active sites are expected to be obtained. For example, a broad band feature appeared in the *in situ* Raman spectra of Ir NPs (Figure 1), similar to those reported previously [1,2]. Rutile IrO_2, in contrast, remained in the shape of E_g, B_{2g}, A_{1g} modes in *in situ* Raman spectra. The *ex situ* Raman spectra also provide evidence for perchlorate adsorption, which may affect the reported assignment of *in situ* spectra [1,2]. Nevertheless, further insight into the OER process will be obtained by *in situ* UV-visible spectroelectrochemistry of IrO_2 and Ir NPs drop-casted on electronically conductive FTO glass.

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Rapid morpho-chemical analysis of cancer cells with a turn-key stimulated Raman imaging system

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Niklas Lüpken[a], Sven Dobner[a], Maximilian Brinkmann[a]
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In the field of cancer research, it is crucial to differentiate between healthy and dying cells, as cancer therapies aim to induce apoptosis only in cancer cells while minimizing harm to healthy cells. However, current imaging methods often rely on exogenous labels that can interfere with cellular processes. Hyperspectral Stimulated Raman Imaging (SRS) has shown great promise as a label-free and particularly fast chemical imaging technique, but existing SRS imaging devices suffer from limitations such as bulkiness and restricted spectral tunability.

We present a compact and portable SRS imaging system capable of acquiring multispectral datasets with high chemical specificity by scanning the spectral range of 700-3100 1/cm in just 100 ms, providing a tenfold increase in spectral tunability over state-of-the-art systems. Leveraging a compact and robust fiber laser fully integrated with a standard widefield microscope, our system overcomes the bulkiness, difficult handling, and fragility of existing SRS imaging systems.

Additionally, we present a balanced photodetector to overcome laser excess noise, which is intrinsic to fiber lasers and typically prevents imaging systems from reaching the shot-noise. By measuring laser noise in a reference beam and subtracting it from the SRS signal after the sample, common laser noise is cancelled out. The detector allowed reaching a signal-to-noise ratio of 21 in imaging MCF7 cancer cells with a pixel dwell time of only 5µs. To reach the same SNR with a standard SRS detector, we required a pixel dwell time of 50µs, proving a reduction of imaging time by a factor of 10.

As a proof-of-principle, we applied SRS imaging for monitoring of cell death in MCF7 breast cancer cells. Cell death was induced using the drugs Staurosporine, Actinomycin, and Camptothecin, typically used in preclinical research to induce apoptosis and as lead compounds in drug discovery. SRS enabled clear visualization of morphological changes, e.g., in the shapes of the nucleus and cytoplasm, as well as changes of the spectral fingerprint during cell death.

In conclusion, we demonstrate significant progress in SRS imaging, introducing a reliable and user-friendly approach for rapid chemical-specific imaging, contributing to the advancement of Raman imaging in biomedical research and medical diagnostics.

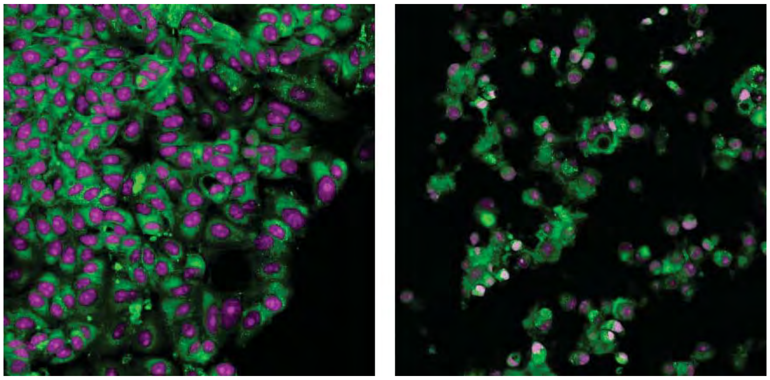

Figure 1: Two-color SRS images of MCF7 cells, taken at 2835 cm^{-1} and 2945 cm^{-1}, showing clear differences in the shapes of the nuclei and cytoplasm before and after treatment with Staurosporine.

Correlative nano-FTIR and AFM-Raman spectroscopy

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scattering-type Scanning Near-field Optical Microscopy (s-SNOM) is a known approach to optical microscopy and spectroscopy bypassing the ubiquitous diffraction limit of light to achieve a spatial resolution below 10nm. s-SNOM employs the strong confinement of light at the apex of a sharp metallic AFM tip to create a nanoscale optical hot-spot. Analysing the elastically scattered light from underneath the tip interferometrically enables the extraction of the optical properties (dielectric function) of the sample directly below the tip and yields nanoscale resolved amplitude and phase images simultaneous to topography [1].

Applying a broadband mid-IR light-source, this microscope enables nano-FTIR spectroscopy, sensitive enough to record spectra from molecular monolayers and individual macromolecules such as proteins (Fig. 1) [2]. Even measurements in liquid environment can be conducted and present a new label-free microscopy method to identify proteins, lipids, tyrosines, etc. in living cells [3].

Separating the collected light by the elastically scattered light and the inelastically scattered light, allows for correlation of s-SNOM data and AFM-Raman data at the same sample location [4].

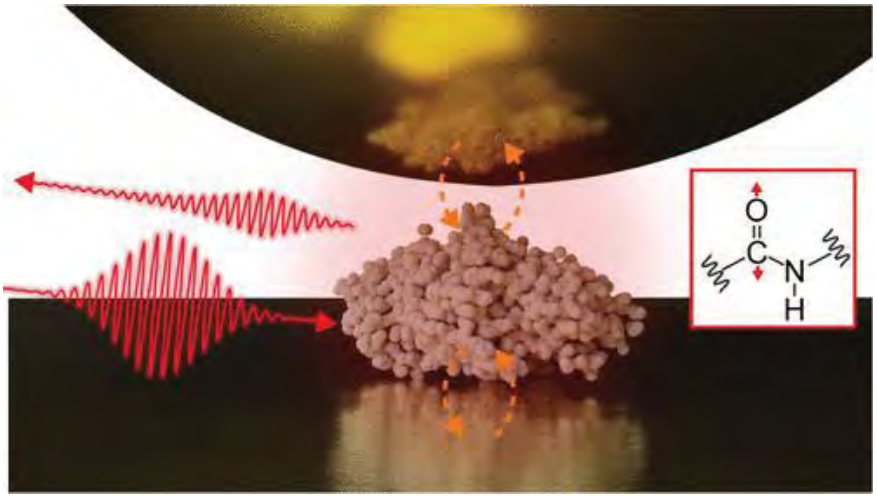

Figure 1: nano-FTIR spectroscopy of a single protein consisting of ~500 amino acid residues [2]

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Laser-induced nano structuring for SERS of Extracellular Vesicles

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Extracellular vesicles (EVs) are micro and nanoscale packages produced by all cells, available in all body fluids, and their content reflects the cell of origin. This makes them valuable biomarkers making the diagnosis of many diseases possible [1]. Raman Spectroscopy (RS) is a label-free tool able to identify molecules using the inelastic scattering of laser light. Compared to RS, Surface-Enhanced RS (SERS) can be 10^6 times more sensitive due to the nanometric patterning of the surface. This positions SERS as an ideal tool to analyse EVs, even if in very low concentrations.

In this work we propose LIPSS (Laser-induced periodic surface structures) as SERS substrates [2]. This is a laser machining technique successfully used by our group for the detection of EVs in preeclamptic patients [3], preeclampsia being a leading cause of mortality in pregnant women worldwide. Nano-structuring of steel wafers by ultrashort laser pulses is achieved and optimized with the aim of achieving the highest SERS signal enhancement, testing different laser parameters such as power and fluence, as well as the thickness of the metal layer used as SERS-active surface. Alternative pillar-like structures are also investigated, obtained by changing the writing beam polarization from linear to circular and elliptic. The enhanced SERS sensitivity of the structures will allow for smaller concentrations of EVs to be detected and assessment of the healthiness of placenta and embryo cells.

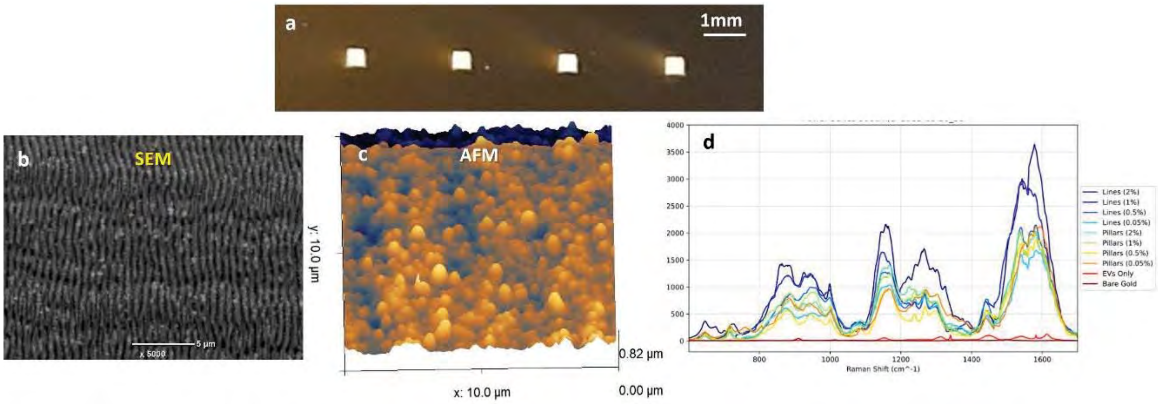

Figure 1: (a-c) LIPSS on a gold coated steel wafer with different magnification. a) picture of the gratings (500µm each), (b) SEM and (c) AFM image of the structures. (d) Raman spectra of EVs from placenta cells on different structures.

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Thousand foci CARS microscopy

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Coherent Raman scattering (CRS) is a label-free method which provides chemical specificity and allows real-time imaging of the sample [1]. The excitation of the sample is typically a single focus which is raster-scanned, allowing for non-descanned signal detection by a single channel detector but limiting imaging speed. Using wide field excitation instead allows rapid imaging but requires much higher pulse energies, does not have sectioning capability, is plagued with coherent interference artefacts, and uses imaging in detection, limiting its applicability to very thin samples. Recently the use of random illumination, generating a speckle wide field excitation and analysing the signal fluctuations, recovered *z* sectioning capability but still relies only on imaging in detection and requires many frames to reduce speckle noise [2].

We present a multifocal [3] coherent anti-Stokes Raman scattering (CARS) approach using 1000 foci which are raster-scanned over 10x10 to 50x50 points. The signal from each focus is isolated in detection imaging and fed into an image reconstruction algorithm. To provide sufficient pulse energy for each focus we used an amplified oscillator together with an optical parametric amplifier with µJ pulse energy and 200 kHz repetition rate, similar to [2]. This approach allows rapid sample imaging with sectioning, while relaxing requirements on detection imaging which only needs to distinguish the signals from the different foci separated by about 20 times the optical resolution. Fig.1 shows example data.

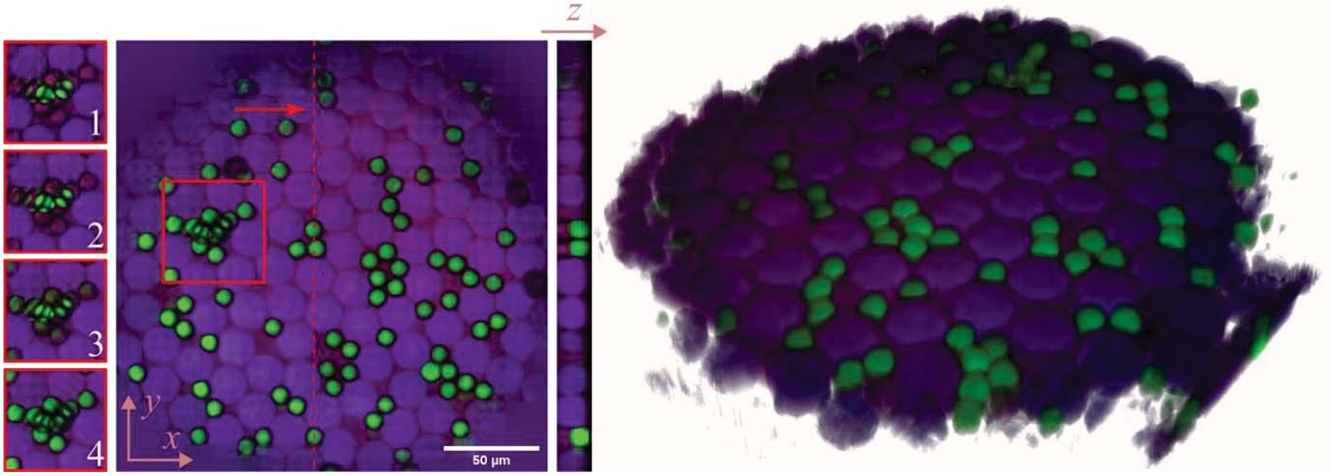

Fig. 1: Single lateral field of view using 20x20 points and 10 z sections. Left: CARS image of PS (10 µm, green) at 3050 cm^{-1} and PMMA (20 µm, blue) beads at 2950 cm^{-1} embedded in oil. The y-z section is taken at the dotted red line. Insets 1-4 are taken with 2 µm *z* axis increments. Right: a 3D render of the data.

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High-rejection and common-path birefringent filter for Brillouin microscopy in turbid media

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Brillouin microscopy represents a promising all-optical and label-free technique for evaluating the 3D mechanical properties of biological specimens [1,2]. However, detecting the Brillouin spectrum remains challenging primarily due to the overwhelming Rayleigh peak, which in biological samples can be more than a million times stronger than the Brillouin peaks in biological samples. Various approaches, including diffraction masks [3], apodization [4,5], absorption cells [6], and dark-field configurations [7], have been explored to suppress the intense Rayleigh peak. Despite offering improved spectral visibility, these methods often introduce system instability, complexity, and reduced throughput efficiency due to multiple optical paths.

Here, we present a method to suppress the strong elastic Rayleigh signal by altering the polarization state of Brillouin scattered light relative to the pump signal. Our common-path Birefringence-Induced Phase Delay (BIPD) filter achieves an unparalleled extinction ratio of up to 65 dB in a single pass. By integrating the filter into a custom multimodal Brillouin-Raman microscope, we demonstrate its efficacy by acquiring Brillouin spectra in highly turbid materials, such as in pure milk (Figure 1). Furthermore, we obtain Brillouin and Raman maps of vertebral bone tissues, demonstrating the filter's ability to enable mechanical investigations of highly scattering samples previously inaccessible using standard spectrometers.

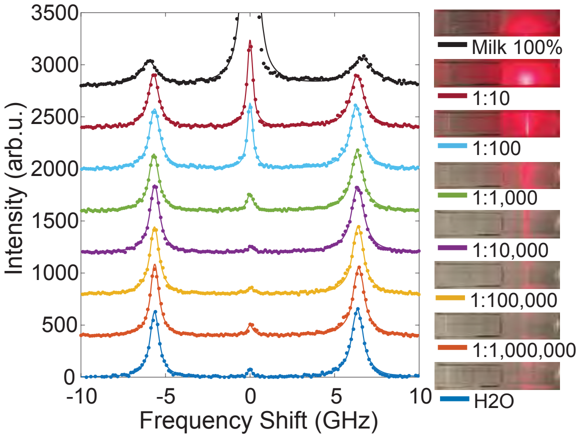

Figure 1 Brillouin light spectra of water-milk solutions at different concentrations. The high suppression capability of the BIPD filter enables the detection of the Brillouin peaks in the depth of pure milk.

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WISER: Wide-field Imaging with Super-resolution Enabled by Raman Signals

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Raman signals, enabling label-free imaging, have significantly impacted various research fields, including advanced materials and biological systems. However, its effectiveness is currently hindered by slow speed and limited imaging area, necessitating point-by-point confocal imaging using a high numerical aperture lens. While novel techniques like tip-enhanced Raman spectroscopy (TERS) have enhanced spatial resolution, they suffer from smaller imaging areas, probe vulnerability, and prolonged image acquisition times. Additionally, the technical expertise required and the cost of TERS instrumentation limit its applicability in demanding areas like high-throughput in-line applications.

We introduce a novel and cost-effective method that addresses some of these challenges by employing wide-field structured illumination combined with hyperspectral imaging. This approach achieves a 28% improvement in spatial resolution without compromising the speed of confocal imaging. Utilising a structured light generator, an imaging interferometer, and a standard microscope similar to a structured illumination setup, our method offers promising results for wider applications.

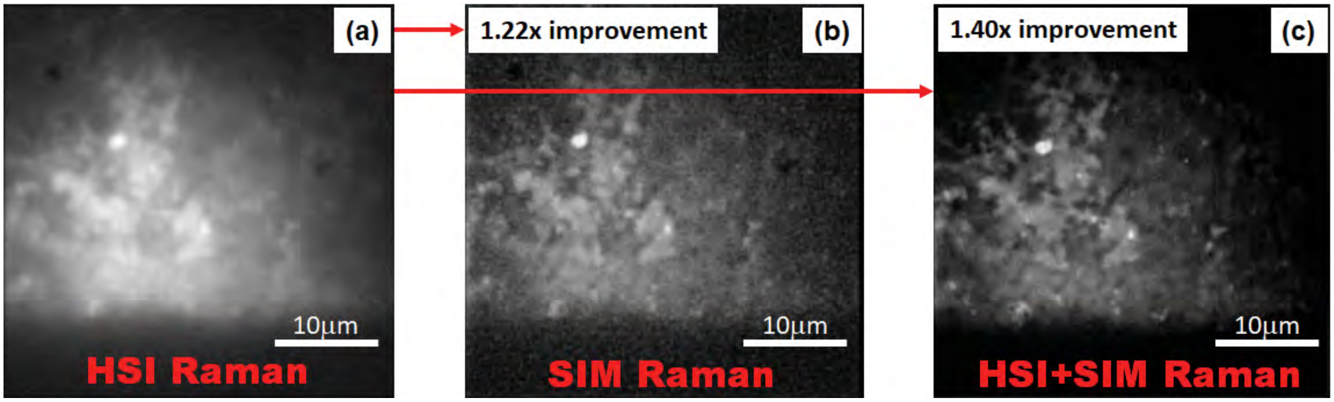

Figure-1: Example measurements of wide-field Raman imaging of nano-diamond containing N-V centres: (a) wide-field hyperspectral image, (b) structured illumination Raman image using a bandpass filter for nanodiamond signal, and (c) image acquired using WISER method.

It's important to note that the current findings demonstrate using spontaneous Raman signals, which are inherently low for many materials. However, this method can be adapted with other enhancement techniques such as resonance Raman.

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Dive right in! Molecular insights into electrochemical surface science.

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Surface scientists have become increasingly aware of the importance of expanding the research focus from classical UHV systems to electrochemical settings that adequately represent realistic working conditions of applications based on solid/liquid reactive interfaces, such as (electro)catalysis or energy conversion schemes. Gathering atomistic understanding about the complex interrelation between charge transfer, chemical conversion and interfacial potential in the present of a solvent is one of the most imminent challenges that surface scientists working with solid/liquid interfaces are facing.

Great efforts are being devoted to the development of novel methodologies to elucidate interfacial electrochemistry mechanisms on the molecular scale. In my talk, I will highlight recent advances to achieve *operando* plasmon-supported Raman nanoscopy coupled with break-junction experiments.[1-3] This approach allows us to gain unprecedented insights into the correlation of chemical, topographic and electronic properties of individual reactive sites at solid/liquid interfaces, such as catalyst structure or (bio)molecular switches in electrolyte under potential control.

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Coupling optical tweezers with tip-enhanced Raman spectroscopy for the investigation of individual supramolecular systems

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Quantitative information on the dynamics and mechanistic principles behind supramolecular systems can be investigated in the single-molecule regime by measuring the nanometer displacements resulting from the application of picoNewton forces in optical trapping (OT) experiments. For example, the mechanical strength of non-covalent interactions can be quantified when studying the reversible breaking/formation of hydrogen bonds on individual host-guest systems or the switching of a macrocycle between binding stations in a molecular shuttle.[1],[2] However, the fundamental chemical mechanisms behind the obtained real-time operational kinetics are not accessible with OT experiments and fundamental questions about the physicochemical processes underlying real-operation remain unanswered. Tip-enhanced Raman spectroscopy (TERS) is a powerful nearfield-based technique providing chemical characterization with single molecule sensitivity and few-nm spatial resolution. The combination of OT-optical force microscopy with TERS would give access to the underlying physicochemical processes triggering specific shuttling events in molecular motors and other types of supramolecular systems.

In this perspective talk I will introduce a hybrid setup being developed at IMDEA nanociencia that merges OT for single-molecule optical force microscopy experiments with TERS. We aim to create a hybrid tool that can disentangle the relation between mechanical, conformational and chemical properties of individual synthetic supramolecular systems and the non-covalent interactions governing their behavior in situ and under physiologically relevant working conditions.

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Visualizing the structural evolution of individual active sites of MoS_2 during hydrogen evolution reactions

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Understanding the structural evolution of individual active sites during the reaction is a longstanding target in surface science, energy science, and catalysis. Nanoscale active sites with specific geometric and electronic structures govern the catalytic performance of electrocatalysts. However, it is still challenging to in-situ precisely characterize the intrinsic nature and evolution of the active site, because the active site is too small for characterization techniques to decipher the local properties, and the realistic conditions are still challenging for theoretical simulations to describe the reaction environment properly. Here, we use electrochemical tip-enhanced Raman spectroscopy (EC-TERS) [1, 2] to in situ monitor the geometric and electronic evolution of individual active sites of MoS_2 during hydrogen evolution reaction (HER). Reconstruction regions of 40 nm with varied lattice and electron density from the active edge site to the pristine MoS_2 were observed during HER. We further revealed the progressive generation of active sites during the electrochemical activation and reaction processes. The synergetic reconstruction around active edges because of the lattice deformation reduces the activation energy barriers and promotes the electrocatalytic reaction. These discoveries offer new insights into our understanding of the active site and its dynamics during electrocatalytic processes. The deep understanding of the active site opens up promising ways toward the rational design of electrocatalysts.

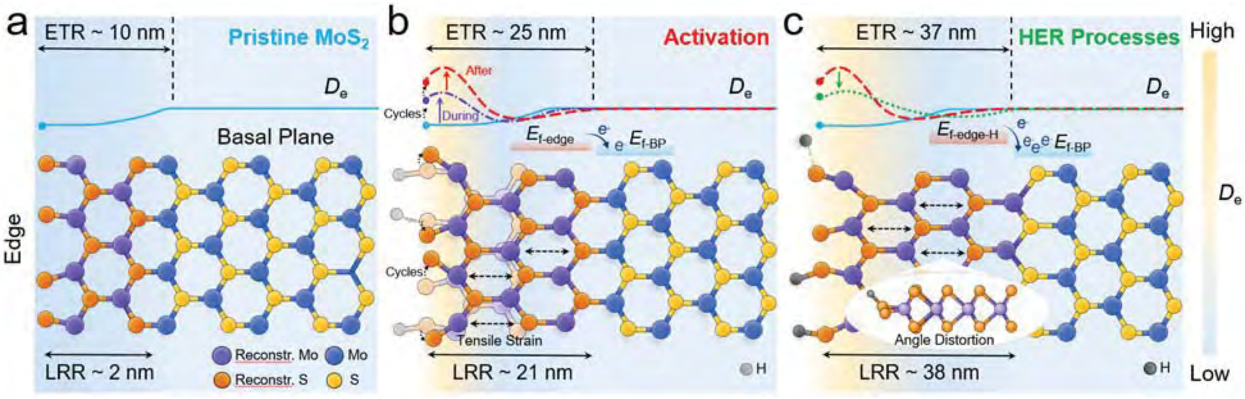

Figure 1: Structural evolution of the active edge site in atomically thin MoS2 on different states

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Electronic interference and Berry-phase acquisition effects in the wave-packet dynamics of an excitation-transfer trimer

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My talk will summarize several interesting features in the dynamics of equilateral electronic excitation-transfer (EET) trimers with distance-dependent excitation-transfer couplings. In the absence of electronic-vibrational coupling, symmetric and antisymmetric superpositions of two single-monomer excitations exhibit purely constructive and purely destructive interference in the EET to the third monomer, respectively. In the former case, transfer is modulated by motion in the symmetrical framework mode. Dynamical distortions in the shape of the framework degrade coherent EET in the former instance, while activating transfer in the latter case. In its symmetrical configuration, two of the three single-exciton states of the trimer are degenerate. This degeneracy is broken by the Jahn–Teller-active framework distortions. I'll show wave-packet dynamics calculations illustrating closed, nearly circular pseudo-rotation on both lower and upper adiabatic surfaces of the degenerate manifold leading to the acquisition of physically meaningful geometric (Berry) phases. Another manifestation of Berry-phase development is seen in the evolution of the vibrational probability density of a wave packet on the lower Jahn–Teller potential comprising a superposition of clockwise and counterclockwise circular motions. Circular pseudo-rotation on the upper cone will be shown to stabilize the corresponding adiabatic electronic state against internal conversion. Strategies for observing EET-interference effects via pump-probe anisotropy measurement on a proposed tetracence tripod will be described. Finally, I'll advocate the further exploration of the sequential pre-resonant impulsive stimulated Raman methods needed to initiate the large amplitude coherent pseudo-rotational motion required for spectroscopic observation of Berry-phase acquisition in this type of EET trimer.

Raman spectrum of the perpendicular phantom state in cis-trans photoisomerization

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Trans-cis photoisomerization is one of the most fundamental photochemical reactions and plays essential roles in nature to drive various biological functions as well as in designing artificial light-energy conversion systems and molecular switches. This prototypical photochemical reaction has generally been described by a model in which the reaction proceeds via a common intermediate having a perpendicular conformation around the rotating CC bond, irrespective of from which isomer the reaction starts. However, such an intermediate was not identified unambiguously, so it was often called the “phantom” state. In this study, we succeeded in the structural identification of the common, perpendicular intermediate of stilbene photoisomerization based on its Raman spectrum. To capture the phantom state with clear structural evidence, we introduced two methyl groups to the central ethylenic part of stilbene, which significantly accelerates the photoisomerization from the trans side. Consequently, the photoisomerization processes from both cis and trans proceed on the ultrafast time scale, which can populate a sufficient amount of the perpendicular state to be observed. Second, we employed ultraviolet FSRS [1] to realize resonance with the transient absorption that had been proposed due to the perpendicular state [2]. The results revealed ultrafast birth and decay of an identical, short-lived transient that exhibits a vibrational signature characteristic of the perpendicular state upon photoexcitation of the trans and cis forms. Complementary ab initio molecular dynamics simulations provided a consistent view that the photoexcited trans and cis forms are relaxed to the ground state through the conical intersection near the perpendicular intermediate. This study fills the last piece of this textbook photochemical reaction [3].

Figure 1: Perpendicular phantom state in cis-trans isomerization

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Tracking the Ultrafast Interplay between Excited State Proton Transfer and Chromophore Ring Twists with Tunable Femtosecond Stimulated Raman Spectroscopy

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The interplay between excited-state proton transfer (ESPT) and ring twisting (RT) motions of organic chromophores on ultrafast/microscopic timescales intrinsically governs macroscopic functionalities of many fluorescent proteins (FPs) as bioprobes and ion sensors in bioimaging and super-resolution microscopy. We have recently investigated the roles of these two processes in various FPs and model chromophores using femtosecond transient absorption (fs-TA) and femtosecond stimulated Raman spectroscopy (FSRS). We showed that a comparable competition of ESPT and RT controls the ratiometric fluorescence of phiYFP, a standalone yellow FP chloride sensor [1], and REX-GECO1, a green-red emission and excitation ratiometric FP calcium sensor [2,3]. In another photoswitchable cyan FP, mTFP0.7, ESPT was found to prevail for the initial dark chromophore, followed by RT in the excited state, leading to the final bright state. At a more fundamental level, we delineated a kinetic competition between ESPT and RT for the cationic GFP chromophores in solution by systematically tuning the photoacidity and RT rate via structural modifications [4].

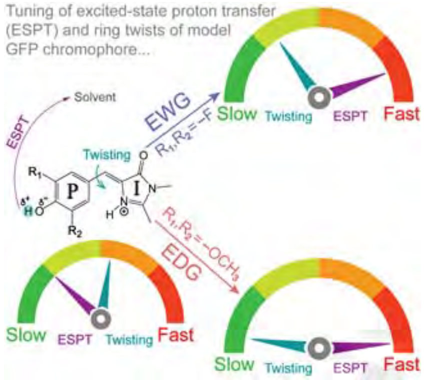

Figure 1: Tuning of ESPT and ring twists of model GFP chromophores with site-specifically incorporated substituents can be guided by an experimental toolset of fs-TA and wavelength-tunable FSRS, aided by quantum calculations, which reveal the bifurcated excited-state pathways for efficient energy relaxation.

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Ultrafast Full-spectral Raman Imaging with MHz Spectral Rate and Single Photon Sensitivity

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Raman has been dramatically advanced in various aspects over the last decades, however the tradeoff among sensitivity, spectral speed, and spectral range is not well solved so far. Here, we develope photoncounting Raman spectroscopy that features a major advantage in non-repetitive single-shot spectra measurement at MHz spectral rate, covering full Raman span (-300-4300 cm-1) covering fingerprint, silent, C-H, and O-H region, simultaneously. Furthermore, we demonstrate full-spectral Raman imaging of metabolic activity of glucose in an intact Caenorhabditis elegans. The technology also exhibits superior performance in fluorescence suppression, and will shine light on a variety of cutting-edge biochemical applications.

Tracking NO-dependent changes of the cytochrome c oxidation state in endothelial cells by resonance Raman imaging

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Cytochrome c (CytC) is a heme protein crucial for life-supporting ATP synthesis. Moreover, it's also responsible for triggering apoptosis. [1] These functions are determined by the oxidation state of the heme-bound iron ion. During the electron transport, free interconversion between ferrous (Fe^{II}) and ferric (Fe^{III}) CytC remains detrimental. In turn, an increase in ferric CytC and its release from mitochondria to the cytoplasm is a necessary step in launching the cascade of events towards cell death. Therefore, tracking the oxidation state of CytC remains an important aspect of cellular physiology.

Herein, methodology based on resonance Raman imaging using 405 nm excitation wavelength was uniquely designed and applied to observe ferrous-to-ferric transition in endothelial cells (ECs). Oxidation of CytC was evoked by the interaction with NO produced by ECs stimulated with calcium ionophore. The observed effect was blocked by L-NAME, a known inhibitor of NO synthases.[2] As further inquired, NO-dependent change in oxidation state may be connected with peroxynitrite activity, being a result of superoxide reaction with NO.[3]

Resonance Raman Imaging allowed for qualitative assessment of a ferrous to ferric CytC ratio in a label-free manner. The applied methodology provided simultaneous spatial characterization and differentiation of ferrous and ferric heme species in live endothelial cells. An exemplary image of a cell showing differentiation between ferrous and ferric CytC is presented in Figure 1.

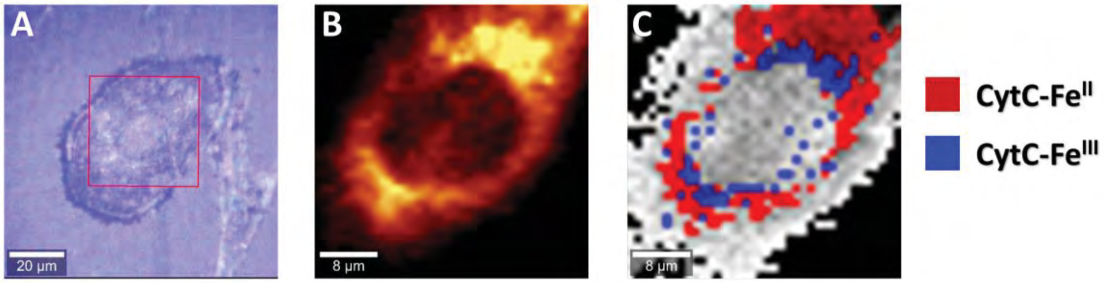

Figure 1: A) visible image of a single EC; B) Resonance Raman image acquired with 405 nm excitation wavelength with distribution of CytC; C) k-means cluster image showing differentiation between ferrous (CytC-Fe^{II}) and ferric (CytC-Fe^{III}) cytochrome C.

Acknowledgements:

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High-throughput cellular imaging with reduced out-of-focus background in multiline illumination Raman microscopy

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Raman microscopy provides label-free visualization of molecular distributions of biological specimens. We have recently developed a line-illumination Raman microscope with multislit detection, named multiline illumination Raman microscope, that enabled simultaneous spatial sampling of 10000-20000 Raman spectra in the wavenumber range of 300 cm^{-1} [1]. However, the background signal from out-of-focus plane caused by overlapping laser beams prevents further improvement of throughput in cellular imaging using multiline illumination Raman microscopy.

In this research, we aimed to improve the throughput of multiline Raman imaging by reducing the out-of-focus background mainly produced by water from the sample buffer solution and the objective immersion medium. We suppressed Raman scattering of sample buffer solution by reducing the thickness of the water layer in the sample by sandwiching it by two coverslips with a 20 μm thick spacer. Additionally, we used heavy water for the objective immersion medium to remove the water background. By combining these two methods, we confirmed the Raman scattering from water was reduced by more than 45% in imaging of living human cancer cells at the high-wavenumber region (2700-3100 cm^{-1}), realizing a 2.1-fold improved imaging throughput using the CH stretching modes with keeping the same SNR. We also theoretically investigated the effect of out-of-focus background reduction with different numbers of illumination lines.

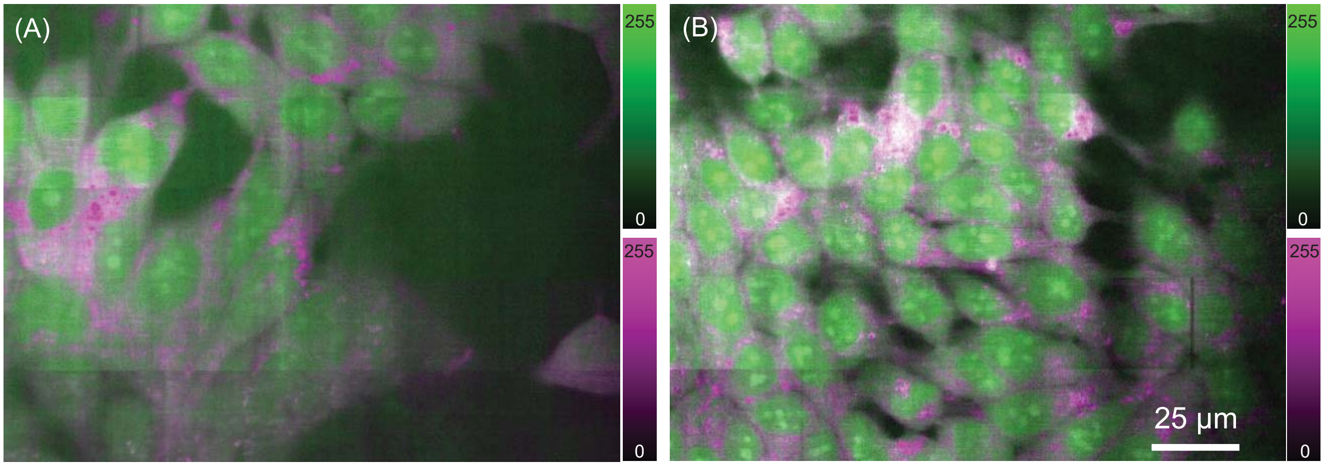

Figure 1: Reconstructed Raman images of HeLa cells without (A) and with(B) the background reduction methods. Green and magenta in the image show the distribution of Raman intensity at 2854 and 2937 cm^{-1}, which can be assigned to lipids and proteins, respectively.

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Molecular indicators of cellular interactions in the blood-brain barrier: 2D and 3D models

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As the central nervous system is limited by the lack of the appropriate models mimicking the stress and drug transport to the brain, there is an ongoing need to develop functional in vitro systems of the blood-brain barrier (BBB) and tools to investigate them beyond currently used bioimaging modalities. Here, we cultured the key cells of the human BBB at 2D and 3D spatial architecture to observe step-by-step interactions between these cells, including a progenitor cell therapy (Fig. 1). We applied Raman and FTIR imaging to observe molecular alternations in the cellular compartments and the whole cellular interior, respectively. We aimed to establish indicators of these interactions to enable fast and label-free detection of the BBB stress (hypoxia, toxicity) and its response to the therapy. The analysis of Raman images indicated that the brain endothelium is the most sensitive to environmental changes and Raman signals of cytochromes located around the nucleus and lipids accumulated in intracellular bodies (LDs) are the most pronounced indicators of cell-cell interactions. For example, fatty acids in LDs became saturated in the progenitor–endothelium co-culture and were formed due to the first physical contact in the endothelium-astrocyte interactions (Fig. 1). In turn, IR imaging recognized the cellular diversity in the 3D architecture of BBB due to conformational changes in proteins toward β-sheets and the presence of A- and B-DNA forms (Fig. 1).

Our work showed the proof of principles that Raman and IR spectroscopy recognize the cells of various phenotypes and their direct interactions. The spectral datasets combined with unsupervised multivariate analysis and prediction models identify the cell communication and junctions.

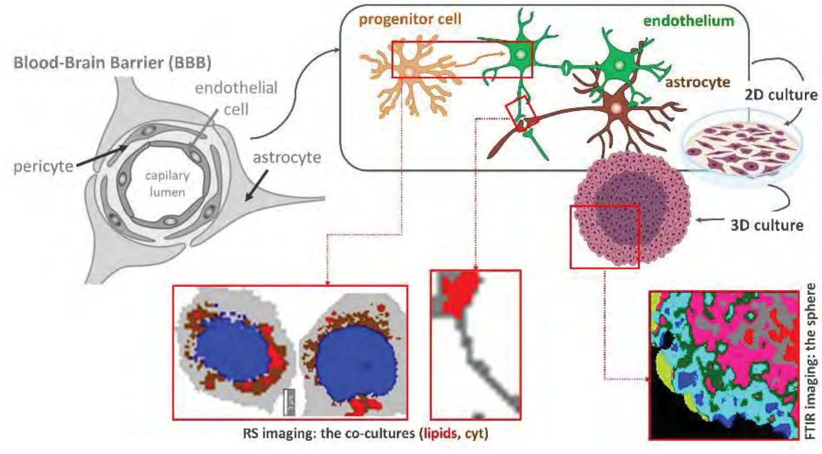

Figure 1: A workflow of the in vitro spectroscopic investigations and exemplary CA maps of RS and IR images.

Acknowledgments

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Probing of Diffusely Scattering Media using Spatially Offset Raman Spectroscopy (SORS)

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This presentation will provide an overview of recent developments in the area of subsurface probing of diffusely scattering (turbid) samples using Spatially Offset Raman Spectroscopy (SORS). SORS is an approach in which the illumination and collection areas on sample surface are mutually displaced by spatial offset Δs (see Fig. 1). This enables deeper sensing than possible with conventional Raman spectroscopy where the illumination and collection zones are overlapped. SORS facilitates the non-invasive interrogation of turbid samples such as biological tissues, opaque containers (paper/plastics/glass) and pharmaceutical powder formulations (tablets/capsules) at depths typically up to several mm's to cm's [1,2,3]. The method yields chemical information on the inner content of materials or containers as well as it enables to characterise their physical nature (eg temperature).

The talk will cover the description of underlying physical phenomena, emerging SORS variants and applications including disease diagnosis and security screening.

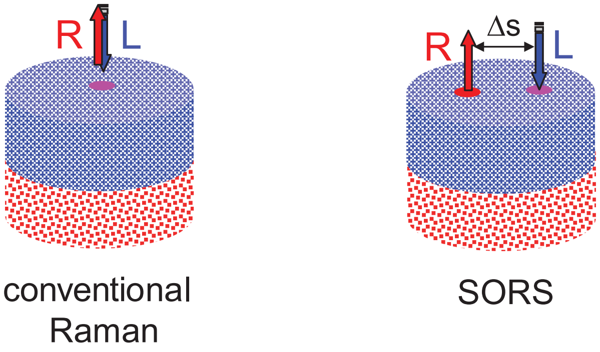

Figure 1: Schematics of conventional Raman and SORS sampling configurations.

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Surface-Enhanced Spatially Offset Raman Spectroscopy: Neurochemical Sensing for Human Health Monitoring

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Human health monitoring is a rapidly developing field, with emerging technologies in point of care, wearable, and electrochemical sensors. Both non-invasive and minimally invasive sensors have been developed that can monitor human health conditions through the skin, and in non-invasively collected biofluids such as urine, saliva, tear fluid, and sweat. While there has been considerable progress made, challenges remain in measuring chemical information from subsurface layers in a multi-layer vertebrate system. Raman spectroscopy combined with multivariate analysis provides a unique solution to this challenging problem, particularly through the combination of surface-enhanced Raman spectroscopy, which allows for highly specific detection at ultralow concentrations, with spatially offset Raman spectroscopy, which allows for targeting subsurface layers (SESORS). We will discuss recent progress in development of SESORS for human health monitoring, specifically towards neurochemical sensing with SESORS [1-2].

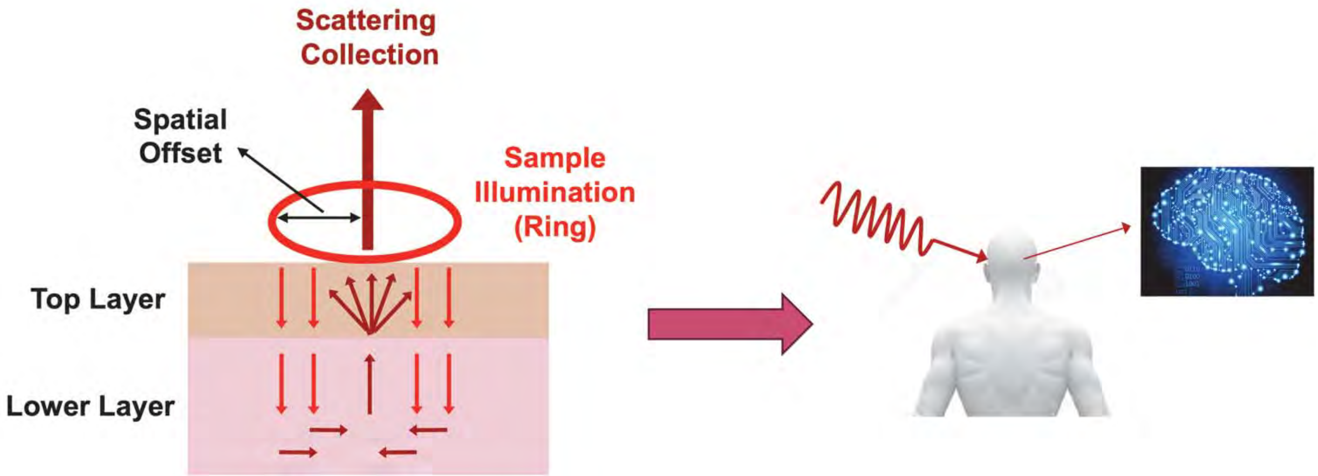

Figure 1: Schematic of the mechanism of SORS (left) for human neurological health monitoring (right).

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Raman spectroscopy is being widely applied in the world and beyond due to its incredible versatility, it is non-invasive, non-destructive and provides a highly detailed chemical probe. Raman is routinely used for pharmaceutical analysis, security purposes, art heritage applications and has even made it all the way to Mars.

Raman has also gained much interest in recent years for its potential application within *in vivo* medical diagnosis. This is especially driven by advances in “deep Raman”, where large volumes can be probed using spatially offset Raman spectroscopy (SORS) or transmission Raman spectroscopy (TRS) approaches. When these approaches are coupled with nanomaterials i.e. surface enhanced Raman spectroscopy (SERS), this provides a set of tools that can rapidly probe the chemical environment in real time in a multiplex fashion i.e. informing on concentration, pH, redox and importantly the biodistribution.

Understanding biodistribution of nanomedicines is animal models is of critical importance, especially when significant amounts of material is delivered off target and only small fractions accumulate in a target location. Here we discuss the latest work and limitations in developing transmission Raman spectroscopy as a tool for rapidly monitoring the biodistribution of Raman nanotheranostics (RaNT) nano-constructs in whole animals and exploring live kinetics. This work is corroborated with secondary techniques, including micro-CT and ICP-MS, to answer the question how quantitative can Raman be?

Applications of Femtosecond Coherent Anti-Stokes Raman Scattering (CARS) for High-Pressure Combustion Measurements

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The application of chirped-probe-pulse (CPP) femtosecond (fs) coherent anti-Stokes Raman scattering (CARS) spectroscopy for measurements in high-pressure combustion systems is discussed. In particular, the application of fs CPP CARS for measurements in a rocket combustion test rig is described. The fuel and oxidizer for these rocket propulsion tests were hydrogen and oxygen, respectively, and temperature was determined from CPP fs CARS of diatomic hydrogen. The molecule CO_2 is of interest for measurements in methane/oxygen combustion, for example. The fs CARS spectroscopy of H_2 [1] and CO_2 is discussed in detail [2]. A high-temperature (up to 1000 K) and high-pressure (up to 70 bar) gas cell was constructed and measurements of both H_2 and CO_2 were performed. The effects of chirp and self-phase modulation are especially severe for the H_2 spectrum due to the wide range of Raman frequencies for the H_2 vibration-rotation transitions. The effects of collisional narrowing on the spectrum of CO_2 were modeled and the procedures for incorporating collisional narrowing effects in the fs CARS spectral models are discussed [2]. The application of broadband dual-pump CARS with nanosecond lasers for highly accurate temperature measurements (to within 1%) in counterflow flames is also discussed [3].

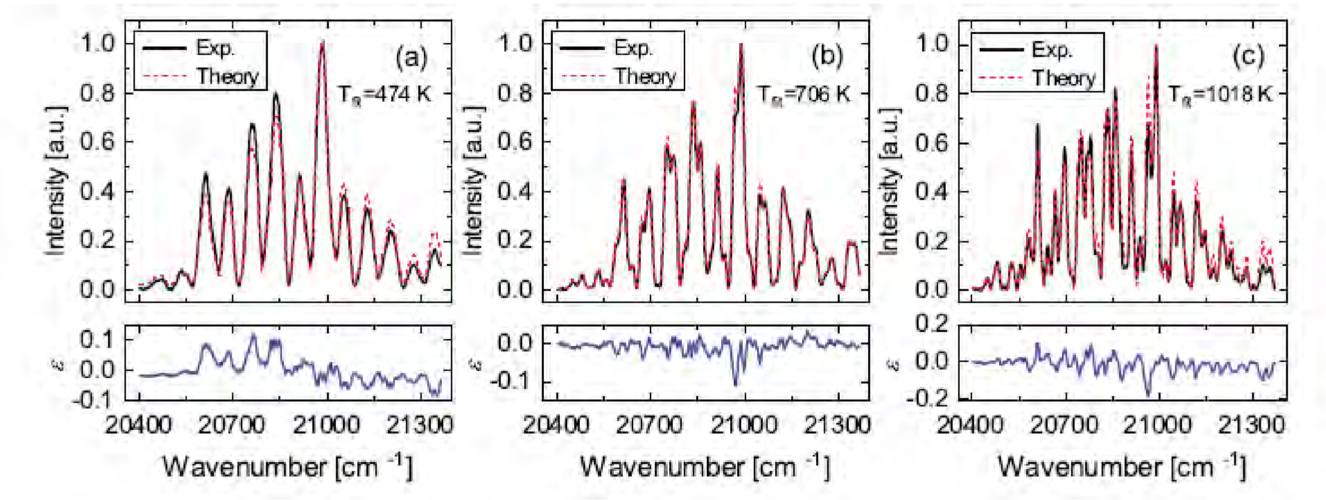

Figure 1: H2 CPP fs CARS experimental spectra and best-fit theoretical spectra acquired at a pressure of 30 bar.

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High-resolution fs/ps coherent anti-Stokes Raman scattering for combustion and propulsion systems

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The design and optimization of next-generation aerospace propulsion and combustion systems requires detailed knowledge of thermodynamic properties such as temperature and gas composition. Novel combustors, such as air-breathing scramjet engines and detonation-based combustors, exhibit significant spatial variation and fast temporal dynamics. Femtosecond/picosecond coherent anti-Stokes Raman scattering (fs/ps CARS) enable gas temperature and species quantification with spatial and temporal precision within reacting flows. The advancement of fs/ps CARS for spatially precise and multidimensional gas-phase measurements and its application to study a high-speed turbulent combustion system will be presented.

The gas temperature and fuel distribution recorded throughout a supersonic combustion ramjet (scramjet) cavity flameholder will be presented, demonstrating the use of fs/ps CARS for quantitative measurements within a challenging high-speed combustion environment. Two measurement advances will be highlighted: ultra-high-resolution detection using counter-propagating phase-matching and the measurement of temperature and species gradients using one-dimensional fs/ps CARS. Counter-propagating (CoP) phase-matching combined with 60-fs pulses for exciting the Raman coherence yields a spatial resolution on the order of 10's of μm, significantly minimizing spatial averaging in the presence of steep property gradients. The extent of the measurement region may be adjusted by controlling the excitation pulse width, as shown in Fig. 1. Also shown in Fig. 1 are temperature measurements using this instrument across a supersonic gas jet exhibiting a ~50 K temperature difference over 80 μm [1]. Two-beam one-dimensional fs/ps CARS has been demonstrated previously in combustion systems [2], and recent work employing an optical parametric amplifier for compact frequency conversion and a planar BOXCARS phase-matching configuration for quantifying temperature gradients within a stationary premixed flame will be discussed.

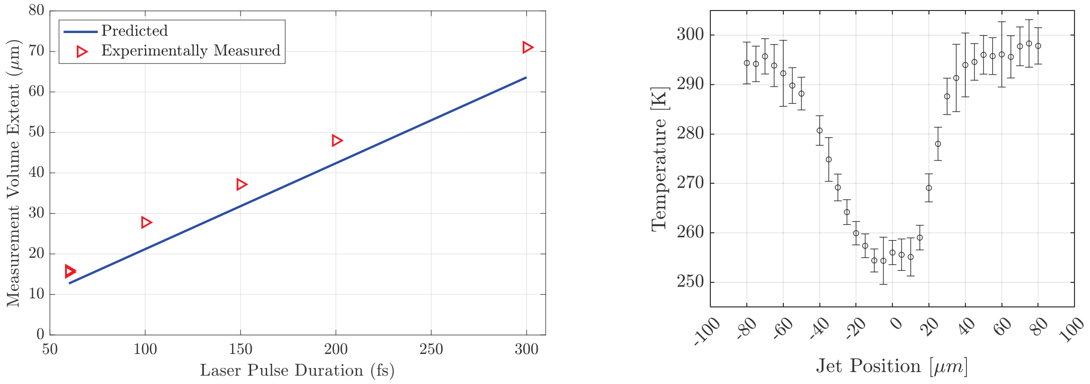

Figure 1: Results using a counterpropagating beam configuration demonstrating the ability to adjust the spatial extent of the CARS measurement region within a gas and the application of this measurement to quantify gas temperature across a microscale (FWHM ~66 μm) jet.

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Spinning molecules to their breaking point, and beyond, with a Raman-based optical centrifuge

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When molecules reach high levels of rotational energy, the molecular structure becomes distorted from a rigid rotor model as the bonds get pulled apart. In the extreme case, a molecule can fall apart directly through centrifugal force. The understanding of molecular behaviour under these conditions is important and examples include simulating and understanding gas temperatures and reactivities on hot exo-planets or energy exchange during atmospheric re-entry of space vehicles traveling at hypersonic speeds. However, quantum mechanical selection rules have historically limited the amount of energy readily deposited into the rotational degree of freedom for experimental studies.

In this talk I will cover our recent development and application of the “optical centrifuge” to molecules CO_2, O_2, and N_2O. In this instrument, a tailored, corkscrew shaped, driving field is generated by shaping a high powered femtosecond laser pulse. This pulse drives molecules through sequential rotational Raman transitions to “super-rotor” states. In the case of CO_2, the CO_2 molecules climbed over 360 rungs of the quantum rotational energy ladder, reaching states well beyond the ground state C-O bond dissociation energy. To populate such states thermally would require temperatures of over 71,000 K. At these energies, the molecules are barely held together and rotate more than 5 full cycles during a single collision interaction. Never before has the entire manifold of possible rotational states up to dissociation been measured for CO_2, and these measurements thus provide a more accurate determination of rotational energy spacings. Further, direct measurement of the rotation-to-vibration energy exchange process has been rare in the literature, but has been directly observed in an ensemble of CO_2 super-rotors.

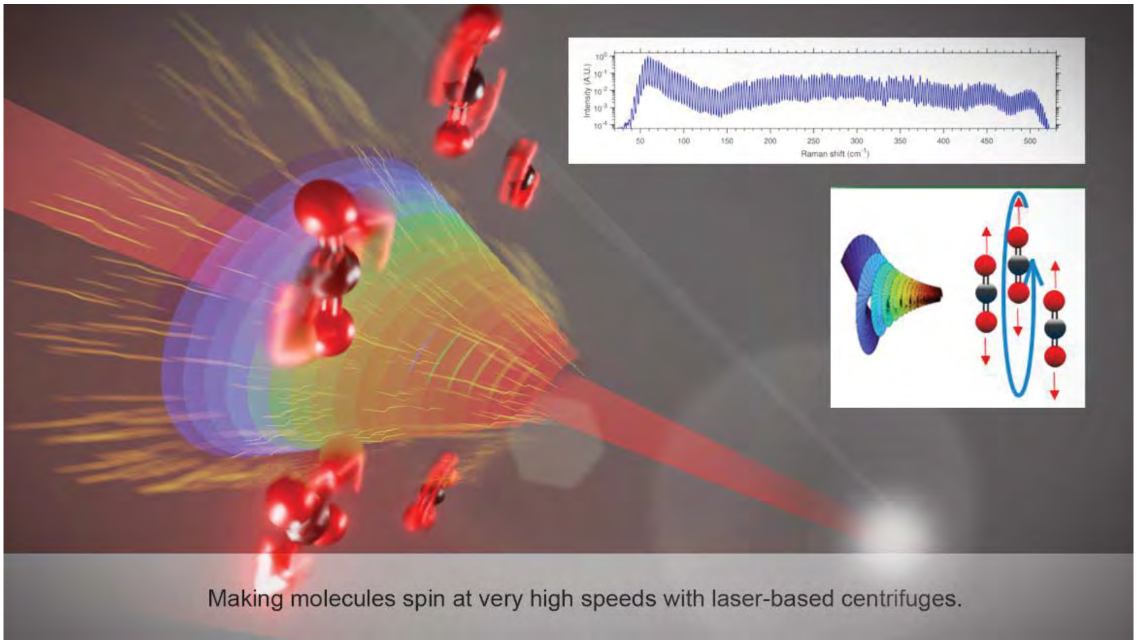

Figure 1. CO_2 molecules trapped and unidirectionally accelerated in the optical centrifuge pulse, adding more than 360 quanta of rotational energy. Coherent Raman scattering of the rotationally accelerated molecules is shown in the upper right inset.

Translational Clinical Raman Spectroscopy

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The rise in cancer due to an ageing society and the rapid spread of life-threatening infectious diseases and antibiotic-resistant germs demands new methods for early diagnosis and personalized therapy. Raman spectroscopic methods have shown their potential in meeting these challenges. The use of such Raman spectroscopic approaches outside specialized laboratories directly at the end user requires easy to use optical instruments with a high Technology Readiness Level (TRL). In this lecture we report on various innovative technological concepts for bringing Raman spectroscopic approaches closer to the clinics.

We will start with introducing novel Raman fiber probes, non-linear Raman microscopes, and endospectroscopic probes for precise surgical guidance and intraoperative histopathological examination of tissue. They aid in a precise determination of tumor margins, staging and grading, information needed for initiating personalized therapy plans quickly.

Furthermore, Raman spectroscopy is also applied to address three important unmet needs in infectious disease treatment: (I) determination of the immune response; (II) rapid identification of the infection causing pathogen and in case of bacterial infections its resistance pattern and (III) response to treatment. The presented Raman approaches - comprising the entire process chain (i.e., from sampling to the final diagnostic result) - reduce the critical parameter of time as compared to standard microbiology.

Equally important as the development of clinically usable spectroscopic devices is the development of tailored sophisticated artificial intelligence based spectral analysis routines. Thus, the presentation will also introduce innovative spectroscopic dataset evaluation algorithms for the translation of spectroscopic data into quantitative diagnostic markers.

Finally, translational infrastructures to overcome the valley of death to apply such Raman spectroscopic approaches for clinical routine will be introduced.

Acknowledgements

Financial support of the EU, the ”Thüringer Ministerium für Wirtschaft, Wissenschaft und Digitale Gesellschaft”, the ”Thüringer Aufbaubank”, the Federal Ministry of Education and Research, Germany (BMBF), the German Science Foundation, the Fonds der Chemischen Industrie and the Carl Zeiss Stiftung.

Macroscopic inelastic scattering imaging technique for intraoperative margins assessment in brain and breast cancer: Uses cases and pilot clinical data

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[b]*Centre de recherche du Centre hospitalier de l'Université de Montréal (CRCHUM), Montreal, Quebec, Canada*

Raman spectroscopy is used for a wide range of materials characterization applications requiring detailed molecular fingerprinting and the quantification of molecular species based on the detection of specific vibrational bonds. In this talk, I will describe how the integration of technologies relying on the detection of spontaneous Raman spectroscopy signal detection can complement current medical practice for surgical guidance and in situ diagnostics. Case studies will be presented in neurosurgical oncology and breast-conserving surgery.

A multicenter study was performed to test whether an intraoperative single-point Raman spectroscopy system could distinguish the three most common types of brain tumors from brain tissue. Nine hundred and eighty-five in situ spectroscopy measurements and colocalized tissue specimens were acquired from 67 patients undergoing surgery for glioblastoma, brain metastases, or meningioma to assess tumor classification. The device achieved diagnostic accuracies of 91% for glioblastoma, 97% for brain metastases, and 96% for meningiomas. These data led to the development of a machine learning model discriminating tumor containing tissue from non-tumoral brain in real time and prior to resection. A similar study was conducted in 20 breast-conserving surgery patients, demonstrating the system can detect invasive breast cancer with more than 90% accuracy.

Here we report the development of a new whole-specimen spectroscopy system to detect residual cancer cells on the surface of surgical specimens using the machine learning models trained using the clinical data acquired with the single-point probe. The field-of-view of the system covers a square area of sides one centimeter, allowing the technology to be used for residual cancer detection at surgical margins. The results of a pilot study are presented in specimens from 8 glioblastoma patients and 5 breast-conserving surgery patients. The instrument was able to detect clusters of cancer cells from the Raman peaks associated with the amino acid phenylalanine and tryptophan as well as the relative concentration of lipids and proteins associated with deformations of the CH_2 and CH_3 molecular bonds. The technique could be applied to common cancers for which margins examination is done including prostate cancer, lung cancer, and gynecological cancers.

Resonance Raman Spectroscopy for *in vivo* Monitoring of Regional Tissue Oxygenation

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Tissue oxygenation refers to the net product of oxygen supply and consumption within a tissue[1]. Insufficient oxygen supply (hypoxia) can disrupt cellular function, and result in life threatening conditions across multiple organ systems[2]. As such, sustaining adequate tissue oxygenation levels during surgery is important for preventing post-operative complications.

Existing techniques for *in vivo* hypoxia monitoring demonstrate a range of drawbacks[3], and there is a clear clinical need for a real time, non-invasive, accurate method of assessing tissue oxygenation. Resonance Raman Spectroscopy (RRS) is a tool with the potential to achieve this. The resonance enhancement of the haemoglobin molecule afforded by using an excitation wavelength near the haemoglobin Soret band at 416 nm allows rapid assessment of its oxygenation status (Figure 1)

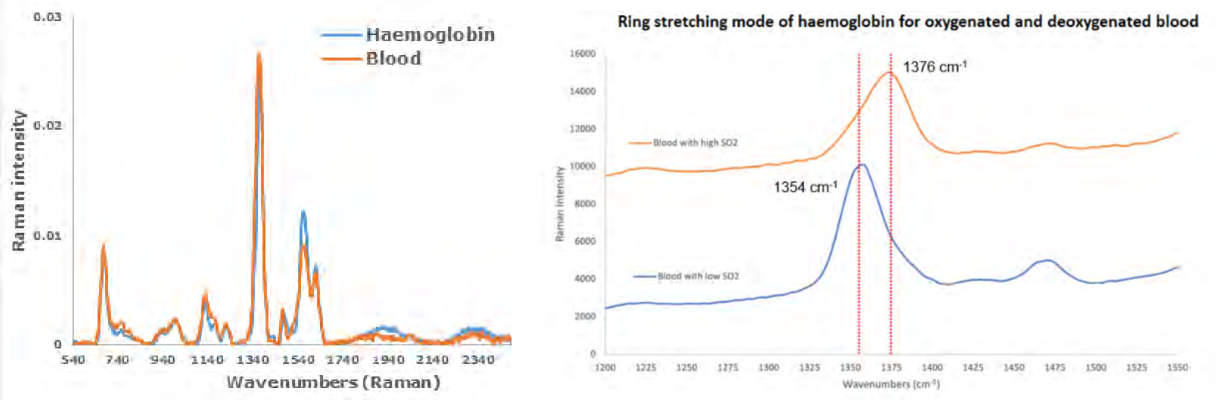

Figure 1(a) Raman spectra of haemoglobin and Human whole blood, showing the selective enhancement of the haemoglobin molecule (b) Shift of the haemoglobin molecule in-plane ring stretching mode for oxygenated (96%) and de-oxgyenated blood (36%)

Previous studies investigating the use of RRS for this task typically focus on a critical care context and as such investigate global oxygenation status measurements[4]. This study investigates the feasibility of a portable RRS system with a custom made fibre optic probe to non-invasively monitor regional tissue oxygenation changes within specific organs in a murine model.

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In-clinic differentiation of skin cancers from benign skin lesions and inflammatory dermatoses with a portable Raman system

Michel Nieuwoudt[1-4], Paul Jarrett[5,6], Hannah Matthews[1-4], Michelle Locke[7,8], Marco Bonesi[1-4,9], Brydon Burnett[1,2], Hannah Holtkamp[1-3], Claude Aguergaray[2-4,9], Hannah Sheridan[1-4], Ira Mautner[2-4,9], Thom Minnee[1-4] and Cather M.Simpson[1-4,9].

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New Zealand and Australia share the unenviable status of having the highest incidence of skin cancers in the world, due to their high levels of UV exposure. Two thirds of fair skinned Europeans there will develop a non-melanoma skin cancer such as BCC (basal cell carcinoma) or SCC (squamous cell carcinoma) in their lifetime, with one in fifteen developing the deadly malignant melanoma. However, Northern European countries such as Denmark, the Netherlands, and Norway are not far behind, taking 3rd, 4th and 5th place, respectively.[1]

If detected early enough, malignant melanoma can have a good prognosis. But for many patients, primary care physicians like General Practitioners are their first point of call. Most have not had extensive dermatology training and tend to over-refer lesions for biopsy, as they do not want to miss a clinically significant lesion that may require urgent treatment. This results in up to 90% unnecessary biopsies, with unnecessary scarring.[2] Portable Raman spectrometers present an ideal opportunity to minimize such unnecessary biopsies; recent advances in semiconductor technologies have led to the availability of small portable Raman systems with fibre optic probes that can be used in the clinical setting.

Here we present a study that used a portable Raman spectrometer to measure a wide variety of benign and malignant skin lesions in the clinic. [3] Chemometric analysis was performed on the Raman spectra to extract latent information about differences in the protein and lipid biomolecules, between healthy and skin cancer tissue (Figure 1) .

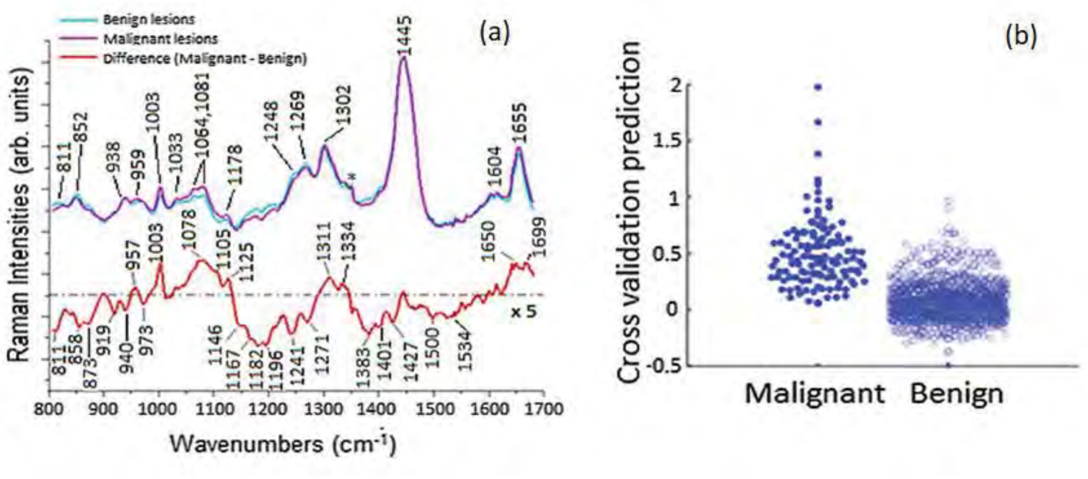

Figure 1(a). Mean spectra of malignant (purple) and benign (light blue) skin lesions, with difference spectrum (red) between the malignant and benign mean spectra, multiplied by a factor of 5 to enhance peak differences, (b) bee swarm plots showing cross-validated differentiation of 532 benign and 96 malignant skin lesions.

Using PLS-DA (partial least squares discriminant analysis), malignant lesions were differentiated from benign ones with AUC (Area under the curve) for a ROC (receiver operator curve) of 0.916, with a specificity of 50% at 100% sensitivity. The results show the feasibility of deployment of Raman spectroscopy in the clinic, in real time, as a non-invasive optical biopsy.

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Electrochemical reactivity analysed by enhanced Raman approaches. Focus on temporal and spatial resolutions.

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The development of electrochemical techniques combined to Raman spectroscopy such as electrochemical Tip- or Surface- Enhanced Raman spectroscopy (EC-TERS or EC-SERS) have attracted considerable attention over the past years by the scientific community. This spectro-electrochemical combination provides an accurate view over electrochemical processes occurring at an electrode by acquiring simultaneously local topography, and vibrational fingerprints about the material or molecules under potential control. Based on the use of an insulated TERS tip, we introduced recently a new approach that we called EC-Tip-SERS.[1] Here, electroactive molecules are adsorbed directly onto a gold tip that acts both as working microelectrode and single hotspot for SERS. Interesting transient mechanistic information can be obtained in this configuration, for example such as those associated to the oxygen reduction reaction towards hydrogen peroxide in water using viologen SAMs as electrocatalyts. Nevertheless, there is here no spatial resolution. The latter can be obtained in TERS,[2] but this requires to handle the Raman signal and STM feedback simultaneously in a home-made design. We will present our latest results and developments in that direction.[3]

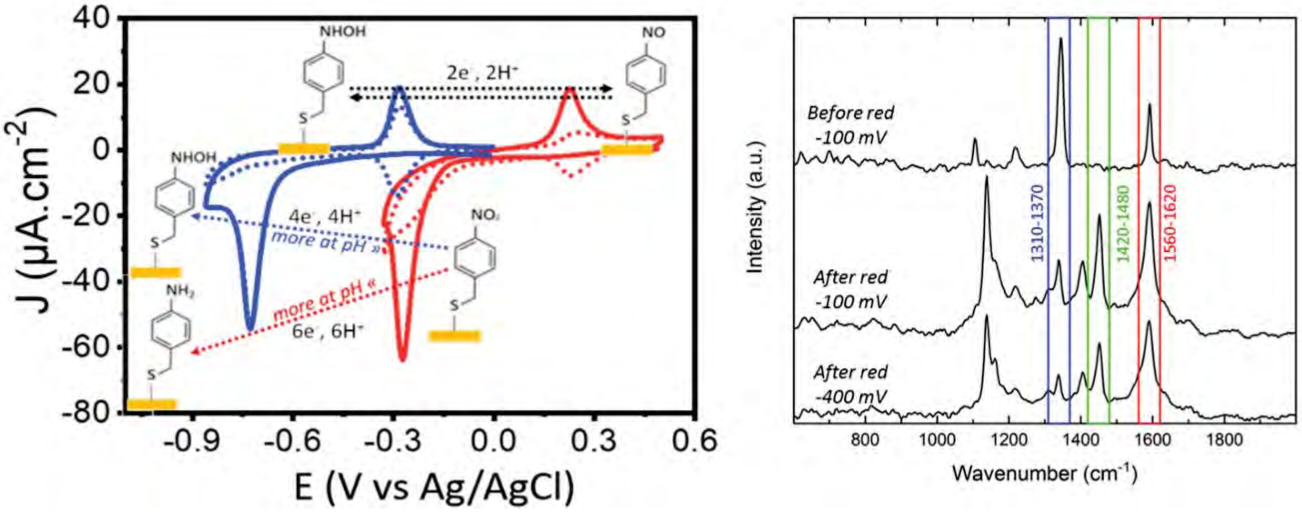

Figure 1: Electrochemical reactivity and Raman identification of a 4-nitrobenzyl mercaptan.

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In situ Raman Reveals the Structure and Dissociation of Interfacial Water

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Understanding the structure and dynamic process of interfacial water at the electrochemical double layer (EDL) is an extremely important topic in electrochemistry/catalysis. The discharge processes of water molecules or hydrated proton are the initiation of the classical hydrogen evolution reaction (HER). Thus, the structure and composition of interfacial water molecules directly affect the HER process. As model catalysts, atomically flat single-crystal electrodes exhibit well-defined surface and electric field properties, and therefore, can be used to elucidate structure-activity electrocatalytic relationships at the atomic level. However, interfacial water is notoriously difficult to probe, during the HER process at single-crystal surface, due to interference from bulk water and the complexity of interfacial environments, such as various water orientations, hydrated ions, and hydrogen bonds network in water molecules.

Herein, by employing electrochemical in situ shell-isolated nanoparticle-enhance Raman spectroscopy (SHINERS) strategy,[1] we systemically investigated the structure and composition of water molecules from interfacial solution phase and electrode phase during the HER process.[2] It was found that the interfacial water is mainly composed of water of trihedrally and tetrahedrally coordinated water and Na+ hydrated water ($Na.H_2O$) at Pd surface. The structure and composition of interfacial water dynamically changes and forms an ordered structure as HER progress. Meanwhile, interfacial solution phase (ion concentration and types) was studied for the influence of interfacial water and HER. We found that the population of Na.H2O in high concentration electrolyte is the highest in interfacial water molecules, while the HER also shows a better performance in the same condition.

We further investigated the electrode phase (crystallographic orientation and electronic structure), to understand the role of ordered $Na.H_2O$, which is more conducive to water dissociation during HER process. The ordered interfacial water structure could accelerate the efficiency of electron transfer. Theoretical calculations also show that $Na.H_2O$ is more susceptible to dissociate HO-H bond than trihedrally and tetrahedrally coordinated water at electrode surface during HER process. We propose a new structure-relationship between the interfacial water and HER, which could help to understand the nature of electrocatalysis.

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In-situ Raman Spectroscopy Study of Electrocatalytic Reactions

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Electrocatalytic reactions at the solid/liquid interface involve complex key information such as charge transfer, species evolution, and energy transfer. *In-situ* studies of electrocatalytic reactions at the solid/liquid interface will provide direct help in revealing specific structure-activity relationships, understanding the essence of electrocatalytic reactions, and guiding the design of efficient catalytic materials. Surface-enhanced Raman spectroscopy technology, as a fingerprint spectroscopy technique with extremely high surface detection sensitivity, can provide direct spectral evidence of trace intermediate species and adsorbed species on catalyst surfaces, unaffected by interference from aqueous solutions, making it very suitable for studying the mechanism of electrocatalytic reactions at the solid/liquid interface.

We have developed and utilized highly sensitive *in-situ* electrochemical Raman spectroscopy for systematic studies of important electrocatalytic processes in energy systems, such as oxygen reduction reaction, hydrogen oxidation reaction, hydrogen evolution reaction, CO_2 reduction reaction, etc., successfully capturing direct spectral evidence of relevant reaction key intermediate species, such as OOH*, OH*, H*, OCCO*, etc [1-5]. Combined with theoretical calculations, through the analysis of direct Raman spectroscopic evidence of relevant intermediate species and surface adsorbed species, correlation structure-activity relationships are established, deeply revealing the essence of electrocatalytic reactions, and providing help for improving catalyst preparation and design.

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Water Harvesting at the Single Crystal Level

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Climate warming induced draughts are an increasing problem worldwide. Water harvesting from the atmosphere using metal-organic frameworks (MOFs) has emerged as an exciting direction for access to clean drinking water, especially in arid environments. Here, MOFs play a pivotal role as a local concentrator of water molecules facilitating their phase transformation from gas to drinkable fluid. While the influence of the crystal structure of MOF crystals is well studied, the impact of particle morphology on water uptake and release are more difficult to access [1]. Common MOF characterization methods often rely on statistical averages over large sets of crystals (bulk), omitting the single-particle level. However, to evaluate the inherent material properties for water harvesting beyond misleading contributions due to the adsorption of further gas molecules, inter-particle condensation or defect sites, single-particles studies are required providing spatial and temporal information to answer what the upper limit for a given material is and how much water is lost due to intra- and inter-particle effects.

To approach these questions we employed our recently developed MOSAIC imaging system dedicated to multimodal and correlative microscopy and spectroscopy [2]. Through spatially resolved fluorescence and Raman spectroscopy combined with a suite of optical confocal imaging techniques, we investigated the local water uptake and release in various materials, including MOF-801(Zr). We found that water uptake can be highly heterogeneous among different particle morphologies, often coinciding with polycrystalline regions and defect sites, thereby modulating the total uptake and kinetics [3]. Importantly, our results highlight the necessity of evaluating materials at the single-particle level by demonstrating that both MOF-801(Zr and Hf) exhibit the capability of harvesting at least 20 fold more water than previously determined through bulk experiments.

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Studies on the growth mechanisms of protective scales on ferritic steels using Raman spectroscopy under *in-situ* and *ex-situ* conditions

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Ferritic Stainless Steels (FSSs) are the most promising interconnect material for both intermediate temperature Solid Oxide Fuel Cells (IT-SOFCs) and Electrolyser Cells (SOECs) thanks to their satisfactory oxidation resistance while being easy and cheap to manufacture. The high need to solve the problem of CO_2 production by burning traditional fossil fuels (coal, oil, or natural gas) resulted in increasingly growing interest in both devices dedicated to electrochemical energy conversion providing high efficiency, ultra-low pollution and fuel diversity [1].

However, high-temperature (HT) oxidation of FSSs still casts a shadow on their further development and system's efficiency. To address this issue properly, not only protective measures have to be taken (i.e. surface engineering), but also comprehensive studies on the structural changes occurring in the entire system with the emphasis put on the early stages of oxidation are of high importance.

The main aim of these studies is to determine the influence of temperature (700, 800, 900 ºC), atmosphere (H_2/H_2O = 10/90 or 50/50), oxidation time (up to 1000 h), and material modifications in terms of chromium content (Crofer 22APU and 22H, AISI 442 and 446) on the structural evolution of scales. To provide a simple, fast, and inexpensive way to study above-mentioned topic independently on the oxidation stage (both initial – *in-situ* studies and further stages – *ex-situ* studies), a versatile approach utilizing methodology outside of the state-of-the-art is used, namely Raman spectroscopy.

For *in-situ* studies, steels specimens of 3x3x5 mm geometry are exposed in HT attachment and simultaneously measured in the function of both temperature and time of oxidation (max. ca. 12 hours) using either 488 or 633 nm laser lines. For *ex-situ* studies, Raman Confocal Imaging is applied on pre-prepared cross-sections of specimens oxidized in aforementioned conditions. First results provide a great insight into structural evolution of scales depending on the alloying elements' (Cr, Al) content that allows to group them into either protective or non-protective materials, as well as to demonstrate the capabilities of such an approach over conventional XRD or TEM methods.

Acknowledgments

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Ultra-sensitive detection of Terbutryn via indirect Surface-enhanced Raman scattering

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The growing use of chemicals in agriculture, including fertilizers and pesticides, significantly contributes to environmental pollution. Among them, triazines, which main role is to control broadleaf and grassy weeds, have been identified as relevant, based on monitoring data from EU freshwaters sources.[1] Terbutryn, a selective herbicide and a triazine compound, has shown moderate toxicity to fishes[2,3] as well as tendency to move from treated soils into water compartments through runoff and leaching. Residual amounts of terbutryn and its metabolites have been found in drinking water[4] and, therefore, its application has been banned in many countries due to the potential bioaccumulation in organisms.[5] Despite these restrictions, terbutryn continues to be detected in water environments. Detection methods commonly used to identify these contaminants often involve costly and time-consuming procedures, besides concerns regarding their effectiveness in achieving low-level detection. Surface-enhanced Raman scattering (SERS) offers unique advantages as detection tool enhancing the Raman signal of molecules located in close vicinity to noble metallic nanostructures, providing highly selective and sensitive detection at the single-molecule level.[6]

For that, a critical factor in the successful translation of the analytical potential of SERS to real applications is represented by the controlled design of reliable and effective SERS-active substrates. Nanofabrication of controlled assembly of spherical gold nanoparticles (AuNPs) generating a dense collection of hot spots on/in sub-micron template units provided SERS substrates with single particle sensitivity for the application presented here. However, the integration of a layer to trap terbutryn close to the Au surface is of vital importance since triazines show poor or no affinity towards gold. Therefore, crystalline porous materials (e.g. covalent organic framework and metallic-organic framework) were growth on AuNPs assemblies. These composites were detected by SERS down to 10^{-10} M of 4-nitro-benzenethiol (4NBT), which was used as molecular probe. Due to the very low Raman cross-section of terbutryn, an indirect SERS strategy was selected using 4NBT. This strategy allowed the detection of terbutryn down to 10^{-9} M, demonstrating the applicability of SERS in combination of hybrid composites for the ultra-sensitive detection of organic contaminants in water.

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Plexcitonic SERS Tags: New Opportunities for Ultrasensitive Biosensing and Bioimaging

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Plexcitonic nanoparticles demonstrate robust light-matter interactions mediated by localized surface plasmon resonances, offering potential applications in photonics, solar cells, sensing, and more. Recently, we investigated these interactions using UV-vis and surface-enhanced Raman scattering (SERS) spectroscopies, supported by finite-difference time-domain (FDTD) calculations.[1] Emphasizing the significance of merging plasmonic nanomaterials with J-aggregates featuring near-zero-refractive index, we utilize nanorattles comprising J-aggregates of the cyanine dye TDBC and plasmonic silver-coated gold nanorods. These structures, encapsulated within mesoporous silica shells, enable J-aggregate adsorption onto the metallic nanorod surface, ensuring high colloidal stability.

Electromagnetic simulations reveal strong confinement of the electromagnetic field within the J-aggregate layer, particularly at wavelengths near the upper plexcitonic mode. Conversely, damping occurs towards the J-aggregate/water interface at the lower plexcitonic mode. This behavior is attributed to the sharp variation in dielectric properties of the J-aggregate shell near the plasmon resonance, resulting in a high refractive index contrast between water and the TDBC shell at both upper and lower plexcitonic modes. Such characteristics contribute to the notable SERS efficiency of the plexcitonic nanorattles under both 633 nm and 532 nm laser illumination. SERS analysis demonstrates detection sensitivity down to the single-nanoparticle level, yielding an exceptionally high average SERS intensity per particle. These findings unveil promising prospects for ultrasensitive biosensing and bioimaging, presenting superbright and highly stable optical labels driven by the robust coupling effect.

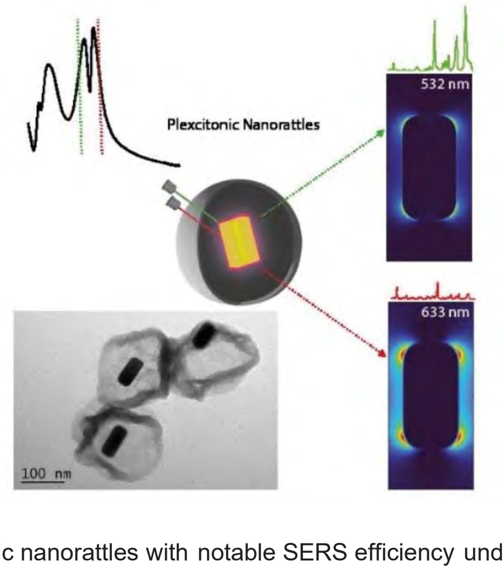

Figure 1: Scheme of plexcitonic nanorattles with notable SERS efficiency under 633 and 532 nm laser line.

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Plasmonic Supercrystals in Microfluidics Devices: Enabling Ultra-Sensitive SERS Detection.

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Surface-enhanced Raman scattering (SERS) substrates have the potential for a wide range of applications in sensing and detection due to their ability to enhance the Raman signal of molecules mainly due to the presence of strong electromagnetic fields generated after the plasmon excitation. Moreover, this effect could be more intense in the case of plasmonic supercrystals due to antenna effects. However, developing SERS substrates faces several challenges such as achieving homogeneous and reproducible enhancement, these need to be overcome to develop reliable and reproducible substrates for use in real-world applications.

Herein, we demonstrate how the employment of microfluidic platforms allows to generate gold plasmonic supercrystals through the slow pervaporation of the solvent of a colloidal dispersion of gold nanooctahedra (**Fig 1A-B**). The characterization of the formed supercrystal reveals a single-domain supercrystal with dimensions of 1.7×0.3 mm^2, and that all the obtained supercrystals present the same orientation. Furthermore, the integration of a plasmonic supercrystal inside a microfluidic platform guarantees the infiltration of any analyte, even without affinity for gold surface, within the plasmonic supercrystals and therefore its ultrasensitive detection. The study of the sensing capabilities of these platforms showed a highly uniform and intense SERS activity, being both key parameters to achieve quantitative and ultrasensitive analysis (**Fig 1C** and **E**). In fact, a limit of detection of 10^{-19}M was achieved for crystal violet. Also, the possibility of combining the capabilities of our SERS sensor with the chromatographic properties of silica nanoparticles to develop a sensor device with charge selectivity was investigated (**Fig 1D**).

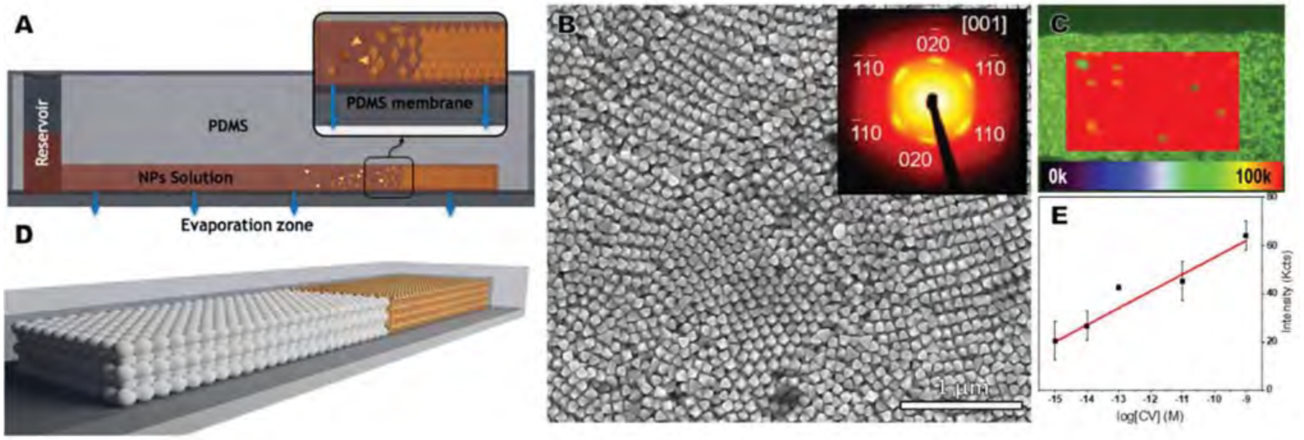

Figure 1: (**A**) Schematic illustration of self-assembly of gold octahedra nanoparticles inside microfluidic platform induced by pervaporation. (**B**) Scanning Electron Microscopy (SEM) image of the supercrystal. Inset corresponds with 2D-SAXS images with the indexation of the strongest Bragg peaks. (**C**) SERS intensity map obtained at 1617 cm^{-1} in the presence of 10^{-7} M of crystal violet. (**D**) Schematic representation of the final SERS Sensor modified with a silica supercrystal for chromatographic separation. (**E**) SERS intensity obtained for different concentrations of crystal violet (10^{-9} - 10^{-15} M).

Nanogap-containing core-shell-like plasmonic nanostructures for precise and sensitive detection of pesticides via SERS spectroscopy

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Every food product released to the market is subjected to multiple tests for the presence of pesticides, which can have a serious negative impact on human health. In the modern world, food products and drugs are routinely quantitatively and qualitatively analyzed by mass spectrometry (MS) whether they are safe to consume. However, these methods face some problems, such as inability to detect certain analytes, too low sensitivity, complex measurement procedure and expensive equipment. Therefore there is a great need to search for alternative analytical techniques. Surface-enhanced Raman scattering (SERS) spectroscopy can be a promising replacement [1], which has already been frequently proven suitable for analyte quantification and detecting even single molecules. However, SERS performance is strictly related to the quality of plasmonic substrate. Therefore, successful and reliable SERS analysis demands meeting numerous requirements imposed on plasmonic nanostructures.

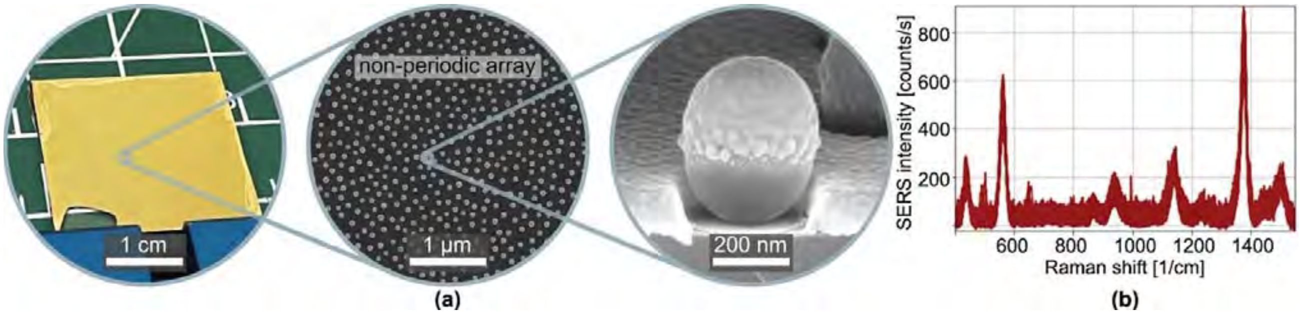

Figure 1: (a) Optical (left) and SEM (centre and right) images of CSLNs (b) 210 SERS spectra of thiram adsorbed on CLSNs from 100 µg/ml solution in isooctane, collected from 200 µm x 200 µm area.

Here, we present novel nanogap-containing core-shell-like nanostructures (CSLNs) that serve as SERS-active plasmonic substrates. A modified nanosphere lithography method was used to fabricate non-periodic arrays of nanoapertures filled with metal-capped dielectric nanospheres (Fig.1 (a), right image). The nanogap formed between a metal cap and the edge of the nanoaperture provides a high SERS signal with a very low relative standard deviation (RSD) over a large sample area. The optical response of CSLNs resembling that for the core-shell nanoparticles exhibits 3 plasmonic resonances, which can be easily both coarse and fine tuned from about 300 nm to 1100 nm by a simple adjustment of the geometrical parameters of the structure. This allows for improved SERS performance by matching the excitation wavelength and/or the energy of the analyte's Raman shifted peak.

CSLNs were successfully used for the SERS detection of the thiram pesticide, which is beyond the capability of direct detection by MS methods. Thiram was identified at low concentrations without additional substrate functionalization (Fig.1 (b)). RSD of less than 8% was achieved in a very rigorous experiment, collecting nearly 700 points over at least 1 cm^2 of the substrate area. A calibration curve was determined to satisfy the requirements imposed by the commercial quantitative detection of pesticides. These results demonstrate the potential of the proposed here new CSLNs-based substrates to develop a universal methodological approach, adaptable to SERS detection of other pesticides and various analytes.

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Novel approaches to broadband coherent Raman microscopy

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Coherent Raman scattering (CRS) microscopy is a powerful nonlinear optical technique for chemical identification of (bio)-molecules based on their intrinsic vibrational spectrum and for high-speed label-free imaging of cells and tissues. Single-frequency CRS microscopy allows the detection of molecules with a specific Raman response but is not sufficient to distinguish different components within complex heterogeneous systems in which chemical species display spectrally overlapped resonances. For this reason, there is ongoing research aimed at extending CRS to broadband detection, combining the speed of coherent Raman spectroscopy with the information content of spontaneous Raman [1].

In this talk we present two innovative approaches to broadband CRS, both in the stimulated Raman scattering (SRS) and coherent anti-Stokes Raman scattering (CARS) modalities. We first present a broadband CARS (B-CARS) system based on a high power ultrafast solid-state laser and white light continuum generation in a bulk material, acquiring high-quality spectra in a 1-ms time, limited by the spectrometer refresh rate, covering both the fingerprint and the CH stretching region [2, 3]. A convolutional neural network is used to denoise the B-CARS spectra and remove the unwanted non-resonant background [4]. Our instrument delivers high quality label-free images of therapy-induced senescent cells and liver tumor tissues.

Next, we present a broadband SRS system equipped with a home-built multichannel lock-in amplifier, simultaneously measuring the stimulated Raman signal over 32 frequencies within 44 μs, allowing for detailed, high spatial resolution mapping of spectrally congested samples [5]. We demonstrate the performance of our SRS microscope by discriminating the relative concentrations of different fatty acids in cultured hepatocytes at the single lipid droplet level and by identifying fibrosarcoma tumor lesions embedded within healthy tissue. Finally, we describe our effort to develop a commercial broadband SRS microscope suitable for a clinical environment [6] based on a compact, turnkey fiber laser system for application to histopathology and label-free tumour identification, staging and grading.

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Super-Resolution Spectral SERS Imaging

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Advances in the understanding of Raman signals detected from the surface of plasmonic nanoparticles has enabled super-resolution SERS imaging. Our group has developed super-resolution spectral SERS imaging to monitor chemical activity to nanometer resolved spatial locations of single particles,[1] in biological systems,[2] and most recently correlated with electron microscopy of the particles. In Figure 1A, the underlying approach is illustrated. Wide-field imaging of nanoparticles in the sample can be recorded simultaneously with the spectra arising from distinct particles on a single CMOS array sensor. By recording movies, temporal fluctuations in the SERS signal can be recorded and used with super-resolution algorithms to identify the nanometer-scale location from where the Raman spectrum arose. Through the synthesis and screening of diverse nanoparticle probes, we have been able to identify optimized probes for single particle detection.[3] Correlated scanning electron microscopy with polarization dependent SERS imaging further provides insight into the nature of the confined fields (Figure 1B) and how to control enhancements in complex nanoparticle shapes. Increased understanding of the interaction of light with plasmonic particles is enabling spectroscopy on nanometer dimensions that holds promise to elucidate chemical interactions in a diverse range of chemical and biological systems.

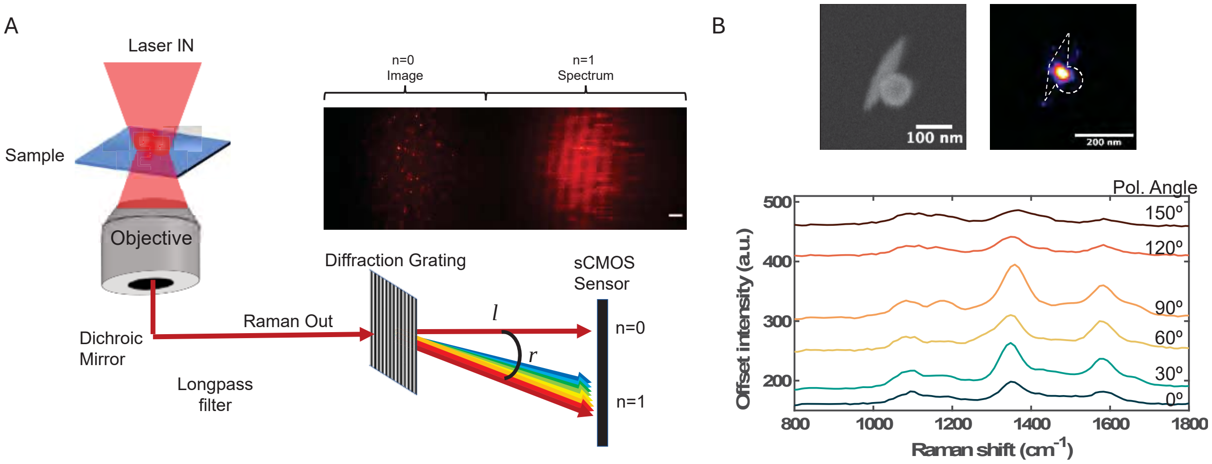

Figure 1: A) Wide-field imaging with a diffraction grating prior to the sensor enables super-resolution imaging with the simultaneous SERS spectroscopy from each nanoparticle in the image. **B)** Correlated SEM and polarization dependent measurements provide new insight into electric field confinement and chemical behavior on the surface of the particles.

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Advancing Electrochemical SERS Using Screen-Printed Electrodes

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Electrochemical surface-enhanced Raman spectroscopy (EC-SERS) has been in existence since the first SERS observation 50 years ago this year. Recently, there has been a significant increase in the use of EC-SERS for scientific studies, largely due to the availability of portable instrumentation at reduced cost. [1] Over the past 15 years, our research group has explored screen-printed electrodes (SPEs) as a platform for routine spectroelectrochemical investigations which combine SERS and electrochemistry. In this talk, I will outline the various ways in which we fabricate screen-printed electrodes for use in EC-SERS measurements and will discuss applications of the technique, including biosensor development and thin film investigations. [2,3]

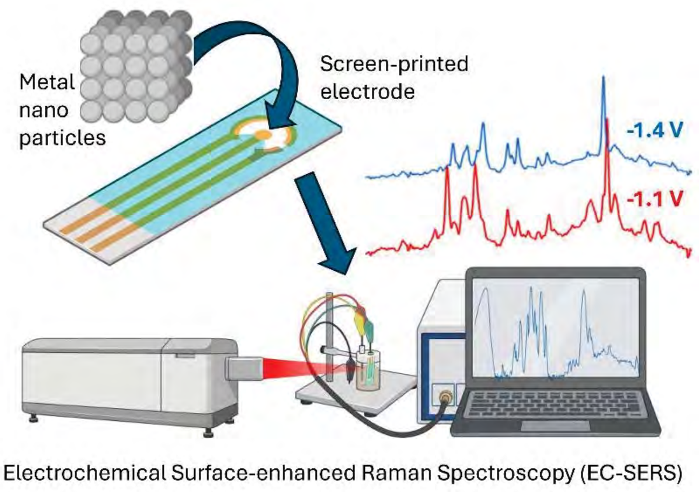

Figure 1. Electrochemical surface-enhanced Raman spectroscopy using modified screen-printed electrodes will be discussed.

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Optimisation and Characterisation of Ultrabright Nanotags for Surface-Enhanced Raman Scattering (SERS)

Ruairí Carland[a], Neil Shand[b], Duncan Graham[a], Karen Faulds[a]
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Surface-enhanced Raman scattering (SERS) is a powerful spectroscopic technique which harnesses the optical properties of plasmonic nanostructures to amplify Raman scattering. Indirect detection by SERS involves the preparation of a plasmonic nanostructure followed by functionalisation with a Raman reporter. Often, this is then subsequently encapsulated in an outer shell, which can support the conjugation of targeting moieties. For many applications an intense SERS signal is crucial, such as the detection of these “nanotags” in complex biological environments. Understanding the relationship between structure and SERS response therefore represents an important fundamental aspect in the preparation of ultrabright SERS nanotags.

One current strategy to increase the SERS response is to employ the use of aggregated nanoparticles.[1-2] However, aggregation is an uncontrolled process that yields a wide range of structures. In this work, we discuss our recent endeavours in optimising and characterising gold nanoparticle aggregates, with the aim of better understanding the relationship between aggregate structure and optical response. Specifically, to determine a combination of structural features that will maximise the SERS response of spectroscopic tags in both dry and ‘in-situ’ conditions.

This will be achieved using two complimentary approaches, asymmetrical-flow field-flow-fractionation (AF4) and correlative SERS/SEM imaging. AF4 facilitates the comprehensive characterisation of specific morphologies in their ‘in-situ colloidal state’, by subjecting polydisperse samples to a high-resolution separation under flow, prior to on-line structural and optical characterisation. Correlative SERS/SEM imaging allows for the direct comparison between structure and SERS response at a single nanotag level; these highly specific dry state characterisations can then be compared against wet-colloidal state characterisations of similar morphologies obtained by AF4 analysis.

By ascertaining the combination of structural features that maximise the SERS response of nanotags, it will become possible to rationally design and develop aggregated plasmonic structures that exhibit improved sensitivity.

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Peculiarities of using different nanostructures for surface-enhanced Raman scattering

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SERS is a commonly used technique to enhance the signal, which allows the analysis of low-concentration samples or even the detection of a single molecule. The SERS effect can occur when the analyte is in close vicinity of a nanoscale-structured metal surface. By using metal surfaces with optimal parameters, the intensity of Raman signals can be enhanced by several orders of magnitude. Our previous research shows that thermal de-wetting of thin metallic layers could be used for SERS application. In this research, we compare the results obtained during the examination of nanostructures created by spark ablation and vapour-liquid-solid techniques, to find the optimal creation parameters for the SERS substrate with a higher enhancement factor, which is suitable to detect the least analyte and will be suitable for further applications. In Figure 1, the Scanning electron microscope images of different nanostructures for the SERS application can be seen.

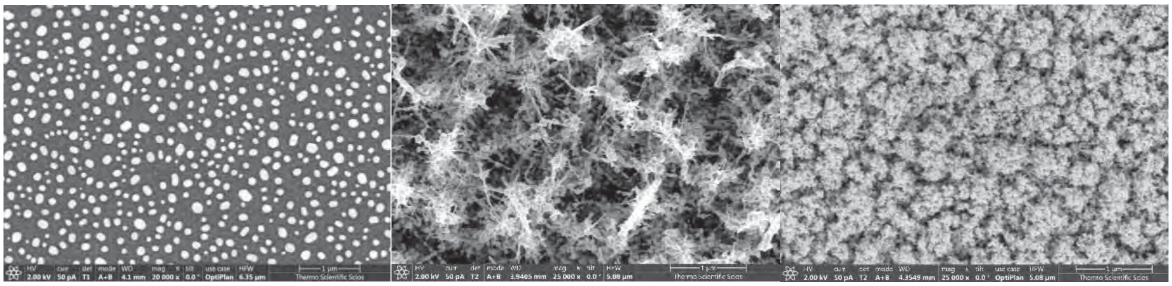

Figure 1: Scanning electron Microscope images of the created and investigated nanostructures: a) Thermal dewetted gold nanostructures, b) Silicon nanotrees covered by gold layer, c) Ag-Au gold nanostructures created by arc-discharge method.

It was earlier shown, that for gold nanoislands created by the thermal dewetting process, the enhancement factor for 10-5 M solution of rhodamine 6G was 90-150. 533 nm excitation light source was used for better performance. These results were compared with silicon nanotrees covered with a thin gold layer fabricated by the vapour-liquid-solid method. By the number of cycles during the creation process, the parameters of the nanostructures could be changed as a result different nanotree densities could be achieved. The calculated enhancement factor of the created nanostructures varied between 5-70 000, it could be optimized by changing the number of cycles during the creation process (the largest enhancement factor was 70 000, for it 4 cycles were used during the creation process, while a 633 nm excitation light source gave the better performance). Also, the previous results were compared with gold-silver nanostructures created by the charge ablation method. This method could precisely control the composition from pure silver to pure gold containing SERS substrates. The enhancement factor of created nanostructures varied between 70-160 000, it could be optimized by the composition change (the optimal composition was Ag20-Au80, and the enhancement factor for this composition was 160 000, while a 633 nm excitation light source gave the better performance. The detection limit of the investigated analyte was found out for the investigated structures as well.

On the Measurements of Surface Enhanced Raman Scattering Spectrum

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Surface-enhanced Raman scattering (SERS) is a highly sensitive spectroscopy technique widely utilized in chemical and biological sensing. It relies on specially designed nanostructured surfaces to significantly enhance the Raman signal of target analytes through local electromagnetic field enhancement and potential chemical enhancement due to charge transfer. Despite the prevalent understanding that SERS hot-spots and electromagnetic enhancement, practical measurements often reveal complexities beyond these factors. Here a comprehensive theoretical framework is presented to facilitate a deeper understanding of SERS measurements in two widely documented categories of SERS substrates [1]: plasmonic colloidal particles, including spherical and spheroid nanoparticles, nanoparticle diameters, and thin-film-based SERS substrates such as ultra-thin substrates, bundled nanorods, and porous plasmonic thin films. Special attention is given to the influence of analyte adsorption, orientation, and the polarization of the excitation laser on effective SERS enhancement factors. The study notably considers the impact of analyte size on the SERS spectrum, exploring scenarios where the analyte is significantly smaller or larger than the hot-spot dimensions. Additionally, the analysis incorporates optical attenuations stemming from the optical properties of both the analyte and the SERS substrates. The outcomes offer insights into various observed phenomena in SERS measurements, including variations in relative peak intensities, reductions in SERS intensity at high analyte concentrations, and significant baseline fluctuations. These findings not only contribute to a better understanding of SERS measurements but also provide valuable guidance for optimizing SERS substrate design, enhancing SERS measurements, and refining the quantification of SERS detection methodologies.

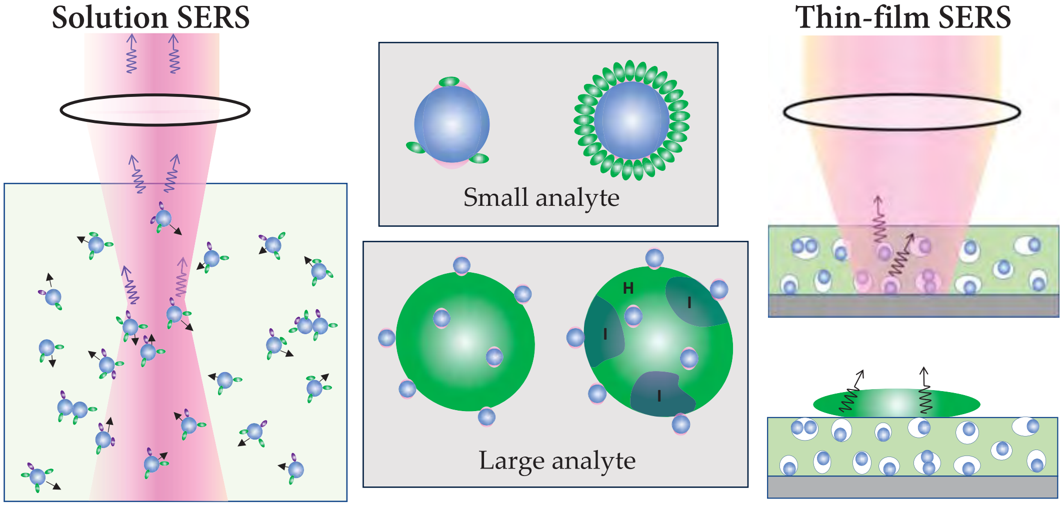

Figure 1: Different SERS measurement configuration with different sized analytes.

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Ab initio modeling of Time-Dependent Raman and Surface Enhanced Raman Scattering

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Raman scattering,[1] a powerful technique with a historical trajectory spanning almost a century, stands as a formidable tool for unravelling the vibrational structures of molecules. Theoretical modelling of Raman signal has profoundly impacted our comprehension of the underlying mechanisms involved in the process. Our effort has been devoted to design a new theoretical strategy to simulate Raman spectra in time domain,[2] drawing inspiration from the pioneering Lee and Heller's theory.[3] This method offers a unique advantage by providing insights into the time evolution of the molecular Raman signal, tracking the dynamics of the incident electric field. It not only provides for the total Raman signal at the process's conclusion but gives transient information. Importantly, the flexibility of this approach allows for the utilization of various incident electric field shapes, enabling a closer alignment with experimental setups.

The vibronic wave function of the molecule is prepared dressing its electronic states with vibrational levels computed at TDDFT[4] level of theory including the Franck-Condon and Hertzberg-Teller expansion[5] of the transition dipole moments. The numerical propagation of the vibronic wave function, when influenced by a light pulse, yields the time evolution of the vibronic coefficients. These coefficients are managed through an Inverse Fourier Transform with the integral restricted to a finite time, to extract the cumulative Raman signal emitted after a specific time interval from the interaction with the pulse.

We exploited resonance and non-resonance condition for porphine molecule in aqueous solution. Moreover, the effect of the vibrational relaxation, which should be taken into account when its time scale is similar to that of the Raman emission, has been included through the stochastic Schroedinger equation approach.[6] In particular, vibrational relaxation processes have a great impact on the Raman response when the pulse is resonant with a molecular excitation.

The strategy has been extended to simulate the Raman signal in presence of a metal nanoparticle (NP), therefore moving toward simulation of surface enhanced Raman scattering (SERS). SERS experiments have taken hold thanks to the enhancement of the local field induced by the surface plasmon resonances on metal NP, that transpose in more intense Raman response. Scientific community widely concurs on attributing the enhancement of molecular response to two principal mechanisms, commonly identified as electromagnetic and chemical interactions. It is generally assumed that the electromagnetic contribution alone accounts for the greatest part of the enhancement of the SERS signal, in particular, when electric fields resonant with the plasmonic peak are employed. Within the framework of a multiscale approach, the system is treated by coupling the quantum mechanical description of the molecule with the polarizable continuum model for the NP.[7] This method is based on the preliminary calculation of the electronic properties of the molecule in presence of the NP that are merged with the molecular vibronic analysis to obtain the vibronic wave function. The molecular electrodynamics in presence of a NP is computed when interacting with an incident electric field.[8] Subsequently, the Raman signal is evaluated through an inverse Fourier Transform of the coefficients' dynamics. Numerical examples on realistic organic molecules are also presented.

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First-principles density functional theory calculation of vibrational resonant Raman spectra in large semiconducting and metallic systems

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Since its first realization, vibrational Raman spectroscopy has become one of the most widely used optical techniques in materials science. It allows for the determination of structural properties and new phases even at extreme conditions, where other techniques fail or are less readily available and it can be also used in the absence of long-range structural order as for liquid or amorphous materials. First-principles calculation of Raman spectra is thus highly desirable, allowing to associate Raman lines to specific microscopic structures. Within density functional theory, the standard approach for the calculation of vibrational Raman intensities in periodic systems, relies on second order derivatives of the electronic densities with respect to a uniform electric field [1,2]. The advantage with respect to other approaches relies in the negligible computational effort required for the evaluation of the intensities, compared to that required for the calculation of vibrational frequencies, allowing for the determination of Raman spectra in large systems up to several hundreds of atoms. However, this technique is limited to the treatment of static electric fields, failing to account for (near-)resonant effects present in semiconductors and metals. In this work, we propose, within the framework of density functional theory, a generalization of the second-order approach for the treatment of non-static electric fields, which keeps the computational advantage and thus allows for the efficient calculation of resonant Raman spectra in large semiconductors and metals.

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Accurate Predictions of Raman and Raman Optical Activity Spectra: Recent Progresses and Challenges

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While Raman spectroscopy is now routinely used for structural characterization in conjunction with computations to fully unleash its capabilities as an investigative tool, it presents a number of well-known challenges from a theoretical perspective, starting from the definition of its core quantity, the polarizability tensor. Experimental conditions can span a wide range of conditions from the far-from-resonance to the resonance regimes. In the former case, analytical forms are available, notably for density functional theory (DFT), which has become the standard for simulations.

However, in absence of reference high-level theoretical models, the validity of DFT-based protocols can only be measured with respect to experiment, a comparison that may be complicated by modelling issues. Furthermore, spectral simulations are done within the harmonic-oscillator approximation, which may cause a cancellation of error in combination with DFT. This problem is further exacerbated for ROA, whose signal is about 10^3-10^4 times less intense than Raman and results from the interaction of more quantities. The harmonic approximation is also unable to predict spectral band-shapes in regions that are now becoming more accessible thanks to progresses in instrumentation, for instance at higher wavenumbers.

In this contribution, we show the recent advances made to predict anharmonic effects in the Raman and ROA spectra of medium-large molecular systems[1]. We will discuss the potential caveats of such an approach, like the problem of resonances, and how recent efforts have paved the way for such calculations to be doable routinely and reliably[2], as illustrated through comparison with experiment. These promising results pave the way toward the study of more complex and flexible biosystems and their environment[3].

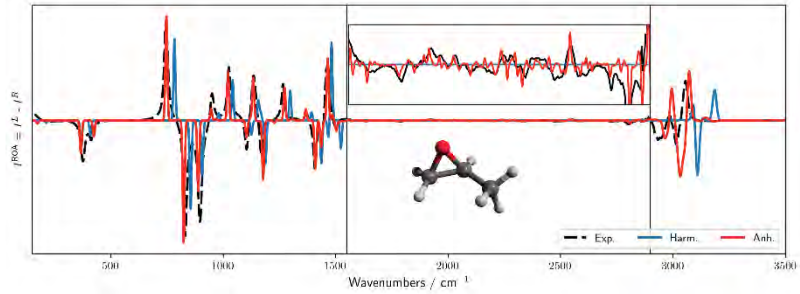

Figure 1: Theoretical and computed Raman optical spectrum of *S*-methyloxirane.

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Combined theoretical and experimental approach for assessment of fine-grain vibrational information in SERS

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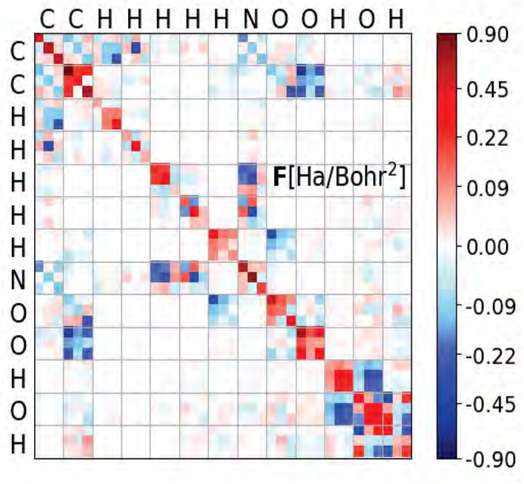
The undeniable application potential of Raman spectroscopy in biophysics is constrained by impossibility to track vibrational frequencies (being eigenvalues of the dynamic matrix) in closed form. To mitigate this limitation, we present results of our running work on molecular binding, proposing a framework for tracking shifts of vibrational frequencies due to different binding geometries.

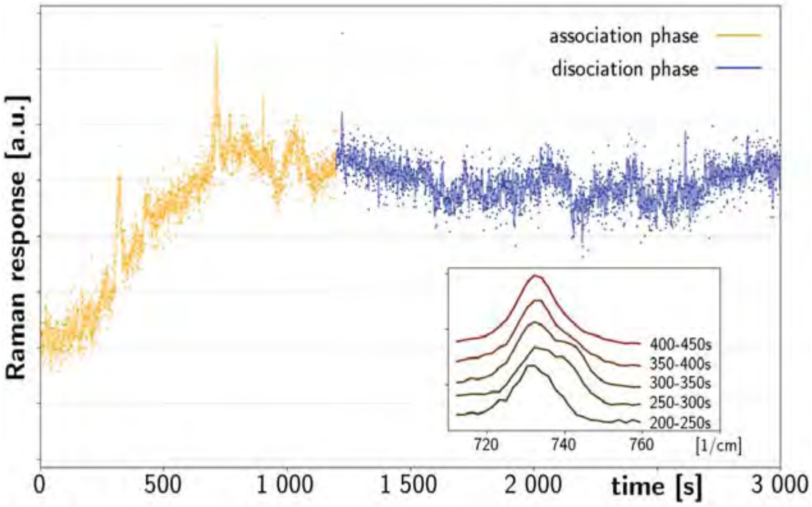


As a demonstration, consider Hessian that corresponds to solvation of glycine (C2H5NO2 diagonal block) with a water molecule (final HOH diagonal block); in the Figure (left), this binding results in non-zero off-diagonal blocks (scaled 10x for brevity of the Figure).

We will provide expansion formulas that approximate the shifts of vibrational frequencies based on the off-diagonal blocks; the presented formalism is independent of the way the Hessian was obtained - the force constants can come from DFT, molecular dynamics, etc., based on user preference.

The examples that we will analyze using the presentes framework cover (de)protonation of aminoacids upon changing pH, effects coming from explicit solvation of biomolecules, phosphate to Ag/Al_2O_3 core/shell nanoparticles binding [**1**], and other.

As an illustration, consider binding assay of adenine to a nanoscale silver substrate in a microfluiding setting; the Figure (right) tracks area of the adenine fingerpeak near 730 cm^{-1}. In the inset, the time-accumulated SERS spectra of the adenine fingerpeak confirm temporary establishment of the adenine-silver complex [**2**] during initial phase of the binding; the situation will be analyzed using the presented formalism.



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CARS Spectroscopic Imaging Using a >50-ps Supercontinuum Light Source

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Over the past decades, coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) have emerged as powerful label-free microscopic platforms [1]. Recent advancements have driven these techniques in spectroscopic imaging. In particular, SRS microscopy boasts microsecond acquisition times within a spectral range of 200 cm^{-1} [2]. On the other hand, CARS microscopy has significantly broadened spectral coverage to approximately 4000 cm^{-1}, encompassing the entire region of vibrational fundamentals [3,4]. Despite the interference of the nonresonant background (NRB), spectroscopic CARS microscopy instead utilizes this NRB as a local oscillator for phase retrieval. This facilitates the accurate extraction of the imaginary part of $\chi^{(3)}$.

In this presentation, I will introduce CARS spectroscopic imaging using a non-mode-locked >50-ps supercontinuum light source for capturing all vibrational fundamentals. I will also focus on second harmonic generation (SHG). While collagen and muscle myosin are well-known harmonophores in biological tissues, we demonstrated the presence of another harmonophore contributing to unique morphologies in biological tissues [5].

As an example, Figure 1 presents CARS and SHG images of the mouse dentate gyrus, highlighting numerous SHG bright spots (yellow) surrounding cell bodies (black), identified as originating from ciliary rootlets in neurons.

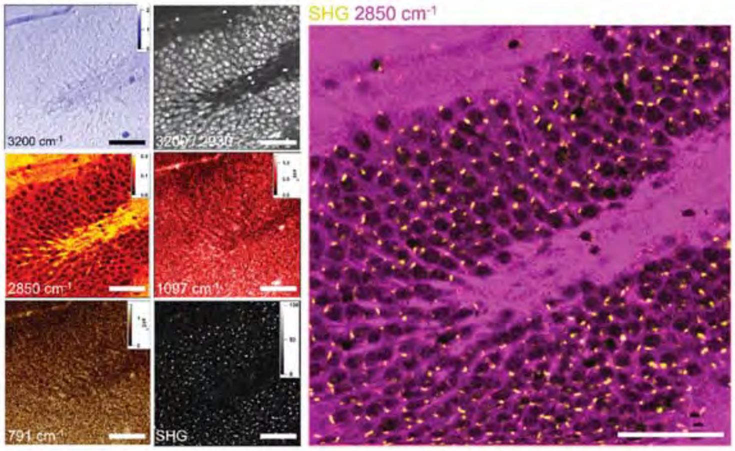

Figure 1: On the left, CARS images at 3200, 2850, 1097, and 791 cm^{-1}, as well as the ratio image of 3200 cm^{-1} to 2930 cm^{-1}. On the right, merged image of the CARS at 2850 cm^{-1} (magenta) with the SHG (yellow).

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Raman Imaging Reveals a Novel Membrane Component in the Sporangium Wall of a Rare Actinomycete

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The rare actinomycete *Actinoplanes missouriensis* produces unique spherical structures called sporangia, which contain a few hundred spores. Recent studies using electron microscopy [1] and lipidome analysis have shown that the wall of the sporangium consists of three distinct layers, but a chemical understanding of the intact *A. missouriensis* sporangium wall remains elusive. In the present work, we used Raman imaging with multivariate curve resolution–alternating least-squares [2] (MCR–ALS) to visualize the constituents of *A. missouriensis* sporangia (Fig. 1) in a nondestructive manner. In addition to proteins and carotenoids that are abundant in the spore and sporangium matrix, we detected a component that is highly localized to the sporangium wall. The MCR–ALS-derived Raman spectrum of this component is similar in many respects to that of lipids. However, it lacks the *cis*-C=C stretching band at ~1650 cm^{-1} and the ester C=O stretching band at ~1740 cm^{-1}, and yet it shows unusual bands at ~1550 and ~1615 cm^{-1}. To obtain more information on the unknown component of the sporangium wall, we performed multimodal nonlinear optical imaging [3], including multiplex coherent anti-Stokes Raman scattering [4] (CARS) and second harmonic generation (SHG). The SHG signal was specifically observed from the sporangium wall, suggesting that the layer containing the unknown component (most likely the thickest middle layer) has a noncentrosymmetric structure. Using a knockout strain of *A. missouriensis*, we found that this component is a type of polyketide, possibly with conjugated chain(s). Raman and other nonlinear optical imaging modalities have enabled the discovery of an unprecedented molecule associated with biological membranes.

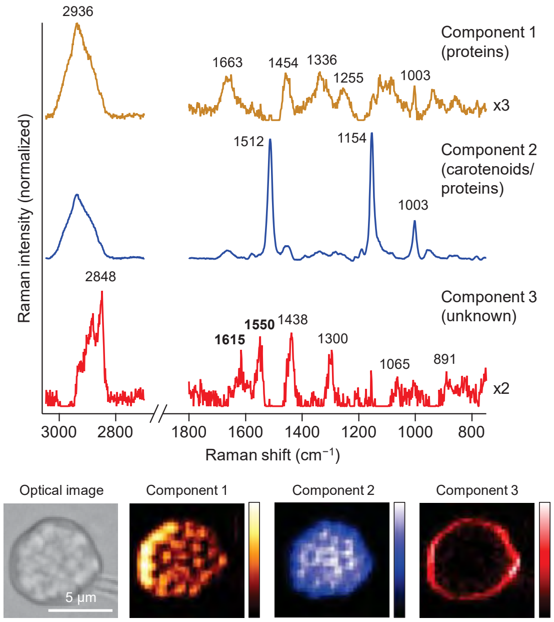

Figure 1. MCR–ALS Raman imaging (632.8 nm excitation) of an *A. missouriensis* sporangium in water.

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Monitoring cellular interactions in Organ-on-Chip models through Raman spectroscopy and multimodal imaging

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Recapitulating cell and tissue dynamics in tissue-engineered constructs that are applied as in vitro test systems or implants can contribute to a better understanding of mechanisms at the cellular interface; ultimately boosting clinical translation. We could demonstrate that time-resolved Raman imaging applied on Organ-on-Chip platforms enabled to monitor cellular dynamics of tumor-immune or host-microbiome interactions at various scales. Data obtained longitudinally or on a large sample size provide comprehensive information that require state-of-the-art analytics to unravel relevant tissue alterations. Therefore, in addition to conventional multivariate methods for spectral processing, algorithms conventionally applied for (spatial) Omics were translated to the analysis of Raman data. The workflow was initially implemented on the tissue scale but could be translated to in vitro models and liquid biopsies. Furthermore, correlative approaches were implemented to enhance the molecular characterization of the samples by integrating multimodal data from additional readouts such as metabolomics, transcriptomics.

Combined Raman Spectroscopy and Holo-Tomography Uncover First Morpho-Molecular Developmental Dynamics in Living Embryonic Colonies

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Early differentiation dynamics in embryonic stem cells (ESCs) are decisive for terminal fate reprogramming[1]. Profiling them noninvasively is fundamental not only to characterize their initial native state and safely monitor aberrations or developmental disorders, but also to maintain biocompatibility for their immense therapeutic potential[2]. Recently, RNA sequencing was exploited to describe the first 96 hours of embryonic development[2], but this procedure requires sample manipulation and destruction, leading to artifacts and impairing further usage of the characterized target. Here, we merge Raman Spectroscopy (RS) and three-dimensional (3D) Holo-tomography (HT) for label-free molecular and morphological profiling, respectively, of living pristine embryonic colonies through the first four days of embryonic differentiation (0-96 hours) (Fig. 1). We present human bias-free systematic data-analysis pipelines to extract quantitative and biologically interpretable information, assembled in a systemic-level chronological timeline with RNA expression profiles of the same *in-vitro* developmental model. We uncover synergistic morpho-molecular rearrangements, starting at an unprecedentedly early time point (2-18 hours). Subsequently, through the transcriptomic window of pluripotency exit (24-48 hours), as ectodermal-like and extra-embryonal endodermal-like multipotent germ layers start being generated unevenly, our RS data witness a phenotypic branching of ESCs into two new states, while HT-derived morphology depicts one transient pluripotency exit state. Machine-learning predictive modelling confirms the generalizability of our results on unseen samples. Our work offers a non-invasive window into unexplored early phenotypic dynamics in living embryonic colonies, maintaining their pristine nature for downstream analyses or medical applications.

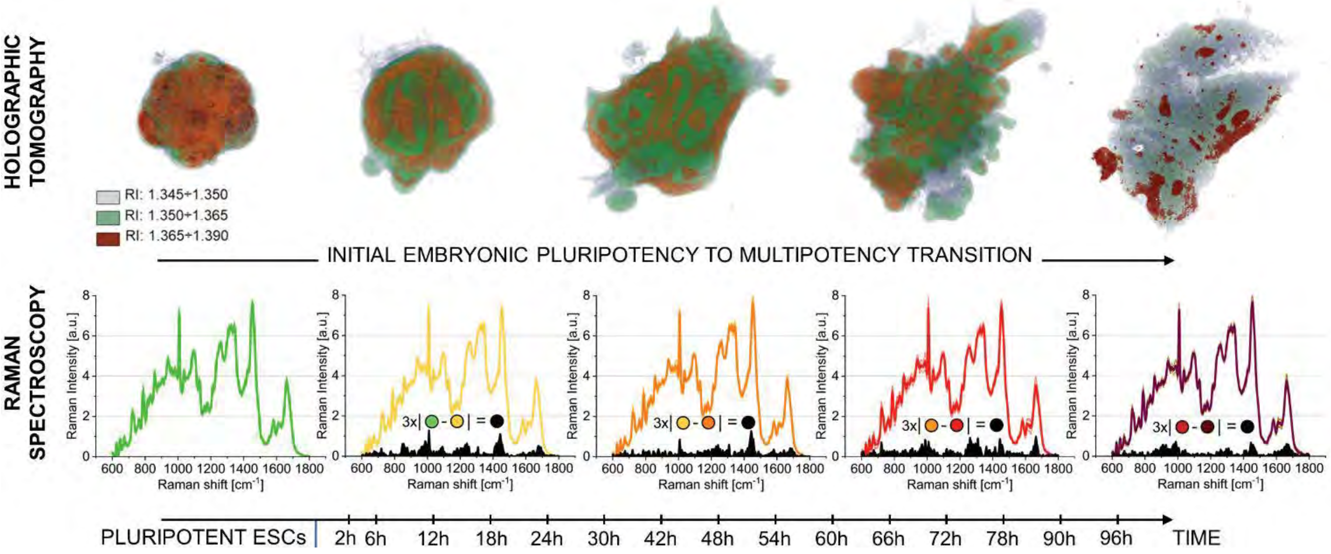

Figure 1: RS and HT representative data measured in living label-free embryonic colonies. On top, phase tomograms, 3D maps of the sample refractive index. Below, Raman spectra (600-1800 cm^{-1} fingerprint region), with absolute differential intensities in black. Circa 500 embryonic colonies were observed to achieve statistical significance of results.

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Mechano-Raman Spectroscopy

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Shear phonons are collective atomic-layer motions in layered materials that carry critical information about mechanical, thermal and optoelectronic properties. Phonon branches with co-directional atomic-layer motions carry unique information about the global structure and hidden interfaces in layered crystals and heterostructures, but they are not detectable in optical Raman measurements due to the very limited electron-phonon coupling. Here we utilize the propagating feature and mechanical coupling between shear phonons and localized plasmonic cavities to successfully realize direct characterization of ground-state shear phonons down to 4 cm^{-1} in energy by introducing mechano-Raman spectroscopy (MRS)[1]. MRS has the ability to characterize the global crystal structure with more than 10^8-fold enhancement and to accurately measure sub-picometre displacements under ambient conditions with a thermal-noise-free feature. The propagating behavior and the capacity of MRS to detect optically hidden interfaces are demonstrated. The broad tunability of plasmons makes the MRS technique a robust tool for extensive applications, including global crystal flaw detection, mechanical sensing and the mechanical modulation of light.

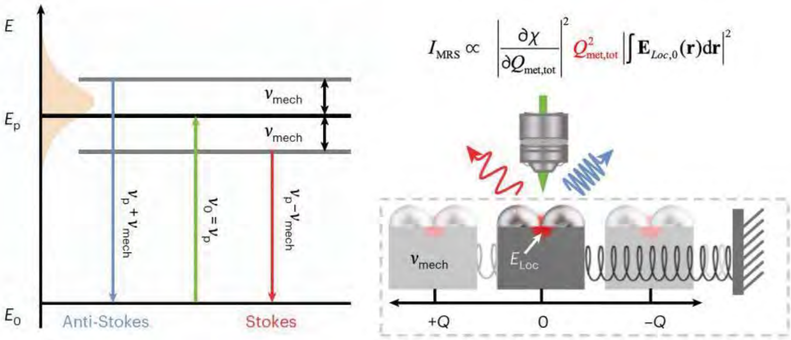

Figure 1: The Concept of Mechano-Raman Spectroscopy (MRS)

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OPEN-SOURCE FOR RAMAN SPECTROSCOPY HARMONISATION

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Raman spectroscopy is becoming a key technology used in the research and development for characterization of materials. Ideally, spectra should be comparable and linked to specific material properties. However, in real life, Raman spectra differ between instruments and depend on the spectrometer, optical path, or sample environment, among others, and only a limited number of calibration standards for Raman spectroscopy are available to date [1]. The CHARISMA project aims to harmonize and standardize characterization by Raman spectroscopy, including hardware, measurement protocols, and in silico methods, enabling end users to share digital spectral data through a FAIR database across domains and across the entire life cycle of diverse products.

We present https://github.com/h2020charisma/ramanchada2, an open-source, MIT-licensed, Python package that collects existing and novel state-of-the-art algorithms allowing the users to process Raman spectra, perform energy and efficiency calibrations and generate synthetic spectra based on user specification and/or data from DFT simulations. ramanchada2 is used as a core dependency of a FAIR Raman database, implemented as a Python API with a cloud backend and HDF5 storage for Raman spectra and metadata, following the Nexus format structure. The metadata query and spectra search API is compatible with the eNanoMapper database and the NanoSafety Data Interface [2]. With spectra and metadata converted to the eNanoMapper datamodel, The Nexus format enables to package multiple types of experiments into the same file, for example XRD ,Raman and biological assays using harmonized structure and terminology.

To facilitate Raman spectra analysis by end users, we are also developing oranchada, an user friendly wrapper of all ramanchada2 functionality. It is available as an add-on for the popular data mining software Orange and includes predefined harmonisation workflows.

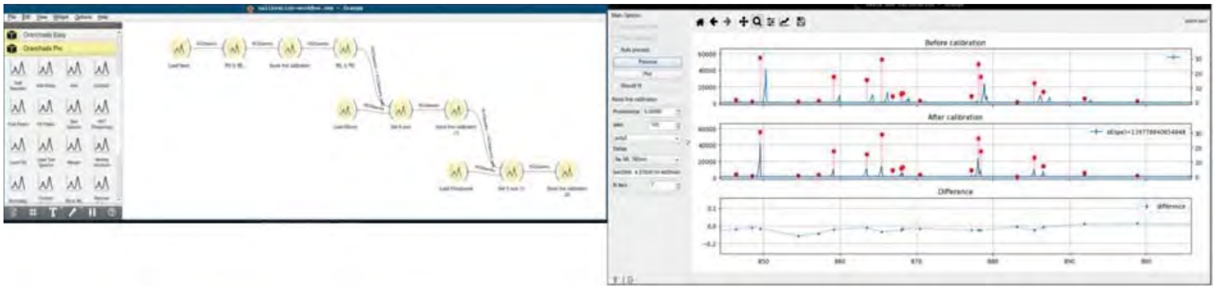

Figure 1: Screenshots of Oranchada https://github.com/h2020charisma/oranchada

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Raman Base: it's high time for revolution in handling and sharing of Raman-spectroscopic data

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The ability to directly and non-destructively probe complex chemical structures and interactions in real-time makes Raman spectroscopy an extremely powerful research tool. However, its efficiency is strongly conditioned by the availability of reference data and the tools to process, share, search, compare, and interpret spectra. All these factors are crucially dependent on the effectiveness of global scientific communication, but scientific articles by themselves are woefully inadequate, especially in terms of sharing and exploring large datasets. A paradigm shift is necessary to trigger substantial progress. The best example of this can be found in molecular biology: a free, open global database of DNA sequences (GenBank), with a dedicated, configurable search engine (BLAST), enabled unprecedented exploration of DNA, using sequences contributed by researchers worldwide. Global RNA and protein databases followed. Consequently, genomics, transcriptomics, and proteomics evolved, enabling many remarkable collaborative advancements, e.g., gene therapy and genetic engineering, the so-called molecular revolution. Raman spectroscopy urgently requires a similar shift towards an effective, global, purpose-built spectral database, to accompany or supersede the traditional pictures of processed spectra and tables of peak assignments. We assume that the willingness to test new approaches requires motivation and trust. We sincerely hope that we can motivate you with our big vision of a global hub for Raman spectroscopy and earn your trust by introducing you to its dynamically evolving precursor, which we named Raman Base.

Raman Base is an open online database of Raman spectra accompanied by a dedicated search engine, as well as a private data repository, and a workspace for efficient data processing and analysis (including AI). It offers everyone a platform to analyze, annotate, browse, compare, download, identify, organize, process, search, share, store, upload, view, and zoom into the details of Raman spectra, free of charge and in a user-friendly manner. We believe that any publication dealing with Raman spectroscopy would greatly benefit from being accompanied by all the relevant Raman spectra, including the raw source data. In Raman Base, these spectra can be easily retrieved by entering the DOI of the associated article and instantly displayed, further investigated, or used as a reference, enhancing their accessibility and usefulness for all interested parties. Our goal is to initiate and spread the discussion about the benefits of a global, centralized database of Raman spectra, gain more users and further support for maintaining and improving Raman Base, including volunteer software engineers, Raman instrument producers, and editors-in-chief of relevant journals. We would love to create a stable, robust virtual infrastructure to provide the integrating element to the global Raman community, and to accumulate big data to establish Ramanomics. We envision Raman Base as the paradigm-shifting global platform for Raman-spectroscopic research; however, we need your support to succeed. Please, visit https://ramanbase.org, where you can register for free and start using Raman Base, or contact us anytime at info@ramanbase.org.

We acknowledge the financial support from Ministry of Industry and Trade of the Czech Republic (MPO), project CZ.01.01.01/01/22_002/0000650 Development of a standardized nanostructured substrate and analytical system for routine analysis by surface-enhanced Raman spectroscopy.

Raman Diffusion-Ordered Spectroscopy

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Measuring the diffusion constant is a well-known method to determine the size (hydrodynamic radius) of molecules, aggregates and particles. Here, we combine the size sensitivity of such diffusion-based methods with the structure sensitivity of Raman spectroscopy by performing Raman diffusion-ordered spectroscopy (Raman-DOSY), the Raman analog of NMR-DOSY, and the complement of infrared-DOSY which we have developed previously [1]. We demonstrate Raman DOSY on samples containing small molecules, proteins, and supramolecules (micelles).[2]

The core of the Raman-DOSY setup is a flow cell with a Y-shaped channel containing two inlets: one for the sample solution and one for the pure solvent.[2] The two liquids are injected at the same flow rate, giving rise to two parallel laminar flows in the channel. After the flow is stopped, the solute molecules diffuse from the solution-filled half of the channel into the solvent-filled half at a rate determined by their hydrodynamic radius. Their arrival is recorded in a spectrally resolved manner by Raman micro spectroscopy. From the time series of Raman spectra, a two-dimensional Raman-DOSY plot is obtained, which has the Raman frequency on one axis and the diffusion coefficient (or equivalently, size) on the other. In the case of mixed samples, the Raman-DOSY plot cleanly separates overlapping Raman peaks arising from molecules of different sizes, and we obtain size and structural information of all compounds in the mixture.

Raman-DOSY is label-free, does not require deuterated solvents, and can be applied to samples that might be difficult to investigate with other diffusion-based spectroscopy methods.

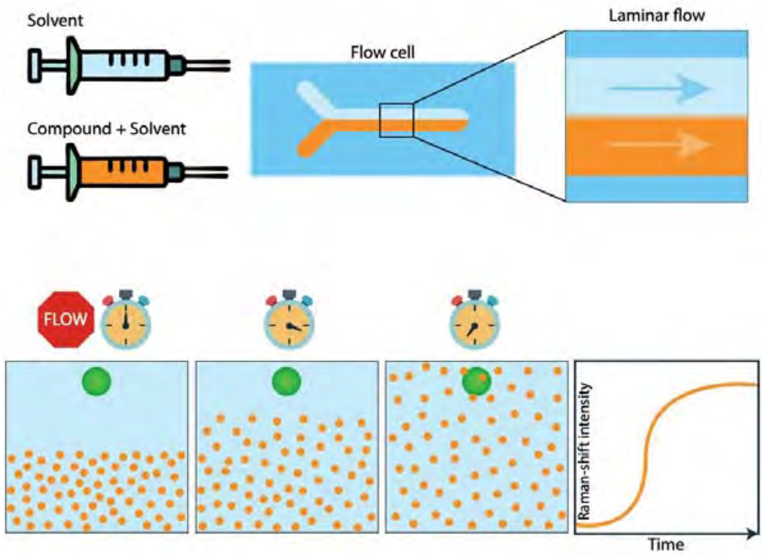

Figure 1: Schematic of a Raman-DOSY experiment.

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Background-free Raman spectroscopic measurements enabled by tailor-made dual-wavelength diode lasers

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In Raman spectroscopy, especially in real-world applications outside the laboratory, weak Raman signals are often masked by multiple orders of magnitude more intense background disturbances such as sample fluorescence and ambient light. Different techniques utilizing the direct relation between Raman signals and excitation wavelengths have been presented to retrieve the Raman signals under such conditions. Shifted excitation Raman difference spectroscopy (SERDS) is based on a physical approach and requires subsequent measurements at two excitation wavelengths. The Raman signals follow the wavelength shift, while background disturbances remain spectrally constant. Subtracting both spectra therefore separates the Raman signals from present background disturbances and fixed pattern noise, e.g., from a CCD detector.

Dual-wavelength laser light sources suitable for SERDS ideally provide alternating emissions with individual spectral widths well below 10 cm^{-1} and spectral distances matching the signal bandwidths of the targets under study, typically about 10 cm^{-1} for solid and liquid samples. Using diode lasers as excitation light source, these functionalities can be specifically designed and implemented at chip level.

In this work, tailor-made distributed Bragg reflector (DBR) ridge waveguide (RW) diode lasers and monolithically integrated dual-wavelength DBR RW diode lasers emitting at around 785 nm for SERDS [1, 2] will be discussed. Moreover, their implementation into compact, portable SERDS sensor systems for on-site investigations will be demonstrated.

The devices reach optical output powers in the 100-mW range. Heater elements implemented close to the DBR gratings allow a rapid adjustment of the spectral distance within a range of 2 nm in 50 ms. To speed up the alternating wavelength operation, e.g., for real-time SERDS measurements, monolithically integrated dual-wavelength devices allow alternating wavelengths operation at switching frequencies in the kHz-range.

The reported devices were implemented into in-house developed turnkey systems [3], delivering the necessary temperature and current control for laser operation in in-house developed portable SERDS sensor systems for real-world investigations. Selected experiments, e.g., on-site soil analysis [4] and discrimination of coloured textiles [5] will demonstrate the capabilities of these diode laser devices and systems.

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Novel Instrumentation for 2D Characterization: Combined Magneto-Optical Magneto-Transport

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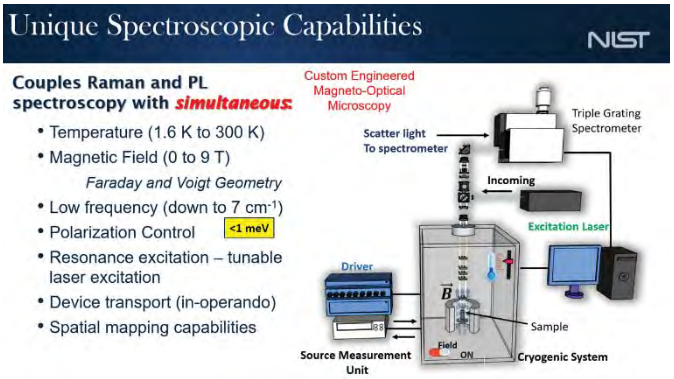
Raman spectroscopy, imaging, and mapping are powerful non-contact, non-destructive optical probes of quasiparticles and fundamental physics in graphene and other related two-dimensional (2D) materials, including layered, quantum materials. An amazing amount of information can be quantified from the Raman spectra, including layer thickness, disorder, edge and grain boundaries, doping, strain, thermal conductivity, magnetic ordering, and unique excitations such as magnons and charge density waves. Most interestingly for quantum materials is that Raman efficiently probes the evolution of the electronic structure and the electron-phonon, spin-phonon, and magnon-phonon interactions as a function of laser energy and polarization, temperature, and applied magnetic field. Our unique magneto-Raman spectroscopic capabilities will be detailed, enabling spatially-resolved optical measurements while simultaneously measuring electrical transport in a back-gated graphene Hall bar device. Raman and electrical data from an hBN-graphene-hBN device operating in the quantum Hall regime will demonstrate our novel capabilities [1]. In addition, unconventional quantization plateaus from a PNP junction created via spatial photodoping by the Raman laser will be presented. Lastly, results from a series of 2D magnetic material systems showing multi-quasiparticle interactions observable in our novel measurement system will be highlighted [2,3].

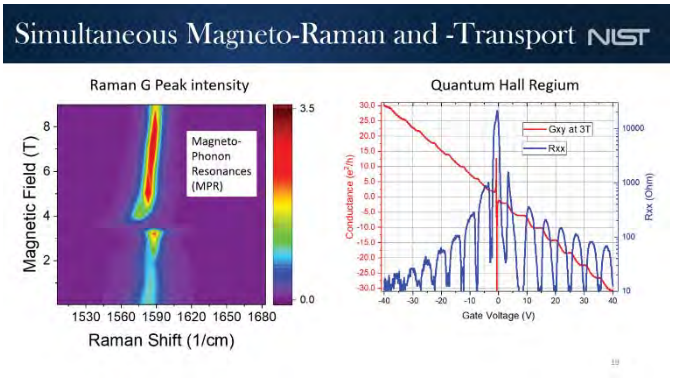


Unique Spectroscopic Capabilities

Couples Raman and PL spectroscopy with *simultaneous*:

- Temperature (1.6 K to 300 K)
- Magnetic Field (0 to 9 T)
  Faraday and Voigt Geometry
- Low frequency (down to 7 cm^{-1})
- Polarization Control <1 meV
- Resonance excitation – tunable laser excitation
- Device transport (in-operando)
- Spatial mapping capabilities

Figure 1: Unique Measurement Capability



Simultaneous Magneto-Raman and -Transport

Figure 2: Operando Raman of Gr/hBN Hall Bar

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Accurate wavenumber and intensity calibration for standardization of Raman spectroscopy

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Accurate wavenumber and intensity calibration is an important step towards the standardization of Raman spectroscopy allowing for correct interpretation of measured spectra. Quantitative Raman spectroscopy, by which we can determine the absolute concentrations of molecules, also requires calibrated spectrometers. The determination of absolute concentrations from Raman spectra requires the knowledge of absolute Raman cross-sections which can be measured on calibrated spectrometers.

In the present work, wavenumber and intensity calibration of a Raman spectrometer was performed with the use of pure rotational Raman bands $(\Delta\nu = 0, \Delta \mathrm{J} = \pm 2)$ of H_2, HD, D_2 and vibration-rotation Raman bands $(\Delta\nu = 1, \Delta \mathrm{J} = \pm 2)$ of O_2 as primary standards in the low-wavenumber and fingerprint region (from −1000 to +1650 cm^{-1}) of the Raman spectrum. Wavenumber calibration was based on reference transition wavenumbers.[1,2] Intensity calibration was based on ratios of accurate theoretical Raman intensities for transitions from common rotational states, to eliminate temperature effects. Wavelength dependent polarizability anisotropy (γ), and their ro-vibrational matrix elements $(\langle\psi_f|\gamma|\psi_i\rangle^2)$ which are needed for the evaluation of rotational Raman intensities of hydrogen molecule and isotopes, were accurately calculated for the purpose.[3,4] Polarization dependence of the spectrometer was corrected to ensure that all these bands have the correct depolarization ratio, $\rho = 0.75$. The calibrated Raman spectrometer was used to measure standard Raman spectra of carbon tetrachloride, benzene, cyclohexane, toluene and benzonitrile, for which the relative Raman intensities and depolarization ratios were determined with carefully estimated uncertainties. Accuracy of the calibration was checked using anti-Stokes to Stokes Raman intensities to determine the temperature of the liquids, using multiple bands spanning from −992 to 992 cm^{-1}. A comparison with measured temperature revealed temperatures to be within 1.5 K, showing Raman intensities were accurate to within 2-3%.[5]

In the high-wavenumber region (from 2400 to 4200 cm^{-1}), all the observed vibration-rotation bands $(\Delta\nu = 1, \Delta \mathrm{J} = \pm 2)$ of H_2, HD and D_2 were used in a least-squares analysis scheme. Weights were applied to data from pairs of bands originating from common rotational states. In this analysis the intensity correction curve and temperature were determined simultaneously, with an uncertainty of about 3%.[6]

Relative Raman intensities of selected liquids (in the fingerprint region) obtained from accurate measurements serve as common laboratory standards for intensity calibration with error within 5%. Our calibrated Raman spectrometer is applicable for experimental determination of Raman cross-sections owing to its high accuracy calibration.[7]

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Semiconducting Nanowires for Metrological Evaluation of Spatial Resolution in Raman Microscopy

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Raman microspectroscopy is a widely used technique across multiple fields where chemical mapping of materials is required with sub-micrometre resolution. Whilst spatial resolution of Raman microscopy is a key performance parameter for such studies, there are currently no documentary standards and very limited reference samples available to address this need. Here we propose and evaluate a new reference sample for metrologically measuring the spatial resolution of a confocal Raman microscope, which has been tested through an interlaboratory comparison involving institutions in 10 countries.

The proposed method of spatial resolution measurement is based on a line-spread function measurement using a nanowire feature that has a diameter much less than the expected point spread function of the instrument [1]. In order to provide metrological traceability it is important that the nanowire features can be uniquely and repeatably identified, so that the nanowire dimensions can be measured using metrological atomic force microscopy (AFM). This is achieved using the dielectrophoresis concept, where inorganic semiconducting nanowires can be controllably deposited across gaps between pairs of metal electrodes [2]. Other requirements of the sample are that it exhibits high Raman scattering contrast with the substrate, and is robust enough to endure repeated laser exposure. A VAMAS (Versailles Project on Advanced Materials and Standards) international interlaboratory comparison study has been conducted to evaluate the proposed sample and associated measurement protocol. We will present the outcomes of this study.

As interest in super-resolution Raman spectroscopy methods grows, there is an increasing need for spatial resolution measurements that can be applied for resolutions < 100 nm [3]. The techniques developed in this work are scalable and we have considered their feasibility for use as a reference sample for tip-enhanced Raman spectroscopy (TERS), including a demonstration using a nanowire with ~ 5 nm diameter.

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Verification of Si Wafer First-order Phonon Peaks for Reliable Calibration of Raman Microscopes

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Raman microscopes are widely used in various fields and their spectral resolutions differ greatly depending on the system and optical components. Thus, the microscopes must be calibrated before measurement to obtain reliable results. Although the first-order phonon peak of Si wafers at ~520 $\mathrm{cm^{-1}}$ is generally used as a calibrant of Raman microscopes, not only is it unclear how the positions of the first-order phonon peaks are comparable over Si wafers of different manufacturers, dopant types (non-doped, n-type and p-type), and crystal orientations (100, 110 and 111), but they also shift with the temperature (~−0.02 $\mathrm{cm^{-1}/K}$ or $\mathrm{cm^{-1}/°C}$) and residual stress (tensile or compressive).

In this study, we examined the changes in the position of the first-order phonon peak at different temperatures (−3, 0, 15, 30, 45 and 60 °C) using a HeNe laser at 633 nm and its plasma lines. Because a comparable linear relationship between the temperature and the wavenumber was obtained regardless of the Si wafer examined as shown in Figure 1, most commercially available Si wafers can be used for the calibration of Raman microscopes. Based on this equation, the wavenumber at 23 °C was calculated as 520.63 $\mathrm{cm^{-1}}$ and is consistent with the value of NMIJ CRM 5606-a (520.45 $\mathrm{cm^{-1}}$ ± 0.28 $\mathrm{cm^{-1}}$) reported previously [1]. Although shifting of the peak was introduced by the laser power due to an increase in temperature at the laser spot, it was less sensitive than broadening of the peak width. A peak shift was observed with a 532 nm laser at 2.1 mW using a 100× air objective lens (numerical aperture 0.9), but this did not occur with a 633 or 785 nm laser even at more than 10 mW. Thus, less laser power should be used to calibrate Raman microscopes using the first-order phonon peak of Si wafers under high-resolution conditions, especially for a 532 nm laser.

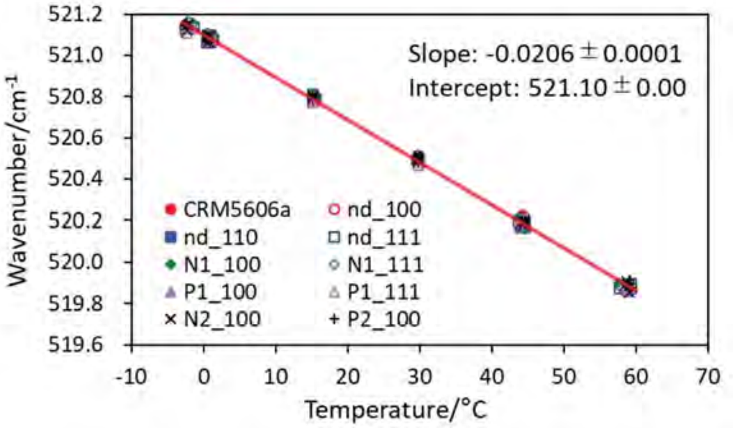

Figure 1: Relationship of temperature and wavenumber for different types of Si wafers (None-doped, nd; n-type, N; p-type, P). Numbers after N and P are ID of manufactures.

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Porphyrin metalation mechanism by ferrochelatase in gram-positive pathogenic bacteria

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In 2015 it was discovered that monoderm bacteria produce heme *b* using a biosynthetic pathway, different from that used by humans [1]. In detail, coproporphyrin ferrochelatase incorporates ferrous iron into coproporphyrin III to give ferric coproheme [2]. This porphyrin is decarboxylated to yield heme *b* by the coproheme decarboxylase enzyme in the final step of this biosynthetic process [3,4].

This seminar will focus on the recent insights into the mechanisms of iron incorporation into the physiological substrates, coproporphyrin III, as obtained by the combination of resonance Raman spectroscopy and X-ray crystallography [2,5-8].

The porphyrin metalation mechanism includes the formation (observed both in the crystal and in solution) of a saddle-distorted, stable intermediate that is predominant at the beginning of the titration. This intermediate species results from the readjustment of the hydrogen bond interactions of the coproporphyrin III propionates (in position 2,4,6,7) with the protein scaffold during the enzymatic catalysis. Once the propionates have established the interactions typical of the coproheme complex, the distortion slowly decreases to reach the almost planar final product. These results provide new and intriguing information on the catalytic reaction pathway. The understanding of the structure-function correlation of enzymatic mechanisms in heme biosynthesis is essential for the development of new therapeutic strategies.

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Deep UV Resonance Raman spectroscopy as potential tool for molecular investigation of biomolecules

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Thanks to the resonance enhancement, deep UV Resonance Raman (DUVRR) spectroscopy benefits of some advantages with respect to the conventional off-resonance Raman technique, such as i) the strong increment of the detection limit which enables to collect high signal to noise Raman spectra even in the presence of very low concentrated samples, ii) the unique selectivity of specific chromophores in the sample based on the fine tuning of the DUV excitation wavelength and iii) the suppression of the interfering fluorescence background which often limits the analysis of Raman signal. This makes DUVRR a powerful method for exploring, with molecular sensitivity, complex systems such as biological molecules, including peptides and proteins, nucleic acids, lipids and small organic species [1]. By opportunely tuning the excitation wavelengths in the deep UV range the Raman signals arising from the nucleic acid bases in DNA and RNA can be selectively intensified, while sugar and phosphate group vibration bands are practically negligible in UVRR spectra of nucleic acid as well as any other interfering signal of the solvent. Similarly, the Raman vibrational bands of amide bonds and aromatic amino acid side chains can be enhanced in the spectra of polypeptides and proteins, thus isolating spectral features very informative on the secondary and tertiary structures of these biomolecules. The detailed analysis of DUVRR spectra of biomolecules can give unique insights on the structural arrangement of specific molecular moieties, their interactions with the solvent environment and the establishment of specific interactions. This has been demonstrated to be a very informative approach for the study, for instance, of the structural stability of nucleic acids in unconventional solvents, in the aggregation processes of proteins or in determining the nanostructure of supramolecular architectures [2-4]. In this contribution, we would like to give a wide overview on the opportunities and advantages offered by the multi-wavelengths DUVRR spectroscopy for the molecular characterization, in situ and operando conditions, of simple and more complex biological species.

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Understanding [NiFe] Hydrogenases by Resonance Raman Spectroscopy

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[NiFe] hydrogenases are metalloenzymes that catalyse the cleavage and evolution of hydrogen (H_2). Thus, they represent valuable model systems for sustainable energy conversion utilizing H_2 as a clean fuel. Besides multiple iron-sulphur (FeS) clusters, [NiFe] hydrogenases contain a unique catalytic metal site featuring sulphur-coordinated nickel (Ni) and iron (Fe) as well as biologically uncommon CO and CN^- ligands (Fig. 1). Since these diatomics give rise to structurally sensitive and spectrally isolated bond stretching vibrations (1900–2100 cm^{-1}), infrared (IR) spectroscopy has long been used to study the [NiFe] site of these enzymes (Fig. 1). However, structural details are difficult to extract by this approach, and no insights into low-frequency vibrations that inform on metal-ligand bonding are possible.

To overcome this limitation, we have introduced resonance Raman (RR) spectroscopy as a complementary technique that selectively probes all metal-ligand coordinates of [NiFe] hydrogenases (Fig.1).[1,2] Here, prominent signals between 400 and 650 cm^{-1} reflect normal modes with dominant contributions from Fe–CO/CN stretching and bending coordinates of the [NiFe] active site. Supported by ^{13}C labelling and normal mode analysis on the density functional theory (DFT) level, these vibrations could be assigned and utilized to explore the structure of the $Fe(CO)(CN^-)_2$ moiety and the [NiFe] site as a whole.[1,2] This way, detailed information on the geometry, spin state, and protonation of two catalytic [NiFe] intermediates was obtained. In addition, signals below 400 cm^{-1} can be assigned to metal-sulphur vibrations, which may reflect Fe–S modes of the FeS clusters or Ni–S modes of the catalytic NiS_4 moiety. In fact, both sets of modes can be selectively probed and analysed by varying the excitation wavelength, as verified by ^{64}Ni labelling and DFT calculations.[2]

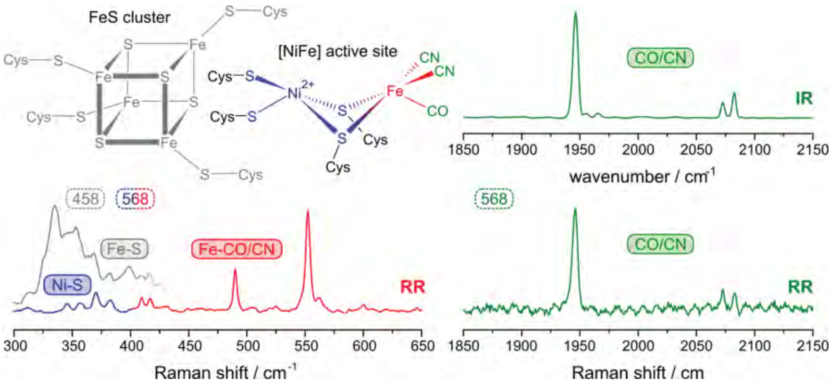

Figure 1: Structural Features and Vibrational Spectra of [NiFe] Hydrogenase

Despite these merits, the RR analysis of [NiFe] hydrogenases is challenging since multiple light-sensitive [NiFe] states can be photoconverted by the probe beam.[1] This complicates their detection and the state-specific assignment of signals, but two strategies can be used to meet this challenge. (1) The assignment of signals to specific [NiFe] states can be facilitated by applying other spectroscopic techniques under conditions mimicking those of the RR experiment.[1] (2) Both problems can be circumvented by expanding RR excitation and detection regimes. This way, photoconversion is limited by increasing the excitation wavelength, and unambiguous assignment of low-frequency metal-ligand vibrations is achieved by extending the RR experiment to high-frequency CO/CN stretch modes that can be matched to state-specific signatures known from IR spectroscopy (Fig. 1).[3]

In total, the presented studies illustrate an integral approach that combines wavelength-dependent resonance Raman spectroscopy with other experimental and computational techniques to gain detailed and selective insights into complex and light-sensitive multi-cofactor enzymes.

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Revealing novel structural and functional features of iron-sulfur clusters in proteins by (SE)RR spectroscopy

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Resonance Raman (RR) spectra of iron sulfur (FeS) clusters embedded into proteins, peptides and model compounds, reveal selectively enhanced modes involving the metal-ligand stretching coordinates that are sensitive to the cluster type, structure and symmetry [1]. Surface-enhanced RR (SERR) spectroscopy offers unparalleled sensitivity for studying FeS containing proteins found in a close proximity of plasmonic Ag surfaces that can act as electrodes. SERRS thus allows coupling of electrochemical with spectroscopic methods for exploration of redox processes and reaction mechanisms of FeS proteins [2].

Here, we show the ability of (SE)RR spectroscopy to unravel remarkable features of FeS clusters found in i) the human and bacterial DNA repair enzymes that house [4Fe-4S] clusters of unknown function [2,3] and ii) the unique non-cubane [4Fe-4S] cluster found in heterodisulfide reductase HdrB [4].

We reveal essential biophysical differences between the full-length and truncated human endonuclease hNTH1, the latter of which is structurally analogous to bacterial enzymes. This implies that mechanistic insights provided by studies of bacterial homologues or truncated forms of hNTH1 as model systems may not be applicable to the physiologically relevant human protein, and that the previously suggested DNA mediated electron transfer role of the FeS in these enzymes requires revising.

By coupling RR spectroscopy with density functional theory (DFT) calculations and homology modelling, we disclose the structural, electronic and vibrational properties of the non-cubane cluster found in the active site of HdrB. We reveal the unique RR fingerprint of the cluster that represents a fusion of [2Fe-2S] and [3Fe-4S] centers, with one cysteine thiolate playing a role of an inorganic sulfide, and its unexpected conformational change in the presence /absence of the substrate [4].

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Investigation of the Co-Aggregation of β-Lactoglobulin and Albumin Whey Proteins using a Multi-Technique Approach

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Soft materials, formed through various thermal treatments [1] and utilizing different protein mixtures, can exhibit diverse physical and viscoelastic characteristics. Whey proteins, known for their nutritional value and functional properties such as gelation, emulsion, or foam formation [2], are commonly used in food formulations. The predominant globular proteins in whey are β-lactoglobulin (BLG) and albumin (BSA), often employed as models due to their cost-effectiveness and propensity to aggregate under various environmental conditions. This study aims to provide molecular and structural insights into the aggregation processes of BLG [3] and BSA whey proteins in both diluted and crowded solutions. Aqueous protein solutions with low pH are examined at different BLG-BSA molar ratios, in a diluted regime to induce fibril formation, and in a crowded regime to facilitate the development of hydrogel networks. A multi-technique spectroscopic approach is used to probe the co-aggregation process at different length scales, as shown in Figure 1. Molecular-scale information is obtained through FTIR, CD, and UVRR spectroscopies, while structural details at the nanoscale are derived from the SAXS technique. The structure of nanoaggregates is influenced by molecular interactions, with branching determining the viscoelastic properties of the system [4]. These properties are further characterized using HD-TG non-linear spectroscopy.

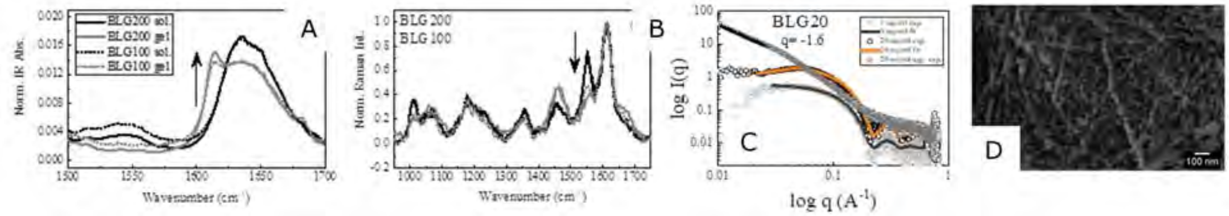

Figure 1: FTIR (A) and UVRR (B) spectra of BLG solution and gel. SAXS curves (C) of BLG monomers and fibrils. SEM (D) image of BLG fibrils.

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Vibrational effects in Metal Organic Frameworks induced by CO_2 uptake

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Metal-organic frameworks comprise a very promising class of materials which seems to fit perfectly on a broad range of application fields, from electrochemical charge storage, catalysis and selective adsorption of gasses, all the way up to superconductors and topological insulators [1]. Raman scattering as well as IR-absorption have been applied many times before [2,3] in order to study the interaction of CO_2 with MOFs via the recorded CO_2 Fermi resonance bands and the changes induced in the vibrational spectra of the host material. Herein we present new results from two MOF subclasses, the zeolite imidazolate frameworks as well as fluorinated MOFs which contain fluorine atoms directed towards the channels of the material, giving emphasis on the low frequency Raman and far-IR vibrations, which are related to motions of entire molecules, as well as pore breathing and/or skeletal swing.

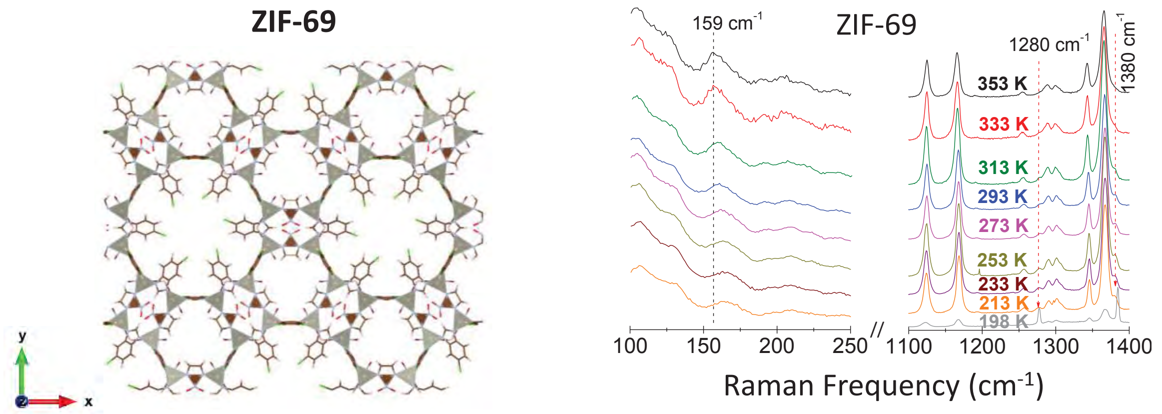

Figure 1. *Structure of ZIF-69, showing a highly porous structure with functionalized chloro-benzimidazole ligand (Cl atoms are denoted with green line). Raman spectra at variable temperatures and CO_2 pressure of 2 bar for ZIF-69. Red dotted arrows designate the CO_2 Fermi dyad peaks [3].*

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High-Pressure Raman Investigations of $AuSn_4$ and its Chemical Pressure Equivalent $PtSn_4$: Structural Transitions, Stability, and Superconductivity Insights

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Recently, $AuSn_4$ and $PtSn_4$ are found to be in the category of topological nodal line semimetals (TNLS). TNLS are systems where two or more bands are exactly degenerate at specific values of the crystal momentum in the first Brillouin zone (BZ) [1]. Both $AuSn_4$ and $PtSn_4$ are isomorphous in structures (orthorhombic space group Aba2). Among these, $AuSn_4$ alone exhibits superconductivity (SC) with a critical temperature of $T_c = 2.4$ K [2,3], as also confirmed by our own $\rho(T)$ measurements. Also, $AuSn_4$ has an interesting combination of both SC and topological properties which has benefits to look for Majorana Fermions which have a practical application to be used in fault-tolerant quantum computation [3]. Further, our high-pressure x-ray diffraction (HP-XRD) and HP Raman studies reveal that $PtSn_4$ can be considered as the chemical pressure equivalent of $AuSn_4$ at approximately 5 GPa. Notably, the HPXRD results for $AuSn_4$ show a structural change when the pressure exceeds 2 GPa. This structural shift is also evident in the HP Raman data, as illustrated in Figures (b) and (c), revealing that $AuSn_4$'s structure becomes unstable at pressures surprisingly lower than the comparable chemical pressure in $PtSn_4$. A recent DFT study showed the possibility of structural phase transitions in $AuSn_4$ due to the atomic displacements induced by the soft modes [4]. Figure (a) illustrates the Raman data, showing narrower, intense peaks at lower pressures. With increasing pressure, there is a noticeable shift in the Raman spectra towards higher wavenumbers in addition to the broadening of the peak. Interestingly, $PtSn_4$ under identical conditions showed only a shift, no broadening. These observations are consistent with our HP-XRD data. We analysed the HP Raman data by deconvoluting with Lorentzian line shapes (see Fig. (b) and (c)). Our analysis shows five modes for $AuSn_4$ at 0.31 GPa, which aligns well with expectations from the group theory predictions. With the increase in pressure, above 1.96 GPa, an additional mode is found to be necessary to describe the Raman spectra. This new mode may be a result of the phase transition observed in the HPXRD studies referenced earlier. Figure c represents the deconvoluted Raman modes for 3.6 GPa where the additional mode (M*) can be clearly seen. With these results, this research provides insights into the structural behaviours under pressure of these two systems. The important aspect of the study is finding the structural comparatives of both systems and finding out the differences in their physical properties like superconductivity in $AuSn_4$ which is not there in $PtSn_4$.

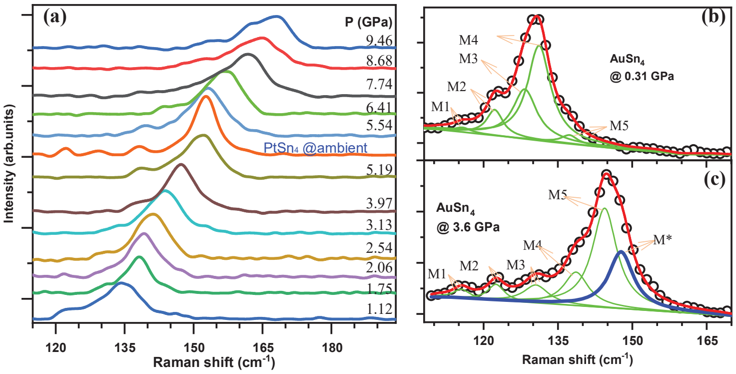

Figure (a) stack plot showing high-pressure Raman plots of $AuSn_4$. Spectrum corresponding to $PtSn_4$ in the ambient condition is also included here which shows a chemical pressure of ~5 GPa. (b) and (c) shows the spectral deconvolution respectively for 0.31 and 3.66 GPa Raman spectra of $AuSn_4$. Note an additional mode M* at 3.66 GPa.

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The Role of Structural Flexibility in Plasmon-Driven Coupling Reactions: A SERS investigation

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The plasmon-driven dimerization of 4-nitrothiophenol (4NTP) to 4-4′-dimercapto-azobenzene (DMAB) is a testbed for understanding bimolecular photoreactions enhanced by nanoscale metals, in particular, regarding the relevance of electron transfer and heat transfer from the metal to the molecule [1-3].

In this talk, we evaluate the influence of microscopic photoheating on the kinetics of a model plasmon-catalysed reaction. Direct measurement of the reaction temperature by nanoparticle Raman-thermometry demonstrated a dominant role of the thermal effect in the kinetic limitations of this multistep reaction [1]. At the same time, no reaction is possible by dark heating to the same temperature [2]. The role of heat was further illuminated by adding a methylene group between the thiol bond and the nitrophenyl, which adds structural flexibility to the reactant molecule [3]. Time-resolved surface-enhanced Raman-spectroscopy proves that this (4-nitrobenzyl)mercaptan (4NBM) molecule has a larger dimerization rate and dimerization yield than 4NTP and higher selectivity toward dimerization. It is concluded that the rate limiting step of this plasmonic reaction is the dimerization step. These results show that plasmon nanoparticles have the unique ability to simultaneously provide charge carriers for bond activation and localized heat for driving bimolecular reaction steps and thereby enhance several steps of complex tandem reactions simultaneously.

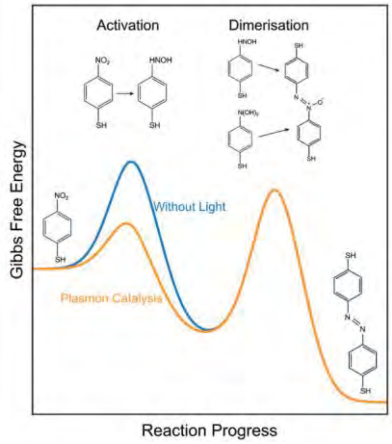

Figure 1: The two activation barriers involved in the dimerization of 4NTP to DMAB: the first barrier is lowered by the transfer of hot electrons. The second barrier is overcome by thermal activation of the intermediates.

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Controlling Excited State Localisation in Bichromophoric Photosensitizers via the Bridging Group

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The photophysical properties of a series of bichromophoric transition metal complexes, illustrated in Figure 1, are explored in a joint synthetic, spectroscopic and theoretical investigation.[1] The studied structures posses both organic and inorganic donor groups, such that both intra-ligand charge transfer (ILCT) and metal-to-ligand charge transfer (MLCT) states are accessible in the visible region, as observed previously.[2, 3]

The influence of a variable bridging group, situated between the organic donor and the acceptor, on the energetic order and character of the excited states is probed using a suite of methods, including steady-state resonance as well as time-resolved resonance Raman spectroscopy. Hereby we demonstrate that by variation of the bridging unit, the nature of the thermally equilibrated excited state can be switched from a short lived (~60 ns) emissive ^{3}MLCT state, to a long-lived (~45 μs) dark ^{3}ILCT/^{3}LC state. Spin orbit coupling (SOC) calculations reveal that the ^{3}ILCT/^{3}LC states are not directly populated. Instead, strong SOC between initially populated ^{1}MLCT states and their triplet counterparts, facilitates rapid intersystem crossing, after which the long-lived ^{3}ILCT/^{3}LC state is populated from the ^{3}MLCT state via internal conversion.

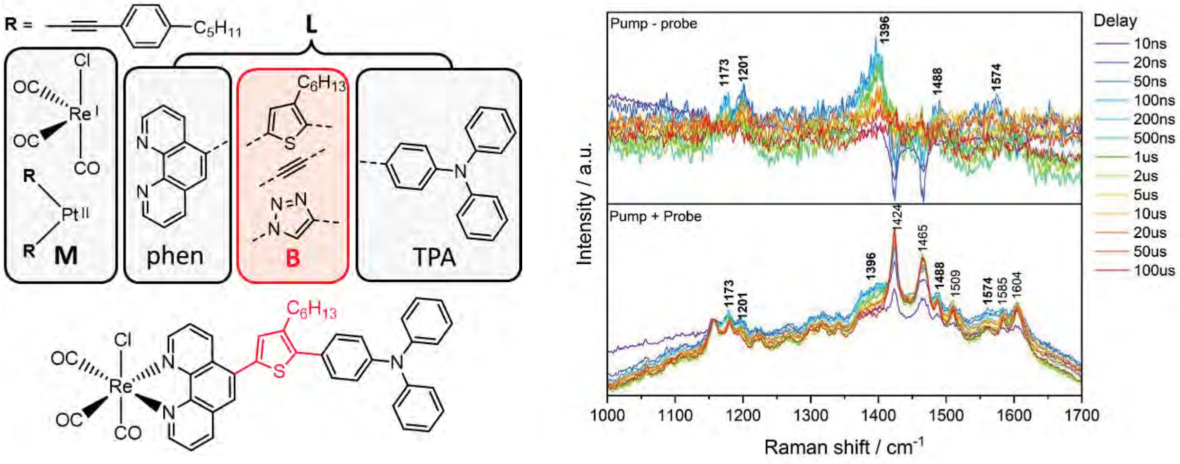

Figure 1: Left: Structure of investigated complexes, with the variable bridging unit indicated in red. Right: Time-resolved resonance Raman spectra of [ReCl(CO)$_3$(phen-thio-TPA)] obtained with 355 and 532 nm pump and probe pulses, respectively.

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Realtime tracking of femtosecond vibrational dynamics in liquid water

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Understanding the properties of aqueous solutions is key to determining the effects of the liquid environment on chemical reactions in biological systems. For this reason, vibrational relaxation and energy dissipation in liquid water have been intensely investigated via pump-probe experiments. However, the time resolution has so far been limited to a few tens of femtoseconds [1], preventing a direct exploration of the crucial role of high frequency stretching modes.

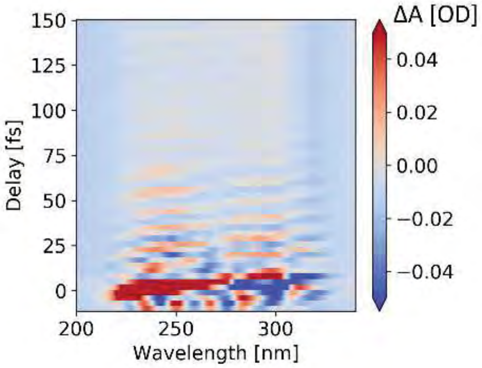

Figure 1: Colormap of the measured transient absorption signal of the UV probe wavelengths as a function of the pump-probe time delay.

Here, we present a time-resolved investigation of the vibrational dynamics initiated in liquid water by a sub-5 fs visible (VIS) pump pulse. The rapid evolution of the vibrational wave packet, created by impulsive stimulated Raman excitation in the ground state of the molecule, is then probed with sub-6 fs time resolution by monitoring the transient absorption using ultrabroadband UV probe pulses (Fig.1) [2]. In our experiment, the VIS pump and UV probe pulses are focused on a thin liquid jet operating in vacuum. Our data analysis reveals an oscillatory transient signal, measured here for the first time in the time domain, with a 10 fs period and a decay time of about 25 fs. These values are in good agreement with those expected for the O-H stretching mode in the ground state of liquid water [3]. These results prove that the investigation of high-frequency vibrational modes critically requires the use of few-cycle pump and probe pulses with sub-period duration, to coherently excite and to sample the oscillation, respectively. Improved statistics in the detection of the high-frequency mode is reinforced by the ultrabroadband nature of the probe, instrumental for investigating the dynamics in different spectral regions. In particular, the blue and red sides of the transient absorption exhibit out-of-phase oscillations, as a result of the strong spectral dependence of the coherent signal on the phase of the probe pulses. Our results pave the way for studying energy dissipation of vibronically hot bio- and organic molecules in aqueous solutions, in which the relaxation dynamics can occur at extremely fast time scales as a result of a dynamical interactions between the water molecules and internal modes of the solute along the OH stretching coordinate.

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Retrieving Genuine Nonlinear Raman Responses in Ultrafast Spectroscopy via Deep Learning

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Stimulated Raman spectroscopy (SRS) harnesses optical nonlinearities to access ultrafast transformations at the ground, for instance, of the optoelectronics properties in novel materials and photochemical transformations in biomolecules[1]. However, given the nonlinear nature, multiple sources contribute to the experimental signals and generate interrelated unwanted components, from random point wise fluctuations to structured baseline signals. Combined to the complexity of SRS lineshapes, these sources of noise cause loss of information and increase the measurement difficulty.

To enhance the resolution of nonlinear Raman techniques beyond these limitations, we have devised a deep neural network (NN) for denoising and baseline removal of raw SRS spectra[2]. By means of multiple convolutional kernels operating in parallel, it is possible to adapt the receptive field of the network to the informative features and treat the multiple spectral scales present in the SRS data. Tested on experimental data, the NN algorithm demonstrated superior performances with respect to standard postprocessing routines, allowing us to obtain noise- and background-free SRS spectra of fluorescent molecules and prototypical heme proteins.

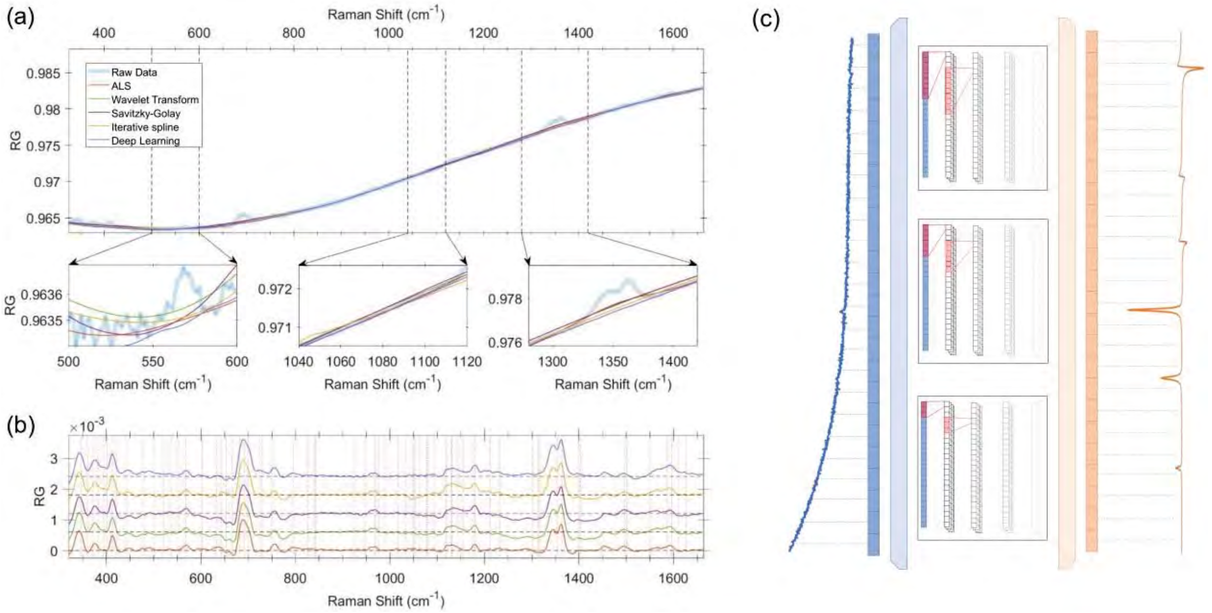

Figure 1: Performance variation of different post-processing routines on SRS data of Cyt C: baseline subtraction from raw data (a) and clean spectra (b). (c) Sketch of the parallel kernel approach.

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Sideband Raman Cooling of an Optical Phonon in Semiconductors

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Sideband Raman cooling of a specific vibration mode in trapped atom and optomechanical systems has produced plenty of opportunities in fundamental physics, precision metrology, and quantum science. Although theoretically proposed over 40 years, the laser cooling of specific lattice vibrations (i.e., phonon) remains challenging owing to solid structures' complexity. In 2016, we experimentally demonstrated the resolved-sideband Raman cooling of an individual optical phonon by strong exciton−phonon coupling in ZnTe nanobelts [1]. Motivated by the potential of atomic thin semiconductors as versatile quantum materials, we extend to two-dimensional semiconductor WS2 in 2022 [2]. As shown in Figure 1, by detuning the laser pump to a lower (higher) energy-resolved sideband to make a spontaneous scattering photon resonate with an exciton at an anti-Stokes (Stokes) frequency, the dipole oscillation of the optical phonon is photoelastically attenuated (enhanced) to a colder (hotter) state. These works open the laser cooling and control an individual phonon mode in semiconductors, which is fundamental to quantum technology based on lattice vibration.

Considering that the lowest cooling temperature is limited by the ratio of the optical transition damping rate to the target phonon frequency in a linear optical process, We theoretically and experimentally demonstrated the nonlinear coherent anti-Stokes Raman sideband (CARS) cooling of a lattice vibration in the semiconductor nanobelt with a single weak continuum laser excitation. They achieved ground-state cooling beyond the traditional resolved sideband limit owing to effectively suppressing the parasitic heating effect. This method is prospective to be extended to the quantum control in cold atoms, molecules, and cavity optomechanics fields. It opens a new insight into nonlinear light-matter interactions at the level of a weak light field.

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Tips, Fields and Molecules

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25 years after the first experimental realization of tip-enhanced Raman scattering (TERS), the method has continued to develop in terms of technology, theory, and applications. This keynote focusses on recent examples that illustrate how different application fields are driving new experimental developments.

A major driving force is the demand for faster acquisition of TERS spectra. It is important to understand that the lateral resolution in TERS is not adjustable and, unlike microscopy, continuous zooming with increasing resolution is not feasible. However, to improve TERS turnover times, one can correlate 'slow' TERS experiments with faster AFM-related techniques. By implementing this pre-filtering step, the number of necessary TERS spectra can ultimately be reduced. Force-distance experiments correlated with TERS data will demonstrate the principle on complex biological and polymer samples.

The tips are crucial for every TERS experiment, and various designs have been developed for specific applications. Liquid environments pose a particular challenge for TERS, and are essential for many applications in biology and chemistry. Under such conditions, particle-based TERS tips are generally unstable. The presentation will showcase a novel method for synthesizing silver islands under aqueous conditions as an example of the ongoing search for the optimal probe.

It is worth noting that even the best tips cannot be precisely controlled down to the atomic level or even just oriented exactly towards the sample. However, the presentation will introduce new approaches for flexibly controlling the field at the tip apex, as it is easier to control the light on the tip than the tip itself.

Proving Non-Thermal Plasmon Catalysis Mechanism Though Anti-Stokes SERS Spectroscopy

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Plasmon-assisted chemical reactions, in which the chemical reactivity of surface-bound reactants is significantly enhanced and altered by the plasmon-excited metallic nanostructure, have attracted tremendous attention as a promising heterogeneous photocatalyst. One hypothesis for the mechanism is the non-thermal vibrational excitation of surface adsorbed reactants: the energetic electrons, called the hot electrons, that are non-thermally excited during the decay of plasmons, transfer energy as a form of vibrational energy to the reactant. However, such a hypothesis has yet to be validated at the quantum state level. Here, we study the steady-state distribution of overtone-excited reactants prepared by plasmons. We find that the anti-Stokes peak of SERS spectra of 4-nitrobenzene (NBT) reactant placed in a plasmon-excited metallic gap, obtained under the illumination of continuous-wave (cw) visible light, is asymmetrically broadened due to the overtone-excited reactants. From the lineshape analysis, we determine that at least 40 % of the vibrationally excited NBTs are in the overtone states. We use the intensity and wavelength dependences of the overtone-state distributions to clarify the thermal and non-thermal contributions to the activation and identify the mechanism of non-thermal activation.

TERS enhancement and ‘quenching’

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Tip-enhanced Raman spectroscopy (TERS) has shown its distinct capability in characterizing materials with high chemical and spatial resolution. In the past years this technique has witnessed an extremely active development, which has shown to be operated at low temperature, ultra-high vacuum or electrochemical environment. [1]

In this talk, we demonstrate our newest insights into the impacts of excitation polarizations and tip-sample distances on the TERS enhancements. To obtain a well-defined sample system, organic thin films of approximately monolayer coverage are thermally evaporated onto ultra-smooth gold films. To gain distinct electric field distributions in the focuses of tightly focused excitation beams, radial and azimuthal polarizations are used (figure 1). [2] To explore the impacts of gap distances, both shear-force feedback and nanoparticle-on-mirror configuration are employed. The Raman enhancement and decrease of organic molecules in the nanogaps are discussed together with the recently reported ‘Raman quenching’ effect. [3] We analyse the results by considering the changes in the molecular geometry, ground state and excited state charge transfer processes, as well as the electromagnetic field enhancements at different nanogaps. Quantum chemistry calculations permit a detailed insight into the interactions of the organic molecules with its optical and chemical surroundings. By combining home-built techniques and first-principle methods, we elucidate the geometrical and electronic factors in understanding the Raman features from the nanogaps. Such an interdisciplinary study paves the way for obtaining insights of charge transfer effects in TERS. The mechanisms and questions disclosed in our studies could be essential for a broad application of TERS in realistic sample systems at ambient conditions.

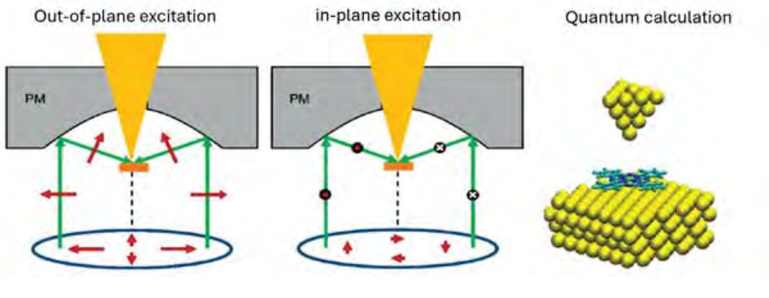

Fig. 1 Comparisons of TERS with out-of-plane and in-plane laser excitations. TERS scheme used in the quantum calculation.

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Acknowledgments

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Amplified plasmonic forces from DNA-origami scaffolded single dyes in nanogaps

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The precise positioning of single quantum emitters inside optical cavities is crucial for the fabrication of advanced photonic devices at the nanoscale. Aligning the emitting dipole with the peak optical field optimises Purcell enhancements that increase emission rates, efficiency, and directionality.[1,2]

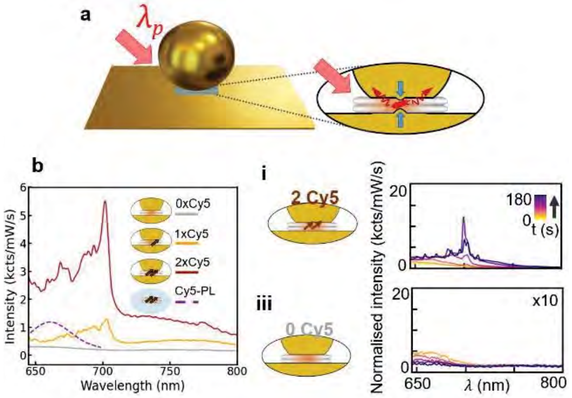

Figure 1. Assembly of NPoM cavities using DNA nanotechnology. a Gold nanoparticle attached to DNA nanostructure (grey) in the NPoM geometry which locates two-quantum emitters in the plasmonic gap. b Typical emission spectra of NPoMs with 0 (grey), 1 (orange) and 2 (red) quantum emitters in NPoM and in solution (purple).

Leveraging DNA origami (DNAo), we demonstrate the ability to nano-position single quantum emitters in ultra-narrow plasmonic gaps, enabling a detailed study of their modified light emission. As a result, the interaction of the tightly confined light of quantum emitters with atoms from the metal surfaces can be studied.

In the presence of 1 or 2 dye molecules inside the DNAo, emission from the NPoM construct acquires strong contributions from surface-enhanced-Raman scattering (SERS) of the dye (**Fig. 1b** and **i.**). Sharp vibrational peaks are observed which vanish when no dye is included in the construct (grey in **Fig. 1b**). This completely differs from bulk solution photoluminescence of the chromophore (purple dashed in **Fig. 1b**). These differences are confirmed by time scans (**Fig. 1i.** and **ii.**), which show the appearance of SERS peaks in the dye-labelled NPoMs, but weak featureless light-emission from the non-dye-labelled NPoMs. The progressive emergence of spectral features is shown to result from interaction of the dye molecule with its metallic environment.[3]

Detailed investigations show that each highly polarizable dye molecule strongly interacts with the neighbouring metal atoms, generating optical forces capable of rearranging atoms within the surrounding metal facet. Our findings suggest that a fundamental reconsideration of light-matter interactions at the nanoscale is required in such extreme naonconfinement systems.[3]

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Investigation of selection rules in tip-enhanced Raman spectroscopy using anisotropic materials

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Tip-enhanced Raman spectroscopy (TERS) provides Raman information with nanoscale spatial resolution by using the near-field light generated through local surface plasmon resonance at the apex of a sharp metallic tip[1]. A consequence of utilizing confined light is the modification of selection rules due to the breakdown of the long wavelength approximation. For example, our previous study showed defect-related Raman peaks in defect-free areas of graphene in sub-nanometer TERS analysis because of the high wavenumber of the near-field light[2]. Samples that have been studied in previous works for TERS selection rules were mostly isotropic, and few works have investigated anisotropic materials that show polarization dependence. Carbon nanotubes (CNTs) allow us to explore the selection rules along with the polarization effect. Here we report the investigation of TERS selection rules in CNTs.

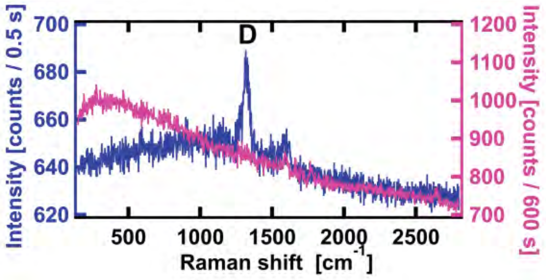

Fig. 1 Raman spectra from CNTs.

When we measured a TERS spectrum by locating a Au tip upon a single-walled CNT, an intense D-band, which is normally attributed to the presence of defects, was obtained (blue spectrum in Fig. 1). In contrast, almost no D-band was observed in the conventional far-field Raman spectrum of the sample (pink spectrum in Fig. 1). TERS imaging of the single-walled CNT in a 40 nm × 20 nm area showed that the D-band was observed across the entire CNT. The non-locality of the D-band is distinct from the defect-related features discussed in a previous study[3]. We attributed these unusual D-bands to the modification of Raman selection rules by highly confined near-field light at the tip-sample junction.

In the theory of CNTs, the polarization of the light should be considered for the electron excitation in a CNT. In our TERS system, CNTs are excited with the near-field light polarized perpendicular to tube axes. In this case, the electronic transition occurs between different cutting line numbers of the band structures in a CNT (Fig. 2). This transition is normally not observed because the signal is weak, however, owing to signal enhancement at a nanogap between the tip and the sample, the Raman signal was observed experimentally[4]. Based on the double resonance Raman scattering which typically activates the D-band, we also suggest that non-vertical electron transitions occur in both electronic excitation and Raman scattering due to the high wavenumber component of the near-field light[2] (Fig. 2). Because the second non-vertical transition during the emission of Raman scattered light plays a role in momentum conservation, which was satisfied with the presence of defects in the case of far-field, the D-band can be observed without the presence of defects[5].

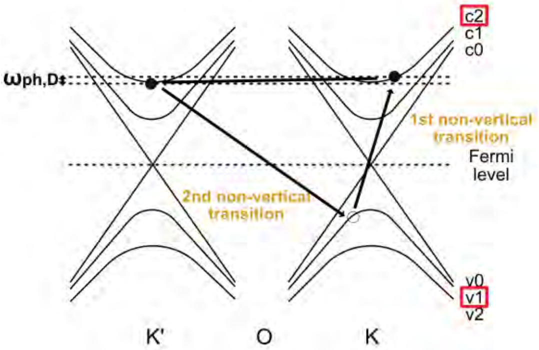

Fig. 2 Mechanism of the activation of unusual D-bands.

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Biochemical surface characterization of virions using Tip-enhanced Raman spectroscopy.

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Tip-enhanced Raman spectroscopy (TERS) is an effective technique for studying surface-bound molecules at unprecedented lateral resolution and chemical specificity. The potential of TERS was used to investigate surface features of SARS-CoV-2. In the process of virus replication, the first step is the attachment of the virion which is the entry pathway into the host cell. It is important to study the surface proteins of the virion as it determines the specific receptors on the cell surface, after which the next process of penetration, uncoating, and replication occurs.

TERS spectra of the SARS-CoV-2 virion were acquired with few 100 ms (100 – 300 ms) and the virions showed amide I bands at 1600, 1616, 1633, 1643, and 1658 cm^{-1} and amide III at 1240, 1272, and 1260 cm^{-1}. In addition to the secondary structure of SARS-CoV-2 virion, lipid spectral features were observed at 1763, and 1740 cm^{-1}. For selected virions, the protein and lipid spectral features of virions observed in TERS spectra were correlated and validated with additional nano-FTIR measurements. N-finder analysis was performed on the SARS-CoV-2 virion spectral datasets to detect the surface's secondary structure proteins and lipid distribution (Figure 1).

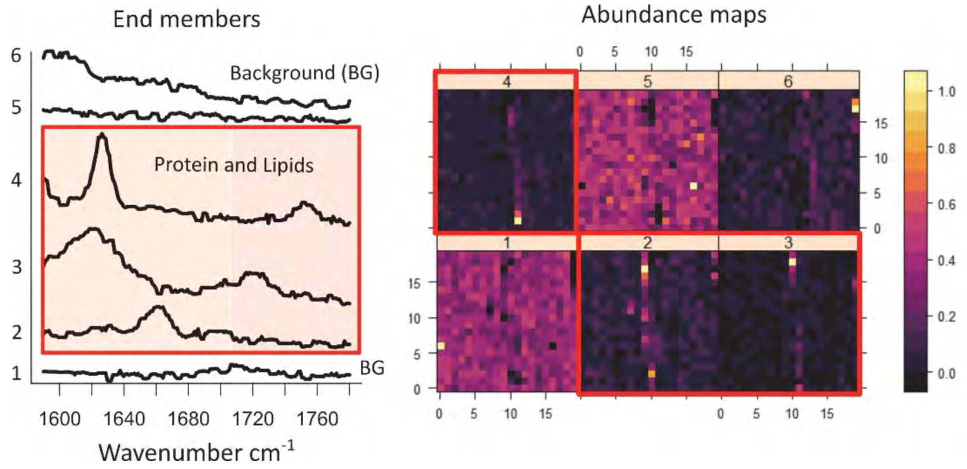

Figure 1: Protein and lipid distribution in SARS-CoV-2 virion.

Overall, TERS can detect the surface protein distribution of the virions with biochemical specificity.

Chirped-impulsive Raman spectroscopy

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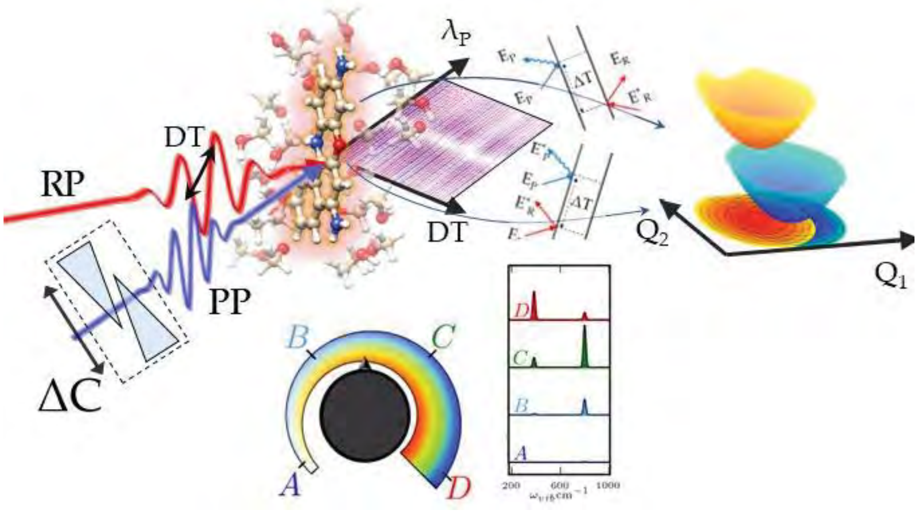
Photophysical and photochemical processes are often dominated by wavepacket dynamics among molecular vibrations pertaining to different electronic states and moieties. Dissecting the complex, often overlapping, spectroscopic signals arising during the journey from the reactant to the photoproduct to obtain structural and dynamical information is a challenge.

We recently demonstrated how chirped pulses, i.e. with a frequency spectrum unevenly spread over the temporal profile, can be the key to wisely direct molecular vibrations and detect the corresponding spectroscopic signals to overcome technical and conceptual hurdles.

Specifically: we demonstrate how to manipulate the ISRS signal using chirp as a control knob to (i) selectively excite desired vibrations and disentangle spectral components arising from different excited states, assigning the measured Raman features to the pertaining potential energy surface; (ii) efficiently retrieve time-dependent dynamics of vibrational excitations without scanning the temporal delay between the pump and probe pulses in a fast and effective way; (iii) map excited-state potential energy surfaces, measuring the sign and the magnitude of their displacements with respect to the ground state equilibrium configuration.



Structural Evolutions in the Excited State of Slip-Stacked Perylene Dye Array probed by Time-resolved Impulsive Stimulated Raman Spectroscopy

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Perylene bisimide (PBI) emerges as a prominent photoactive molecule within varied molecular assemblies. Notably, slip-stacked PBI arrays exhibit intriguing photophysical phenomena, encompassing excimer formation, charge transfer, and singlet fission processes. The electronic and structural couplings between adjacent PBI moieties wield considerable influence over exciton localization and delocalization dynamics. In the pursuit of comprehending fundamental structural alterations in the excited state, this study employs time-resolved vibrational spectroscopy on slip-stacked PBI arrays spanning from dimer to pentamer, offering insights into the development of localized excited states.In the ground state, Raman spectroscopy unveils foundational structural intricacies of PBI arrays up to pentamer. Especially, the 110 cm^{-1} mode, associated with the intermolecular out-of-plane motion of the PBI ring, intensifies without experiencing a frequency shift from monomer to pentamer, a phenomenon corroborated by TDDFT calculations. Excited state Raman spectra, acquired via impulsive stimulated Raman spectroscopy (ISRS), reveal the presence of low-frequency ~100 cm^{-1} modes. The initial observation of a lower frequency (94 cm^{-1}) in the dimer compared to the 110 cm^{-1} in the ground state implies a looser intermolecular structural coupling in the excited state. Conversely, a slight blueshift from dimer to pentamer in the same mode suggests a relatively stronger coupling in the excited states, consistent with the observed exciton localization processes in time-resolved electronic spectroscopy. Additionally, a 40 cm^{-1} mode, particularly evident in PBI trimer and pentamer, is attributed to collective phonon modes involving vibrational motions of the entire array.

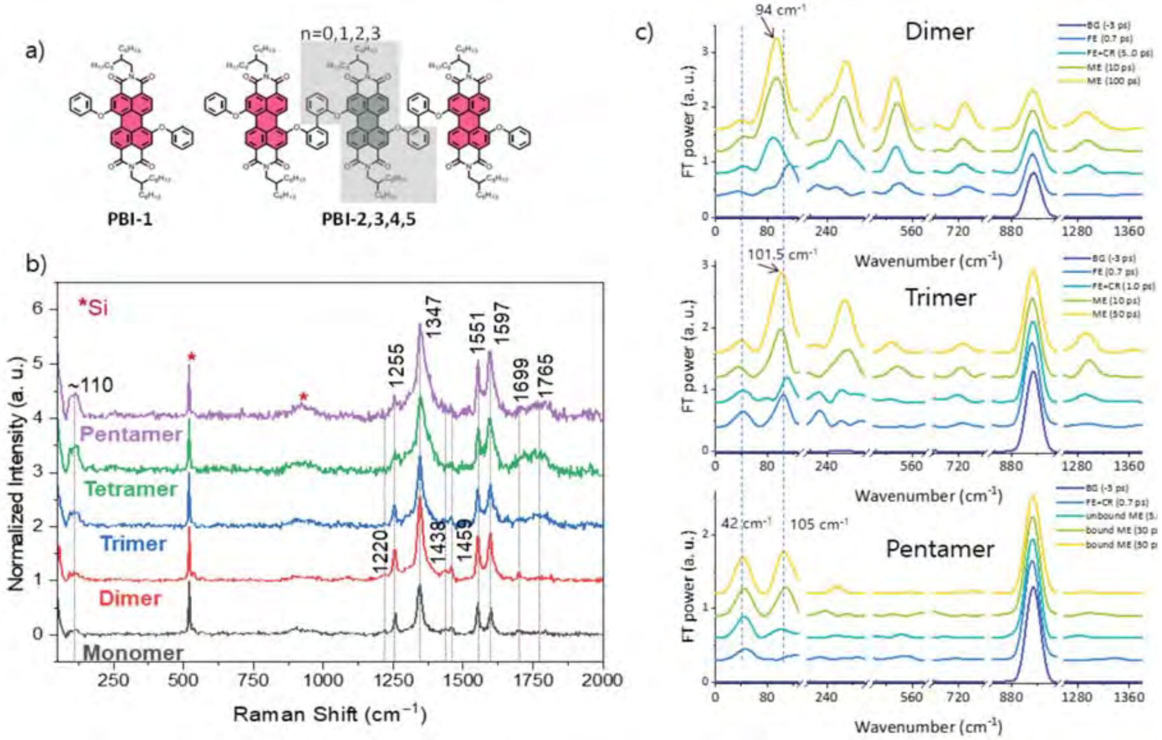

Figure 1. a) Molecular structure of slip-stacked PBI arrays; b) Raman spectroscopy of slip-stacked PBI arrays (excitation wavelength at 473 nm, the data is normalized at 520 cm^{-1}); c) Impulsive stimulated Raman spectroscopy (ISRS) of slip-stacked PBI arrays.

Probing the Dynamics of Molecular Switches using Ground- and Excited-State Resonance Raman Spectroscopy

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Resonance Raman spectroscopy is a well-established technique for probing the ultrafast dynamics of molecules in electronically excited states. The relative intensities of the resonance Raman bands reveal the vibrational modes with the largest Franck-Condon activity in the resonant state, and therefore indicate which vibrational displacements dominate the initial motions of a wave packet upon optical excitation [1]. Variation of the resonance Raman intensities for different electronic absorption bands reveals differences in the topology of the excited-state potential energy surfaces that influence the photochemical reactivity of the molecule. This contribution describes broadband stimulated resonance Raman measurements for prototypical photochromic diarylethene molecular switches in both the ground and excited states. Ground-state resonance Raman measurements probe the dynamics following excitation into the first two excited states (S_1 and S_2) at the equilibrium ground-state geometry [2], whereas excited-state resonance Raman measurements probe the dynamics following excitation into higher-lying electronically excited states (S_n) at non-equilibrium geometries [3]. We describe recent efforts to map these initial wave packet dynamics onto the reaction coordinate for two different photoswitch molecules. Insights from these measurements reveal new details about the reactions of molecular switches and provide valuable benchmarks for comparison with theory.

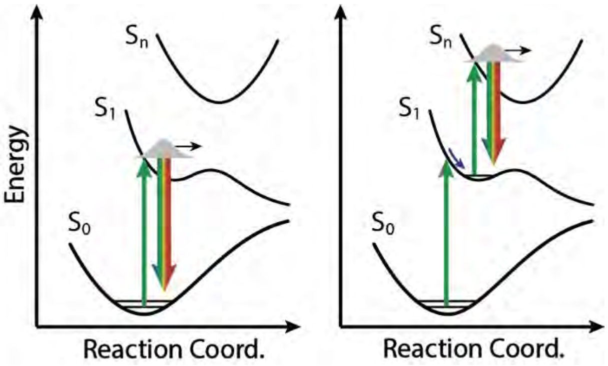

Figure 1: Resonance Raman spectroscopy probes the wave packet dynamics in the upper electronic state.

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Vibrational Spectrum of Excited State Following Ultrafast Internal Conversion Through Conical Intersection

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Transient absorption (TA) signals measured by short-enough femtosecond pulses commonly show modulations of signal intensity due to nuclear wave packets (NWPs) in both the ground and excited states, which offer the vibrational spectrum in similarity to a resonant Raman spectrum. We have demonstrated previously that time-resolved fluorescence (TF) can also provide the vibrational spectrum of the emitting species exclusively, if recorded with a time-resolution higher than the periods of vibrations [1].

When a molecule is excited to a higher electronic state, S_1 fluorescence is observed entirely, the well-known Kasha's rule, due to the ultrafast internal conversion (IC). The NWPs created in the initial state are then carried over to the S_1 state. While some NWPs orthogonal to the IC coordinate remain unaffected, the NWPs coupled with IC may undergo variations during the ultrafast IC. In these days, it is well established that conical intersections (CI) should be responsible for the ultrafast IC, and CIs generally exist in the photophysics and photochemistry of polyatomic molecules. However, revealing the details of the nonadiabatic quantum dynamics through a CI is challenging. In this work, we have used TF to record ultrafast ICs and the vibrational spectrum of S_1 state. Figure 1 shows an example of such experiment [2]. BODIPY dye (PM650) was excited to S_2 (and S_3) state, TF signal is detected, which is modulated by NWPs in S_1. Because the passage through a CI modulates the phases and amplitudes of the NWPs, the resulting vibrational spectrum of S_1 is different from the ordinary resonant vibrational (Raman) spectrum of the S_1 state. To reveal the quantum dynamics of IC, we have performed quantum chemical calculations and various molecular dynamics simulations. In particular, multi-configuration time-dependent Hartree (MCTDH) with linear vibronic coupling was utilized to propagate the initial NWPs and the resulting NWPs in S_1 and the corresponding vibrational spectrum was obtained, which matches with the experiment. More importantly, we can identity the vibrational modes that are coupled with the CI and cause the ultrafast IC.

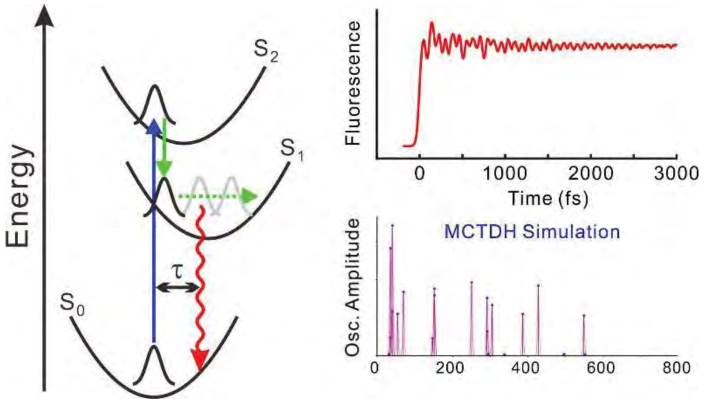

Figure 1: The schematic (left) of the experiment. TF (top right) was detected following S_2 excitation. MCTDH simulation was utilized to obtain the vibration spectrum of S_1 state (bottom right) following ultrafast IC.

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Structural changes of chromophores upon intramolecular charge transfer in the excited states

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Intramolecular charge transfer (ICT) has been of great interest in chemistry and related fields due to its extensive applications. The structural changes of chromophores upon ICT can be the driving force of the drastic changes in the photophysical properties including the emission quantum yields. Recently, we have shown the skeletal vibrations of push-pull chromophores obtained from femtosecond stimulated Raman spectroscopy (FSRS) can sensitively probe the structural changes of π-conjugated backbones (stilbene, biphenyl, and styrylpyran) upon the ICT in the excited states [1]. In this work, we report the ICT dynamics of thioflavin T and stilbazolium derivatives obtained from FSRS and impulsive stimulated Raman spectroscopy (ISRS). Thioflavin T is a well-known imaging probe of amyloid fibrils, where the emission increases are strongly related to the inhibited structural changes of the thioflavin T backbone. The detailed structural changes of thioflavin T with ICT were obtained in ISRS by the appearance and disappearance of deformation modes of dimethylaniline and benzothiazole backbones in the 200-650 cm^{-1}. Two kinetic components ~370 fs and 3.4 ps represent two-step structural changes of thioflavin T, such as the bend of benzothiazole and the twist of dimethylaniline. Structural changes of stilbazolium derivatives upon the ICT in the excited state will also be discussed based on FSRS measurements. Two isomers of N-methylpyridinium synthesized for the inhibited rotation of dimethylaniline upon ICT would present that time-resolved vibrational probes rather than time-resolved electronic probes are required to observe ultrafast structural changes of chromophores during the ICT in the excited states.

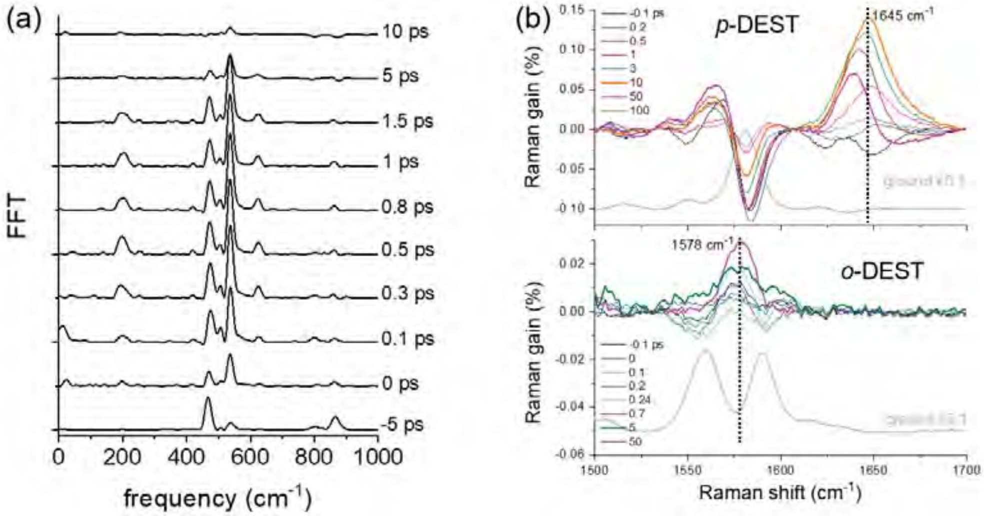

Figure 1. (a) ISRS of thioflavin T in 1-propanol, (b) FSRS of stilbazolium derivatives, *p*-DEST and *o*-DEST in methanol obtained with 405 nm excitation.

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Time-resolved resonance Raman spectroscopy reveals the mechanism of the water oxidation catalyst Ru-hbpp

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[c]*Zuse Institute Berlin, Berlin, Germany*

The artificially catalysed water oxidation reaction (WOR) is key to catalytic water splitting as a strategy for efficiently producing hydrogen as a clean and sustainable fuel, necessary for addressing current challenges of ever-growing energy demand[1]. The Ru-hbpp (*in,in*-$\{[\mathrm{Ru^{II}(trpy)(H_2O)}]_2(\mu\text{-bpp})\}^{3+}$) binuclear metal-complex is the successor of the very first molecular catalyst for the WOR, the so-called blue dimer. Ru-hbpp has been improved in efficiency and stability[2]. Moreover, it acts as a perfect model catalyst for providing insight into the complex mechanism of the WOR by means of various experimental methods. Structural and kinetic properties of the catalytic reaction, however, are often difficult to obtain with common methods. In contrast, resonance Raman (RR) spectra encode detailed information on specific reaction intermediates. The only limitation to access this information is that data analysis is complicated by different levels of resonance enchantment of different intermediates and their spectral similarities.

In the current study we demonstrate a self-developed analytical tool based on non-negative matric factorization (NMF) for processing complex time-resolved RR spectroscopic data. Using Ru-hbpp as a model system, we followed the WOR with RR spectroscopy in real time at ambient conditions. Subsequent mathematical analysis let us distinguish spectral features of specific intermediates and correlate them with kinetic traces in an unbiased manner[3]. Kinetic and structural information on selected RR-active intermediates was extracted with support of DFT calculations and orthogonal spectroscopic methods such as time-resolved UV-Vis spectroscopy. Combining theoretical and experimental information, we suggest a catalytic reaction mechanism, thereby providing valuable insight into understanding of catalysing the WOR in general.

As a result, this work presents a useful analysis strategy for processing time-resolved resonance Raman spectroscopy data, which was successfully applied to uncover mechanism of water oxidation reaction catalyzed by Ru-hbpp.

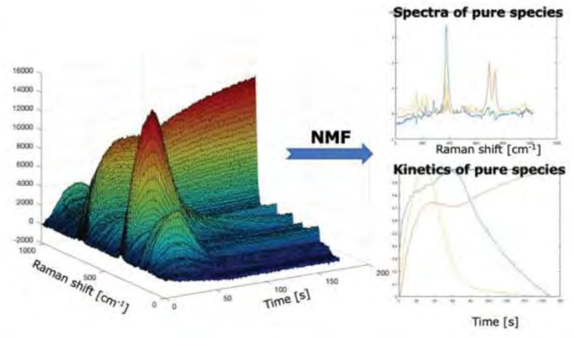

Figure 1: NMF analysis of TR RR dataset

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Time-Domain Raman Spectroscopy: Encoding the temporal information in the spectral Domain via chirped pulses

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Impulsive Stimulated Raman Scattering (ISRS) is a pump-probe technique that exploits the joint action of two ultrashort pulses to coherently stimulate and read out vibrational oscillations directly in time domain [1]. Critically, a full scan of the time delay between the pump and probe pulses along the entire dephasing time is required, implying long acquisition times as well as extended exposure of the sample to multiple ultrashort pulses. This typically hinders the signal-to-noise ratio that can be practically achieved in complex experiments and ultimately renders ISRS ineffective to study irreversible processes and specimens susceptible to photodegradation. To address these limitations, in this presentation we discuss an experimental protocol based on using a largely chirped probe pulse for recording Raman vibrations without the need to scan over the pulses delay, referred to as chirped-based impulsive stimulated Raman scattering (CISRS) [2]. The proposed scheme has been experimentally benchmarked on a fluorescent oxazine dye, namely Cresyl Violet, showing that CISRS is an effective technique to capture both ground and excited-state vibrations.

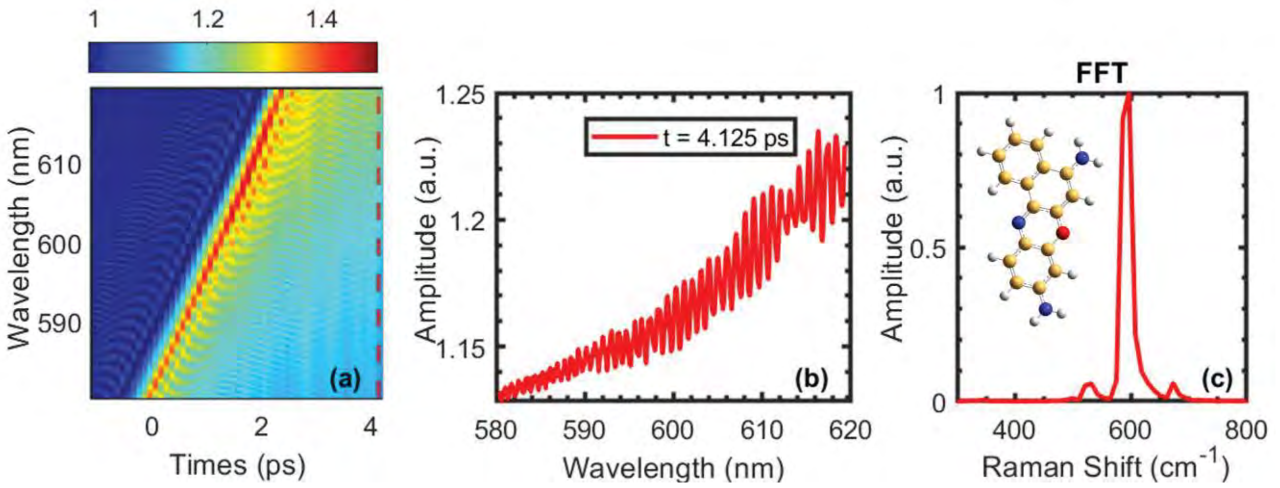

Figure 1: a) Differential transmission map of Cresyl Violet dissolved in water acquired with a largely chirped probe (5800 fs^2). b) The differential transmission at a fixed pump-probe delay (4.125 ps) shows that, in view of the pulse chirp, the ISRS oscillations are encoded in the spectral domain, i.e. they can be monitored as a function of the probe wavelength. c) Raman spectrum of Cresyl Violet obtained after converting the wavelength axis in the time axis by exploiting the definition of chirp and Fourier transforming the resulting oscillations.

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Recent developments in coherent Raman imaging.

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Coherent Raman scattering encompasses coherent anti-Stokes Raman scattering (CARS), coherent Stokes Raman scattering (CSRS) [1] and stimulated Raman scattering (SRS) [2]. These three light-matter interaction processes have in common their ability to populate vibrational states of molecules that creates a modulation of the refractive index; however, they differ by the way the refractive index modulation is read. This leads to different technical implementations and different detection abilities when they are used to generate chemical images of samples. In this talk I will review how coherent Raman processes can generate images both in the time domain [3] and the spectral domain. I will also discuss what limits the imaging speed in the conventional point scanning scheme [4] and why wide field imaging can possibly breakthrough this limit [5]. Finally, I will present recent effort to push these contrast mechanisms in flexible endoscopes [6].

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Stimulated Raman scattering imaging of single-cell drug exposure and drug response

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The quest for novel cancer treatments necessitates intricate biological models that mimic the complex environment of human tumors more accurately. These models are crucial for the discovery and development of drugs that can effectively target cancer cells without inducing high toxicity in normal cells. However, traditional chemical assays mostly measure the ensemble response, neglecting potential differences between cells in heterogeneous tissue and the contribution of microenvironmental factors to drug responses. This challenge underscores the need for advanced imaging techniques that can probe drug dynamics at the single-cell or subcellular levels within these complex models. In this context, stimulated Raman scattering (SRS) microscopy emerges as a powerful tool due to its innate ability to direct probe drug distribution and the phenotypic response of single cells. This presentation will highlight how SRS imaging contributes to the field by enabling precise measurements of intracellular drug concentrations and responses at the single-cell level. We demonstrate that SRS can quantitatively assess the distribution and efficacy of EGFR inhibitors within the subcellular compartments of both simple cell culture and 3D tumor spheroid models. This approach not only enhances our understanding of drug behavior in more physiologically relevant settings but also paves the way for the development of more effective drug screening methods.

Advances of stimulated Raman scattering microscopy with novel light sources and Raman probes

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Stimulated Raman scattering (SRS) microscopy provides molecular vibrational contrast with high sensitivity, finding various applications including label-free imaging to Raman probe imaging [1]. The principle of SRS microscopy involves the lock-in detection of the SRS signal to detect a single vibrational mode, whose vibrational frequency is specified by the optical frequency difference between pump and Stokes laser pulses. Enhancing the performance of SRS microscopy requires the development of light sources with low noise and wide wavelength tunability, as well as novel Raman probes.

This talk will discuss recent advances in SRS microscopy facilitated by the introduction of novel light sources and Raman probes. I first introduce our integrated SRS and fluorescence microscope [2], whose SRS part is based on the high-speed wavelength tunable laser, based on the spectral filtering of Yb fiber laser pulses [3-5], synchronized with a picosecond Ti:sapphire laser. This system enables simultaneous video-rate imaging of SRS and fluorescence with frame-by-frame tuning capability of vibrational frequency, fluorescence excitation wavelength and detection wavelength, and was successfully applied to high-speed super-multiplex imaging with polyyne-based Raman probes [2]. More recently, the introduction of a fiber optical parametric oscillator [6] has extended the wavenumber tunability from 300 cm^{-1} to 1400 cm^{-1}, expanding the wavenumber range including fingerprint and CH-stretching region. To further enhance the sensitivity, research is underway to develop a squeezed light source that aims to reduce noise in balanced-detection SRS by 2.8 dB [7]. The presentation will also cover the development of activatable Raman probes for multiplex enzyme sensing [8], and photoswitchable Raman probes for superresolution imaging [9]. We anticipate that these technical innovations significantly expand the applications and capability of SRS microscopy.

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Fast and few: high-speed compressive Raman imaging

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Raman imaging is recognized as a powerful label-free approach to provide contrasts based on chemical selectivity. Nevertheless, Raman-based microspectroscopy still have drawbacks precluding high-speed chemical imaging. The main issue is the inherent high data throughput in microspectroscopy: fast spectral imaging is challenging for dynamic and large-scale imaging due to its data acquisition, processing and representation change (from vibrational resonances amplitudes to chemicals concentration) procedures.

In this contribution, I will introduce the concept of compressive Raman imaging: by exploiting the sparsity [1] and redundancy [2] in Raman data sets, one can considerably simplify and speed up the spectral image acquisition, reaching speeds compatible with video-rate imaging [3] by detecting just a handful of photons. I will discuss the different ways of performing compressive Raman, in particular focusing on challenges for bio-imaging, and also show more recent results applied to long-time imaging of electrochemical systems [4]. Compressive Raman imaging tackles the challenge of democratizing chemical imaging to non-specialists of vibrational spectroscopy: that is, provide a framework in a “blind” manner, due to a simpler workflow analysis.

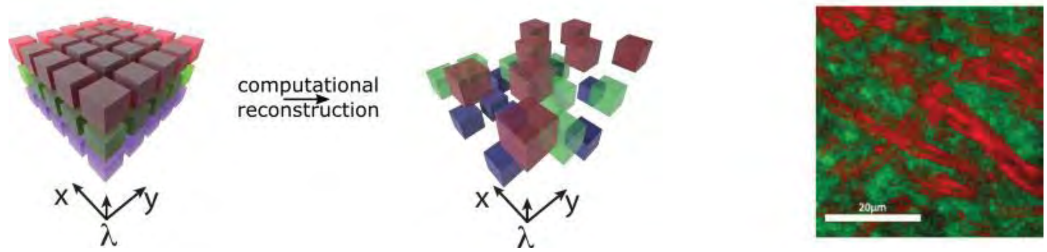

Figure 1: (left-middle panel) Conceptional description of the compressive Raman framework, where the hyperspectrum is sparsely sampled and the information is reconstructed aided by advanced algorithms. (right panel) one example of biological application of this technology: lipid-rich (red) and protein-rich (green) chemical images of brain tissue.

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Polymer, Water and Salt Concentration in Complex Coacervates determined by Femtosecond Stimulated Raman Microscopy (FSRM)

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Complex coacervation is a liquid-liquid phase separation phenomenon that can occur when two oppositely charged polyelectrolytes are combined in a water-salt solution. Depending on the concentrations in the mixture, particularly the salt concentration, this mixture separates into two phases: A polymer-rich phase and a polymer-depleted phase [1,2]. Complex coacervation is observed in biological and synthetic systems [1,3] and is thought to have played a role in the origin of life [4]. Especially for biologically coacervates, a quantification of the components in the two phases using microscopic amounts is highly desired [5]. This proof-of-principle study demonstrates the feasibility of employing femtosecond stimulated Raman microscopy (FSRM) [6] for characterizing a “classical” complex coacervate.

The complex coacervates were prepared dissolving the polyelectrolytes PDADMAC and PSSS, and the Raman active salt NH_4SCN in water. With FSRM, it is possible to characterize the composition of the two phases regarding their polymer, salt, and water content as shown in Figure 1. By measuring different complex coacervate samples obtained by varying the salt concentration, a phase diagram could be constructed, which will be discussed in relation to theoretical predictions.

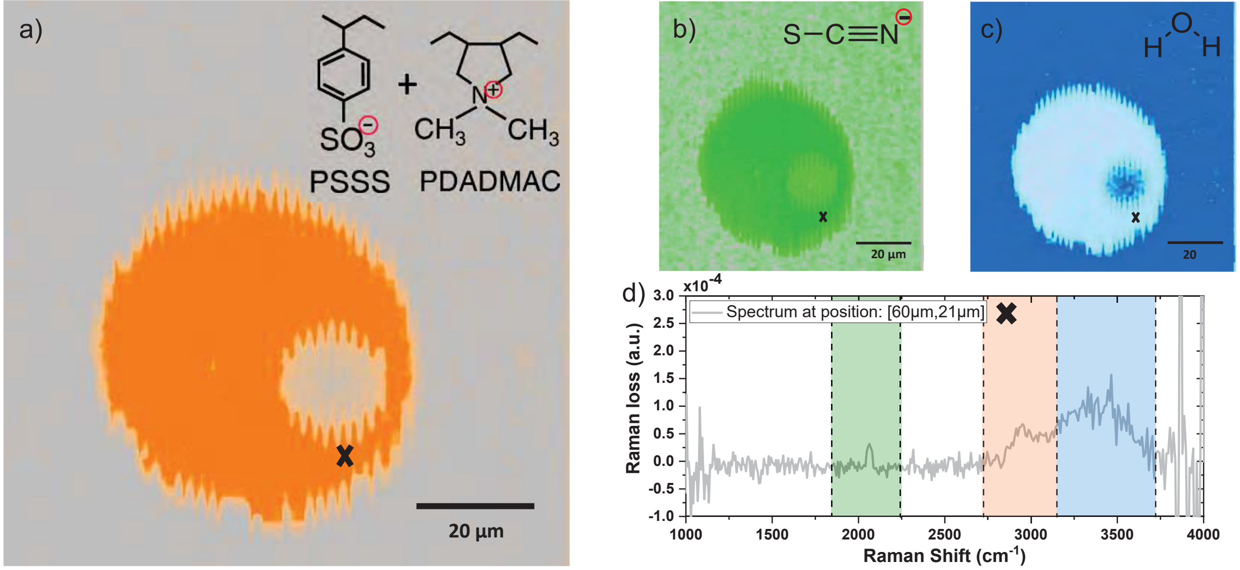

Figure 1: FSRM chemical maps of a PSSS/PDADMAC-NH_4SCN coacervate in water. The map has a spatial resolution of 1 µm and was constructed from 10000 FSRM spectra recorded in ~17min. The maps are color-coded representing the C-H stretching signal of the polymers (a) the CN stretching mode of $S\text{-}CN^-$ (b), and the stretching modes of H_2O (c), as shown in the Raman spectrum (d).

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A plug-and-play broadband coherent Raman platform

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Coherent Raman imaging (CRI) emerges as powerful label-free imaging technique, offering sub-cellular spatial resolution and molecular-specific contrast by detecting the intrinsic vibrational fingerprints of cells and tissues[1,2].

We present a fully engineered broadband Coherent Raman Platform (CRP) designed to achieve state-of-the-art performance in multiplex stimulated Raman scattering (SRS) microscopy with unprecedented ease of use and long-term reproducibility. The CRP comprises an all-fiber dual-wavelength self-synchronized laser and a detection unit based on a compact multichannel lock-in amplifier, ensuring low noise SRS performance over the entire CH spectrum (2800-3100 cm^{-1}), parallelizing detection across 38 spectral channels in 2 μs. Additionally, the system is equipped with an epi-detection module for TPEF and SHG signals.

Moreover, the CRP combines a broadband label-free approach for chemometric analysis of biological specimens (Fig. 1a) with artificial intelligence tools, enabling users to unleash the full power of hyperspectral data. Such a system finds broad application in biomedical sectors where traditional exogenous labeling is a limiting factor, such as in live cell imaging, metabolomics, and histopathology[3] (Fig. 1b).

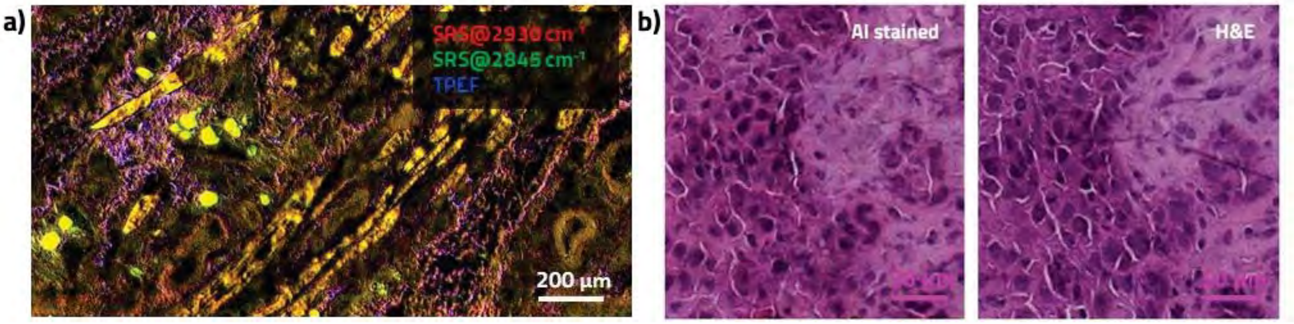

Figure 1: a) Image of human head and neck tumour tissue obtained combining SRS signal @2845 cm^{-1} (green), SRS signal @2930 cm^{-1}(red) and two-photon excitation fluorescence (TPEF) signal (blue). b) Comparison of AI-generated H&E-like image from SRS data with the very same tissue area H&E stained.

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Multimodal imaging analysis using Stimulated Raman Scattering Microscopy and Secondary Ion Mass spectrometry to visualise topical drug penetration into the skin

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Assessing the penetration of drugs into the skin is crucial for evaluating the effectiveness of topical formulations. However, existing methods for studying the pharmacokinetics of drugs in the skin can be tedious, time-intensive, and invasive. Stimulated Raman scattering (SRS) microscopy is a rapid, non-invasive, and label-free chemical imaging technique which can map drug distribution in skin. However, the SRS signal sensitivity is limited due to attenuation with depth into the skin. In this study, secondary ion mass spectrometry (SIMS), a more sensitive technique, has been used to validate the SRS signal detection limit of 4-cyanophenol (CP) (a model 'drug'). In-vitro permeation studies were performed on porcine skin after application of different concentrations of CP solutions. Following skin treatment, SRS and SIMS images were obtained from identical tissue sections. Feature-based image registration was conducted, along with line profile analysis to determine average intensities across corresponding SRS-SIMS images. Additionally, SRS-SIMS data were collected from a skin homogenate spiked with known concentrations of CP to inform the observed results and assess the detection limit of the chemical. Line intensity profiles of the registered images showed that, while SRS was able to detect CP ~150 µm into the epidermis, SIMS could extend this depth to ~500 µm. In the skin homogenate, the SRS signal of CP was linearly proportional to concentration up to ~0.04 mM. In summary, the multimodal image registration utilizing SRS and SIMS showcased the genuine potential of the former to evaluate drug permeation into the skin and to compare the performance of various topically applied formulations.

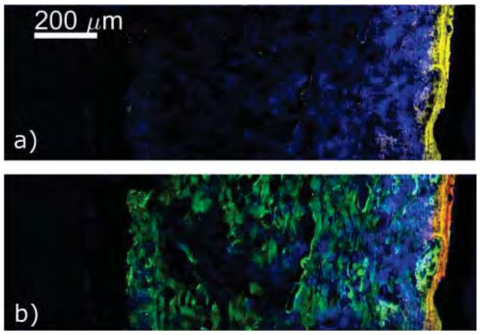

Figure 1. 40 µm thick porcine skin section treated with 170 mg/mL CP for 6 hours a) Overlay of CP-SRS (yellow) and CP-SIMS (blue) channels, b) Overlay of CP-SRS (yellow) and Amide I-SRS (green), and CP-SIMS (blue)

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Spectral focusing SRS microscopic analysis of pharmaceutical co-crystallization

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Poorly water-soluble drugs are problematic in drug development and can lead to poor bioavailability. One possibility to overcome this challenge is to alter the crystal structure. Co-crystals consist of more than one compound (often two compounds) at a stoichiometric ratio in a single crystal structure. During manufacturing and storage, it is essential to have adequate analytical techniques to characterize these co-crystals, as, for example, any unwanted solid-state form presence can lead to lower solubility and lower bioavailability.

In this study, spectral focusing SRS microscopy was used to study co-crystallization during liquid-assisted milling. Indomethacin and nicotinamide (IND-NIC) were prepared as a 1:1 molar ratio by liquid-assisted milling using ethanol as the liquid (added in every 4 min). SRS microscopy was revealed to be an excellent tool to study the co-crystallization process and to get insights into the chemical and solid-state composition of samples at different time points of milling (**Figure 1**). The images revealed that co-crystallization had already started within 1 min. It was also shown that the amount of ethanol strongly influences the co-crystallization process, and indeed if milling was performed without adding additional ethanol fewer co-crystals were observed after 3 min. Furthermore, SRS microscopy revealed that the sample was mostly co-crystalline after 12 min of milling. These findings were also supported by spontaneous Raman scattering microscopy analysis of the same samples. However, spectral focusing SRS microscopy has some clear advantages over spontaneous Raman scattering microscopy. Image acquisition with spectral data is rapid, and images cover a large enough area to get a more representative overview of the sample. Furthermore, the lateral spatial resolution is below micrometer and the method is non-destructive. As a matter of fact, unlike spontaneous Raman scattering microscopy, SRS microscopy revealed traces of IND particles in the images even after 24 min.

Overall, spectral focusing SRS microscopy was demonstrated to be an excellent tool to study co-crystallization of pharmaceuticals. Also, other pharmaceutical solid-state samples are well suited to analysis using this technique.

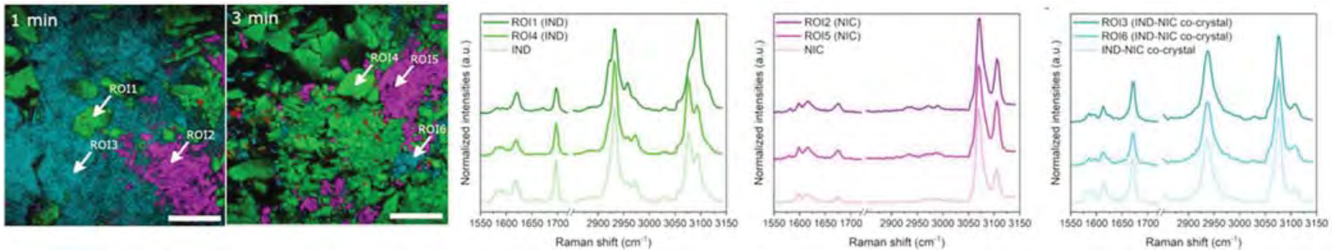

Figure 1: False colour classical least squares (CLS) images at time points of 1 min and 3 min of milling and SRS spectra extracted from areas indicated by arrows along with reference spectra (green is IND, magenta is NIC and cyan is IND-NIC cocrystal). Scalebars: 50 μm.

Droplet deposition Raman spectroscopy: its aspects and potential applications

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The droplet deposition Raman spectroscopy is based on the liquid droplet deposition of analyte on a suitable (generally hydrophobic) solid surface. When drying, the flow in a liquid sample carries dispersed material to the droplet edge, and it forms coffee-ring or small spot patterns where the analyte is pre-concentrated [1,2]. This approach itself as well as in combination with SERS can receive considerable attention in the field of sensitive Raman detection using analyte pre-concentration.

This contribution will introduce experimental aspects and some advanced applications of droplet deposition. Two main advantages will be principally emphasized: the sensitivity of detection of various analytically important molecules and the drying-induced segregation of the components from the mixtures (such as body fluids). For example, recently, we reported that the spatial segregation of lipids and carbohydrates from pure infant formula into dried patterns was confirmed by Raman spectral mapping [3]. Lipids tended to accumulate in the coffee-ring, and carbohydrates formed the thin layer in the central part of the ring (Figure 1). The same separation in the dried pattern was also observed for melamine-blended infant formula, where melamine was detected only from the thin central layer together with carbohydrates due to the melamine glycation by lactose. Therefore, we could profit not only from the efficient pre-concentration and drying-induced spatial separation but also from the chemical reaction potential of molecules of interest present in a complex solution or suspension.

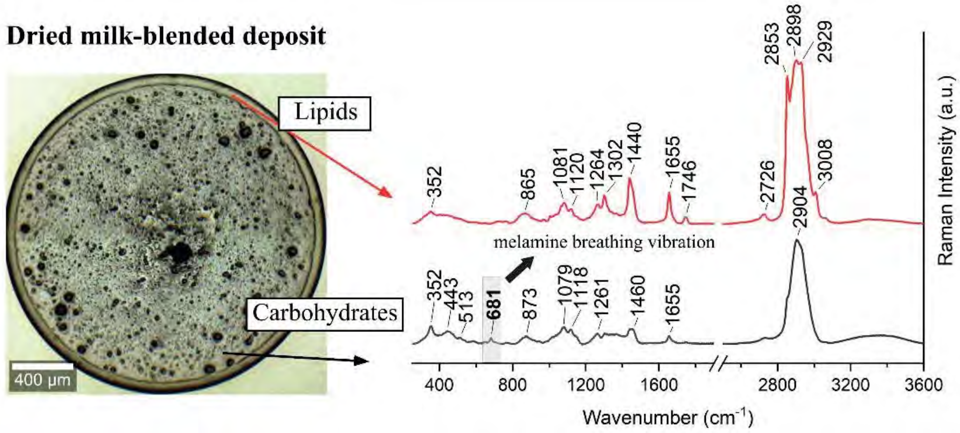

Figure 1: Drying-induced segregation of lipids and carbohydrates from infant formula.

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Experimental assignment of glycosaminoglycan vibrational bands

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Condensed-phase vibrational spectrum assignment generally relies on the comparison with either experimental results from chemically similar structures or with theoretical data. While theoretical calculations can provide the best basis for comparison for small and rigid structures, in the case of flexible molecules, such as glycans, strong differences become apparent between the theoretical and experimental data already at the disaccharide level. With the advances made in cryogenic gas-phase IR spectroscopy, it has become one of the most attractive approaches to generate spectral libraries for comparison.[1,2] In messenger-tagging IR spectroscopy, mass-selected ions are trapped at cryogenic temperatures (~40 K) in low-pressure He atmosphere with 1-5% N_2 gas. Under these conditions, N_2 molecules and the trapped ions form adducts, *i.e.,* a tagged ion, which can be detected as a 28-Da shift in a time-of-flight mass analyzer. With resonant IR excitation, the ions lose the tag due to their increased energy, and the depletion of the signal can be followed as a function of the wavelength, yielding the IR spectrum.

Here, we present the spectra of sulfated glycosaminoglycans (GAGs) with ^{32}S and ^{34}S, and compare the changes in their spectral features. Based on the results, we can directly assign the asymmetric and symmetric $–SO_2$ stretching vibrations, and gain insight into the changes of molecular properties within a wide range of GAG structures. Therefore, this approach allows to shift from relying on theoretical data for the band assignment in GAG spectra to using them merely to support the results.

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New spike removal algorithms for Raman spectroscopy: A comparative approach based on synthetic spectra

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Spikes are typically produced when cosmic rays collide with the charge-coupled device (CCD) during spectrum acquisition. This interaction generates electrons, which are subsequently read alongside the charges produced by the Raman scattered photons' energy, leading to the appearance of spikes in the Raman spectrum. Characteristically, these spikes manifest as extremely narrow, high-intensity peaks at positions that do not correlate with the spectral wavenumber. If not addressed, these anomalies could introduce significant errors in further data analysis processes such as normalization, calibration, spectral search, or dimensionality reduction [1]. Hence, the elimination of spikes is crucial in the preprocessing stage of Raman data analysis [1, 2]. Spike detection algorithms can be categorized depending on whether they rely on several spectra for comparison among them or they can be used with a single spectrum. The first ones require a minimal difference between adjacent spectra, whereas the second type offers greater versatility. In this study, several single-spectrum algorithms were implemented, including a neural network based one and compared among them and with literature [3,4,5,6]. The sensitivity of each method is evaluated based on synthetic spectra: Different spike positions within different Raman peaks and different levels of noise. Furthermore, their suitability in terms of ease of implementation and computational time is also discussed.

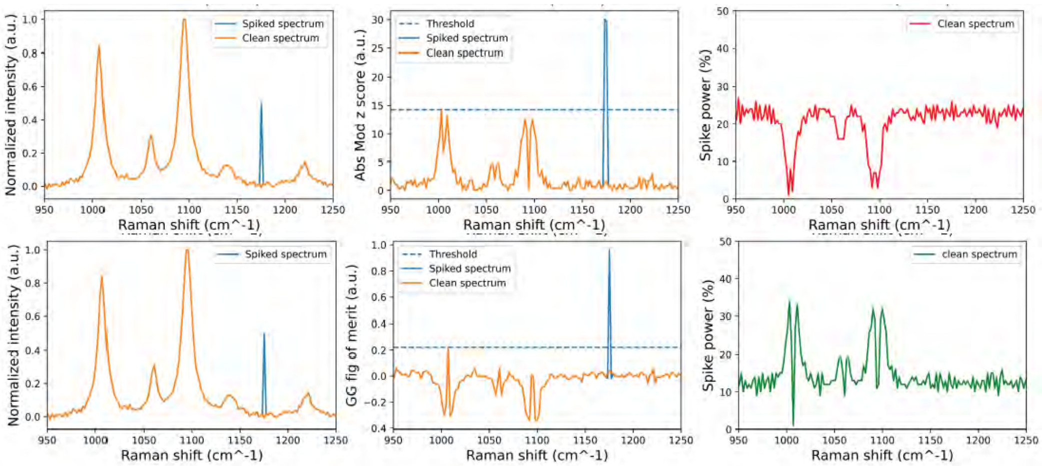

Figure 1: Columns 1,2,3: Spectra (orange) with spike (blue line), figure of merit and calculated sensitivity, respectively for algorithms [4] (first row) and [5] (second row).

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In vivo Resonant Raman microscopy study of *Artemia salina* (Linnaeus, 1758); carotenoids profile as proxy for variability in environmental conditions

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Artemia salina (Linnaeus, 1758) has been used for ecotoxicity testing, both in field and laboratory settings [1]. In one study [2], nanoparticles of titanium dioxide (regularly used in sunscreens) have been shown to potentially have negative impacts on *A. salina.* Having that in mind, *A. salina* carotenoid profile could be used as a proxy for monitoring the water quality and sunscreen contamination. Within recent monitoring project devoted to characterization the salt water bodies from a balneary resort [3] we noted an intriguing variability of the *A. salina* population and pigmentation in two adjacent lakes benefiting from similar environmental conditions. Here we report for the first time the in vivo characterization of *A. salina* pigments using resonance Raman spectroscopy and, based on these results we further explored the carotenoid profile of this crustacean as a proxy to readout the organic and inorganic content variability of salt waters from the resort during cold and warm seasons. The in-vivo carotenoids characterization is feasible using RR spectroscopy, Lorentz deconvolution of the main carotenoid ν_1(C=C) band revealed two peaks corresponding to canthaxanthin and astaxanthin carotenoids. Furthermore, we propose carotenoids profile could be correlated with the sulphate levels, which have already been shown to have significant variations [3].

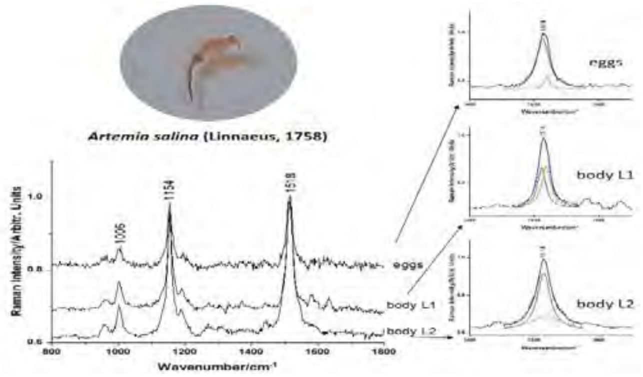

Figure 1: In vivo Resonant Raman spectra of *A. salina* eggs and body from two lakes

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Exploring thermotropic phase transition of dried phospholipids by droplet deposition Raman spectroscopy

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Phospholipid properties and their behaviour under diverse conditions are crucial for understanding cell membranes in general. An internal region of a lipid bilayer is formed of hydrocarbon chains with varying degrees of conformational disorder strongly depending on the temperature. Liposomal suspension was used to study phase behaviour upon heating where one or several intermediate phase transitions can be observed. The transition temperature T_m at which the main phase transitions from a gel to a liquid crystalline phase occurs is different for each lipid. Raman spectroscopy as a method providing unique information based on the vibrational motion of molecules appears to be particularly sensitive to lipid conformational changes present during the thermotropic phase transition. Raman spectra obtained from the gel and liquid crystalline state of lipids directly reflect changes related to the structural alternations during the transition.

Here we explored the thermotropic phase behavior of different dried phospholipids (differing in acyl chain length and type of the head group) by droplet deposition Raman spectroscopy. The method is based on drying-induced pre-concentration of the studied analyte into dried patterns, coffee-ring for phospholipids [1,2]. Classical Raman spectra from dried deposits were then acquired in the temperature range covering the main phase transition. Upon heating, changes in spectral bands (intensity ratio, wavenumber shift) were observed directly related to the phospholipid phase transition (Figure 1).

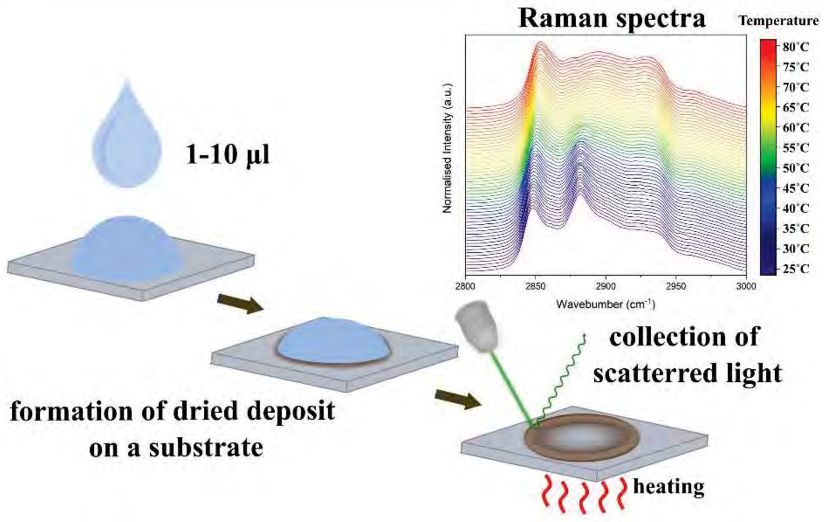

Figure 1: Monitoring thermotropic phase transitions of dehydrated phospholipids by droplet deposition Raman spectroscopy.

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Inkjet-Printed SERS Substrate for Multiplex Detection and Quantification of Biorelevant VOCs

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We report the fabrication of a low-cost paper-based SERS platform with enhanced sensing capabilities. The sensor consists of a highly concentrated glycerol/ethanol dispersion of Au nanostars inkjet-printed on hydrophobic chromatographic paper forming circular sensing spots of 1 mm in diameter. The Au ink exhibits high stability for 1 year. Interestingly, we demonstrate that the modification of the paper surface with hydrophobic ligands improve the sensing capabilities either in gas or liquid phase by confining the analyte solution in the Au sensing platform. It contributes to both concentrate the analyte, as well as, to decrease the sample volume to just a few µl. The substrate has been optimized by analysing the amount of the nanoparticles and the number of printing cycles. A high spot-to-spot reproducibility even for Au spots from different printed papers was observed.

The paper-based SERS platform was tested towards the detection of the fungicide thiram in the liquid phase. Additionally, applying a digital protocol for SERS analysis, a good linear correlation between the digital counts (or positive events) and the analyte concentration was obtained at the single-molecule SERS regime. In both cases, the quantification region threshold was 10^{-11} M. The great sensitivity performance of the inkjet-printed SERS substrate is reflected by the low sample volume needed (only 2 µL).[1]

In addition, we demonstrated an operational SERS strategy to detect gaseous Raman weak-intensity aldehydes that have been considered as a biomarker of lung cancer. To enhance the adsorption of gaseous molecules, the nucleophilic addition reaction with the Raman-active probe molecule p-aminothiophenol (4-ATP) pre-grafted on the SERS substrate, the gaseous aldehyde molecules were sensitively captured to detect at the ppb (parts per billion) level. Interestingly, integrating machine learning with surface-enhanced Raman scattering (SERS) accelerates the development of practical sensing devices. Such integration, in combination with direct detection or indirect analyte capturing strategies, is key to achieving high predictive accuracies even in complex matrices. Herein, we demonstrate the simultaneous detection and quantification of up to three bio-relevant aldehydes.

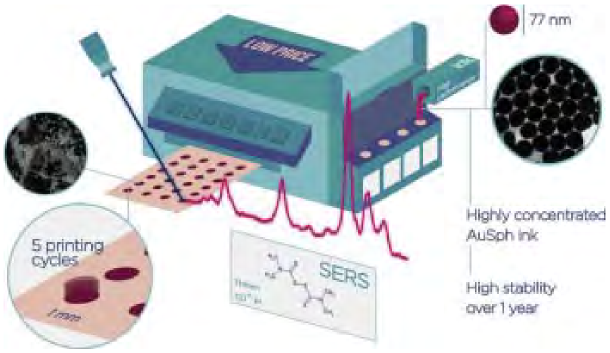

Figure 1: Schematic representation of the proposed inkjet-printed SERS substrate.

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Deciphering the molecular pH-gating mechanism of bacterial urea channels utilizing a novel Raman Spectroscopy approach

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Chronic gastric infection with the bacteria *Helicobacter pylori* (Hp) affects more than 50% of the world population. It is the largest etiological agent of gastric diseases such as gastritis, peptic ulcer disease and stomach cancer [1]. Standard treatment struggles with increasing therapy failures, due to antibiotic resistance of the bacteria. Thus, finding alternatives is of great importance. One of the key processes during *Hp* colonization is the local neutralization of acidic gastric juice by the bacteria. Survival of the bacteria in an acidic environment with a pH as low as pH 1 depends on the expression of the inner membrane urea channel *Hp*UreI [2]. Given the intrinsic porosity of the outer membrane, the periplasmic (pp) side of *Hp*UreI with its pp loop 1 (PL1) and 2 (PL2), which should constitute its pH sensor, represents a perfect and easily accessible therapeutic target. However, the development of therapeutic approaches requires a molecular understanding of the pH-gating mechanism of *Hp*UreI, which is still pending. In contrast to *Hp*UreI, *Ss*UreI, a homologous channel from *Streptococcus salivarius* (*Ss*), is described as a pH-independent channel [3]. Nevertheless, *Ss* plays a crucial role in maintaining pH balance in oral biofilms and has a significant impact on dental health, including dental caries, calculus formation, gingivitis, and periodontitis.

Using vibrational spectroscopy in combination with yeast complementation assays and *in vitro* protein permeability characterization we work towards the aim to elucidate the pH-gating mechanism of UreI channels. Thereby, pH-dependent Raman spectra of purified UreI channels in detergent micelles and mixed lipid/detergent micelles are compared to UreI channels reconstituted into lipid vesicles. Furthermore, these protein-containing vesicles are spread on glass to form supported lipid bilayers and investigated with a novel Raman Spectroscopy approach in total internal reflection (TIR) configuration.

Contrary to existing literature, our experiments show that *Ss*UreI is a pH-gated urea channel, similar to the homologous UreI channels in *Helicobacter pylori*. This finding is crucial for understanding the pH-gating mechanism of bacterial urea channels and challenges our understanding of the acid acclimation of *Streptococcus salivarius* via urea metabolism. Moreover, our results emphasize the great potential of Raman spectroscopy in membrane protein research. Vibrational spectroscopy can overcome the traditional hurdles in structural biology to study lipid-protein interactions.

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Mapping Selenium Nanoparticles Distribution *in-vitro* through Confocal Raman Microspectroscopy

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Selenium nanoparticles (SeNPs) has recently gained large attention thanks to their antimicrobial, anti-cancer and anti-inflammatory properties.[1] Selenium is an essential trace element in the human diet and plays a key role as a biochemical constituent in different proteins. SeNPs appear to show biocompatible properties, with promising uses in biomedical applications, such as drug delivery and imaging.[1,2] In addition to these attractive properties, SeNPs possess a specific Raman signal located outside the typical Raman fingerprint region of cells.[3] These characteristics make SeNPs ideal for Raman imaging purposes, yet their potential has never been explored. In our work, we monitored for the first time the distribution of SeNPs inside human dermal fibroblast (HDF) cells using Confocal Raman Microspectroscopy. SeNPs have been produced through chemical reduction of selenium precursor. The synthesis resulted in the formation of stable nanoparticles of around 75 nm in diameter. Cytotoxicity of SeNPs have been tested against healthy HDF cells for short and long exposure times, resulting in a decrease in viability only for the highest concentration tested (15 µg/mL) after 72 h. Raman spectra have been acquired from fixed cells treated with different concentrations of SeNPs at various time points, to assess the uptake dynamic and localization of SeNPs inside cells. The Raman maps have been then reconstructed from the originals Raman spectra, without the use of complex calculations. The obtained maps showed a homogeneous distribution of SeNPs in the cells, which appears to be directly proportional to the concentration and exposure time. Eventually, to verify the NPs localization, 3D Confocal Raman Microspectroscopy and TEM analysis have been performed, demonstrating the distribution of the nanoparticles within cells, and more specifically within lipid vesicles.

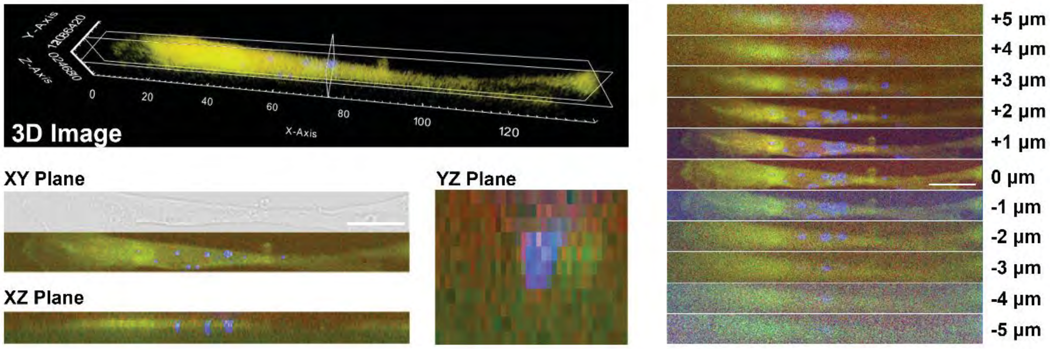

Figure 1: 3D confocal Raman image of HDF cell treated with SeNPs (blue spots)

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RAMAN DATA HARMONISATION

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The industrial and academic use of Raman spectroscopy is becoming wider as the number of specialized techniques and device types increases, costs are reduced, and Raman data relevance is enhanced due to measurements in more realistic conditions [1] as well as faster and improved analysis, supported by data science and artificial intelligence. All this has fostered Raman use and the development of Raman active products, as well as standards and norms for terminology, calibration, performance validation, or data analysis [2],[3]. EU-funded CHARISMA Project aims to contribute to this landscape and support academic research and industrial implementation of Raman through harmonization and FAIRification (making data Findable, Accessible, Interoperable, and Reusable) to minimise data variability, and thus increase quality and trust and foster machine readability and automation. We review the standardisation landscape and present our developments, which include protocols for full data calibration, with specific samples and algorithms for the full optical path; a NeXus data format that enables data, metadata and processing information from multiple experiments to be stored together in the same file; and open-source user-friendly software.

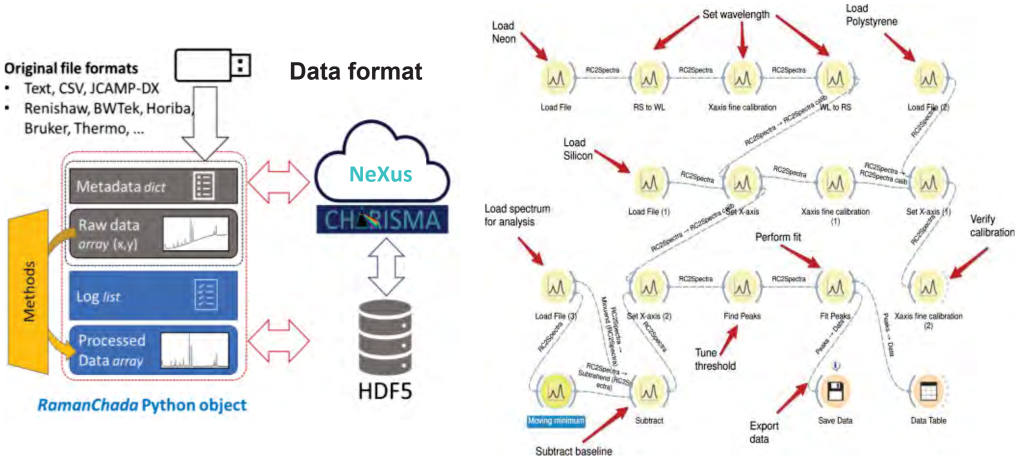

Figure 1: Calibration and storage in NeXus format in Orange data mining software with oranchada add-on.

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Exploring beam profile effects in Raman spectroscopy

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Raman spectroscopy finds applications across various fields, particularly in the analysis of biological samples, owing to its non-invasive and non-destructive nature in providing chemical information. A notable portion of Raman microscopes are confocal, and thus provide a detailed examination of a fine optical volume within the sample. However, an important but often forgotten consideration lies in the laser beam profile, which affects confocality, spatial and axial resolution, as well as signal intensity. Optimizing or understanding the beam profile is essential for obtaining reliable Raman spectra.

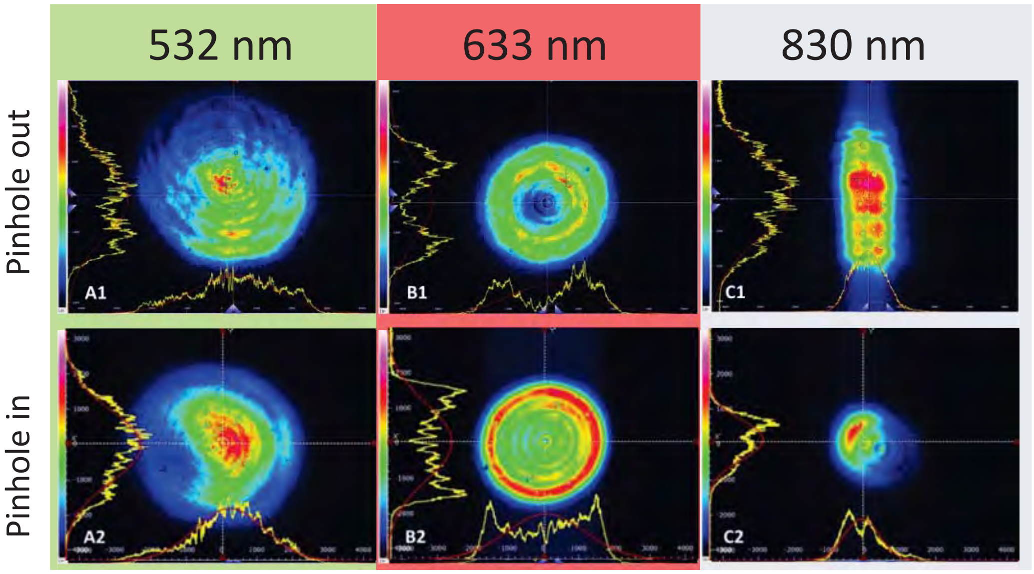

Figure 1: Example Power intensity distributions measured at the sample position for one instrument using 532 nm, 633 nm and 830nm lasers. Panels A1-C1 are the results when no pinhole is used whereas panels A2-C2 are the results for the same lasers when the pinhole is used.

Here we present a method to characterise the laser beam profile and show that it impacts the power density at the focal point and that it is affected by beam path elements (such as the use of a pinhole). The beam profile of the laser delivered to the objective lens significantly influences the energy distribution in the generated focal volume. Our study reveals the impact of optical elements on the beam profile and the consequent aberration effects. These aberrations can lead to distortions in the power density at the focal spot and result in unexpected, localised heating effects on the sample. We propose a protocol for identifying optical elements contributing to these aberrations, emphasizing their importance in achieving precise Raman measurements.

Super-Spectral-Resolution Raman Spectroscopy using angle tuning of a Fabry-Perot Interferometer with Applications to Diamond Identification

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Raman spectroscopy is an extremely powerful laser-based method for characterizing materials based on their unique inelastic scattering spectrum. Ultimately, the power of the technique is limited by the resolution of the spectrometer. Here we introduce Super-Spectral-Resolution Raman Spectroscopy (SSR-RS), a method which greatly improves the spectral resolution of the spectrometer. A Fabry–Perot (F-P) Etalon filter (Finesse > 30, FSR = 2 cm^{-1}), mounted on an angle-tunable motor, is added to the classical micro-Raman setup, and Raman spectra are automatically acquired for many different angles of the F-P filter as shown in Figure 1(a). In previous published super-resolution Raman experiments, the mirror distances (d) with the F-P were varied [1, 2]. Here, the F-P etalon itself is fabricated as monolithic structure, plane and parallel by design through a set of innovations developed by Light-Machinary. With a low-resolution grating of 150 g/mm, which by itself exhibited a best-case resolution of 40 cm^{-1}, we applied the SSR-RS technique to diamond to obtain the ”Inter-theta-gram” data shown in Figure 1(b), from which a linewidth fit parameter of less than 1 cm^{-1} was derived. To baseline SSR-RS, we used the super-spectral-resolution method to extract the linewidth of the laser excitation itself and obtained a laser linewidth of better than 0.007 cm^{-1}. In essence, the modelling aspect of SSR-RS is a kind of “point-spread-function” fitting of the spectrum. Further investigations were carried out on the spectra of a natural diamond compared to a lab-grown diamond, where in both cases, a spectral resolution improvement of at least 40X of the original spectrum has been obtained. Using SSR-RS, we were able to observe differences between natural vs. lab-grown diamond due to different Raman shifts and Raman shift linewidths.

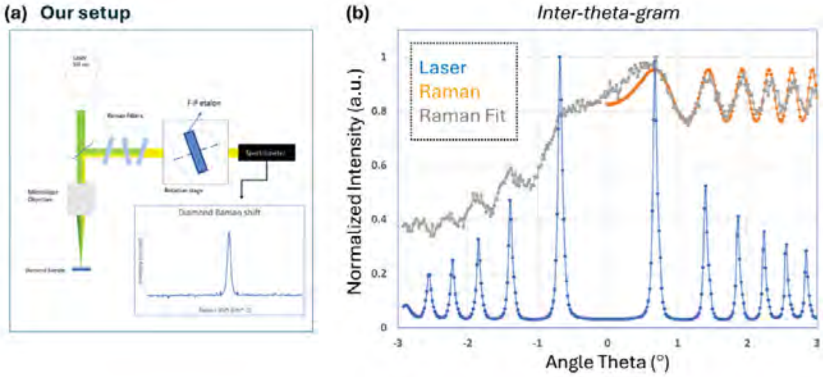

Figure 1: Super-Spectra-Resolution Raman, (a) Our setup, (b) and Laser and Diamond Raman data are taken vs angle to form an “Inter-theta-gram”, with fit of Raman peaks shown as well.

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Angle-Resolved Polarized Raman Methodology for Determining Lattice Plane Orientation of 2D Materials

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The experimental determination of crystal and molecular orientation in anisotropic materials is crucial to studying their direction-dependent properties. Polarized Raman spectroscopy offers a highly precise method for the optical characterization of phonon symmetry (and other associated quasiparticles) with diffraction-limited lateral resolution.[1] Here, we introduce a unique 180-backscatter Raman technique and a precise method for polarization control, employing two sets of half-wave plates (HWP) and two polarizers. With our polarization protocol, we utilize a polarimeter to analyze the ellipticity and orientation of the incident HWP, as they vary with rotation. We leverage the symmetric E_{2g} and asymmetric A_{1g} phonons in molybdenum disulfide (MoS_2) to determine the configuration of HWP combinations that select parallel and perpendicular polarizations. Having validated this protocol with known crystalline planes of silicon (Si), we apply this method to characterize various research-relevant 2D anisotropic materials such as black phosphorus, bismuth selenide (Bi_2Se_3), rhenium disulfide (ReS_2), and tungsten ditelluride (WTe_2). We reveal the importance of accounting for instrumental depolarization effects for accurate description of sample phonon symmetry. This work will lead to the development of documentary standards within ISO for quantitative measurement of crystal and molecular orientation through polarized Raman spectroscopy.

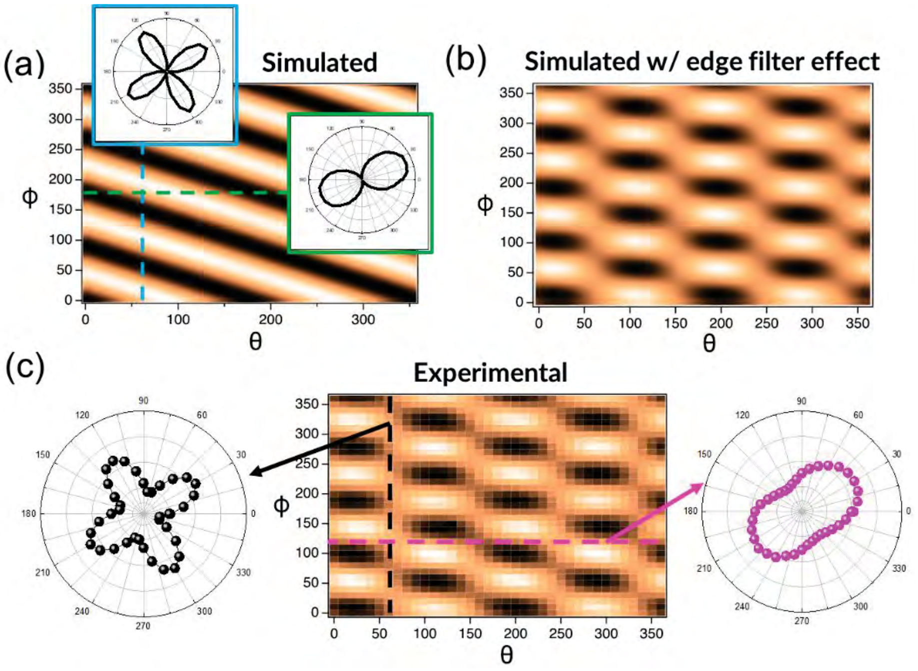

Figure 1:

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Difficulties of harmonising spectra in multichannel Raman systems.

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Raman data is intrinsically linked to the device where the data is acquired. The recent efforts to harmonise and better define the way that units shall be calibrated is an important step in the right direction.

Nevertheless, the number of parameters that affect the Raman signature e.g. detector response, data offset, optical path variations, environmental consideration, etc and the heavy data processing that follows, introduces challenges at the time to use data in other devices. A clear approach on how to calibrate the unit, analyse the noise, resolution and artefacts, define data pro-processing, and non-linear factors associated with the data and use the correct chemometrics modeling, helps to resolve these problems.

This presentation will focus on practical solutions on how to outcome these variations and how to produce data that can be easily used along a broad number of devices and instrument configurations.

New Microspectral and Laser Engineering Solutions for Nanometer Optical Measurements

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Andrey Pogonyshev[a], Nikita Tolkach[a]
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To conduct modern research with nanoscale spatial resolution it is essential to combine probe-optical methods with a new microspectral and laser engineering solutions. This can be achieved with the help of scientific equipment produced by NT-MDT BV – one of the leading developers of optical probe equipment and high-end scientific instruments for experimental nanophotonics research.

For 30 years NT-MDT BV has been producing devices for nanotechnology research. Today the company is a leader in the original, high-quality and high-tech scientific developments. NT-MDT BV is committed to creating modular technologies for creating the next generation of scanning probe microscopes (SPM). These technologies will allow a university or company to begin its scientific activities with a cost-effective core product based on SPM. Subsequently, it is possible to purchase additional modules for the SPM and create a large multi-user research center or combine the SPM with related technologies. In the course of developing these modular solutions, the company has also created ultramicrotomy for nanotomography and spectrometric instruments that combine the world of imaging with the world of chemical analysis.

Currently, we have developed a highly professional probe-spectral measuring system of a new generation. This system is designed to provide the most flexible architecture and provides many opportunities for implementing the most complex scientific experiments. The research can be carried out using both optical spectroscopy and probe nanoscopy.

The spectral part of our measuring system is designed for convenient integration with an atomic force microscope. This makes it quite easy to work in the spectral signal amplification modes of the probe – TERS and TEPL. Already now the functionality of the system is expanding into the field of implementing multi-photon processes and also allows them to be recorded with high time resolution, with the ability to construct hyperspectral maps of nonlinear probe-optical interactions.

The NT-MDT BV team is committed to expanding the production of spectral and probe equipment. Therefore, we have already developed and independently mass-produce solid-state thermally stabilized lasers in the visible and near-IR ranges in the power range from 10 to 100 of mW. Our lasers are distinguished by high power stability, narrow emission linewidth, Gaussian beam profile and high suppression coefficient of laser side modes. We offer versions of lasers with an open beam at the output and with radiation introduced into an optical fiber. The design feature of our laser sources is based on the integration of specially selected laser diodes with reflective volumetric Bragg gratings. The purpose of such integration is to isolate and stabilize a single laser mode in the emission spectrum.

The main area of application of lasers produced by NT-MDT BV is Raman and photoluminescence spectroscopy of stationary states. We actively use our lasers for combination with probe-optical and microspectral research methods. These lasers can be used not only with our equipment, but also with devices from third-party manufacturers.

Low Frequency Raman Spectroscopy as a Method of Investigating Pharmaceutical Polymorphs

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Low frequency Raman spectroscopy is very sensitive to crystalline states because of the presence of intense phonon modes in the spectra.[1] This makes it possible to readily determine differing polymorphs and detect rapid solid state transformations such as dehydration.[2] We have recently coupled low frequency Raman with a spatially offset optical configuration.[3] This allows one to observe sub-surface species and to utilise the power and speed of low frequency Raman to measure solid state transformations below surface levels. This would permit one to characterise and determine the nature of degradation processes in a completely non-invasive fashion in tableted systems as well as glass transitions.[3,4]

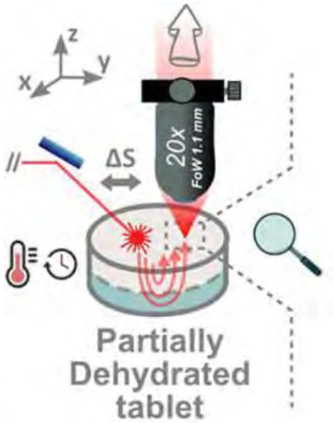

Figure 1: Spatially offset configuration

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Future Perspectives for Low-Frequency Raman Spectroscopy in Pharmaceutical Applications

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With advances in narrow wavelength band filters such as volume Bragg gratings, low-frequency Raman (LFR) spectroscopy has re-emerged as an attractive tool to probe solid-state characteristics of pharmaceuticals due to its exceptional structural sensitivity and typically stronger signal response when compared to the Raman fingerprint region [1]. Here, three novel LFR applications beyond, for example, now routinely adapted characterization of polymorphic forms, are highlighted by implementing this spectral domain for surface-enhanced Raman measurements (small-scale structural sensing of drug molecules and, ultimately, the biomolecular corona interface), in-line pseudo 3D imaging of subcutaneous drug delivery systems via spatially offset measurements, and performance analysis of lipid-based oral formulations during the lipolysis (*in operando* with small angle X-ray scattering). These studies encapsulate some of the future directions for the technique that may help to unravel its true potential, and, ultimately, help to incentivize its broader use within the research community.

Figure 1: Photograph of LFR system coupled with synchrotron small angle X-ray scattering beamline at MAX IV illustrating the analytical flexibility of the technique.

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Coherent Raman imaging of complex solid-state profiles of pharmaceuticals

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Complex solid-state characteristics of pharmaceuticals can critically impact their quality and therapeutic performance, but can be difficult or impossible to fully understand and control with industry-standard analysis methods. Coherent Raman imaging, offering rapid, label-free and (sub)micron chemically and solid-state specific imaging of solid samples in a range of media and with minimal/no sample preparation, is well suited for detailed solid-state analysis of active pharmaceutical ingredients (APIs), excipients and their formulations.

This presentation features a case study in which stimulated Raman scattering (SRS) with fast spectral focusing was employed for detailed qualitative and quantitative solid-state imaging of lactose. Lactose is a widely employed excipient for tablet and inhalation formulations, despite its complex solid-state behaviour that strongly affects pharmaceutical performance. Several commercially available tabletting and inhalation grades of lactose were imaged with SRS, supported by correlative sum frequency generation (SFG) detection. With the aid of multivariate spectral analysis, the inter- and intra-particulate distribution of up to four solid-state forms (amorphous and three crystalline forms) of lactose were imaged with (sub-)micron resolution (Figure 1). In addition, trace levels of forms could be detected well below detection limits of established solid-state analysis methods, and the quantitative compositions of the forms could be estimated. An important consideration was the effect of crystal orientation on the spectra. Overall, SRS analyses provided substantially more solid-state detail about the different lactose grades than available from the manufacturers or traditional analysis methods. Coherent Raman microscopy has wide-ranging potential for better understanding and optimisation of the solid-state behaviour of pharmaceuticals.

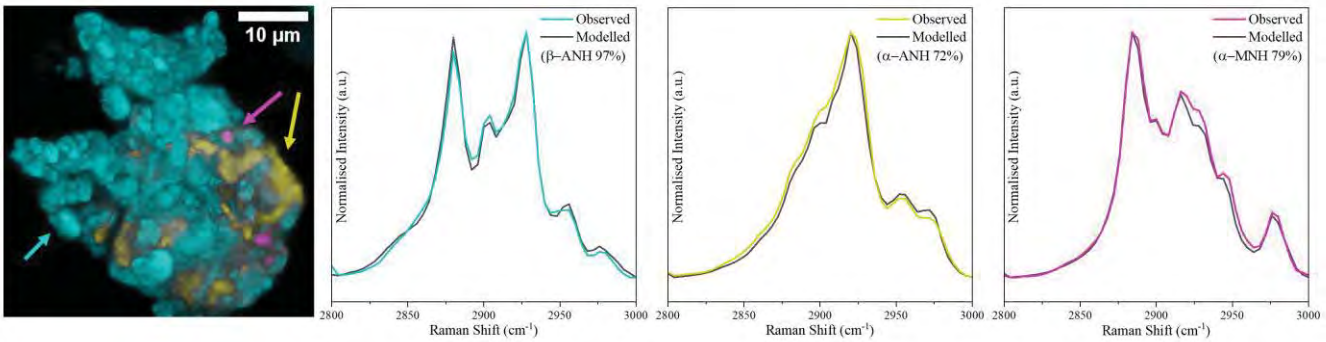

Figure 1: False colour maximum projection image of lactose solid-state form distribution in a Lactohale 400 particle. Solid-state assignments are based on supervised linear unmixing analysis of the SRS spectral data. Spectra extracted from regions of interest (coloured arrows) are compared with reference model spectra for each of the forms: β-anhydrate (cyan), α-anhydrate (stable) (yellow), and α-monohydrate (magenta).

Raman Spectroscopic Analysis of Biogenic Gases and Drug Target Interactions

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The excellent chemical selectivity of Raman spectroscopy can be exploited for the investigation of antimalarial drugs and their molecular interactions with biological target structures [1-5].

Weak bio-molecular interactions, such as π-π interactions, hydrogen bonding etc., cause often only small changes in the molecular force constants and the observed wavenumber shifts in the spectra and are difficult to be resolved with conventional Raman spectrometers [1, 4, 5]. Thus, parallelized Raman difference spectroscopy was applied to analyze the molecular interaction of the antimalarial active agent chloroquine with the target structure hematin with high spectral resolution [1]. A shift of -1.1 cm^{-1} was observed in the core-size marker band $\nu(C_\alpha C_m)_{asym}$ peak position of the chloroquine-hematin-mixture compared to pure hematin [1]. The oxidation-state marker band ν(pyrrole half-ring)$_{sym}$ exhibited a shift by +0.9 cm^{-1} [1]. The results indicate a hydrogen bond between the quinolinyl moiety of chloroquine and the oxygen atom of ferric-protoporphyrin IX hydroxide (Fe(III)PPIX-OH) and are supported by density functional theory (DFT) calculations [1]. The consequence is a reduced electron density within the porphyrin moiety and increased core size [1].

The molecular interactions of the antimalarial drug artesunate with the target structure β-hematin were investigated by means of resonance Raman excitation, two-dimensional correlation analysis of stepwise increasing artesunate molar ratios, DFT calculations of the structures of β-hematin and its mono- and di-meso-alkylated complexes, as well as normal coordinate structural decomposition analysis [2]. Very small changes in the peak properties could be identified and interpreted [2]. Various changes in peak properties of distinct Raman bands with a high ratio of $C_\alpha C_m$-stretching of C_mH-bending-vibrations arose, because alkylation of the hematin unit takes part at the C_m-positions [2].

The permeation behavior of the promising antimalarial candidate ferroquine through parasitophorous membranes was investigated, which result in a strong enrichment at the site of action inside *Plasmodium falciparum* and efficient interaction with the lipophilic site of the malaria pigment formation [3]. DFT calculations on the drug's micro-speciation supported the assigned shifts in peak positions of resonantly enhanced high-wavenumber Raman signals at λ_{exc} = 257 nm [3]. FQ is fully protonated in polar mixtures like the host interior, the parasite's cytoplasm, or digestive vacuole and only present as a free base in nonpolar ones, such as the host's and parasitophorous membranes [3].

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Raman spectroscopy methods for studying pharmaceutical multiparticulate and liposomal formulations

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The formulation of medicines faces significant challenges to meet the requirements for safe and accurate administration. Multiparticulate (MP) formulations have a strong potential to address these challenges because they combine dose flexibility with ease of administration. Similarly, the approval of the first liposomal drug formulation in 1995 has provided momentum for a large area of nanomedicine. With these new developments, the need for analytical quality control methods and related standards to support product development, manufacturing and regulatory approval is becoming increasingly important. It would be advantageous to develop more rapid and non-destructive techniques, which could be deployed on the manufacturing line and provide real-time information.

We developed a method to measure the relative concentration of nanoparticles in a solution with Raman spectroscopy. Firstly, we used PS nanoparticles of different sizes as a model system, to test the measurement method. We then studied liposomes to measure the concentration of different components such as lipids and active ingredients. Measurements were performed on samples within closed vials, indicating this is a technique suitable for in-line measurements.

Understanding the physical stability of formulations during storage as a function of time and environmental parameters such as humidity and temperature is important to manage their commercialisation, supply chain and use. Current techniques for studying MPs include scanning electron microscopy (SEM) and confocal laser scanning microscopy, whose resulting chemical information is somewhat limited. We have employed advanced methods such as environmentally-controlled SEM to monitor temperature- and humidity-induced changes in-situ, and a range of Raman spectroscopies including stimulated Raman scattering (SRS) microscopy to identify and map the different ingredients at the surface and inside the multiparticulate formulations.[1]

These techniques allowed us to identify and monitor specific changes to the formulations and distribution of individual ingredients. We envisage these techniques to be useful in furthering the validation and development of a range of future medicines formulations.

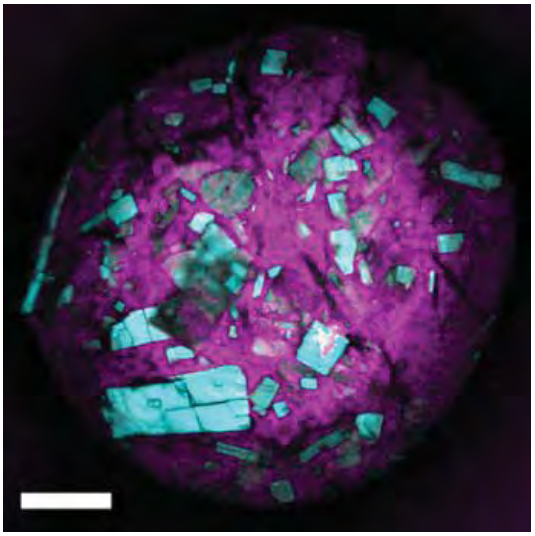

Figure 1: Stimulated Raman scattering microscopy maximum projection image of a multiparticulate formulation, with the drug in cyan and an excipient in magenta. Scale bar represents 50 µm.

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Spectroscopic, molecular properties and stability study of secondary metabolite- Natural Deep Eutectic solvent mixture

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Natural Deep Eutectic Solvents (NADES) are an emerging class of solvents that has recently gained significant attention in the pharmaceuticals, cosmetics, and food industries [1]. The present work aims to characterize NADES, which is comprised of Menthol, Myristic Acid, and Curcumin (MMCC), using spectroscopic techniques and computational methods. The main problems associated with Curcumin are its poor water solubility, low bioavailability, and high degradation rate. Enhancement of the solubility and bioavailability of this promising molecule is crucial for potential clinical application, which requires the development of efficient media. Raman, SERS, UV-Vis and IR spectra are recorded and correlated with the computational data. The MMCC are characterized using Density Functional Theory (DFT) using B3-LYP/6-31+G (d,p) level of theory. The molecular, chemical and vibrational properties of MMCC are obtained from theoretical calculations. The theoretically computed Raman and IR vibrational wavenumbers are found to be in agreement with experimental Raman, SERS, and FTIR spectra. Solubility and stability of Curcumin, extract and standard samples in NADES are tested. The Men-Myr-Cur solvent can extract more Curcumin from the raw turmeric rhizome and slow degradation of the Curcumin is observed in Men-Myr-Cur solvent.

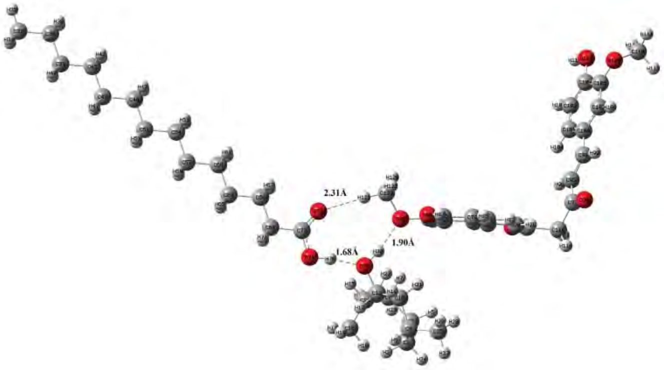

Figure 1: Optimize structure of Men-Myr-Cur

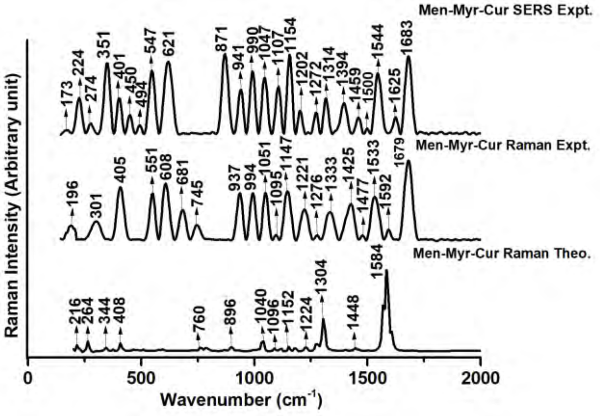

Figure 2: Theoretical and experimental Raman and SERS spectra of Men-Myr-Cur in the spectral range (0 -2000) cm^{-1}

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Spectroscopic properties of agomelatine - loaded hydrogels

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According to WHO, one of the most common mental disorders in the world is depression which affects c.a.5% of adults which makes it of great social importance [1]. Agomelatine (AGM) acts on melatonin and serotonin receptors. It is successfully used in treatment of depression, approved for clinical use in the European Union in 2009. Unfortunately its characterized by low bioavailability after oral administration is a significant inconvenience [2]. The solution to this problem may be transdermal administration of the drug in the form of e.g. a gel, and in the future, the use of 3D printing methods to produce new forms of drugs as additively produced microneedles. The main advantages of transdermal path are: increases patient compliance, preventing the drug from degradation in the gastrointestinal tract and first-pass effects of the liver and sustaining therapeutic drug level [3]. Raman spectroscopy, due to its high sensitivity, lack of special preparation of the sample and non-destructive nature, is increasingly used in the pharmaceutical industry, including for the identification of active substances, excipients, identification of polymorphic forms, or its distribution in the final form of the drug [4].

The main aim of the presented research was to determine the impact of temperature to spectroscopic properties of agomelatine and two types of gels containing AGM (with and without ethanol) for skin application. Ethanol gels and suspensions with AGM differ visibly. Micro-needle (MN) transdermal systems containing AGMs were also made using the Digital Light Processing (DLP). The Raman spectroscopy allowed to determine the polymorphic form of agomelatine in all drug forms studied and its distribution in the 3D printed solid drug form.

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UV- and Fiber-Assisted Raman Spectroscopic Drug Monitoring

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In pharmaceutical analytics and medical theranostics exists a hight demand for accurate and efficient drug monitoring. Conventional approaches, encounter challenges regarding their speed, sensitivity, and bedside applicability. This situation underscores a pressing need for advancements in real-time, accurate detection and quantification of pharmaceutical compounds[1].
Recent innovations in Raman spectroscopy, particularly those exploiting fiber[2-4]- and deep-ultraviolet (UV) enhancement techniques[5, 6], represent a significant leap forward in addressing these challenges. These advancements may facilitate the sensitive and precise monitoring of drug levels, thereby offering new approaches for the treatment of sepsis. Specifically, these developments have enabled the sensitive detection of antibiotics in body fluids such as urine[2, 5] and plasma[6], marking a step towards rapid therapeutic drug monitoring (TDM), which is critical in the treatment of severe infections with antibiotic regimens[7].
Moreover, fiber-array-based Raman spectroscopic imaging[8, 9] was applied for the analysis of multiple active pharmaceutical ingredients (APIs), regarding their particle size, shape, and distribution[8]. This technique was also applied for the direct detection and quantification of the APIs in the antimalarial tablet Riamet® [9] and has proven to be promising as an *in-situ* counterfeit and substandard detection tool. Thus, these advancements underscore the versatile applicability of enhanced Raman spectroscopy for drug monitoring.

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Raman Spectroscopy of 2D Transition Metal Dichalcogenides Interacting with Metals

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The research on Transition Metal Dichalcogenides (TMDs) has progressed significantly, prompted by the increasing interest for this kind of materials as potential candidates in future ultrathin optoelectronic devices. The most prominent member of the TMD class is molybdenum disulphide (MoS_2), a layered semiconductor with a gap in the visible region, which can be exfoliated down to the monolayer 2D form. Since the discovery of the exceptional optoelectronic properties of 2D TMDs, Raman spectroscopy has been extensively used as a tool to characterize their structure and thickness [1].

The future application of TMD films in real devices requires high crystalline quality, stability under operating conditions and engineered functional metal contacts. In this framework a detailed understanding of the interface properties of TMD-metal heterosystems is necessary. Moreover, the deposition of TMDs on metal surfaces—either by exfoliation or bottom-up growth—has been recently explored as a promising method to obtain mono-layer TMD films of a relatively large-area. The interaction with metals is known to have profound effects on the electronic properties of 2D TMDs. Recent works on TMD-metal interfaces and metal-assisted synthesis methods have shown that also their Raman spectra are significantly affected by the interaction with metals, especially for TMDs grown by vapor phase techniques [2,3]. However, a clear understanding of how such interaction modifies the TMDs vibrational properties is still lacking.

Here we discuss how the Raman response of single- or few-layer TMDs, such as MoS_2, WS_2 and MoS_2/WS_2 heterostructures, deposited in UHV by Pulsed Laser Deposition on Ag(111), Au(111), Au(100) and Ag(110) surfaces, is affected by interaction with the metal and how it modifies upon exposure to ambient conditions or after transfer of 2D TMDs on different substrates, such as silicon oxide [4,5].

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Study of the performances of colloidal and solid SERS substrates made up of PDA-coated silver and gold nano-objects

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SERS technique has been widely used by several research groups for the trace detection of various organic dyes, pesticides, and biomolecules down to the single-molecule level. Anyway, the need for stable, reusable, and efficient SERS sensing chips remains crucial for the possibility of label-free trace detection of organic molecules in real environments. Nowadays, the composites formed with polymers and metallic NPs have attracted great interest due to their multifunctionality and are potential alternatives for developing efficient and scalable SERS substrates.

In this work we carefully studied the SERS properties of different SERS chips made up of metal nano objects coated with an ultra-thin layer of polydopamine (PDA). PDA is a versatile biopolymer with great adhesive properties that spontaneously self-polymerizes from dopamine (DA) in weak alkaline media and in the presence of oxygen. Both gold nanostars (GNS) and silver nanospheres (AgNS) were used as metallic enhancing substrates.

The SERS tests were performed on PDA-coated colloids as well as on solid glassy chips decorated with the nano objects by means of self-assembled monolayer approach and subsequently covered by a thin PDA layer, as illustrated in Figure 1 for gold based substrates. In all cases, the SERS response was investigated at different PDA thicknesses in the range 0-10 nm.

For all the SERS substrates a detailed characterization of the morphology has been carried out by TEM and SEM microscopies. The thicknesses of the different PDA layers were estimated by combining profilometry, AFM and ellipsometry measurements. The main plasmonic resonances were derived from UV-vis-NIR absorption measurements.

The SERS measurements were performed on PDA@GNS by using Rhodamine6G as Raman reporter while for PDA@AgNSs Methylene Blue was used. The SERS performances were tested in terms of i) surface reproducibility by mapping the SERS signals at micron and millimeter scales, ii) stability over time and iii) enhancement factors. The results suggest that 1-2 nm thick PDA layers can improve SERS performances. Possible applications in the detection of emerging pollutants exploiting adhesive properties of PDA are discussed.

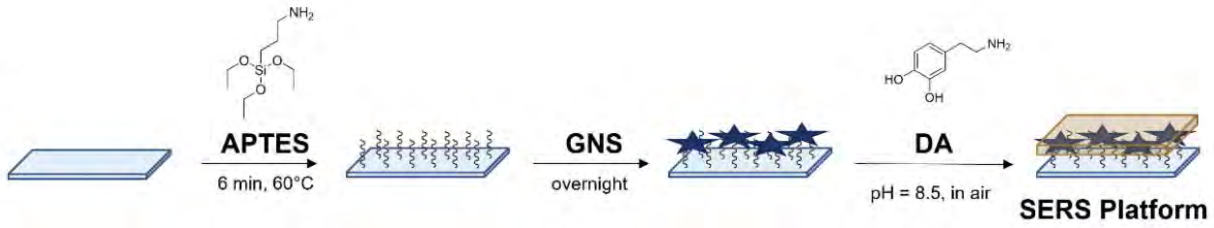

Figure 1 Scheme of the synthetic path to obtain PDA@GNS glassy SERS substrates.

Determining the Crystal Orientation of TMDs by Angular-Resolved Second-Harmonic Generation

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Second-harmonic generation (SHG) is a unique tool for the investigation of transition metal dichalcogenide (TMD) flakes and can even be used to determine their orientation in an experiment [1,2]. We present our studies on the angular-resolved detection of the SHG in the back-focal plane (BFP) of a microscope objective in monolayer WSe_2 and $MoSe_2$. We first analyze the signal produced by the center of monolayer flakes and demonstrate how edges influence the angular SHG radiation pattern. Moreover, we find that we can determine the crystal orientation of the flake from the ellipticity of the detected SHG back-focal plane image. We compare these BFP images with the 6-fold pattern recorded while simultaneously rotating the incident polarization and analyzer. As we show in figure 1, We observe that when the incident field is polarized in the armchair direction, the ellipticity of the SHG-BFP pattern is increased compared with the zig-zag direction.

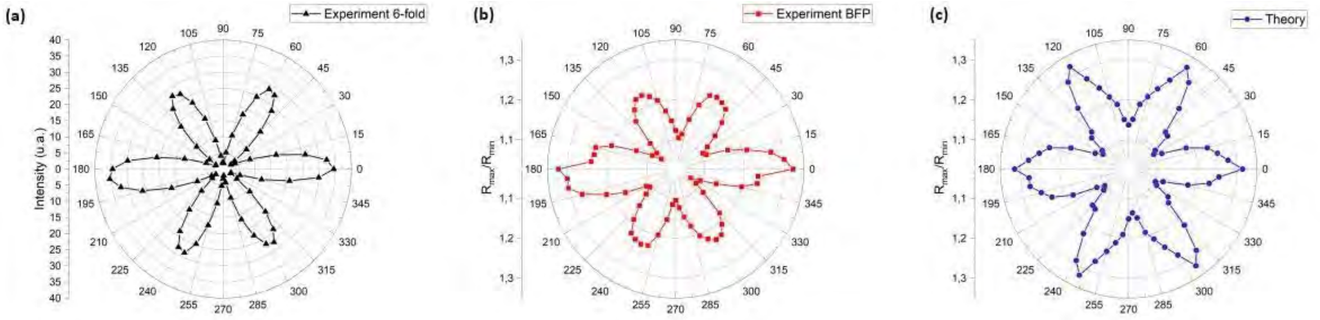

Figure 1: Second-Harmonic generation polar plot. Figure (a) shows the polar plot measured with analyser parallel related to incident bean detected in spectrometer. Figure (b) shows the polar plot based in the ratio of major radius and minor radius of the ellipses measured by second harmonic back focal plane. Figure (c) shows the polar plot based in the ratio of major radius and minor radius of the ellipses generated in our simulation.

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Surface Resonant Raman Scattering from Cu(110)

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We report the first evidence of Raman scattering from surface phonons of a pristine metal surface [1]. Our study reveals a Raman-active surface vibrational resonance on Cu(110) with a surprisingly large scattering efficiency. With the incident photon energy close to the energy of the Cu(110) surface state electronic transition [2], the Raman scattering from the surface optical resonance can be significantly enhanced, while any contribution from bulk phonons is absent. Density functional theory-based calculations of the electronic band structure show that the observed Raman signal can be unambiguously attributed to the coupling of this specific mode with electronic surface states, and provide insight into the mechanism causing the strong enhancement of the Raman scattering. Furthermore, we show that resonant surface Raman scattering can be used to analyze vibrations caused by adsorbates on metal surfaces.

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Superlattice nanowires: a versatile platform for phonon engineering

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Phonons are the vibrations of the atomic lattice responsible for heat and sound transport in materials. Thus, the ability to manipulate and control phonons lie at the heart of thermal management. [1] To effectively manage heat, it is imperative to engineer materials at the nanoscale, a task demanding sophisticated fabrication and/or growth techniques. Superlattices (SL), characterized by their compositionally modulated periodic heterostructures have been used to explore novel phononic properties. [2] In particular, SL structures with nanoscale periodicities are a model system to study the cross-over from coherent to incoherent phonon transport. [3] Efficient phonon engineering in SLs necessitates smooth interfaces between layers, relative to the characteristic phonon wavelengths. However, achieving such smooth interfaces poses a significant challenge during the growth process, creating a bottleneck in the fabrication of nanoscale SL structures. Nanowires (NWs), on the other hand, are quasi one-dimensional structures which allow the growth of axial and radial heterostructures otherwise difficult in planar structures. [4] SL NWs are, therefore, a powerful platform to manipulate heat transport using coherent phonon effects.

In this study, we aim to showcase the remarkable adaptability of NWs as a platform for effective phonon engineering. Specifically, we focus on the investigation of SL NWs composed of group III-V semiconductors, namely GaAs/GaP and InAs/InP. [2,5] Group III-V semiconductor NWs are ideal material systems to explore nano-photonic and electronic properties. [6] However, their phononic properties remain unexplored. Using inelastic light scattering techniques such as Raman spectroscopy and Brillouin interferometry, we show that the phononic and electronic properties of SL NWs can be tuned by controlling the SL periodicity. Our results are also corroborated with the help of ab-initio calculations.

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Raman studies from InAs/GaAsSb Quantum Dot Heterostructures

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We use Raman spectroscopy to investigate strain coupling in self-assembled InAs Quantum Dot (QD) systems, leading to enhanced absorption efficiency through vertical ordering in multilayers. Our study focuses on phonon analysis of two-layered InAs/GaAsSb QD heterostructures using low temperature polarized Raman scattering. At Sb content > 14-16%, a shift in band alignment from type I to type II is observed, resulting in room temperature emission at 1.7 µm. Samples were grown using molecular beam epitaxy (MBE) [1], with two dot layers separated by a 6.5 nm spacer layer and capped with 4 nm $GaAs_{1-x}Sb_x$ material, as shown in Figure 1. High-resolution transmission electron microscopy (HRTEM) reveals a 'lenslike' shape for the QDs, with larger sizes observed for higher Sb content. *Nextnano* software simulations show a strain field distribution along the depth, with both upper and lower QDs experiencing compressive strain, reduced in magnitude for higher Sb content. Raman measurements were carried out at 77 K with 532 nm excitation source and analyzed using a HR800-UV confocal micro-Raman spectrometer. Spectra in $z(xx)\bar{z}$ geometry reveal optical and interface (IF) phonons, with peaks attributed to InAs, GaAs, and $GaAs_{1-x}Sb_x$ QDs. Peaks 5 and 3 are GaAs LO and TO modes. Using the simulated strain profile, we have calculated the strain induced shift for the different optical modes following a method by Cardeira et al [2]. The InAs LO peak is blue shifted to ≈ 261 cm^{-1} due to the large compressive strain which agrees very well with observed one at ≈ 260 cm^{-1}(Peak2) consistent with simulations. Frequency positions of modes in sample B are shifted to higher energy compared to sample A due to lesser strain. Shoulder peaks at 4 and 1 have been assigned to GaAs-like IF and InAs-like IF modes observed at lower frequency side of GaAs / InAs LO phonons based on our calculation using the classical dielectric model using a ellipsoidal/spheroidal shape for the QDs.

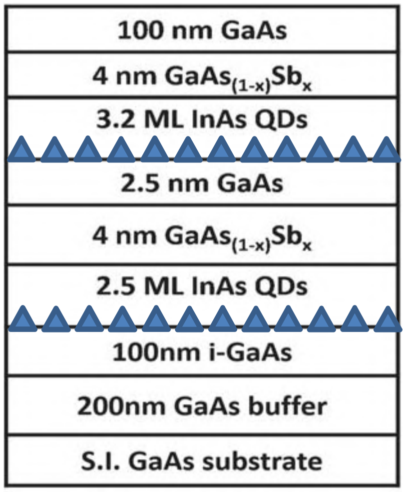
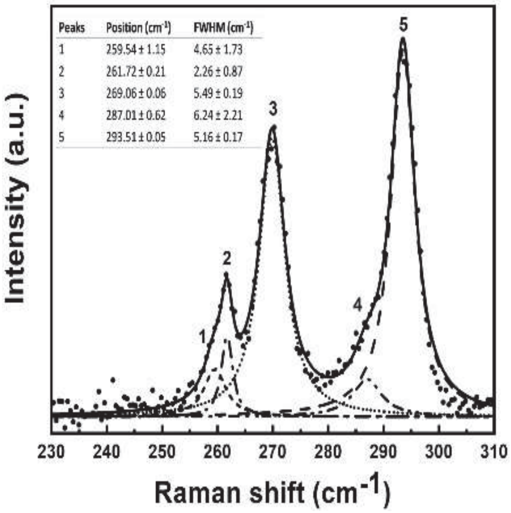


Figure 1 The Bilayer QD Heterostructure



Peaks	Position (cm^{-1})	FWHM (cm^{-1})
1	259.54 ± 1.15	4.65 ± 1.73
2	261.72 ± 0.21	2.26 ± 0.87
3	269.06 ± 0.06	5.49 ± 0.19
4	287.01 ± 0.62	6.24 ± 2.21
5	293.51 ± 0.05	5.16 ± 0.17

Figure 2 Raman spectrum for sample A

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The Interaction between Fluorescent Molecules and Plasmonic Nanoparticles: From SERS to SEF

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The electric field intensity can be locally enhanced thanks to metallic nanoparticles (or surfaces) that give rise to localized surface plasmon polaritons (SPPs). This mechanism is the basis for electromagnetic enhancement (EM), which plays a crucial role in amplifying Raman signals within Surface Enhanced Raman Spectroscopy (SERS).

Raman scattering, however, is not the only process affected by the increase in the electric field intensity; fluorescence can increase as well, resulting in Surface Enhanced Fluorescence (SEF).

While SERS peaks at very close distances from the metal surface, fluorescence is often quenched at such proximity, necessitating distances of the order of a few nanometers to tens of nanometers for SEF[1]. The distance from the metallic surface, however, is not the only factor affecting these phenomena; particle material, morphology[2], as well as excitation wavelengths are also involved.

Here, the interaction between a fluorescent molecule and a plasmonic nanoparticle is studied to deeply investigate the balance between these two processes. Plasmonic nanoparticles of different materials (silver and gold spherical nanoparticles) as well as different morphologies (gold nanorods) are employed. Additionally, different distances as well as different excitation wavelengths are explored. All these parameters are evaluated to obtain a deeper understanding of all the complex mechanisms involved in the interaction between a fluorescent molecule and a plasmonic nanoparticle.

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Nanoscale Chemical Analysis of Thin Film Solar Cell Interfaces using Tip-Enhanced Raman Spectroscopy

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In the realm of thin film solar cells, Sb_2Se_3-based cells have gathered significant attention over the last decade due to their non-toxic nature, bandgap of 1.0 - 1.2 eV, high absorption coefficient and the highest reported power conversion efficiency (PCE) of 10% [1]. However, despite the substantial interest and promising performance of Sb_2Se_3 thin film solar cells, the PCE efficiency is still significantly lower than the theoretical value. This is primarily due to the limited chemical and structural understanding of the layer interfaces, where chemical defects such as newly formed compounds or structural defects such as inhomogeneous layer separation can critically influence PCE. Since the interfacial regions in these solar cells have nanoscopic dimensions, their analysis demands nanoanalytical tools. However, conventional analytical techniques fail to meet this challenge since the information from the individual layers is averaged, precluding true nanoscale analysis.

In this study, we overcome these challenges with tip-enhanced Raman spectroscopy (TERS) and apply it to investigate the absorber (Sb_2Se_3) and buffer (CdS) layers interface in a Sb_2Se_3-based thin film solar cell [2]. Hyperspectral TERS imaging with 10 nm spatial resolution revealed an intermixing of phases instead of a sharp boundary between the CdS and Sb_2Se_3 layers. Intriguingly, this interface exhibited an unexpectedly large thickness of 295 ± 70 nm attributable to the Sb_2Se_3 layer roughness. Furthermore, TERS measurements provided compelling evidence of CdS penetration into the Sb_2Se_3 layer (Figure 1), likely resulting from unwanted reactions with the absorber surface during the chemical bath deposition process. Furthermore, nanoscale TERS mapping confirmed the coexistence of ZnO, which serves as the uppermost conducting layer, and CdS within the Sb_2Se_3-rich region for the first time. Overall, this study demonstrates the potential of TERS for nanoscale investigation of thin film solar cell interfaces and provides novel insights into their intricate interface structures and compound intermixing [2].

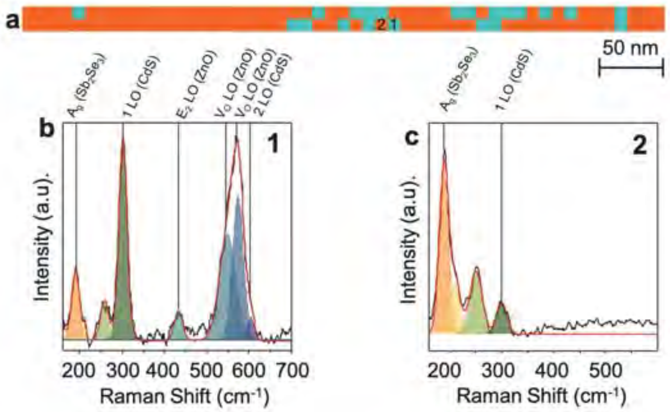

Figure 1: a) Overlay of the TERS maps of Sb_2Se_3 (orange) and CdS (blue) Raman signals. Step size: 10 nm. (c) TERS spectrum measured at the pixel marked as 1 in Panel a. Strong CdS (300 cm^{-1}) and ZnO (433 cm^{-1}, 549 cm^{-1} and 574 cm^{-1}) signals are observed at this position. (d) TERS spectrum measured at the pixel marked in Panel a. A strong Sb_2Se_3 signal (190 cm^{-1}) is observed at this position.

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Raman Spectroscopy Unlocks the Unique Vibrational and Electronic Properties of Linear Carbon Atomic Wires

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Carbon atomic wires are 1-dimensional carbon molecular systems. They represent one of the finite realizations of the elusive allotrope Carbyne, the ideal infinite chain of sp-hybridized carbon atoms with two possible structures: semiconducting polyyne with alternated single-triple bond and metallic cumulene with all double bonds. In finite systems, the length and the terminations affect the wire structure in a significant way, thus opening the way to tailor the electronic and optical properties by controlling their structure [1]. For these reasons, carbon atomic wires are attractive for a wide range of applications, including electronic devices [2,3].

In this framework, Raman spectroscopy turns out to be fundamental for both understanding the experimental synthesis of these systems and performing ad advanced spectroscopic investigation of their intriguing properties [4-7].

Here, we discuss how Raman spectroscopy, including surface-enhanced Raman scattering (SERS) and UV resonant Raman spectroscopy, unveils the peculiar structure-dependent properties of these systems. Exploiting tunable UV source at synchrotron light sources, we were able to resonantly enhance the Raman process at each vibronic peak of size-selected carbon atomic wires. These studies allowed us to retrieve the fine structure of the vibronic levels in the ground and excited state and to investigate size-dependent electron-phonon coupling and anharmonic effects in these systems as a function of wire length and termination [5,6].

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SERS-based Assay Platforms for Rapid and Accurate Diagnosis of Infectious Diseases

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The global impact of the COVID-19 pandemic has led to significant challenges in both social and economic aspects worldwide. Currently, the standard diagnostic method for COVID-19 involves RT-PCR, which identifies RNA within the virus. However, the diagnostic process, including sample preparation, gene amplification, and detection, takes approximately 3-4 hours. While rapid immunodiagnosis kits utilizing antigen-antibody reactions have been introduced to expedite the diagnosis, their adoption as the standard method is hindered by their low limit of detection and poor accuracy [1]. One critical issue with commercialized immunodiagnostic kits is the risk of false-negative results, which can exacerbate the spread of COVID-19. To address this challenge, we have developed innovative SERS-based assay platforms to measure SARS-CoV-2 lysates with exceptional sensitivity quantitatively. This presentation will showcase two SERS-based assay platforms, including a SERS-based lateral flow assay (LFA) immunodiagnostic strip equipped with a portable Raman reader [2] and an Au-nanoparticle-internalized nano-dimple SERS-PCR sensor. Our SERS-based assay platforms exhibit significant potential in overcoming the limitations of low sensitivity and quantitative analysis constraints inherent in conventional antigen tests for SARS-CoV-2 detection. The outcomes of our study highlight the feasibility of clinical applications that can substantially enhance the detection limit and accuracy compared to currently available commercialized COVID-19 immunodiagnostic kits.

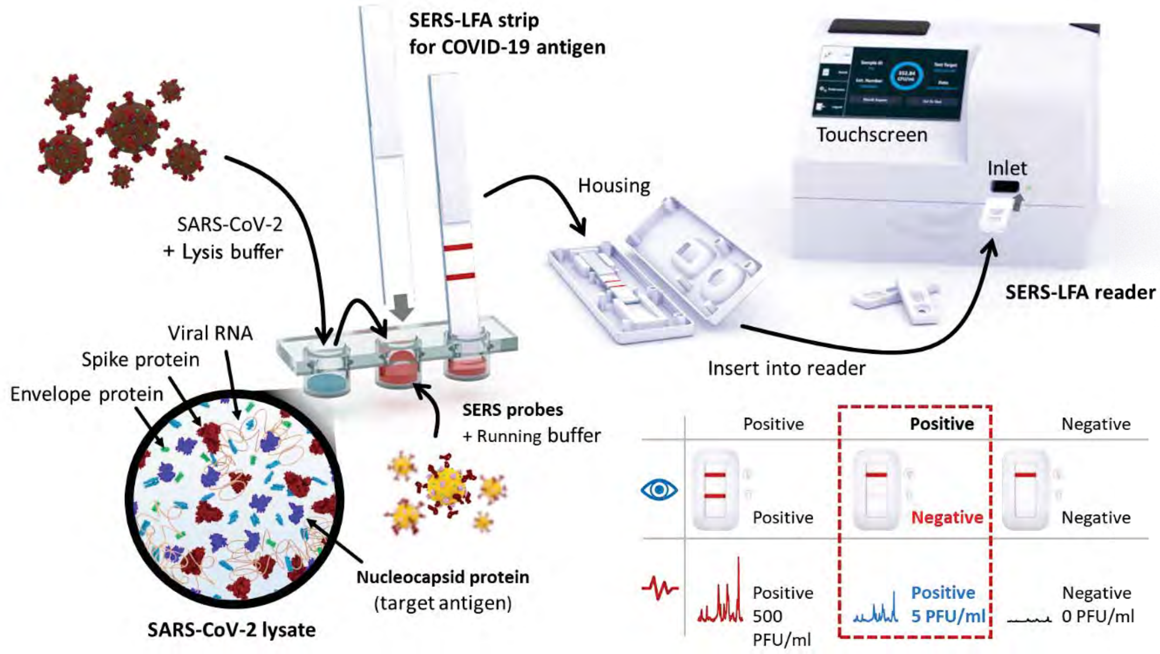

Figure 1: Illustration of SERS-LFA system for on-site diagnosis of COVID-19

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Sensitive and Selective Bioanalysis using SERS

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Surface enhanced Raman scattering (SERS) is an analytical technique with several advantages over competitive techniques in terms of improved sensitivity and multiplexing. We have made great progress in the development of SERS as a quantitative analytical method. Many bioanalytical detection methods exist, with fluorescence spectroscopy tending to dominate, however SERS has the advantage that it is both sensitive and has the ability to multiplex which is limited when using techniques such as fluorescence. We have developed approaches to both identify and quantify the presence of multiple analytes within a mixture e.g. pathogenic DNA sequences, bacteria using SERS combined with data analysis techniques.

Here we demonstrate the development of new bioanalytical assays based upon SERS which have been used successfully for the detection of bacterial pathogens using modified SERS active probes. Biomolecule functionalised nanoparticles have been designed to give a specific SERS response resulting in discernible differences in the SERS which can be correlated to the presence of specific pathogens. In this presentation the simultaneous detection and quantitation of 3 pathogens within a multiplex sample will be demonstrated. We will also explore the use of SERS active functionalized nanoparticles for the detection of biomarkers using SERS based lateral flow immunoassays (SERS-LFIA). In this approach we have developed an assay for the detection of a biomarker related to drug induced liver injury (DILI) in patient samples in combination with a portable Raman spectrometer. This has potential for the triaging of patients for treatment after paracetamol/acetaminophen overdose.

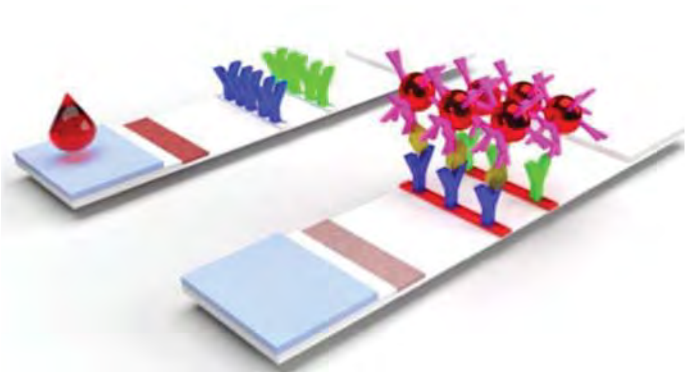

Figure 1: SERS-LFIA for DILI

Plasmonic nanopores for single-molecule Raman sequencing

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Proteins are the workhorses in biological systems that can be used to characterize cellular functions and progress of diseases as biomarkers. The fact that proteins cannot be amplified results in a serious lag of proteomics behind genomics and transcriptomics, hampering not only mechanistic studies but also clinical applications. Compared to DNA, proteins are folded 3-dimentional structures and consist of 20 proteogenic amino acids with different charges. These features limit these single-molecule sequencing methods, because they can detect 4 at maximum of the 20 proteinogenic amino acids.

Surface-enhanced Raman spectroscopy (SERS) as a powerful label-free biomedical detection method with single-molecule sensitivity. In fact, each amino acid molecules have their own fingerprint Raman scattering signals that can be used for sequencing. But the Raman signals of single amino acids were too weak to be detected even by SERS. In the tremendous amount of literature of SERS-based protein detections in past decades, the SERS spectra of proteins are mostly occupied by SERS signals of aromatic amino acids and backbones, while those of non-aromatic amino acids are invisible.

In this talk, we will present our recent work on plasmonic nanopore-enhanced Raman biosensors that has demonstrated manipulation of single biomolecules and single-molecule detection (Figure 1). The plasmonic bowl-shaped nanopore under electric bias generated electro-osmotic sheath flow that unfolded single DNA and directed it toward the plasmonic hot spot for SERS detection.[1] On the other hand, by trapping single gold nanoparticle in a gold nanopore to generate a single plasmonic hot spot in the nanogap, the Particle-in-Pore sensor achieved detecting single aromatic and non-aromatic amino acid residues within single peptides.[2] Our results suggest that the signal dominance due to large spatial occupancy of aromatic rings of the peptide sidechains can be overcome by the high localization of the single hot spot. With reference to a protein database, our works have paved a ground-breaking way to nanopore-enhanced Raman identification and sequencing of single proteins.

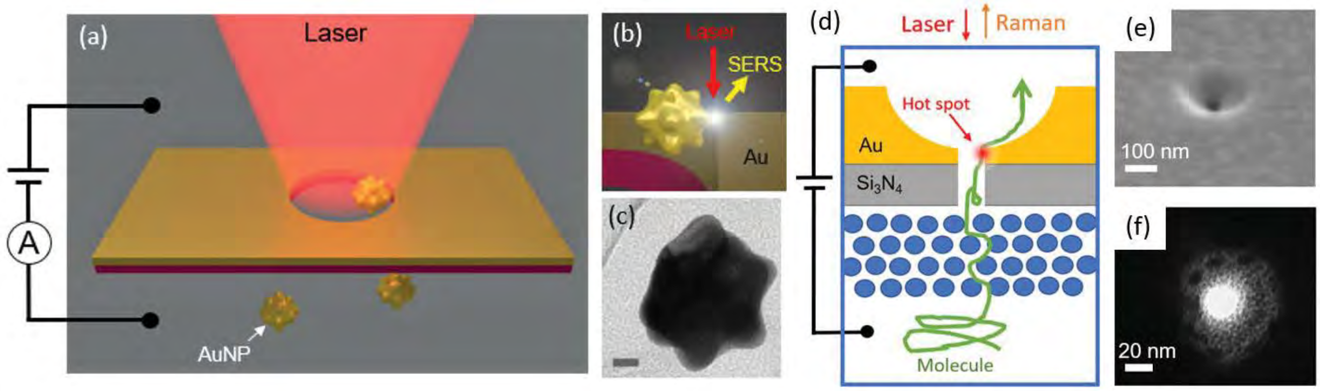

Figure 1: (a-c) the plasmonic particle-in-pore sensors; (d-f) the plasmonic bowl-shaped nanopore sensor.

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SERS Dipstick Assay for Simultaneous Multiplex Subtyping of Breast Cancer Derived Small Extracellular Vesicles

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Breast Cancer pertains to be second most deadly cancer in the world and accounted for 31% of all female cancer cases in 2023 [1]. Early diagnosis is paramount in all cases for early intervention and treatment; however, this remains a challenge as current medical imaging only accounts for when the tumors have already formed [2]. Hence, there remains to be a demand for a point-of-care device that is affordable, rapid sensitive, specific and user-friendly [3-4]. A new surface-enhanced Raman scattering (SERS) dipstick assay design is thus proposed for the quantitative detection of EpCAM, a cancer specific biomarker on small extracellular vesicles (sEVs). Due to the heterogeneous nature of sEVs, targeting only one specific surface protein of sEVs can limit the detection ability of the assay [2,4]. Hence, sEVs were captured by the three most common surface proteins, CD9, CD81 and CD63 on the surface of the assay, as illustrated in figure 1 to account for varying surface expression between different cancer subtypes [2]. The assay has the ability to be used in multiplex quantitative detection through SERS which is crucial for diagnostic and treatment monitoring purposes [4].

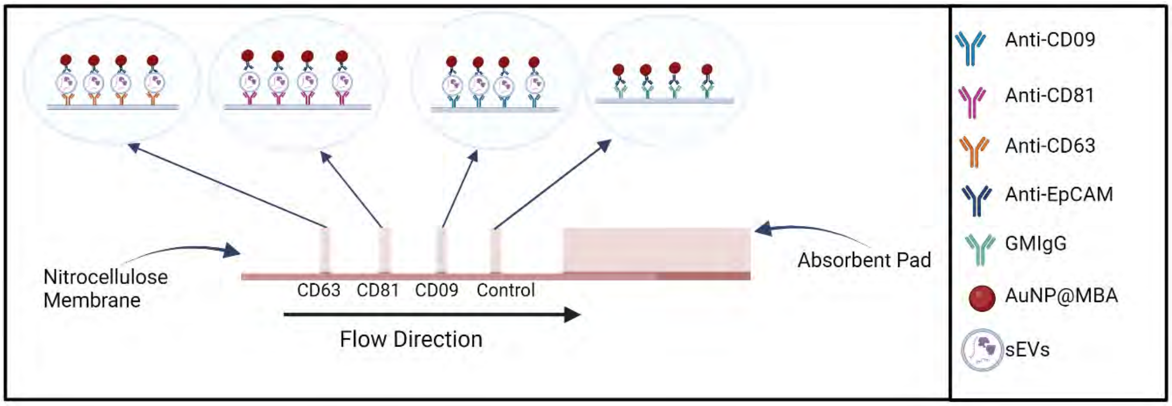

Figure 1: Schematic illustration of the assembly of the SERS test strips for detection and profiling of cancer sEVs.

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Applications of surface enhanced Raman scattering (SERS) spectroscopy for detection of DNA

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The deoxyribonucleic acid (DNA) is the carrier of genetic information for all living organisms. Even a small mutation in DNA sentence can cause serious diseases. Therefore, developing sensitive, fast and precise methods for detection of DNA mutations is very important from a practical point of view. A promising method for detection of specific DNA fragments is surface-enhanced Raman scattering (SERS) [1,2]. In SERS spectroscopy, if molecules of some analytes are located at or near the surface of nano-structured plasmonic materials, the efficiency of the generation of Raman signal can be increased by many orders of magnitude. In some cases, the efficiency of the generation of Raman signal in the SERS effect is so large, that it is possible to record a good quality Raman spectrum even of a single molecule. Therefore, SERS spectroscopy is one of the most sensitive analytical tools. In this presentation some methods of detection of various DNA fragments using SERS spectroscopy will be described. We focus especially on developed by us methods based on hybridisation-induced rearrangement of the alkanethiol linking moieties via which capture single stranded DNA chains are attached to the surface of the plasmonic SERS substrate and the rearrangement of the chains of 6-mercaptohexan-1-ol which is also usually chemisorbed on the surface of the SERS-active plasmonic metal to eliminate the direct interactions of nitrogenous bases of DNA with the metal surface. The scheme of the SERS DNA sensor utilising the rearrangement of the alkanethiol chains (alkanethiolate linking moiety of capture single stranded DNA and 6-mercaptohexan-1-ol) during DNA hybridization is show in Figure 1. Such rearrangement is indicated by a characteristic change in the measured SERS spectrum. Developed new SERS DNA (bio)sensor has been tested on clinical samples and is characterized by the low detection limit at the level of μg/L, wide analytical range from *ca.* 7 μg/L to *ca.* 70 mg/L and high selectivity.

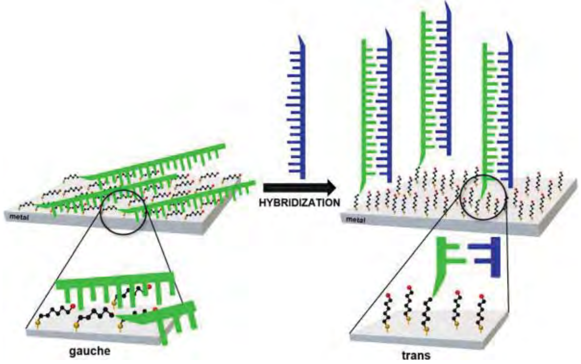

Figure 1: Scheme of the SERS DNA sensor based on rearrangement of the alkanethiol chains (alkanethiolate linking moiety of single stranded DNA and 6-mercaptohexan-1-ol). Reprinted from ref. [2]. CC BY license.

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SERS Biosensors for Early Diagnosis and Treatment Guidance in Plants

Molecular analysis is essential for precise and data-driven agriculture, but its use in agricultural settings is limited due to the current burdensome analytical paradigm. Nanotechnology can revolutionize molecular analysis by decentralizing it and making it easily accessible. This paradigm shift can result in more sustainable and efficient agricultural practices.

This work presents the development of a nanosensor technology for *in-situ* detection of infected plants. Our platform uses homogeneous surface-enhanced Raman scattering (SERS) sensing to detect viral infections in plants. The sensors are incorporated in gel networks, enabling sample preparation-free analysis in the field using a “touch-and-detect” method. The gels efficiently uptake genetic material, while the sensors give a specific and sensitive response to the target sequence. We recently demonstrated this approach to detect viral infections in tobacco plants [1]. This sensing approach is versatile and can detect plant disease biomarkers (DNA), plant pathogens (viral RNA), and/or small molecule contaminants (pesticides). This presentation will focus on the demonstration of these sensors to detect early viral infections in plants to guide treatment and mitigation strategies.

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Maximizing label-free SERS sensitivity and spectral resolution for therapeutic drug monitoring application: SPE prior to a lab-on-a-disc

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The demand for rapid and decentralized drug analysis methods, particularly for Therapeutic Drug Monitoring (TDM), is ever-increasing in clinical settings [1]. The demand for a compact, all-in-one, sample-to-answer solution for analysing small molecules within complex matrices is persistent and challenging in analytical chemistry [2]. This study introduces a novel method to enhance the integration and automation of a robust Surface-Enhanced Raman Spectroscopy (SERS) assay without the need for labels. The aim is to enhance control, reproducibility, and sensitivity in quantifying methotrexate (MTX), anticancer drug, in human serum. The approach combines a miniaturized solid-phase extraction (SPE) technique for efficient drug separation with a centrifugal microfluidics disc design containing a sensitive nanopillar-based SERS substrate(CD-SERS). This automated platform enables precise sample wetting, analyte migration, and localized analyte distribution on the SERS substrate, improving the accuracy and specificity of analyte detection. Additionally, a robust algorithm for data analysis based on Partial Least Squares Regression (PLSR) enhances the accuracy of MTX quantification in commercial human serum. Results indicate that combining SPE with CD-SERS improves the signal-to-noise ratio, and the implemented PLSR model achieves Limit of Detection (LoD) and Limit of Quantification (LoQ) values of 2.90 and 8.92 µM for MTX, respectively. The study validates the combination of SPE with CD-SERS against HPLC and immunoassay using MTX clinical patient samples, demonstrating its potential for clinical application with a significant recovery rate (80%) in patient samples. Overall, this approach presents a promising strategy for integrating and automating label-free SERS assays for point-of-care clinical use.

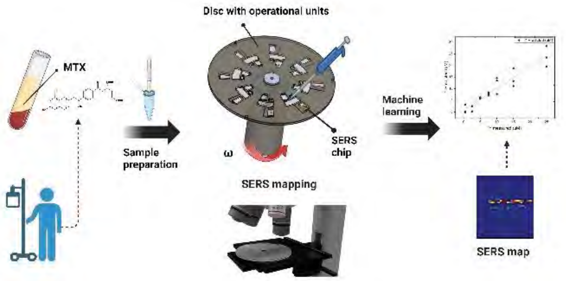

Figure 1: Overall procedure of the drug quantification assay implemented in this work

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MULTISPECTRAL OPTICAL SENSOR FOR MONITORING PSYCHOLOGICAL STRESS

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The human skin is a complex organ responsive to physiological and psychological states. The usefulness of skin metabolites as a source of biomarkers was demonstrated for dermatologic, systemic diseases, and psychiatric conditions (major depressive disorder, schizophrenia)[1]. It was shown that psychological stress-induced skin reactions can include changes in cytokine secretion acute/chronic secretion of corticosteroids[2] and changes in volatile components[3] in the skin. In the current study, we aim to individuate stress-related skin response with a high-throughput multimodal combination of optical techniques.

Raman, reflectance, and fluorescence spectra were recorded on the palm skin of healthy volunteers using a multimodal fiber-probe device developed by our group and previously described[4]. The cognitive-emotional stress was induced with the Montreal Imaging Stress Task[5]. Electrodermal activity, heart rate, and heart rate variability were monitored throughout the experiment.

A multimodal approach achieved 100% accuracy in classifying “no-stress” and “stress” conditions. The Raman bands associated with several stress biomarkers such as cortisol, lactic acid, and ceramides correlated with the spectral shifts observed in our experimental conditions. These preliminary results confirm the potential in application of our multispectral optical sensor for the non-invasive detection of psychological stress and psychiatric disease conditions.

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The impact of Raman spectroscopy on the understanding of artists' materials and the preservation of works of art

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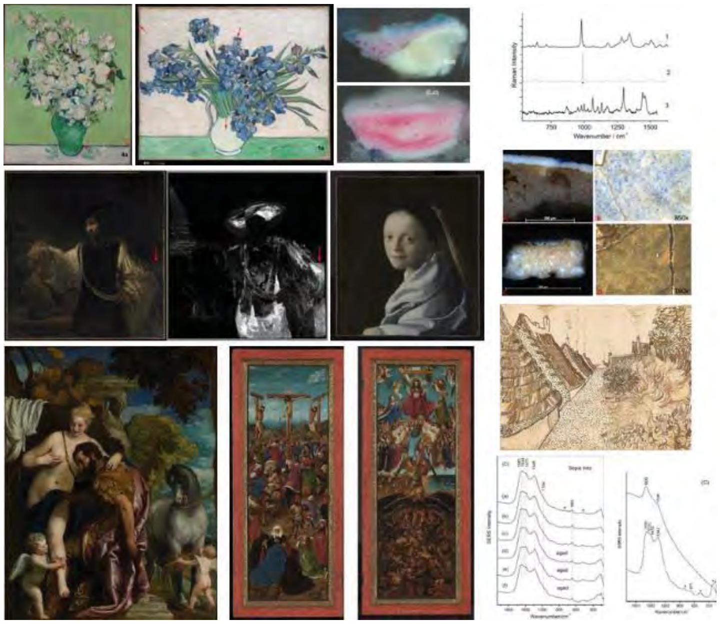
Since pioneering works in the 1980s and 1990s, such as those of Guineau[1] and Coupry et al.,[2] and the pivotal publication of the first libraries of natural and synthetic pigments[3,4] and of a catalogue of minerals often found in corroded metal and glass artifacts,[5] Raman spectroscopy has become an indispensable technique in museums' and other cultural institutions' laboratories to characterize and identify materials and deterioration products in paintings, drawings, prints, manuscripts, photographs, and other objects, such as sculpture and ceramics.

Works of art are typically composed of heterogeneous materials consisting of inorganic and/or organic compounds structured in complex ways. Deterioration processes often involve interactions at the interfaces of these components, with degradations in one triggering subsequent chemical reactions and physical changes in another. The talk with discuss what we learnt from the Raman analysis of paintings by van Gogh, Rembrandt, Vermeer, Veronese, van Eyck, and other artists, and how the results are not only crucial for increasing our appreciation of the masterpieces but also for preserving them for future generations. [6,7,8,9,10,11]



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Investigating the effect of proximity in a Gradient-SERS nano/micro-cavity device on the Raman signal intensity

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Here we investigate the effect of proximity of a Surface Enhanced Raman Scattering (SERS) substrate on the intensity of the Raman signal from an analyte layer. Our research hypothesis began with efforts to characterize monolayers of polyelectrolytes using regular Raman spectroscopy. We were not able to detect Raman scattering from a single organic monolayer. SERS in principle could provide enough enhancement however, SERS substrates were not always readily compatible with the aqueous Layer-by-Layer polyelectrolyte deposition process. Therefore, inspired by Tip-Enhanced Raman Scattering (TERS) [1], we investigated what happens if we deposit the monolayer onto the concave side of a lens and then contact it to the top of a SERS substrate, to create a SERS "sandwich" (with the lens), see Figure 1(c). We call this nano/micro-cavity sandwich device a "Gradient SERS" (G-SERS) sandwich. When contact is made, in the center of the device, the two surfaces produce "Newton's Rings", due to optical interference. We observe strong SERS enhancement of the Raman signal especially at the point where the analyte contacts the SERS substrate (see point 24 in Figure 1(b)) and compare this signal to the Raman signals from different "rings" (see other points in Figure 1(a,b)). In the center of the rings, where the lens is the closest to the SERS substrate, the intensity is highest. As we get further from the center, the lens gets further from the SERS substrate, and the intensity gets lower. However, we also observe an additional Raman enhancement due to cavity enhanced Raman scattering (CERS) [2], when the cavity resonance crosses one of the Raman transitions. We aim to understand the G-SERS mechanisms as the distance between the analyte and substrate is varied.

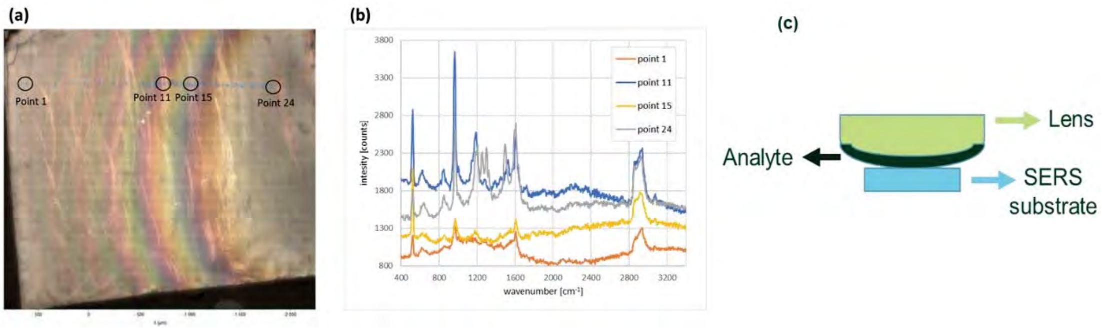

Figure 1: Gradient-SERS (G-SERS). (a) Optical interference between top analyte coated lens and bottom SERS substrate results in Newtons rings. (b) SERS enhancement depends on position along the gradient of the SERS nano/micro-cavity sandwich. (c) Schematic of G-SERS sandwich.

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New substrate for Organic Surface-Enhanced Raman Spectroscopy (OSERS): High Crystallinity Small Molecule Organic Semiconductor Single Crystal

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Enhanced Raman spectroscopy based on organic surfaces have gradually emerged as a promising branch of SERS with higher selectivity, despite the enhancement mechanism being still controversial [1]. Herein, a novel small molecule organic semiconductor SERS substrate was suggested for the first time as a high-crystallinity p, p'-diaminoquaterphenyl sheet-like single crystal exhibiting a herringbone configuration orientation. Femtosecond transient absorption spectroscopy was employed to demonstrate the presence of semiconductor intrinsic photo generated free charge carriers in this organic single-crystal micro sheet (OS MS), and further observation revealed significant charge transfer between the OS MS and probe molecules (e.g., methylene blue, MB) under pump light excitation. When different excitation light matches different OS MS-probe molecular systems, significant high selectivity SERS phenomena can be observed, indicating that organic semiconductors may have the similar photo-induced charge transfer (PICT) mechanism as inorganic semiconductors.

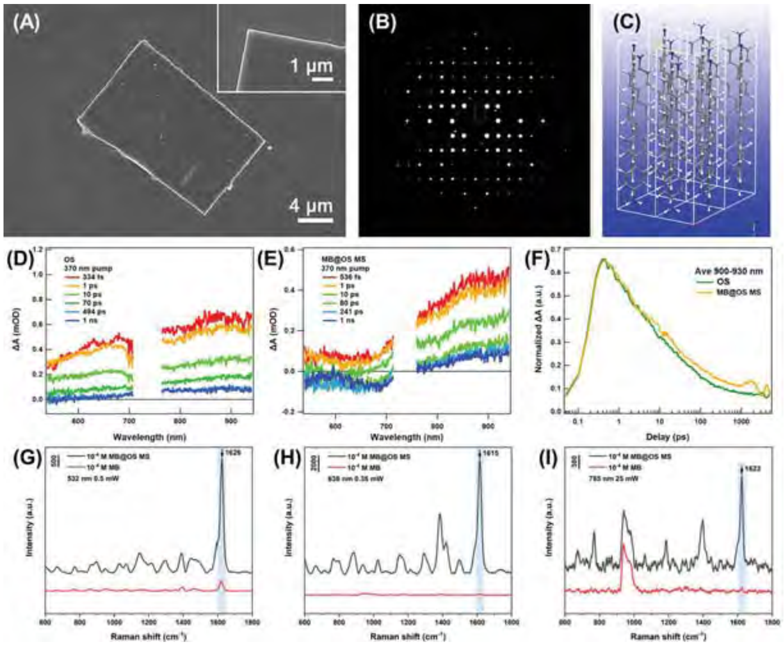

Figure 1: (A) SEM of OS MS. (B) Slice view of 3DED projection images of the OS MS (hk0). (C) Single-crystal packing structure of the OS MS. (D, E) TA spectra of OS MS and MB@OS MS recorded with a pump wavelength of 370 nm. (F) TA dynamics of OS MS and MB@OS MS recorded with a pump wavelength of 370 nm and probed at ave 900-930 nm. (G-I) Raman spectra of MB@OS MS at (G) 532 nm, (H) 638 nm, and (I) 785 nm excitation laser.

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Excitation Wavelength Optimization for SERS: Enhancing Analytical Performance and Decoding the Molecular Electronic Structure Affected by Metallic Nanostructures

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Surface-enhanced Raman scattering (SERS) has evolved into a formidable analytical tool over the past fifty years, finding wide-ranging applications across various fields including chemistry, biology, medicine, and materials science. The broad spectrum of SERS applications is primarily attributed to its exceptional sensitivity, which is capable of detecting even single molecules. This remarkable sensitivity chiefly arises from the significant enhancement and localization of the electromagnetic field by nanostructures. Positioning a molecule within such "hot-spots" can amplify the SERS signal by more than ten orders of magnitude. Nonetheless, the enhancement of the electromagnetic mechanism is not the sole factor affecting the SERS signal intensity. Concurrently, a multitude of other physical and chemical effects can alter not just the intensity but also the characteristics of the SERS spectra. Particular interactions, like charge transfer between the metallic nanostructure and the molecule or its chemisorption on the surface, markedly influence the spectra's features. Furthermore, selecting the excitation wavelength impacts both the SERS spectrum's intensity and the distribution of relative band intensities. This impact is due to the degree of tuning to the plasmon resonance of the nanostructure, which is responsible for the electromagnetic enhancement effect, and to the resonance of the molecule itself. Such complexity, while complicating the analytical applications of SERS, simultaneously fosters a deeper comprehension of the interactions between molecules and the surfaces of plasmonic nanostructures.

Here, we focus on the impact of tuning the excitation wavelength to the electronic transitions of the molecule on its detectability and the analysis of SERS excitation profiles. High-resolution SERS excitation profiles for malachite green[1] and several porphyrin derivatives[2] deposited on both aggregated and separated gold nanorods show that even a slight detuning of the excitation wavelength from the electronic transition energy (by 10–20 nm) can dramatically reduce the contribution of resonant Raman enhancement to the total intensity of the recorded SERS signal. This reduction thereby decreases the chances of detecting small amounts of substances and their individual molecules.[3] Moreover, we demonstrate that analysing SERS excitation spectra allows for an analysis of the interaction between molecules and the nanostructure, particularly enabling an assessment of the electronic structure of molecules adsorbed on nanostructures or positioned in hotspots.[4] The observed shifts in electronic transitions for these dyes suggest that maximizing the SERS signal, and thus its detectability to the single-molecule level, necessitates an understanding of these changes and appropriate tuning to the resonance of the adsorbed molecule. This proposed methodology for optimizing the analytical applications of SERS demonstrates the potential of using SERS excitation profiles in physicochemical studies and enhancing the understanding of phenomena related to the SERS process.

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High-Performance SERS Sensors with a Nanoparticle-on-Liquid-Mirror (NPoLM) Nanophotonic Resonator Structure

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Surface-enhanced Raman scattering (SERS) has been a widely used method for sensing various substances. SERS sensors generally employ noble metal nanostructures which support localized surface plasmons, leading to hotspots with high field enhancement. In general, higher field enhancement requires reducing the dimensions of the hotspots, which makes the delivery of analyte into the hotspots more challenging. Consequently, there is a trade-off between the field confinement/enhancement in hotspots and the analyte delivery efficiency, which often limits the performance of conventional SERS sensors. In this work, we demonstrate a new type of SERS sensors which employ liquid-metal-based nanophotonic resonators to effectively overcome the aforementioned trade-off and achieve high SERS performance.

As shown in Fig. 1a, the nanophotonic resonator is essentially a nano-patch antennas consisting of a gold nanoparticle (Au-NP) separated from a room-temperature liquid metal (e.g., liquid gallium or liquid eutectic gallium-indium alloy) ground plane by a thin layer (a few nm) of adsorbed analyte molecules [1]. Exceedingly high field confinement/enhancement is achieved in the nanometric gap between the Au-NP and the liquid metal ground plane (see Fig. 1b), which in turn substantially enhances the Raman scattering signals from the analyte molecules in the nanometric gap (i.e. the hotspots). As the liquid metal is introduced to cover the Au-NP and form the nanometric hotspots only after the efficient adsorption of analyte molecules on the Au-NP surface, this nanoparticle-on-liquid-mirror (NPoLM) SERS sensor architecture effectively eliminates the trade-off between field confinement/enhancement and analyte delivery efficiency. Another advantage of the NPoLM architecture is that its resonance wavelength is sensitively dependent on the Au-NP size. This is in sharp contrast to the plasmonic resonance of a bare Au-NP which is only weakly dependent on the NP size. Therefore, the resonance wavelength of the NPoLM resonators can be tuned to match the excitation laser and/or Raman signal wavelengths by tailoring the Au-NP size. We developed a rapid thermal annealing (RTA) based process to cost-effectively produce Au-NPs on sapphire substrate (Fig. 1c), and optimized the Au-NP size distribution to achieve high performance of the NPoLM SERS sensors for 785 nm excitation laser wavelength. As shown in the example in Fig. 1d, our NPoLM SERS sensors can achieve two orders of magnitude or more SERS performance improvement in sensing trace amount of analyte molecules compared to the conventional SERS substrates with the Au-NPs alone (i.e., without introducing the LM).

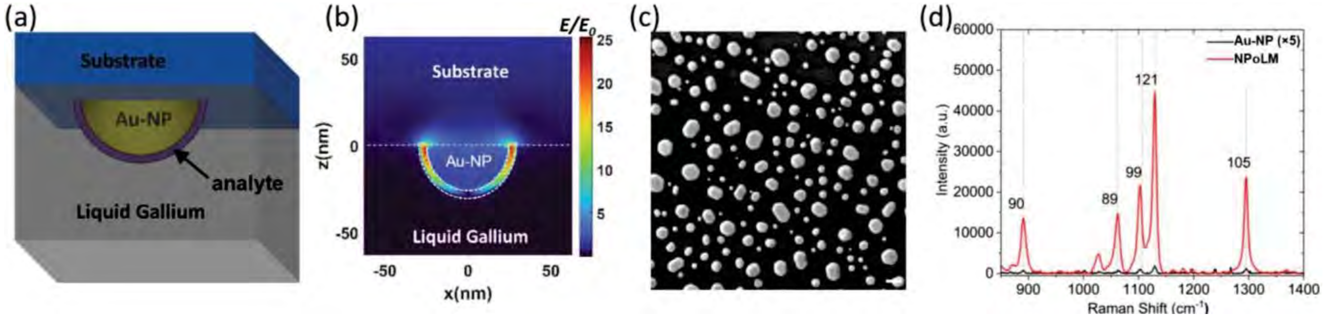

Figure 1: (a) Schematic of the NPoLM SERS sensor structure (a single unit cell). (b) Simulated electric field enhancement profile of a NPoLM resonator at its resonance wavelength near 785 nm. (c) SEM image of RTA-produced Au-NPs on sapphire substrate. The scale bar at lower-right corner is 100 nm. (d) SERS spectra of self-assembled monolayer of 1-octadecanethiol molecules obtained using the conventional SERS substrate with Au-NPs alone (i.e., before introducing the liquid metal, black curve) and using the corresponding NPoLM SERS sensor (i.e., after introducing the liquid metal, red curve). The Au-NPs SERS spectrum is multiplied by a factor of 5 for better visibility in the graph. The numbers near each Raman peak indicates the performance improvement of the NPoLM SERS sensor with respect to the conventional SERS substrate with Au-NPs alone.

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Surface enhanced Raman scattering of bioaerosols: where are we now?

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Bioaerosols are microscopic airborne biological particles such as bacteria, fungal spores, pollen, viruses, and their derivatives. The global spread of the COVID-19 pandemic and the rise of antibiotic resistance in healthcare settings are examples of how bioaerosols have emerged as serious public health concerns in both outdoor and indoor settings. Identifying and quantifying bioaerosol components is crucial for assessing risks and setting appropriate exposure limits. Specifically, new research opportunities have arisen thanks to the proliferation of real-time (RT) methods for autonomous, online detection and characterization of bioaerosols features. Effective online bioaerosol monitoring is hindered by the complexity, diversity, and great spatiotemporal variability of bioaerosols, as well as their mixing with abiotic components, both internally and externally. Traditional methods, such as culture-based methods, depend on immediately gathering bioaerosols before performing time-consuming laboratory analysis, prohibiting the high-resolution time-based characteristics required for an online approach.

Because of its high sensitivity and specificity, and ability to be performed in complex biological mixtures, using portable and relatively inexpensive devices, surface enhanced Raman scattering (SERS) spectroscopy has become an appealing method for online bioaerosol monitoring [1] However, as technology progresses, it becomes increasingly difficult to standardise processes so that the results are consistent and comparable. Because of the lack of standardised methodologies, SERS sensing of bioaerosols remains challenging.

Here we present state of the art in the use of SERS spectroscopy to examine bioaerosols. A critical evaluation of the experimental aspects involved in the collection of SERS spectra is presented, and the elective applicability and weaknesses of various experimental setups are highlighted, helping to provide a solid foundation for real-time bioaerosol characterization using SERS spectroscopy.

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On-site Surface Enhanced Raman Scattering detection of miRNAs by an automatized platform.

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The development of miniaturized Surface Enhanced Raman Scattering (SERS) devices and the availability of portable Raman spectrometers promote the spreading of a precision medicine approach by enabling on-site cancer biomarkers detection [1]. In this work, SERS chips integrating Ag-decorated porous silicon (pSi)-PDMS membranes were exploited for the development of a setup for the automatization of SERS analysis of miRNAs. The sensor relies on a two-step hybridization assay [2] to specifically capture and sensitively detect the target miRNA thanks to a Raman reporter introduced in the second hybridization. The functionalization and incubation conditions, reporter type and labelling site along the DNA probe were evaluated to optimize the sensing protocol, that was then adapted to the automatic setup. The platform (Figure 1a) enabled to automatically run the microfluidics and the Raman spectrometer, including spectral data analysis. The performances of the system were tested by analysing miRNAs (miR-214, 320a and 222) in buffer solutions at several concentrations. The automatized sample injection allowed to concentrate the analyte on the SERS substrate surface by a dynamic incubation procedure. As a result, the target miRNAs were detected with remarkable SERS intensity at picomolar concentrations (Figure 1b). Moreover, the sensitivities of the assay performed with the new setup or with a bench Raman microscope were comparable. The in-chip quantification of miRNAs involved in breast and lung cancer by an entirely automatized procedure was thus achieved, demonstrating the possibility of on-site SERS sensing thanks to the robust and easy to handle platform.

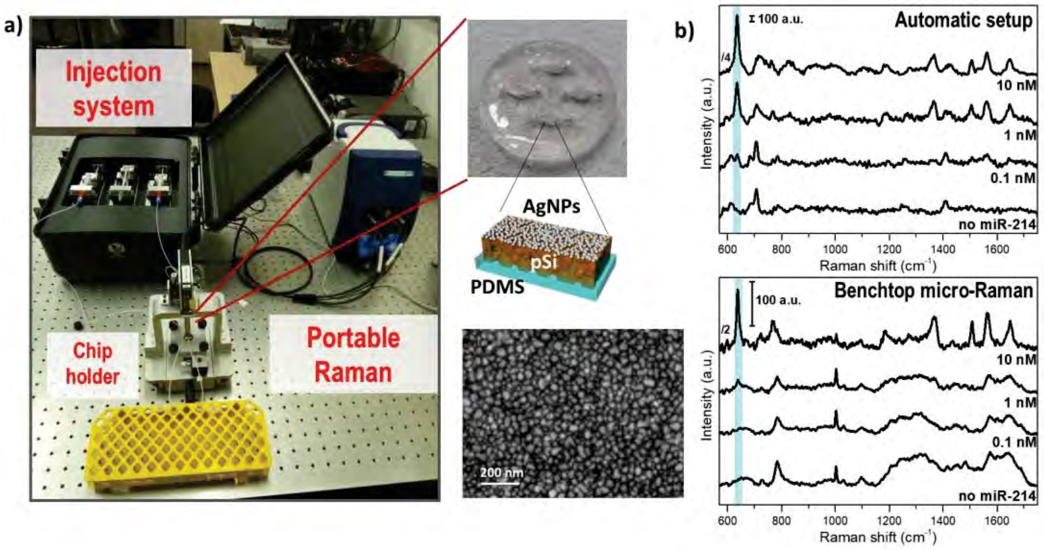

Figure 1: Microfluidic and Raman integrated setup for automatized SERS analyses, SERS chip and FESEM micrograph of the Ag nanostructures; b) SERS detection of miR-214 ($\lambda_{exc.}$= 532 nm) by the automatic and portable setup (top) or through the laboratory-scale procedure (bottom). The main SERS peak of the Raman reporter is highlighted.

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Initial Collisions Promoting Bimolecular Chemical Reactions Examined with Picosecond Time-resolved Raman Spectroscopy

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Bimolecular reactions in solutions are initiated by a "collision" between reactant molecules, with enough collision energy and an appropriate site of impact. It is not easy, however, to study bimolecular chemical reactions with picosecond time-resolved Raman spectroscopy, even though the method provides detailed information on the molecular structure of the reactant, intermediate, and product species. The reactant molecules diffuse in the solvent molecules before they collide with each other to start a reaction. The diffusion motion is not controllable. Starting a bimolecular reaction in synchronous with the probe light pulse with a timing jitter smaller than a few picoseconds is a challenging task.

Aromatic molecules often react with carbon tetrachloride by extracting the chlorine radical from carbon tetrachloride, when the aromatic molecules are photoexcited to the first excited singlet (S_1) state (Figure 1). The reaction time is approximately 3 ps for *trans*-stilbene, biphenyl, and *p*-terphenyl [1,2]. The aromatic molecules are always located next to carbon tetrachloride, another reactant, in carbon tetrachloride solutions. We can start a bimolecular chemical reaction that proceeds in 3 ps with the irradiation of a picosecond light pulse, by using this series of photochemical reactions.

$$\mathrm{CCl_4 + Ar \xrightarrow{h\nu} \cdot CCl_3 + Cl\text{-}Ar\cdot}$$

Figure 1: Photoinduced bimolecular radical reaction between aromatic molecule and carbon tetrachloride.

We study photoinduced bimolecular radical reactions of *trans*-stilbene derivatives with *para*-substitutions and *α*-terthiophene with carbon tetrachloride by using time-resolved spectroscopy. A C-Cl stretch vibration of the trichloromethyl (CCl_3) radical was recorded with nanosecond time-resolved infrared absorption spectroscopy, indicating the formation of the radical products. The result with picosecond time-resolved Raman spectroscopy [3] showed mode-specific acceleration of vibrational dephasing for the S_1 state of *α*-terthiophene in carbon tetrachloride, suggesting mode-selective interruption of vibrational motion caused by the bimolecular chemical reaction.

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Mechanism of vibrational energy transfer from OH stretch to OD stretch in H_2O/D_2O mixtures; the fs-IR pump – stimulated Raman probe study

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Femtosecond infrared pump – stimulated Raman probe spectroscopy (fs-IR-SRS) is a new time-resolved vibrational spectroscopy developed in the IPC PAS, Warsaw. [1] Thanks to the multiplex advantage of Raman spectroscopy and features of the stimulated Raman process, the technique provides sub-100 fs time resolution, spectral resolution better than 10 cm^{-1} and at least 200 cm^{-1} to 4000 cm^{-1} probe bandwidth. Henceforth, this is an excellent method to study vibrational relaxation pathways, intermolecular interactions and processes of chemical systems in their electronic ground state.

In the studies of vibrational energy relaxation pathways in H_2O/D_2O mixtures, we observed almost instantaneous (faster than 100 fs) energy transfer from the excited OH stretch mode in HDO to the OD stretch mode (see a comparison of time traces for bleaching and induced absorption of OH stretch, 3350 cm^{-1} and 3121 cm^{-1}, and OD stretch, 2559 cm^{-1} and 2321 cm^{-1}, respectively, in Figure 1).

De Marco *et al.* observed a similar effect with Ultrafast Broadband 2DIR Spectroscopy [2] and pointed at OH stretch coupling to HOD bend overtone as the dominant channel of the energy relaxation process. In this work, we consider this channel and others involving OD stretch + libration as well as direct excitation of OD stretch + DOD bend mode. [3] We support our analysis with ab initio molecular dynamics simulations.

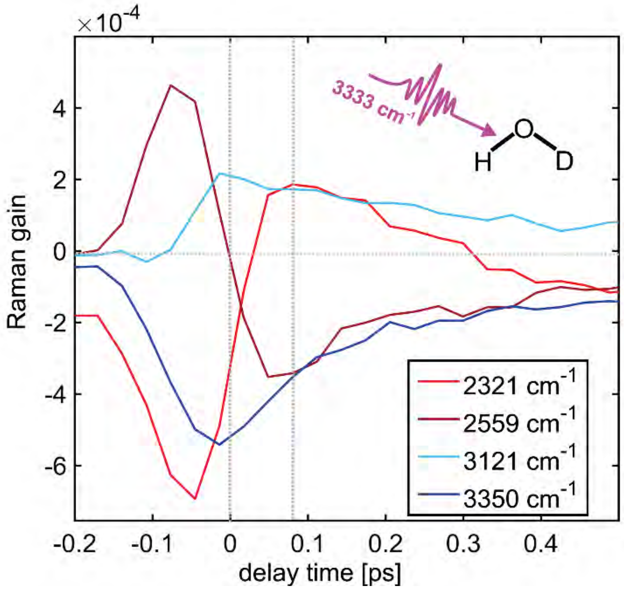

Figure 1 - Time traces of FSRS signal at 2321 cm^{-1}, 2559 cm^{-1}, 3121 cm^{-1}, 3350 cm^{-1}after pumping 17 mol % HDO in D_2O sample at 3333 cm^{-1}.

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Measuring and mapping the interfacial thermal conductance of graphene/hBN by pump-probe microscopy

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Ultrafast optical pump-probe is a well-known and powerful tool for studying excited-state dynamics. The understanding of the electronic cooling pathways can show important material properties, such as electron-electron, electron-phonon, phonon-phonon interactions and energy dissipation to the environment. This last step is usually overlooked in typical pump-probe experiments due to its longer time scale, but it can reveal how heat dissipation occurs, for example between 2D materials and different surfaces. Here, we present an optical pump-probe microscopy of graphene exfoliated over transparent silicon dioxide (SiO2) and partially over hexagonal boron nitride (hBN), as can be seen in Figure 1b. Measuring the optical transient response over tens of picoseconds at every point of the sample, shown in Figure 1a, we were able to determine and to map the interfacial thermal conductance of graphene, as shown in Figure 1c. The transient response was modeled with a temperature-dependent optical conductivity model [1] combined with a three-temperature model [2] modified to include the heat dissipation from graphene to the substrate. Our work opens opportunities for mapping and testing the heat dissipation of graphene on different substrates and to engineer more sophisticated heterostructures for heat management for future electronic devices.

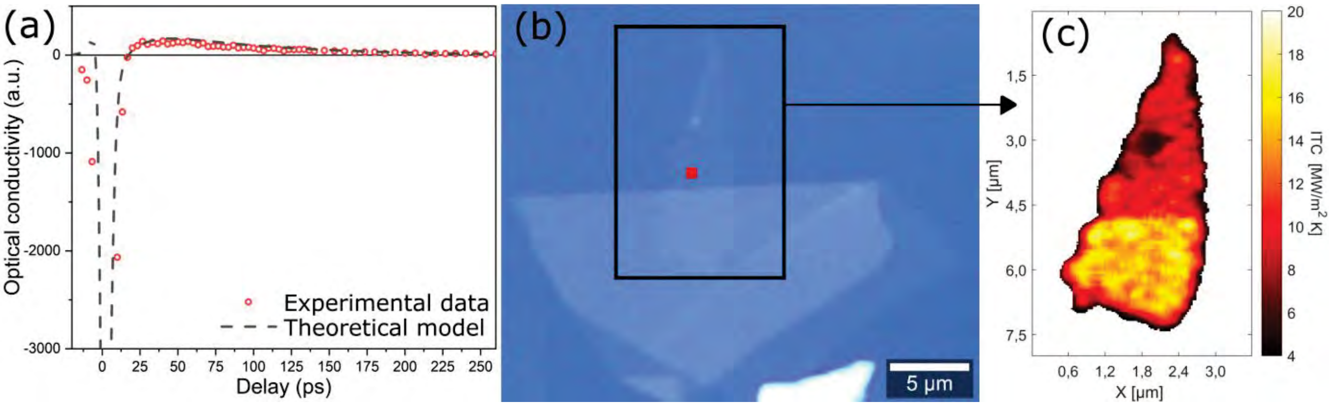

Figure 1: (a) Transient response measured on the red area shown in optical image. (b) Optical image of the sample. (c) ITC map obtained by measuring the transient response of each point of the black rectangle.

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Time-resolved Raman spectroscopy on bulk and monolayer MoS_2

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MoS_2 belongs to the class of graphene-like, layered materials called transition metal dichalcogenides (TMDs). Semiconducting TMDs, like MoS_2, show an indirect to direct bandgap transition when the system is exfoliated down to the monolayer structure, which makes them promising materials for future applications in optoelectronics and related fields [1]. Unravelling the relaxation pathways of photoexcited electrons in such systems is of great applicative interest. Remarkably, the structural and dimensional tunability of electronic properties makes TMDs benchmark systems to study the interplay between electronic and vibrational degrees of freedom.

Time-resolved Raman spectroscopy (TRRS) can provide direct access to the electron-phonon coupling dynamics, as well as the incoherent phonon relaxation [2]. The aim of our experiment is studying the deexcitation dynamics of MoS_2 after impulsive optical pumping of the excitonic bandgap. At the SPRINT-NFFA facility, we have carried out a TRRS study on the system, monitoring the temporal evolution of two specific Raman active features, namely the out-of-plane A_{1g} mode and the in-plane E^1_{2g} mode, after the photoexcitation. Equilibrium Raman spectroscopy studies have linked the modification of such phonon lineshapes to doping effects, providing the background for studying the optically induced, transient doping of the system [3,4,5]. To explore the effect of the indirect-to-direct bandgap transition on the system relaxation dynamics, the experiment was performed on both bulk and monolayer MoS_2, revealing significant differences in the phenomenology and relaxation timescales. The role of the substrate on the monolayer deexcitation dynamics was explored by selecting substrates with different electrical and thermal properties.

After presenting the results of the TRRS study, I will discuss their perspective integration in a multi-messenger, time-resolved spectroscopy study, coupling the time resolved photon and electron spectroscopies available at the SPRINT-NFFA facility (CNR-IOM@Elettra, Trieste, Italy).

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Exploring Phonon Dynamics in Germanium by Time-Resolved Raman Spectroscopy

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Most of the modern technologies rely on the fast response of small and high-speed semiconductor devices. Improving high-speed devices requires a clear understanding of the various microscopic processes occurring on a picosecond or even a femtosecond timescale [1]. Accessing the picosecond timescale is only feasible through ultrashort optical pulses as high-speed electronic instruments are not fast enough to detect ultrafast dynamics.

Many experiments based on the so-called pump-probe technique have been developed to investigate carrier dynamic information [2]. These experiments typically involve two ultrafast laser pulses. The first pulse, the pump, excites the material out of equilibrium, bringing electrons from the valence to the conduction band. The second pulse, called probe, monitors the evolution of carrier dynamics as a function of the delay time between pump and probe.

We use a pump-probe setup capable of performing both time-resolved Raman spectroscopy (TRRS) and transient reflectivity (TR) measurements while monitoring either Raman spectra or changes in reflectivity. In the TRRS scheme, we employ a triple spectrometer then collects Raman spectra as a function of delay time, which allows to track the time evolution of optical phonon temperature. The TR setup, instead, allows to access the dynamics of different energy transfer mechanisms due to both electron-phonon and phonon-phonon interactions.

Extensive research has been devoted to study the dynamics of hot carriers and phonons in group III-V materials [3]. However, the understanding of hot carriers' relaxation in group IV semiconductors has lagged behind, mainly because of the indirect band gap of silicon and germanium.

In our work, we carried out TRRS and TR room temperature measurements in intrinsic crystalline germanium. We investigated non-equilibrium optical phonons along with different carrier's energy transfer mechanisms. We observe the rise of phonon temperature due to the energy relaxation of the photoexcited carriers with a subsequent decay rate of 3 ps [4][5]. Additionally, TR-based measurements revealed coherent acoustic phonon oscillation [6].

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Transient stimulated Raman scattering spectroscopy and imaging

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Stimulated Raman scattering (SRS) has been developed as an essential quantitative contrast for chemical imaging in recent years. However, while spectral lines near the natural linewidth limit can be routinely achieved by state-of-the-art spontaneous Raman microscopes, spectral broadening is inevitable for current mainstream SRS imaging methods. This is because those SRS signals are all measured in the frequency domain. There is a compromise between sensitivity and spectral resolution: as the nonlinear process benefits from pulsed excitations, the fundamental time-energy uncertainty limits the spectral resolution. Besides, the spectral range and acquisition speed are mutually restricted. In this talk, we will introduce a novel time-domain excitation strategy that bypasses all these fundamental conjugations. We encoded the vibrational oscillations in the fluorescence signal [1] or stimulated Raman loss (SRL) signal [2] by femtosecond pulse-pair sequence excited vibrational wave packet interference. The Raman spectrum was then achieved by Fourier transform of the time-domain signals, which features the natural-linewidth-limit spectral line shapes, laser-bandwidth-determined spectral range, and improved sensitivity. Its advantages and drawbacks in biomedical imaging applications will be discussed in detail.

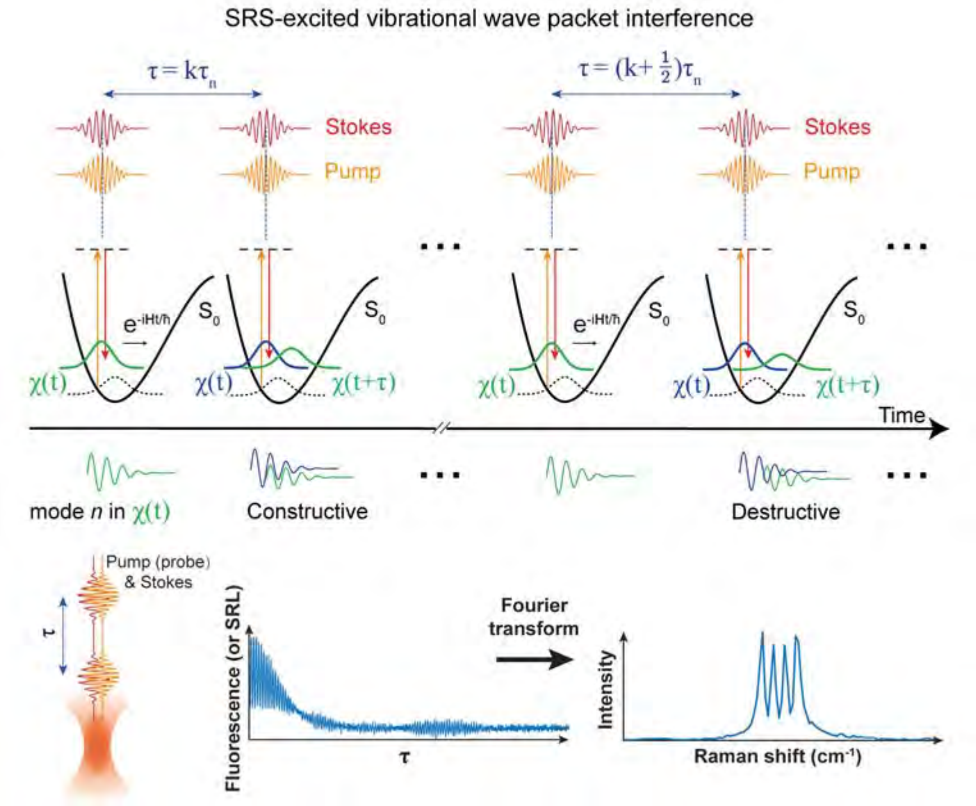

Figure 1: Principle of transient stimulated Raman spectroscopy.

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Auger-assisted secondary hot carrier transfer in a type I MoS_2/$PtSe_2$ heterostructure probed by transient absorption spectroscopy and two-dimensional electronic spectroscopy

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We present transient absorption spectroscopic study on the carrier dynamics of a type I heterostructure constructed with 1L MoS_2 and 2L $PtSe_2$. By exclusively exciting the small bandgap $PtSe_2$, we observed exciton signals of MoS_2 from hot carrier transfer from $PtSe_2$ to MoS_2 within 70 fs. Importantly, we also found a secondary charge transfer process at delays of several tens of picosecond that can be attributed to hot carriers that are regenerated in the $PtSe_2$ by intralayer Auger scattering. Blue shift of the exciton peaks within 100 ps probes the dynamic buildup of an internal electric field across the heterostructure interface due to charge transfer.[1]

More recently, the ultrafast carrier dynamics of the heterostructure is probed by two-dimensional electronic spectroscopy (2DES) operating at 100-kHz repetition rate with shot-to-shot detection. The results show charge transfer between MoS_2 to $PtSe_2$ after optical excitation, evidenced by the cross peaks between MoS_2 exciton and $PtSe_2$ excitation. A few 100s of femtosecond later, the signal is dominated by hot charge transfer from $PtSe_2$ to MoS_2 by analysing the position of the cross peaks (stars in the figure). The work uncovers two-step (primary and secondary) charge transfer processes and reveals Auger-assisted hot carrier transfer mechanism in type I heterostructures. This finding suggests the possibility for designing optoelectronic and photocatalytic devices by optical sub-bandgap excitation in type I heterostructures made of two-dimensional materials, largely expanding the scope of their applications.

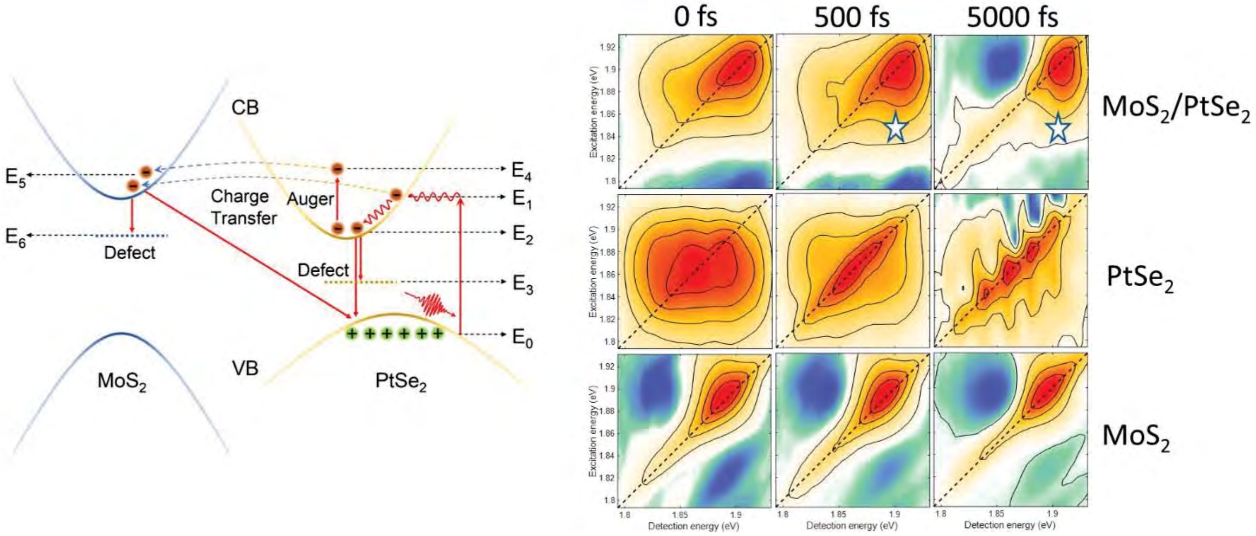

Figure 1: Scheme of the primary and secondary charge transfer in a type I heterostructure (left) and 2DES spectra (ΔT/T) at different pump-probe waiting times for the three samples (right).

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Atomic-Selective Impulsive Vibrational Spectroscopy Upon Core-Level Resonant Excitation

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Combining the specificity of vibrational spectroscopy with the selectivity of resonant X-ray radiation has the potential to deepen our atomic-level knowledge of complex systems. Here, we present a proof-of-principle implementation of impulsive vibrational spectroscopy (IVS)[1] upon core-level resonant excitation in the extreme ultraviolet (EUV) range. The experiment was performed at the FERMI free electron laser at Elettra, Trieste, by tuning the EUV pulses on resonance with the $4d_{3/2}$ and $4d_{5/2}$ orbitals of a trigonal Tellurium single crystal, impulsively stimulating coherent phonons in the system. The transient modulation of the optical reflectivity was monitored as a function of the time delay using energy-dispersed probe pulses centred at 795 nm. Figure 1a shows a typical transient reflectivity map obtained upon 46.2 eV excitation, which is characterised by pronounced coherent oscillations with a period of 118.2 cm^{-1} (Figure 1b), corresponding to the A_1 phonon mode of the crystal[2]. A chirped-detection scheme[3] was employed to demonstrate the pump photon energy dependence of the phonon amplitude across the Te $N_{4,5}$-edge by encoding the temporal information in the probed wavelengths, drastically reducing the acquisition times. Our results represent the first step towards the implementation of low-frequency element- and orbital-selective vibrational spectroscopy in more complex systems.

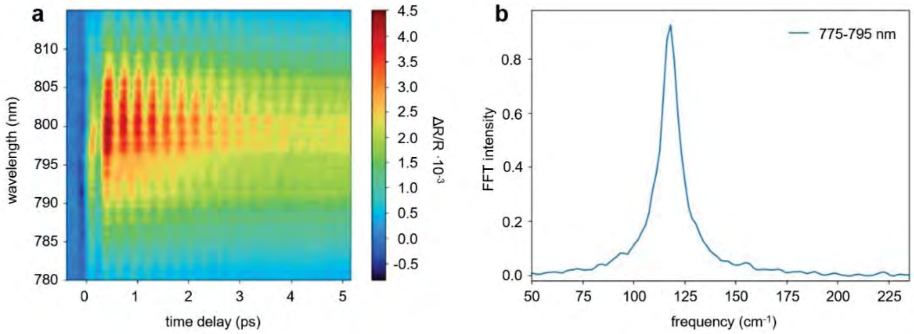

Figure 1: (**a**) Chirped-corrected transient reflectivity map in tellurium single crystal upon EUV pump and wavelength dispersed optical probe. (**b**) Fast Fourier transform of the averaged time trace in the probe range 775-795 nm.

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Temperature dependence of stimulated versus spontaneous Raman scattering: an outlook towards ultrafast thermometry

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Owing to their sub-picosecond temporal resolution, coherent Raman spectroscopies (CRS) have been proposed as a feasible expansion of Spontaneous Raman (SR) thermometry, offering the potential to track dynamics of mode specific vibrational energy content during out-of-equilibrium processes[1,2]. Building on a quantum description of matter with a perturbative expansion of the molecular density matrix[3], in this presentation we will derive and compare the temperature dependence of SR and CRS experiments. In the case of the latter, we will illustrate that the presence of multiple laser fields stimulating vibrational coherences introduces additional quantum pathways, leading to destructive interference of coherent Raman signals. This ultimately reduces the thermal sensitivity of single spectral lines, nullifying it for harmonic vibrations and temperature independent polarizability. Theoretical results are benchmarked comparing the spontaneous and stimulated Raman response of different systems, namely Sapphire and Calcium Fluoride (Fig. 1). Building on the derived results, we will show how harnessing anharmonic signatures such as vibrational hot bands to enable coherent Raman thermometry[4].

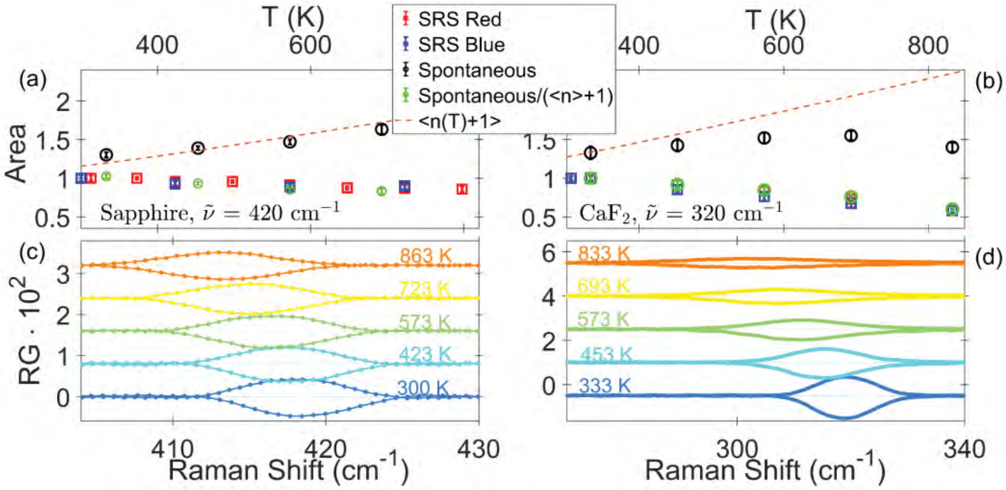

Figure 1: Temperature dependence of spontaneous and stimulated Raman responses. Experimental responses have been measured for two crystals, namely Sapphire and CaF2 and are reported evaluating the peak areas in panels a-b, respectively. Panels c-d show the corresponding SRS spectra, measured for a probe pulse red/blue shifted with respect to the Raman pump (corresponding to gains/losses).

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Raman micro-spectroscopy reveals the spatial distribution of fumarate in cells and tissues

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Aberrantly accumulated metabolites elicit intra- and inter-cellular pro-oncogenic cascades, yet current measurement methods require sample disruption and lack spatio-temporal resolution, limiting our ability to fully characterize their function and distribution. In this talk, we discuss how Raman spectroscopy can detect accumulated fumarate in living cells *in vivo*, and can distinguish between Fumarate hydratase (Fh1)-deficient and Fh1-proficient cells based on fumarate concentration [1]. Moreover, Raman imaging reveals the spatial compartmentalization of fumarate within cellular organelles in Fh1-deficient cells. These results suggest Raman microspectroscopy could be adopted as a valuable tool for small molecule metabolic imaging, enabling *in situ* non-destructive evaluation of fumarate compartmentalization.

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Broadband Coherent Raman Imaging for Disease Diagnosis

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Coherent Raman Scattering (CRS) imaging modalities, such as Coherent Anti-Stokes Raman Scattering (CARS) and stimulated Raman scattering (SRS) enable the rapid investigation of large specimen for disease diagnostics, i.e., to identify disease specific marker molecules or to localize compositional changes. For complex diagnostic tasks, e.g., tumor margin detection, grading and staging of tumors or the recognition of infectious diseases at an early stage, coherent Raman imaging at a single or few vibrational resonances is not sufficient.

Broadband CARS (BCARS) and broadband SRS instead enable the acquisition of parts or the full vibrational spectrum within time frames as short as an individual laser pulse. While broadband SRS can be used to measure the Raman spectrum directly, phase retrieval using the Kramers-Kronig algorithm is needed used to reconstruct the imaginary part of the BCARS spectrum corresponding to the spontaneous Raman spectrum. By simultaneously probing a significant part of the spectrum, broadband CRS is ideally suited for fast composition diagnostics on complex tissue and cell samples, but broadband laser sources are crucial. In this contribution we first introduce present results of whitelight generation aiming at high spectral power density and low noise as key requirements for BCRS. For clinical applications, compact and robust laser sources are needed. Here, fiber-based lasers are ideal and the implementation in microscopic and endoscopic devices is shown, which provides significant advantage in comparison to single band CRS. This is demonstrated in biomedical applications such as single cell identification and tumor margin detection. Even more information can be extracted by a multimodal approach, combining BCRS with second harmonic generation and two photon excited fluorescence and fluorescence lifetime imaging. But since disease induced changes are small, to fully exploit the information content of spectral data, machine learning and AI based data analysis algorithms are crucial and need to be used.

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Visualization of topical drug delivery with label-free chemical imaging

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Stimulated Raman scattering (SRS) microscopy is capable of real-time examination of formulated products and their permeation into the skin, allowing the detailed, time resolved investigation of chemical absorption. The high sensitivity modulation transfer detection mechanism permits imaging which is free from emitted fluorescence, with the added benefit that the signal intensity is linear with concentration, simplifying quantitative analysis. SRS has shown excellent promise as a tool for the study of topical drug formulations themselves, in addition to the “metamorphosis” of applied formulations on skin and the real-time disposition of actives and excipients, including penetration pathway and nanoparticle distribution.[1,2] In addition to unique mechanistic information, it provides a means of assessing topical drug bioavailability and bioequivalence.[3] SRS can be performed simultaneously to other optical imaging modalities such as second harmonic generation (SHG) and fluorescence microscopies to image connective tissues and other endogenous species. Workflows have also been developed to perform correlative SRS-SHG-secondary ion mass spectrometry imaging (SIMS).[4] This combination of spectroscopic imaging approaches facilitates powerful visualisation, combining high chemical specificity and sensitivity with sub-micron spatial resolution.

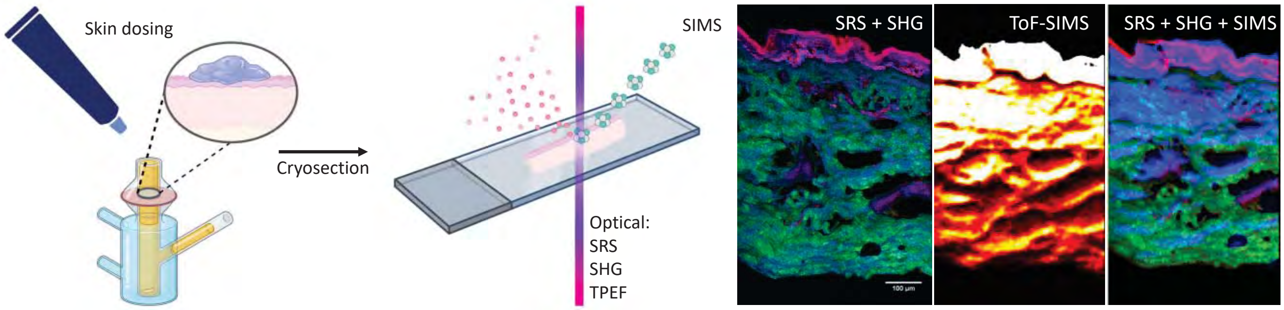

Figure 1: Methodology and example data from combining non-linear optical microscopies with mass spectrometry imaging for high resolution visualisation of drug distribution within the skin.

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Heterogeneous structure and order of protein condensation

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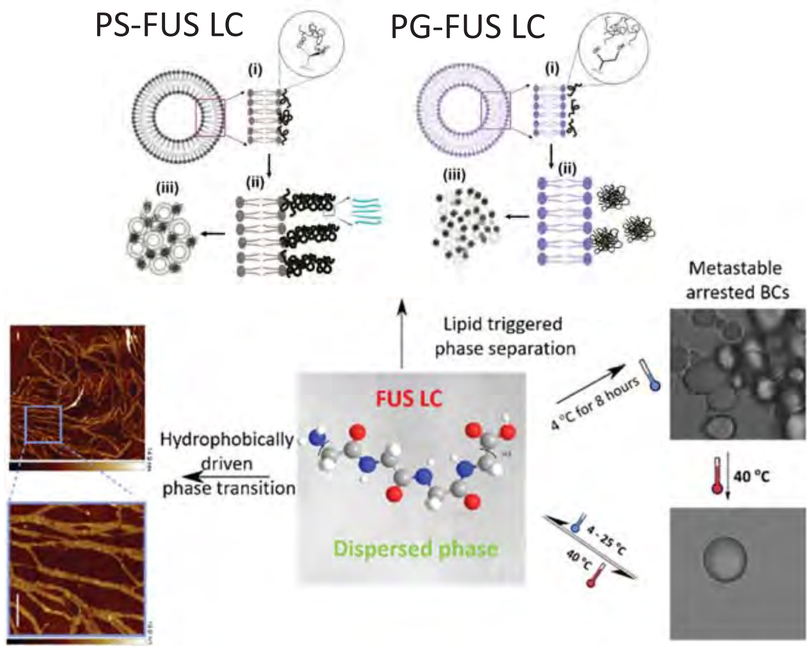
Formation of membraneless liquid-like biomolecular condensates (BCs) *via* liquid-liquid phase separation of disordered proteins is responsible for many behaviors in cells, specifically stress granule formation, chromatin organization, and RNA transcription. Structural changes of these BCs can be triggered by external stimuli such as pH, salt, or temperature as well as surface interactions. Often external stimuli can facilitate aberrant phase transitions or non-native multivalent interactions of these disordered proteins, which eventually leads to solidification. Here, I will present our studies of the structural and biophysical properties of FUS condensation and other proteins (BSA, and Nucleoporins) over time, after thermal shock[1–4], and at lipid interfaces [5] using nonlinear Raman microscopy and IR spectroscopy to reveal the diverse molecular states that FUS inhabits. We find that proteins take on a disordered state at short times and age into solid-like particles over time. Moreover, we find that interfaces can catalyze FUS assembly at concentrations less than 50-fold required in bulk.



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Deciphering the sum-frequency generation spectrum of collagen type I in the carbon-hydrogen stretching range

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In this study, we conducted experiments and simulations to uncover the nature of the vibrational features observed in the sum-frequency generation (SFG) spectrum in the CH-stretching range of collagen type I. To achieve this, we developed an all-reflective laser-scanning SFG microscope, enabling direct measurements of individual tensor elements $\chi_{ijk}^{(2)}$ of fibrillar collagen. Through polarization-sensitive SFG, we identified achiral tensor elements that sensitively report on the symmetric and asymmetric methylene (CH_2) stretching modes of collagen. Additionally, we conducted computational modeling based on crystal structure information obtained from the Protein Data Bank[1] and using the bond polarizability derivative model[2] to uncover the mode symmetries. Our analysis revealed that the bands at 2917 cm^{-1} and 2942 cm^{-1} correspond to the symmetric CH_2 stretching of the pyrrolidine rings of proline and hydroxyproline, as shown in Figure 1. The asymmetric stretches of the methylene bridge group are found at 2870 cm^{-1} and in the range of 2980 cm^{-1} to 3030 cm^{-1}. These findings help predict the protein's nonlinear optical properties from its crystal structure, thus establishing a connection between protein structure and SFG spectroscopic measurements.

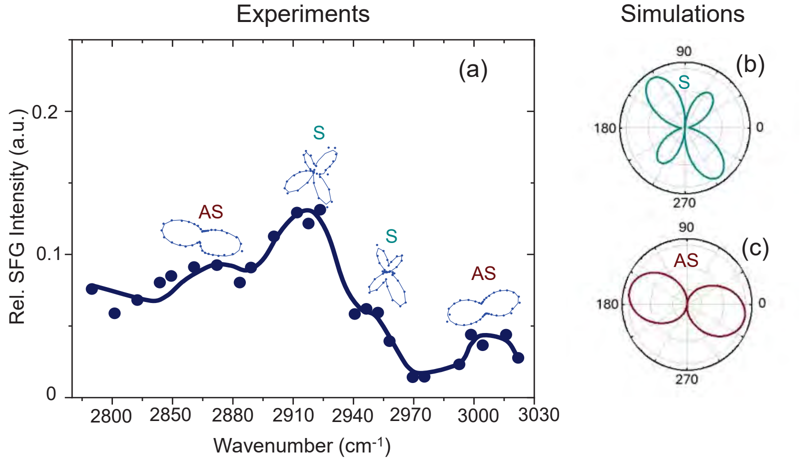

Figure 1: Spectral and polarization-dependence of the SFG signal for the tensor element $\chi_{XYX}^{(2)}$. (a) Experimental spectrum and polarization plots presented at the frequencies 2860 cm^{-1}, 2917 cm^{-1}, 2942 cm^{-1}, and 3016 cm^{-1}. Dots show the experimental data points whereas the thick solid line is a guide for the eye. (b) and (c) simulated polarization-dependence for the tensor element $\chi_{XYX}^{(2)}$ using the crystal structure of collagen type I for the symmetric (S) and asymmetric (AS) methylene modes respectively.

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Raman study of liquid-liquid phase separation, constitution of viral factories, and reovirus assembly

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More complex dsRNA viruses replicate in special cytoplasmic inclusions called viral factories. Viral factories are highly dynamic structures belonging to a broader class of biological condensates formed by liquid-liquid phase separation. They exhibit droplet-like behavior including mobility, fission and fusion, and shape change. Such objects can be studied with proven imaging methods such as transmission electron microscopy and electron tomography, which provide ultrastructural information in unsurpassed detail. On the other hand, information on the dynamic parameters of viral factories, such as internal viscosity and mass exchange with the surrounding cytoplasm, can be studied on live infected cells by combining advanced methods of time-resolved fluorescence and holotomographic microscopies [1]. However, none of these powerful imaging methods provide direct information about the chemical composition of viral factories and its changes during their constitution and virus assembly.

Here, we demonstrate the ability of confocal Raman microscopy to provide highly required information on the chemical composition of viral factories during liquid-liquid phase separation and virus assembly. In a case study, replication of avian reovirus was studied using a combination of confocal Raman microscopy and a wide range of different electron and optical imaging methods. We show that, despite some limitations and difficulties, confocal Raman microscopy can provide another piece of the puzzle allowing a deeper understanding of these processes.

Acknowledgement

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The integration of electrophysiology and Raman spectroscopy for biological and drug dynamics investigation

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The drug launch in the market entails a costly and lengthy process, about 2 billion dollars over 10 years. Preliminary drug screening *in vitro* exploits the combination of fluorescence microscopy and live/dead staining. Then, the functional toxicity of electrogenic cells prior to or following drug treatment is investigated.[1] Such approach does not include the structural drug cytotoxicity such as phenotypical, metabolic and morphological changes, resulting in undetected drug cytotoxicity. With the purpose of investigating both functional and structural toxicity, we integrated a platform recording extracellular electrical activity with a non-invasive label-free optical imaging, Raman spectroscopy (Figure 1).

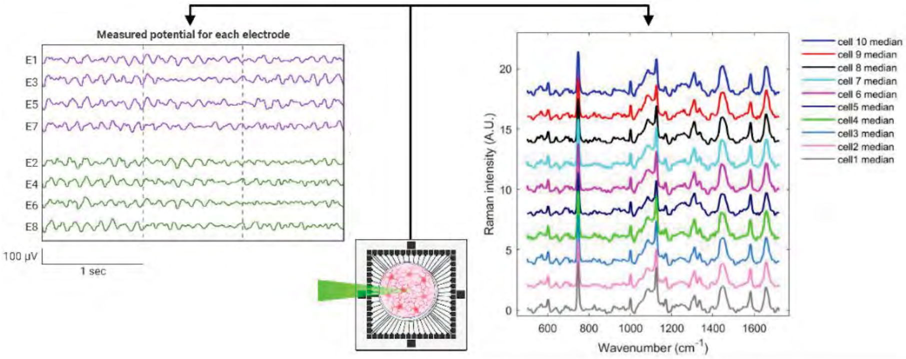
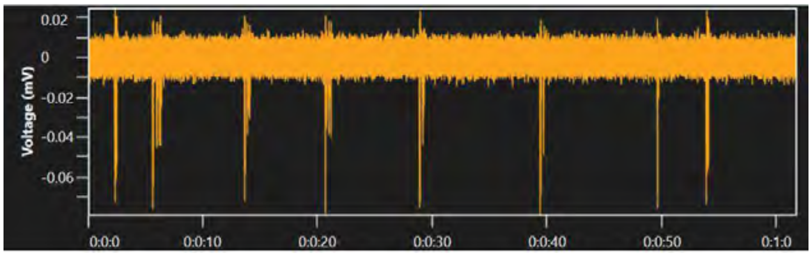


Figure 1: The schematics of an integrated platform recording electrical and Raman activity from an electrogenic cell layer.

We showcase the technique using electrogenic primary neuronal rat cells while exploring biological dynamics associated with cytochrome C, lipids and proteins, suggesting its strong potential for a fine understanding of both functional and structural cytotoxicity under physiological or exogenous triggers (Figure 2).



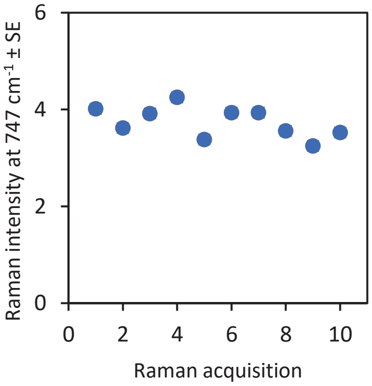


Figure 2: The electrical activity of primary neuronal rat cells and the Raman scattering of cytochrome C corresponding to the electrode of interest.

Overall, we provide the first integrated platform for sequential electrical and Raman measurements, highlighting the potential advantages of such a tool for the screening of structural and functional toxicity drug mediated.

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Stimulated Raman Scattering Spectromicroscopy: Advanced Label-Free Detection of Cetuximab in *ex vivo* Tumor Models

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Head and neck squamous cell carcinoma, originating from the mucosal epithelium in the oral cavity, pharynx, and larynx, stands as the sixth most prevalent global cancer imposing a pertinent health risk.[1] Though recent decades have witnessed significant advancement in targeted therapy,[2] the timely selection of the individual treatment remains challenging. Also, label-free monitoring and quantification of the effect of drugs on cancer cells is still evolving. In this context, we have investigated the effect of cetuximab on tumor cells in a multi-layered tumor oral mucosa model[3] by Stimulated Raman Scattering Spectromicroscopy. Employing SRS[4] along with data processing based on a linear decomposition algorithm, we extracted protein, lipid, and DNA profiles of tumor cells, unravelling cellular changes induced by cetuximab. Going a step further in the analysis of the SRS maps, we also applied the SIMPLEX minimization algorithm to hyperspectral Raman images in the wavenumber range from 2,800 to 3,050 cm^{-1} enabling us to localize the drug and generate drug concentration maps in a label-free manner. This work demonstrates a proof-of-concept using simple and sensitive algorithms for label-free detection of drugs and cellular organelles within a cellular environment using Raman-based spectromicroscopy techniques.

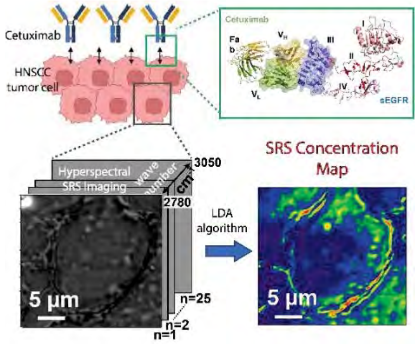

Figure 1: Schematic representation of the cetuximab concentration in tumor oral mucosa models derived from the application of an linear decomposition algorithm on hyperspectral Raman images.

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Template-Assisted Arrays of Plasmonic Nanoparticles for Surface-Enhanced Raman Scattering (SERS) Sensors

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Surface-Enhanced Raman Scattering (SERS) has emerged as a powerful analytical technique for highly sensitive molecular detection. This study introduces a novel approach to enhance SERS performance through the development of template-assisted arrays of plasmonic nanoparticles. The proposed method leverages the controlled fabrication of well-ordered nanoparticle arrays using templates, enabling precise tuning of nanoparticle size, shape, and interparticle spacing.

Nanoparticles are strategically arranged on diverse substrates such as vertically grown carbon nanotubes, patterned silicon substrates produced using semiconductor lithography, anodized aluminum oxide (AAO) substrates, and heat-treated nickel foam. The well-organized plasmonic nanoparticles on these substrates generate stronger SERS signals by creating numerous areas of heightened local field enhancement, known as hot spots, formed between particle-particle or particle-template structures.

The fabricated SERS sensors show excellent sensitivity and reproducibility, with a significantly improved signal-to-noise ratio compared to conventional approaches. The template-based array provides a versatile platform for tailoring SERS substrates to specific molecular targets, with great potential for applications in biosensing, environmental monitoring and chemical analysis.

This work not only contributes to the fundamental understanding of plasmonic nanoparticle behavior but also presents a practical and scalable methodology for the development of high-performance SERS sensors. The template-based approach introduces new avenues for the design and optimization of SERS substrates, paving the way for advancements in the field of molecular sensing and detection.

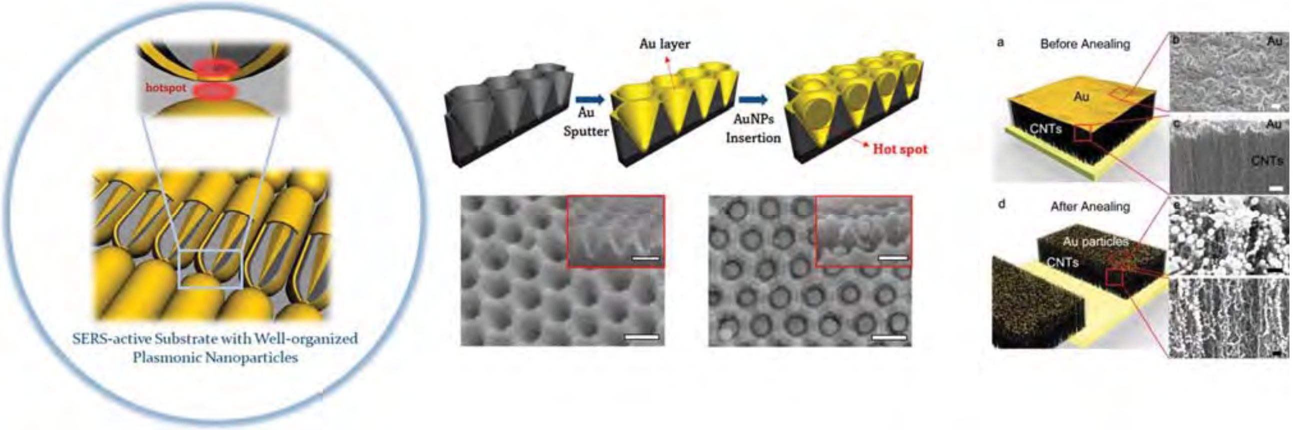

Figure 1: Examples of SERS-active Substrates using Template-Assisted Arrays of Plasmonic Nanoparticles

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SERS Applications in Liquid Biopsy for Cancer Diagnosis

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Liquid biopsy, referring to the detection of tumour-derived biomolecules in the circulation, has garnered significant attention due to their capability of providing a snapshot of the molecular landscape of cancer for individualized disease management. Therefore, non-invasive analysis of circulating biomarkers has the benefit of rapid and little inconvenience to the patients. However, the translation of liquid biopsy to the clinical applications is still challenging due to the rare amount of cancer-relevant circulating biomolecules against an abundant background of non-target biomolecules. To overcome this technical hurdle, we have proposed a new optical tool by utilizing plasmonic nanomaterial and surface-enhanced Raman scattering (SERS) for liquid biopsy analysis towards cancer diagnosis and treatment response.

In this talk, I will present our recent results in this evolving field—from the synthesis of cutting edge plasmonic nanomaterials [1-2] to the design of biosensor platform [3], and clinical evaluation of the new SERS optical tools for liquid biopsy analysis [4-6] with the focus on the detection of circulating tumour cells (CTCs) and extracellular vesicles (EVs). By taking the advantages of the sensor platforms (e.g. microfluidic device) in fast sample preparation and the unique properties of plasmonic nanomaterials, we target simultaneously and selectively detecting and monitoring of the phenotypic profiles of CTCs and EVs in response to drug treatment.

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Probing intracellular interactions with SERS in the presence of a fluorescent dye

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The use of fluorescent dyes for distinguishing and targeting organelles is at the center of many studies at the single cell level. The optimization of fluorophore concentrations and combinations for multichannel imaging possible in live cells is the starting point for analysing diverse physiological processes. The combination of fluorescence with vibrational imaging opens the possibility of characterization of structure and molecular composition of different organelles or other subcellular structures. In particular, surface enhanced Raman scattering (SERS) spectroscopy is a sensitive tool to monitor intracellular interactions of plasmonic nanoparticles.[1,2] Combining fluorescence and SERS microscopy requires careful consideration of the experimental conditions to avoid high backgrounds and fluorescence quenching. We aim to understand how fluorophores and nanoparticles may interact in the intracellular environment and what their potential effects are on cellular biochemistry.

In this work, we used gold nanoparticles for SERS based sensing the effect of a fluorescent dye of intravesicular localization along the endolysosomal pathway *in vivo*. We used LysoSensor Green DND-189 and evaluated the interactions with gold nanoparticles inside lysosomes by SERS spectroscopy. Depending on the concentration of LysoSensor and localization of the nanoparticles along the endolysosomal pathway, the SERS signals show differences in the interactions with different biomolecules such as proteins and lipids. To delineate the spectral contributions by the dye itself, we collected its Raman and SERS spectra at different pH values to compare them to the spectra obtained in the living cells. Gold nanoparticle distribution and aggregation in the cells and the cellular ultrastructure were imaged by soft X-ray tomography in combination with the distribution of lysosomes by fluorescence microscopy. Understanding how different probes interact in living cells can help the interpretation of multimodal imaging results.

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Observation of DNA strand interaction with SERS

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Surface-enhanced Raman spectroscopy (SERS) has demonstrated its ability as powerful tool that can provide us information about the structure and the conformation of biomolecules such as DNA. One can probe the interaction between two biomolecules and extract some evidences of the conformation changes induce by the interaction. It is of primary importance to understand such interaction to improve the performances of sensors that are based on the capture of analyte by a bioreceptor. In addition, molecular interactions are the basis of many biological mechanisms. It is therefore important to have a better understanding of these phenomena and to be able to answer to specific questions as: how does the interaction take place? is it dynamic or static? is there any specific conformation for the interaction?

In this work, we focus on the interaction between two DNA complementary strands as well as strands containing mismatch in their sequences. To do this, we to study the interaction between a DNA sequence consisting of 20 Bases of poly-Thymin (PolyT) with its complementary poly-Adenin (PolyA). The PolyA strand is grafted at the surface of the gold nanostructured surface (Hamamatsu commercial SERS substrate [1]) using a thiol group at the 5' extremity of the DNA strand. We assume that we form a monolayer of PolyA. Some solutions of PolyT with different concentrations (10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) are successively deposited on the SERS substrate. We performed Raman mapping on the surface and we recorded 400 spectra using a 633 nm excitation wavelength. One can observe the 735 cm^{-1} band assigned to the ring breathing mode of the PolyA and some variations of its intensity depending on the position on the map. By changing the concentration, we observe a decrease of the average SERS intensity of this band as well as a decrease of the standard deviation of the intensity of this band. We interpret this intensity change by some modification of the orientation and flexibility of the PolyA DNA strands interacting with the PolyT [2]. The increase of the concentration of Poly-T induced a loss of flexibility of the PolyT/PolyA molecular complex. We performed similar experiments by introducing a mismatch inside the PolyA sequence. A C base is inserted at different positions in the sequence of polyA and we can observe some modification of the interaction between the PolyA and the PolyT strands. This study provides a new approach for the reliable quantification and structural analysis of biological molecules.

This work was supported by the European project DeDNAed (H2020-FETOPEN2018-2020, n° 964248).

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SERS temperature measurements link cell fate to temperature increase during cancer photothermal therapy

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Nanoparticle-mediated photothermal therapy is a promising cancer therapy that induces tumour cell death by elevating local tissue temperature using near-infrared (NIR) radiation and light absorbing nanoparticles.[1] Maintaining the correct therapeutic temperature increase inside the tumour is crucial during treatment- too low, and cell death does not occur, too high, and surrounding 'off-target' healthy tissue is damaged. This challenge is compounded by the variability of nanoparticle delivery efficiencies observed in clinical trials and the lack of technologies to accurately measure temperature during treatment. In this talk, we address this challenge with dual-functional gold nanoparticle clusters that exhibit a strong NIR absorbance and generate surface enhanced Raman scattering (SERS) to enable simultaneous plasmonic heating and temperature read out via anti-Stokes thermometry.[2] These dual-functional clusters were applied to heat in vitro cancer cell cultures and measure temperature increase within the laser spot via SERS. Following heating, the extent of cell death and the associated cell death pathway was assessed using flow cytometry and rationalised by the temperature increase measured with SERS. This work demonstrates the first application of SERS to link temperature increase during photothermal therapy with cell fate and highlights the importance of measuring temperature directly within the laser spot, i.e. the temperature elevation experienced by the cells during treatment. As this technology matures, it could be applied to ensure consistent, therapeutically effective and safe temperature elevations are achieved in solid tumours during photothermal therapy in the clinic.

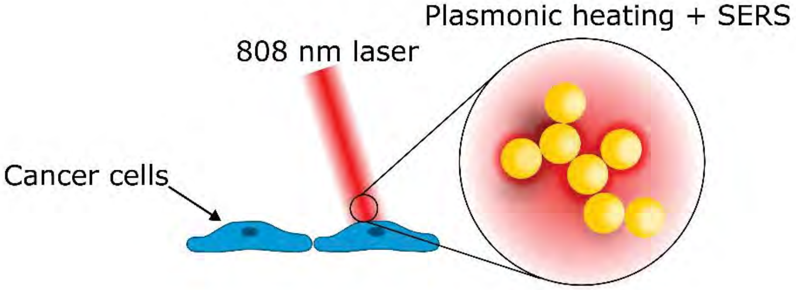

Figure 1. Gold nanoparticle clusters absorb 808 nm laser light and heat cells. SERS spectra generated by the clusters was used to measure temperature increase experienced by cells to improve understanding of cell response to nanoparticle mediated photothermal therapy.

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Sensitive pathogens detection using nanozyme based catalysis amplification on Ag-PSi SERS scaffold

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Food safety and the prevention of foodborne illnesses depend greatly on the quick and accurate identification of pathogenic microorganisms. A bifunctional Pt@Au core-shell nanozyme was designed for rapid identification of two major food pathogens (*Escherichia coli* and *Staphylococcus aureus*) based on a label-free surface-enhanced Raman scattering (SERS) approach. Herein, we utilize their capacity to imitate natural enzymes to oxidize 3,3′,5,5′-tetramethybenzidine (TMB) into an oxidized product (oxTMB), a highly active Raman-reporter-molecule. The number of signal molecules may be considerably increased by catalyzing the conversion of Raman-inactive molecules into reporters using the ultrathin Pt shell. Additionally, the Au core and silver decorated pours silicon (Ag-PSi) act as an active SERS substrate to boost reporter molecules' signals, prominently enhancing the detection sensitivity. The bifunctional Pt@Au nanozyme coupled to SERS immunoassay achieved low detection limits of 3.4 and 4.5 CFU mL^{-1} for *Escherichia coli* and *Staphylococcus aureus* in overall 60 min assay duration. Furthermore, the signal amplification, inherent physical features, platform’s selectivity and overall shelf-life were thoroughly evaluated while depicting satisfactory performances. Finally, the practicality of the developed SERS bioassay was elucidated in various products (raw milk, tahini, fish, ground water, and lettuce) spiked with different pathogens concentrations that revealed recovery values of 90-115% with respect to conventional practices. The successful validation of actual samples' analysis emphasizes the platform's reliability, robustness and suitability for practical use, including on-site or bed-side operation. It can also be used by regulatory agencies for improved management of the health risks associated with consuming contaminated and for environmental analysis as well as other biosensing fields.

Keywords: Nanozyme, Pt@Au, TMB, Ag-PSi, SERS, Pathogens.

The Problems with Diagnostic Methods based on Untargeted SERS of Biofluids

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The literature is flooded with an increasing number of papers reporting diagnostic methods, for a variety of conditions, that are based on untargeted SERS analyses of biofluids such as serum, plasma, urine and others. Most of these papers have in common a similar structure [1]: i) they report differences between spectra of two (or more) groups of subjects, often assessing these differences by using statistical tests, ii) they discuss these differences on the basis of a biochemical interpretation of SERS bands, and iii) they propose and validate one or more multivariate predictive models based on those spectral differences, reporting diagnostic figures of merit such as accuracy, sensitivity, specificity and AUC. A critical analysis shows how, unfortunately, each of these steps is often affected by some serious methodological issues: statistical tests are inadequate, biochemical interpretation is erroneous, and predictive models are incorrectly validated, leading to an gross overestimation of models performance. Guidelines are proposed to adopt a more rigorous methodological approach, in order to limit the propagation of incorrect information about the usefulness of untargeted SERS analysis of biofluids for diagnostic purposes.

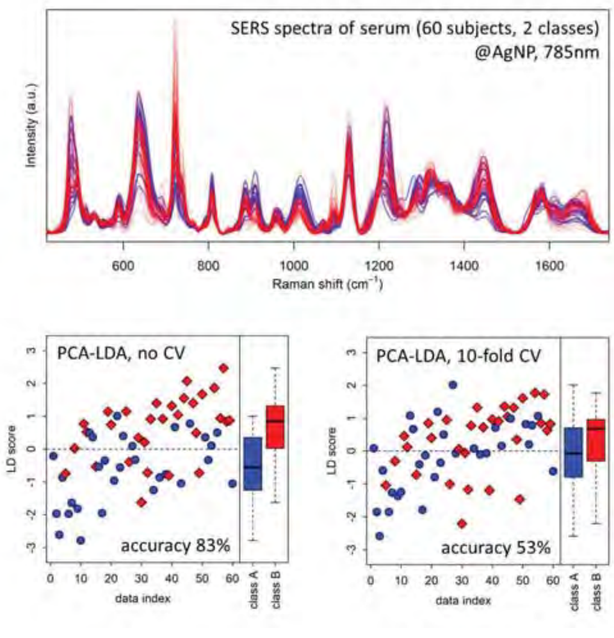

Figure 1: an example of performance overestimation for a predictive model based on SERS spectra of serum

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Development of integrated SER-microfluidic device for the detection of small extracellular vesicles from breast cancer patients' plasma

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Cancer-derived small extracellular vesicles (sEVs) are lipid bilayer vesicles (50-200 nm) secreted from cancer cells to the extracellular environment.[1] They carry distinct biomolecules (e.g., proteins, glycans and miRNA) from their parental cells, which can provide valuable information for cancer diagnosis and prognosis. However, the heterogeneity and low numbers of sEVs in plasma sample prevent them from being used in clinical application. Recently, microfluidic technology has gained great attention for isolating/capturing sEVs from human plasma/serum. Moreover, surface-enhanced Raman scattering (SERS) has emerged as a promising candidate for sEVs analysis due to its ultra-sensitivity, stability, rapid readout, and multiplexing capability.[2] Thus, the integration of microfluidic device with SERS could offer new opportunities for the detection of sEVs in clinical application. In this study, we focus on developing an integrated SERS-microfluidic device to capture the sEVs on the device surface and profile surface proteins on cancer-derived sEVs from cancer patient's plasma using SERS.[2] The isolated sEVs from breast cancer (BC) patients' plasma were characterized by Nano-flow cytometer (NanoFCM) and transmission electron microscopy (TEM) to ensure the presence of sEVs in plasma sample. The microfluidic device with a Polydimethylsiloxane (PDMS) layer was fabricated on the glass slide. The captured sEVs were detected by fluorescent imaging, which showed a specific capturing of sEVs on the glass surface. Furthermore, SERS nanotags conjugated with anti-EpCAM antibody were used to quantify the EpCAM expression level on sEVs from both healthy donor and breast cancer patient's sEVs. It was discovered that EpCAM expression level is much higher on pre-operational patient than the post-operational patient and healthy donor, demonstrating the promising application of integrated device with SERS in clinical settings.

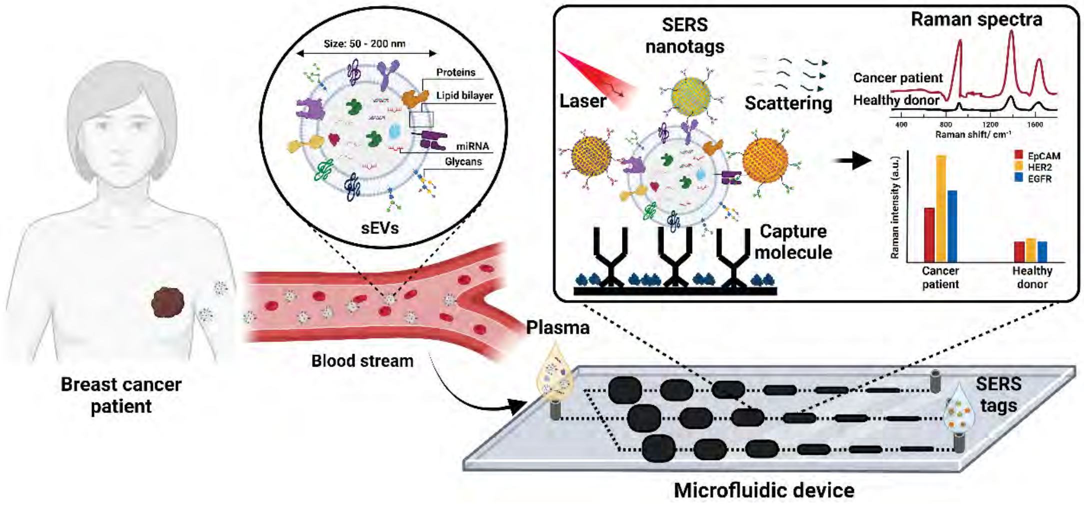

Figure 1. Schematic of SERS - microfluidic device for breast cancer-derived sEVs protein profiling.

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From Development to Detection: Dendritic Nanostructures in SERS for Advanced Biomolecular Analysis

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Surface-enhanced Raman spectroscopy (SERS) is a powerful technique that has shown great promise in the field of biomolecular detection for various applications in medical diagnostics and research [1]. By using silver sulfate as a precursor instead of silver nitrate, we developed dendritic nanostructures with sensitive SERS detection capabilities (Figure 1a). Silver sulfate acts as a growth agent and a mild capping agent, simplifying the fabrication process and enhancing substrate stability. We integrated these dendritic nanostructures with electrochemistry to create a platform for the potential-dependent SERS analysis of biological molecules. As an example, we have utilized dendritic structures to detect and analyze DNA tagged with an alkyne molecule. The significance of the alkyne tag lies in its location within the biologically silent region of the Raman spectra. We leveraged 2D correlation-based analysis [2] to study the effective changes in the Raman mode of DNA bases and the alkyne tag (Figure 1b). In future work, we will focus on bioanalytical detection schemes employing this SERS platform in clinical-relevant research, highlighting the potential of dendritic nanostructures in enhancing the sensitivity in SERS applications and their analysis in the complex sample matrices.

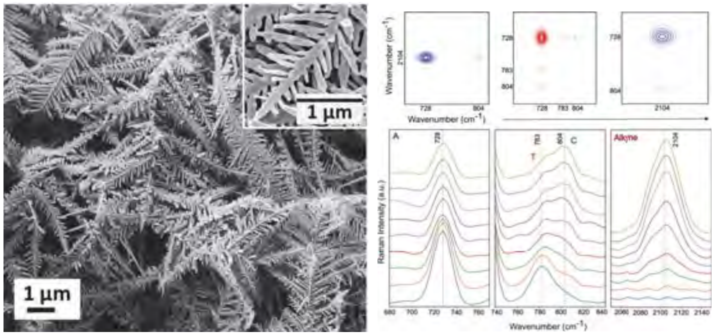

Figure 1: a) SEM image of as synthesized dendritic nanostructures, b) 2D correlation analysis and related spectral changes in the alkyne tagged DNA.

Acknowledgement: The Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) supported this work under grant 465289819.

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Collaborative Strategies for Advancing Microplastic Research

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Plastic pollution is globally recognized as an environmental challenge affecting ecosystems and human health. More recently, there has been growing concern about small fragments at the micro and nanoscale, which originate from the breakdown of larger plastic litter. This concern has driven research efforts to understand the sources, distribution, fate, and impact of these particles. Effective solutions to the challenges posed by micro- and nanoplastics require collaborative research efforts and interdisciplinary approaches. Recognizing this need, the COST Action CA20101 “Plastics Monitoring Detection Remediation Recovery” (https://ca-priority.eu/) – acronym PRIORITY – serves as a pivotal platform for fostering collaboration among scientists, policymakers, and stakeholders [1]. COST Actions, such as PRIORITY, offer networking strategies that enable researchers to share expertise, resources, and best practices, facilitating the development and implementation of innovative solutions to address plastic pollution. By leveraging the collective knowledge and resources of diverse research communities, COST Actions play a crucial role in advancing our understanding of microplastic pollution and supporting global efforts towards its mitigation and remediation.

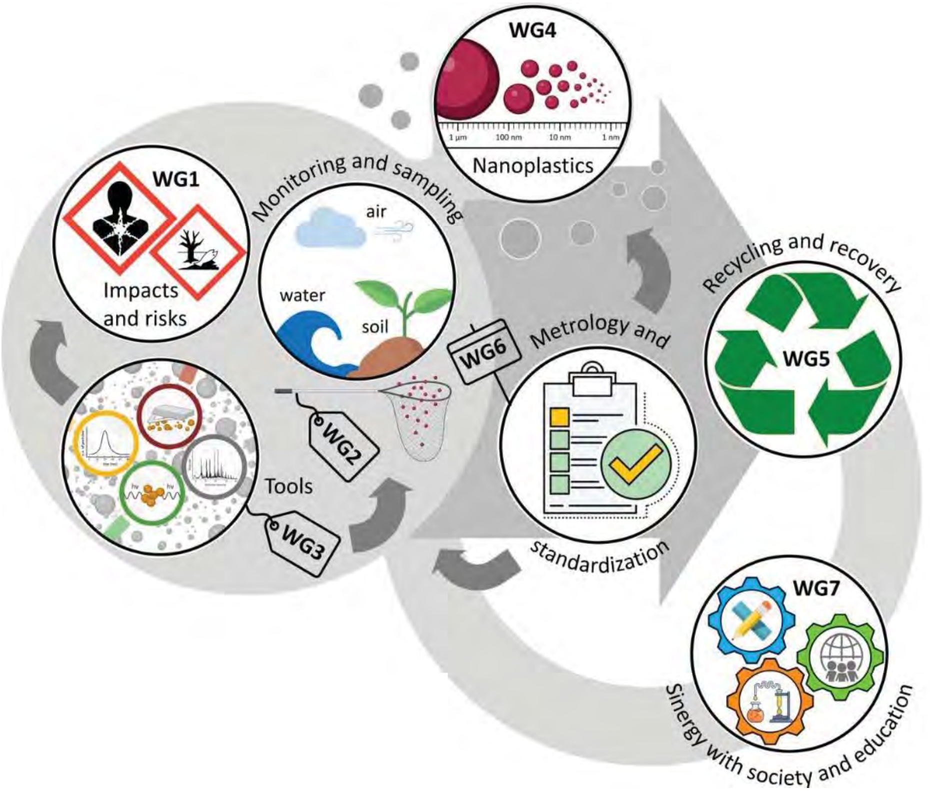

Figure 1: PRIORITY Working Groups Scheme

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Microplastics detection in seawater using 2D Raman mapping of a filter (membrane) with residue

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Nowadays, microplastics (MPs) – synthetic particles less than 5 mm in size – are widely present in the environment, and seen as a potential global threat to biodiversity, food safety and public health [1]. The problem is especially acute for seawater, since it is one of the main sources of foodstuff, and, at the same time, it accumulates a huge amount of plastic waste. As a result, MPs have already been found in the tissues and organs of many marine species, as well as in sea salt. To control and correct the situation, it is necessary the systematic extensive monitoring of MPs in seawater is including their size/type distribution, spreading over the water area/depth, weathering, degradation, interaction with chemical pollutants etc.

Raman spectroscopy is an effective analytical technique for this task [1]. It is a rapid, non-contact and non-destructive method that uses monochromatic light to excite molecules in a material, and the spectrum of inelastically scattered light (Raman spectrum) to recognize the types of these molecules, intra- and intermolecular bonds. The method has high selectivity and sensitivity, allowing to identify different types of MPs, even if their number are low, they are mixed, or the irrelevant (matrix) components are present. In addition, one can observe minor changes in the MPs chemical composition and structure. Combination with scanning optical microscopy (Raman microscopy) yields the distribution of locally measured Raman spectra over the sample (hyperspectral imaging) that in turn can be used for simultaneous visualization of the MPs chemical composition, geometry and spatial distribution (chemical imaging).

Our Raman microscope can perform the analysis of MP at a very small footprint so it can be used in portable labs on ships in the ocean.

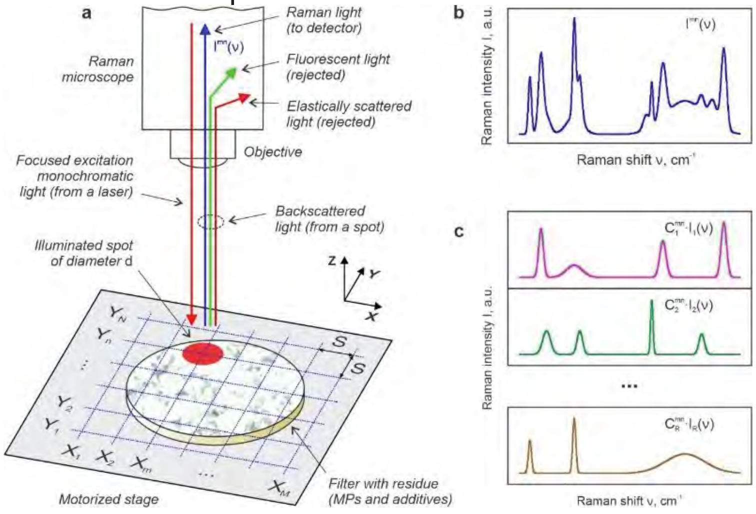

Figure 1: 2D Raman mapping: (a) general measurement scheme; (b) model Raman spectrum for the mixture “obtained” from one illuminated spot; (c) “pure” Raman spectra for individual components present within the spot

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Evaluation of Raman and Fluorescence Techniques for Detection and Identification of Microplastics in Environmental Samples

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To assess the potential for toxicological effects of microplastics on the environment, it is required to not only quantify total concentrations, but also characterize the polymer type, size, surface, additives, and shape [1]. To this end, laser-based imaging techniques such as Raman, Stimulated Raman Scattering microscopy (SRS), and fluorescence microscopy after Nile Red staining can be used [2,3]. Challenges, however, are specifically the detection of particles of <10 µm, avoiding false positives, and finding an optimum in terms of measurement speed, chemical specificity, minimum particle size, quantification, and sample preparation. Comparisons between these different methods on the same samples have not yet been published.

In this work, part of the sample preparation, spontaneous Raman, SRS, Deep-UV Raman, fluorescence staining and (fluorescence) microscopy and combinations of these techniques were optimized and evaluated for microplastics in riverine suspended matter. The optimized Nile Red staining allows coarse categorization into polar and non-polar materials based on their fluorescence index. A deep-UV Raman microscope was constructed which allows identification of coloured plastics, even those pigmented with carbon black. Since spontaneous Raman mapping is very slow, Raman point measurements are preferred with pre-selection based on fluorescence imaging. SRS is several orders of magnitude faster than spontaneous Raman mapping but requires multiple scans at different z-heights and multiple wavenumber settings. The chemical specificity, minimum particle size, measurement speed, and quantification of the various methods will be discussed.

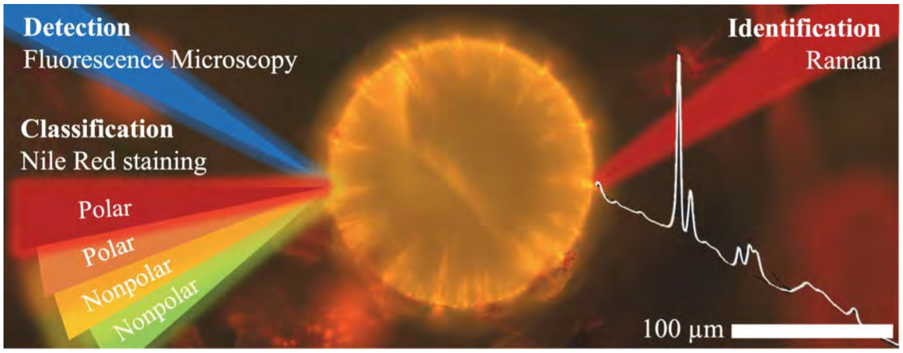

Figure 1: Illustration of spectroscopic approaches, applied to a polystyrene particle found in Rhine suspended matter

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Combination of asymmetric flow field-flow fractionation and Raman spectroscopy enabled by dielectrophoresis to quantify and identify nanoplastics in water

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Identification, quantification and characterization of micro (MPs) and nano-plastics (NPs) in food matrices have been gaining a lot of attention due to their suspected toxicological effect on human health. While several studies have been conducted on MPs, for NPs big challenges have to be taken in consideration because of their diversity in size (1 nm – 1 µm, ISO/TR 21960:2020), shape, type of polymers, and their low concentration in matrices. Most of the detected MPs, and of the expected NPs, are made of synthetic polymers such as polyethylene, polypropylene, polystyrene, polyethylene terephthalate, and poly(methyl methacrylate). Raman microspectroscopy (µ-Raman) can be applied to identify this wide variety of polymers due to their highly specific fingerprint spectra, and for the analysis of water-dispersed NPs. To overcome its limit of resolution, µ-Raman has been coupled with high resolution microscopy, or combined with optical tweezers, which trap the NPs in the focus of the laser beam, enabling the spectral acquisition [1]. In this context, µ-Raman enabled by optical tweezers was coupled online with field-flow fractionation to have NPs separation and characterization [1]. However, these methods have been tested only on reference materials and with a size range from 200 nm to 5 µm, leaving unexplored NPs made of all the other polymers and with smaller sizes. In addition, optical methods for the quantification of NPs are still missing.

In this work, we propose a new method that combines asymmetric flow field-flow fractionation (AF4), for NPs separation and quantification through a UV detector, and µ-Raman spectroscopy, for NPs identification enabled by dielectrophoresis. For this purpose, nano-polyethylene terephthalate (nPET) and nano-polypropylene (nPP) with diameters of 90 nm and 180 nm respectively, as measured by dynamic light scattering (DLS) technique, were dispersed in water with different concentrations and separated by size with AF4. The UV detector, coupled with the AF4, allowed to construct calibration curves from the elugrams of the separated particles in order to quantify the concentration of the NPs suspended in the medium. Then, the single fractions were collected and injected into a dielectrophoretic cell to locally accumulate the particles, filling the focal volume of the µ-Raman and allowing the acquisition of the NPs spectra in the medium despite their low concentration. This work aims to develop and standardize an analytical method to quantify and identify NPs that could be applied to more complex matrices, such as food (e.g. drinking water, milk) or environmental samples.

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Blue, naturally degraded nanoplastics abundance and their detection via two Raman-based approaches

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Nanoplastics (NPs), which are defined as plastic particles less than 1 µm in size, have recently raised global attention due to their potential to significantly harm our planet's environment and biodiversity. The detection of NPs in natural ecosystem is impeded by the absence of validated and standardized methodologies. However, regulatory bodies have initiated approaches under development according to ISO/IEC Directives, where, regarding technical implementation, vibrational spectroscopy methods gained significant interest. As already well-consolidated, Raman technology is a powerful analytical method for macro- and microplastics (MPs) detection and sorting [1] with high potential to become a standard technique for controlling both MPs and NPs.

Herein, by making use of two Raman-based approaches we demonstrate an effective and feasible detection of naturally degraded blue nanoplastics. We primarily targeted blue NPs due to their high abundance, as a consequence of abundant blue MPs reported in various environmental ecosystems [2], as showed in many recent studies. We found that blue NPs can be easily tracked based on the widely-used copper phthalocyanine (CuPc) pigment in plastic industry, which exhibits particular resonance Raman (RR) signal under appropriate laser excitation. Thus, our approach (Fig.1 a) was to demonstrate RR detection of single blue NPs particles. Further, combining the RR of blue NPs with surface-enhanced Raman scattering (SERS), we were able to push the nano detection limit well beyond the resolution boundaries of micro-Raman spectroscopy (Fig.1 b). The SERS measurements were performed using nanostructured SERS substrates fabricated by means of colloidal lithography, technique that allowed us to produce customized and effective SERS substrates. Moreover, by combining SERS with our previously developed Raman database [1] containing information on naturally aged plastics, and our established detection methodology, an automated approach to detect NPs could be implemented.

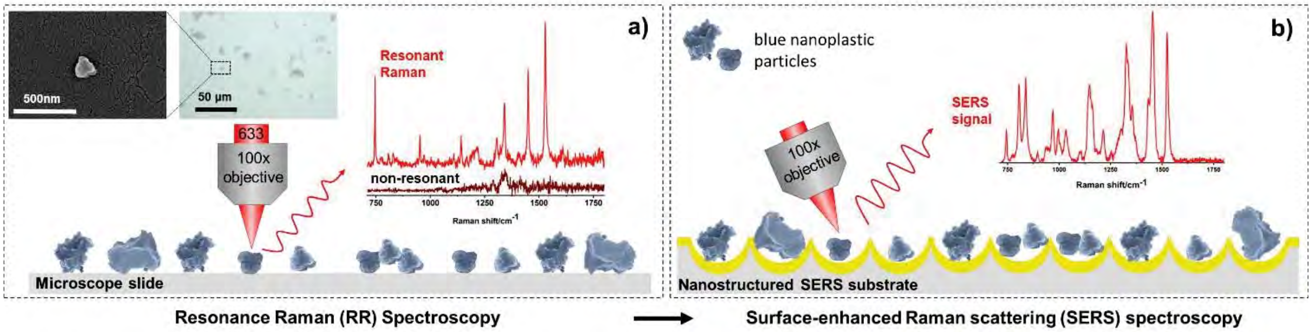

Figure 1: Graphical abstract illustrating the detection of blue nanoplastics via two Raman-based approaches: RR and SERS spectroscopy.

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Development and validation of a microRaman spectroscopy method to detect small microplastics in milk powder/infant formula

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The presence of microplastics in various food products has raised significant concerns regarding potential health risks to consumers. Among these products, milk, being a staple in many diets, has garnered attention due to its widespread consumption and nutritional significance. Studies have documented the occurrence of microplastics in milk samples sourced from diverse geographical locations, highlighting the ubiquity of this issue. The sources of microplastic contamination in milk are multifaceted, encompassing environmental pollution, agricultural practices, and processing methods. Factors such as plastic packaging, equipment used during milking and processing, as well as contamination from the environment, contribute to the introduction of microplastics into milk. Furthermore, the potential health implications of microplastic ingestion through milk consumption remain a subject of ongoing research and debate. While studies have suggested the transfer of microplastics across the gastrointestinal tract and their potential to accumulate in tissues, the long-term health effects are yet to be fully elucidated. Various analytical techniques, including spectroscopic methods, microscopy, and spectrofluorimetry, have been employed to detect and quantify microplastics in milk samples. These techniques offer insights into the types, sizes, and concentrations of microplastics present in milk.

In this work, a new methodology was developed to accurately determine and characterize small microplastics (100-5µm) in milk powder (infant formula) using micro-Raman (µRaman) technology, combining enzymatic digestion, organic matter removal in alkaline conditions, chemical digestion using microwave and a final filtration step through a silicon (Si) filter. The present methodology was developed and validated for different polymers using both commercially available reference materials with defined size and morphology produced from bottom up approach (polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC) and a more representative polydisperse reference material obtained by cryo-milling procedures (PET)). As for PET microplastics, size, number and number distribution was previously evaluated in an inter-validation study involving two different laboratories with different microRaman instrumentations in order to provide a reference number for such material. This material was further used for spiking procedure in the milk matrix to optimize the different sample pre-treatment steps, and to demonstrate a negligible effect on the particle characteristics and polymer compositions of the MP samples. The analytical procedure was further validated in terms of MP recovery rate and quantification sensitivity with the calculation of LOD and LOQ. This methodology will be further applied in commercial infant formula from local supermarket to evaluate the MP contamination on real samples.

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Time-Gated Raman Spectroscopy for Fluorescence Reduction in Recycled Polymers

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Raman spectroscopy is a critical analytical tool in the challenge of plastics recycling. The fluorescence background can be very strong relative to Raman scattering in polymers. Over the years, different methods and techniques have been developed to mitigate the fluorescence: use of continuous wave (CW) NIR laser as an excitation source where fluorescence can be reduced [1], and mode-locked laser [2] as one of the first time-gated methods for background suppression. A strong candidate for temporal fluorescence reduction is Raman spectroscopy using a time-gated technique [3], cleverly utilizing the differences in lifetime between fluorescence and Raman photons. Using a pulsed laser and synchronizing the resulting inelastic scattered photons, this method can significantly reduce fluorescence in the spectrum. While in the past its use was limited by its cost and complexity, the Raman acquisition has been simplified using a CMOS single-photon avalanche diode array (SPAD) detector [3]. Here, we report time-gated time-resolved Raman spectroscopy can also be useful for identification and characterization of plastics recovered containing dyes and additives with a strong fluorescence background. As an example, in fig. 1 we can observe time-gated Raman spectra of a multilayer polymer with fluorescence background reduction (using 532 and 775 nm pulsed lasers), in comparison of CW Raman spectrum (785 nm laser excitation) with fluorescence masking the Raman scattering. In short, using this technique, the Raman fingerprint can be obtained from samples that are difficult or unfeasible using CW techniques. Enabling its use in mechanical and chemical recycling.

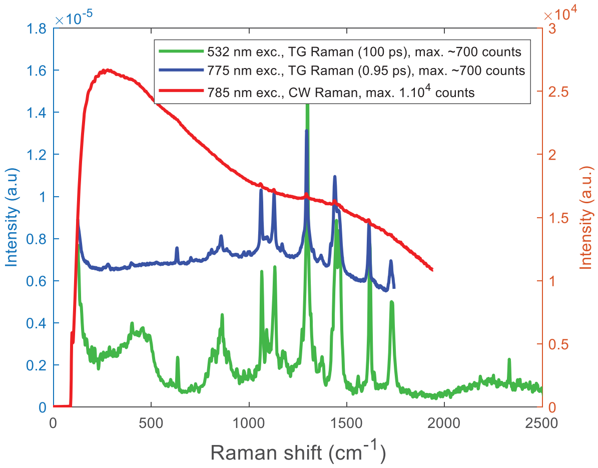

Figure 1: TG-Raman and CW Raman spectra of a multilayer polymer

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A wide-field Fourier-Transform Raman Microscope for fast microplastics detection

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Microplastics (MPs) contamination constitutes a global environmental problem: MPs accumulate in the aquatic food web and represent a risk for the human health [1]. Spontaneous micro-Raman (μ-Raman) Spectroscopy has emerged as an effective and non-destructive technique for MPs assessment, allowing detection of fragments smaller than 10um [2]. However, due to its point-by-point scanning acquisition and spectra detection by a dispersive spectrometer, μ-Raman is limited by its long measurement time (>20h for 100k pixels image). Moreover, μ-Raman is affected by the fluorescence background overlapping with the Raman signal and hindering the detection of MPs in highly fluorescent field samples.

Here we demonstrate fast MPs detection by a novel wide-field Raman microscope, where spectra are obtained by the time-domain Fourier-transform method [3], while the wide-field approach enables fast parallel collection of Raman spectra over all pixels of the image (~10 min for a 100k pixel image). Moreover, the time-domain method allows acquisition of background-free Raman spectra, rejecting the fluorescence background by proper sampling of the interferograms. The system's capability to detect and classify MPs is first validated by measuring known polymers' fragments synthesized in different sizes and shapes. Its application for environmental MPs assessment is demonstrated through measurements of particles directly filtered from seawater samples (Figure 1).

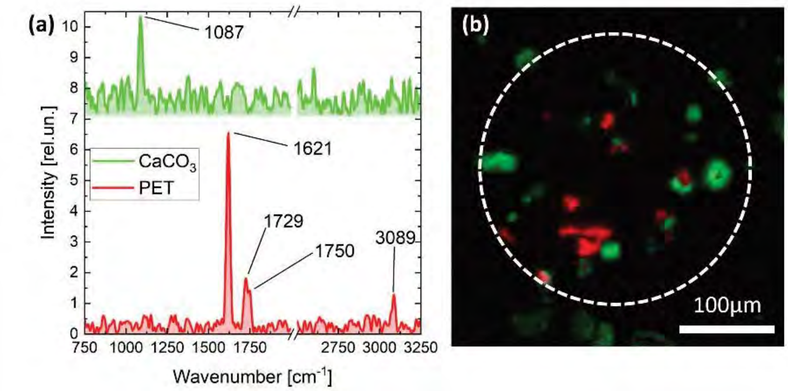

Figure 1: Widefield Raman map of particles filtered from a seawater sample. **(a)** Endmember spectra retrieved by N-FINDR analysis distinguishing between Polyethylene Terephthalate (PET) MPs and $CaCO_3$ grains. **(b)** Composite map of endmember spectra shown in panel (a).

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Label-free detection of viruses with SERS through analyte localization and polymer-enabled capture

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Virus detection is highly important; the last several years, since the onset of the SARS-CoV-2 pandemic, have highlighted a weakness in the field: the need for highly specialized and complex methodology for sensitive virus detection, which also manifests as sacrifices in limits of detection made to achieve simple and rapid sensing. Surface-enhanced Raman spectroscopy (SERS) has the potential to fill this gap, and two novel approaches to the development of a detection scheme are presented in this study (Figure 1). First, the physical entrapment of vesicular stomatitis virus through substrate design to localize virus analytes into SERS hotspots is explored. Then, the use of non-specific linear polymers as affinity agents to facilitate polymer-enabled capture of the virus for SERS detection is studied. This work shows great promise for further explorations of label-free virus detection methods involving thoughtful substrate design and unconventional affinity agents.

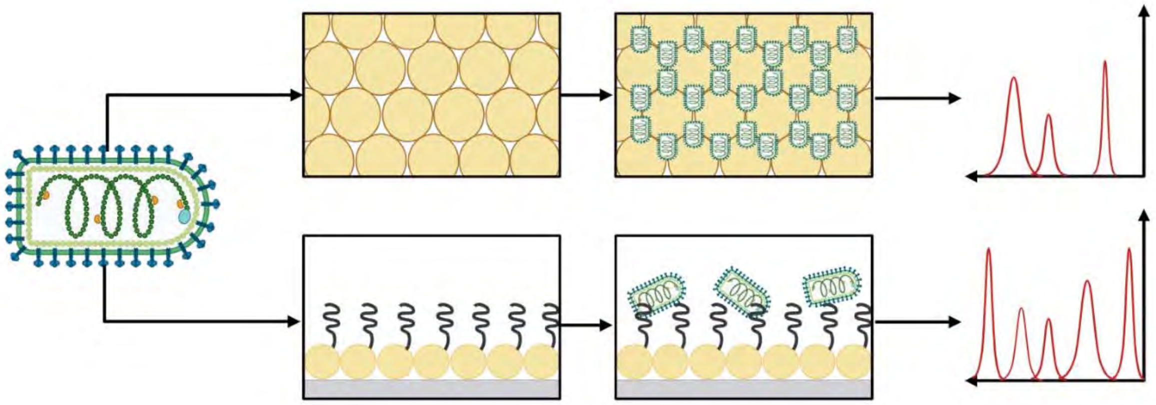

Figure 1: Schematic of SERS-based virus detection schemes

Arrayed Nanoplasmonic/SERS Molecular Biosensing of Cancer Biomarkers for Rapid and Low-Cost Liquid Biopsy

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Plasmonics refers to the interactions of light with conduction band electrons in metallic nanoparticles and nanostructures. Recent advances in lithography-based planar nanofabrication enables rigorous control of the shape, size, and arrangement of these nanoscale features with unprecedented precision. In this talk, I will explore our decade-long pursuit in nanoplasmonics of these "patterned" structures on a substrate from three aspects: material choice, substrate dependence, and arrayed far-field coupling. Specifically, we have demonstrated the use of gold, gold-silver alloy, and nanoporous gold for tailored nanoplasmonics [1-3]. In addition, we have employed advanced nanofabrication to precisely control the removal of a portion of the substrate dielectrics to optimize the plasmonic performance [4]. We have further investigated arrayed behavior of far-field plasmonic coupling in various designs [5-6]. These nanoplasmonic plasforms have found potential applications in the context of chemical/biosensing of key physiological analytes and cancer biomarkers such as mutated DNA [7-9], micro-RNA (miR) and small extracellular vesicle (sEV) including exosomes [10-13]. These new technologies can potentially outperform existing liquid biopsy technologies based on circulating tumor cells and circulating DNA for early detection, diagnostics, therapeutic monitoring, and surveillance.

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SERS-based method for detecting and identifying pathogenic microorganisms - bacteria and viruses.

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Surface-enhanced Raman spectroscopy provides a unique vibrational signature of the scattered molecules. SERS as an ultrasensitive, label-free and non-destructive technique, reveals specific information down to the molecular level and thus will offer valuable information for biological systems analysis and monitoring. We present its application for detection and identification of pathogenic bacteria from clinical and environmental samples, including viruses including SARS-CoV-2 [1-3]. The developed SERS-based sensors challenge the current standard method of bacterial detection and identification in terms of sensitivity, selectivity, cost, and time of analysis. The direct SERS analysis of bacteria (even a single bacteria cell) is performed directly from Perspective nanostructures incorporated into a microfluidic module. The recorded SERS data of bacteria are categorized (assigned to particular bacterial species) using data analysis software based on a SERS database created for bacteria [4-5]. The long-term incubation of bacteria was eliminated, and the total analysis, including the numerical analysis of recorded SERS data, did not exceed 15 minutes. Coupling of plasmonic nanostructures with microfluidic systems ensures miniaturization of the developed methods for their further applications. This invention opens a new path in microbiological diagnostics for sensitive, simple, quick, and on-site detection of pathogenic microorganisms, including environmental and clinical microbiology (hospitals, health centers), food industry and environmental protection.

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Sensitive detection of pathogens using nanozyme catalysis amplification SERS assay on Ag-Psi

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Introduction: Target bacteria identification is crucial for maintaining a healthy food supply, as well as minimizing foodborne diseases, food damage, farm animal death, and financial loss for food or food-related firms and farmers [1]. In this study, an optical biosensor is used to detect and quantify *Escherichia coli*, which serves as a model indicator bacterial specie. This investigation's sensing capabilities are extremely precise, detecting and discriminating bacteria quantities using the SERS (surface enhanced Raman spectroscopy) test. It is an effective approach for increasing molecules' inherently modest Raman scattering cross-sections, allowing highly sensitive detection down to single molecules during interaction with noble metallic particles.

Experimental: The Ag-PSi nanostructure was created by deposition of silver over newly etched porous silicon (pSi) (620 mAcm^{-2} A for 5 second) [2]. Bacterial detection was accomplished by contact with particular antibody functionalized Au@Pt and filtering. Filter unreacted conjugate antibody was used to capture antibody functionalized AgPSi for detection using Au@Pt nanozyme activation of SERS active chromogenic substrate.

Results and Discussion: The results reveal that this approach of detecting particular bacteria from a food sample containing a combination of several bacteria species is exceedingly accurate and can distinguish between the different bacteria kinds with very high precision, with detection limits as low as log10^{1}. The bioassay results are consistent with a standard approach. This technology is easier to construct, more stable, and simpler to prepare and operate than the standard method now in use.

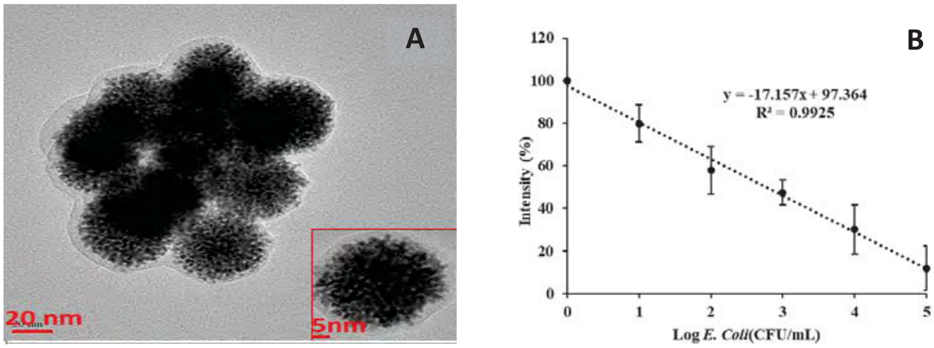

Figure. (A) TEM image of Au@pt nanoparticle and (B) Linear calibration curve for E. coli detection.

Conclusion: Overall, the presented sensing concept has the ability to detect and differentiate extremely low amounts of bacteria (log10^{1}) in food samples, providing an inexpensive and simple detection method. As a result, this sensing platform is a useful tool for identifying the bacteria *E. coli* as a potential point-of-care diagnostic platform in hospitals. It can also be used by regulatory agencies for improved management of the health risks associated with consuming contaminated and for environmental analysis as well as other biosensing fields.

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Tandem SERS and MD profiling of salivary biomarkers for point-of-care sensors detecting head and neck cancer and infections

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In the successful therapy of important and widespread illnesses such as cancer and infections preventing complications and boosting the probability of survival and wellness of the patient, early diagnosis and precision medical results are crucial. An important goal to pave the way for simple access to point-of-care diagnostics is related to the development of liquid biopsy diagnostic devices with the attempt to provide trust-worthy, non-invasive, and suitable assays identifying biomarkers from biofluids. Among the possible non-invasively collectible biomatrices, saliva is one of the best candidates, as it is easily accessible and easy to transport and store. Combining SERS biosensing techniques with molecular dynamics (MD) can be a valuable strategy to deal with the complexity of the SERS dataset. All-atom MD computations are in fact powerful tools for the atomistic characterization of proteins and offer a thorough understanding of the molecular drivers behind protein adsorption on the metal surfaces' highlighting structural hotspot that accounts for SERS signal differences. In this scenario, the combined SERS-MD workflow was used to disentangle specific disease-related biomarkers present in a biological fluid [1]. Currently detected predominantly using an expensive enzymatic immunoassay, lysozyme (LYZ) and interleukin-8 (IL-8) have already been shown to be differentially expressed in several disease states of head and neck cancer or infections. Overall, this preliminary study, by combining SERS and MD techniques, showcases the needed tool to address the challenging recognition and interpretation of disease-specific markers in body liquids, paving the way to point-of-care SERS-based technologies for health monitoring.

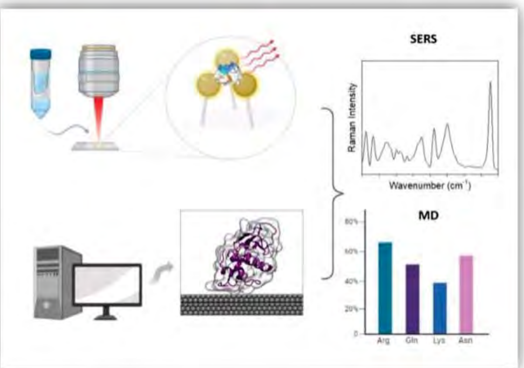

Figure 1: SERS/MD simulations combined workflow for identification of salivary markers.

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Emerging SERS platforms based on MoS_2-2D flakes for the characterization of extracellular vesicles derived from ovarian cancer

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Extracellular Vesicles (EVs) play a crucial role in allowing intercellular communication. Additionally, EVs contain a wide variety of biomolecular cargo that may be useful for the identification of potential biomarkers related to different stages of cancer disease. Despite their promise, the inherent challenges caused by the small size and heterogeneity of EVs hinder the development of effective characterization protocols. To address these issues, surface-enhanced Raman spectroscopy (SERS) is a good alternative to elucidate the unique fingerprint related to the biomaterial in cancer EVs.[1] Transition metal dichalcogenides 2D materials such as MoS_2, are especially attractive as SERS substrates due to their tunable electronic and optical properties in sensing technology.[2] Moreover, recent studies have demonstrated that MoS_2 can interact with the vesicle membrane producing the adsorption of EVs through Coulomb and Van der Waals interactions.[3] For this reason, MoS_2-flakes were used as a potential label-free SERS platform for the identification of EVs derived from ovarian cancer. The samples were collected from donors with early-stage [FIGO (I/II)] with high-grade serous carcinoma (HGSC) and extracted by Size Exclusion Chromatography (SEC). Finally, the analysis of the unique fingerprints of the EVs and the characterization of the signals related to the bio-cargo were obtained by SERS.

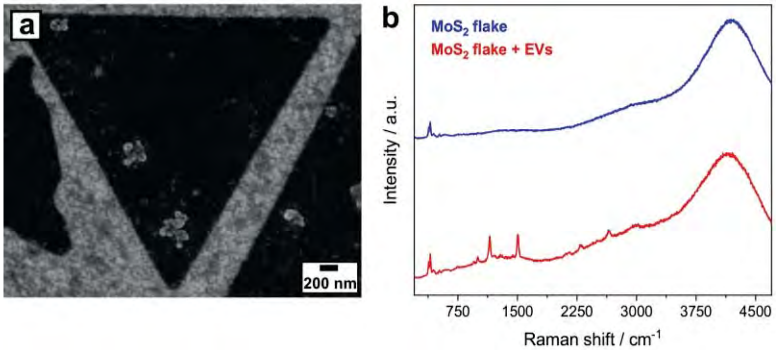

Figure 1. a) SEM image of MoS_2 flakes and b) associated Raman spectra with and without EVs.

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Intraoperative Tumor Diagnosis in Head and Neck Cancer with Raman Spectroscopy (RAMAN-HNSCC): A Prospective Clinical Trial

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Head and neck squamous cell carcinoma (HNSCC) is the seventh most prevalent cancer type globally, with approximately 890,000 new cases diagnosed annually, leading to approximately 450,000 deaths. Surgical resection is a predominant curative treatment modality for early and selected locally advanced stage HNSCC cases, with the primary objective of achieving complete tumor resection while minimizing morbidity and preserving functional and aesthetic aspects [1].

In the context of surgery for HNSCC, the successful outcome heavily depends on complete tumor resection at the cellular level. The standard approach involves tissue biopsies and subsequent histological examination, which is both time-consuming and subjective for intraoperative decision-making [2].

Raman spectroscopy, a biophotonic technique, is a non-invasive, non-ionizing method, that can effectively differentiate between tumorous and non-tumorous tissues ex vivo [3, 4, 5]. The RAMAN-HNSCC trial (DRKS00028114) assesses the feasibility of Raman spectroscopy for real-time in vivo tumor differentiation compared to histopathology. Secondary objectives include validating measurements, ensuring safety, and assessing usability. The study aims to evaluate the accuracy and precision of Raman spectroscopy as a diagnostic method, holding promise for improved intraoperative tumor diagnostics in HNSCC, impacting patient outcomes positively by preserving critical function and aesthetics.

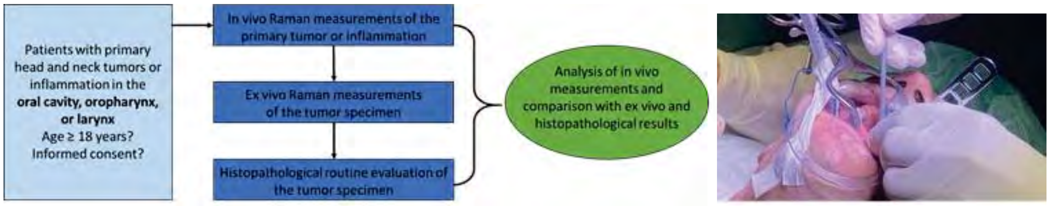

Figure 1: Workflow for the clinical study RAMAN-HNSCC (left), Recording Raman spectra in an oropharyngeal carcinoma using the Raman probe developed at the Leibniz Institute of Photonic Technology, Jena, Germany (right)

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Label-Free Albumin Quantification Using Surface-Enhanced Raman Spectroscopy

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Accurate, label-free quantification of proteins is essential for *in vitro* studies aimed to model the effects of changing the (micro)environment of biological tissues. Surface-enhanced Raman scattering (SERS) is sensitive to low concentrations of solutes relevant to tissue studies. In the Verpoorte lab, a tissue of interest is endothelium, the layer of cells lining blood and lymph vessels (vascular system) in the body. This layer serves as a barrier between the circulatory system and organs, and when healthy, selectively allows the passage of molecular and cellular species required by the body. In chronic diabetic kidney disease, however, high glucose concentrations increase blood vessel permeability, resulting in undesired leakage of albumin, a protein that transports many species in the blood. We report here the first steps in the development of a quantitative SERS-based method for the determination of albumin, applied to endothelial cell-layer permeation studies in a microfluidic device. The approach uses gold nanoparticles (AuN) and the Raman-active pyrazine carbonitrile (PCN) as a reagent that competitively binds to albumin and the AuN to form a complex. The more albumin present, the smaller the Raman signal due to PCN bound to AuN. Initial experiments seem to indicate that accurate quantification of physiologically relevant albumin concentrations ranging from 44 μM to 0.4 μM is possible. The data obtained compares well with a state-of-the-art spectroscopic method, which uses fluorescently labelled albumin as a tracer to directly investigate the permeability of a human umbilical vein endothelial cell (HUVEC) layer cultured in a microfluidic device. [1] However, reproducibility and changes in Raman spectra are a common challenge in SERS spectroscopy. [2] We performed several experiments, including time-resolved Raman spectroscopic analysis and computational methods, to elucidate the nature of the species contributing to the spectra obtained. We used comparisons of multivariate (spectral) curve resolution, use of a synthetic Raman reference compound, and density functional theory (DFT) to attribute spectral changes to gold surface interactions. Ultimately, we found that these interactions aid quantification efforts in complex media. We anticipate that this assay will stimulate more sophisticated physiological readout of organ-on-a-chip systems.

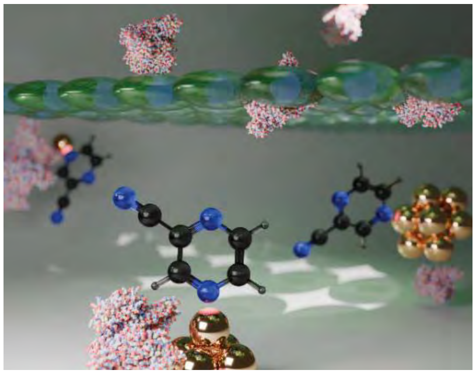

Figure: Albumin, a protein shown here in pink, passes from the top through a diabetically compromised endothelium, between cells (in green). Pyrazine carbonitrile (blue-black molecules underneath layer) interacts competitively with variously sized gold clusters and albumin.

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Advanced Spectroscopic Analysis of Modified Metallic Surfaces for Biomedical Applications

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Controlled modification of metallic surfaces enhances their antibacterial, corrosion-resistant, and biocompatible properties for biomedical applications.

In our studies, we used precise methods to deposit various nanoparticles (NPs) onto titanium surfaces to improve their utility in implantology [2]. The next step is the determination of the adsorption process of plasma proteins (amino acids) and bacteria, which is crucial post-implantation due to the influencing of cellular response and implant biocompatibility.

The application of advanced electrochemical and spectroscopy methods enabled the assessment of corrosion inhibition and adsorption processes [2]. Employing spectroscopic methods, particularly nanospectroscopy imaging techniques like AFM-IR, offers a novel approach in corrosion research [1,2].

Figure 1 illustrates AFM topographies (A and A') and AFM-IR intensity maps (B and B') of the reference Ti and AgNPs/Ti surfaces with deposited Escherichia coli bacteria. Understanding the behavior of modified systems requires an in-depth characterization of the nanostructure of the prepared surfaces with high spatial resolution. Detailed characterization of modified metallic materials at the nanostructure level is essential for the preparation and application of bioactive materials.

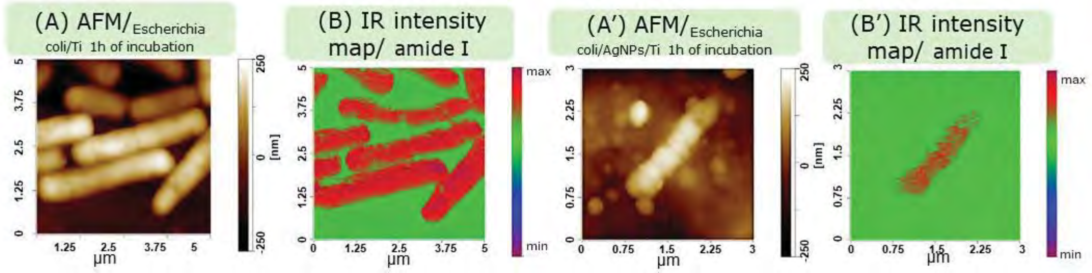

Figure 1: The AFM topographies (A, A') and AFM-IR intensity maps (B, B') of amide I region for the Ti and the AgNPs/Ti samples with Escherichia coli bacteria deposits which were obtained after 1 hour of incubation

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Probing Materials at the Nanoscale: a Journey in Tip-Enhanced Spectroscopy.

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The past two decades have been very active in the development of tip-enhanced spectroscopy and of its application to a variety of materials and biomaterials with intricate properties at a nanoscale level. We present here our journey in this dynamic field applied to the study of nanomaterials and what knowledge it has brought to the field, its promises, its limitation and its challenges.

In particular, we will focus on our most recent work applied to the study of 2D materials and how combination of Tip-enhanced Raman spectroscopy (TERS) and atomic force microscope (AFM) modes can be utilized jointly to decipher the optical and mechanical properties of 2D flakes of transition metal dichalcogenides with thickness of less than 1 nm. The presence of surface defects, growth of additional layers and surface functionalization are examples of nm features that can be revealed optically and mechanically from the combination of an Atomic Force Microscope and a confocal microscope.[1-3]

Current developments in machine-learning methods are also interesting tools that can be applied to analyse thousands of spectra collected a typical confocal or TERS mapping. We will present few examples on how such methods can be applied to Raman spectroscopy measurements and how it can help to decipher rich spectra.[4]

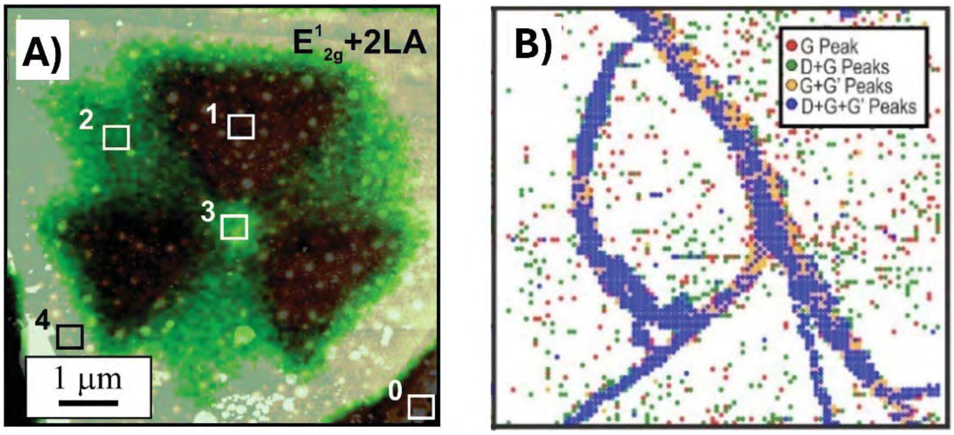

Figure 1: (A) TERS map of substructures in WS_2 flakes (B) deep learning analysis of a TERS map of multiple carbon nanotubes.

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Layer Hybridisation and Exciton-Phonon Coupling in $MoSe_2$ / WSe_2 Heterobilayers

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Transitional metal dichalcogenides (TMDCs) have attracted considerable attention due to their robust excitonic features and potential applications in valleytronics, as well as mixed light-matter states such as exciton-polaritons[1]. The stacking of TMDC monolayers to form heterobilayers provides opportunities to tailor material properties, which is crucial for a wide range of applications spanning from biosensors to solar cells and transistors. The appeal of two-dimensional heterostructures lies in their ability to inherit the physics of the individual monolayers. However, merely combining the properties of the respective monolayers is insufficient for accurately characterizing the resulting heterostructure, especially in the presence of strong cross-layer processes[2].

Here we investigate the cross-layer interactions by studying exciton-phonon coupling in a WSe_2/$MoSe_2$ heterobilayer (Fig. 1(a)) using resonant Raman spectroscopy and found an additional exciton-phonon coupling channel within WSe_2/$MoSe_2$, Fig. 1(b). This observation is facilitated by a cross-resonance resulting from the interaction between the two layers, evident in the resonance profile of the A_{1g} mode in MoSe2. Consequently, the A_{1g} mode in $MoSe_2$ exhibits scattering interactions not only with the A exciton in the $MoSe_2$ layer but also with the A exciton in the WSe_2 layer, Fig. 1(c). To record the resonance Raman profiles, we take resonance Raman profiles on monolayers of WSe2 and $MoSe_2$ and compare the changes in Raman intensity with Raman profiles recorded at heterobilayers. We support our findings with density functional theory (DFT). Calculations employing the frozen phonon method and DFT reveal a predominant exciton-phonon coupling between the A exciton in WSe_2 and the A_{1g} phonon mode in $MoSe_2$.

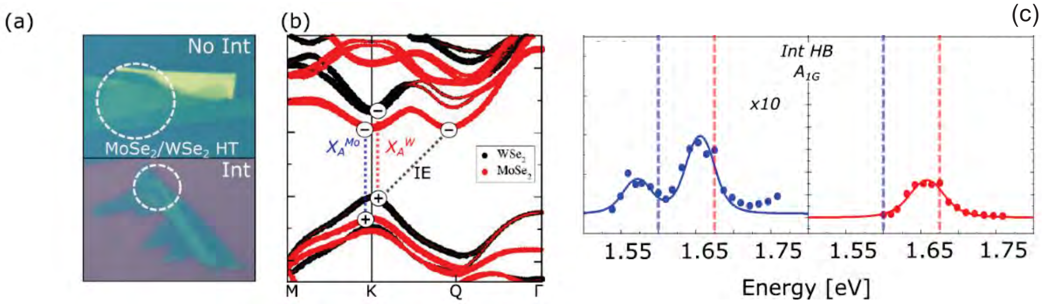

Figure 1: (a) Optical image of MoSe2/WSe2 heterobilayers. (b) Illustration of the type II staggard band gap configuration, blue(red) Xa showing the direct excitonic transition for monolayer MoSe2(WSe2), IE showing the indirect excitonic transition. (c) resonance Raman profiles of a heterobilayer (blue) and monolayer $MoSe_2$ (red)

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Polarization-angle resolved Raman spectroscopy of α-Ga_2O_3 grown on m-plane sapphire

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Gallium oxide has received great research interest as a competitive material for power electronics and solar blind UV photodetectors due to its wide bandgap [1]. Among the different polymorphs of Ga_2O_3, the alpha phase possesses the largest band gap at room temperature (~5.3 eV) [1]. In this work we study the Raman tensor elements of alpha-Ga_2O_3. Heteroepitaxial alpha-Ga_2O_3 films with a thickness of up to 1.4 µm were grown on m-plane Al_2O_3. The edges of the films were prepared by ion mill polishing to obtain optically smooth cross-sectional surfaces. We report polarization angle resolved Raman measurements on the a-, c- and m-planes of alpha-Ga_2O_3 with high angular resolution. Joint data modeling of the different planes in parallel and crossed polarization enables the quantification of the Raman tensor elements of all Raman active modes. Deviations from expected scattering intensities are discussed in terms of shear strain and lattice tilt due to lattice mismatch in the heteroepitaxial growth [2, 3].

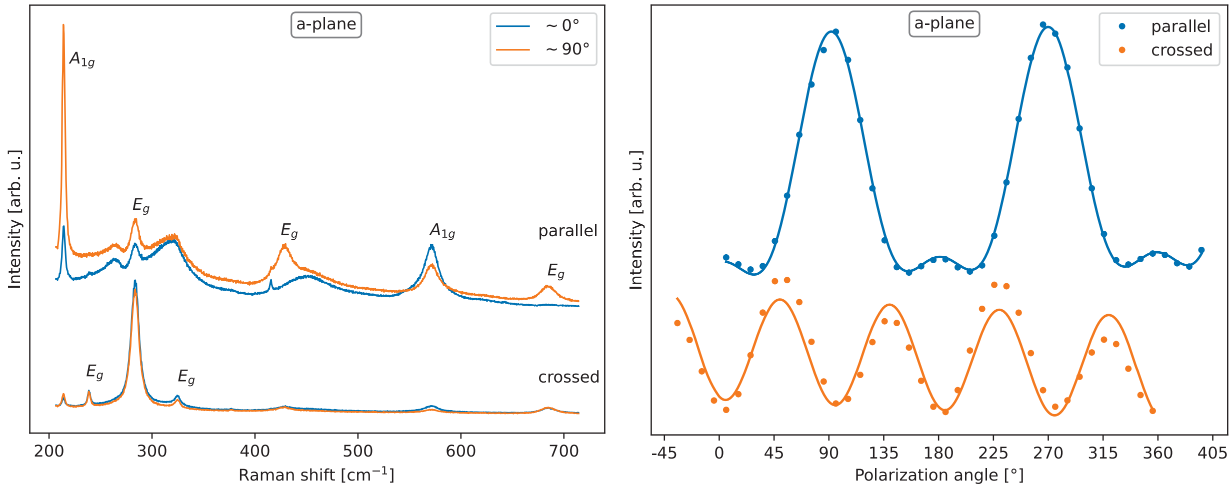

Figure 1: Left: Polarization-angle resolved Raman spectra of a-plane alpha-Ga_2O_3 at approximately 0° and 90° polarization-angle. Upper spectra measured with parallel polarization and the lower spectra with crossed polarization. Visible are the seven alpha-Ga_2O_3 modes and additional features. Right: The corresponding intensities of the A_{1g} mode at 218cm^{-1} for the polarization-angle measurements on the a-plane shown left.

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Magnetic Field Dependent Raman Scattering in 2D Materials and Thin Films

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Raman scattering is not usually considered to be magnetic field dependent. However, a few publications have become available presenting this observation in 2D materials. Ji et al. [1] observe a decline of the A_{1g}-peak's intensity in MoS_2 with external fields up to 9 T. In contrast, Wan et al. [2] observe a modulation of the peak's intensity, due to a rotation of the light's polarization. We will present our recent polarization resolved and magnetic field dependent Raman experiments on a variety of materials, including TMDCs like WSe_2, conventional semiconductors such as silicon, and thin films like amorphous tellurium. For some of these we also study the heterostructures with $Fe_{3-5}GeTe_2$, which exhibits perpendicular magnetic anisotropy (PMA) above 350 K [3].
A proper understanding of this effect would give access to a facile way to probe the local (effective) field at the interface of magnetic-nonmagnetic hybrid heterostructures.

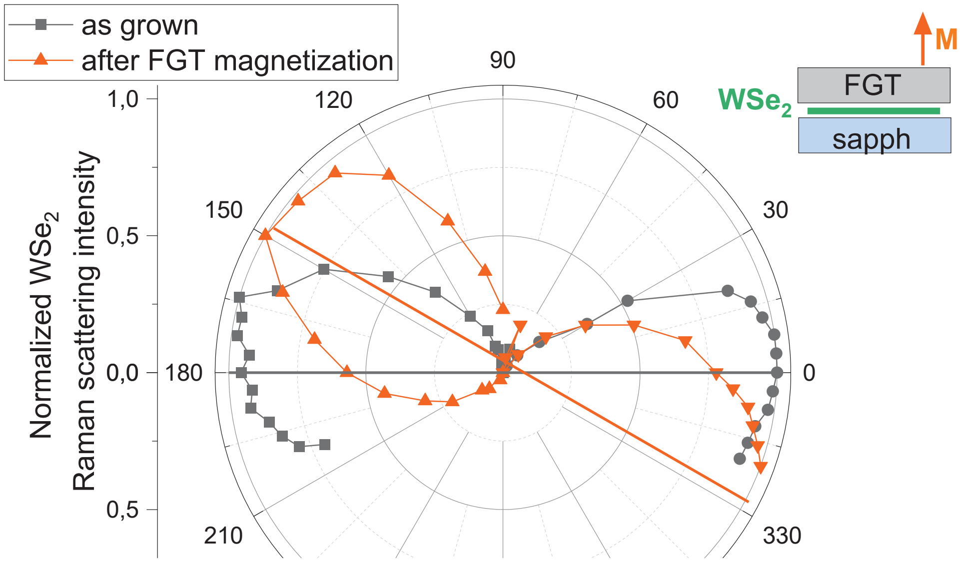

Figure 1: Polarization angle resolved A_{1g}-peak amplitudes in a FGT/WSe_2 heterostructure after excitation with fixed linear polarization. Two measurements are shown: the heterostructure with the as-grown FGT, without PMA (black) and after magnetization by an external field (orange). Both measurements are made under the same conditions.

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Dynamics of Fractionalized Spins in Quasi 2D Magnetic $V_{0.85}PS_3$

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Abstract

Transition metal phosphorous tri-chalcogenides (XPS_3, X = V, Mn, Fe, Ni) with strong in-plane covalent bonds and weak van der Waals gap between the layers of magnetic atoms have appeared as an intriguing candidate for exploring the quasi-2D magnetism [1]. Interestingly these materials show emergence of quite different magnetic ground state on varying transition atoms such as Ising-type (T_N ~ 123 K, FePS3), *XY*/Heisenberg-type ($NiPS_3$ /$MnPS_3$, T_N = 155 K / 78 K) [2]. One of the particular ground states conjectured in these systems is the quantum spin liquid (QSL) state.

QSL, a state characterized by exotic low energy fractionalized excitations and statistics is still elusive experimentally and may be gauged via indirect experimental signatures. Remnant of QSL phase may reflect in the spin dynamics as well as quanta of lattice vibrations, i.e., phonons, via the strong coupling of phonons with the underlying fractionalized excitations. We will be presenting our in depth Raman spectroscopic studies on single crystals of $V_{1-x}PS_3$, uncovering the spin fractionalization into Majorana fermions deep into the paramagnetic phase gauged via the emergence of a low frequency quasi-elastic response along with a broad magnetic continuum marked by a crossover temperature T^* ~ 200 K from a pure paramagnetic state to fractionalized spins regime. Also the anomalies in the phonons self-energy parameters in particular phonon line broadening and line asymmetry evolution at this crossover temperature is also observed, attributed to the decaying of phonons into itinerant Majorana fermions [3].

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Quantitative Studies on the Microstructures of Ternary CaO-Al_2O_3-SiO_2 Glasses, Melts and the Correlation with Viscosities

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Microstructure of CaO-SiO_2-based glassy samples with various Al_2O_3 contents were examined quantitatively by Raman spectroscopy and ^{27}Al MAS NMR. Sequence of multiple cluster models of aluminosilicate system modified with Ca^{2+} and Na^+ cations have been designed, and Raman spectra simulation were carried out after geometric optimization by quantum chemistry (QC) *ab initio* calculation [1].

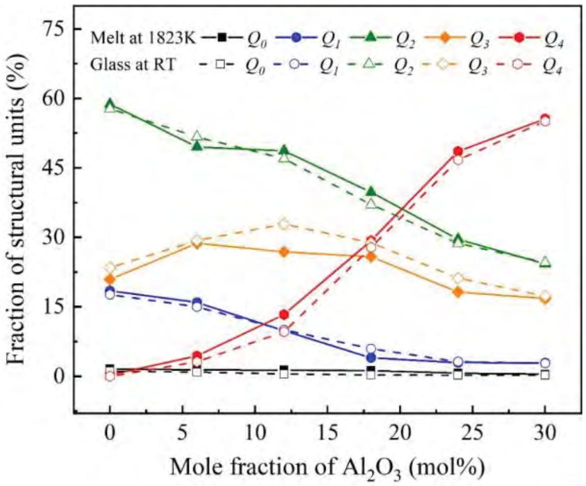

Figure 1

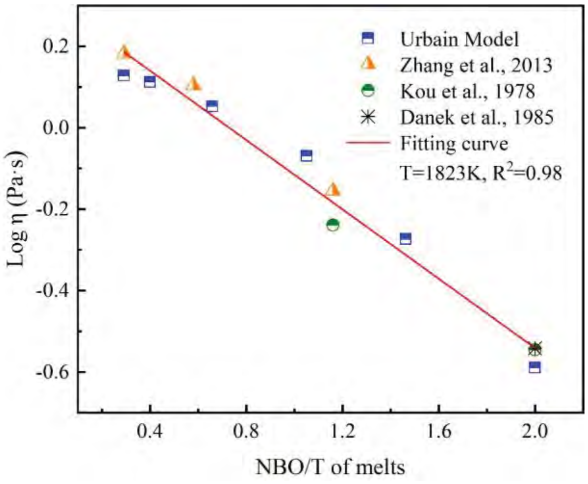

Figure 2

Figure 1: The quantitative distribution of primary Q_i (i=0-4) species with the increasing Al_2O_3 content

Figure 2: The quantitative linear relationship between the logarithm of viscosities and NBO/T of melts. The blue icon presents viscosity values obtained by Urbain model at 1823 K and red line is the corresponding fitting curve with the function of $\log\eta(Pa\cdot s) = -0.43(NBO/T) + 0.31$

The evolution of microstructure and its correlation with the viscosity of CaO-SiO_2 based melts, incorporating various Al_2O_3 additives, have been investigated by employing *in situ* high temperature Raman spectroscopy at 1823 K and viscosity model [2]. Furthermore, a quantitative analysis of the relationship between viscosity and structure was conducted based on the average number of non-bridging oxygen per network-forming tetrahedron (NBO/T). It provides valuable insights for examining and predicting viscosity behaviour of aluminosilicate systems.

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Investigating YAG-Pits in Intraocular Lenses using Raman microscopy supplemented by micro-CT and SEM

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Posterior capsule opacification (PCO) is the most frequent sequela of cataract surgery, which consists of removing the lens of the eye and replacing it with an artificial Intraocular lens (IOL). A widely accepted treatment of PCO is Nd:YAG laser capsulotomy (removing the opacification with a laser). The procedure is safe and effective, but iatrogenic damage of the IOL due to an improper laser focus is possible. These permanent damages to the IOL, so-called YAG-pits, might affect the optical quality of the IOL and are the subject of this study. Using Raman microscopy the degree, type of chemical damage and size of YAG-pits (created under controlled conditions) are assessed. Notably, a larger "spectral" damage area (meaning a material change seen by Raman) then "visual" damage area is commonly observed. To supplement the Raman measurement, both SEM and micro-CT are used. Where SEM shows the YAG-pits with high resolution on the surface, whereas micro-CT measures the volume of the YAG-pits. This way several damage metrics are generated and asses across IOL-types and YAG-parameters.

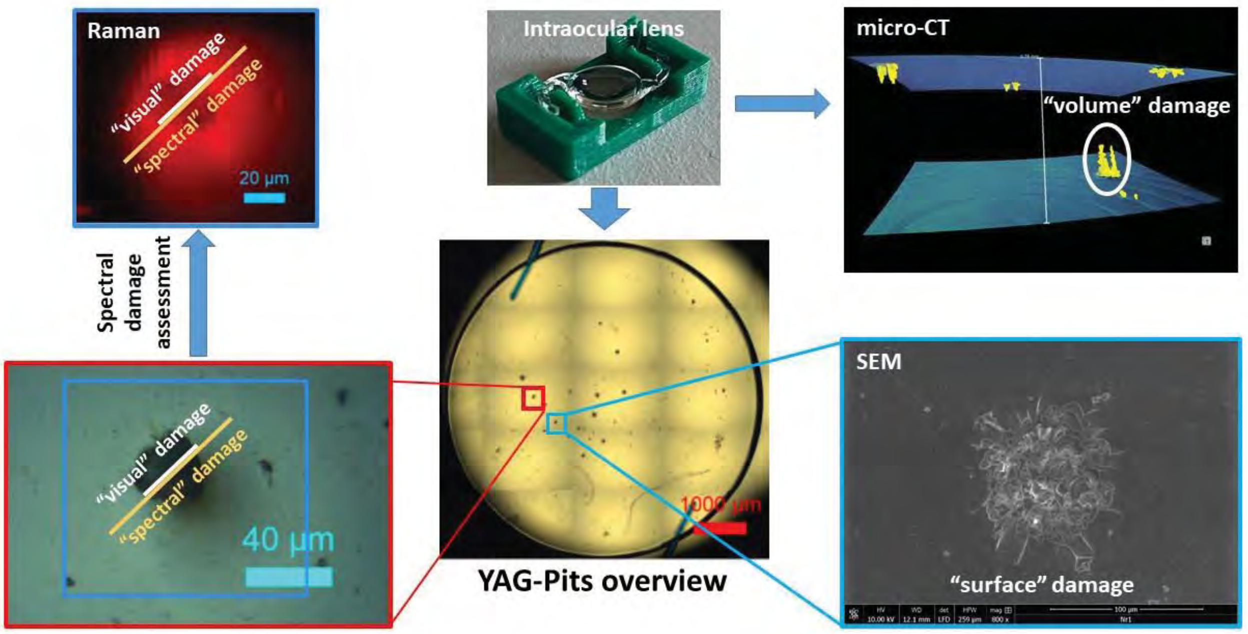

Figure 1: Summary of our approach to combine Raman microscopy with SEM and micro-CT, in order to create a variety of damage metrics for the YAG-pits.

Structure and thermodynamic stability of the aqueous diborate ion up to hydrothermal conditions: A Raman spectroscopic and Ab-Initio investigation

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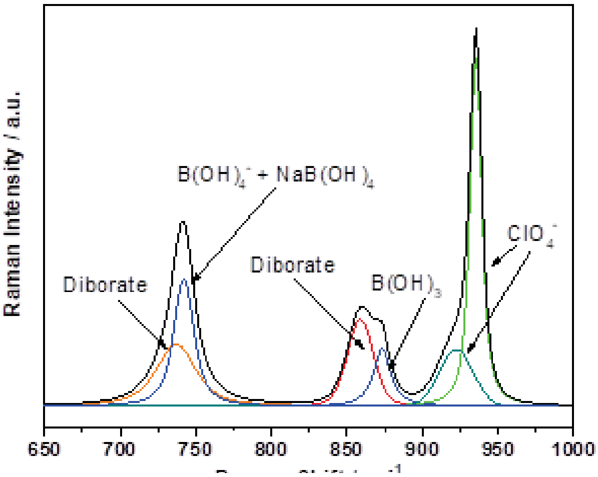
Accurate boron speciation data in high temperature water are needed to model the primary coolant chemistry of pressurized water reactors under boron “hideout” conditions. We report first spectroscopic evidence for the presence of a hydrothermally stable diborate ion from our Raman spectroscopy study combined with computational predictions.

A novel titanium sapphire flow cell[1] was used obtain the reduced isotropic Raman spectra of aqueous sodium borate solution with and with excess NaOH or NaCl/LiCl from 25 to 300 °C at 20 MPa by measuring the parallel and perpendicularly polarized Raman signal. The spectra showed the presence of borate, boric acid and an unknown peak which doesnot correspond to any previously reported polyborates. The peak intensity of the unknown species increased with temperature. Based on the classic high-temperature potentiometric titration study by Mesmer et al[2]. the new band was postulated to arise from a diborate ion, $[B_2(OH)_7]^-$ or $[B_2O(OH)_5]^-$. Ab initio density functional theory (DFT), together with chemical modelling studies, suggest that the most likely diborate species is $[B_2(OH)_7]^-$. The inconsistency in the calculated formation constant with temperature and composition suggested that there could be another diborate species present, mostly likely the $[B_2O_2(OH)_4]^{2-}$ ion pair.



A representative peak fitting and peak assignments for the Raman spectrum of 1 molal $Na(BOH)_4$ (aq), at 250 °C and 20 MPa

This work contributes to understanding of polyborate speciation under hydrothermal conditions. The results are being used to develop advanced models for predicting conditions that cause borate species to concentrate under oxide deposits on the fuel elements of pressurized water nuclear reactors (PWRs), in order to avoid power losses associated with boron “hideout”

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Determination of cinnamon adulteration by Fourier Transform (FT) Raman spectroscopy combined with chemometrics

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Food adulteration in the herbs/spices sector has become a huge problem [1]. Cinnamon is one of the oldest spices and its essential oil is used in the food, cosmetic, and pharmaceutical industries [2]. It has been demonstrated that some cinnamon essential oil components are antibacterial and anti-inflammatory, which has triggered a lot of interest in using cinnamon essential oil in food flavouring, food preservation and complementary medicine [2]. The most common types of cinnamon are Cinnamomum verum (Ceylon) and Cinnamomum cassia, the former being considered as the ‘’true cinnamon’’ due to its higher cost and sweeter taste compared to the latter type and thus cassia is often substituted for ‘’true cinnamon’’ [3]. Moreover, in contrast to Ceylon, cassia cinnamon contains significant amounts of coumarin, a substance that exhibits toxic effects to humans and hence poses serious concerns for public health [3]. Considering these issues and the inherent complexity of the food production chain, the development of fast and efficient screening tools to ensure proper quality control and cinnamon authentication is of great importance [4].

Although the most commonly adopted analytical methods to investigate cinnamon adulteration are high-performance liquid chromatography (HPLC) and gas chromatography (GC), they require complex sample preparation and experimental procedure [5,6]. On the other hand, vibrational spectroscopy has been successfully applied for rapid screening of Ceylon cinnamon substitution by cassia due to its non-destructive nature. Studies are either performed on the extracted cinnamon essential oil or directly on the cinnamon powder using fourier transform infrared spectroscopy (FTIR) [6,7] or by surface-enhanced Raman spectroscopy (SERS) [2]. However, the extraction of essential oils and the use of SERS substrates require some additional sample preparation steps that limit the inherent advantages of spectroscopy.

This work reports for the first time a systematic FT-Raman study on a large number of commercial cinnamon powder samples purchased on-line and in shops to detect the substitution of Ceylon cinnamon by cassia. All Raman measurements were performed on the raw material without additional preparation needed. The Raman data were analysed by principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) and a chemometric model for the discrimination between the two cinnamon species was constructed. As a step forward, the transfer of this newly developed method to the handheld Raman instrumentation can facilitate official control of samples.

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Multiplex pathogenic bacteria detection in milk with the nanoparticle-assisted porous silicon-based SERS microarray biosensor

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The ongoing threat of milk-related pathogens poses a substantial risk to human health, necessitating the development of efficient detection methods. This study explores the creation of a SERS based multiplex biosensor to meet the demand for simultaneous detection of multiple pathogens, thereby reducing measurement time and enhancing food safety. The biosensor utilizes an indirect immunoassay, leveraging gold nanoparticles as selective Raman reporters and a silver nanoparticle-modified porous silicon microarray (Ag-pSi) as the SERS substrate. In the initial phase, experiments focused on optimizing physical parameters and conditions, leading to the successful construction of an assay capable of detecting bacterial cells, specifically *E. coli*, *S. aureus*, and *B. cereus*, within the concentration range of log1 to log5 CFU/mL, with detection limits of 6, 6, and 5 CFU/mL, respectively. The assay's selectivity was validated through experiments involving common interfering pathogens. Subsequently, the developed assay was applied to detect specified bacterial cells spiked in various milk qualities and presented recovery values ranging from 85% to 105%. Notably, the overall assay timing was maintained under 90 minutes. These findings underscore the efficacy of the multiplex detection approach for bacterial pathogens in diverse samples, representing a significant advancement toward the realization of a portable SERS biosensor.

Diagnosing arsenic-induced biochemical responses in rice using Raman spectroscopy

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Rice (Oryza sativa) is the primary crop for nearly half of the world's population. Groundwater in many rice-growing parts of the world often has elevated levels of arsenite and arsenate. At the same time, rice can accumulate up to 20 times more arsenic compared to other staple crops [1]. This places an enormous amount of people at risk of chronic arsenic poisoning. In this study, we investigated whether Raman spectroscopy (RS) could be used to diagnose arsenic toxicity in rice based on biochemical changes that were induced by arsenic accumulation. We modelled arsenite and arsenate stress in four different rice cultivars grown in hydroponics over a nine-day window. Our results demonstrate that Raman spectra acquired from rice leaves, coupled with partial least squares-discriminant analysis, enabled accurate detection and identification of arsenic stress with approximately 89% accuracy. We also performed high-performance liquid chromatography (HPLC)-analysis of rice leaves to identify the key molecular analytes sensed by RS in confirming arsenic poisoning. We found that RS primarily detected a decrease in the concentration of lutein and an increase in the concentration of vanillic and ferulic acids due to the accumulation of arsenite and arsenate in rice. This showed that these molecules are detectable indicators of biochemical response to arsenic accumulation. Finally, a cross-correlation of RS with HPLC and ICP-MS demonstrated RS's potential for a label-free, non-invasive, and non-destructive quantification of arsenic accumulation in rice [Fig. 1].

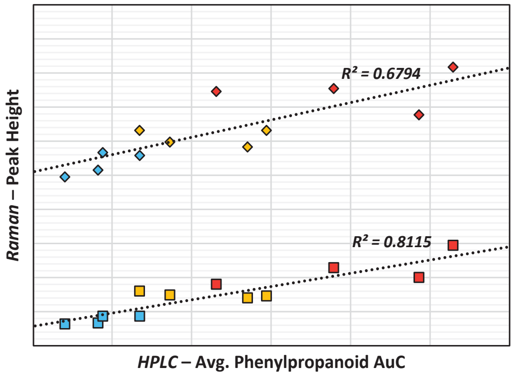

Figure 1: Scatter plot comparing increases in two Raman peaks (1604 cm^{-1} – diamonds; 1632 cm^{-1} – squares) with increases in phenylpropanoid content.

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Raman Spectroscopy Sensor for Gas Detection: Implications for Planetary Exploration and Industry

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Raman spectroscopy is a powerful technique for characterising the composition and structure of mineral targets. Besides solid and liquid targets, its high specificity makes its application to gas analysis a compelling use case. In the context of planetary exploration, Raman spectroscopy could be used to measure gas composition and detect minor components in atmospheric samples, including methane and other hydrocarbons. Moreover, major atmospheric constituents such as nitrogen and oxygen are diatomic symmetric molecules, which are non-absorptive and therefore cannot be observed by IR spectroscopy. Raman Spectroscopy can therefore play a vital role in unravelling the chemical compositions of atmospheres in the context of *in situ* planetary exploration.

Additionally, this technology could also offer opportunities for industrial processes monitorization, being particularly interesting the case of hydrogen due to its potential as a fuel source in zero-emission mobility solutions.

It is well known that the lower density of gases compared to mineral or liquid solutions results in a weaker Raman response, making it challenging to apply the technique for gas detection applications. In this study, we evaluate a sensor concept which improves the efficiency of the Raman detection of gases. This sensor increases the interrogated volume of gas by confining the light and gas inside hollow-core fibers, facilitating multiple interactions with the analyte. This greatly improves the limits of detection of gases compared to standard Raman spectroscopy setups. This technology will allow quick and quantitative measurements of gas (see Figure 1), facilitating the detection of a variety of gas mixtures in a wide range of temperature, pressure, and humidity environments, with potential applications for in situ planetary exploration missions or other Earth applications.

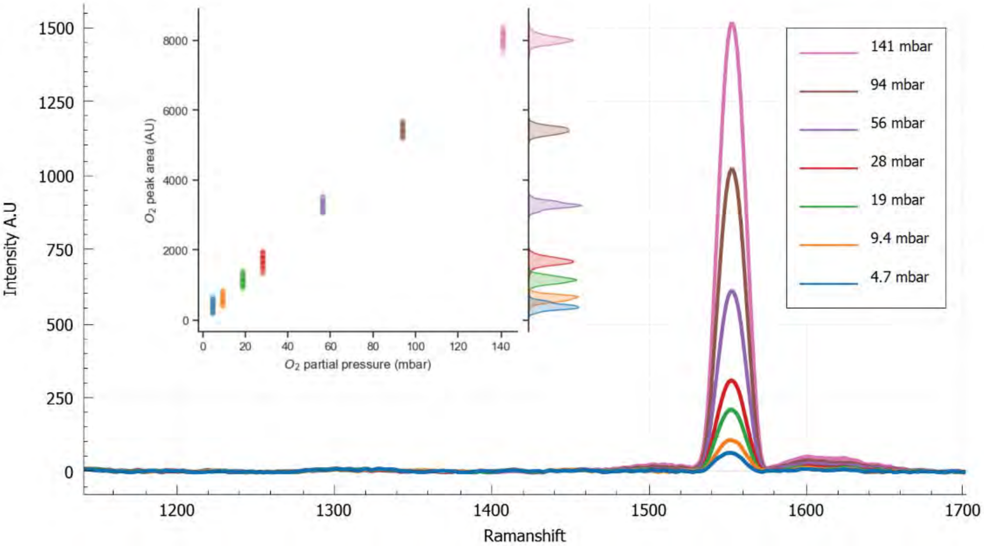

Figure 1: Comparation of the oxygen Raman peak of a N_2-O_2 mixture at different partial pressures. The measured area shows a clear correlation with the partial pressure of oxygen inside the chamber.

Decoding SERS performance via a comparative analysis of five commercial SERS substrates

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Surface-enhanced Raman spectroscopy (SERS) plays a pivotal role as an optical sensing technique in a variety of fields including medicine, microbiology, and environmental analysis [1]. Notably, planar SERS substrates are preferred for their seamless integration into lab-on-chip systems and superior reproducibility compared to colloidal SERS. The evaluation of these substrates involves metrics like enhancement factor, sensitivity, and reproducibility, with various experimental and post-processing factors influencing their outcomes. One crucial aspect is the illumination area which translates to the number of hotspots generating the signal. In this study, Raman mapping was employed on five commercially available SERS substrates to examine the impact of the illumination area on the SERS performance and signal reproducibility [2]. The results indicate that a larger illumination area contributes to a more stable and reproducible signal, especially in the case of irregular nanostructures. This heightened signal stability corresponds to increased sensitivity. Consequently, adjusting the illumination area allows for achieving a more reproducible signal on a SERS substrate with a random structure, albeit at the cost of resolution. Ultimately, when selecting a SERS substrate for a specific application, the trade-off between a more uniform structure offering superior resolution and a larger illumination area enhancing signal reproducibility should be carefully considered.

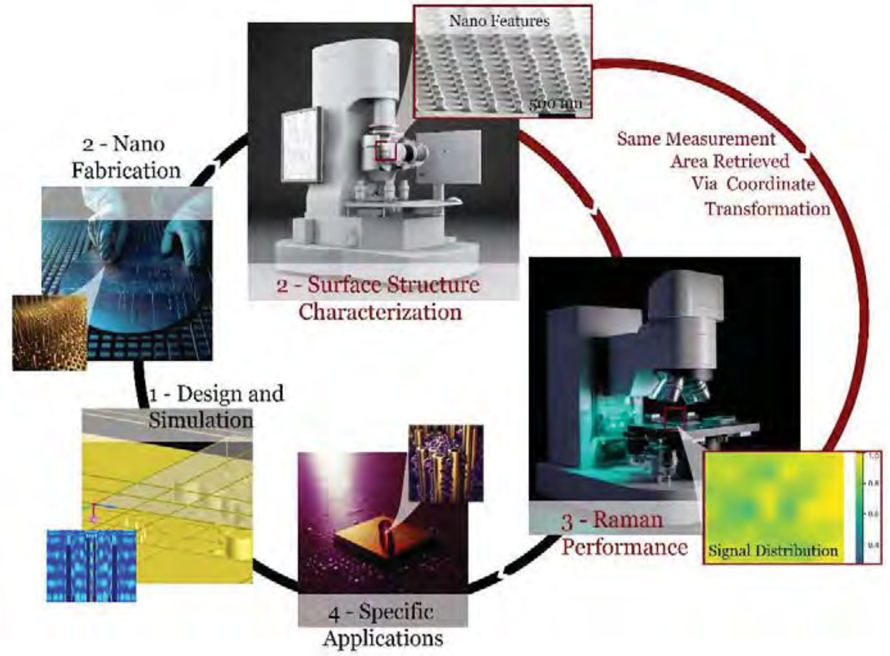

Figure 1: The cycle of SERS design, fabrication, and testing: this study, shown in dark red, allows testing the suitability of a SERS substrate for specific applications, guiding further adaptations or fine-tuning.

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Improvements in SRS light sources: 10x faster imaging and 100x faster tuning

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We have successfully developed an advanced light source tailored for Coherent Raman microscopy and other nonlinear microscopy modalities, including SHG and multiphoton fluorescence. In comparison to the prevailing SRS light source available in the market (APE's picoEmerald S , our innovative solution offers several notable enhancements.

One prominent advantage of the new light source lies in its significantly accelerated tuning speed. We have achieved a 100-fold increase for random wavelength access, including laser output power control and dispersion compensation. Switching to different Raman bands now takes about 1 second. As is state of the art, the light source setup includes both spatial and temporal overlap of Stokes and Pump laser beams to provide the application with a single laser beam. The temporal overlap can automatically adjust to the dispersion properties of the external optical setup (microscope).

To speed up image acquisition and improve signal, we have changed the repetition rate from 80 MHz to 40 MHz, resulting in doubled pulse energy. This in combination with a subharmonic modulation frequency of 20 MHz leads to a significant reduction of the pixel dwell time. The theoretical improvement is a factor of 8 all while maintaining the same Signal-to-noise ratio within the image. The laser is based on proven solid state OPO technology and thus intrinsically shot noise limited from 5 MHz onwards. The output pulse lengths and bandwidths of 2 ps and about 10 cm^{-1} respectively combine optimum spectral resolution with high sensitivity.

The OPO's redesigned optical cavity also allows for a wider wavelength coverage. The Signal beam is now tunable from 660 nm to 1020 nm (up from previously 700 nm to 990 nm) which corresponds to an Idler tuning range of 1040 nm to 2340 nm (up from 1080 nm to 1950 nm). To enhance SHG and TPF efficiencies even further, the new light source now allows to selectively increase the output bandwidth, which offers the opportunity to compress the pulse duration down to a few 100 fs.

We directly compare the image quality of the two light sources side by side coupled into the same microscope and will present CH- and fingerprint images of relevant biological samples.

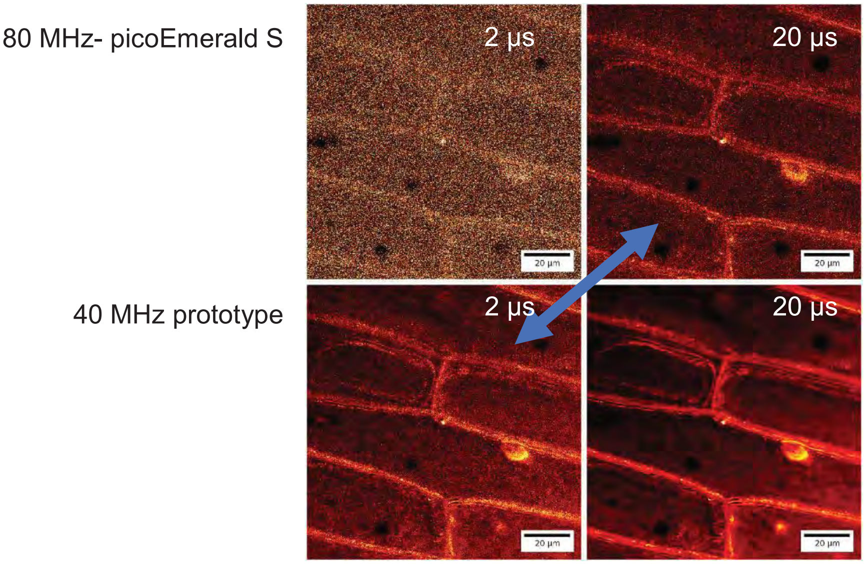

Figure 1: comparative SRS-images of onion cells @ 880 cm^{-1} with 2µs and 20µs pixel dwell time respectively with the 80MHz picoEmerald S and the new 40 MHz prototype (20 mW Pump – 946 nm / 9 mW Stokes – 1032.0 nm)

Raman Spectroscopic Probes of Photonic Materials for Solar Energy Conversion

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Photonic materials, including plasmons and polaritons, are highly promising catalysts for driving energetically unfavorable chemical reactions with sunlight, due to their large optical cross sections and ability to modify potential energy landscapes. However, the efficiencies of most plasmon-driven and polariton-driven processes are quite low, likely due to the lack of mechanistic understanding of the underlying physical processes. Here I'll discuss our use of Raman spectroscopies to advance our fundamental understanding of these systems. First, I'll describe our development of ultrafast surface-enhanced Raman spectroscopy (SERS) to probe the contributions of plasmon-generated hot electron transfer, heating, and vibrational energy transfer on timescales relevant to photocatalysis. [1-4] Second, I will talk about our efforts in mapping out reaction coordinates in polaritonic systems, quantifying the degree of mode-specific activation. These efforts in developing a fundamental understanding of polariton and plasmon-mediated processes in molecules will ultimately aid in the rational design of cost-effective photonic materials capable of driving industrially relevant chemistries using solar radiation.

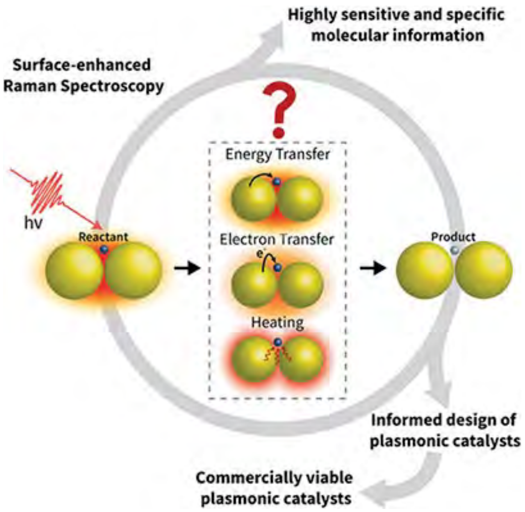

Figure 1: Raman probes of fundamental mechanisms in plasmon-driven photocatalysis [1]

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Plasmonics for Photochemistry of Ions and Molecules

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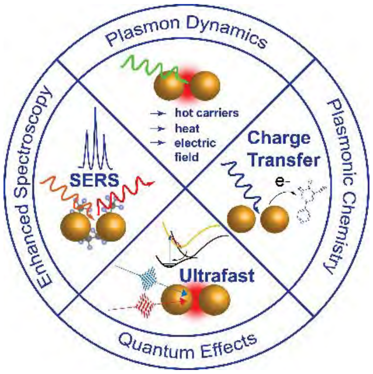
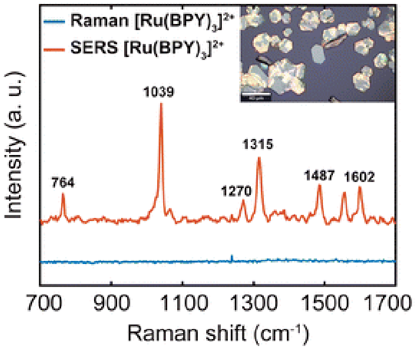
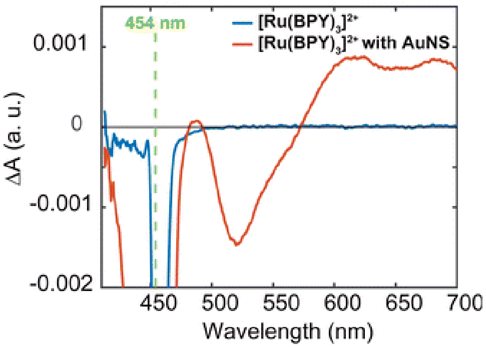
Plasmons hold great promise for photon conversion and coherent energy transport at the nanoscale. Following visible light excitation of collective conduction electrons near the surface of nanostructures, hot charge carriers are created via non-radiative relaxation pathways that are then harnessed to drive localized molecular transformations of individual molecules. Limited knowledge of the precise mechanisms underlying plasmon-mediated dynamics in nanogaps currently restricts widespread technological applications, which in turn has generated substantial fundamental research interest in surface molecule dynamics. This talk demonstrates the direct observation of the molecular anion radical probed using continuous wave single nanoparticle pump-probe surface-enhanced Raman spectroscopy (SERS) within optically confined near-field plasmonic substrates.[1] Corroboration of the anion radical species formed within the solid-state is provided by open-shell density functional theory (DFT), resonance Raman, and electron paramagnetic resonance spectroscopy (EPR). Wavelength tunability of plasmon-driven electron transfer as well as the spectrally resolved polarization response of individual nanoparticles is correlated with far-field scattering and high-resolution transmission electron microscopy (TEM).[2,3] Implications for speeding up/slowing down excited-state dynamics in high-field confinement are discussed using spectroscopic visualization of kinetic lifetimes in transient absorption spectroscopy (fs-TA).[4,5,6] Utilization of this new energetic pathway can be applied to basic energy research with the potential for profound impact on next generation light harvesting nanoscale devices.







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Plasmon-Molecule Coupling in Vibrational Circular Dichroism Spectroscopy

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Vibrational circular dichroism (VCD) is the extension of CD into the infrared spectral region. VCD is used to assign absolute configuration and conformational population distribution of enantiomers. One challenge with applying this spectroscopy method is that VCD signals are 4 to 5 orders of magnitude lower than the parent infrared absorption signals [1]. As a result, high concentrations of molecules and long acquisition times are required for VCD analysis. We are exploring plasmonic nanostructures with tunable chiroptical activity for plasmon-enhanced VCD detection of chiral molecules. Nanosphere template lithography (NTL) provides an approach to explore the dependence of chiroptical behavior on the structural properties. Using copper mask NTL, both gold (Au) and aluminum (Al) structures with different symmetric and asymmetric features were produced with optical responses tunable across a broad spectral range from the ultraviolet (UV) to the infrared (IR) [2, 3]. Understanding the influence of plasmons on VCD spectral features of molecules is important for assessing the information content of the VCD spectra. By tailoring the chiroptical behavior of the plasmonic nanostructures, the origin of VCD spectral features have been studied. For achiral molecules, VCD spectral signatures indicate the influence of the chiroptical activity of the plasmonic nanostructures. For chiral molecules, strong VCD signal enhancements are observed, demonstrating the potential for plasmon-enhanced VCD platform development.

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Raman Snapshots of Ultrafast C-H Bond Activation in Water

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Triggering C-H bond activation reactions inside enzyme-like nanocavities will enable development of green methods to carry out organic transformations in water. Recently it was shown that host-guest charge transfer interactions inside cationic nanocages can be used to polarize bonds for photo-induced C-H bond cleavage in water via an ultrafast proton-coupled electron transfer (PCET) step [1,2,3]. Here we use structure-sensitive femtosecond stimulated Raman spectroscopy to resolve the individual electron and proton transfer reactions at the C-site for the first time, and demonstrate a key role of the pre-organized water-cluster during the C-H bond photoactivation. Resonance-selective Raman snapshots of the photogenerated methyl-substituted bithiophene substrate radical cation reveal rich vibrational dynamics of the modes during bithiophene planarization leading up to proton transfer to the solvent water molecules in ~30 picoseconds [4]. Our work establishes the significance of pre-organization of proton acceptor after initial charge transfer to the supramolecular host, and therefore opens up dynamics-based guidelines for developing new methods for carrying out radical based reactions inside water-soluble nanocavities.

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Absolute excited-state molecular geometries revealed by resonance Raman signals

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Ultrafast photoreactions are governed by multidimensional excited state potential energy surfaces (PESs), which describe how the molecular potential varies with the nuclear coordinates. Nature has tailored electronically excited PESs, in which the molecular geometry is specifically modified from the ground state (GS) equilibrium configuration to efficiently convert the absorbed light energy into nuclear rearrangements, driving the system photochemistry and determining the biological functions by bond length modifications, torsional re-orientations, formation or rupture of chemical bonds. This can be rationalized by the displacements between PESs and, critically, their sign determines if these changes move closer or away two functional groups, ruling ES properties and dynamics. Their knowledge is thus of utmost importance. Such displacements are encoded in the Franck-Condon overlap integrals, which in turn determine absorption spectra and complex-valued Raman excitation profiles (REP). Conventional spectroscopic approaches probe transition amplitudes [1], only accessing the REP's absolute value, and hence are not effective for determining the sign of ES displacements. Herein we introduce an experimental technique [2], based on the detection of the broadband impulsive Raman response at selected temporal delays and probe chirps, to directly measure complex REPs along desired normal modes. The key to achieve this task is in the linear dependence of the signal on the Frank-Condon overlaps, brought about by resonant probe and off-resonant pump pulses, which critically enables time domain sensitivity to the phase of the stimulated vibrational coherences. Our results, experimentally benchmarked on Rhodamine B [2], provide the tool to unambiguously determine the sign of ES molecular displacements, revealing the first steps of photoreaction processes [2-3].

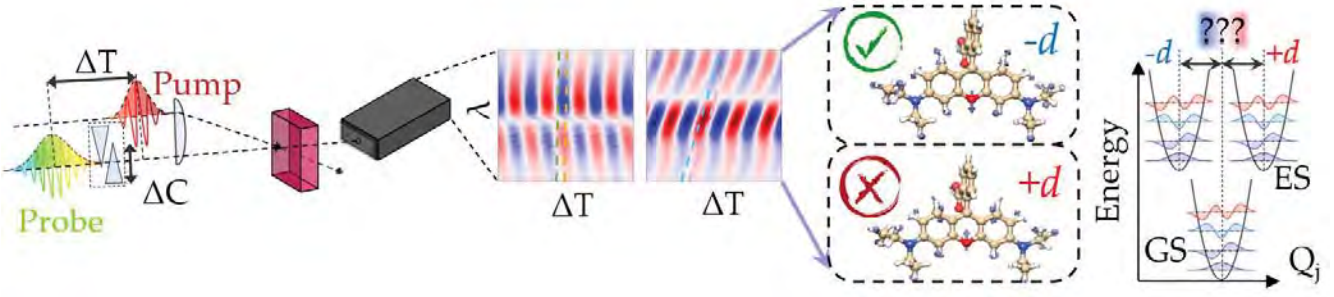

Figure 1: Sketch of the non-degenerate pump-probe experimental scheme which, by measuring impulsive Raman excitations, accesses the sign of Rhodamine B excited-state displacements along its normal modes.

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Stretching Long Lived Excited States Using Molecular Design, A Time Resolved Resonance Raman Study.

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Long-lived excited state behaviour is an important attribute for many photophysical applications, such as photovoltaic cells, molecular sensing, photodynamic therapy,[1] and photocatalytic reactions. As such, we have synthesised and characterised a series of Re and Ru complex dyes. We designed these with both metal and organic electron donor groups and bipyridine acceptor group(s). A previous study,[2] on a similar architecture has observed excited state switching between metal-ligand charge transfer (MLCT) and intra-ligand charge transfer (ILCT) states. We are investigating the tuning of both these transitions by using different substituents on both the bpy ligand, to tune the ILCT, and the metal to tune the MLCT. In order to understand the myriad of CT states in these structures resonance Raman spectroscopy has been used to evaluate the state populated on photoexcitation. Transient resonance Raman and absorption spectroscopies have been used to observe the relaxation from the initially formed state to the long-lived excited state. All of these are performed in conjunction with DFT and TD-DFT calculations to support the spectroscopic findings.

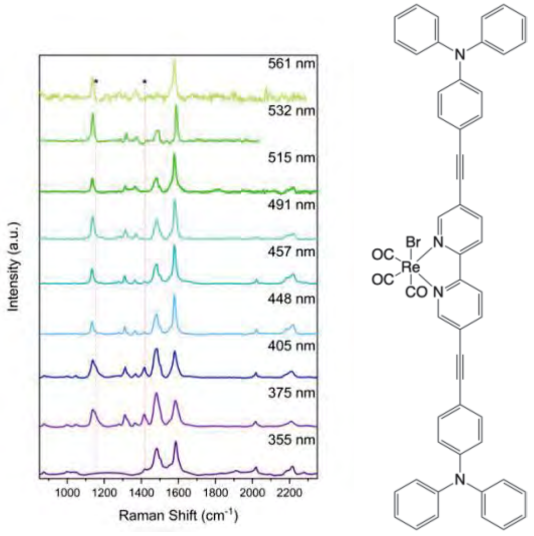

Figure 1: Example resonance Raman plot of a rhenium complex.

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Photoactivation mechanism of Orange Carotenoid protein resolved by fs to millisecond stimulated Raman spectroscopy

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Orange Carotenoid Protein (OCP) is a unique photoprotective, water-soluble protein predominantly found in cyanobacteria [1]. It plays a critical role in non-photochemical quenching (NPQ), a protective process that dissipates excess absorbed light energy as heat, safeguarding photosynthetic organisms from photodamage. The vibrational characteristics of optically excited echinenone in various solvents and the Orange Carotenoid Protein (OCP) in red and orange states were systematically investigated through steady-state and time-resolved spectroscopy techniques. Time-resolved experiments, employing both Transient Absorption (TA) and Femtosecond Stimulated Raman Spectroscopy (FSRS), reveal distinctive components corresponding to different states in the OCP photoactivation process. The time-resolved studies indicate more defined vibrational signatures during the initial 140 fs of carotenoid evolution in OCP, an absence of a clear vibrational signature for the relaxed S1 state of echinenone in OCP, and more robust signatures of a highly excited ground state (GS) in OCP. Differences in S1 state vibration population signatures between OCP and solvents are attributed to distinct conformations of echinenone in OCP and hydrogen bonds at the keto group forming a short-lived intramolecular charge transfer state. The vibrational dynamics of the hot-GS in OCP show a more pronounced upshift compared to echinenone in solvents, thus suggesting an unusually hot form of GS. The study proposes a hypothesis for the photoactivation mechanism of OCP, emphasizing the extraordinarily high level of excitation in longitudinal stretching modes as a driving force. The study underscores the importance of vibrational analysis in understanding the intricate processes involved in OCP photoactivation.

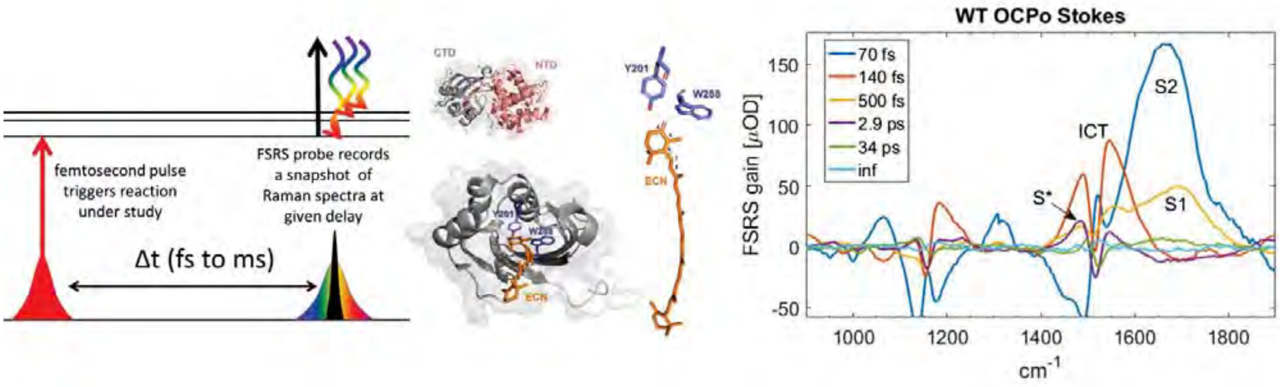

Figure 1: Stimulated Raman method, OCP protein and Raman signatures of its excited states.

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Label-free 3-D molecular imaging of living tissues using Raman Spectral Projection Tomography

1

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The ability to image tissues in three-dimensions (3-D) with label-free molecular contrast at mesoscale would be a valuable capability in biology and biomedicine. Here, we present Raman spectral projection tomography (RSPT) for volumetric molecular imaging with sub-millimeter spatial resolution.
We have developed a RSPT imaging instrument capable of providing 3-D molecular contrast in transparent and semi-transparent samples. Alongside the instrumentational development a computational pipeline for multivariate reconstruction was established to extract label-free spatial molecular information from Raman projection data.
We show imaging and visualization of phantoms of various complex shapes with label-free molecular contrast. Finally, we apply RSPT as a novel tool for imaging of molecular gradients and extracellular matrix heterogeneities in fixed and live tissue-engineered cartilage constructs and explanted native tissues. RSPT imaging opens new possibilities for label-free molecular monitoring of tissues.
These findings show that the imaging approach can serve as a platform for minimally invasive molecular analysis and a means to meticulously track molecular gradients within living tissues with high precision in both contrast and resolution[1].

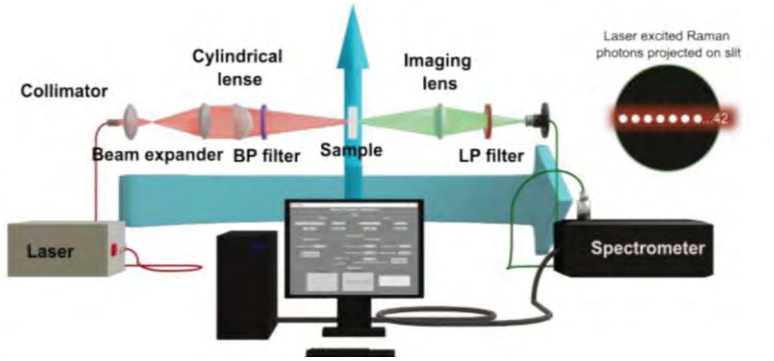

Figure 1: Schematic illustration of the excitation and detection configuration for RSPT: A 2W 785 nm laser is beam shaped into a line using a cylindrical lens. The Raman scattered light is collected using a lens and imaged onto a line array comprised of 43 fiber cores coupled to a spectrometer. The sample is positioned for rotation around a 360° angle and z-axis displacement.

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Cellular and tissue bioimaging by SERS spectroscopy using gold nanotags with cyanine dyes

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Development of new tools for bioimaging cells, tissues and organs is important to improve the quality and completeness of medical diagnostics and treatment. Application of molecular spectroscopy methods for bioimaging involves the use of tags such as molecular dyes or hybrid nanotags. Surface-enhanced Raman scattering (SERS) can be used with plasmonic nanotags to image targeted cells or tissue and track molecular processes in the area of interest [1]. Here, we present a comprehensive research of SERS tags based on anisotropic gold nanoparticles (NPs) modified with cyanine dyes, including their detailed study in suspensions, in-vitro testing on cells and detection in tissues after their native biodistribution reached in result of intravenous injection.

A wide range of core-shell nanotags has been obtained, differing in the morphology of the core, the cyanine used and the technique of its immobilization (non-specific adsorption or covalent conjugation). Preliminary SERS measurements in the solutions allowed us to optimize the tags composition, reaching a signal level sufficient for their detection in tissues.

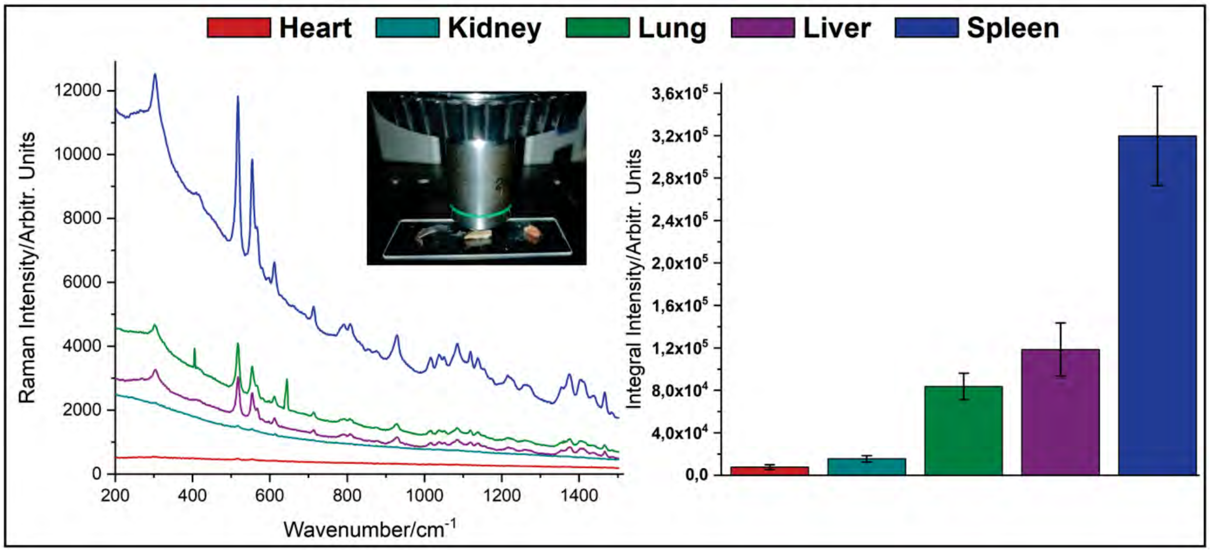

Figure 1: Raw SERS spectra of organs harvested from the animal injected with AuNPs@Cy7 tags (left); diagram of tags biodistribution obtained from the SERS spectra

In-vitro testing of the nanotags on cells showed that they undergo to endocytosis regardless of the core morphology and nature of coating polymer. The obtained SERS maps along with scanning electron microscopy images revealed the tendency of tags to localize in the cells cytoplasm and form small agglomerates which demonstrated "hot spots" effect.

This work was supported by Russian Science Foundation, grant № 22-73-10052. The authors would like to thank the Research Park of SPbU.

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Quantification of biomolecules in a liquid droplet formed by liquid-liquid phase separation using the Raman band of water

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[Introduction] We have quantified intracellular environments and biological molecules using the Raman band of water [1–5]. In this presentation, we show our recent Raman results on evaluating the chemical properties of liquid-liquid phase separation (LLPS) in solutions and living cells.

[LLPS] LLPS is a phenomenon in which a highly concentrated liquid state (droplet) of specific biomolecules is generated. LLPS has been used to explain various biological phenomena, including the pathogenesis of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). It has been proposed that fibrosis of causative proteins is involved in the pathogenesis of those diseases, and the fibrils are generated by the liquid-to-solid phase transition of the droplets formed via LLPS. We have proposed that Raman microscopy is useful for studying LLPS [3,4]. Especially we have demonstrated a label-free method to evaluate the protein concentration in a single liquid droplet using the Raman band of water.

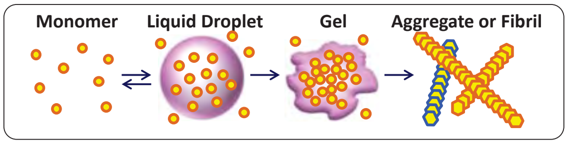

Fig. 1. The concepts of protein droplets, gels, and aggregates.

[LLPS of FUS LC] We applied the concentration quantification method to LLPS of the low-complexity (LC) domain of FUS, which is one of the ALS-associated proteins (Fig. 2). The Raman bands due to FUS LC were observed only inside the droplets and only water Raman bands were observed outside the droplets, confirming the condensation of FUS LC in the droplet. The protein concentration inside each droplet was quantified using the outside water Raman band as an intensity standard. This new quantitative method is based on the fact that the concentration of water molecules outside the droplets is constant regardless of the sample. We made a calibration line by plotting the intensity of the FUS LC Raman band normalized to the water Raman band as a function of the FUC LC concentration. Then, the protein concentration in the droplet was determined without labeling by comparing the calibration line and the protein Raman band inside the droplet normalized by the outside water band. Applying this method, we detected changes in the protein concentration with varying solution environments such as pH and salt concentration (Fig. 3). We found that FUS LC was highly concentrated to be 13–15 mM in the droplets, and the inside protein concentration decreased as the conditions become less favorable for LLPS. The obtained results can be explained in terms of the phase diagram.

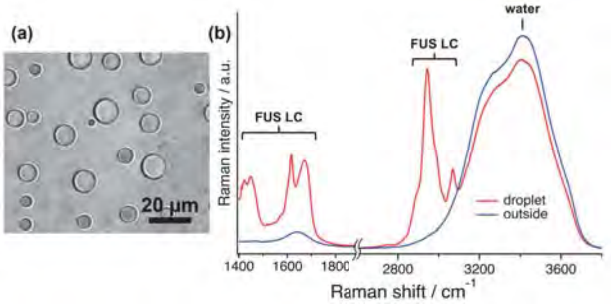

Fig. 2. (a) A bright field image of FUS LC droplets. (b) Raman spectra of inside and outside of the droplet.

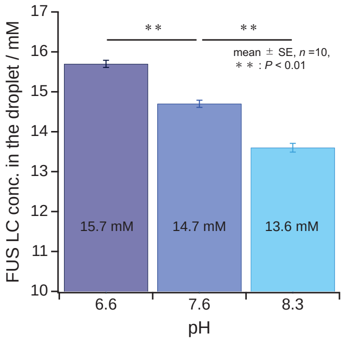

Fig. 3. Protein concentration in the droplet with varying pH.

[LLPS in a living cell] We applied the quantification method to droplets in living cells. Concentrations of proteins and other components of intracellular droplets were successfully identified. In particular, nucleic acids were found to be highly concentrated in several types of intracellular droplets. These results are difficult to obtain using other optical methods. The results of quantifying intracellular droplets will be presented in the presentation.

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Detecting biochemical variation in brain organoid maturation stages through Raman microscopy

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Brain organoids serve as valuable in vitro models for exploring brain development, understanding neurological disorders, and facilitating drug testing[1]. However, their full potential is hindered by several challenges, including resource-intensive production processes and limited standardization, leading to variations among organoids[2]. Addressing these challenges requires non-destructive techniques that enable dynamic studies and cost reduction.

To overcome these obstacles, our study proposes a label-free, non-invasive approach using spontaneous Raman Spectroscopy (RS). Employing a 785 nm laser source, we collect RS data from multiple cortical organoids at different developmental stages using a custom-made high-throughput multi-modal spontaneous Raman microscope. Following, preprocessing and normalization, we leverage machine learning tools such as Random Forest[3] classification to identify and extract essential features associated with each maturation stage. These features encompass variations in lipid, protein, and glycogen content, providing valuable insights into developmental processes.

In conclusion, our methodology allows for the distinction between different maturation stages of brain organoids without compromising their growth and integrity, providing a unique perspective to observe dynamic changes over time. Implementing Raman Spectroscopy as a non-invasive tool for brain organoid analysis holds tremendous promise by streamlining conventional methods and introducing a standardized approach for accurately discerning and monitoring various maturation stages. Additionally, it facilitates longitudinal studies, enabling a deeper understanding of brain organoid development and responses to external stimuli or drug testing.

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Macroscopic biomedical imaging of chemical contrast using short-wave infrared Raman scattering

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Current Raman scattering-based imaging applications are mostly limited to microscopy scales, hindering its application on a larger scale for example tissues, whole animals, or during medical procedures in humans. Wide-field Raman imaging could overcome these spatial constraints, yet tissue autofluorescence poses a major challenge to the endogenous chemical contrast. Here, we demonstrate the Raman scattering wide-field imaging in the shortwave infrared (SWIR, 950-1700 nm) range as a novel approach for macroscopic biomedical applications. This approach overcomes previous limitations and achieves high chemical contrast imaging, enabling novel biomedical applications. With fields of view surpassing 50 cm2, we showcase (Figure 1) the versatility of SWIR Raman imaging by monitoring body composition dynamics in living mice, non-invasively detecting liver lipid content in metabolically challenged mice, and identifying calcified areas and lipid-rich deposits in human atherosclerotic plaques [1]. Our work enables non-invasive, label-free, in vivo imaging of chemical contrast across micro- and macroscopic scales, opening up new directions for pre-clinical and clinical research.

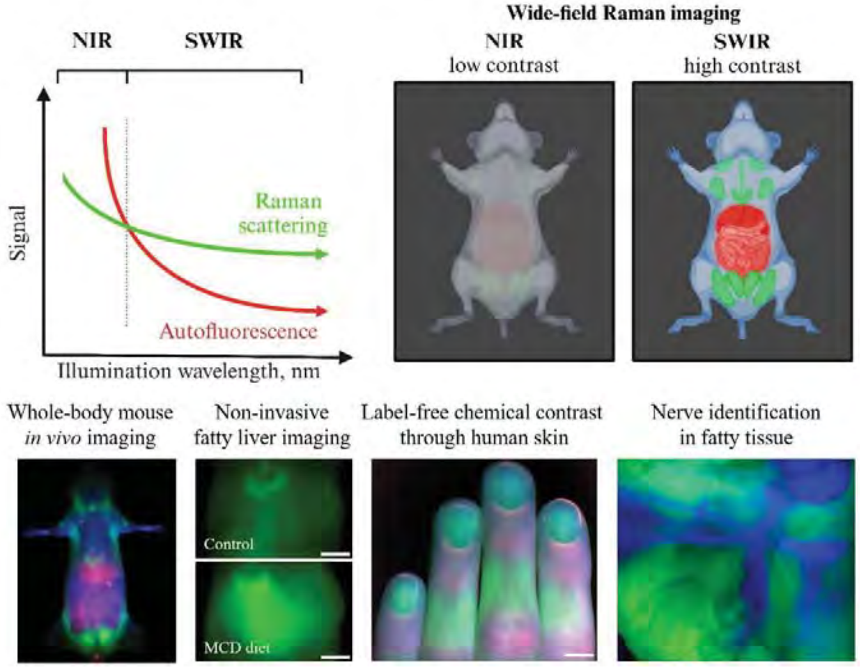

Figure 1: Demonstration of main advantages of wide field Raman imaging in SWIR spectral range and proof-of-concept biomedical applications – imaging of a whole mouse, a liver through skin, human fingers, a nerve.

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Terahertz Raman Coherence in Biomolecules

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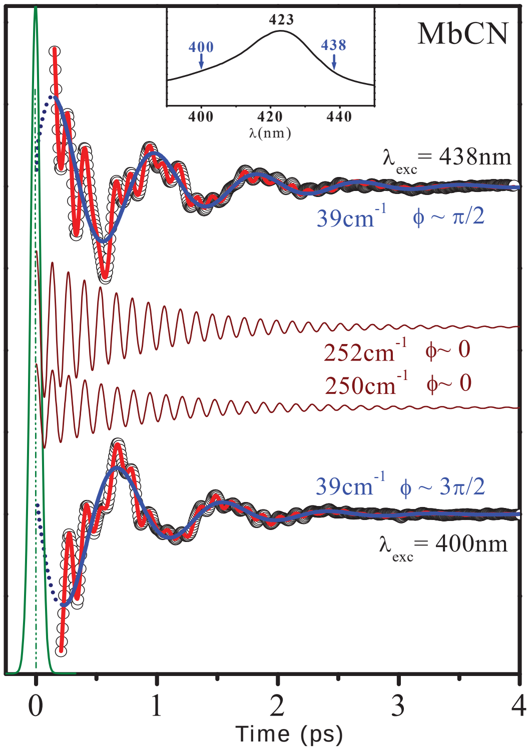
Biomolecules engage in a wide range of fundamental biophysical processes that underpin life. For example, heme proteins are involved in electron transport, catalysis, small molecule binding and transport, as well as molecular sensing, signalling and genetic regulation. Another type of biomolecule, the green fluorescent protein (GFP), has emerged as a cofactor-free fluorescent probe of gene expression and protein motility that is used in the study of cellular physiology. GFP has an optically driven reaction cycle that depends on deep proton tunneling along its “proton wire”, offering unique opportunities for studies of laser-triggered biological proton tunneling [1]. The role of protein vibrational dynamics, which facilitates all of these biophysical processes, will be discussed in the context of both time and frequency domain resonance Raman spectroscopy.

One of the most biologically relevant and important spectral regions is located between 1-10 THz (~33-330 cm^{-1}), which encompasses ambient temperature (~200 cm^{-1}). However, for samples in aqueous phase, standard infrared and Raman experiments are not able to successfully access frequencies below ~5 THz because of interference from the strong absorption and quasi-elastic light scattering of water. In contrast, stimulated Raman coherence spectroscopy has detected coherent vibrational responses in protein-chromophore systems down to ~1 THz [1-3]. It is noteworthy that these low frequency responses exhibit damped oscillatory behavior rather than overdamped diffusive decay as sometimes assumed [4]. Under resonant conditions, the coherence signals generally display the predicted amplitude and phase changes that depend on the laser pulse carrier frequency as well as on the photochemical processes that may be induced [5]. On the other hand, there are still observations that remain puzzling and are not understood. For example, the phase of the 1.2 THz response shown in the Figure.



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Coherent THz Hyper-Raman: Spectroscopy and Application in THz Detection

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In a series of experiments we have demonstrated a new nonlinear optical effect in the THz interval of frequencies. The latter is based on the use of femtosecond optical pulses and intense, sub-ps, broadband terahertz (THz) pulses to generate a THz-optical Four- and Five-Wave Mixing in the investigated material. The spectrum of the generated signal is resolved in time and wavelength and displays two pronounced frequency sidebands, Stokes and anti-Stokes, close to the optical Second Harmonic central frequency $2\omega_L$, where ω_L is the optical central frequency of the fundamental beam, thus resembling the spectrum of standard Hyper-Raman scattering, and hence we named this effect 'THz Hyper-Raman' – THYR (see figure below). We applied this technique to several crystalline materials, including α-quartz and gallium selenide. In the first material, we find that the THYR technique brings spectroscopic information on a large variety of low-energy excitations that include polaritons and phonons far from the Γ-point, which are difficult to study with standard optical techniques [1]. In the second example, we show that this new tool offers some advantages in detecting ultra-broadband THz pulses. In this paper we review these two recent results, showing the potentialities of this new THz technique [2].

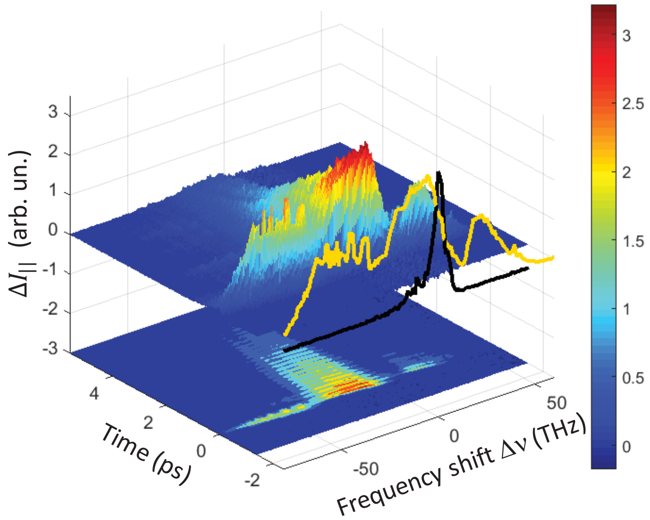

Figure 1: 3D diagram of the THYR signal for the || polarization combination in a quartz crystal as a function of time and frequency shift Δν. A cut of the signal at $t = 0$ is reported in front of the diagram (yellow curve) together with the LO-SHG spectrum (black curve). Below the 3D diagram a contour plot of it is reported, highlighting the signal oscillations lasting for about 4 ps.

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Raman Spectroscopy of Metal-Organic Framework in the terahertz region

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Metal-Organic Frameworks (MOFs) have revolutionized the field of crystal engineering, and solid-state chemistry is unique materials, which can transform their crystal structure from a dense, nonporous to a highly porous, open state and vice versa as a response to external stimuli. They are bistable or multistable crystalline materials with long-range structural ordering, a reversible transformability between crystalline phases, and permanent porosity [1]. MOF exhibit wide variety of properties attractive for a variety of applications.

We report the results of a Raman scattering study of a low-wavenumbers (terahertz) spectral region of Metal-Organic Framework soft and highly porous crystals. On example of switchable Metal-Organic Framework DUT-8 belonging to the class of pillared layer MOFs [2], we demonstrate the advantages of a low-wavenumbers spectral region to determine MOF's pores state and properties. Different crystals of the DUT-8 series with various pore sizes were studied. The Raman spectra comparison showed a significant difference in the low-wavenumber region for open (23 cm^{-1}) and closed (59 cm^{-1}) pore forms of DUT-8(Ni) [3]. This characteristic spectral feature can be a basis for rapid, and routinely applicable Raman-based techniques for the characterization of different switchable MOF phases and for in situ evaluation and analysis of them by the spectral profile.

Based on spectral profile Raman spectroscopy facilitates in situ investigations on switchable DUT-8(Ni) under hydrostatic pressure in different pressure transmitting media due to the distinct differences in the characteristic lattice vibration frequencies of the closed and open pore phases [4].

The phase transition under treatment of water vapour was observed in DUT-4 MOF. In *situ study* of DUT-4 with confocal Raman and PXRD under ambient conditions revealed a first-order phase transition between the original ordered and distorted phases. The threshold behaviour of the transition, as well as the effect of exposure time and humidity, has been confirmed experimentally. Raman spectroscopy prove that the transition is reversible and the initial state could be restored by keeping under vacuum conditions [5].

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Visualization of Marine Degradation Process of Poly(ε-caprolactone) using Raman Spectroscopy

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Recently, "microplastics," very small pieces of plastic waste, have become a major environmental problem. In order to solve the problem of marine plastic wastes, it is necessary to clarify the mechanisms of marine biodegradation and to replace currently used plastics with biodegradable plastics that can be biodegraded in the marine environment. Therefore, we have investigated the visualization of the changes in crystallinity and intermolecular hydrogen bonding of poly(*ε*-caprolactone) (PCL) film, a biodegradable polymer, during marine degradation using both high- and low-frequency regions of Raman spectroscopy combined with quantum chemical calculations (QCCs) based on the Cartesian Coordinate Tensor Transfer (CCT) method. Raman mapping measurements in the low-frequency and C=O stretching regions were used to investigate the strength of CH--O=C hydrogen bonds and changes in crystallinity during the marine degradation process of PCL. The Raman peak at around 60 cm^{-1} was found to reflect the CH--O=C hydrogen bonds based on quantum chemical calculations. It is considered that the peak shift of the 60 cm^{-1} band could be used to observe changes in the hydrogen bonding and to assess the disorder of the crystal structure of PCL.

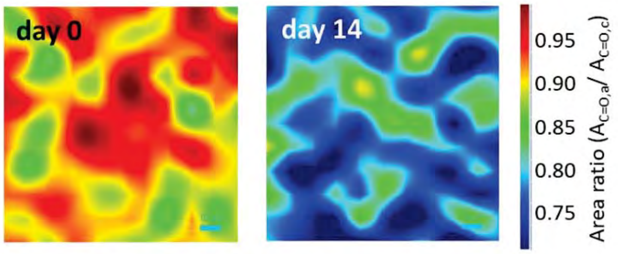

Figure 1: Raman mapping of quenched PCL films developed by area ratio, $A_{C=O,\ amorphous}/A_{C=O,\ crystalline}$ during marine degradation.

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The Blue Project: the contribute of the Raman Spectroscopy for Investigating The Plastic Marine Litter

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Plastic marine litter is a serious threat to our oceans, causing a multitude of problems for marine life, ecosystems, and even human health. Indeed, the plastic debris entangles and injures marine animals like seabirds, turtles, and mammals. They can become trapped in plastic bags, six-pack rings, or fishing gear (ghost fishing), leading to restricted movement, starvation, and even death. Furthermore, the plastic pollution disrupts and damages marine ecosystems. Coral reefs, crucial for marine biodiversity, can be smothered by plastic debris. Plastic bags can also alter the composition of seafloor sediments, impacting bottom-dwelling organisms. The presence of marine litter can improve the transfer of pollutants like pesticides and industrial chemicals improving the bio-accumulation in the marine animals and moving up the food chain, potentially impacting human health. At the end, the presence of plastic marine litter can damage fishing gear and reduce fish stocks creating a negative economic impact.

In the last few years, an ever-increasing sensitivity towards this problem has led to the focus of various studies on this problem, on how to intercept marine plastic litter, on how to remove it, but also simply on how to recognize and quantify it. The Blue Project, supported by ESA ESTEC, has investigated the potential of the LIDAR technique for the remote sensing of plastic marine litter, including also plastics located in the first layers of the water column which represents a considerable fraction of the plastics entering the sea. Especially, LIDAR techniques (fluorescence, airborne backscatter, Raman scattering) was applied in targeted laboratory and at-sea experiments for exploring the detection and characterization of plastics.

In this work, the contribution of the Raman spectroscopy to this project will be described as also the experimental approaches for future application not only in the lab characterization but also in situ determination. Indeed, a specific Raman database was built based on real samples collected in Pacific Ocean, underlining the difference and the difficulties in the recorded spectra. Furthermore, different approaches in the characterization were applied to determine the sensibility of the analysis in real conditions. At the end a new approach for measuring the Raman cross section of the polymers were tested to support the eventually future developments.

Optimized Raman Spectroscopy for Nanoplastic Detection

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As interest in microplastics has grown, there has been an increasing focus on the hazards of nanoplastics in particular. Compared to microplastics, there is still a significant lack of reliable analysis methods that can quickly analyse nanoplastics. Our laboratory has been conducting research based on Raman spectroscopy over the past few years to address this issue. In this presentation, I would like to introduce nanoplastic analysis methods using surface-enhanced Raman spectroscopy [1,2] and the analysis methods currently under development in our laboratory. Our results are not only limited to laboratory-scale analysis but also aimed at developing practical and versatile analysis methods that can be widely used in various environments.

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Optical Tweezers combined with Raman spectroscopy for micro/nanoplastics and car tyres micro/nanoparticulate analysis in liquid ambient.

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Hybrid instruments combining Optical Tweezers (OT) with Raman spectroscopy (Raman Tweezers, RT) are becoming increasingly popular, as they enable contactless measurements of size and composition of particles trapped in liquid, such as micro- and nano- plastics (MNPs). Here we show optical trapping and chemical identification of sub-5 µm plastics, down to the 50 nm range by RT [1]. Analysis at the single particle level permits to unambiguously discriminate plastics from organic matter and mineral sediments, overcoming the capacities of standard Raman spectroscopy in liquid, intrinsically limited to ensemble measurements. Applications are shown on both model particles and naturally aged environmental samples, made of common plastic pollutants. The analysis is extended to samples of tire and road wear particles collected from a brake test platform, where we highlight the presence of sub-micrometric agglomerates of rubber and brake debris, thanks to the presence of additional spectral features other than carbon [2]. Challenges and research oriented towards the exploration of the full potential of RT as a tool capable to overcome the technological gaps in microplastics pollution studies [3] will be finally illustrated, with focus on portable setups. We acknowledge funding by IFREMER through the project MERLIN-MICROPLASTIQUE (17/1212947B) and by the European Union (NextGeneration EU) through the projects PNRR-SAMOTHRACE (ECS00000022) and PRIN2022-PLASTACS (202293AX2L)

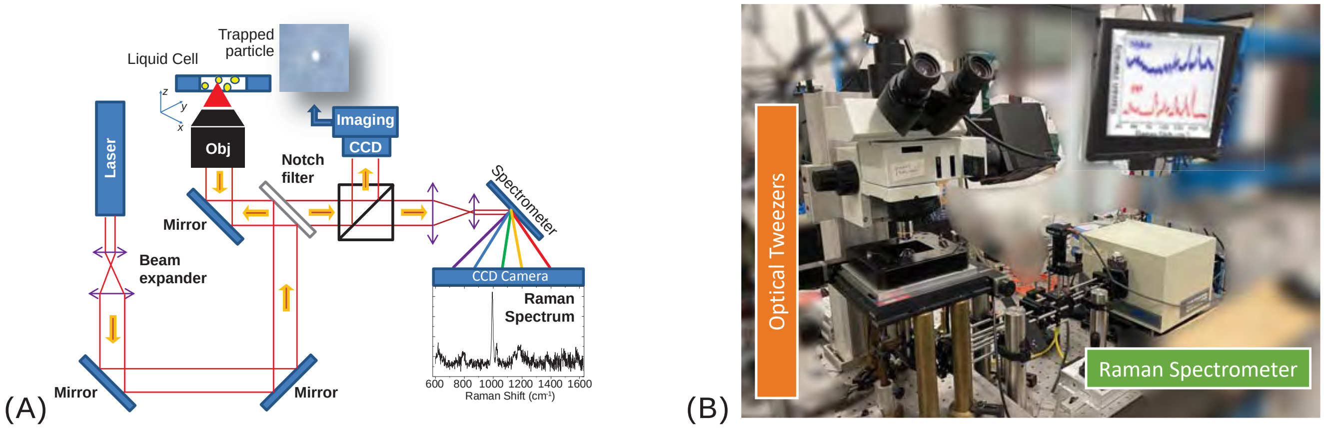

Figure 1: (A) Schematic and (B) picture of the Raman Tweezers setup

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Fat Analysis in Live Cell and Tissue with Raman Spectroscopy

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When we talk about obesity, we concern about fat accumulation but usually do not consider fatty acids composition in the fat. In a body, fat consists of triacylglycerols (TGs) which are saved in adipocytes for energy source. TG consists of 3 fatty acids (FAs) that bond to glycerol with ester bonding. When muscles require energy, adipocytes release FA into blood vessels and FAs are delivered to the body parts. Although the fat metabolism is an efficient energy stock system, it is not the perfect one, especially for the body which keeps too much fats. It was reported that excess concentration of saturated FAs, such as stearic (SA) and palmitic acids (PA), induced death of hepatic cells in culture [1]. It suggests that a cell may have its specific response to a specific FA. As we had been interested in the cellular response to FAs and their metabolism, we developed Raman techniques for quantitative analysis of FAs' composition of fat in live cells and tissues [2]. A ball lens top hollow optical fibre Raman probe (BHRP) has confocal like optical property that allows one to measure subsurface tissues in a totally non-invasive manner [3]. The fatty acid composition was successfully analysed noninvasively in subcutaneous fat tissues of live hamsters using BHRP which was designed to have a long (~400 μm) working distance (WD). The relative concentrations of linoleic (LA) and decanoic acids (DA) which were fed by peroral administration were monitored quantitatively. The result suggested that the subcutaneous adipocytes liked to hold LA with about 10 times higher rate than DA [4]. In contrast, a hepatic cell model showed quite different responses. In a non-alcoholic steatohepatitis (NASH) liver, the hepatic cell accumulates excess fat. We employed a liver cell model (HepG2) to study the fat accumulation in the cells. The cells were cultivated with excess amount of PA, SA, oleic acid (OA) and LA [1]. It has been reported that saturated FAs, PA and SA, induce the cell death but OA does not. We cultivated HepG2 cells with PA, SA, OA and LA, and monitored the FA compositions in the live cells. The cells cultured with LA, as well as PA and SA, showed the quick reduction of their populations. The Raman analysis of survived cells indicated that the HepG2 cell harvested LA quickly and generated LA rich lipid droplets in the cell on the 3rd day. However, the survived cells on the 5th day held a lower concentration of LA and a higher concentration of OA than those in the cells on the 3rd day, suggesting that the cells which could convert LA into OA survived in the high LA cultivation. The result may suggest that LA accumulation induces cell death and inflammation in liver. To improve the analytical accuracy for FA compositions, we study on multivariate analysis for Raman spectroscopy. Since Raman spectroscopy is not an absorption spectroscopy, Beer-Lambert law is not applicable. The spectral treatment techniques must be improved to analyse concentrations of FAs with different chain length.

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Raman microspectroscopy and Raman imaging for drug efficacy testing and disease monitoring

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Non-invasive imaging techniques such as Raman microspectroscopy and Raman imaging (RMS) and Fluorescence Lifetime Imaging Microscopy (FLIM) are promising tools for marker-independent in situ monitoring and biomarker development. Whereas FLIM is especially sensitive to metabolic changes by targeting the endogenous fluorophores NADH and FAD, RMS can access various cell and tissue structures due to their unique molecular-sensitive spectral fingerprints. Combined with advanced 3D in vitro models, non-destructive investigations of (patho-)physiological cellular processes can be obtained.

We implemented RMS and FLIM for ex vivo tissue analysis and in in vitro models, such as Organ-on-Chip platforms and demonstrated that RMS enables the identification and visualization of major subcellular structures such as nuclei, proteins, or lipids, as well as structural and non-structural extracellular matrix proteins such as collagens and glycol- or matricellular proteins [1-4]. In addition to quantitative image-based assessment, analysis of the extracted spectral information can further identify alterations in molecular composition, e.g. changes in lipid composition and oxidation were demonstrated upon culture duration or external stimulation. Moreover, FLIM enabled to investigate the metabolic balance between glycolysis and oxidative phosphorylation in tissue spheroids and was sensitive to detect early signs of apoptosis [5].

Overall, our results showed that both, RMS and FLIM, provide real-time insights on tissue dynamics and should be further established and developed as complementary tools in tissue diagnostics and 3D in vitro culture systems.

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O-PTIR spectroscopy to study ochronosis of tissues in alkaptonuria

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Alkaptonuria (AKU) is a rare, genetic disorder characterised by an absence of the homogentisate 1,2-dioxygenase (HGD) enzyme. This halts the tyrosine and phenylalanine metabolic pathway leading to an accumulation of homogentisic acid (HGA) in the urine and serum. Consequently, a dark brown or black HGA-derived pigment forms and is deposited into connective tissues in a process called ochronosis. Ochronosis typically affects collagen rich tissues, particularly joint cartilage, and causes decolouration and changes to the properties of the tissues making them hard and brittle. This leads to early and severe ochronotic osteoarthritis which is painful and debilitating. There is currently no cure although a recently approved drug ‘Nitisinone’ can halt pigmentation and slow disease progression [1].

HGA and HGA-derived pigment are the main cause of the pathology that manifests in AKU. One of the biggest unanswered questions is the mechanism by which pigment is deposited into tissues and cartilage [2]. This is central to the pathophysiology of ochronosis and disease progression. A better understanding of this interaction could lead to new therapeutic targets that aim to prevent or remove pigmentation that causes pain and poor quality of life in AKU patients.

Consequently, careful study of tissue samples from humans and animals with AKU is required. Currently, one of the best approaches for studying early tissue ochronosis is using AKU mouse models including models deficient in HGD, such as Hgd tm1a$^{-/-}$. Unlike in humans, ochronosis is not easily visible so staining of tissues is required. This utilises Schmorl’s stain which stains ochronotic pigment a blue-green due to the pigment reducing ferricyanide anions to ferrocyanide anions [3]. However, little investigation has been conducted into alternative methods of identifying pigmented areas in tissues.

One potential alternative to staining the tissues is using optical photothermal infrared (O-PTIR) spectroscopy enabling quick identification of pigmented tissue areas without the cost and time involved with staining samples. [4] Furthermore, as it is non-destructive, tissue samples can then be utilised for other purposes. This work has investigated the ability of O-PTIR spectroscopy to distinguish between pigmented and non-pigmented areas in tissue samples as an alternative to histological staining.

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Molecular signature during the formation of Blastema in regenerating Annelid Using Resonance Raman spectroscopy and phasor approach

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Understanding the “molecular signature of life” and what activates the “dormant state” and its transition to the “growing state” is a long-standing question in biosystems. Recently there have been a few studies on the “spectroscopic signature of life” in single living fission yeast at 1602 cm^{-1}; carrot seeds germination, germinating mung bean, activated ameba cyst with carotenoids Raman bands of β-carotene at 1525 v_1(C=C), 1158 v_2(C-C) and 1005 v_3(CH_3) were considered as the “signature of activation of life” [1,2,3]. *Aelosoma viride* is tasked with building entire body segments out of their single starting cell at their amputated region and undergoing epimorphic regeneration; therefore, these annelids are the most suitable for the study of regeneration. A time-dependent study with Resonance Raman spectroscopy at 532nm has been acquired from the regenerated site of freshwater *A. viride*. The Raman spectra at different hours post-amputation (hpa) of *A. viride* were recorded to elucidate the molecular composition in the regenerating newly formed stem cells. The Raman spectral detection at wound healing at 6 hpa (hour post amputation), blastema bud initialization at 12 hpa, and blastema bud at 24 hpa displayed high-intensity peaks of Carotenoids “Zeaxanthin” at 1527 cm^{-1}, 1159 cm^{-1}, 1008 cm^{-1}. A sudden disappearance of the carotenoids was detected post-wound healing, but reappearance of carotenoids was detected at the blastema formation. We evaluated the interplay of reactive oxygen species and carotenoids and their vital role in the anterior regeneration of *A. viride*. In vivo intracellular imaging of blastema bud was made possible by applying Two-Photon Fluorescence Lifetime Imaging in combination with the phasor approach. These findings indicate that the Raman signature of carotenoids can be used to detect the process of newly derived stem cells in the study of regenerative medicine.

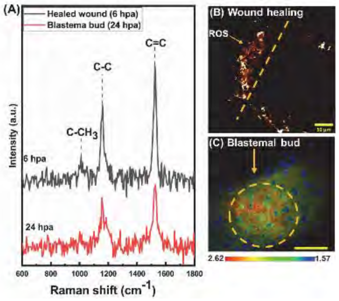

Figure 1: (A) The Raman spectroscopic signature at wound healing (6 hpa) and budding blastema cells at 24 (hpa). (B) Detection of Reactive Oxygen Species at the wound healing at the anterior regenerated segment. (C) Two-Photon Fluorescence Lifetime Imaging label-free direct screening of the Blastema bud at 24 hpa.

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Raman spectroscopy reveals periodic lattice distortion in twisted heterostructures of 2-dimensional materials

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Raman spectroscopy has been extensively used in 2-dimensional (2D) materials research. It is often used to measure doping, strain, and thermal properties in addition to structural properties such as defects. When two layers of the same or different 2D materials are stacked to form heterostructures, the physical properties are modulated due to the interaction between the two materials. Such heterostructures have been extensively studied as the alignment of the bands in the constituent materials allow for manipulation of optoelectronic and transport properties. Several factors such as the band offset between the bands of the two constituent materials and the twist angle between the crystallographic directions of the two layers determine the physical properties of these structures. It has been demonstrated that the phonon spectrum as well as the electronic band structure and optoelectronic properties change systematically as the twist angle between two layers of a given set of materials is varied. Furthermore, at very small twist angles, atomic-scale lattice reconstruction [1] is observed and should be accounted for in describing the physical properties of heterostructures. Recently, Raman spectroscopy revealed that moiré superlattices made from single layers of MoS_2 and WSe_2 with a fairly large lattice mismatch (~4.5%) exhibit a pair of torsional distortions with opposite chirality irrespective of the twist angle [2]. The whirlpool-shaped periodic lattice distortions introduce fuzziness in the Raman spectra and universal redshifts to the intralayer excitons for all twist angles. Both of these modulations become weaker as the twist angle increases but do not disappear, whereas they are turned off when the constituent layers are not tightly coupled. In a lattice matched (~0.24%) heterostructures of $MoSe_2$ and WSe_2, a series of moiré phonons are observed, and the low-frequency Raman spectra show a rich array of interlayer shear and breathing modes that evolve with the twist angle [3]. Furthermore, the interlayer excitons that appear strong in the twisted heterostructures with the twist angles near 0° or 60° have different energies and photoluminescence excitation spectra for the two cases, which results from different electronic structures and carrier relaxation dynamics. These results demonstrate that the details of the lattice interactions as well as the twist angle should be considered in designing heterostructure-based devices.

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Unveiling the charge transfer / recombination pathway of sensitizing photocycloaddition with lead halide perovskite nanocrystals

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Precisely tracking the evolution of reaction intermediates in photocatalytic processes provides essential mechanistic understandings to achieve rational design of catalytic cycles with improved energy efficiency and chemical selectivity. The present research employs transient absorption and time-resolved stimulated resonance Raman spectroscopy to study the dynamics of key intermediates in photocycloaddition reactions of enones catalyzed by perovskite nanocrystals (PNCs). By comparing the experimental Raman spectra with quantum computational results, an anion radical is identified as the intermediate product of photoinduced electron transfer from the PNCs. The anion intermediate then undergoes a charge recombination process to generate the triplet excited state that initiates [2+2] cycloaddition with olefin partners. Through analyzing the kinetic data of the intermediates, the electron transfer/charge recombination mechanism has been proven to be the primary sensitization pathway. With this insight, we introduced sacrificial electron donors to compete with charge recombination so the anion intermediate could be preserved to produce cyclopentyl motifs through a [3+2] cycloaddition. Raman spectral features also indicate that the coordination between enones and the Lewis acid sites on the PNC surface affects the electronic properties of the substrate and facilitates sensitization.

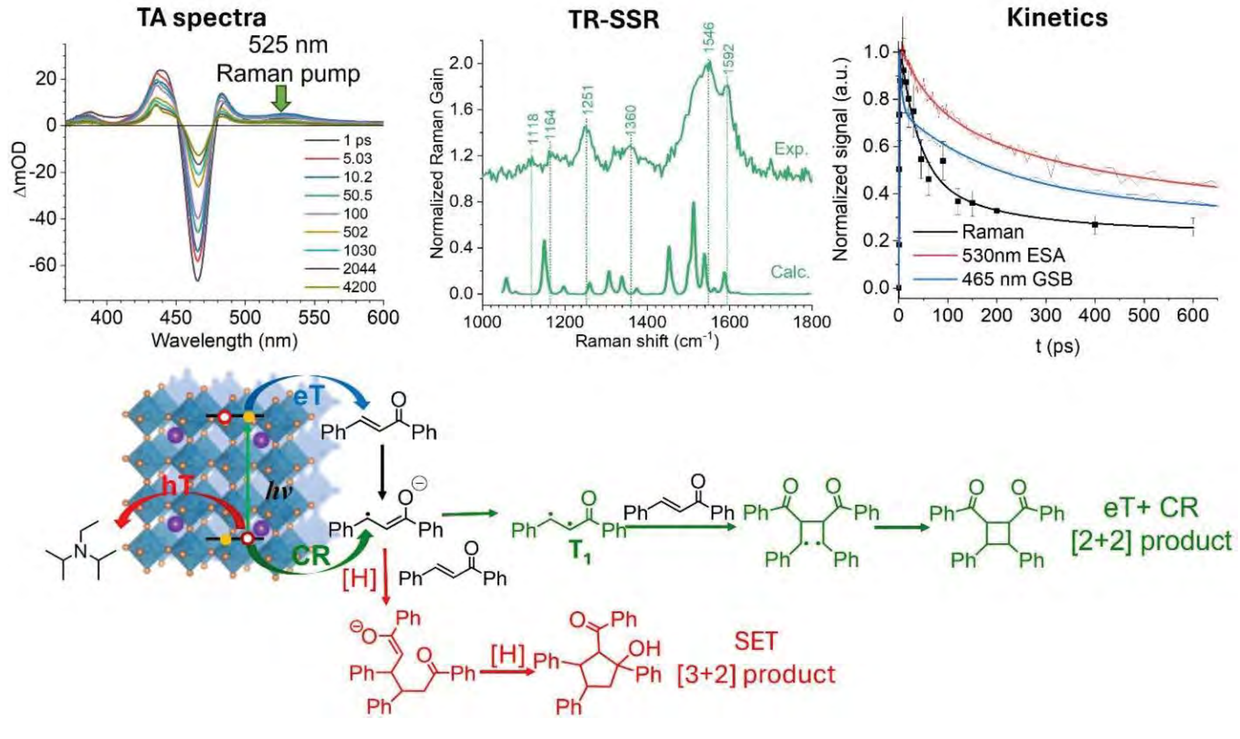

Figure 1: Understanding and modulating photocatalytic cycloaddition reactions with TA, TR-SRR, and kinetic analysis.

Angle-Resolved Polarized and Temperature dependent Raman Scattering of 2D Hexylammonium Lead Iodide Perovskite

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3D hybrid halide perovskites are promising materials for future electronics and optoelectronics and applications in spin-electronic devices due to their unique properties, such as high coherent emission, high defect tolerances, strong spin-orbit coupling, and strong light-matter interactions. However, there is a challenge in the applicability of these conventional perovskites due to their poor environmental stability. Lowering the dimensionality of these materials has been the key to this problem, offering more stable performance and better moisture resistance. This is attributed to the hydrophobic organic spacers that passivate the inorganic layers in 2D hybrid organic-inorganic perovskites (2D HOIPs). 2D HOIPs are composed of inorganic layers made of metal halide octahedra separated by organic cations layers. This work presents angle-resolved polarized Raman spectroscopy measurements of 2D HOIP hexylammonium lead iodide $[(HA)_2PbI_4]$, that includes phonon modes down to 20 cm^{-1}. Polarized Raman spectroscopy is a very effective method for assigning the phonon symmetry, which can be assigned using computational methods we describe [1]. The polarized Raman scattering measurements were carried out in the backscattering geometry at different temperatures. We utilized two halfwave plates to control the incident and the scattered light polarization, such as the sample can be rotated under parallel and cross-polarization configurations. The theoretical predictions combined with the experiments revealed the anisotropy of the of Ag and Bg Raman active modes below 110 cm^{-1}. Temperature dependance of the low frequency modes suggests a phase transition close to 125 K. The modes which suggest a phase transition are correlated to inorganic framework motions. This transition temperature has been reported before at 127K revealed by PL measurements [2].

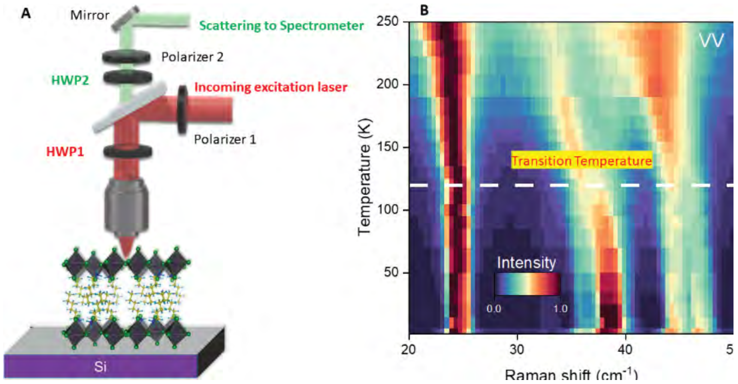

Figure 1: A. Experimental setup. HPW is a half wave plate. B**.** Temperature dependent Raman map.

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Structural Phase Transitions in Layered Hybrid Organic-inorganic Metal-halide Perovskites by Raman spectroscopy: Effect of Transition Metal and Organic Cation

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In recent years, layered hybrid organic-inorganic metal halide perovskites (2D HOIPs) have garnered increasing attention for their potential in spintronic and optoelectronic fields due to their chemical and structural versatility. By modifying their organic and metal cations, as well as the number of inorganic layers and perovskite phase, it is possible to tailor their optical, electrical and magnetic properties[1]. However, research on these layered compounds is still in an early stage compared with their tridimensional counterparts. One key aspect for a further development of these materials is determining the absence or presence of structural phase transitions[2], which could lead to new properties such as ferroelectricity or ferroelasticity[1], and even affect to others such as the optoelectronic properties. In this regard, Raman spectroscopy stands out as a valuable non-destructive and sensitive technique for monitoring structural changes related to the rearrangement of the inorganic lattice and organic cations[3]. In this study, we used temperature-dependent micro-Raman spectroscopy to investigate how transition metals (Mn^{2+}, Co^{2+} and Cu^{2+}) and organic spacers (alkyl- and aryl-ammonium) influence on the emergence of phase transitions. Our analysis of the vibrational dynamics revealed that only the crystals containing ethylammonium cation (EA) showed structural phase transitions in the studied temperature range. Specifically, EA_2MnCl_4 perovskites exhibited one phase transition at 230K associated to a change in the conformation of the organic molecules, which is also reflected on the photoluminescence emission. In contrast, in the case of the EA_2CoCl_4 and EA_2CuCl_4 crystals, one (240K) and two phase transitions (240K and 330K) appeared, respectively. These transitions involved the rearrangement of both the organic moieties and inorganic lattice, with the latter attributed to a higher structural flexibility. Therefore, this work demonstrates that the emergence of structural phase transitions in 2D HOIPs depends not only on the selection of the organic cations but also on the structural flexibility of the inorganic lattice[4]. Thus, the chemical design towards the modulation of phase transitions could be exploited in devices adding new functionalities or enabling active switching.

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Tracking chemical interactions of Caspofungin on silver nanoparticles through SERS spectroscopy for antifungal applications

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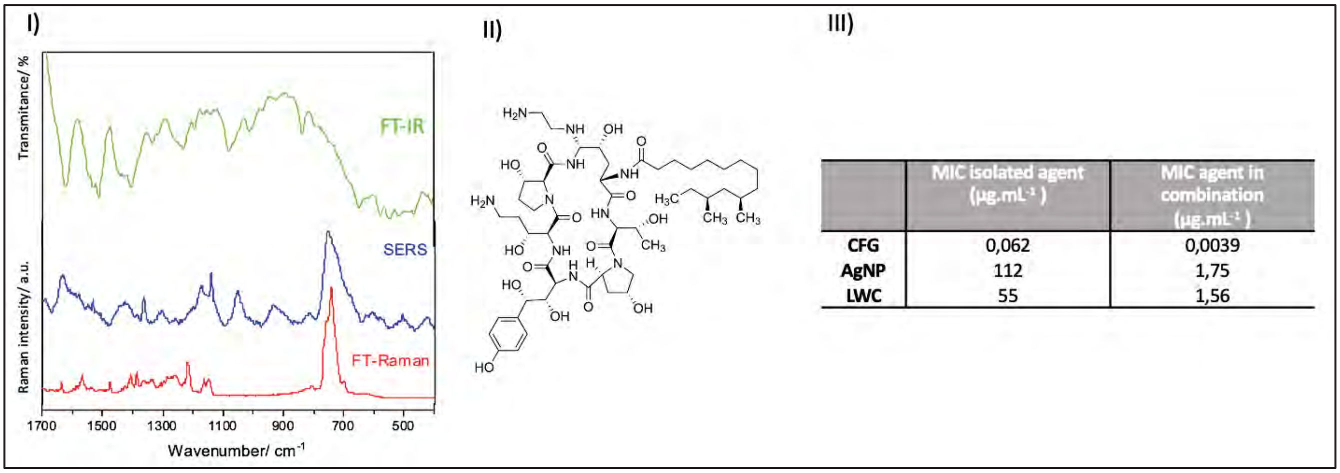
Caspofungin (CFG)(Fig.1-II) and other echinocandins act on the fungal cell wall by inhibiting the synthesis of β-1,3-D-glucan, resulting in disruption of the cell wall and death of the fungus, as for instance *Candida spp*. Reports of decreased susceptibility of *Candida spp.* from CFG indicates the risk of therapeutic failure, and elevations in the minimum inhibitory concentrations (MIC) of antifungals. Silver nanoparticles (AgNP), due to their lower both toxicity and production costs, as well as noteworthy large surface area, have been receiving special attention for their inhibitory potential against fungus and bacteria [1]. In this work, CFG was used to combat *Candida albicans* in vitro, coadsorbed with chitosan (LWC) on AgNP surfaces, that led to significant decline in MIC values (Fig. 1-III). Moreover, the synergy involving such a ternary mixture, with all components having some antifungal effect, was monitoring by checkerboard method analysis [2]. The surface-enhanced Raman scattering (SERS) spectrum of CFG was obtained to characterize its adsorption on AgNP surface (Fig. 1-I).



	MIC isolated agent (μg.mL^{-1})	MIC agent in combination (μg.mL^{-1})
CFG	0,062	0,0039
AgNP	112	1,75
LWC	55	1,56

Figure 1: I) Infrared, Raman, and SERS spectra of Caspofungin; II) Caspofungin molecule; III) MIC for three antifungal agents, isolated and in combination against *Candida albicans*.

The observed spectral changes, in comparison with Raman and FTIR spectra, indicate AgNP can act in the transport of the drug for delivery to *Candida spp.* Cell. The effective chemical interactions of CFG with AgNP surfaces can justify the observed decrease in the MIC values, shown SERS analysis is the paramount tool to track this process.

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Metal-organic frameworks for efficient mephedrone detoxification or supervised withdrawal – synthesis, characterization and *in vivo* studies

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Metal-organic frameworks (MOFs) stand out as revolutionary porous materials owing to their adjustable properties, porous characteristics, and versatile applications. Combining attributes of inorganic metals and organic acids, their structure facilitates diverse applications, including drug delivery systems (DDS) [1]. These carriers exhibit low cytotoxicity, high biocompatibility, and ample drug packing capacities.

In contemporary times, drug addiction remains a pervasive issue, with synthetic cathinones (SCs) being among the most prevalent and abused substances. Notably, mephedrone (4-methylmethcathinone, 4-MMC), a prominent SC, shares pharmacological similarities with amphetamines [2]. Overdosing on 4-MMC can lead to symptoms like arrhythmia or hypertension, for which β-blockers like propranolol (PRO) offer antiarrhythmic effects and palpitation prevention. Leveraging MOFs as carriers for PRO during 4-MMC overdose emerges as a modern, rational approach, offering both protective effects and efficient drug delivery.

In our study [3], we synthesized MOF materials, incorporated PRO, formed PRO@MOF composites, and characterized them through various techniques including PXRD, FTIR, RAMAN, BET, and SEM. We evaluated PRO release profiles from the composites into water and a solution mimicking human body fluid, alongside *in vivo* cytotoxicity tests. The mechanism of PRO loading within the MOF network was also analyzed using theoretical methods. This approach heralds an innovative and secure system for detoxifying the body post-drug use.

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Classification of THC and CBD in cannabis oils using Raman spectroscopy combined with chemometrics and machine learning

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The growing cannabis industry necessitates accurate and efficient analytical methods for monitoring cannabinoids in cannabis products. Raman techniques have emerged as a promising tool to address the industry's demands[1,2]. This work presents the development of a Raman spectroscopic method for classifying the two predominant cannabinoids, namely delta-9 tetrahydrocannabinol (THC) and cannabidiol (CBD), in edible and complex cannabis oil products. A miniaturized quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction was optimized for cleaning small-volume samples (i.e., sample drops of 0.050 mL). Raman spectral profiles of the target analytes and representative matrices were collected prior to analysis of various commercial cannabis oils. For each sample type, analyses were performed in at least triplicates, and about 240 Raman spectra were gathered for method development and validation. The developed method yielded detection limits of 16 mg/L for THC and 36 mg/L for CBD, with extraction recoveries acceptably between 75 and 102%. The QuEChERS extraction enabled the acquisition of high-quality Raman spectra, revealing distinct features of THC and CBD in highly complex samples. Principal component analysis (PCA) simply demonstrated clear discrimination between THC and CBD (for example, see Figure 1). Furthermore, commonly used machine learning models, including k-nearest neighbors classifier, logistic regression, and random forest classifier, were applied for data analysis. Preliminary studies have shown that these models achieved accuracy and precision of over 95% for predicting the dominant cannabinoid type in oil samples. The results were also compared with conventional HPLC analysis, confirming validity of the proposed method. This work holds potential for several cannabis testing applications, such as regulatory compliance and quality control.

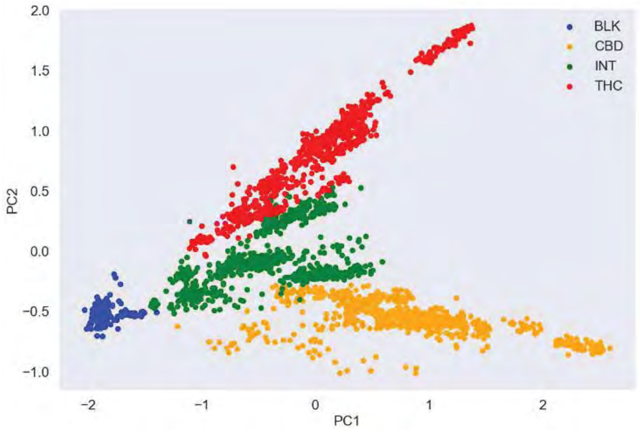

Figure 1: PCA of the Raman spectra of different target analytes and standards at vary concentrations in coconut oils. BLK (blue) = Blank; CBD (orange) = CBD dominant; INT (green) = intermediate CBD/THC ratio; THC (red) = THC dominant.

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SERS in complex bio samples – from drug monitoring to microbial communication

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In order to allow for a sensitive detection of drugs, biomarkers or metabolites in complex bio matrices and samples, such as blood plasma, saliva or cultural supernatants, targeted and untargeted SERS detection schemes are available. In the case of untargeted and direct SERS-based detection schemes, the high affinity of a target analyte towards the SERS-active metallic sensor layer is exploited; whereas for targeted detection schemes, the enrichment of the analyte molecules on the surface of the SERS sensor is performed by e.g. metal-organic frameworks or recognition elements such as aptamers. Within this presentation, we will introduce our recent results in bio spectroscopy applying powerful SERS-based detection schemes. To illustrate the potential of SERS in therapeutic drug monitoring, the antibiotic ceftriaxone was spiked in fresh blood plasma samples as well as microdialysates and the role of proteins within this detection scheme is discussed. In microdialysate samples, detection down to 1.4 µM is achieved. [1] Furthermore, we showed the SERS-based detection of pyrazinoic acid (POA), a metabolite of the tuberculosis-relevant prodrug pyrazinamide (PZA). To be specific for POA, gold nanoparticles equipped with a Prussian blue modification were applied, complexing the POA molecules via Fe (II). This scheme has a high potential in assessment of PZA resistance in cultural supernatants of *M. tuberculosis* bacteria, as only sensitive bacteria convert PZA into POA. [2] Also, we studied the SERS signature of saliva to verify the detection of salivary biomarkers, i.e. interleukin-8 and lysozyme by combining SERS with molecular dynamics simulations. [3] Recently, we developed silver SERS substrates with improved stability and tested their application capabilities towards cultural supernatants to identify signalling molecules relevant in microbial communication.

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Design and functionalization of the metal nanoparticles for future biomedical approaches.
Micro- and nano-spectroscopy studies.

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Nowadays, metal nanoparticles have become very attractive due to their interesting features and quite uncomplicated functionalization which allows for the modification of their properties [1]. Thanks to spectroscopic methods such as Raman and FTIR and their surface–enhanced techniques (SERS and SEIRA) controlling the nanoparticles' modifications at each step of the fabrication is possible. The new insights at local interactions (in the nanoscale) on nanoparticle surfaces is provided by a new technique that combines atomic force microscopy with infrared absorption spectroscopy (AFM–SEIRA).

In recent years, AFM–SEIRA supported by the SERS and SEIRA techniques became our routine strategies for the characterization of the drug/nanocarrier conjugates and surfaces functionalized by the nanoparticles for biomedical applications [2,3]. In our approach, we have firstly characterized drug/gold nanoparticles interactions (Fig. 1A) and subsequently introduce them to *in vitro* model of non-small cell lung cancer cells. Engagement of fluorescent microscopy was beneficial to visualize cell morphology and cellular components that colocalize with nanoparticles uptaken by the cells (Fig. 1B). Finally, Raman spectroscopy allowed us to asses precise conjugates distribution in the cytoplasmic area of the cells and reach their effect on cells` biochemical composition (Fig.1 C, D).

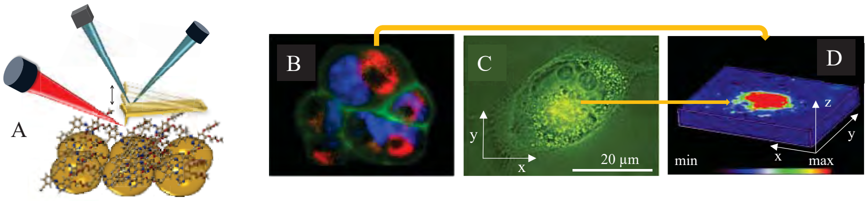

Figure 1: AFM–SEIRA experimental scheme (A). The fluorescent (B) and microscope image (C) of H1299 cells treated with erlotinib/AuNPs conjugates containing 10mg/L of AuNPs and 0.1 µM of erlotinib for 24h. The cells were stained – nucleus (blue), mitochondria (red), and actin (green). The Raman 3D maps (D) of the characteristic conjugates spectral range (2106 cm^{-1}–1925 cm^{-1}) visualize the conjugates inside the cell.

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Surface-enhanced Stokes and anti-Stokes Raman from metallic nanostructures

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There are two types of inelastic Raman scattering; the Stokes (S) and the anti-Stokes (aS) scattering. The aS/S ratio is used in normal Raman spectroscopy for thermometry. This is because the normal Raman intensity is directly proportional to the population of the vibrational states, which are governed by the Boltzmann distribution. However, there are several reports in the literature [1-3] that indicate that for molecules adsorbed in nanostructured materials the aS/S ratio does not necessary follow the expected from the Boltazmann distribution. This phenomenon is related to the conditions of extremely enhanced local fields experienced by the species adsorbed on those materials [4]. In this work, we explored the behaviour of 4-mercaphenol and 5,5′-dithiobis(2-nitrobenzoic acid) adsorbed on both silver and gold metallic nanostructures. The nanostructures were fabricated using a self-assembly approach from suspensions of synthesized nanoparticles. The surface-enhanced Raman behaviour was measured at different conditions (such as laser excitation energy and power). The results indicated nonlinearities (Figure 1) that might be correlated to several different contributions, including local plasmonic resonances, thermal decomposition and nonlinear Stokes-anti-Stokes generation.

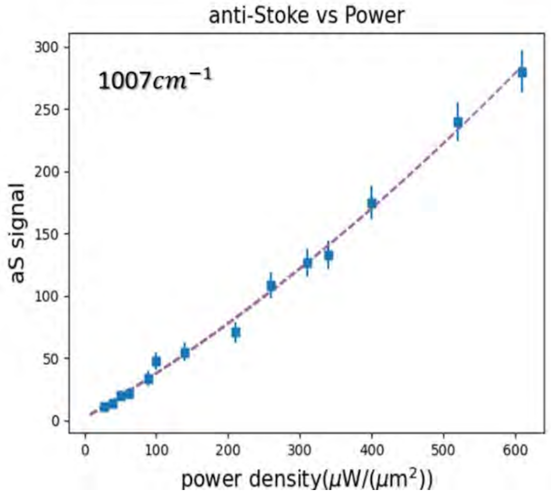

Figure 1: Example of aS signal intensity dependence on the laser power.

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Improving SERS Sensing by Understanding Metal-Molecule Interactions

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Abstract

Our planet is populated by an estimated 8.1 billion highly interacting individuals enjoying generally long lives. Along with the advantages of the modern world come however many issues, mainly related to human health, that bring substantial strain to the medical systems. In both rich and developing countries, people in growing numbers are subjected to non-communicable diseases due to aging and to communicable diseases due to population densities in urban setting, that lead to easy spreading of pathogens. In both scenarios, the healthcare systems have a hard time keeping up with prevention, diagnosis, treatment, and management, mostly because of the high costs of these procedures. There is therefore the need to develop and implement diagnostic methods that are truly low cost, rapid, robust, and deployable, and can be provided to all people, regardless of geographic location or socioeconomic status.

Our group combines fundamental concepts of nanomaterials and surface chemistry, physical chemistry and spectroscopy, and sensor development to address these issues. In my talk I will discuss how only by truly understanding the metal-molecule interactions at the plasmonic nanoparticle surface can SERS be leveraged to produce reliable sensors that are quantitative, accurate, and reproducible. While SERS has been for many years deemed an unreliable technique, it was because much of the research effort has been directed toward the identification of a “universal substrate” that can provide diagnostic results with zero sample pretreatment. Now, it is important that we abandon this concept and adopt the same strategies utilized for other identification techniques, such as mass spectrometry, where detailed sample pretreatment and detection flowcharts are defined before analysis can begin. I will therefore, in conclusion, argue that if we want to obtain truly low cost, rapid, robust, and simple diagnostic tools that can be easily employed by the healthcare providers, we need to do the heavy lifting work at the source, in our research laboratories.

Benchmarking SERS Sensors: A Path to Standardized Evaluations

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Surface-enhanced Raman scattering (SERS) offers unparalleled sensitivity for detecting and identifying chemical and biological species. This exceptional sensitivity results from the resonant excitation of coupled plasmonic nanostructures that generate highly enhanced and localized electromagnetic fields. The strength of this enhancement is particularly dependent on the structural morphology and optical near-field coupling. Minor changes in the nanostructures can lead to significantly different SERS responses. SERS substrates range from high-precision nanofabricated planar nanostructures to solution-synthesized colloidal silver or gold sols of varying size, shape and morphologies. The development of new SERS sensors is an active and vibrant research field, leading to a wide range of nanostructure designs with different performances. Commercial SERS substrates, used with handheld Raman analyzers, promise the detection of environmental and industrial toxins, narcotics and warfare agents and have reached the end-user market.

Despite advances, comparing the performance of different SERS sensors remains challenging. To date, there are no standardized or internationally accepted reference measurements, established through rigorous metrological validation that allow for comparisons between different types of SERS sensors. Establishing comparability between different substrates is particularly important for regulated areas, such as law enforcement operations or clinical analysis. As noted in a recent editorial piece in ACS Sensors [1], the field can benefit from standardization. While there are many variables that can influence the SERS performance, a straightforward approach to establish inter-comparability of different SERS sensors is to establish a SERS reference standard and defined measurement parameters with a set of standardized analyte molecules. It is important to note that the SERS reference standard does not need to have high enhancement performance. Ideally, a good reference standard should be exceptionally stable with excellent uniformity, affordable and widely available (e.g. not dependent on a single or just a few suppliers), which are the same general criteria for all of the physical reference materials used in analytical chemistry. Alternatively, SERS sensors can also be compared to another stable reference sample with all of the above characteristics. A good reference candidate is a Si wafer with a specific orientation and measured under certain defined conditions (e.g. polarization and wavelength). A polystyrene film maybe be another good alternative.

In this presentation, we evaluate the comparability of SERS sensors using the two proposed methods. We will present the benchmarking data between a set of commercial and custom designed SERS sensors to a potential SERS reference standard and to Si wafer. We will show the uniformity and stability of a SERS reference standard have introduced significant uncertainty associated with this method. We hope to engage researchers in the field of SERS to guide the development of SERS reference standards that will allow comparability of the performance of different types of SERS sensors.

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Comparison of chemically synthesized gold nanobowls, nanourchins and nanospheres composed with graphene oxide as ultrasensitive SERS platforms

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Engineering of plasmonic properties of gold nanostructures expands the field of their applications from photocatalysis and photothermal effects to ultrasensitive surface-enhanced Raman spectroscopy (SERS). It is generally agreed that the nanoparticles having spikes or sharp edges are able to generate strong electromagnetic fields upon plasmonic excitation, being beneficial for SERS. We study template-free synthesized gold nanobowls (AuNBs)[1], nanourchins (AuNUs)[2] and nanospheres (AuNSs) deposited on graphene oxide (GO) for SERS applications. The gold nanoparticles are combined with pristine GO or graphene oxide conditioned in ammonia ($GONH_3$) or graphene oxide conditioned in sodium hydroxide (GONaOH). The SERS properties of the hybrid supports were studied using rhodamine 6G (R6G) as the SERS probe. The 633 nm laser line is used, which falls out of the molecular resonance with R6G. The results indicate that AuNBs show largely higher enhancement factors when compared to AuNUs and AuNSs. Furthermore, the GO materials are able to modify the SERS enhancement by 1 order of magnitude. We explain the influence of the GO material by three factors: (1) enabling or disabling the charge transfer between gold and R6G, which is crucial for the chemical part of SERS enhancement; (2) causing the aggregation of gold nanoparticles and formation of hot spots; (3) dipole contribution to the electromagnetic enhancement through the abundance of polar groups on the surface.

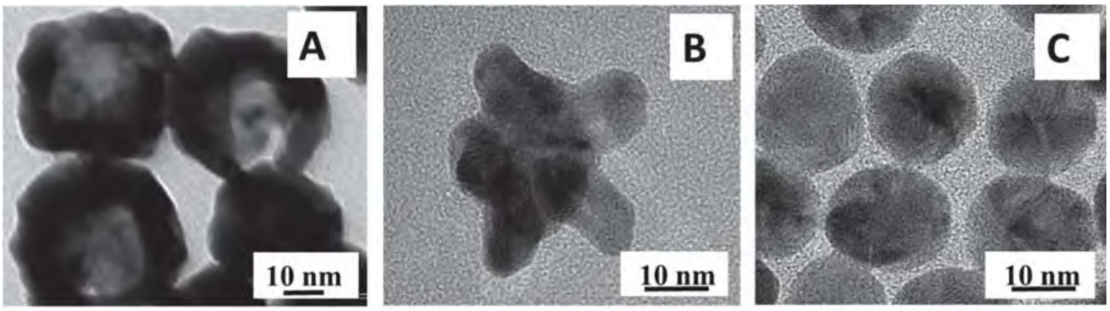

Figure 1: HRTEM images of: gold nanobowls (AuNBs) (A), gold nanourchins (AuNUs) (B) and gold nanospheres (AuNSs) (C).

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Novel hybrid magneto-plasmonic nanoclusters for ultrasensitive detection through SERS effect

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In the last decades, researches have been focused on the design and development of nano-sized platforms suitable for biomedical applications, including drug delivery, biosensing and theranostic. To this end, different kinds of nanomaterials were studied due to their remarkable properties, such as large surface area, permeability, superparamagnetism, and localised surface plasmon resonance, making them unique in their functioning. However, the synthesis of multi stimuli responsive materials still relies on long and complex procedures to obtain highly heterogeneous nano constructs which implies the use of organic solvents and are characterized by low reaction yield.[1]

In this scenario, the present work is focused on the development and optimization of novel hybrid iron oxide-gold system with extraordinary magnetic and plasmonic properties, exploitable as versatile, low-cost diagnostic nanoplatforms. The system is characterised by easiness of preparation with organic solvent-free synthesis, and high yield of a nanocomposite that is readily engineered according to application. To this end, magnetite NPs, synthesised using Massart coprecipitation method, were further decorated with Turkevich citrate capped AuNPs, after the formation of a shell designed to promote Au bonding. Then, sonochemical assisted in-situ AuNPs growth was implemented to improve the plasmonic properties of the systems, as attested by Vis spectroscopy (Figure 1a).[2] Thiol-gold high chemical affinity was exploited to further functionalize the hybrid system with PEG-derived molecules, which has the dual scope of stabilizing the hybrid dispersion in different type of fluids and providing binding site for further decorating with a wide range of functional application-dependent compounds (ranging from antibodies to small molecules). Morphological characterization attested the formation of desired hybrid magneto-plasmonic nanocluster (Figure 1b). Finally, the feasibility of these NPs to work as SERS substrate was preliminary demonstrated through micro-Raman experiment using Raman-active molecule (4-mercaptobenzoic acid) as model target (Figure 1c). However, thanks to the versatility of functionalization, we foreseen the potential applications of highly stable magneto-plasmonic nanoclusters as biosensing element in sandwich-like assay for the target detection of analytes in biofluids, as well as functional filler to introduce magneto-plasmonic properties in polymer matrices exploitable for tissue engineering applications.[3]

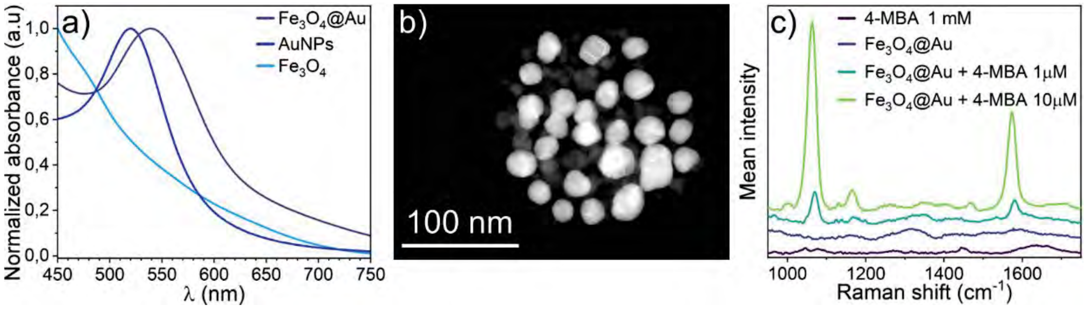

Figure 1: Synthesized hybrid magneto-plasmonic nanoprobes: (a) versus magnetite NPs and AuNPs Vis spectra, (b) STEM micrography, and (c) SERS effect for 4-MBA detection (at 1 and 10 µM 4-MBA concentration) against 4-MBA 1 mM solution.

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Nanoscale Characterization Elucidating Defects in 2D Semiconducting MoS_2 Nanolayers using Tip-Enhanced Raman Spectroscopy

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ABSTRACT

Modulating physical and chemical properties of two-dimensional (2D) transition metal dichalcogenides (TMDC) by defect-engineering induced by oxygen plasma is actively pursued. In this work, exfoliated 2D MoS_2 layers treated by medium power oxygen plasma for different times (0, 10, 20, 40, and 60 s) are investigated using Kelvin Probe Force microscopy and tip-enhanced Raman spectroscopy (TERS) besides micro-Raman and photoluminescence (PL) spectroscopy. Under oxygen plasma, defects (mono- and di-sulfur vacancies) and chemical oxidation is predominant from 0s (native defects) up to 40s, while etching becomes dominant beyond 40 s, for mono- (1L), bi- (2L), and tri- (3L) layer MoS_2 with optimal defect density for four- (4L) and more layers. While Raman spectra exhibited lattice distortion (broadening of phonon bands) and surface oxidation by the presence of sub-stoichiometric molytrioxide MoO_3 (*i.e.,* MoO_{3-x} or MoS_xO_{2-x}) the increased spectral weight of trions and quenching in PL spectra are observed with treatment time. The localized nanodomains (~20-30 nm nm) and aggregated vacancies as nanovoids and intermixed MoS_2/MoO_{3-x} alloy are identified in near-field Raman spectra. The atomic force microscopy also showed defects aggregation and Kelvin probe force microscopy revealed the work function (WF) increase from 4.98 eV to 5.56 eV, corroborating the existence of MoO_{3-x} phase which enables doping and shift Fermi level. We also highlight the unique interaction between the gold substrate and the formed MoO_{3-x} facilitating Mo^{6+} cation reduction to lower oxidation (*i.e.*, Mo^{4+}) thereby yielding intermediate oxidation states responsible for lower WF (*ca.* theoretical 6.3 eV for stoichiometric MoO_3). Strong correlations among the work function, vibrational and optical responses are established while exploring the oxygen plasma-induced defects and changing the landscape on oxygen doping at the nanoscale with varying MoS_2 layers, which are useful for heterogeneous electrocatalysis and applicable to other 2D TMDCs.

KEYWORDS: MoS_2, oxygen plasma, chemical oxidation, etching, KPFM, TERS.

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Electromagnetic enhancement and charge transfer synergistic of noble metals and metal-oxides as SERS platform for detection of organic dyes

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Detecting trace amounts of organic dyes is crucial due to the serious threat posed by the wastewater discharge to the ecosystem. Surface-Enhanced Raman Spectroscopy (SERS) stands as a powerful and non-destructive method for rapid fingerprint recognition. The efficacy of the SERS technique is significantly influenced by the choice of substrate. Integrating metal oxides with noble metals notably enhances the SERS activity. Corrugated noble metal surfaces leads to electromagnetic enhancement due to formation of "hot-spots", and the structural variations in metal oxides contribute to chemical enhancement via interfacial charge transfer, amplifying the overall SERS signal [1]. The objective is to detect low concentrations of methyl orange (MO) and rhodamine B (RhB) in the presence of silver (Ag), a noble metal, and zinc oxide (ZnO). To achieve this, both chemical and physical routes were utilized for synthesis of Ag-ZnO composites [2, 3]. The nanoparticles and nanocomposites synthesized using both methods were examined using field emission scanning electron microscopy, revealing spherical Ag nanoparticles and hexagonal ZnO nanorods in the chemical process. Nanocomposites synthesized by colloidal dispersion displayed clusters of Ag nanoparticles attached to hexagonal ZnO nanorods. Transmission electron microscopy confirmed these findings. Whereas, the morphology of thin film nanocomposites fabricated using RF and DC magnetron sputtering, showed a non-uniform distribution and increased agglomeration with higher Ag content. Pristine and annealed films were subjected to synchrotron XRD to study potential structural modifications. The hexagonal wurtzite structure of ZnO was evident in samples with low Ag content, but increased Ag content suppressed this crystalline behavior. Using the UV-Visible spectroscopy, the presence of band edge from the metal oxide and the plasmonic resonance originating from the noble metal were observed for the nanocomposites prepared using both techniques. However, the metal oxide and noble metal composition altered the plasmonic resonance. These synthesized SERS substrates with tunable optical properties were then utilized for detection of organic dyes, for concentration as low as 10^{-9}M for rhodamine B and 10^{-6}M for methyl orange. The experimental results were supported by the Finite-Difference Time-Domain (FDTD) simulations, conducted for Ag nanoparticles and Ag-ZnO nanocomposites. The inputs for the size and gap between nanoparticles were obtained from the FE-SEM and TEM results of the Ag-ZnO nanocomposites. The shift in the surface plasmon resonance peak was observed in the extinction spectra by varying the gaps between the nanoparticles. The electric field distribution around the metal nanoparticle was also studied. Both experimental and theoretical calculations, based on plasmonic absorption and electric field enhancement, demonstrated the capability of Ag-ZnO nanocomposites as effective SERS substrates for optimized detection of organic molecules.

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High-Order Resonance Raman Spectroscopy

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Linear Raman spectroscopy is a standard analytical tool used in countless applications, but the response from spontaneous Raman scattering is typically weak. Using resonance Raman and coherent processes with phase matching leads to directed beams and larger signals as well as femtosecond time resolution if ultrashort laser pulses are employed. If the signal is fully phase-resolved as a function of all excitation-pulse delays, this leads to “coherent multidimensional electronic spectroscopy” that can be used to analyze not only electronic but also vibrational dynamics and their mutual coupling [1].

A powerful theoretical concept for the description of such methods is perturbation theory. Experimental challenges occur because higher spectroscopic orders overlap with lower-order ones leading to artifacts, and cascading lower orders may appear along directions of desired higher orders. This has been problematic for early attempts at fifth-order Raman spectroscopy [2]. While using electronic resonance can avoid “cascading artefacts”, the question remains how to separate nonlinear orders without overlap.

Here we show how to isolate nonlinear response systematically up to arbitrary orders [3–6]. Our comprehensive analysis reveals that, astonishingly, the effect of summing up tens of thousands of Feynman diagrams that enter the nonlinear response can be described by a simple analytical equation that separates all the orders. We have applied this scheme to obtain multi-particle correlations in squaraine polymers, the light-harvesting complex LHCII, the laser dye cresyl violet, CdSe/ZnS quantum dots, Si nanocrystals in a matrix, an RNA-templated merocyanine dimer, and plasmon–exciton hybrid systems (“plexcitons”). The procedure is generally applicable, easy to implement in any laboratory, and requires only straightforward intensity variation and appropriate linear combination of the obtained raw data. We perform simulations with our own open-access software package [7].

The systematic extension of the coherent resonance Raman concept to high orders of perturbation theory allows one to systematically increase the number of interacting particles in complex systems and reconstruct their dynamics.

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Ultrafast exciton dynamics mediated by vibronic couplings

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The ultrafast dynamics of non-equilibrium excitations in functional materials and nanostructures, triggered by light-matter interaction, rely on a complex interplay between electronic and nuclear motion. Vibronic couplings may significantly influence the initial energy flow and the motion of charges following photoexcitation. Detailed understanding of the underlying physics of photoinduced ultrafast phenomena is crucial for controlling nanoscale energy and charge transport, for the rationale design of efficient new materials and toward the development of quantum technologies. Most of the mechanisms underlying these processes occur on timescales of only few 100s of femtoseconds or even shorter, thus demanding methods combining high time resolution and the ability to unravel coupling mechanisms. We employ two-dimensional electronic spectroscopy with <10 fs time resolution to gain detailed insight into the light-induced ultrafast dynamics in technologically relevant materials and the fundamental role of vibronic couplings in these dynamics. In this talk I will give an overview of some of our recent results. Specifically, in thin films of aggregated quadrupolar molecules, we recently discovered intermolecular conical intersections governing the ultrafast energy relaxation within < 50 fs after photoexcitation [1]. The passage of the optically launched wavepacket through the conical intersection marks the transition from coherent propagation towards localization driving the first step in the ultrafast energy relaxation dynamics in these molecular aggregates. In halide perovskites crystals, we recently unveiled exciton Rabi oscillations driven by low frequency coherent phonon fields [2] indicating the importance of nonadiabatic couplings for coherent exciton dynamics in these materials. Our results suggest strategies to control ultrafast coherent dynamics in functional materials.

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Using Time-Domain Raman Spectroscopy to Expose Vibronic Mixing in Chlorophylls

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It has been shown that carefully designed femtosecond spectroscopy experiment enables measuring time-domain Raman signals both in ground and excited electronic states with high accuracy and fidelity [1]. Furthermore, employing polarization control to measure Raman signals (vibrational coherences) in time domain, allows for selectively extracting particular vibrational signals, for example, only those pertaining to the vibronic coupling in the molecules. In the presented work we use this sensitivity to study vibronic coupling in chlorophyll molecules.

Electronic-vibrational (vibronic) coupling has been suggested to play an important role in photosynthetic processes, such as energy transfer and charge separation. Even though this coupling in molecules has been suggested almost a hundred years ago, it has proven to be a highly elusive phenomenon to investigate. It can, however, be revealed by studying coherence signals. To this end we employ polarization-controlled 2D electronic spectroscopy together with advanced analysis that allows to extract full information about coherences, including their nature, which in turn directly reports on vibronic mixing.

In 2013 Raimers and co-workers proposed that vibronic mixing is ubiquitous in chlorophyll family of molecules [2]. We sought for experimental confirmation and refinement of these ideas by studying two chlorophyll molecules. We started with low temperature studies of Chl c1 [3] and then carried out a following study on Chl a. By analysing coherences observed in 2DES experiments we found clear evidence of vibronic mixing of the two lowest electronic states, Qy and Qx. Importantly, we distinguished multiple vibrational modes that are involved in vibronic mixing. Results obtained in our studies together with numerous less direct indications of vibronic mixing in a large range of chlorophyllides clearly establish that vibronic mixing is ubiquitous and should be taken into consideration when studying various functions chlorophyllides perform in biological systems.

In principle vibronic mixing allows for subtly adjusting molecular quantum states to match resonances important for energy and electron transfer functions. Presence of the ubiquitous vibronic mixing in chlorophyllides and also in excitonic photosynthetic systems rises an intriguing question, if it is an unavoidable consequence of the properties of the porphyrin-type molecules, or an optimization mechanism, which nature employs to finely tune primary processes of photosynthesis.

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Exploring Excited State Landscapes with Surface Enhanced Hyper-Raman Scattering

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Surface-enhanced hyper-Raman scattering (SEHRS) is the two-photon analog of surface-enhanced Raman scattering (SERS), which is a spontaneous non-linear scattering exhibited by molecules in a plasmonic field. Due to the complementary selection rules as compared to Raman and IR, this spectroscopic tool provides additional information on molecular vibrations. SEHRS has strong enhancement that enables the analysis of molecules at very low concentrations, high sensitivity can probe local environment changes and orientation of molecules and as it employs higher wavelengths in near IR region contributes to bioimaging. SEHRS in conjunction with SERS and theoretical studies could reveal the non-Condon effects which are highly difficult to probe otherwise. In this talk, we discuss the relevance and potential of SEHRS in understanding chemical systems in molecular regimes and the challenges and limitations associated with it.

Origin of unique hyper-Raman signals of sugar and model compound

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Hyper-Raman (HR) spectroscopy has been known as a unique method to study molecular vibrations because the HR signal intensity is related to hyperpolarizabiity and different from IR and Raman spectroscopy. The unique selection rule of HR spectroscopy is beneficial in detecting the IR and Raman inactive modes of highly symmetric molecules. It is interesting to consider the uniqueness of HR in the case of low-symmetry molecules such as biomolecules. In this study, we report unique information on sugar and a model compound obtained from HR spectroscopy.

Figure 1a shows the 532 nm-excited HR (thick) and Raman spectra (thin) of 50 mM glucose in phosphate-buffered saline (pH 7.4) containing 100 mM phosphate and 100 mM NaCl [1]. The bands at 1540 cm^{-1}, 1616 cm^{-1}, and the others in the HR spectrum are absent in the Raman spectrum and, thus, not readily assignable to the normal modes of glucose. We chose trifluoroethanol (TFE), having the CHOH group, as a simplified model compound. TFE (50 mM in aqueous solution at pH 13.2) is found to exhibit a similar HR pattern as glucose (Fig. 1b, thick) even though the Raman spectrum (thin) is not similar to each other. Considering the laser power dependence, the isotope effects, and the concentration dependence, we concluded that the radical anions of TFE and a TFE dimer were responsible for the unique HR signals [2]. Considering the calculated energy level of the electronic states, the double (pre)resonance effect with the one- and two-photon absorption bands would largely enhance the HR signal of the very minor species otherwise not detectable. Due to the similarity of the spectral pattern, the anion radical of glucose could explain the HR signal. This study exemplifies that HR spectroscopy provides unique information that Raman spectroscopy is inaccessible.

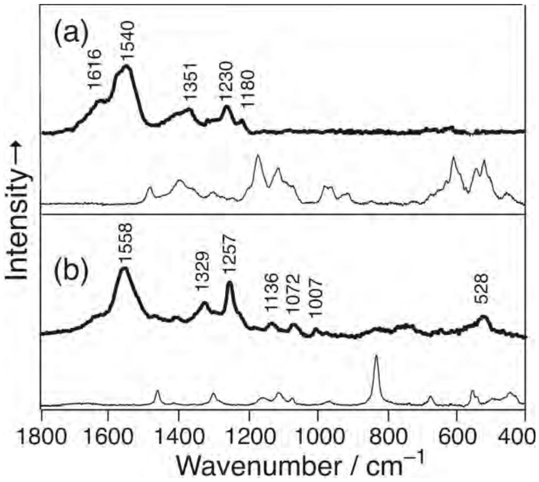

Figure 1: 532 nm-excited HR(thick line) and Raman spectra (thin line) of 50 mM glucose in phosphate-buffered saline (pH 7.4) (a) and 50 mM trifluoroethanol in aqueous solution at pH 13.2.

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Observation of Coherent Anti-Stokes Hyper-Raman Scattering

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We have first observed coherent anti-Stokes hyper-Raman scattering (CAHRS) signals [1]. Hyper-Raman (HR) spectroscopy has large potential as a tool complementary to Raman spectroscopy. Indeed, recent studies on HR spectroscopy have shown that HR spectroscopy offers information on molecular structures unobtainable from Raman spectroscopy [2, 3]. However, the extremely small cross-sections of HR signals have prevented HR spectroscopy from being applied to various fields. We have circumvented this disadvantage by employing a coherent Raman technique. CAHRS spectroscopy was theoretically proposed several decades ago [4, 5], but was not achieved experimentally. The CAHRS process, analogous to the CARS process, is a fifth-order nonlinear optical process based on six-wave mixing as shown in Fig. 1. In practice, a picosecond near-infrared laser (pump, ω_1) and a femtosecond visible laser (Stokes, ω_2) drive vibrational coherences of hyper-Raman active modes with the frequency of $2\omega_1 - \omega_2$, giving rise to a CAHRS signal whose frequency is $4\omega_1 - \omega_2$.

A CAHRS spectrum from benzene liquid around 700 cm^{-1} (Fig. 2a) obviously shows a spectral feature of the vibrational resonance at the 670 cm^{-1} a_{2u} band due to the CH bending mode. It should be emphasized that the 670 cm^{-1} a_{2u} band is HR and IR active but Raman inactive due to the selection rules, manifesting that the signal surely originates from the CAHRS process. Also, the dependences of the signals on the incident laser powers and polarizations support the origin of the signals. We further demonstrate that CAHRS signals from benzene have much higher signal-to-noise ratios than spontaneous HR signals by a factor of 10^2 in a short exposure time (~1 sec). Incident laser pulse energies are ~μJ, much less than the threshold of breakdown of liquids, enabling stable measurements. The results suggest that CAHRS spectroscopy can markedly shorten the measurement time of HR spectra. We believe that CAHRS spectroscopy can be an appealing tool in various fields, including material and biomedical sciences.

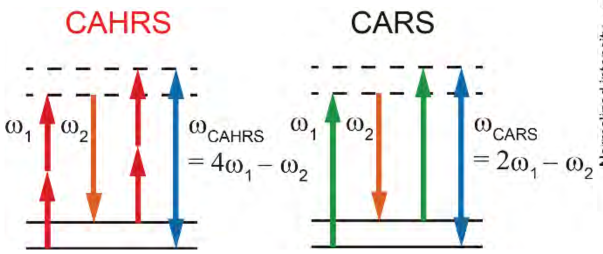

Figure 1: Ladder diagram for the CAHRS (left) and CARS (right) process

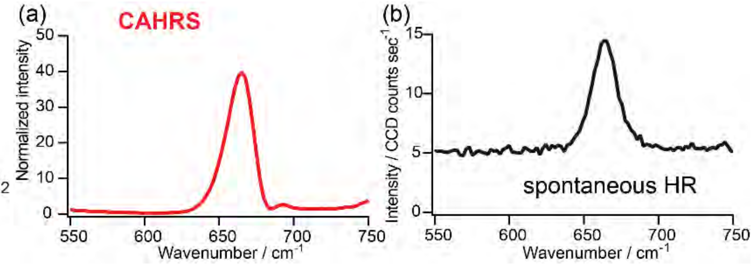

Figure 2: CAHRS spectrum (left) and spontaneous HR spectrum (right) from benzene liquid around 700 cm^{-1} region

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Supermultiplexed and Superdeep Surface-Enhanced Raman Scattering Bioimaging

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Multiplexed bioimaging has important applications in many fields, such as the acquisition of a full picture of immune checkpoints (ICPs) networks within the tumor microenvironment in order to develop combined treatment strategies for maximizing immune response. Non-invasive localization of deep lesions remains a long-standing pursuit for clinical applications. However, it is challenging to realize in vivo deep bioimaging via the optical modalities, particularly for those using the safe laser irradiance below biological maximum permissible exposure (MPE). We will address these two challenges via the surface-enhanced Raman scattering (SERS) technique in this talk.

We will first report supermultiplexed volume-active SERS (VASERS) nanotag platform [1], where Raman reporters are arranged in 3D volumetric electromagnetic hotspots. By examining various bio-orthogonal Raman reporters, we have developed frequency modulation guidelines and achieved 32 resolvable colors in the Raman-silent region, the largest number of resolvable SERS colors demonstrated to date [2]. These VASERS nanotags show great potential for imaging ICP networks in breast and gastric cancer tissues to predict the optimal combination of antibody drugs for immunotherapy.

Next, we will report non-invasive bioimaging of deep lesion deeply hidden in either ex vivo thick tissues or in vivo mice model with gap-enhanced Raman tags (GERTs) [3-6]. We have developed a home-built transmission Raman system with a laser power density of 0.264 W/cm^2 (< MPE) to achieve the detection of GERTs through up to 14-cm-thick ex vivo porcine tissues, as well as in vivo imaging of “phantom” lesions labelled by GERTs in an unshaved mouse. Furthermore, we demonstrate a universal method to achieve the depth estimation of lesions. This work indicates the potential of SERS bioimaging for non-invasive clinical cancer diagnosis.

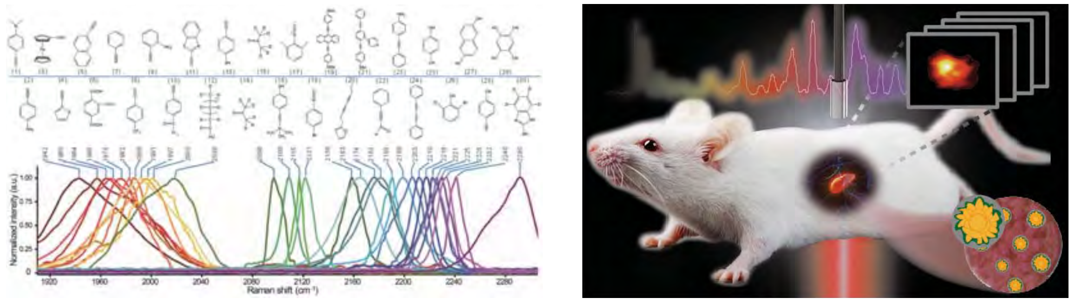

Figure 1: (Left) supermultiplexed bio-orthogonal VASERS nanotags; (right) schematic of non-invasive in vivo imaging of deep-seated tumor through living mice by using a transmission Raman setup and ultra-bright GERTs.

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High-throughput Raman-activated Cell Sorting and Sequencing via FlowRACS 3.0 and RACS-Seq/Culture

A single cell is the atomic unit of function and the basic step of evolution for life forms on Earth. Thus, rapid production of the single-cell “metabolic phenome” and its matching full “genome” plus “culturome” can address “who, what, why and wealth” in a microbiome at the ultimate precision. To fulfill this promise, we established the Ramanomics Technology Platform (RTP), by inventing novel instruments such as Flow-mode Raman-activated Cell Sorter (FlowRACS) and Raman-activated Cell Sorter coupled to Sequencing or Culture (RACS-Seq/Culture). In this talk, I will introduce two new technologies: (1) Rapid, label-free profiling of heteroresistance to antimicrobials by high-throughput Raman Flow Cytometry via the FlowRACS 3.0 instrument. (2) Linking ecosystem function (e.g., biopolymer degradation for environmental remediation) to single-cell *in-situ* metabolism, to the underlying single-cell full genome and eventually to the individual enzyme genes responsible for the ecosystem function *in-situ*, directly from microbiota samples, which is accomplished by a phylogeny-metabolism dual-targeted single-cell sequencing approach (FISH-RACS-Seq) via the RACS-Seq instrument.

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The formation of vascular lipid droplets and their ATGL-dependent lipolysis - spectroscopic and functional insight

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Lipid droplets (LDs) are dynamic organelles that serve as a site for the storage and release of lipids, however, their role in the endothelial cells (ECs) has long remained unexplored. Historically, LDs were considered as a passive deposition of lipids and to have a detrimental effect on ECs e.g. in atherogenesis. This perception has shifted, as results allowed us to understand that endothelial LDs' formation and their subsequent lipolysis play a vasoprotective role.

We studied the formation of LDs in isolated murine aorta subjected to various pro-inflammatory factors such as tumour necrosis factor (TNF), lipopolysaccharides (LPS), angiotensin II (AngII), hypoxia, or excess oleic acid (OA). All used compounds led to the increased formation of LDs. The analysis of Raman spectra of LDs in the isolated aorta stimulated by TNF, LPS, AngII or hypoxia uncovered the abundance of highly unsaturated lipids and absence of phospholipids and cholesterols. Additionally, by comparing the Raman signature of endothelial LDs under hypoxic or OA-overload conditions in the presence or absence of atglistatin, it was shown that atglistatin did not affect the biochemical composition of LDs.

Interestingly, the TNF-induced formation of LDs in the aorta was associated with cytosolic phospholipase A_2 ($cPLA_2$)- and adipose triglyceride lipase (ATGL)-dependent prostacyclin (PGI_2) release. Blocking the $cPLA_2$- and diacylglycerol O-acyltransferase-1 (DGAT-1)-dependent formation of vascular LDs, as well as inhibiting their ATGL-dependent lipolysis, resulted in the impairment of PGI_2 release. These results indicated that the vascular ATGL-$cPLA_2$-PGI_2—dependent pathway is linked to TNF-induced formation of LDs.

Since various diseases might alter the dynamics of LDs formation and ATGL-dependent lipolysis, our results suggest that pharmacotherapy focused on normalizing vascular LDs formation and lipolysis may provide a vasoprotection effect in vascular inflammation.

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Inside or outside the cells? The journey of a microplastic monitored by Raman microscopy

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The ubiquitous pollution of the ecosystems by microplastics (MPs) directly originates from the release and degradation of plastic waste in the environment. The identification of the particles collected in aquatic media, soils, air, or biological samples such as fish stomach, has been well documented by FTIR spectroscopy, Raman microscopy, and more recently pyrolysis-GC-MS. However, their detection in cells and tissues have been limited by the difficulty to image unlabeled MPs in living systems. Raman microscopy can fill this gap by providing label-free imaging of environmental MPs in biological systems at a single particle and subcellular level. In this study, mouse bone marrow derived macrophages and human primary blood neutrophils, an adherent and a circulating immune cell type respectively, were exposed to weathered MPs. MPs were irradiated by UV for 5 months in water to induce accelerated ageing of the polymer, generating unlabeled weathered micro- and nano-plastics (< 1 µm). Using line-illumination spontaneous Raman microscopy, we identified two modes of interaction between MPs and immune cells, depending on MP size and cell type. The localization of micro- and nanoplastics in different cell compartments by Raman microscopy could bring valuable information in toxicological studies to monitor the fate of environmental microplastics.

Raman imaging of plant leaf cuticle adaptations in extreme alpine environment

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Plant leaf cuticle sits as the external multifunctional surface at the interface with the atmosphere, protecting inner tissues from environmental stressors and dehydration. Recently, the power of Raman spectroscopy revealed insights into plant cuticles of different plant species (e.g. Norway spruce, tomato, Arabidopsis) by bridging chemical to structural data and visualising the multifaceted structure composed of chemically different layers [1, 2]. However, studies of the plant leaf cuticle of alpine species adapted to extreme environmental conditions are scarce [3]. Herein, we close this gap by studying chemical and structural variations of alpine plant leaf cuticles across different species and growth forms (dwarf shrubs, conifers and herbaceous species). Distinct patterns of lipids, carbohydrates and phenolic components of the cuticle as well as the transition to the epidermal layer are visualised. Based on the underlying Raman spectra we distinguished different groups of aromatic components and lipids and can suggest different roles in e.g. mechanical and UV-protection. The correlation between specific chemical compounds and their functions against environmental stressors gives us an insight into how diverse plant species cope with environmental changes.

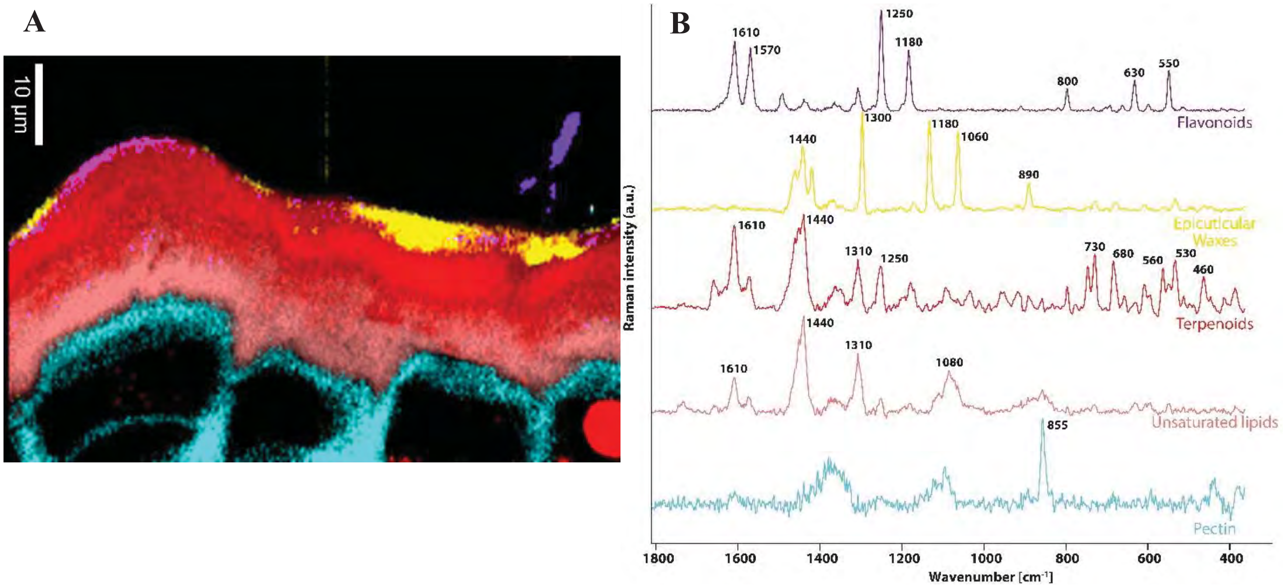

Figure 1: A. Raman imaging of *Kalmia procumbens* leaf cuticle. Two layers (the outer more aromatic [red] and the inner more lipidic [pink]) and the epicuticular waxes [yellow] form the cuticle. **B.** Corresponding spectra.

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Raman Imaging Algorithm for Analysing Therapy-Resistant Cancer Cells

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Glioblastoma commonly known as Glioblastoma multiforme is a primary glial tumour and most malignant form of brain cancer. With a high mortality rate of only about 5 percent, patients surviving more than five years. Despite significant advances in diagnosis and therapy, the outcome of gliomas remains unfavourable. The concept of cancer stem cells (CSCs) has emerged and provided new insights into GBM resistance and management. The complex relationship between the microenvironment and the behaviour of therapy resistant cancer cells and CSCs offers a promising direction for understanding cancer biology and development of target therapies. Yet their identification and characterization pose significant challenges in oncological research due to their small abundance and the lack of uniform and dependable biomarkers for CSCs across various types of cancer. A recent discovery, termed the Hydrogel Activated Reprogramming Phenomenon (HARP), reveals that cancer cells, when cultured on hydrogel substrates, can demonstrate stem cell-like features. This finding indicates that hydrogels could serve as a valuable tool for exploring CSCs properties [1]. Furthermore, integrating hydrogel-based cell culture with Raman microscopy, a non-destructive, label-free imaging method offering detailed spectral data, might enhance the identification and profiling of CSCs. When combined with chemometric techniques, this approach could provide precise analyses across diverse cancer types. Line-illumination Raman microscopy, renowned for its rapid imaging capabilities, has garnered interest for its ability to visualize the molecular composition within individual living cells [2-3]. Nonetheless, for effective chemometric analysis that differentiates cell types based on spectral and spatial data, it's critical to establish data normalization methods that counteract experimental biases, including inconsistencies in substrates and laser intensity variations in Raman imaging [4-5].

In this study, we introduce the HARP effect and Raman analysis of cancer cells (synovial sarcoma) on hydrogel platforms and the detection of molecular marker for chemotherapy resistant glioblastoma cancer cells. Subsequently, we describe a data preprocessing workflow designed to mitigate artifacts caused by hydrogels and line-scanning Raman microscopy. Our standardization strategy presumes that the surroundings of individual cells mirror experimental artifacts akin to those observed in the cells themselves. At first, our methodology involves segmenting Raman images into two distinct categories: individual cell and background regions (cell-free hydrogel areas), using an advanced hyperspectral image noise reduction framework. Next, we adjust each pixel in the Raman images of individual cells by deducting a locally computed, weighted Gaussian mean background, determined from the non-cell region. The Gaussian function's weights are fine-tuned using a Bayesian optimization algorithm to minimize the average spectral discrepancy among individual cells within a Raman image. We demonstrate that this preprocessing technique for Raman images effectively reduces spectral variability between different substrate types and across biological replicates. Additionally, we apply the algorithm to identify potential Raman markers in chemotherapy-resistant glioblastoma cells. In conclusion, our approach for local background correction promises to identify effective Raman biomarker in study of therapy resistant cancer cells.

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Cannabidiol as a potential cell response modulator in radiotherapy – spectroscopic perspective

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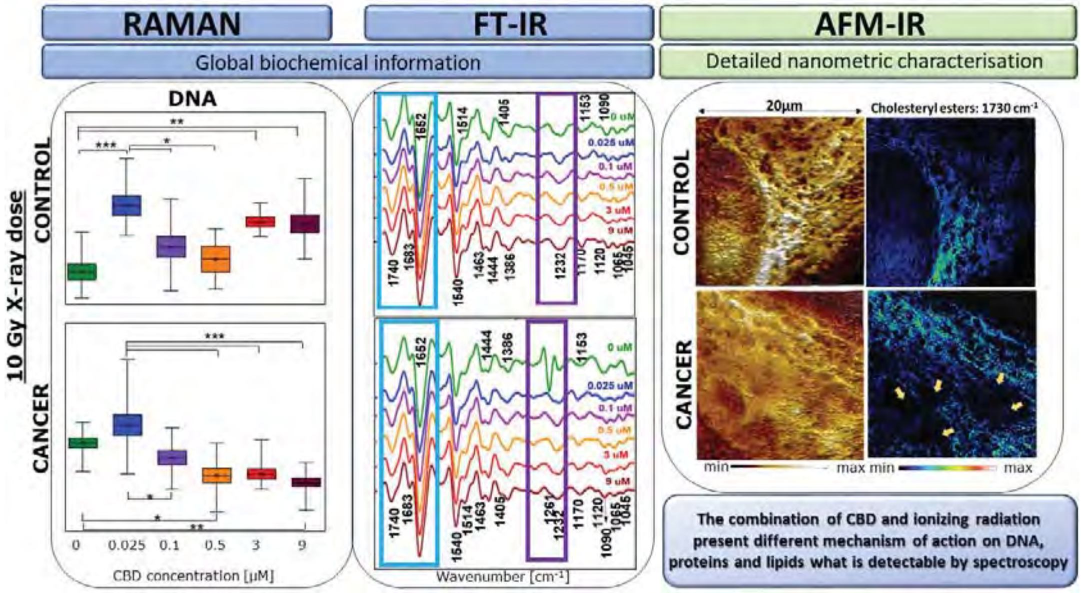
Nowadays, cannabidiol (CBD) has been of interest to many researchers due to its proven anti-inflammatory, neuroprotective, and antioxidant properties.[1] Interestingly, CBD affects cancer cells by inhibiting their growth.[2] The use of CBD as a supporting agent was already successful for glioblastoma treatment, however the application of CBD during the radiotherapy of peripheral nervous system (PNS) has not yet been investigated.

Since spectroscopic techniques provide overall information about the biochemical changes present in various samples, they also allow to track the effectiveness of applied therapy. For that reason, Raman, FT-IR and AFM-IR (Figure 1) were implemented to investigate the potency of a combination of CBD and ionizing radiation in PNS tumor therapy. The presented study allowed for the determination of types of molecules (mainly DNA, ratio of α-helice and β-sheet proteins and cholesteryl esters), that are modified in cancer and normal cells by CBD and significantly influence their radiosusceptibility. Such modification in cell response to ionizing radiation was confirmed by comet assay.



The combination of CBD and ionizing radiation present different mechanism of action on DNA, proteins and lipids what is detectable by spectroscopy

Figure 1: Presentation of exemplary results obtained from Raman, FTIR and AFM-IR data.

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A single-system solution for multimodal label-free imaging

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Multimodal measurements are vital for us to fully characterise and understand our samples, ultimately advancing our scientific endeavours. Traditionally, these measurements would be conducted on multiple instruments, making accurate repositioning of samples and correlation of results challenging, especially at the micron scale.

Here, we present a solution for performing multimodal measurements: Raman spectroscopy, second harmonic generation (SHG), fluorescence lifetime imaging (FLIM), photoluminescence (PL) spectroscopy, brightfield microscopy and darkfield microscopy on a single instrument. In this case, all techniques are performed using the same optical pathway ensuring that they are co-located. This enables one measurement area to be defined for all techniques, making correlation trivial.

To demonstrate the advantages of this system, we investigate two scientifically interesting samples: 1) molybdenum disulphide (MoS_2) flakes; and 2) a whole tooth section.

The MoS_2 flakes showed features on a submicron scale which would make correlation when transferring between instruments extremely challenging. Here, multimodal analysis was performed to obtain detailed information about MoS_2 flake properties. Raman spectroscopy provided information about layer number; SHG provided information about crystal lattice orientation; and PL spectroscopy and FLIM highlighted regions subjected to strain. The detailed information provided by the correlative analysis enabled the quality of the MoS_2 flakes to be accurately assessed.

The whole tooth section presents a much larger sample size so accurately locating small sub-areas when transferring between multiple instruments would be challenging. Here, correlative Raman spectroscopy and SHG were used to analyse the intersection between enamel and dentine in the tooth. Specifically, Raman measurements provided information about the chemical composition of the different tissue types, in particular the presence of collagen and phosphate, whilst SHG provided information about the collagen microstructure. The results demonstrated a high level of collagen with a fibrillar structure in dentine, and a lack of collagen but high phosphate content in the enamel. This was consistent with the current understanding that enamel is composed almost entirely of mineral, whilst dentine also contains organic material.

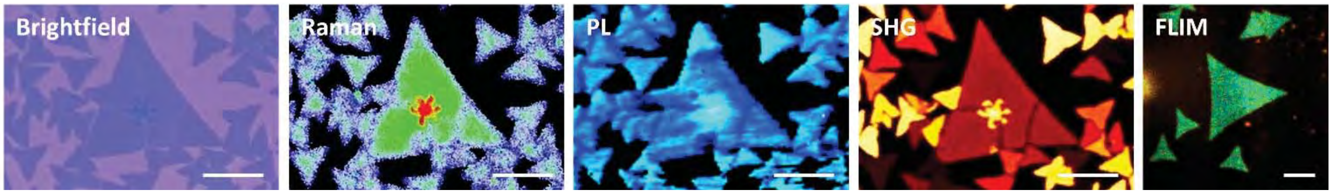

Figure 1: Correlative brightfield microscopy, Raman spectroscopy, PL spectroscopy, SHG and FLIM analysis of MoS_2 flakes, scale bar shows 10 μm.

Evaluating the solvation energy of the proton in super-acidic imidazolium ionic liquids using Raman Spectroscopy

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Understanding the levels of acidity accessible in non-aqueous solvents is paramount for developing new chemical syntheses and applications in non-aqueous media [1]. The highest level of acidity (lowest pH) accessible in a media is often expressed as the proton transfer activity coefficient, which is related to the free energy of transfer when the proton is transferred from one solvent to another, experiencing difference in solvation [2]. Proton solvation is particularly interesting to study in stable room temperature ionic liquids (RTILs), resulting from the combination of organic cations (often derivatives of N,N′-substituted imidazolium, N-substituted pyridinium, quaternary ammonium or phosphonium) and inorganic or organic anions (e.g. carboxylates, sulphates, halogens), that were recognised as an alternative to volatile organic solvents (VOCs) for inorganic and organic syntheses, catalysis, electrolytes and microextraction, , especially for their capacity to dissolve sugar-based biopolymers such as lignin [3-6]. The presence of small fractions of molecular impurities in RTILs has major impacts on their physico-chemical properties, especially the acidity levels accessible in these solvents, which need to be addressed to develop more robust applications. Experimental values for the transfer activity coefficient of proton are essential to confront the computed values, as determining free energies of proton solvation, either experimentally or theoretically, in non-aqueous solvents is one of the most discussed questions in physical chemistry [7]. For the proton, values for the transfer activity coefficient can only be experimentally estimated from extra-thermodynamic models applied to electrode potentials, solubility or spectroscopic measurements [8,9]. Here we report on the novel approach estimating the free energy of solvation for proton in stable N,N′-substituted imidazolium ionic liquids using far-field classical Raman spectroscopy and the acidity function proposed by Hammett [10] in different RTILs. Results obtained for RTILs built from four different cations (C_2, C_4, C_6 and C_8 N-alkyl, N'-methyl imidazolium) and 4 different anions (OTf^-, NTf_2^-, BF_4^- and PF_6^-) will be discussed.

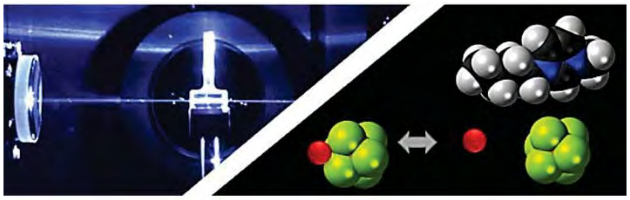

Figure 1: Raman spectroscopy to evaluate the solvation energy of proton in RTILs.

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Dynamic and Controllable Characterization of Biological Macromolecules at the Single-Molecule Level using Optical Tweezers-Coupled Raman Spectroscopy

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It is challenging to characterize single or a few biomolecules in physiological milieus without excluding the influences of surrounding environment. For example, intrinsically disordered proteins (IDP) lack stable secondary structures as monomers in aqueous environments, but sometimes self-assemble into oligomers with various structures and further grow into amyloid fibrils, which are associated with neurodegenerative diseases. However, the molecule mechanisms remain unclear due to the challenge of detecting the transient, low-populated, and heterogeneous oligomers among predominating monomers.

Here, we develop an in situ optical tweezers-coupled Raman spectroscopy to visualize and control the hotspot between two Ag nanoparticle-coated silica beads, generating the adjustable and reproducible surface-enhanced Raman spectroscopy (SERS) enhancements with single-molecule level sensitivity. This dynamic SERS detection window is placed in a microfluidic flow chamber to detect the passing-by proteins, which precisely characterizes the structures of three globular proteins without perturbation to their native states. It resolves the structural variations of alpha-synuclein arisen from its transient species in the low population at physiological concentration, which are buried under the averaging signals in the conventional bulk measurements but crucial for the initiation of its amyloid aggregation associated with Parkinson's disease [1]. Furthermore, we have constructed a simple plasmonic junction between two silver nanoparticle-coated silica microbeads to exert optical plasmonic trapping upon on/off laser excitation to control the formation of dynamic nanocavity with high turnover efficiency, reaching single-molecule level sensitivity to unveil rare pH-dependent amylin (hIAPP) species [2].

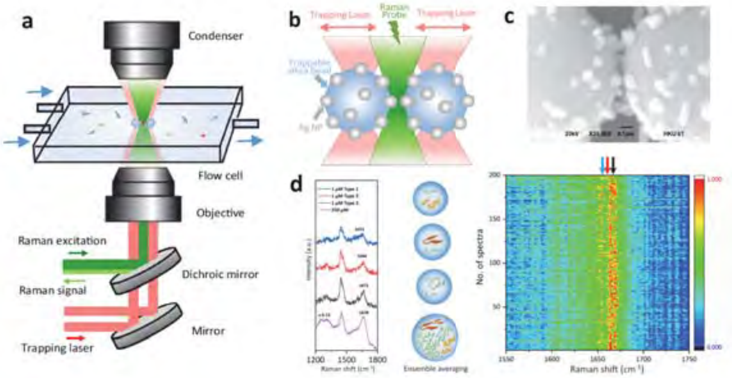

Figure 1: a. Optical tweezers-coupled Raman spectroscopic platform. **b.** Illustration of controllable hotspot. **c.** SEM image of Ag nanoparticle-coated silica beads. **d.** Spectra of alpha-synuclein.

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Exploring Excitation Resonance in Chirally Pure Nanotubes by Full Spectrum Raman Excitation Mapping

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The wavelength of the incident laser can be important in Raman scattering (RS) because of resonance. This effect is prominent in single walled carbon nanotubes (SWCNTs) because they have chirality-dependent strong optical absorption peaks in the visible and near infrared wavelength ranges. We have developed a simple and fast technique to obtain the RS spectra and Raman excitation profiles (REPs) of many bands simultaneously in one shot over a broad range. We call this full spectrum Raman excitation mapping (FS-REM).[1][2]. The experimental Raman excitation maps (REMs) contain rich, detailed information originating in the electronic and vibrational properties of the sample. Samples of different chiralities of SWCNT show different maps because of their specific vibrational modes and different electronic resonances. We experimentally show the variation of the most prominent Raman bands, as well as less studied bands, for several examples of current state-of-the art chirally pure SWCNTs separated by aqueous two-phase systems or by polymer wrapping [species labelled by indices including: (6,5),(7,5) and (8,3)]. For every sample, every band has its own resonant structure, and there is quantum interference between resonances. Since each peak has its own resonant structure, peak intensity ratios - such as the D/G ratio widely used as a measure of crystallinity in graphite carbons - must be interpreted with some care in resonant RS. While the general features of the maps can be understood simply in the broadest of pictures, the experimental maps show complicated structures. We believe FS-REM has potential application for more sensitive and selective chemical fingerprinting, and being able to quickly obtain REMs opens up some possibilities we are beginning to explore including a more refined metrology of absolute peak intensities, and practical real-time REM.

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From SERS to PIERS: Unveiling the power of light at the interface of Ag nanoparticles and thin TiO_2 film

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The innovative integration of semiconducting, photosensitive, and plasmonic materials has opened doors to novel analytical techniques like Photo-Induced Enhanced Raman spectroscopy (PIERS). This novel approach leverages photo-activated semiconductor substrates adorned with metallic nanoparticles (MNPs), offering significant advantages over the widely used Surface-Enhanced Raman Spectroscopy (SERS). In PIERS, photoactivation of the substrate with UV light induces charge transfer between the substrate and the analyte molecules, leading to increased chemical enhancement, and at least an order of magnitude higher sensitivity compared to SERS.

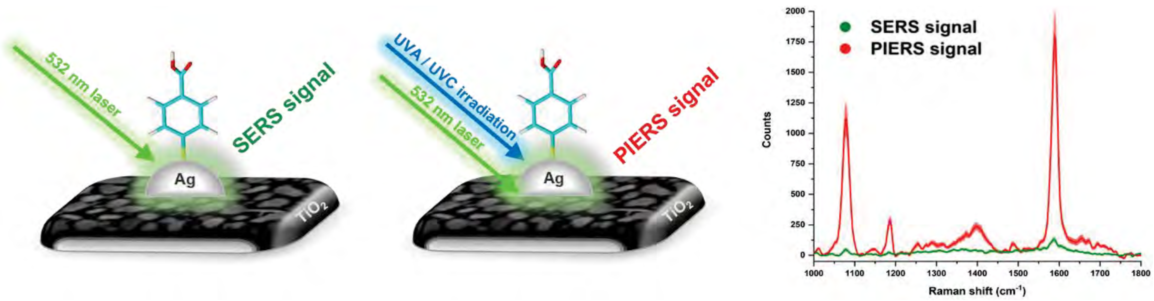

Figure 1: Schematic representation of SERS and PIERS.

Ag-TiO_2 nanoplatforms for SERS and PIERS applications were fabricated using rapid and reproducible sol-gel and photoreduction methods under controlled conditions. Tested nanoplatforms with localized surface plasmon resonance (LSPR) in the UV and Vis regions are characterized by a long-lasting and robust PIERS enhancement factor, up to 20 times higher than SERS, far surpassing the capabilities of most substrates presented in the literature to date. Our results showed that the PIERS signal enhancement is highly sensitive to the structure and plasmonic features of the Ag-TiO_2 interface and photo-irradiation conditions [1,2]. Additionally, we discovered that the presence of a Schottky potential barrier at the Ag-TiO_2 tight junction and the overlap of the semiconductor's absorption band with LSPR of MNPs affect the charge accumulation in the nanoplatforms, which has a positive effect on the PIERS lifetime. The obtained results provide insight into the mechanisms of charge transfer and accumulation in the heterostructure of AgNPs and anatase-TiO_2, which are responsible for induction and prolonged PIERS effect.

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Dual-mode Multichannel Instrument for Broadband Stimulated Raman Imaging

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Coherent Raman scattering (CRS) microscopy is a powerful technique for label-free molecular identification. Stimulated Raman scattering (SRS) is the preferred CRS modality due to the lack of non-resonant background. In SRS, synchronized pump and Stokes pulses impinge on the sample and the stimulated Raman gain (SRG) of the Stokes or the stimulated Raman loss (SRL) of the pump are measured. Since SRG/SRL signals are small ($10^{-5}\div10^{-4}$), they are detected by high-frequency modulation of the pump/Stokes and synchronous demodulation of the Stokes/pump by a lock-in amplifier (LIA). Single-frequency SRS microscopy is however not sufficient to distinguish different components within complex heterogeneous systems. For broadband SRS microscopy, which requires signal demodulation at multiple frequencies, a multichannel LIA (m-LIA) is a crucial component [1].

Here, we present a dual-mode, m-LIA based instrument (shown in Figure 1) for the simultaneous acquisition of the SRS spectrum at 76 Raman shifts in parallel. The developed platform can work in two different modalities: (i) in fast acquisition mode, it acquires the SRS spectrum in ≈20μs, detecting SRG/SRL signals down to $5\cdot10^{-5}$ with an optical power of only 10 μW per channel; (ii) in high-resolution mode it can reach a minimum detectable SRG/SRL signal of $3\cdot10^{-6}$. The core innovation enabling these results is based on a double demodulation scheme. The first demodulation is performed in the analog domain by a custom CMOS front-end and enables low-noise extraction of the SRS signal; the second is performed in the digital domain by 76 custom-designed parallel FPGA-based Digital Signal Processing (DSP) chains [2]. The hybrid modality is preferable to a fully analog one since the second demodulation in digital domain is insensitive to offset and 1/f noise coming from the electronic chip. On the contrary, a fully digital approach would require 76 fast digital LIAs working in parallel, to handle the huge amount of data coming from 76 fast ADCs (with a sampling frequency higher than 20MSps), dramatically increasing the system complexity.

The innovative architecture of this spectrometer allows (i) overcoming the limit set by the electronic noise of the instrumentation and (ii) handling optical signals modulated up to 10MHz with optical excitation power down to 10μW. As shown in Figure 2, our double-demodulation technique over-performs standard LIAs with single demodulation approach: the measured offset is as low as a few tens of μV and insensitive to temperature-related drifts, reaching a minimum detectable signal of 3ppm.

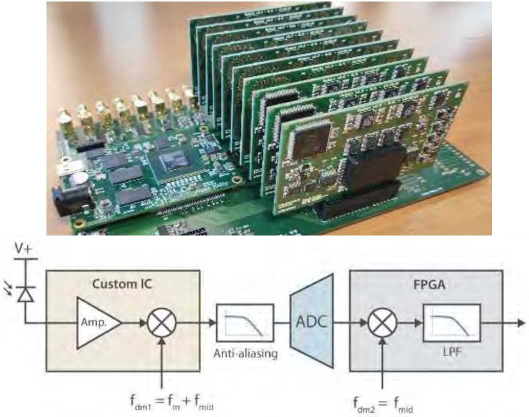

Figure 1:76-channel platform (top)
Simplified single channel architecture (bottom)

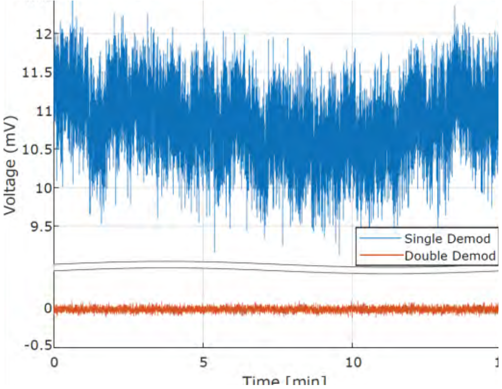

Figure 2: Voltage output offset of a standard LIA ($\overline{V} = 10.8mV$, $\sigma = 387\mu V$, red line) and of our double-demodulation system in high resolution mode ($\overline{V} = 0.4\mu V, \sigma = 18\mu V$, blue line)

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Exploring Chemical-Physical Interactions Between Carbon Atomic Wires and Silver Nanoparticles Using SERS and Synchrotron-Based UV Resonant Raman Spectroscopy

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Carbon Atomic Wires (CAWs) are linear sp-hybridized carbon chains consisting of alternating single and triple bonds, possessing strong π-electron conjugation and remarkable optical, mechanical, and electrical properties that are modifiable by the chain lengths and terminations[1]. Despite their remarkable properties, CAWs face stability issues[1] and low physical production yields[2], posing challenges for detection and characterization via conventional Raman spectroscopy[3]. Stability can be enhanced by adsorbing CAWs onto the surfaces of metal nanoparticles[4]. Detection limits can be overcome using Surface-Enhanced Raman Scattering (SERS) with metal nanoparticles as signal enhancers, or Resonant Raman (RR) spectroscopy by exciting these systems at one of their absorption peaks[5]. Notably, RR has been successfully conducted at the IUVS beamline at the Elettra synchrotron (Trieste, IT), using the synchrotron radiation as a tunable excitation source (tuning step of 1 nm) from 200 to 272 nm, precisely within the CAWs optical bandgap range[5].

This study combines SERS and RR spectroscopy to explore the chemical-physical interactions between silver nanoparticles (AgNPs) and CAWs, elucidating the role of NPs in stabilizing these systems. By gradually diluting a colloidal solution of AgNPs in water with a solution of size-selected CAWs (i.e., same chain length and terminations), we monitored Raman peak intensities during the dilution process (Fig. 1). Intensity is influenced by various parameters, with self-absorption (SA) of the sample being one of the most important: since both CAWs and AgNPs absorb within the Raman excitation source's spectral region, some incoming and emitted photons are absorbed, reducing Raman spectra intensity. When NPs and CAWs interact, their absorbance at certain wavelengths is slightly perturbed, varying the SA compared to NPs and CAWs alone. We measured these variations and the relative SA by comparing Raman intensities for samples containing both NPs and CAWs with reference samples (absence of CAWs, NPs, or both). This method enables the correlation of AgNPs-CAWs interaction strength with SA variations. By conducting these experiments with CAWs of varying lengths and terminations, we correlate CAWs' chemical structure with their ability to interact with metal nanoparticles. This innovative approach can be expanded to study other systems, facilitating the exploration of diverse nanostructured interacting systems.

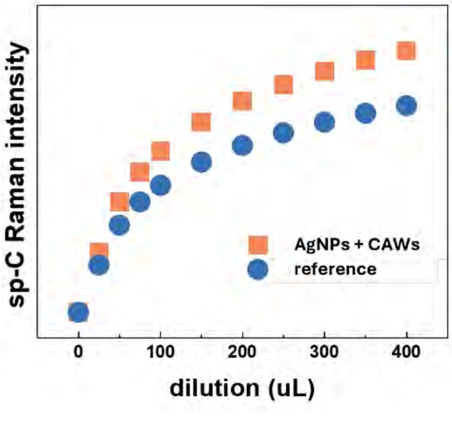

Figure 1. Evolution of the Raman intensity collected at 226 nm of the sp-carbon peak of CAWs at different dilutions in the presence (orange) and absence (blue) of AgNPs. The differences in the two curves manifest the interaction between AgNPs and CAWs.

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Spontaneous Raman flow cytometry using time delay integration

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Flow cytometer is a powerful tool to analyze biological cells, with applications spanning across biomedical research and industry[1]. Fluorescent labels on cells are commonly used for cell analysis and their ability facilitates high throughput analysis. However, these labels can potentially induce photodamage or alter the cellular conditions.

Replacing fluorescence spectroscopy with Raman spectroscopy enables label-free analysis and provides rich chemical information in flow cytometry. However, the low cross-section of Raman scattering necessitates a relatively long exposure time, which makes it challenging to be implemented in high throughput measurement with a high-speed flow. The use of cell trapping is one of the ways to implement Raman spectroscopy in flow cytometry. The cell motion is arrested to realize long enough exposure for Raman detection but the throughput is limited at 4.5 events/s in non-resonant regime[2]. The use of coherent Raman scattering can shorten measurement speed and the throughput of up to 2000 events/s was achieved[3], but it also limits the detection wavenumber region.

In this research, to simultaneously realize a high flow rate and enough long exposure time, we implemented time delay integration in spontaneous Raman flow cytometry. Figure 1 shows the optical setup. An excitation light-sheet was employed to illuminate samples along >800 μm of the flow path. The cell flow was aligned along the confocal slit placed at the entrance of the spectrophotometer. The flow rate was controlled to match the charge transfer rate of the CCD, resulting in signals from each cell being integrated separately along the illuminated area. We tested high throughput measurement of polystyrene beads by the system and a throughput of 77 events/s was achieved. We also demonstrated the measurement of biological cells. The discrimination between HepG2 cells with and without supplied free fatty acid was successful.

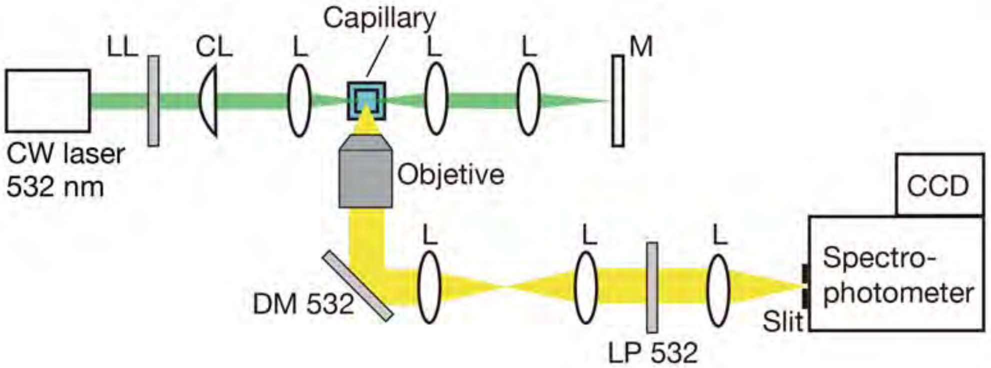

Figure 1: Optical setup. LL, CL, L, M, DM, and LP are laser line filter, cylindrical lens, lens, mirror, short-pass dichroic mirror, and long-pass edge filter, respectively.

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A multimodal wide-field Fourier-transform Raman microscope

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State-of-the-art Raman microscopes are based on point-scanning frequency-domain detection and have long pixel dwell times (of the order of fractions of one second), which makes it challenging to acquire high-spatial-resolution images over large sample areas (e.g., 100um x 100um). In addition, the weak Raman signal is often overlapped with an intense fluorescence background (such as, e.g., from endogenous fluorophores in cells/tissues [1]) which hinders its detection.

In this work we present an innovative compact wide-field Raman microscope based on time-domain Fourier-transform (FT) spectroscopy [2], obtained by adding an ultrastable common-path birefringent interferometer to a commercial microscope. Thanks to the FT approach, the system acquires in parallel spectra on all pixels of a 2D detector, enabling Raman imaging with diffraction-limited spatial resolution and image acquisition times up to 100 times shorter than with standard point scanning microscopes, while the spectral resolution can be decided by changing the scan length.

The time-domain approach offers the unique advantage of disentangling fluorescence and Raman signals through dedicated sampling strategies; as a result, the microscope is able to produce separately fluorescence and Raman maps, allowing multimodal acquisition across the same field of view [3]. We demonstrate the potentiality of the system by showing high resolution Raman images of plastic microbeads, while the multimodal operation is proven by capturing both fluorescence and Raman maps of a few-layers WSe_2 sample, as shown in Fig. 1. Fluorescence maps enable to identify the highly strained regions, while Raman-shift variations of 3 wavenumbers enable distinguishing the monolayer areas from the bilayer ones.

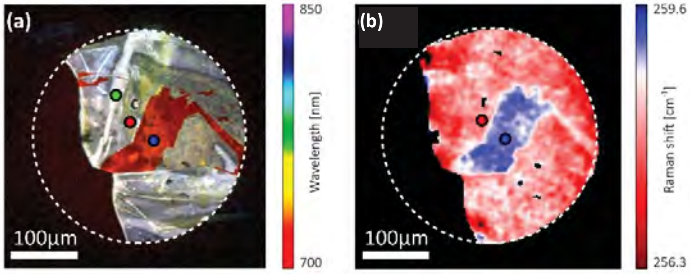

Figure 1: Florescence and Raman image of WSe_2. (a) False-color RGB image obtained from the fluorescence map. The circles indicate respectively 1-layer (1L, green and red) and 2-layer (2L, blue) WSe_2. (b) Peak map of the A_{1g} Raman mode. 1L and 2L are also indicated (red and blue circles respectively).

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Standardization and Harmonization Effort on Microplastics Analysis by Spectroscopic Methods

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Plastic pollution is recognised as a severe anthropogenic issue globally, where complex physio-chemical transformation processes (such as aging, degradation and fragmentation) producing Microplastics (MPs, 5000-1 µm) and, subsequently, Nanoplastics (NPs, <1 µm). Plastic debris comes in a huge range of sizes, polymer types, levels of physicochemical degradation and associated chemicals, therefore a combination of different sampling, sample processing and analytical techniques is needed. To date, many different analytical protocols, methods and techniques have been developed and applied to samples containing multiple polymer types and certain fractions of particle size. However, validated standardized methods remain unavailable at this point.

The most common techniques to reliably identify MPs are micro-Spectroscopy (Infrared or Raman) or Thermo-Analytical methods [1]. While the mass-based thermo-analytical methods can determine the overall mass of different polymer types (LOD in the µg range), the particle-based spectroscopic methods provide information on the polymer type, number, size and size distribution, and potentially identify a single polymer particle in the sub-micron range. While several studies provided a path forward to harmonization, there is an urgent need for collaborative method development, (certified) reference materials and inter-laboratory studies (ILS) to validate and harmonize the various methods (including how to report results) [2]. These efforts and tools are needed to enable a better assessment of data quality and to develop a more efficient and reliable measurement infrastructure in support of (i) ECHA's proposed restriction targeting intentionally added MPs in consumer products, (ii) the Marine Strategy Framework Directive (MSFD), (iii) the new Drinking Water Directive (DWD) that mentions MPs explicitly, and (iv) the new Circular Economy Action Plan (CEAP) adopted in March 2020.

In this talk, an overview of the more recent standardization and harmonization activities will be presented, including i) European projects (eg. EUROqCHARM, 21GRD07 PlasticTrace) that critically review the state-of the-art of the analytical methods and take harmonisation one step further with the preparation of suitable reference materials for QA/QC and harmonisation of methodologies; ii) ILC studies to validate the performance of spectroscopy methods (µ-IR, µRaman) and thermogravimetric methods (eg. VAMAS TWA45); iii) International initiatives and cooperation at the ISO level (eg. ISO/TC 147/SC 2/JWG 1) to set out the key principles for the investigation of microplastics in drinking water and water with low content of natural suspended solids using vibrational spectroscopy, with a main focus on sample preparation, measurement methods, data processing, and method validation.

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Raman Acoustic Tweezers

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Acoustic trapping allows to confine and manipulate millimeter and submillimeter particles in air [1] or in a fluid [2] using sound waves in the ultrasonic range. Recently, acoustic tweezers based on arrays of low-cost ultrasonic transducers have been reported [3, 4]. By opposing two dome-shaped arrays of transducers it is possible to create an acoustic standing wave, capable to trap millimetric particles and liquid droplets in its nodes. A great variety of particles can be trapped, with density as large as 6 gr/cm^3 [5]. In the case of liquids, volumes from few microliters to tens of nanoliters are only needed. In this presentation, we show the results of a novel setup which couples a low-cost acoustic tweezers and a compact Raman spectrometer (Raman Acoustic Tweezers, Raman-AT). The advantage of this combination is a container-less setup to carry out Raman spectroscopy in which particles do not chemically interact with any substrate and Raman spectra are not affected by any spurious background signal. We will show as it is possible to obtain Raman spectra of very small nanoplastics (50 nm in size) dispersed in a 1 μL water droplet, whereas the same drop deposited on a coverslip gives a Raman spectrum which is indistinguishable from the signal of the glass. Another interesting application is in the fact that due to solvent evaporation, the dispersion becomes more and more concentrated, allowing to detect the Raman signal of the dispersed material. An example of this phenomenon is given by the evolution of the Raman spectrum of a drop of a graphene dispersion in water. The combination of Raman spectroscopy and acoustic tweezers, allows the design of portable setups which can be used for environmental monitoring directly on-site. We acknowledge funding by the European Union (NextGeneration EU) through the projects PNRR-SAMOTHRACE (ECS00000022), PRIN2022-PLASTACTS (202293AX2L), PRIN-ENANTIOSELEX (2022P9F79R), PRIN2022-COSMICDUST II (2022S5A2N7).

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Fast microplastic detection by Raman microscopy coupled to essential information selection

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Microplastics, small fragments of plastic with a size of up to 5 mm, are a significant environmental threat due to their widespread presence in both aquatic and terrestrial ecosystems. They are bioavailable to various organisms across the food web and their environmental impact is a recent concern with ongoing research. Vibrational spectroscopy is a powerful tool for microplastic identification, but it is challenging to detect microplastic fragments due to their small size [1]. As minor compounds, microplastics may give very small contributions to the total recorded signal and it is difficult to extract such contributions by the direct application of multivariate curve resolution (MCR) techniques to the whole datasets collected. Essential spectral pixel selection is a promising approach for reducing imaging data without loss of relevant information and thus for the detection of minor compounds [2]. Recent developments in this sense (i.e., Essential information selection in the Fourier domain – EIFD) allow essential spectral pixels to be recognised while measurements are ongoing dramatically accelerating the data acquisition process [3]. Here, we used EIFD coupled to miniRaman microscope [4] for fast detection of microplastics: we started acquiring images of samples containing microplastics with an acquisition time of 100 ms per pixel. If some image pixels were found to be essential, we remeasured them with an acquisition time of 5000 ms. The reduced dataset of essential pixels (Fig. 1a,b) was finally used for MCR decomposition which yielded spatial distribution maps for all the components underlying the specimens under study (Fig. 1c). This strategy allowed us to decrease the measurement time significantly (approximately 50x) and simultaneously detect microplastic fragments in complex scenes.

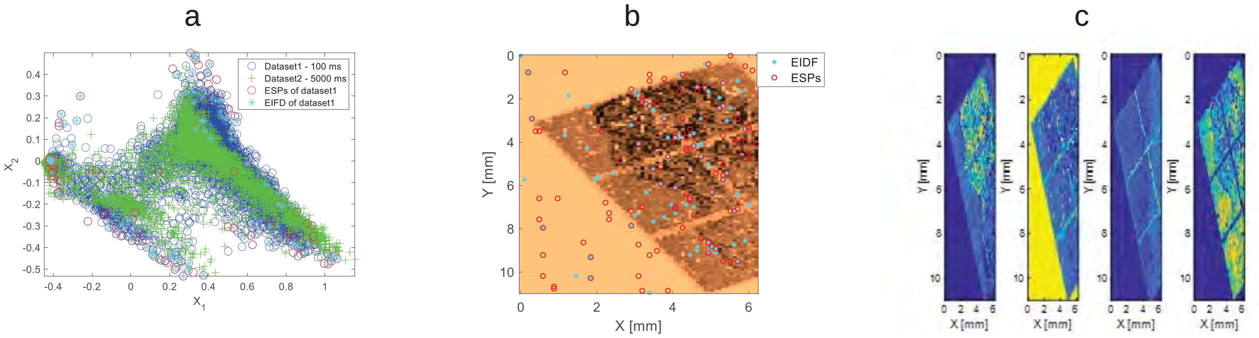

Figure 1: Essential pixels distribution (a,b). Spatial distribution of mixture components (c).

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Stable Isotope Raman Microspectroscopy: Applicability for Analysis of Microbial Degradation of Microplastics

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Stable isotope-based analytical methods gain increasing relevance in different scientific fields. Here, a combination of Raman microspectroscopy with the stable isotope approach – stable isotope Raman microspectroscopy (SIRM) can offer a non-destructive, quantitative spatially-resolved analysis. SIRM provides characteristic fingerprint spectra of samples with the spatial resolution of an optical microscope, containing information on stable isotope-labeled substances (based on red-shift of bands) and the amount of a label. This method requires no or limited sample preparation and can be performed *in situ* without interference of water. It provides information on the carbon metabolism / flow and the cell activity [1], and hence can be suitable for the analysis of the microbial degradation of a most prominent emerging pollutant in the environment and food – microplastics.

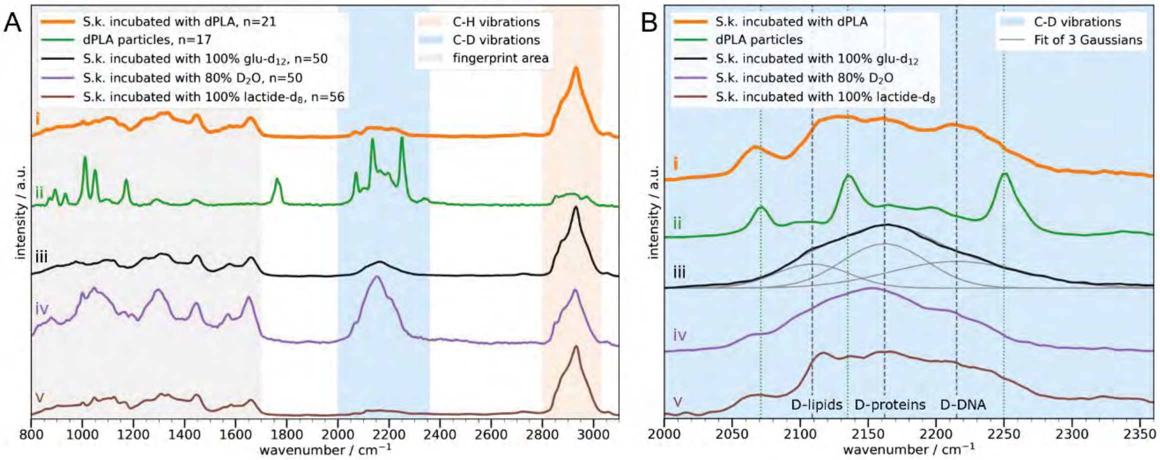

Figure 1: A. The mean spectrum of single-cell spectra of *S. koreensis* incubated with dPLA (i) in comparison to a mean spectrum of: dPLA particles (ii) as well as *S. koreensis* cells labeled with glucose-d$_{12}$ (iii), 80% D$_2$O (iv) or lactide-d$_8$ (v). B. The C-D region with Gaussian fits assigned to D-lipids, D-proteins, and D-DNA

The use of deuterated, instead of ^{13}C-labeled polymers, turned out to be a very suitable alternative (i.e., price, availability, pronounced Raman shift). We selected perdeuterated D polylactic acid (dPLA) as model plastics and traced the deuterium label during incubation experiments into microbial biomass of the environmental bacterium *Sphingomonas koreensis* using C-D vibrations (appear in the Raman-silent region of undeuterated biomass, Fig. 1) [2]. Single-cell analysis was the key to detect phenotypic heterogeneity, and to classify *S. koreensis* cells in two clusters: one showed a significantly stronger deuteration level than the negative *Escherichia coli* control, whereas the other was non-labeled. In summary, we demonstrated that the application of SIRM for the analysis of the biodegradation of D-labeled (micro)plastics can provide unambiguous direct information on the deuterium assimilation by microorganisms at the single-cell level.

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Automated analysis of small microplastic particles in environmental and food samples with Raman microscopy enhanced by Machine Learning methods

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The analysis of microplastic particles (MP) is, especially in the range below 10 µm, a complex multi-step process that includes sampling, sample treatment, measurement and data analysis. For each step, several standard operation protocols has been developed, but mandatory standards do not yet exist. Thus, comparability of results is an issue, even if the same methods are used. Result deviations can occur at each point in the process, but to ensure precision and accuracy, it is necessary to evaluate the contribution of each step.

We will focus here to measurement and data analysis of MP by Raman microscopy. For the measurement of MP < 500 µm, we filter all particles on silicon filters. The measurement of the filter is done by a combination of optical particle detection and Raman spectroscopy. The comparability of the results of different labs is an important issue than there can be very many particles, up to several thousands, on a filter. It is necessary to have an interlaboratory test that compares the results. We developed such a test by fixing defined particles in a defined area on a filter that can be sent from laboratory to laboratory. First results will be presented. Furthermore, we will show statistical subsampling strategies [1] and their error limits. For the data analysis, we use the open source program GEPARD [2, 3]. For the assignment of the spectra, a common method is an automated library search that uses comparison algorithms to assign the spectrum with the best match. Hence, the results often requires a manual follow-up control, which is time-consuming and prone to introducing human bias. As an alternative, we developed a machine learning-based approach for the assignment of the spectra. We trained a model to identify several of the most common polymer types and compare its performance to the routinely used spectral database analysis. First results will be shown.

In the second part of the lecture, we show results on selected samples from different environmental compartments. These are the determination of MP along a river, in a sewage treatment plant (influent, sludge, effluent), in an agricultural soil and in the atmosphere. As an example for MP analysis in food samples we show an inspection along a production line for mineral water (influent, filling, product). All these examples show that the presented combination of methods is very well suited to determine MP in various environmental areas.

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Real world case study quantifying microplastics in drinking water with Raman microscopy

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Microplastic pollution and its potential impact on human health is an area of significant concern, with drinking water being one of the main sources of human exposure. Identification of the amount and type of microplastics is essential to understanding their origin and prevalence in drinking water. Raman spectroscopy offers the high resolution and chemical specificity required to analyse microplastic particles.

This talk will showcase a real-world case study on microplastics in drinking water, including sampling and analytical methodology for microplastic identification using Raman spectroscopy [1]. Samples were obtained from a water treatment plant in Denmark over a five-day period, with sampling performed at the water inlet and outlet, to understand the effectiveness of the water treatment process. Darkfield microscopy was used to determine the amount and morphology of the microplastics present. An increase in the number of smaller particles at the outlet identified the breakdown of particles during the treatment process, as shown by the increase in particles below 5 µm in Figure 1.

Raman spectroscopy was used to chemically identify the particles. The number of microplastic particles was approximately 7,000 times higher than previously estimated using µ-FTIR [2]. The presence of nanoplastics was also investigated, with quantitative results demonstrating the presence of particles down to 0.45 µm. These results suggest that particles below 10 µm are not completely removed during water treatment, this corresponds to an estimated human intake of 1533 microplastics per year from the drinking water studied [1].

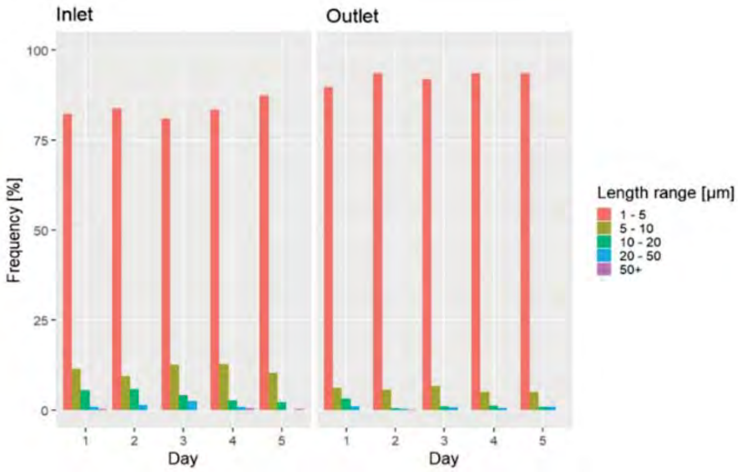

Figure 1: Diagram showing the distribution of microplastic size at the inlet and outlet over a five-day sampling period. The majority of microplastic particles are in the 1-5 µm length range.

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Advancing Microfiber Analysis using Femtosecond Stimulated Raman Microscopy (FSRM)

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Microplastics are omnipresent in the environment. Among these small particles, fibers are the predominant form reported in environmental samples [1]. An important source is laundry of textiles, as synthetic fibers make up a major part of global fiber production and a significant number of fibers is released into the effluent water during a washing cycle [2]. Recent studies, however, suggest an overestimation of synthetic fibers in environmental samples [3], emphasizing the need for accurate qualitative and quantitative measurement techniques. Raman microscopy, frequently used for assessing microplastic contamination, offers insights into particle number, morphology, size, and chemical composition. However, spontaneous Raman scattering microscopy faces several drawbacks such as the usually low Raman scattering cross section, resulting in long acquisition times, and interference from fluorescent background. Hence, there is growing interest in exploring non-linear Raman techniques like CARS [2] and SRS [3, 5]. Each method presents certain drawbacks; for instance, distortion by a non-resonant background or only partial spectral coverage.

Here, we present Raman imaging of microfibers of both natural and synthetic origins using femtosecond stimulated Raman Microscopy (FSRM). FSRM [4,5] enables rapid acquisition of complete Raman spectra free from distortions caused by fluorescence or a non-resonant background. As an example, FSRM imaging of nylon and polyester fibers is demonstrated (Figure 1), showcasing the capability to discern between different materials without the need for prior “chemical” knowledge.

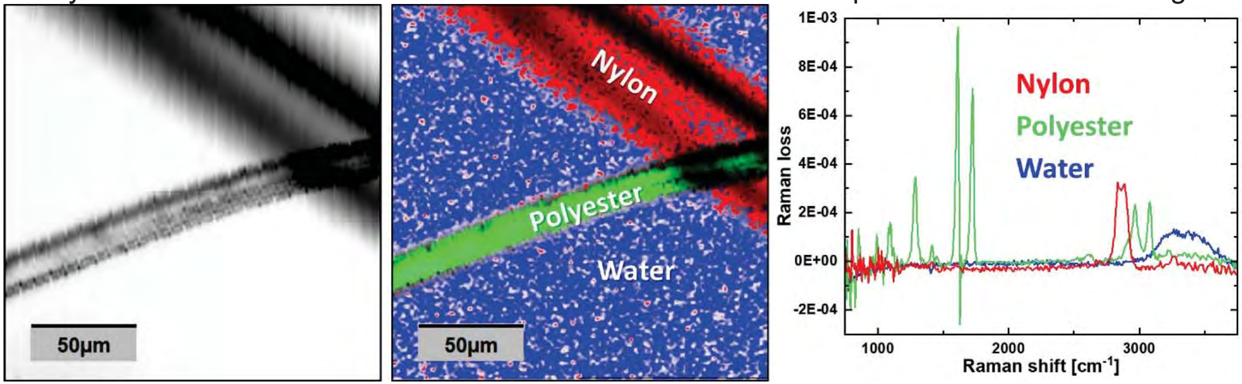

Figure 1: FSRM imaging of polyester and nylon fibers dispersed in water. Acquisition of the 200x200 µm^2 micrograph with a resolution of 1 µm took approx. 7 minutes. The transmission map on the left was generated employing the FSRM data. In the false color image (center) the chemical constituents are discriminated based on the assignment of their Raman signature to a cluster using a fuzzy c-means clustering algorithm. Representative Raman spectra are shown on the right.

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Correlative techniques to probe micro- and nanoparticles and their human health implications

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The presence of micro- and nanoparticles in the world has notably increased in recent years, especially those related to cosmetics products and plastic materials. Several scientific publications study the impact of such particle size range on human health [1,2]. A single technique cannot provide a full characterization of the particle, nor a complete understanding of its impact on humans. Multiple correlative techniques are required for a comprehensive analysis of the particles, which can vary in size, shape, and composition. This study outlines an investigation into the detection, characterization, and spatial mapping of these particles, emphasizing their presence in water sources and cosmetic products.

Microplastics coming from water sources are considered a primary human exposure pathway, becoming crucial to be analyzed, characterized, and identified as an important vector of human contamination. Cosmetics are composed of a complex mixture of components, including micro- and nanoparticles that, in contact with the skin surface, can easily penetrate and play a key role in the body, either by protecting the skin or affecting essential mechanisms. Both cases deal with complex matrices and deserve the combination of several techniques. Correlating Atomic Force Microscopy (AFM), which facilitates high-resolution imaging, enabling the visualization of surface morphology, mechanical properties, and spatial distribution of nanoparticles, and Raman spectroscopy, which discerns molecular composition, structural integrity, and chemical interactions, providing insights into the identity and behavior of nanoparticles, we were able to fully characterize these particles down to the nanoscale, gaining insights into their morphology, chemical composition, and distribution.

A key aspect of our study involves the application of nanoGPS technology, facilitating data correlation across diverse microscopy techniques. This method enables precise spatial localization of particles, allowing comprehensive integration of information from various analytical methods.

Our findings underscore the necessity for advanced analytical techniques in assessing health risks associated with micro and nanoparticle exposure.

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Abstract

Amyloid fibrils are insoluble, non-crystalline protein aggregates that are commonly associated with many diseases, such as Alzheimer's Disease and Type II Diabetes. Amyloid fibrils are also polymorphic: they differ in molecular structure, how they grow, self-propagate, and aggregate. Investigating how different fibril polymorphs aberrantly interact with their local biological environment is critical to understanding the clinical variations of diseases observed in patients. Unfortunately, investigating this is challenging, as there is a lack of bioanalytical methods to monitor the structure and dynamics of fibrils directly in cells or tissue. Here, we discuss our group's work in addressing this problem. We are developing novel structural characterization and chemical imaging tools using Raman spectroscopy to monitor the toxic activities of amyloid fibrils directly in their native biological environments. For example, we recently developed methods to measure important dihedral and bond orientation angles in fibrils. To determine the molecular-level structure of different polymorphs, we use these angular measurements as experimental constraints in molecular dynamics simulations. To probe the supramolecular structure of amyloid polymorphs, we have investigated low-frequency modes between ca. 6 – 200 cm^{-1}. We are working towards translating our methodologies towards Raman imaging applications to directly probe amyloid fibril structures in brain tissue with high-resolution. Our work provides a significant step forward towards establishing structure-toxicity relationships of different amyloid polymorphs.

Insights into Hydration and Hydrogen-Bonding in Aqueous Solutions of Ionic Liquids using Deep UV Raman Spectroscopy

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UV Raman spectroscopy was utilized to investigate inter- and intra-molecular Hydrogen-bonding in aqueous solutions of various model methylimidazolium-based Ionic Liquids (ILs) in the water-rich regime. Through a direct spectral subtraction procedure implemented in the region of the Raman spectra dominated by the CH and OH stretching vibrations, solute-correlated (SC) spectra were obtained, highlighting spectral modifications induced by the solute on the surrounding hydration water. The combined analysis of SC-UV Raman spectra and diagnostic Raman bands of the methylimidazolium [MIM] cation allowed for insights into Hydrogen-bonding features and aggregation of [MIM][X] solutions with different anions ([X] = [Cl], [HSO4], [NO3], [TfO]). Interestingly, the proton-acceptor capability of the anions changed in the order [Cl] ~ [HSO4] > [NO3] > [TfO], affecting the strength of double-ionic cation–anion H-bonds.

The analysis revealed the formation of cation–anion H-bonds with increasing IL concentration, particularly evident for [MIM][Cl], where these bonds were stronger than cation-water H-bonds. The strength of water-anion H-bonding depended on anion basicity, with [Cl] ~ [HSO4] > [NO3] > [TfO]. The data also suggested that chloride ions perturbed a higher number of water molecules compared to other anions. Additionally, the energy of H-bonds involved in hydration indicated a significant decrease in hydration enthalpies going from [Cl] to [TfO]. Notably, for $[NO3]^-$ and $[TfO]^-$based ILs, cation-water H-bonds were found to be stronger than double-ionic cation–anion ones, indicating a preference for water to hydrate the NH group.

Comparisons between spectral features observed for different Protic Ionic Liquids (PILs) and Aprotic Ionic Liquids (AILs) hinted at the potential modulation of water H-bonding and aggregation by changing the cation type. Further studies will elucidate these findings and explore additional PIL and AIL systems to provide a comprehensive understanding.

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Orientations and structures at model protein-protein interfaces from chiral nonlinear vibrational spectroscopy and molecular dynamics simulations

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Globular proteins are essential molecular machines, often forming functional complexes with other proteins via non-covalent protein-protein bonding. These protein-protein interactions are critical for most biological processes, especially in signal transduction, enzymatic reactions, and immune responses. Abnormal interactions can lead to diseases such as neurodegenerative conditions and cancer. Understanding these elementary molecular mechanisms at the protein interface is crucial for developing protein-based therapeutic agents, new functional biomaterials, and diagnostic tools.

In our model, the two partners are photoactive yellow protein (PYP), a sensory protein containing the common “Per-Arnt-Sim (PAS) domain” [1] known for mediating protein-protein interactions, and a self-assembled thin film of naturally occurring polymers of either the positively charged lysine (PLL) or the negatively charged glutamic acid (PLGA). Homodimers and heterodimers based on PYP maintain the function of a PAS domain and, therefore, constitute a crucial model system. Nevertheless, the target protein of PYP has not yet been identified. Here, we prepared homogenous polyelectrolyte layers with the spray-assisted layer-by-layer technique. PYP was then adsorbed onto the topmost layer of the multilayer structure. The structure of the topmost layer with and without PYP was then investigated by chiral and achiral broadband vibrational sum-frequency generation spectroscopy (BB-VSFG). BB-VSFG is a label-free analytical technique, allowing the study of slight structural and orientational changes of interfacial molecules with high sensitivity, even at the protein-protein interface. We studied the orientation of PYP monolayers with a high repetition rate BB-VSFG spectrometer [2,3] in multiple polarization combinations and the vibrational regions of 1500-1700 and 2800-3600 cm^{-1}. We found that the topmost PLGA layer forms a random coil structure [4], while the topmost PLL film has an anti-parallel β-sheet structure. PYP showed different orientation when adsorbing onto PLL or PLGA. Our experimental findings were compared to molecular dynamics simulations to extract the orientation of PYP on surfaces. We found structural similarities of our protein-protein model system, i.e., β-sheet PLL and PYP, with the structure of PYP and a large protein complex found in mice as voltage-gated K^+ channels. Our methodology provides a promising route for revealing orientational preferences of molecular groups during in situ protein-protein interactions and may help identify target proteins in PAS-domain signaling pathways.

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The method of assignment of low-frequency bands in Raman spectra of human hair keratins

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In this work, we propose a method of assignment of low-frequency vibrational bands of human hair keratins. The method is based on the polarization sensitivity of Raman bands of secondary structure elements of proteins. Besides, it can be used in the measurements of spectra with the help of Raman microscopes not equipped with polarization-sensitive elements. In this case, the Raman spectra of human hair measured at different orientations of the sample relative to the exciting radiation should be compared.

It is known that the low-frequency spectral range contains information about collective vibrational modes, such as vibrations of the protein chain or intermolecular vibrations of protein macromolecules. Low-frequency Raman bands are usually assigned to both vibrations of the secondary and tertiary structure of the protein. However, there is no unambiguous interpretation of the observed low-frequency lines.

Human hair is an appendage of the skin and consists mainly of fibrillar proteins – keratins. The main part of the hair (cortex) is formed by coiled-coil structures of α-keratins, which are oriented predominantly along the axis of the hair. The cortex is surrounded by a protective shell – the cuticle, which consists of keratins in a β-sheet conformation and disordered structures. Therefore, human hair is a convenient object for studying vibrational lines of the secondary structure of keratins in the low-frequency spectral range.

The intensity of high-frequency characteristic Raman bands of α-helices depends on the orientation of the hair relative to the exciting depolarized radiation: when focusing the laser beam on the end of the hair after obtaining its cross section according to [1] and when focusing on the side surface of the hair. The corresponding spectral difference in low-frequency spectral interval can be used to reveal the low-frequency bands of α-helices.

Raman spectra were measured using a DXR Raman microscope (Thermo Fisher Inc.) with excitation at 780 nm.

A comparative analysis of the Raman spectra of the cuticle, measured when focusing exciting radiation on the end of the hair and on its side surface, showed the low-frequency Raman markers of α-helices at 150 and 221 cm^{-1}. Comparison of the Raman spectra of cuticle and cortex confirms this result: characteristic bands of keratin α-helices appear in the spectral intervals of 110-165 and 200-240 cm^{-1}. Spectral differences observed in an interval 270-350 cm^{-1} can be attributed to vibrations of β- and disordered structures.

UV- and thermo-induced changes in low-frequency Raman spectra of hair are also discussed.

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Spectroscopic characterization of the coproporphyrin ferrochelatase from *Corynebacterium diphtheriae*

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Heme *b* is an iron porphyrin essential for bacterial pathogens to survive and infect the host. Unlike humans, monoderm (Firmicutes and Actinobacteria) Gram-positive bacteria produce heme *b* by using the coproporphyrin-dependent biosynthesis (CPD) pathway [1]. In the penultimate step of the CPD pathway, the coproporphyrin ferrochelatase (CpfC) catalyzes the insertion of ferrous iron into coproporphyrin III (cpIII), producing iron coproporphyrin III (coproheme). In the final step, the coproheme decarboxylase generates heme *b* by a two-step decarboxylation of the propionate groups at positions 2 and 4 of coproheme, forming vinyl groups [2]. Understanding the biosynthetic path of heme *b* in pathogens is essential for developing mechanism-based therapeutic drugs.

Our group previously investigated the CpfC of the firmicute *L. monocytogenes (Lm)* [3,4]. Here, the UV-vis electronic absorption and resonance Raman (RR) characterization of the wild-type (WT) CpfC from the actinobacterial *C. diphtheriae* (*Cd*CpfC) will be discussed.

Unlike *Lm*, but in common with the human ferrochelatase (fC), *Cd*CpfC contains a [2Fe-2S] cluster, a widespread inorganic iron cofactor whose role in fCs is still under debate. The apoprotein RR spectra are characterized by the bridging and terminal sulfur-iron stretching modes. Since these vibrations are sensitive to the type, configuration, symmetry, and nature of the ligands [5], insights into the structure of the cluster in solution have been obtained.

Moreover, the substrate (cpIII) and product (coproheme) are stabilized inside the active site of the protein by several hydrogen-bond interactions established between polar residues and the propionate groups of the porphyrin ring, in a similar way to *Lm* [3, 4].

Finally, the RR spectra of the CO adducts of the WT and selected variants of *Cd*CpfC complexed with coproheme will be discussed in comparison with those of *Lm*CpfC [6], providing information on the interactions of the distal polar residues with the iron-bound ligand.

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Key residues in the active site of a bacterial ferrochelatase

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Coproporphyrin ferrochelatases (CpfCs) catalyze the insertion of ferrous ion into the porphyrin along the coproporphyrin-dependent (CPD) heme biosynthesis pathway. The latter is unique to Gram-positive bacteria, as eukaryotes use the well-characterized protoporphyrin-dependent (PPD) biosynthetic pathway to accumulate heme *b* [1]. During an infection, heme biosynthesis is a highly efficient strategy for bacteria to survive since this cofactor is otherwise sequestered in high-affinity proteins of the host [2].

In the active site of all ferrochelatases, a conserved glutamate-histidine distal pair was identified as the iron binding site and was shown, in the human enzyme, to be the essential acid-base couple for the initial proton abstraction of the pyrrole nitrogen atoms, necessary for the insertion of divalent metals. On the other hand, the proximal ligand coordinating the porphyrin iron is not conserved through taxa and phyla and its catalytic role is still unclear [3].

Here we revisited the active site of coproporphyrin ferrochelatase by resonance Raman and electronic absorption spectroscopies, and biochemical characterization using the physiological product from the CPD pathway, coproheme. This porphyrin is characterized by four propionate groups, in contrast to previously-used porphyrins of the PPD pathway, having two propionate and two vinyl groups [4,5]. In particular, we compared the wild-type CpfC from the firmicute *Listeria monocytogenes* bacterium to variants on the distal site, exchanging the His or the Glu residues [6]. We also investigated selected variants on the proximal site, where the proximal Tyr was replaced by a His (i.e., the most common ligand in heme proteins), a Met or a Phe (as in human and actinobacterial ferrochelatases, respectively). Moreover, the binding of exogenous ligands, such as imidazole, was studied to give insights on the iron incorporation process.

The understanding at the molecular level of the role of these key residues in the heme pocket of ferrochelatases from pathogens is essential for the development of mechanism-based therapeutic drugs.

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A study of synchrotron-based UV-resonance Raman spectra of N-acetylamino saccharides – In combination with their ATR-far ultraviolet spectroscopy study

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Ultraviolet resonance Raman spectroscopy (UVRR) has so far been used for the structural analysis of proteins. Still, if the excitation light is extended to shorter wavelengths, it can be used to study the structure of sugars and lipids. In the present state, FUV spectroscopy rarely has been studied on sugars and lipids. Still, the complementary relationship between FUV spectroscopy and UVRR allows us to effectively promote the investigation of the relationship between electronic state and molecular structure. For example, FUV spectral data to determine the excitation wavelength of UVRR. On the other hand, it is expected that the UVRR excitation wavelength dependence will provide helpful information for analysis, such as attribution to the detailed structure of the bands in the FUV spectrum. We have developed the FUV spectra for bio-molecules using the attenuate total reflection technique [1] and interpretation of the FUV spectra using quantum chemical calculation. [2] In the present study, FUV spectra and UVRR were measured for N-acetylamino sugars, as seen in the sugar chains. In this presentation, we consider the assignment of FUV spectra based on quantum chemical calculations and discuss the excitation wavelength dependence of the UVRR spectra of N-acetylamino sugars.

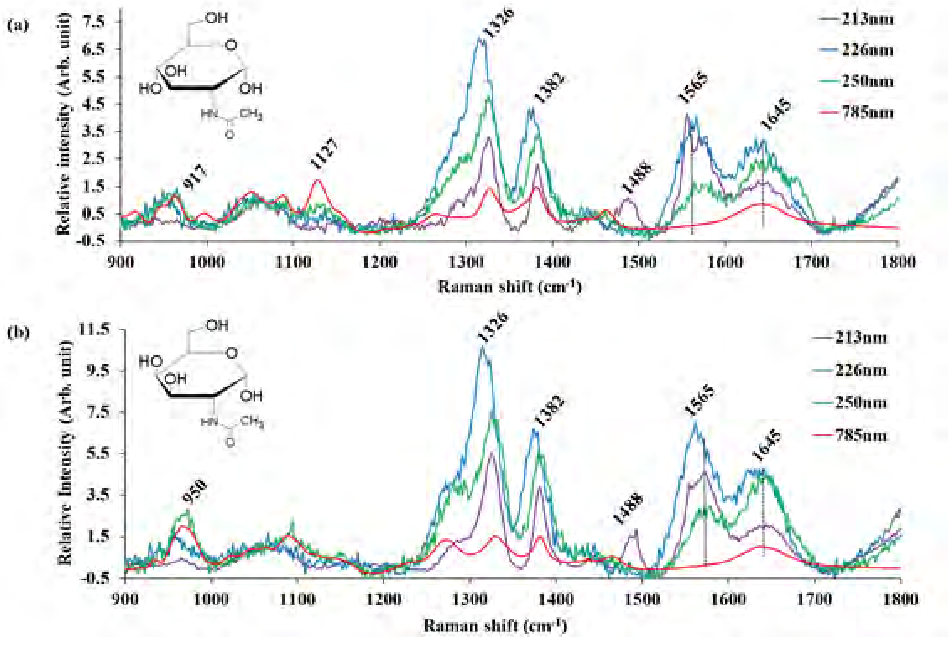

Figure 1: Raman spectra of (a) GlcNAc and (b) GalNAc aqueous solutions excited with different wavelength.

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Deep UV Raman spectroscopy for probing active eukaryotic viruses

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Deep Ultraviolet Resonance Raman Spectroscopy (DUVRR) is an emerging and powerful analytical tool that can be used for detection and characterization of biological samples in a label-free and real time approach. The excitation in the Deep UV provides specific resonance enhancement of biological moieties, especially protein, DNA and RNA structures (1, 2), allowing to efficiently isolate and detect biological Raman markers in the spectra. It is well known that DUV radiation is a genotoxic agent. Prolonged UV light exposition induces damage to the genomes of viruses, breaking bonds and forming photodimeric lesions in RNA (1-3). These damages prevent both transcription and replication which leads to viral inactivation. Even though the effects of UV radiation on DNA of microorganisms have been well-recorded, its impact on RNA and RNA modifications is less known. A sample that is prone to such a damage are viruses, and with the SARS-COV-2 (RNA virus) outbreak, the need of fast tools for viral characterization and classification became even more necessary.

In this work, we will show how to obtain stable, high quality and information-rich DUVRR spectra of active Vesicular Stomatitis Virus (VSV) without affecting its viability, RNA and protein integrity. By opportunely tuning the excitation wavelength, we can detect and assign in the vibrational spectra specific markers of lipid, protein and RNA components useful to elucidate biochemical characteristics of active VSV virus. In addition, we can study the effect of antivirals and DUV irradiation in the mechanism of inactivation of VSV viruses in order to find the most promising antivirals and damaging wavelength for the active VSV virus, respectively. These information can further be exploited to build effective countermeasure tools for pathogenic viruses

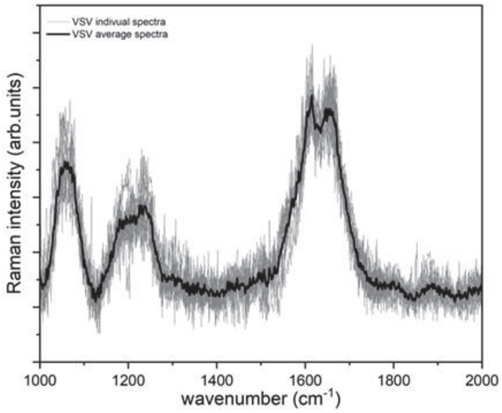

Figure 1. First Deep UV Resonance Raman spectrum of an active eukaryotic virus obtained with 213 nm as excitation wavelength.

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Dimensional Reduction of UV Resonance Raman Spectra of Proteins

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UV Resonance Raman (UVRR) spectroscopy reveals vibrational structures of aromatic side chains and backbone of proteins. In this presentation, we will present UVRR spectra of proteins in different conformations. The large data sets are analyzed with statistical methods to help reveal subpopulations within the ensemble. This combination of vibrational spectroscopy and statistical tools can be applied to a broad range of systems.

Interference Effect in the Tip-Enhanced Raman Spectra from Two-Dimensional Materials

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In this talk I will discuss the use of nano-Raman spectroscopy to study two-dimensional (2D) materials. When addressing 2D systems, the nano (tip-enhanced) Raman spectroscopy (TERS) technique [1] requires considering some new theoretical aspects, including field coherence [2-8] and field distribution [9,10]. Our state-of-the-art results in graphene and transition metal dichalcogenides will be presented, exploring the connection between micro- and nano-Raman metrology (see figure 1) [6,11]. Also, various aspects such as defects, homojunctions, twisted-bilayer structures, localized emissions at bubbles, wrinkles, and borders, as well as substrate effects will be considered. I will conclude by outlining some perspectives for nano-Raman spectroscopy in 2D systems.

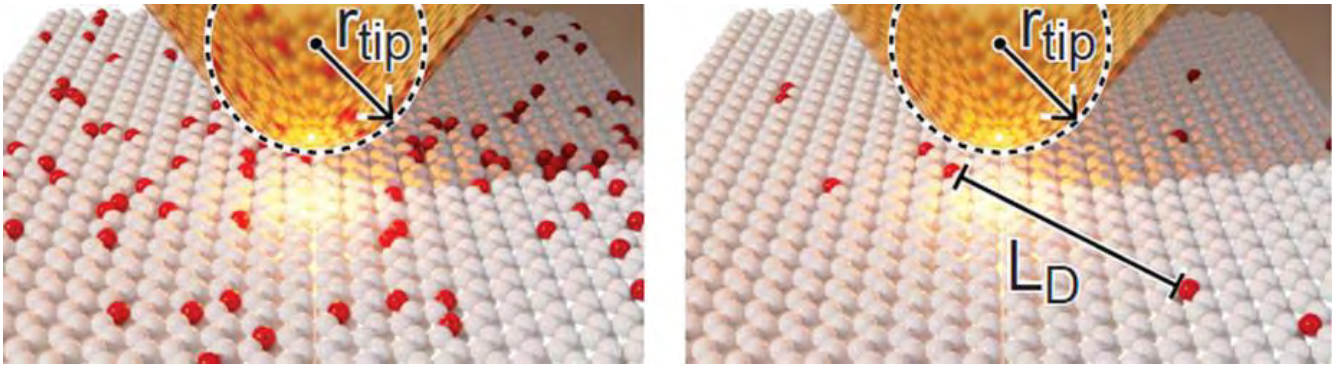

Figure 1: Schematics of a TERS tip probing defects in graphene with high (left) and low (right) defect concentrations [11].

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Raman signature of soliton network in twisted bilayer materials

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The phonon spectra of twisted bilayer graphene (tBLG) and twisted bilayer MoS2 (tBLM) demonstrate the signature of the presence moiré potential. [1] We study the evolution of the phonon bandstructure as a function of twist angle is examined using a band unfolding scheme where the large number of phonon modes computed at the Γ point for the large moiré supercells are unfolded onto the Brillouin Zone (BZ) of one of the two constituent layers. In addition to changes to the low-frequency breathing and shear modes, a series of well-defined sidebands around high-symmetry points of the extended BZ emerge due to the twist angle-dependent structural relaxation. The all-atom results are rationalized by introducing a nearly-free-phonon model that highlights the central role played by solitons in the description of the new phonon branches, which are particularly pronounced for structures with small twist angles, below a buckling angle of about 3.75 degrees.[2] In addition, a machine learning-based approach is developed to provide a continuous model between the twist angle and the simulated Raman spectra of tBLGs.[3] Findings from the theory are compared to experimental data from collaborators when available. [4–6]

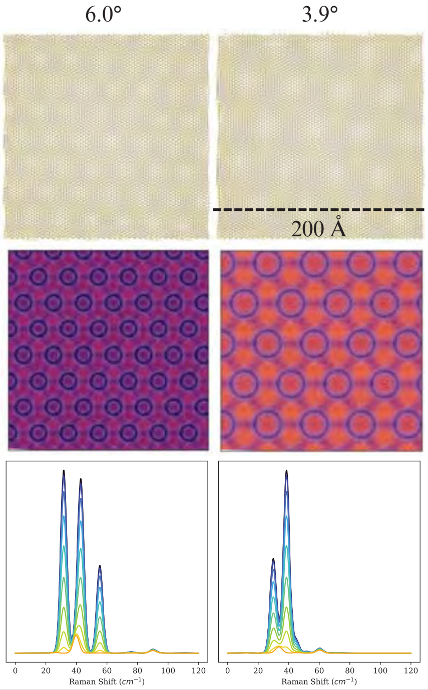

Figure: Twisted bilayer MoS_2 systems for two different angles, showing strong reconstruction (middle panel) and effect on calculated Raman spectra (bottom panel).

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Disentangling Doping and Strain Effects at Defects of Grown MoS_2 Monolayers with Nano-optical Spectroscopies

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The role of defects in two-dimensional semiconductors and how they affect the intrinsic properties of these materials have been a wide researched topic over the past decades. Optical characterization such as photoluminescence and Raman spectroscopies are important tools to probe the physical properties of semiconductors and the impact of defects. However, confocal optical techniques present a spatial resolution limitation lying in a μm-scale, which can be overcomed by the use of near-field optical measurements. Here, we use tip-enhanced photoluminescence and Raman spectroscopies to unveil the nanoscale optical properties of grown MoS_2 monolayers, revealing that the impact of doping and strain can be disentangled by the combination of both techniques. A noticeable enhancement of the exciton peak intensity corresponding to a trion emission quenching is observed at narrow regions down to 47 nm of width at grain boundaries related to doping effects. Besides, localized strain fields inside the sample lead to non-uniformities in the intensity and energy position of photoluminescence peaks. Finally, two distinct MoS_2 samples present different nano-optical responses at their edges associated with opposite strains. The edge of the first sample shows a photoluminescence intensity enhancement and energy blueshift corresponding to a frequency blueshift for E_{2g} and 2LA Raman modes. In contrast, the other sample displays a photoluminescence energy redshift and frequency redshifts for E_{2g} and 2LA Raman modes at their edges. Our work highlights the potential of combining both tip-enhanced photoluminescence and Raman spectroscopies to probe localized strain fields and doping effects related to defects in two-dimensional materials.

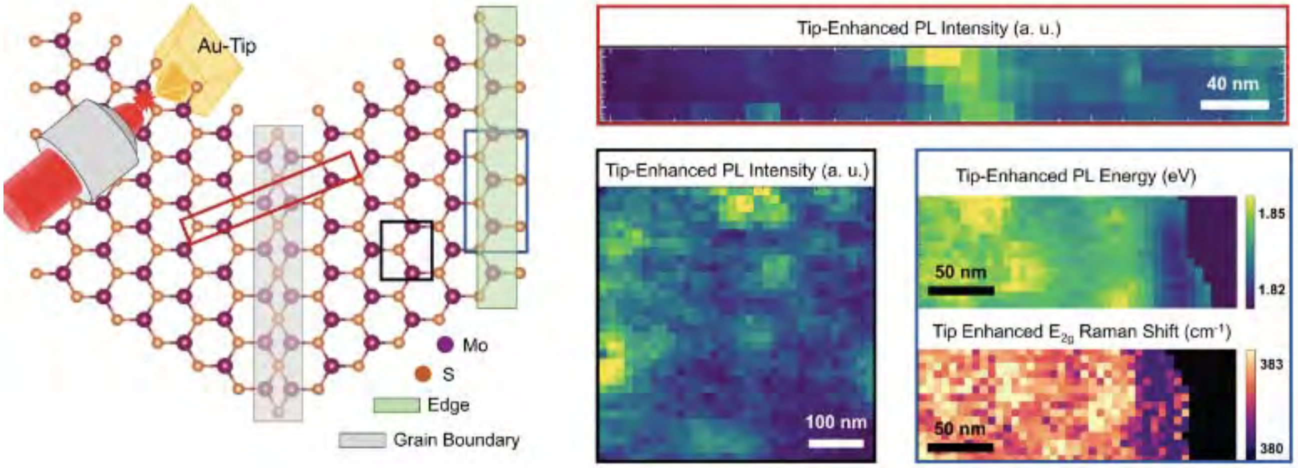

Figure 1: Illustration of tip-enhanced photoluminescence and Raman spectroscopy in different defects in MoS_2.

Magnetic Excitation-Phonon coupling in two-dimensional antiferromagnet above Néel temperature: A Raman study

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The dynamics of coupled quasi-particle excitations can be crucial for understanding and engineering the novel states of matter in two-dimensional (2D) magnetic materials. A strong correlation between different degrees of freedom exists in $NiPS_3$, a van der Waal antiferromagnet, which could lead to new bound states with hybrid attributes [1–3]. Characterizing and identifying the excitations corresponding to these bound states is essential as they allow manipulation of various optical and electronic functionalities. Raman spectroscopy is a non-destructive and highly sensitive optical technique for probing such excitations and their correlations in 2D materials [4]. Here, we report the survival of spin wave excitation and its coupling to phonons in $NiPS_3$ well above its Néel temperature (T_N ~ 155 K), at least up to $2T_N$ through Raman scattering measurements. Our measurements reveal as the spin wave excitation evolves with temperature, it couples from one phonon to another, exhibiting an intriguing signature of magnetic excitation-phonon coupling at high temperatures well above the T_N. This coupling manifests as a distinct Fano asymmetric lineshape in the Raman phonon modes, which evolves with temperature. The Fano anomaly in the phonon mode has been reported recently due to its interference with the electronic continuum [5]. However, our extensive Raman and magnetic measurements demonstrate that the Fano anomaly in the Raman phonon mode has a magnetic origin, not an electronic one. Furthermore, these observations are consistent even after the magnetic dilution of $NiPS_3$ by Zn doping at the Ni site. The survival of the coupling and magnetic excitation in the paramagnetic phase of $NiPS_3$ highlights its potential significance in exploring high-temperature magnonics and spintronic applications.

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Correlative polarization-sensitive Raman, PL, and SHG imaging of 2D materials

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2D materials exhibit unique qualities that show great promise for new designs in optoelectronics, nano-photonics, and bioengineering [1,2]. Understanding these materials on the nanoscale is essential to unlocking their potential and a combination of Raman, photoluminescence (PL) and second harmonic generation (SHG) imaging has emerged as a powerful approach for studying their structural and electronic properties [3]. The polarization-dependent optical response of 2D materials is particularly interesting for the structural insight it provides into 2D materials such as TMDs and graphene [3]. Consequently, there is an increasing demand for dedicated, high-performance instruments that offer not only polarization sensitivity, but also precise polarization control.

This presentation will highlight the latest advances in fully integrated systems that feature improved polarization performance and control.

In addition, examples of their application in a range of correlative Raman microscopy experiments will be provided: Besides their aptness for the requirements of 2D material characterization, the utility they can offer other scientific fields such as geology, life sciences, and pharmaceutical research will be illustrated.

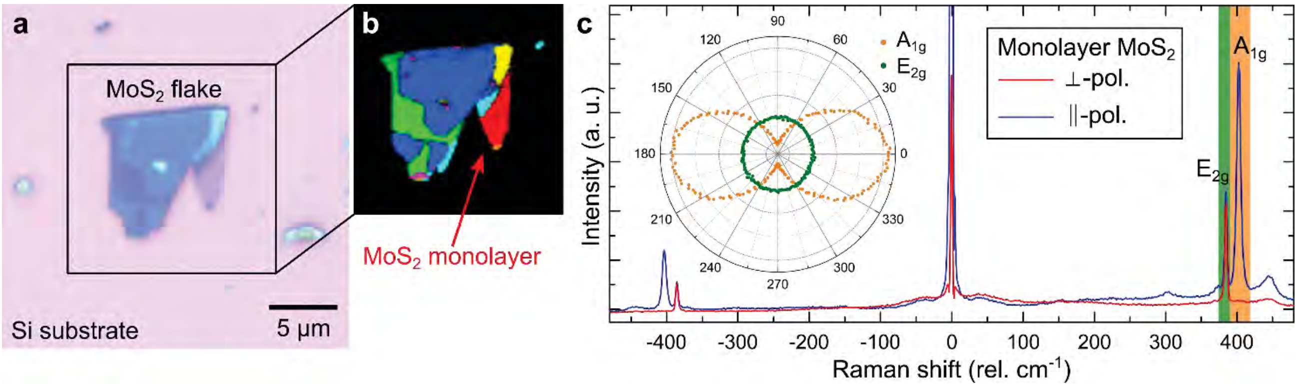

Figure 1: Raman study of TMD MoS_2 utilizing precise polarization control. a) Optical image of a MoS_2 flake on a Silicon substrate. b) False color-coded Raman image with layer number distinguished by Raman spectra. c) Raman spectra of monolayer MoS_2 recorded under parallel (blue) and cross (red) polarization. The distinct polarization dependency of the A_{1g} mode at 403 rel. cm^{-1}, in contrast to the E_{2g} mode at 385 rel. cm^{-1}, is revealed by the detailed polarization plot.

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Phase Transitions of Cesium Lead Bromide Perovskite Quantum Dots Studied by Temperature-dependent Photoluminescence and Raman Spectroscopies

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Lead halide perovskite quantum dots (QDs) have tunable band gaps, high extinction coefficients, narrow emission line widths, and long charge carrier diffusion lengths. However, more studies are needed on the phase transitions of lead halide perovskite QDs. Herein, temperature-dependent Raman and photoluminescence (PL) analyses were conducted to study pure optical properties and structural characteristics of cesium lead bromide (CsPbBr$_3$) perovskite QDs.

In this study, monophasic QDs were synthesized by using the hot-injection with high size-uniformity (10.4 ± 0.8$_5$ nm). Temperature-dependent Raman and PL spectra were measured at 10 K intervals from 180 to 420 K. The phase transitions can be divided into five regions (γ-Pm, gradual phase of γ-Pm → $P2_1/m$, β-$P2_1/m$, α-$P2_1/m$, and Im$\bar{3}$) by analysing the PL changes in intensity, peak shift, and width (**Figure 1**). The γ-Pm-CsPbBr$_3$ QDs exhibit PL blueshift as the temperature increases from 180 to 230 K because of the influence of thermal expansion. Interestingly, a gradual phase transition region of Pm → $P2_1/m$ at 240 – 280 K was found where the PL redshifts and the intensity increases, indicating a higher quantum yield will be driven. In addition, starting from 240 K, the Raman peak intensity at ~75 cm^{-1} grows up. This can be attributed to the narrowing of the Pb-Br-Pb bonding angle distribution in the [PbBr$_6$]$^{4-}$ octahedron. As the temperature increases to 290 K, PL intensity decreases concurrently with the Raman peak shift from 134 to 125 cm^{-1}, showing that the vibration of Cs$^+$ cation is highly affected by the transition to the β-$P2_1/m$ phase [1]. The purple and black regions encompass the reported α-$P2_1/m$ and Im$\bar{3}$ phase, respectively [1]. At 361 K, recent reports show the transition from orthorhombic (β-$P2_1/m$) to tetragonal (α-$P2_1/m$) [1-2]. The Raman signals were not clearly observed due to the thermal activation. At 403 K, the transition undergoes from tetragonal (α-$P2_1/m$) to cubic (Im$\bar{3}$) [1-2].

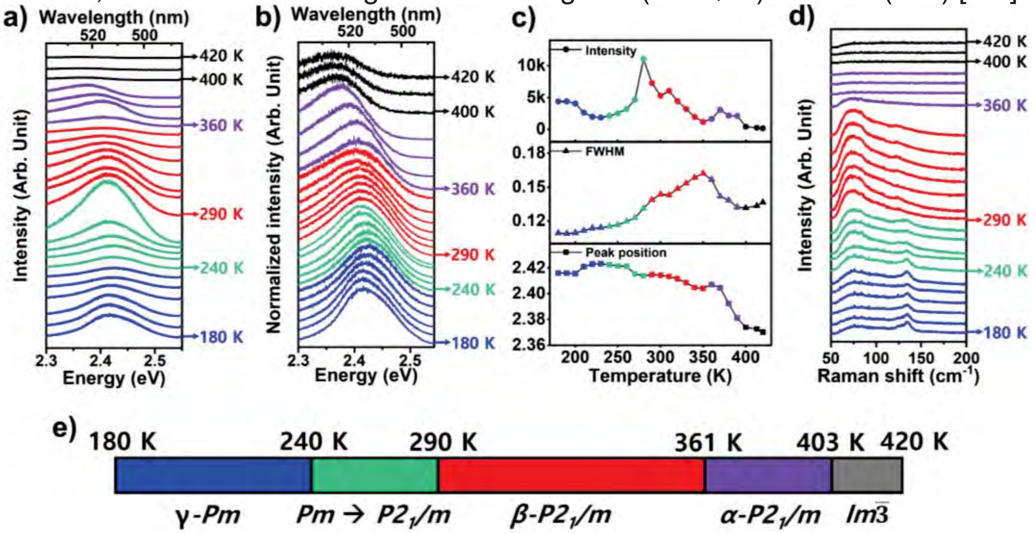

Figure 1: a) Temperature-dependent PL and b) normalized PL spectra. c) temperature-dependences of intensity, FWHM, and peak energy of PL. d) Raman spectra. The temperature interval is 10 K from 180 K to 420 K. e) Summary of phase transition of CsPbBr$_3$.

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Synchronous Chiral Discrimination and Identification of Aromatic Molecules by Chiral-Label-Free SERS

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Molecular chirality is a crucial characteristic of biological processes, and enantiomers of a chiral molecule may demonstrate striking differences in terms of physiological responses. The investigation of techniques for discriminating enantiomers is extremely important in various fields of science and technology, such as catalytic chemistry, biotechnology, and pharmaceutical science. A versatile and robust chiral discrimination strategy for small compounds is of significant importance in various fields. However, most current methods lack either the sufficient sensitivity for recognizing the chirality of the target molecules or their molecular specificity information. We have succeeded in developing a versatile and chiral-label-free SERS-based chiral discrimination sensing system for small aromatic molecules, where the molecular structural specificity and enantioselectivity can be given synchronously in a single SERS spectrum [1-3]. Vertically aligned Au nanorods (NRs) arrays modified with the achiral mixed-thiol self-assembled membranes were employed to differentiate and identify small aromatic molecules in solution by SERS. The key to success in chiral discrimination for this strategy is attributed to the synergy of the CT contribution and the EM enhancement of SERS.

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Development of a SERS biosensor for the detection of miRNA

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The development of Surface Enhanced Raman Scattering (SERS) nanosensors for the detection of biomarkers such as microRNAs (miRNAs) represents a significant advancement in biomedical technology [1]. To afford this, SERS requires low-cost materials and process capable of offering stability of the substrates where the Raman signal enhancement takes place and the design of functionalized biosensors for specific biomarkers and bioreceptors [2-3].

In this work, a novel SERS biosensor is presented for the specific detection of a miRNA biomarker associated with chronic obstructive pulmonary disease (COPD). Key features of this SERS biosensor include: 1) Molecular Beacon (MB) probe designed for the specific miRNA detection, as the biorecognition element of the sensor: the MB probe is labeled with a Raman reporter, enabling the detection of miRNA binding events through SERS signal changes; 2) platform of assembled silver nanowires (AgNWs) which enhance the Raman signals from the labeled MB probes and provide an ideal surface for sensitive and specific detection of miRNA biomarkers; 3) signal-off mechanism, where the presence of the target miRNA leads to a distinct change in the distance between the Raman reporter label and the SERS surface: this change results in a measurable SERS signal alteration, providing a sensitive detection mechanism; 4) sub-femtomolar detection limits, through optimization of the sensing surface: this exceptional sensitivity enables the detection of trace amounts of the biomarker, crucial for early disease diagnosis and monitoring; 5) specificity, ensuring minimal cross-reactivity with other biomolecules; 6) regeneration, allowing for multiple cycles of measurements without compromising sensitivity or specificity [4].

Overall, this SERS biosensor offers a highly sensitive, specific, and cost-effective platform for the detection of miRNA biomarkers associated with various diseases, including COPD. Its innovative design and exceptional performance pave the way for advancements in medical diagnostics and personalized healthcare.

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Understanding the Capping Agent / Metal Interactions in Colloidal Nanoparticles for the Direct SERS Measurement of Biological Analytes

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Surface-enhanced Raman spectroscopy (SERS) is based on a plasmonic, near-field effect, and thus, the intensity of the generated signal is a function of the distance between the analyte and the plasmon-sustaining surface. In other words, detection is enabled if analyte adsorption is thermodynamically favored. When colloidal nanoparticles are utilized as the plasmonic substrates, it must be kept in mind that their surfaces are never chemically bare; rather, they are populated by a multitude of adsorbed species. Consequently, the energetics of these adsorbed species will determine (i) the distance with which the analyte will be able to interact with the surface, and (ii) the type of interaction that will be established at the surface, ultimately dictating the measurement outcome, both in terms of spectral profile and signal quality.

Pre-adsorbed species on the surface of colloidal nanoparticles can be synthesis by-products (*e.g.*, oxidated or unreacted reducing agents) and stabilizers. Because stabilizers typically represent most of the surface adsorbed species in a colloidally stable sol, failure to consider their energetics when implementing direct SERS protocols might result in failure to observe the desired SERS signal. As a result, a rational analytical protocol based on colloid-SERS must consider not only the association constants between the analyte and the plasmonic surface (*i.e.*, the metal), but also those between the surface and pre-adsorbed species, and those between the analyte and pre-adsorbed species.

Despite its importance, the study of the interaction between colloidal sol stabilizers and plasmonic surfaces is an underexplored area of SERS research. In this presentation, an integrated analytical approach for the elucidation of capping agent interactions and associated thermodynamic quantities will be discussed, with a focus on how it can be leveraged for *ad hoc* SERS analytical protocol design for targets of biological and clinical interest. In our view, the study of the fundamental surface interactions taking place in a SERS sample can be instrumental in furthering the adoption of SERS as a mature and reliable analytical technique for everyday applications.

Detection of DNA Hybridization with Raman Spectroscopy

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Raman scattering is an inelastic light scattering process in which the energy of the incident photon is partially transferred to the excitation of the normal vibrations of the molecule. Since these vibrations are influenced by the composition, topology and atomic environment, the Raman spectrum is a kind of vibrational fingerprint that allows to identify the bonding configuration of the functional groups constituting the molecule, and even to track changes in the bonding structure. The latter can be utilized to monitor chemical reactions, like hydrogen bond formation during the hybridization of deoxyribonucleic acid (DNA).

DNA hybridization is technique used in molecular biology to measure the degree of genetic similarity between DNA sequences. In involves mixing of a known (single stranded) test DNA sequence with the sample DNA; followed by the denaturation of the sample DNA and the re-establishment of the hydrogen bonds (hybridization) with the test strand. The success of the latter process depends on the complementarity of the test and sample strands, and its positive outcome is a clear confirmation of the presence of the complementary sequence in the sample DNA.

In most cases, the occurrence of hybridization is detected by the well-known polymerase chain reaction and other techniques. Here we report on a Raman spectroscopy-based method in which the test strand is labelled with an alkyne group and the hybridization is detected through the changes in the parameters of the alkyne Raman peak, occurring due to the hydrogen bond formation. It was found that the variation of the peak parameters allows to distinguish between the complete (match) and partial (mis-match) hybridizations of the test and sample DNA strands allowing to identify the presence of mutations in the sample strand, making the method capable of the detection of single-nucleotide polymorphism (substitution of a single nucleotide at a specific position in the DMA sequence).

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Raman spectroscopy detects the label-free up-regulation of carotenoids within the life states of unicellular organisms and plant seeds

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Given the complexity of biosystems, carotenoids are known to participate differently depending on their surrounding nature. Carotenoids are known to serve as regulators in plant growth; on the other hand, they are considered to be signaling molecules in unicellular organisms. This study evaluates the molecular functions of carotenoids in different biosystems molecularly. We measured the Raman spectroscopic signals of intracellular carotenoids in marine biosystems such as Zebrafish larval, Triop-shrimp (Triops *longi-caudatus*), and segmented freshwater worm (*Aeolosoma A. viride*). To trace the metabolic changes of carotenoids molecularly; we evaluated the Raman signals of carotenoids during the seed inhibition, germination, and development throughout their life cycle. Since seed germination is a complex process and carotenoids trace origins from carrots; we have investigated seeds from carrots and green mung beans. Sharp Raman bands at 1519 and 1156 cm^{-1} of β-carotene were detected during the germination of mung bean seeds; revealing β-carotene up-regulation towards the core signaling the seed embryo to germinate. Raman signals of β-carotene dominated the root area and lutein at 1523 and 1157 cm^{-1} was highly accumulated in the photosynthetic region in carrot seeds. Raman signals of astaxanthin carotenoids were detected in the zebrafish larval after 72 hours post-fertilization. The detection of carotenoids in simpler models like carrot (*Daucus carota*) and mung bean (*Vigna radiata*) could be a non-invasive method to understand their role in complex bio-systems. Our results suggest that Raman spectroscopy and Raman mapping could be a non-invasive method for the detection of the migration of carotenoids in different bio-systems during their life states.

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Shining Light on Imperfection in Nanoimprint Lithography: Nanodome SERS for SARS-CoV2 Nasal Spray Detection

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Nanoimprint Lithography (NIL) is promising tool in SERS substrate development, which can lower the cost per measurement [1]. However, NIL often lacks fidelity between the template and the final structure, which is a hinderance towards use in quantification [2]. Here, we look at depression angle in the substrate plane, *via* white light spectroscopy in transmission. Principal component analysis was performed over a range of 0-1°. Confidence ellipses overlapped signifying (at $p=0.05$) that the performance of the sensing platform is insensitive to small imperfections up to 1° in planar angle misalignment. However, plane angle mismatch of up to 10° was also investigated and asymmetric Fano-like dips were noted beyond 4° signifying interaction between localised and propagating plasmon-polariton excitations. Thus, for optimal sensitivity impinging photons should interact with the substrate plane <4°. The plasmonic sensor signal uniformity was evaluated at an RSD of 13% *via* Raman mapping of gelatine (a mucal lining approximation) and gellan fluid (n=10), which has promise as a nasal spray constituent to combat SARS-CoV2 infection. The substrate is Fe-metallised for prospective application in the emerging area of bio-magneto-plasmonics. While the current study looks at periodic nanostructure arrays it is potentially generally applicable to SERS platforms with different kinds of imperfections.

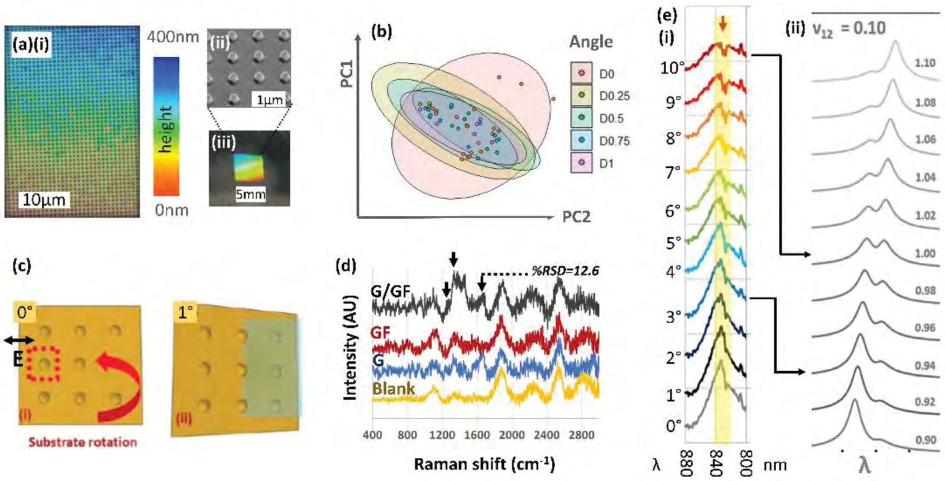

Figure 1 **(a)(i)** Colour-coded optical image of Fe-metallised nanodomes with **(ii)** SEM micrograph, **(iii)** zoom-out (real iridescence). **(b)** Principal component analysis (PCA), PC1 vs PC2, for white light transmission spectra (WLTS) and sample rotations up to 1°, as in **(c)** –spectral range 800-880nm is input into PCA model. **(d)** 5-point smoothed Raman spectra (average=100) of blank Fe nanodome substrate (B), with gelatine (G), gellan fluid (GF) and combined (G/GF). GF bands marked: Amide-III (1267cm^{-1}), CH_2 (1334cm^{-1}), Amide-I (1666cm^{-1}). **(e)(i)** WLTS from 0°-10° for 800-880nm, **(ii)** correlated with spectral patterns from a coupled two-oscillator model. v_{12}=coupling parameter. Yellow band in (i) highlights Fano feature. Numbers on right hand side are respective fractional oscillator spectral positions.

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Analyzing human plasma by Raman spectroscopy at 532 nm: Role of carotenoids in human disease study

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[a]University of the Basque Country (Basque Country, Spain); [b]CIC nanoGUNE BRTA (Basque Country, Spain); [c]IKERBASQUE (Basque Country, Spain)

Characterization of human plasma is a common procedure in the study of various human diseases. Raman spectroscopy as a non-destructive and unlabeled method has provided many references for this body fluid and shows good performance for early diagnosis of various diseases [1]. In particular, Alzheimer's disease (AD), the most prevalent neurodegenerative disease, has also been investigated that way. So far, all published work on this topic used 785 nm as excitation wavelength [2], which is suitable for biosamples from several reasons. Due to its significantly higher Raman cross-section, 532 nm excitation light—despite higher fluorescence background—can provide different valuable information to better understand the plasma composition and find new biomarkers that enable faster and easier diagnosis.

The presented study focuses on the characterization of human plasma by Raman spectroscopy using 532 nm excitation wavelength to assess its potential for the diagnosis of AD. For this purpose, plasma samples from 41 subjects, including healthy control and patients at various stages of the disease, were analyzed. The analyses were performed with vacuum-dried drops of 1 µl deposited on an aluminum foil substrate. To enhance signal-to-noise and capture more variability, three replicates were analyzed per sample.

Interestingly, the results revealed that the most important Raman signal belonged to carotenoids, which are not a typical biomarker for AD. Due to capillary forces and related Marangoni flows during the drying process of the droplets, different constituents of the plasma are distributed at different areas with respect to their rheological properties. At the outer edge of the drop, the Raman signals are dominated by biomolecules other than carotenoids, and towards the outermost edge, the carotenoid signal almost disappears. At the outer edge, a higher accumulation of AD-related biomarkers occurs.

Preprocessed Raman spectra were explored through Principal Component Analysis to study the main variances contained. For predicting patients' status, classification models based on partial least squares discriminant analysis were developed. Variable selection was used to identify specific Raman regions with discriminative power that can be associated with biomolecules and thus with patient's condition.

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Reversible Temperature Sensing using Blue-Winged Grasshopper *Coloracris azureus* Wings

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Raman spectroscopy is a powerful technique for material characterization, which is broadly employed in many fields, *e.g.*, surface enhanced Raman scattering (SERS), analysis of carbon-based materials, and biological materials.[1] The characterization of biomaterials with Raman spectroscopy can not only reveal the chemical structures of the materials but also accessibly probe the interaction between the chemical compounds and the surrounding biological environment. Here, using Raman spectroscopy and other complementary optical technologies, we investigated a reversible temperature sensor based on blue-winged grasshopper *Coloracris azureus* wings.[2] The hindwings of the blue-winged grasshopper *Coloracris azureus* can act as a reversible, power-free bio-thermometer, transitioning from blue to purple/red in a 30—100°C temperature range. Using microspectrophotometry, light microscopy and Raman microscopy, we show that the blue color of the wings originates from pigmentary coloration, based on a complex of astaxanthin and proteins. The thermochromic shift from blue to red, induced by a temperature increase, is attributed to a denaturation of this carotenoprotein complex, upon which astaxanthin is released. This process is reversible upon a subsequent temperature decrease. The color changes are both swift and consistent upon temperature change, making the grasshopper's wings suitable as direct visual sensors on thermally dynamic, curved surfaces. The potential possibilities of sustainable, power-free temperature sensors or microthermometers based on biomaterials are demonstrated.

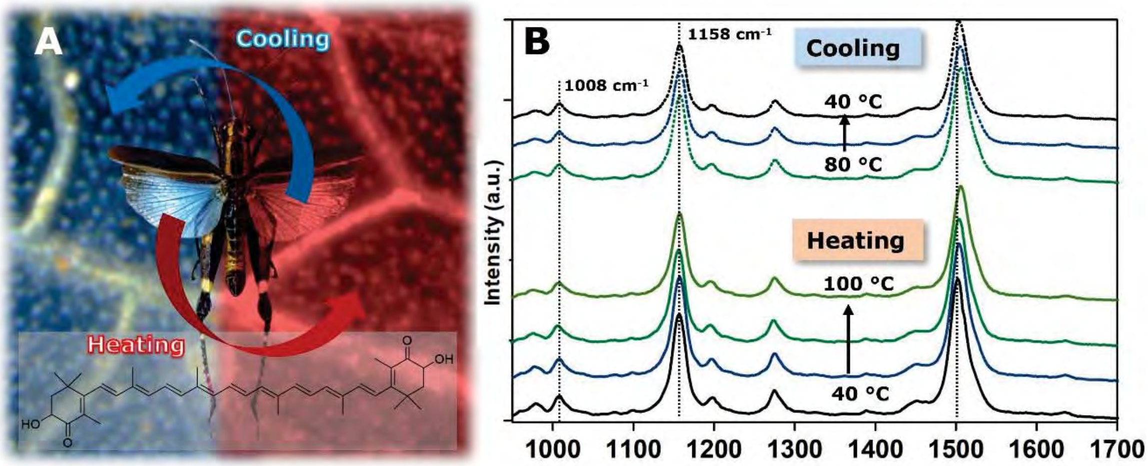

Figure 1: A) Reversible temperature sensor using blue-winged grasshopper *Coloracris azureus* wings. B) Raman spectra of the blue wings during heating (40 to 100°C) and cooling (80 to 40°C).

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iSERS: from correlative single-particle experiments to single-cell imaging and ultrasensitive POCT

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Immuno-SERS (iSERS) is based on molecularly functionalized noble metal nanoparticles (SERS nanotags) for identification and localization of target proteins using SERS-labeled antibodies.[1] This talk starts with the chemistry and physics of single SERS nanotags. Specifically, structure-property relations of various nanotag designs are studied via correlative single-particle LSPR/SERS/SEM experiments and compared with results from computer simulations.[2] Gold nanostars and gold core/satellite superstructures are clearly outperforming other, more common particle candidates; their high brightness in combination with the spectral multiplexing potential of vibrational Raman scattering can be exploited in both single-cell iSERS imaging[3] and ultrasensitive point-of-care testing (POCT). The latter was long hampered by the lack of suitable compact instrumentation for rapid measurements. To this end, we designed and built a custom-made iSERS reader for lateral flow assays (LFAs) which is 2-3 orders of magnitude both faster and cheaper compared to research grade confocal Raman microscopes.[4] Finally, in addition to bioanalytical applications of SERS-based LFAs[5] we also present fundamental studies for improving the accuracy at low analyte concentrations in a combined experimental and computational approach.

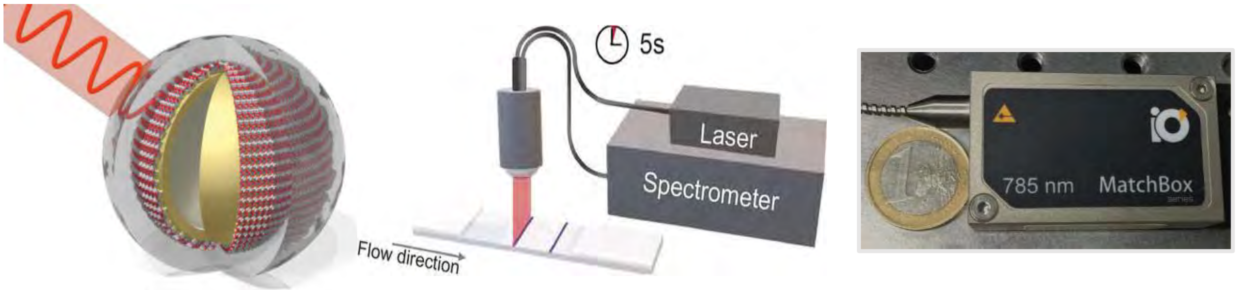

Figure 1: (left) SERS nanotag for red laser excitation (middle/right) Compact & fast SERS reader for POCT

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Surface-enhanced Raman Spectroscopy (SERS) for clinical monitoring and visualizing heterogeneous photocatalysis

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In recent times, Surface-enhanced Raman spectroscopy (SERS) finds many diverse and important analytical applications starting from forensic science, food and environment safety, sensing, photocatalysis, cancer theranostics etc. Noble metal (Ag, Au, Cu etc.) nanostructures have the ability to confine incident electromagnetic radiation and generate massive amount of electromagnetic field in their neighborhood, which can enhance the Raman signals of analyte molecules near to the surface, leading to the phenomenon of SERS. In this presentation, I will discuss some of the recent results from our laboratory on SERS using hybrid bi- and tri-metallic alloy substrates made up of Ag, Au and Cu and their applications in the multiplexing detection of anti-cancer drugs.[1,2] Also, the hybrid alloy plasmonic nanostructures are the ideal adsorbent to carry out the important photocatalytic reactions. Along with the catalysis, the progress of a chemical reaction can also be simultaneously monitored via in-situ SERS. When plasmonic nanostructures are excited with suitable light source, hot electrons and hot holes are produced, which are responsible for suitable tailoring of the reaction rate for various reactions. Thus, the energetics and dynamics of the hot carriers, are key factors in plasmonic catalysis. In this presentation, the role of the hot carriers in Ag-Cu and Ag-Au alloy substrates to enhance the rate, for the C-C coupling reaction of Bromo-thiophenol (BTP) to the desired product Bi-phenyl dithiol (BPDT), will be highlighted using liquid phase SERS measurements.

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Polymer-functionalized multiwalled carbon nanotubes characterized by tip-enhanced Raman spectroscopy

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Tip-enhanced Raman Spectroscopy (TERS) is considered a very useful technique for optical characterization of surfaces and nanostructured materials since this technique can provide chemical and structural information with nanometric spatial resolution [1-3]. Multiwalled carbon nanotubes (MWCNTs) are very versatile nanostructures that can be dispersed in organic solvents or polymeric matrices giving rise to new nanocomposite materials showing improved mechanical, electrical and thermal properties. Moreover, MWCNTs can be functionalized with polymers in order to be employed as specific chemical sensors. In this context, TERS is strategic since it can provide useful information on the cooperation of the two components at the nanoscale for the optimization of the macroscopic properties of the hybrid material. Nevertheless, efficient TERS characterization relies on the geometrical features and material composition of the plasmonic tip used [3]. In this work we show how TERS can be exploited for characterizing MWCNTs mixed with conjugated fluorene copolymers. Notably the distribution of the polymer with respect to the MWCNTs was imaged at the nanoscale (Figure 1) while the careful analysis of the TERS spectra can give useful information for the understanding of the polymer/CNT electronic interactions at the local scale [4].

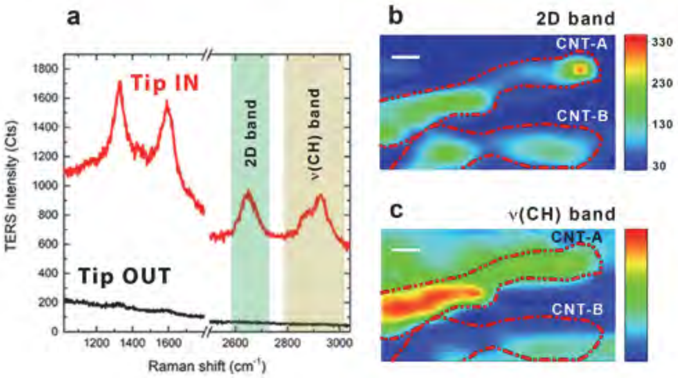

Figure 1: (a) Raman signal of polymer functionalized MWCNTs on gold substrate observed with (red line) and without (black line) the TERS tip in close proximity to the sample surface. TERS imaging of (b) the 2D band of the MWCNT, (c) the CH stretching mode of the polymer. Red dashed lines in the two maps are the contours of the MWCNTs localized from the images in panel (b), while the scale bar is 25 nm.

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50 Years of SERS and its paradigm for Blue Bioeconomy

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On celebration of 5o years since the discovery of the surface-enhanced Raman scattering (SERS) within the widening Raman spectroscopy community, this contribution discusses firstly the metanalyses results concerning “SERS + reproducibility”, as the propagated citations unfairly blamed SERS for lack of reproducibility rather than understanding dependencies. Secondly, while using SERS as a riding horse for expanding the field of nanotechnology, the production, description and probing of new plasmonic nanomaterials suitable for improved SERS sensing is a currently expanding field. Developing new nano-engineered substrates, there are several “popular” molecules for testing the SERS enhancement factor, among them, rhodamine 6G is widely preferred for SERS test [1], providing a specific SERS signature (pattern). In addition, a similar pattern has been observed in SERS spectra recorded on β-carotene at sub micromole concentrations in various biologic or aqueous samples [2, 3,], which obviously, does not comprised R6G. Since the SERS spectral pattern recognition is the key for developing automated algorithms incorporated in current technology for expanding sensing applications, comparing two distinct molecular species for their similar SERS spectral signature pattern is crucial to avoid undesired interpretations of data. A round robin experiment of the SERS similarity of R6G and β-carotene will be discussed, with consequences in both quantitative and qualitative biosensing relying on SERS.

Finally, using SERS, our recent research illustrating a new paradigm of SERS sensing, highlights results devoted to environmental applications, aquatic SERS sensing towards blue bioeconomy [4] and sustainable reuse of resources. Our approach related to the huge potential of reusing the generated waste biomaterial of aquatic origin into added value products, combining SERS with other Raman techniques, and validated by complementary techniques, illustrates the power of SERS tools for sensitive detection, the frequent SERS spectral pattern of β-carotene discussed above, in such media, imposing correct interpretation of physical meaning of data, for proper decision making and answers provided to various raised analytical questions.

“*Render to Caesar what is Caesar's…*”, the well-known quote customised for this topic would require: “… and to SERS, what is due to it”.

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Enhanced Raman scattering in metasurfaces coupled with Hexagonal Boron Nitride

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Nanostructured metal metasurfaces can confine light into hotspots, enabling surface enhanced Raman spectroscopies and highly efficient sensing [1]; however, oxidation and electro-chemical interactions with analytes often lower signal strength and reproducibility. Hexagonal Boron Nitride (hBN) is a wide-bandgap van der Waals material with remarkable insulating properties, ease of fabrication, and extraordinary surface morphology. It is found in applications spanning from large area encapsulation of electronic and optical components to quantum and nonlinear optics, and nanophotonics. Here, we couple bulk hBN supporting Fabry–Pérot resonances with Al-based metamaterial, Figure 1(a). We show that this hybrid platform exhibits both cavity-based enhancement and preservation of metasurface hotspots, thus leading to enhanced multiphoton processes in the visible and near-infrared range. We map Raman signals in two hBN-enriched metasurface devices; Figure 1(b) shows Raman scattering enhancement at 532 nm excitation, with 150 nm of hBN covering the 30 nm thin metamaterial. We investigate how the enhancement depends on the hBN thickness and excitation wavelength. Experimental results are in good agreement with numerical predictions: Figure 1(c) plots the electromagnetic field enhancement in the platform presented in Figure 1(b). Our findings open up a new route towards cost-efficient, scalable, durable plasmonic platforms which can be used in surface enhanced Raman spectroscopy and applications of boosted light-matter interaction at the nanoscale.

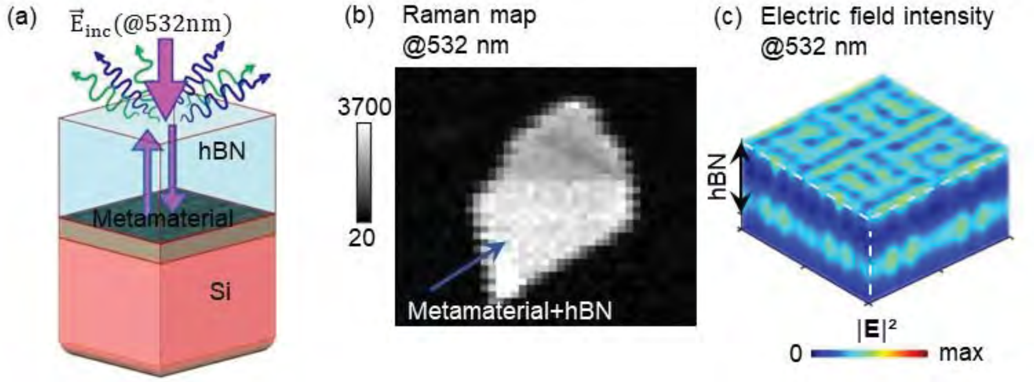

Figure 1: (a) Sketch of the Metamaterial covered by a hBN flake. (b) Raman map at 532 nm, showing the scattering enhancement in the part above the metamaterial. (c) 3D distribution of the electric field intensity at 532 nm shows the metasurface hotspots-governed enhancement.

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Synergistic interaction of Gold-deposited Tungsten Disulfide ($AuWS_2$) Nano-composites for Surface-enhanced Resonance Raman Spectroscopic (SERRS) Studies of Charge-transfer (CT) Complexes

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The synergistic interaction of analyte molecules with active sites of surface-enhanced Raman spectroscopy (SERS) enhancing substrates efficiently accelerates charge transfer (CT) resonance-initiated chemical enhancement. Thus, new strategies for the development of metal-free, two-dimensional (2D) SERS sensing platforms can be envisioned [1]. The unique composition-dependent metallic, semiconducting, and magnetic peculiarities of 2D materials extend their unique features towards advancing SERS platforms benefiting from the coupled chemical and electromagnetic enhancement mechanisms [2].

Herein, a nano-composite substrate composed of gold-deposited tungsten disulfide ($AuWS_2$), which combines charge transfer (CT) and molecular resonance excitations, is proposed. The systematically shifted XRD and Raman peaks validate the optimal Au doping of WS_2 introduced by the controlled decoration of gold nanoparticles (AuNPs) over exfoliated WS_2 nanosheets. AuNPs deposition generates strongly localized electromagnetic fields via the combination of localized surface plasmon resonance and surface-molecule complex formation. The preferential charge transfer from WS_2 to Au through the Au-WS_2 interface produces p-doped WS_2 and reveals strong signal enhancement in the Raman resonances of adsorbed methylene blue (MB) molecules while satisfying the molecular resonance excitation condition. Furthermore, the excitation wavelength used enables the mode-selective intensity amplification of specific Raman modes. For these modes, systematically altered signal intensity ratios (η) of the high- and low-energy vibrational modes satisfy a re-radiation enhancement condition. Accordingly, the lowest detectability down to 2.19×10^{-12} M MB concentration and the maximum intensity enhancement factor of 3.26×10^{7} are achieved by $AuWS_2$ substrates with optimal surface-enhanced resonance Raman spectroscopy (SERRS) performance.

The overlap of the laser excitation line with the absorption spectrum of CT $AuWS_2$ substrates induces CT resonance, which is further exploited for efficient three-fold resonance detection of methyl orange (MO), rhodamine B (RhB), and 3,3′-diethylthiatricarbocyanine iodide (DTTCI) analytes with detectability of 2.5×10^{-10} M, 1.71×10^{-11} M, and 3.91×10^{-15} M concentrations, respectively [3]. Thus, the synergistic interplay of triply coupled resonance, achieved by combining localized surface plasmon, CT, and molecular resonances paves new avenues for significant advancements for the design of highly sensitive hybrid SERRS sensors.

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Detection of Arsenite Contamination in Water: Aggregation-Aided SERS Facilitated by Colloid Cross-linking

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Naturally or industrially sourced arsenic often contaminates water, rendering it unfit for consumption due to its high toxicity. As per the World Health Organization (WHO), even a concentration of 10 parts per billion (µg L-1) in drinking water can pose a risk to human health [1]. Arsenic is present in the environment in both pentavalent (As^{5+}, arsenate) and trivalent (As^{3+}, arsenite) states, with arsenite generally regarded as more hazardous than arsenate in terms of toxicity [2]. This study presents a simple, selective, and highly sensitive method for the detection of arsenite (As^{3+}), utilizing aggregation-aided surface-enhanced Raman scattering (AA-SERS) with modified silver colloids. The presence of arsenic significantly enhances the SERS intensity of mercapto-compounds attached to the surface of colloidal silver nanoparticles. Cross-linking facilitates colloid aggregation, forming a network of arsenic atoms and glutathione 'bridges', as confirmed by UV-Vis absorption spectroscopy, TEM, and Raman imaging. The novel application of 2-mercaptopyridine enabled the attainment of the lowest detection limit for As^{3+} (~0.5 ppb) reported thus far by the SERS technique. Efficient 'direct' sensing of arsenite ions was achieved through our protocol, which is relatively simple and does not rely on data extrapolation compared to other techniques.

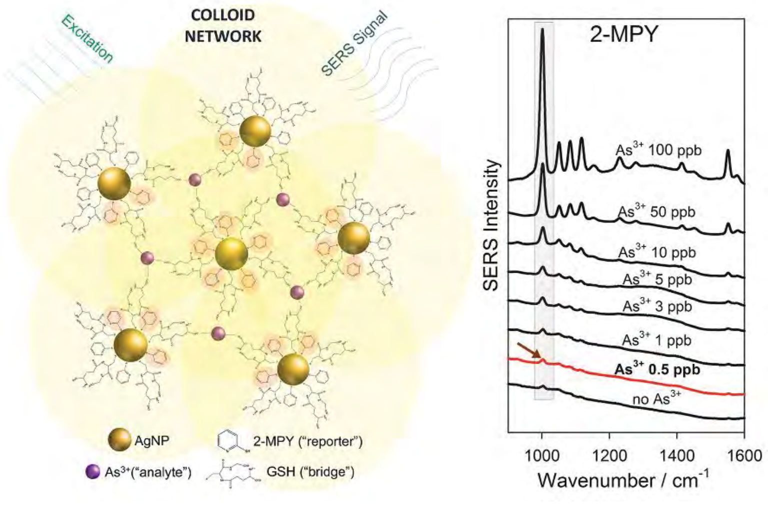

Figure 1: Colloid cross-linking facilitated by As^{3+} and glutathione 'bridges' producing SERS spectra of 'reporter' 2-mercaptopyridine dependent on As^{3+} concentrations. The ring breathing mode is highlighted and the arrow marks the SERS detection limit for As^{3+}.

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Understanding Skeletal Growth and Microplastic Uptake in Corals

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Coral reefs face multiple environmental threats, such as coral bleaching, changing weather patterns, rising ocean temperatures, and contamination from micro-plastic pollutants. Understanding the effect of these threats on corals and to protect reef environments requires an interdisciplinary approach and tools that allow non-destructive *in situ* chemical characterisation and detection.

I will give an overview of our work on developing and applying Raman and CARS methods to study corals and understand (a) the contribution of skeletal organics to coral biomineralisation and (b) the incorporation of microplastics within coral skeletons.

We have established methods to characterise microplastics, suspended in water [1] and in flow conditions [2]. Here we characterise skeletal growth under different nutrient exposures across different species within the stony coral family. Using specific combinations of Raman peaks we characterise their growth and image the centre of calcification (CoC) as well as organics incorporated into the skeletal matrix. We have established a novel analytical pipeline that uses CARS microscopy combined with machine learning based image analysis to segment microstructural features within coral skeletons unravelling chemical information of the skeletal organic matrix. We further apply the developed methodology for microplastic detection and differentiation by comparing CARS and two-photon excitation fluorescence (TPEF) images to separate microplastics from bio-organic and other particles. Initial results also show that CARS and TPEF are promising to visualise microplastic incorporation into coral skeletons. Overall this work makes a strong case for Raman scattering based techniques to provide insight into coral growth mechanisms and help understand the impact of environmental conditions and microplastic pollution on reef environments.

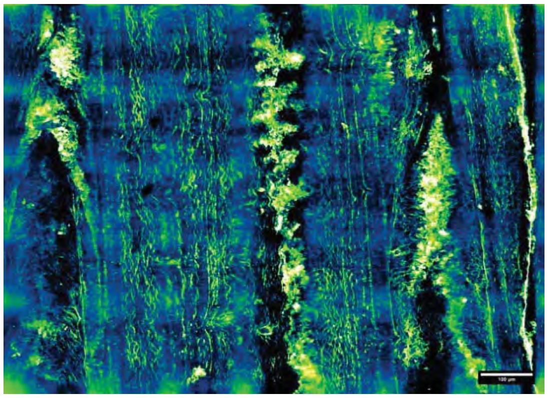

Figure 1: CARS image of a stony coral mapped at 2845 cm^{-1}. The image highlights the organics incorporated in the centre of calcification (middle) and in the coral skeleton layers. Scale bar: 100 µm

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Harnessing Raman Spectroscopy for the Analysis of Plant Diversity

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Diverse plant ecosystems tend to capture resources more efficiently, cycle nutrients more quickly and are more stable over time [1]. Techniques to rapidly assess plant biodiversity are important as we study and respond to rapid changes in ecosystem biodiversity caused by human development and climate change. Plant spectral diversity has recently emerged as a biodiversity metric that exploits the dissimilarity of leaf-level spectra which capture functional and phylogenetic differences among plant species and can be used as a metric for biodiversity and a measure of critical ecosystem function. In this study, we propose the use of Raman spectroscopy as a tool for proximal sensing of leaf chemistry that can be used to assess biodiversity. Raman spectra from 11 vascular plant species commonly found in forest ecosystems, specifically angiosperms (both monocots and eudicots) and pteridophytes (ferns), were acquired in vivo and in situ using a Raman leaf-clip sensor [2]. We achieved an overall accuracy of 91% for correct classification of a species within a plant group and identified lignin Raman spectral features as a useful discriminator for classification. The results demonstrate the potential of Raman spectroscopy in contributing to plant biodiversity assessment.

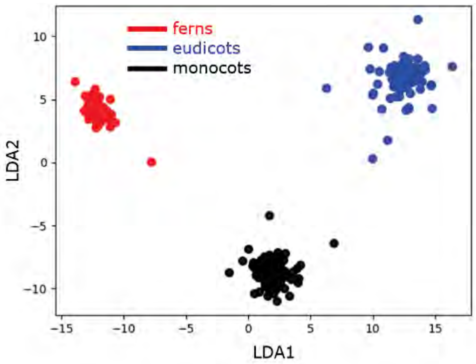

Figure 1: Linear Discriminant Analysis (LDA) scatter plot shows the effective discrimination and separation of plant species (Ferns in red, Monocots in black and Eudicots in blue) based on distinct features observed in the Raman spectra. 4 species of ferns, 3 species of eudicots and 4 species of monocots, each with 3 biological replicates were used in this study.

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Raman study on northwest's Italian crops: from laboratory to on-site analysis

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Climate change, increasing pollution, and globalization, all phenomena that are dominating the current worldwide scenario, are heavily affecting one of the flagships of the Italian economic sector, the agro-industrial one. Thus, developing on-site, fast, and user-friendly sensing methodologies is essential for the knowledge and understanding of the environment [1].

The aim of our research is the realization of non-contact optical sensing campaigns in crop supply chain to identify spectroscopic fingerprints connected to the process quality and, possibly, to animal welfare. This activity is developed in the context of the *Nord Ovest Digitale e Sostenibile* (NODES) project [2] and, in particular, of Spoke 6 "Primary Agroindustry", aimed to improve the sustainability, circularity, and resilience of the agro-livestock system.

The present work illustrates the use of Raman spectroscopy as main sensing technique, on the characterisation of 5 different kernel types of maize variety, *Cinquantino Bianco*, *Chatillon*, *Nostrano Valtidone*, *Ottofile Mantovano* and *Quarantino Cremonese*, which are traditional crops of the Italian northwest's areas. The aim is to correlate Raman fingerprints with some chemical parameters, such as the polyphenols' content, to access the grain quality. Our experimental approach is developed on two interconnected levels. At first, we investigated the kernel response with a benchtop Raman spectrometer, XploRA Plus HORIBA Scientific, probing different parts of the kernel, both the external and inner one on cut-in-half grains and, thus, performing mapping acquisitions from the core to the boundary of the grain itself. The micro-investigation allowed us to understand the composition of the kernels and to obtain reference Raman features. In a second instance, we investigated the integer kernels with a portable Raman spectrometer, i-Raman Plus, BWTEK. By exploiting the reference features identified through the micro-Raman investigation, we were able to properly analyze the spectra collected with the portable instrument by means of a multivariate analysis. The results are complemented by UV-vis-NIR analysis performed by portable Stellarnet and Avaspec spectrometers. Absorbance spectra allow to create a chemometric model to identify grain samples and their wellness. The Raman spectroscopic findings seem to positively correlate to the known content of bioactive molecules (i.e. flavonoids and carotenoids) present in the different maize varieties. Future steps will include (i) the development of a more sophisticated machine learning algorithm, (ii) the realization of a data collection campaign performed on-site.

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Finanziato dall'Unione europea NextGenerationEU | Ministero dell'Università e della Ricerca | Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA | NODES Nord Ovest Digitale E Sostenibile

Industrial-grade Raman spectroscopy for in-situ natural gas composition measurements

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The Natural Gas (NG) networks are becoming increasingly complex year after year. Supplier diversification, the direct injection of regasified LNG and biomethane and the incoming injection of hydrogen contribute to making the gas blends flowing in the national networks heterogeneous. The NG distributors and the final users are deeply interested in determining the thermodynamics properties of the blends to be used in industrial and civil facilities as an energy vector. Currently, common measurements require the use of consumables, trained personnel and employ invasive approaches, as the sample to be measured needs to be extracted from the gas stream and then destroyed by the interaction with the instruments.

Raman spectroscopy is an inherently non-invasive and multi-species approach as it only requires a laser beam to pass through the gas flow to measure its components. A self-standing industrial-grade system has been developed to meet the NG sector requirements. Such a system is able to determine the concentration of hydrocarbons up to butanes, nitrogen, carbon dioxide and hydrogen. Other NG components, typically in trace amounts, are not the subject of analysis for this type of system, as it employs spontaneous Raman decay.

The system realizes the typical two-axis 90° geometry; were on one axis, the radiation from a laser diode is collimated and then focused inside a gas cell, and on the other axis, the scattered radiation is collected and refocused into the focal plane of a dedicated diffraction grating spectrometer. A picture of the instrument is shown in Figure 1 (left side). The spectra of a hydrogen enriched NG and a typical NG mixtures are reported on the right side of Figure 1. The spectral broadness of the laser source significantly affects the acquired spectra as the FWHM of the hydrogen emission peak at -4156 cm^{-1} results to be 76 cm^{-1}.

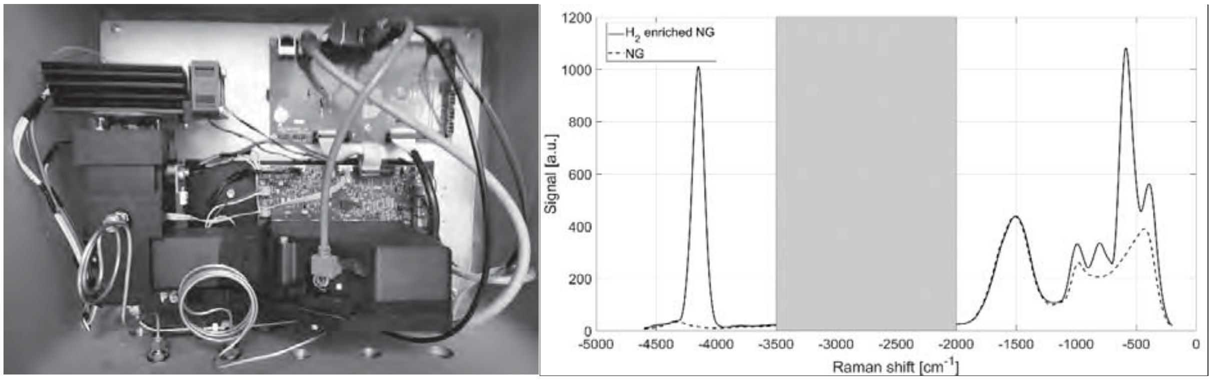

Figure 1: A picture of system on the left side, hydrogen enriched NG and NG spectra acquired by the system during the validation process.

The system has been validated within the industrial temperature range from -20°C to 50°C by complying the NG sector regulations for thermodynamics properties calculation (OIML R 140). The heating value, which represents the main parameter of interest, has been calculated from the components concentration calculation whit a relative error in the range of ±0.5%.

Discrimination of fresh and frozen-thawed Atlantic Salmon using Micro-Raman spectroscopy and chemometrics.

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Non-destructive measurements such as Raman spectroscopy are useful tools for food products as it allows greater sampling of the food product to occur with minimal loss of the sample being analysed. Any losses related to sample analysis are particularly significant for high-value food products, such as aquaculture [1]. The importance in understanding the effects of freeze-thawing processes on the biochemical nature of fish cannot be underestimated as aquaculture are perishable commodities and frozen storage is the common technique employed for preservation of fish in the commercial supply chain [2]. The consumer demand for Atlantic Salmon is high due to their omega-3 fatty acid content and antioxidant properties hence the requirement for screening purposes as the nutritional quality of Atlantic Salmon is known to be affected by the freeze-thawing process [3]. The aim of this work was to employ Resonance Raman spectroscopy along with chemometrics and physicochemical analysis to discriminate fresh and frozen-thawed Atlantic Salmon. Classification of fresh, once frozen-thawed and twice frozen-thawed Atlantic Salmon fillets employing micro-Raman spectroscopy and a developed PLS-SVM (Partial Least Squares-Support Vector Machine) model exhibited a classification accuracy of above 64% into their appropriate classes while the physicochemical analysis (colorimetric, drip loss and protein solubility analysis) indicated that the Atlantic Salmon fillets displayed signs of protein denaturation as well as carotenoid and muscle degradation after each thaw cycle. Overall, the use of Resonance Raman spectroscopy has shown potential to track biochemical changes that can be used to differentiate fresh and frozen-thawed Atlantic Salmon.

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Novel Particle-In-Well technology for single molecule peptide sequencing by surface enhanced Raman spectroscopy (SERS)

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Protein sequencing is a major tool in health science for detecting hormones, enzymes and several types of biomarkers. Although several approaches for single molecule detection have been recorded, the analysis of protein structure including sequencing and modification detection is still a challenge. For example, nanopore technologies have been undergone substantial improvements for single molecule detection, but their usability for identifying and differentiating amino acids is limited.[1]

The major advantage of our Particle-In-Well technology is the fingerprint sensitivity of any molecules as well as the capability of providing a platform with unlimited time and accumulation for SERS signal detection. By trapping single gold nanoparticle in a single gold nanowell (Figure 1), the Particle-In-Well array system is serving as an intense plasmonic hot spot for the analyte adsorbed on the gold nanoparticle, while SERS provides the tool for enhanced fingerprint information source for single molecule detection. In this regard, the analyte is adsorbed on the surface of gold nanospheres, providing only one molecule trapped under laser illumination. Furthermore, the one by one tracking of the building blocks of molecular chains is feasible due to the molecular diffusion in the hots pot. This makes it possible to tackle the amino acid residue of single peptide and opens the way towards the next generation single molecule protein sequencing.[2]

The Particle-In-Well method has already demonstrated successful pyrdine/pyridine-d5 discrimination and has been used for oligonucleotide and peptide fragment detection, including Hypoxia-Inducible Factor (HIF)-1 peptide fragments. The analysis of the collected data is carried out by machine learning model. When fully implemented, it will provide a huge advantage for medical research by providing a simple way for monitoring biomarker variation or modification during therapeutic development.

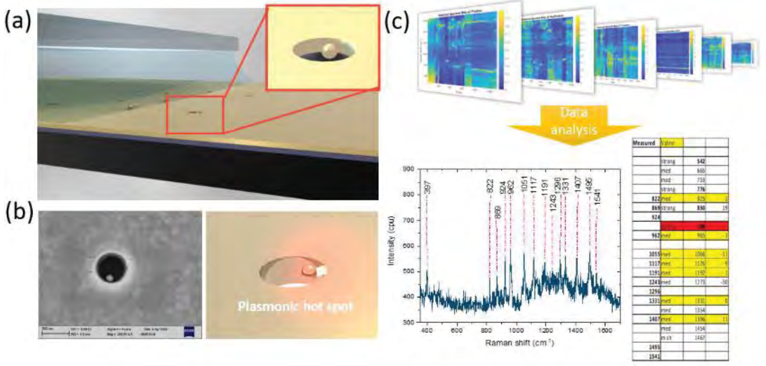

Figure 1: (a) Representation of capillary entrapment of gold nanoparticles (b) plasmonic hot spot of Particle-in-well system (c) data analysis by manual peak assignment

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Classification Of Environmental Microfibers Using Stimulated Raman Microspectroscopy

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Microplastics are found in nearly all environments, including oceans, bottled water, air, food, and human blood. Microfibers are cylindrical-shaped microparticles often reported as the most common and abundant in the environment. Their small size (often < 15 μm in diameter and relatively long length) and weight allow easy and fast distribution even using aerial pathways. Due to their long degradation time and high surface-to-volume ratio, plastic microfibers can become an efficient vehicle for accumulating pollutants, potentially harming human health and the environment. Traditional microscopic techniques for identifying environmental microparticles often overestimate the presence of microplastics, with visual inspection proving unreliable [1]. Although micro-FT-IR and spontaneous Raman microscopy offer classification methods, their effectiveness is limited by microfibers' small size and autofluorescence. These limitations can be overcome using stimulated Raman scattering (SRS) microscopy.

We present the analysis of microfibers from diverse environments: airborne fibers, surface seawater and coastal sediments, deep-sea sediment, fish guts, desert sand, and drinking water, utilizing our broadband SRS microscope. By comparing the SRS spectra with a custom-built reference library of the environment's most common natural and synthetic polymer microfibers [2]. Our results show that most microfibers were of natural origin, underscoring the importance of spectroscopy for their characterization. We found that airborne microfibers can travel 10s of km, typically originating locally; however, the distance can increase significantly following multiple air resuspension events.

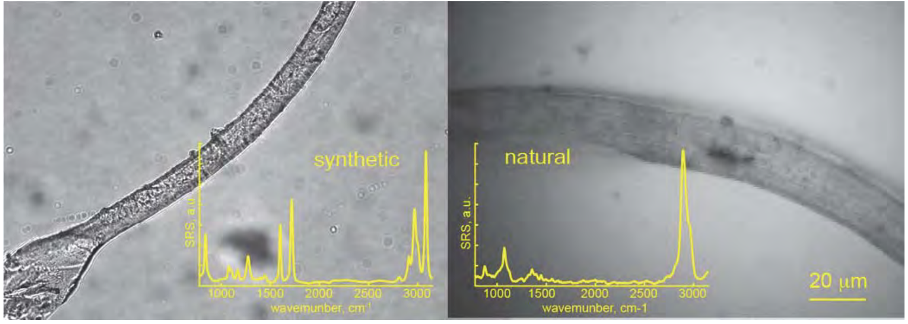

Fig 1. Microscopic images of environmental microfibers and corresponding SRS spectra

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Raman-based Detection of Antibiotics and Metabolites in Pharmaceutical Formulations and Clinical-relevant Matrices

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Raman spectroscopy is a powerful tool for bioanalytical detection methods due to providing molecular-specific fingerprint information. Furthermore, the inherently weak Raman signal can be enhanced by several orders of magnitude via the application of plasmonic-active nanostructured surfaces, i.e., using the SERS technique. Consequently, analyte molecules in the µM range or lower can be detected with high specificity.[1] However, due to the different complexity of the sample matrix, especially the strong interference of the highly complex matrix, customizing the detection strategy targeting less interference by background ingredients becomes more necessary for SERS application in future.

To develop Raman- and SERS-based detection schemes for sample matrices of different complexity were developed, i.e., from pharmaceutical formulations to clinical matrices.[2] For interfering substances in pharmaceutical formulations, the SERS signal of the analyte can be significantly enhanced simply by the dilution method, while protein precipitation and microdialysis can be used in protein-rich body fluids to avoid the blocking effect of protein corona.[3] For the interference of highly concentrated metabolites, it has become possible to use metal-organic frameworks to enrich analytes.[4] These three solutions are facing gradually increasing complexity scenarios to provide innovative SERS strategies in the pharmaceutical industry, therapeutic drug monitoring and rapid detection of antituberculosis drug susceptibility tests.

Acknowledgement: Funding “EXASENS” (13N13856) and InfectoGnostics (13GW0096F) by BMBF, Germany as well as the project CI 344/3-1 and project 465289819 by DFG, Germany is gratefully acknowledged.

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Coherent Stokes Raman scattering microscopy (CSRS)

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I report about the first implementation of laser scanning coherent Stokes Raman scattering (CSRS) microscopy [1]. Blue excited CSRS has the potential to become the coherent Raman technique with the highest spatial resolution, because the emission wavelength is redshifted and falls within the visible range. Thus, CSRS does not suffer from low transmissions as UV emitted coherent anti-Stokes (CARS) scattering radiation nor is it hampered by absorption artefacts as stimulated Raman scattering (SRS). The reason why CSRS was not used before for biomedical imaging is that the signal suffers from a strong fluorescence background. Within this talk, I will show how to suppress the fluorescence background even under visible excitation by narrow bandpass filter and a lock-in based demodulation. Near background free CSRS imaging of polymer beads, human skin, onion cells, avocado flesh and the wing disc of a drosophila larva are presented.

In a second part, I will explain and demonstrate numerically that CSRS solves a major obstacle of all coherent microscopy techniques by sending a significant part (up to 100%) of the CSRS photons directly into the backward direction under tight focusing conditions. This discovery will pave the way for the efficient investigation of thick, nontransparent samples and enable numerous technological advances such as epi-detected coherent Raman multi-focus imaging, real-time laser scanning based spectroscopy or efficient endoscopy.

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Metabolic Nanoscopy for Studying Aging and Diseases

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ABSTRACT

Metabolism is a complex biochemical process in living organisms that involves different biomolecules and consists of various reaction steps. To understand the multi-step biochemical reactions involving various components, it is essential to elucidate in-situ dynamics and the correlations between different types of biomolecules at subcellular resolution. In this context, we have developed and integrated deuterium-probed picosecond stimulated Raman scattering (DO-SRS), multiphoton fluorescence (MPF), and second harmonic generation (SHG) into a single microscopy system to study metabolic changes in aging and diseases.

By developing A-PoD and PRM algorithms, our current metabolic nanoscopy provides ~59nm resolution with hyperspectral volumetric imaging capability. Combined with deuterated molecules (glucose, amino acids, fatty acids, water molecules, etc.) as probes, the metabolic heterogeneity of the brain, adipose tissue, liver, muscle, retina, kidney, lung, and ovaries (in Human, Mouse, and Drosophila tissues) is quantitatively imaged. This metabolic nanoscopy dissects the molecular events and cellular machinery in living organisms during aging and disease progression, offering powerful tools potentially for disease detection, diagnosis, assessing therapeutic efficacy & resistance, as well as for mechanistic understanding of scientific fundamentals in neurodegenerative diseases, cancer, drug delivery, and aging processes.

Short Bio

Lingyan Shi is currently an Assistant Professor in the Shu Chien-Gene Lay Department of Bioengineering at UC San Diego. Her Lab's research focuses on developing high-resolution, high-speed metabolic nanoscopy for studying aging and diseases. She discovered the "Golden Window" for deep tissue imaging and developed metabolic imaging platforms including "DO-SRS" and "STRIDE". Shi group at UC San Diego transformed SRS into a super-resolution multiplex nanoscopy using A-PoD and PRM algorithms, uncovering lipid metabolic dynamics in various organ tissues during aging processes. Dr. Shi holds 8 awarded patents. She received the Blavatnik Regional Award for Young Scientist in 2018, the Hellman Fellowship Award in 2021-2022, the "Rising Star Award" from Nature Light Science & Applications in 2021, the "Advancing Bioimaging Scialog Fellow" by RCSA and CZI, the "David L. William Lecture & Scholarship" Award from the Kern Lipid Conference, and the "Sloan Research Fellow" Award in Chemistry in 2023, and the 2024 rising star award by BMES-Cell and Molecular Bioengineering (CMBE) society.

Expanding the Limits of Coherent Raman Scattering Microscopy for Bioanalytical Applications

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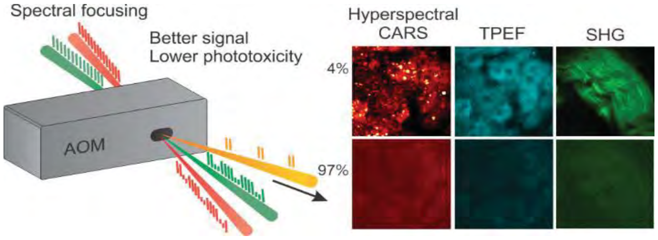
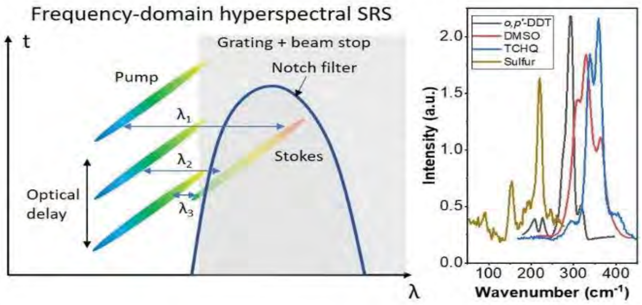
In recent years, coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) imaging methods have become widely utilized for chemical analysis. Hyperspectral CARS and SRS further enhance the chemical selectivity of high-speed Raman imaging by providing a spectrum at each pixel. Pushing the technical boundaries of hyperspectral CARS and SRS microscopy would enhance their utility for broader applications. Our work has propelled the frontiers of both CARS and SRS microscopy techniques by advancing both sensitivity and spectral coverage.

First, we developed a pulse-picking CARS microscopy that enables flexible and independent control of laser pulse peak power and average power. This approach improves the sensitivity of CARS microscopy without introducing more heat perturbation. It also facilitates optimal integration of hyperspectral CARS with other widely applied nonlinear optical imaging modalities such as two-photon excitation fluorescence and second harmonic generation. Applying pulse-picking, we assessed the photo-perturbation of laser pulses in various imaging conditions and established the safe power range for live cell imaging.

Second, we demonstrated a frequency-domain hyperspectral SRS microscopy for the detection of low wavenumber Raman transitions. Such low-wavenumber Raman peaks can be measured using time-domain impulsive SRS methods. However, the requirement of ultrashort laser pulses and interferometry renders complexity in configuration and high susceptibility to sample scattering for time-domain SRS. Frequency domain SRS, despite having relatively simple optical configurations and being widely used in biological sciences, currently neglects the low-wavenumber region below 500 cm^{-1}. We modified frequency-domain SRS microscopy based on spectral focusing allowing it to cover a spectral range from below 100 to 4000 cm^{-1}. This expanded spectral coverage enables us to image compounds with feature Raman transitions in the low-wavenumber region.





INSPIRE: In Situ Pump-Probe IR and Raman Excitation for High-Resolution Complementary Vibrational Imaging

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Vibrational spectroscopy-based high resolution chemical imaging techniques have attracted increasing attention. Mid-infrared photothermal imaging (MIP, aka O-PTIR [1]) has shown promise by breaking the resolution limit of the widely-used FTIR technique. To further push the resolving power, we developed photothermal relaxation localization (PEARL) microscopy that utilizes location-dependent temporal features of the photothermal effect [2]. PEARL breaks the diffraction limit of the probe beam and is widely applicable to general molecules or structures with optical absorption, including electronic absorption and vibrational absorptions. Furthermore, by unifying the pump-probe detection scheme in mid-infrared photothermal imaging and stimulated Raman scattering (SRS), we developed INSPIRE, a single beam probed complementary vibrational imaging platform. By frequency-domain extraction, INSPIRE allows simultaneous fingerprint and high-wavenumber imaging with spectral complementarity, potentially opening new possibilities in various fields.

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Real-time Wide-field broadband CARS microscopy

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Coherent anti-Stokes Raman Scattering (CARS) is a powerful non-linear vibrational imaging technique that provides label-free chemical maps of cells and tissues in their native state at higher speed than spontaneous Raman. This method involves the coherent excitation of vibrational modes in molecules using two ultrashort pulses, called pump and Stokes, which are spatially and temporally overlapped. Broadband CARS (B-CARS) exploits a narrowband pump and a broadband Stokes beam to excite several vibrational modes simultaneously. In standard implementations, the signal is generated in a small portion (i.e. a pixel) of the sample at once, at the tight focus of high-numerical-aperture objectives and collected with a spectrometer. The image is then formed sequentially, pixel by pixel, by raster scanning the beam with respect to the sample. Even considering low pixel dwell times (≈5ms), it takes minutes to perform imaging on large sample regions.

Here we present a novel implementation of BCARS based on wide-field (WF) illumination. It is powered by an Ytterbium-amplified laser, providing ≈270-fs pulses at 1035 nm, with 40-W average power and 2-MHz repetition rate. The spectrum of the CARS pump is narrowed using an etalon to ≈10 cm^{-1}, ensuring sufficient spectral resolution. The Stokes beam is formed via supercontinuum generation in a bulk YAG crystal. This is a compact, robust, stable, and reliable technique that generates broad spectra in the 1100-1500 nm range[1], entirely covering the so-called fingerprint vibrational region (500-1800 cm^{-1}), the richest in chemical information. The Stokes is subsequently amplified via a non-collinear optical parametric amplifier (NOPA). After amplification, a 4-f pulse shaper system with a slit in the Fourier plane selects a single wavelength of the Stokes beam. In this way, we acquire single-shot WF images at a single CARS wavelength over a large field of view (≈100 μm) using a two-dimensional sCMOS camera[2].

We tested our WF-CARS setup on solvents (toluene, DMSO) and plastic beads. By tuning the Stokes beam, we can build three-dimensional hypercubes over the whole spectrum in seconds. At a single vibrational frequency, our microscope enables video-rate imaging. This will pave the way for real-time study of rapid biological dynamics in cells and tissues, speeding up the diagnostic processes.

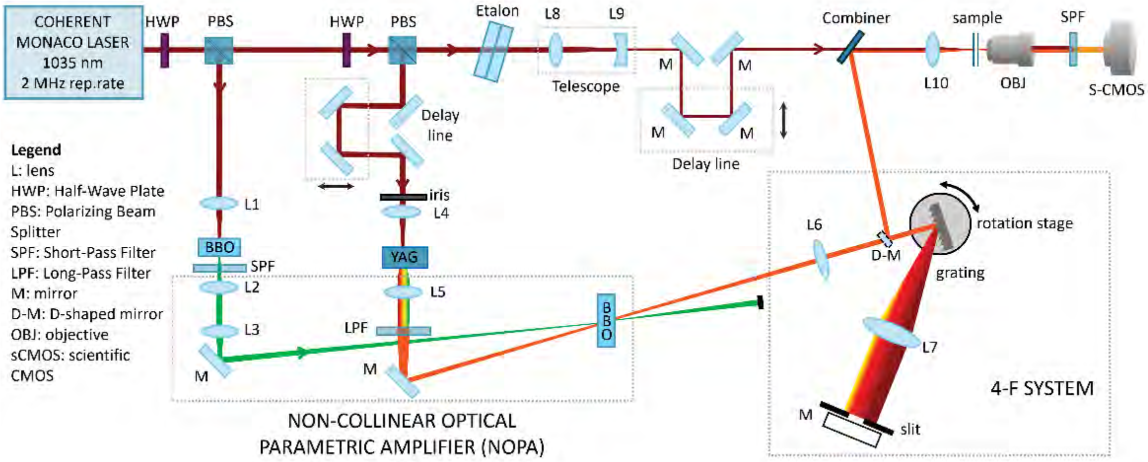

Figure 1: Schematic representation of the WF-CARS setup.

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Coherent Raman Spectroscopy: Quo vadis?

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Although the potential of Coherent Raman Spectroscopy (CRS) in the area of biomedicine, life sciences, and material sciences has been well demonstrated, its wide-spread practical application is still rather limited [1]. The two main CRS techniques are Coherent Anti-Stokes Raman Scattering (CARS) and Stimulated Raman Scattering (SRS) spectroscopy or microscopy. Although many technological challenges have been addressed to date, showing how to improve the resolution, sensitivity, and selectivity of CRS, significant efforts are still needed to increase the awareness of these techniques in the academic community, develop reliable protocols, and extend them to practical applications. For this purpose, it is also necessary to initiate national and international research networks that can significantly contribute to the development of CRS approaches in areas that have so far made little use of CRS alongside other Raman spectroscopic methods. Additionally, it is necessary to consider certain issues of CRS methods and potential solutions that have yet to be addressed. Here, we outline the vision for future development that casts CRS techniques in a new light following technical enhancements, along with the preliminary results obtained.

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Neural endoscopes for vibrational spectroscopy in the mouse brain

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In the past fifteen years, there has been a notable research emphasis on optical methods for interfacing with brain tissue, largely driven by advancements in genetically encoded optical actuators and fluorescent indicators of neural activity operating within the visible spectral range [1]. Recently, the research community has extended its interests in label-free near-infrared optical neural interfaces, reflecting a concerted effort to provide a translational perspective to the field. In this framework, the ability to gather Raman signal from deep regions of the mouse brain would greatly complement existing methods, and it would enable the use of vibrational spectroscopy in a wide range of animal models of neural diseases, including neoplastic, traumatic and neurodegenerative conditions. However, available methods are based on optical system made by several waveguides aimed at decoupling excitation light from collected scattered photons, resulting in large implant cross-sections not suitable for the brain of small animals.

In this presentation, we will first describe low-invasive methods to collect spontaneous Raman signal from the brain tissue, based on both flat-cleaved and tapered multimodal optical fibers. In the case of tapered optical fibers (TFs), a widely extended light delivery and collection surface is exploited to increase the ratio between the Raman signal generated in the brain tissue and the probe's background, enabling *in vivo* detection of spectral fingerprints related to brain tumors and traumatic brain injuries [2]. For flat-cleaved optical fibers, we instead employ wavefront shaping at the fiber's input to scan the excitation light at the fiber output and obtain spatial-resolved detection of Raman signal of a few micrometers over a 50µm light-guiding core.

The second part of the presentation will instead delve into the fabrication of plasmonic nanostructures on the optically active surface of the two types of neural implants. In particular, we will describe a dewetting method to nucleate gold nanoislands with very small nanogaps, supporting the excitation of localized surface plasmon resonances [3,4,5]. The resulting strong electromagnetic field enhancement generated on the waveguide allows for enhancing the Raman signal generated by neurotransmitters molecules, reaching a detection limit of 10^{-7}M in an endoscopic configuration, with all incoming and outcoming signals multiplexed into the same waveguide.

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Improving Accuracy of Quantification in SERS-based Lateral Flow Assays: Experiment and Computational Modelling

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Lateral Flow Assay (LFA) is a widely used platform for point-of-care testing. However, the conventional LFA based on naked-eye detection has limited sensitivity and no quantification capability, which can lead to false-negative results. This challenge was improved by the SERS-based readout using nanotag-labelled detection antibodies. This approach not only allows quantification but also provides access to the low-concentration regime that is inaccessible to conventional LFA. Additionally, the conventional LFA workflow based on parallel immuno-reactions has an inherent systematic error, such as the Hook effect: the decrease in signal at high antigen concentrations.[1] This occurs by a parallel reaction workflow with multiple antibodies conjugated to nanoparticles as the tag reagent. As SERS opens up the field for highly sensitive detection, an additional systematic error has been discovered recently: the formation of non-quantitative immuno-complexes (Figure 1A). The latter occurs due to multiple antigens reacting with multiple antibodies conjugated to nanoparticles, like the Hook effect. We confirmed the possibility and influence of this error through simulations based on the Advection-Dispersion-Reaction equation (ADRE) (Figure 1B).[2] Based on the simulations, we hypothesised that this systematic error could be revealed using SERS readout and overcome by a step-wise approach in the LFA workflow, similar to ELISA. In other words, we expected to improve quantification accuracy by performing step-wise LFA, which results in the more accurate quantitative formation of '1:1' immuno-complexes. Overall, our SERS-based two-step approach yielded a >100-fold improvement in the detection limit compared to the conventional one-pot reaction. This highlights the importance of isolated immuno-reactions in mitigating such systematic errors, thereby improving the reaction accuracy and the detection sensitivity of SERS-based LFA.

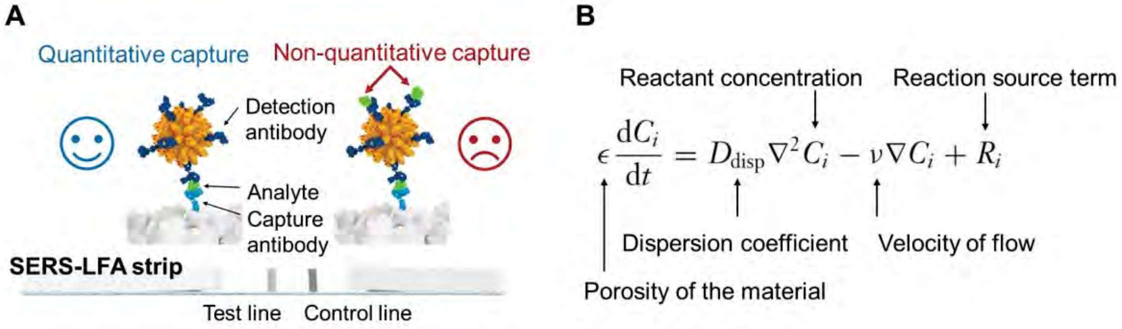

Figure 1: A. Two different types of immuno-complexes formed on the test line of SERS-LFA. **B.** Advection-Dispersion-Reaction equation for computational modelling of immune-reactions on the stirp.

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3D Hydrogels with Plasmonic Gold Nanostars for SERS Biosensing and Bioimaging

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[a]*CIC biomaGUNE, Basque Research and Technology Alliance (BRTA), 20014 Donostia-San Sebastián, Spain*

Biocompatible hybrid materials with integrated plasmonic nanoparticles have been of great interest for surface-enhanced Raman scattering (SERS)-based diagnostic platforms and therapeutic models. [1,2] In this work, we developed and explored different methods for incorporating size- and shape-controlled plasmonic gold nanostars with hydrogels, leveraging the chemical control provided by bottom-up synthesis.[3] Scanning and transmission electron microscopy and UV-vis spectroscopy were implemented to characterize the size, morphology, and spatial position of the prepared nanostructures in bulk hydrogel discs and 3D printed scaffolds. We show that the hydrogel-nanocomposites are biocompatible with MDA-MB-231 breast cancer cells. Furthermore, the cell-containing nanocomposites could be applied for both with-label and label-free SERS biosensing and imaging. Overall, the nanocomposite platforms developed herein may be useful for SERS studies in 3D matrices, possibly also including the monitoring of biological systems over time (4D).

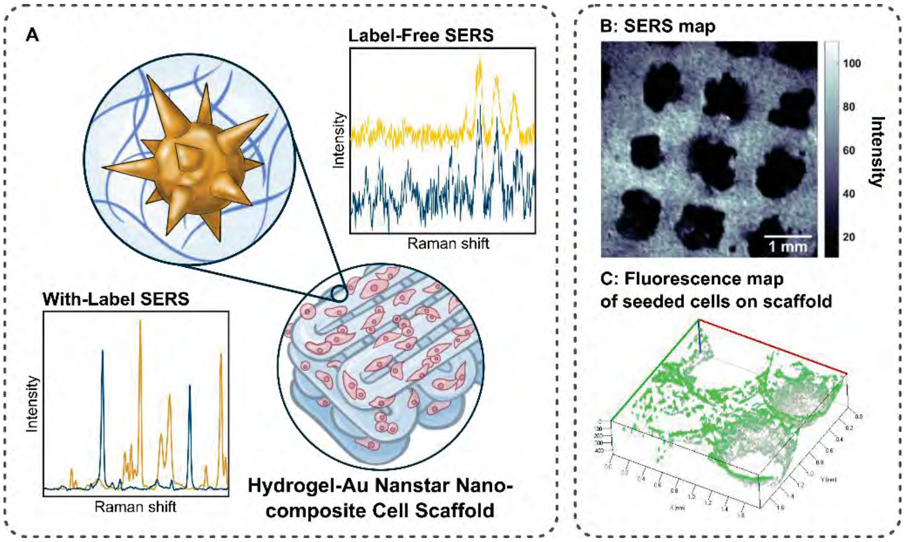

Figure 1: (A) Schematic of the gold nanostar nanocomposite scaffold for with-label and label-free biosensing and bioimaging. **(B)** SERS map of the nanocomposite scaffold with a Raman tag. **(C)** Fluorescence confocal microscopy map of cells on the scaffold labelled with a live (green)/dead (red) stain.

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LipoGold tags as game-changer multifunctional probes for SERS-based applications

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Despite Surface Enhanced Raman Scattering (SERS) finds widespread use in ultrasensitive in-vitro and in-vivo diagnostics and imaging, its potential is limited by the challenging preparation of SERS tags. These tags, usually metallic nanoparticles (NPs) or NP clusters functionalized with Raman-active molecules (RRs), often entail complex synthetic processes, exhibiting low colloidal stability, and suffering from poor reproducibility. Here, we propose "LipoGold Tags," a novel innovative platform exploiting gold NPs clusters formed on lipid vesicles' membranes, drastically simplifying the SERS tag preparation. [1] Raman-active molecules embedded in the lipid bilayer experience a significant electromagnetic field enhancement, optimizing the Raman signals. The study systematically explores concentrations of Raman-active molecules and lipid vesicles, confirming the functionalization of these LipoGold tags with biomolecular probes through a sandwich assay in cell cultures, ensuring signal and specificity maintenance. Then, the capability of functionalized LipoGold tags to specifically detect intracellular GM1 alterations, distinguishing between cell cultured fibroblasts from healthy donors and infantile GM1 gangliosidosis patient was tested. The proposed approach not only outperforms traditional methods but also presents potential as a groundbreaking advancement in producing highly efficient and versatile SERS probes.

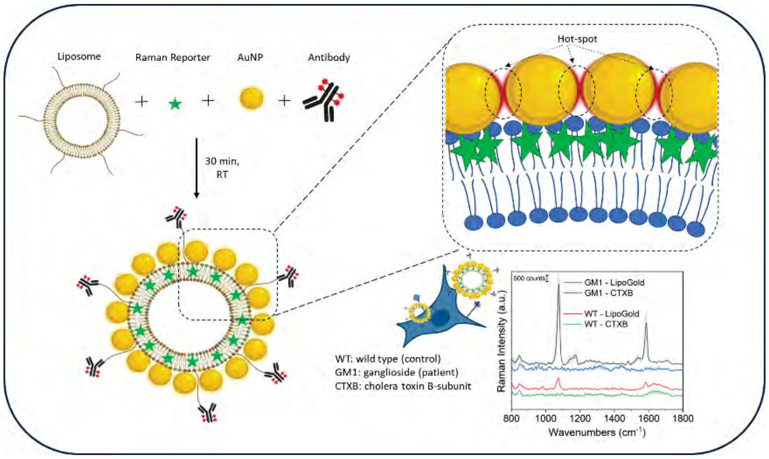

Figure 1: Schematical representation of LipoGold Tags synthetic preparation.

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CRISPR/Cas-assisted surface-enhanced Raman spectroscopy (CRISPR-SERS) for biodetection

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Nucleic acid detection and analysis technologies are essential for the early diagnosis of diseases and the identification of genetically modified crops. Currently, a variety of highly sensitive nucleic acid detection and analysis technologies have been developed in disease diagnosis and food safety. CRISPR/Cas strategies have the advantages of high accuracy in the target nucleic acid identification, and the cleavage function of the target sequence and the enzymatic activity of nearby single-stranded DNA or RNA.

Surface-enhanced Raman spectroscopy (SERS) is a powerful optical technique that can amplify the Raman signal near the plasmonic or semiconductor substrates, remarkably improving detection sensitivity. Integrating SERS with CRISPR/Cas technology can fully use the specificity of CRISPR/Cas for nucleic acid analysis and the high sensitivity of SERS technology, which is promising as a next-generation molecular diagnostics tool [1]. Here, we will present several CRISPR-SERS gene analytical platforms, which have been applied for analyzing cervical cancer-related human papillomavirus (HPV) [2, 3], transgenic crops (35S promoter and nos terminator)[4, 5], and African swine fever virus genes (Figure 1)[6], etc., which will be useful in the fields of disease diagnosis and food safety.

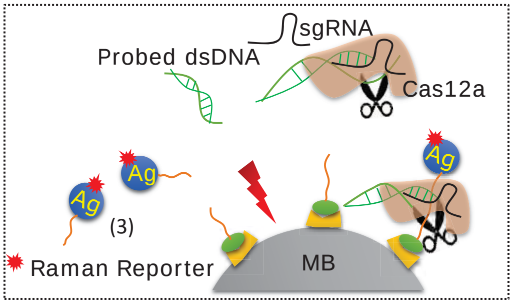

Figure 1: A CRISPR/Cas12a-assisted surface-enhanced Raman assay.

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Plasmonic thin films fabrication by additive manufacturing for biomedicine

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Lab-on-a-chip (LOC) devices have become widespread to study complex cellular processes and to achieve personalized disease modelling.[1] They merge microfluidic, microelectrodes and sensors, and make it possible to grow and monitor living cells or tissues. The core of the architecture is about the microelectrodes design. The established technique for thin film patterning is physical vapor deposition (PVD), which includes sputtering, electron-beam, and pulsed laser deposition methods.[2] Despite being able to produce detailed and well-resolved microelectrodes, it is an expensive, delicate, and time-consuming method. The spray coating technique, on the other hand, allows thin metallic layers to be produced easily and quickly. The advantages of this technique are manifold: a large area deposition can be achieved using less material and at the same time keeping the material properties unchanged.[2] Furthermore, by using appropriate shadow masks, it is possible to obtain microelectrodes with resolutions of a few µm.[3] It is herein presented the application of inkjet printing technology to produce water-soluble shadow masks, which will be used to produce thin nanostructured plasmonic films with a thickness of a few hundreds nanometers. The presented approach is used for fabrication of metal microelectrodes with desired geometry, in an easy, fast, and greener way than traditional PVD techniques. In addition, spraying gold nanoparticles (AuNPs) produced by laser ablation in solution (LASiS) enables the formation of nanostructured films suitable for the occurrence of the SERS effect, allowing the detection of surface-adsorbed molecules. This would make it possible to exploit a further method - in addition to the established electrical and optical methods - to monitor the health and growth of living cell systems grown on the surfaces of these devices.

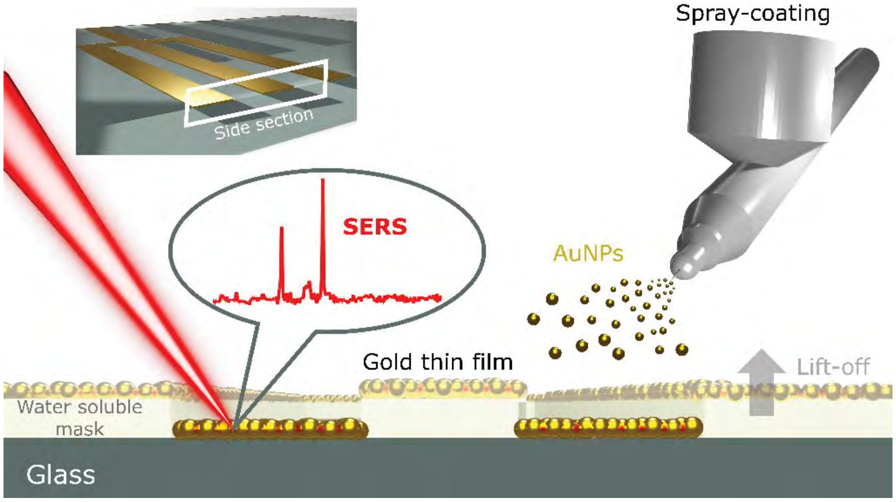

Figure 1: Schematics of the plasmonic microelectrode and the required fabrication steps.

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Chemical and structural characterization of tannin-furanic foams using UV Resonant Raman scattering, FTIR imaging, X-Ray micro-Tomography and solid-state NMR

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There is an urgent demand in plastic-free materials with the aim of reducing the impact of microplastics in the environment as well as to contribute in reducing global warming. Condensed tannins are gaining an enormous interest in material, they constitute the starting point to create tannin-furanic foams, a rigid material that could be considered as alternative to the plastic like extruded polystyrene or polyethylene. Among of the various one, mimosa tannin (from the black wattle tree, Acacia mearnsii) is one of the most promising for industrial applications. Co-polymerization of condensed mimosa tannin, with polyfurfuryl alcohol, generates a solid foam, potentially usable for industrial applications [1]. A precise and accurate characterization of such materials, besides the determination of the macroscopic characteristics, such as density, compression resistance and porosity, requires also the determination of the chemical constitution as well as morphological characterization of the bulk material at the microscopic scale. The peculiar hetero-polymeric composition of such materials therefore requires, for an accurate chemical characterization, a multi-technique approach that allows guessing the chemical conformation based on a joint analysis of all the techniques applied [2,3]. Here we show how the use of vibrational spectroscopy techniques such as UV Resonant Raman, ATR-FTIR, micro-FTIR imaging, supported by X-Ray micro-Tomography, solid state NMR and quantum mechanics simulations, is able to provide a complete chemical and morphological characterization of such materials, as well as to describe the presence of chemical inhomogeneity among the micrometric length scale. The micro chemical compositions of different tannin foams were compared to establish the existing correlations among chemical constitution, synthesis protocols and foams macroscopic properties.

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Raman Spectroscopy: An Important Analytical Tool in the Optimization of Plant-Based Protein Extraction

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This study explores the structural properties of components of semi-refined flaxseed extract (SFE) utilizing Raman spectroscopy coupled with principal component analysis (PCA). The flaxseed meal samples were subjected to pulsed electric field (PEF) prior to alkaline extraction [1]. Through Raman analyses, the secondary and tertiary structures of the extracts were evaluated. The results of PCA analysis shows distinct clustering patterns, effectively distinguishing the spectral features between PEF treated and non-treated extracts. This research provides valuable insights into the structural changes induced by PEF treatment in flaxseed protein extracts, demonstrating the potential of Raman spectroscopy combined with exploratory PCA in characterizing complex biomolecular systems.

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Polymer lifecycle monitoring – coupling Raman with DSC for recyclability investigation.

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Global warming and resource depletion are at the origin of the switch from a linear to a circular economy. This is especially true when it comes to talk about Polymer industry. Indeed, the reduction of petrol resources at the origin of most of polymer materials forces this industry to change their strategy from a linear to a circular approach. Consequently, monitoring the full lifecycle of polymer materials becomes critical.

One of the key factors to evaluate in circular economy concept is the recyclability of the material. Indeed, a recyclable material should offer performances close to the original one. This level of performances is important to commit on the ability of the recycled material to replace the original in the process of reuse. Evaluating the crystallinity and the molecular structure are so important. Thus, the ideal techniques are DSC for crystallinity and Raman spectroscopy for molecular structure. By design, the optical window of the DSC system enables an easy access to the crystals for Raman measurements.

During this talk, we will present why Raman spectroscopy is a complementary technique to DSC to understand the recyclability of polymers.

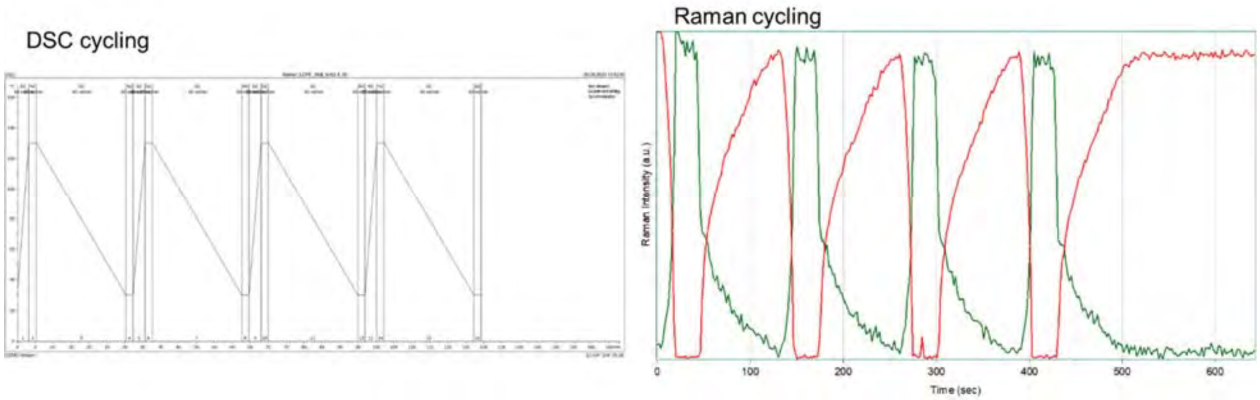

Figure 1: Left: DSC cycling. Right: Raman bands intensity monitoring over cycling.

Raman spectroscopy and machine learning for the quantification of lactic acid in wine malolactic fermentation

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Malolactic fermentation (MLF) is the process by which lactic bacteria convert sour-tasting malic acid, naturally present in grape must, into the rounder and more palatable lactic acid (and carbon dioxide); this step makes it possible to produce stable wines characterized by greater balance and persistence in the mouth [1]. A careful monitoring of malolactic fermentation allows a meticulous determination of the concentration of malic and lactic acid; this allows to achieve high quality standards and the production of wine according to the regulations [2]. In order to monitor the dosage of malic and lactic acid, several analytical methods can be used such as HPLC (high performance liquid chromatography); this work aims instead to investigate an alternative and less expensive way to follow malolactic conversion: Raman spectroscopy (RS). It is important to note that RS has several advantages when applied in the field of oenology, in particular:

- it is a non-destructive technique that allows the study of fruit and must samples,
- it allows on-site measurements if a portable Raman apparatus is available,
- has limited interference from water signals,
- it is a faster method than HPLC, requiring only a few minutes of analysis for a statistically significant amount of replicates.

Raman analysis is therefore a valid candidate for approaching the study of malolactic fermentation. Specifically, the aim is to monitor the fermentation process under examination by determining the concentration of lactic acid in samples in Nebbiolo grape must for the preparation of Barolo wine. Using a portable Raman apparatus, it is possible to acquire the Raman spectra of the samples directly in the cellar. HPLC measurements were used to train the machine learning model which, by PLS (partial least squares) regression, employs the Raman spectra (after a pre-processing of the data) and returns the lactic acid concentration associated with each sample. This method provides results comparable with those given by HPLC; moreover, contrary to the latter, it allows quick and easy monitoring of malolactic fermentation on site. It proves to be an accurate and robust method leading to the determination of a predictive model characterized by an: $R^2 = 0.78$. This research was funded by Regione Piemonte with the QualShelL PSR project [3].

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In-vivo classification of THC and CBD contents in cannabis plants using hand-handled Raman spectrometer

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We present an innovative approach to the in-vivo classification of THC/CBD-rich cannabis plants through a novel miniaturization strategy for Raman spectroscopy. By developing compact Raman spectrometers that utilize patented technology based on cheap non-stabilized laser diodes, densely-packed optics, and small pixel size sensors without cooling, the study achieves performance comparable to more expensive, research-grade systems. This miniaturization is facilitated by real-time calibration of Raman shift and intensity using a built-in reference channel. The miniRaman spectrometer effectively records high-quality Raman spectra of fresh cannabis and its products without the need for sample or environment preparation, identifying characteristic peaks of primary phytocannabinoids such as THC, CBD, and CGB and avoiding time-consuming HPLC analysis.

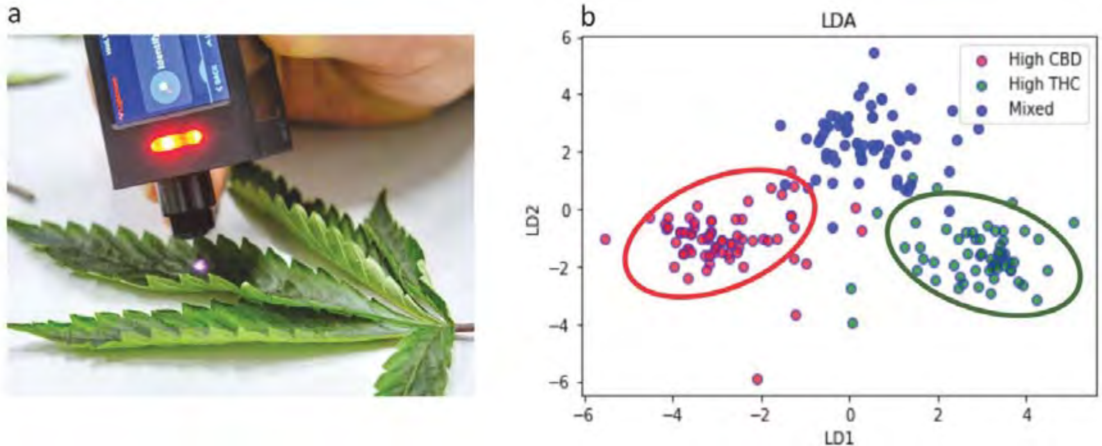

Figure 1: Measurement procedure (a) and results of the classification of THC and CBD in cannabis leaves.

Through spectral deconvolution and chemometrics, quantitative analysis becomes possible, significantly reducing the influence of fluorescence for more precise analysis [1]. The application of this technology allows for the identification of THC or CBD-rich plants with a high accuracy rate of 92%, demonstrating the potential of Raman spectroscopy aided by machine learning for rapid, non-destructive cannabis classification.

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Raman Spectroscopy analysis as a tool to study inorganic acids interactions in ATPS.

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In this work, it is investigated for the first time, the Raman spectroscopy analysis to elucidate key interactions between polyethylene glycol (PEG) and arsenic acid (H_3AsO_4) using Aqueous Two-Phase Systems (ATPS). The ATPS were formed using Na_2SO_4 and PEG 1000 g/mol to be able to transfer a 72% of H_3AsO_4 to the polymer rich phase. The experimental results indicate that H_3AsO_4 molecules experience a different environment in the polymer rich phase. To get an insight into the molecular environment that arsenic acid molecules are immersed in, several ATPS were formed and characterized using different initial concentrations (Figure 1 left) to be able to follow the H_3AsO_4 signal as neutral molecules. Based on the results of the Raman spectra, a characteristic behaviour can be explained: a displacement on the characteristic signals of the arsenic acid as neutral molecules is observed in the polymer rich phase (Figure 1 right). There is also a displacement in the polymer chain signal; all these results suggest that in the polymer rich phase, an interaction between H_3AsO_4 and PEG takes place, and it is already reported that Raman spectroscopy can be employed to study the hydrogen bond through molecular vibration information and molecular dynamics simulation for the extraction of phosphoric acid using ATPS [1]. So, the shift in Raman frequencies provides a tool for determining the conformation of a molecule formed by hydrogen bonds and solvation effects [2,3].
After analyzing all the results, we propose a solvation mechanism between PEG-H_3AsO_4 in which hydrogen bonding interactions predominate.

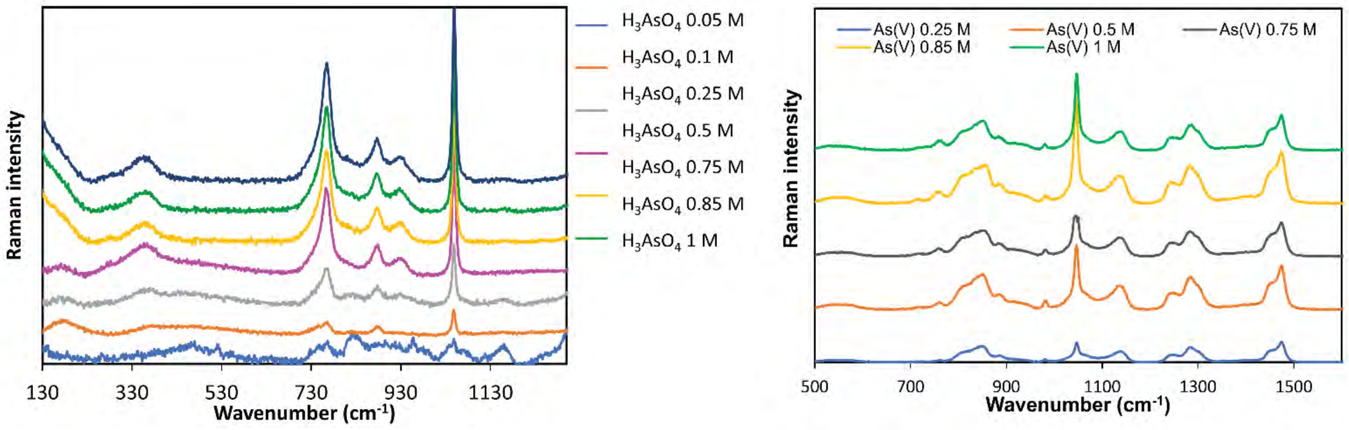

Figure 1: Raman spectra of H_3AsO_4 at different initial concentrations (left) and Raman spectra of the polymer rich phase extracting H_3AsO_4 (right).

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Advancing Raman spectroscopy through machine learning for molecular recognition and ultra-sensitive detection

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Abstract: Raman spectroscopy, renowned for its capability to provide molecular fingerprinting through the inelastic scattering of photons, holds immense potential for diverse applications ranging from bio-medical to pharmaceutical industries, food quality control, and material science. However, the inherently weak Raman scattering phenomenon has historically posed a challenge and limited its applicability, particularly in analyzing bio-samples with weak scattering profiles. Due to weak Raman scattered profiles and low signal-to-noise ratio (SNR), some tiny important features might get overlaid in the noise due to low signal-to-noise ratio (SNR). Moreover, Raman spectra, reflecting sample information in the molecular fingerprint region, often contain numerous features that are difficult to analyze collectively. If we apply some perturbation of time, pH, temperature, etc., these features are non-linear with respect to these perturbations, which makes it even more difficult for analysis purposes. To address these challenges, Machine Learning (ML) emerges as a promising solution. Recent advances in ML algorithms offer efficient solutions to handle low SNR and complex nonlinear problems within a fraction of a second. We have studied the Artificial Neural Network (ANN), which assisted Raman and SERS in the rapid peroxide and fatty acid analysis of edible oil with temperature perturbation. Herein, we developed a Raman method to measure the various trans fatty acids in a food matrix lower than 2% with 94% accuracy.[1] In another study, we analyze the Raman spectrum of various pharmaceutical samples with low SNR and find the meaningful pattern hidden in the noise through powerful and emerging Deep Learning (DL) tools. Herein, we developed a fully ML-automated method for the detection and identification of samples with low SNR Raman spectroscopy.[2]

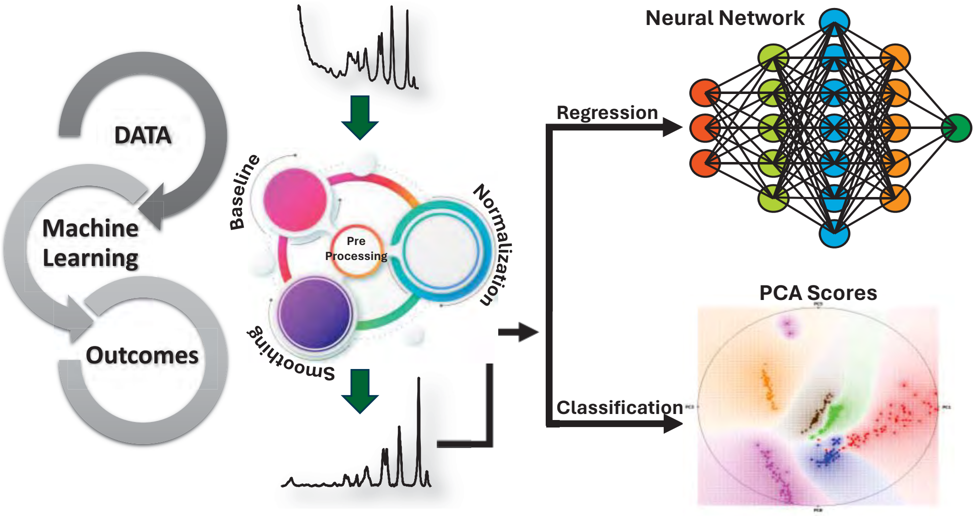

Figure 1: Harnessing Machine Learning for Enhanced Raman Spectroscopy

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A resonance Raman study on *Chlamydomonas reinhardtii* and *Cyclotella meneghiniana* under low, *environmental- realistic* Hg(II)-exposure

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Heavy metal pollution, notably mercury (Hg), poses significant threats to aquatic ecosystems[1], yet its specific impact on the pigments of microorganisms remains inadequately explored [2]. Using resonance Raman (RR) spectroscopy with laser excitation at 532 nm, we investigated the physiological responses of Chlamydomonas reinhardtii and Cyclotella meneghiniana to Hg(II) exposure, simulating realistic environmental conditions with short-term exposures (2 hours, 24 hours) and low concentrations (10 nM, 100 nM). Comparative analyses revealed subtle changes in the main carotenoid spectral profiles. Lorentz fitting of the $\nu_{(C=C)}$ stretching mode identified two principal components: lutein and beta-carotene in Chlamydomonas reinhardtii, and fucoxanthin and diadinoxanthin in Cyclotella meneghiniana. As observations - a specific decrease in intensity in exposed cells, alongside with an increasing trend in full-width at half maximum (FWHM) indicative of altered carotenoid pathways under environmental stress [3]. Our study reveals on the physiological responses of photosynthetic microorganisms to heavy metal pollution and accentuates the utility of resonance Raman spectroscopy in elucidating molecular changes under environmental stressors.

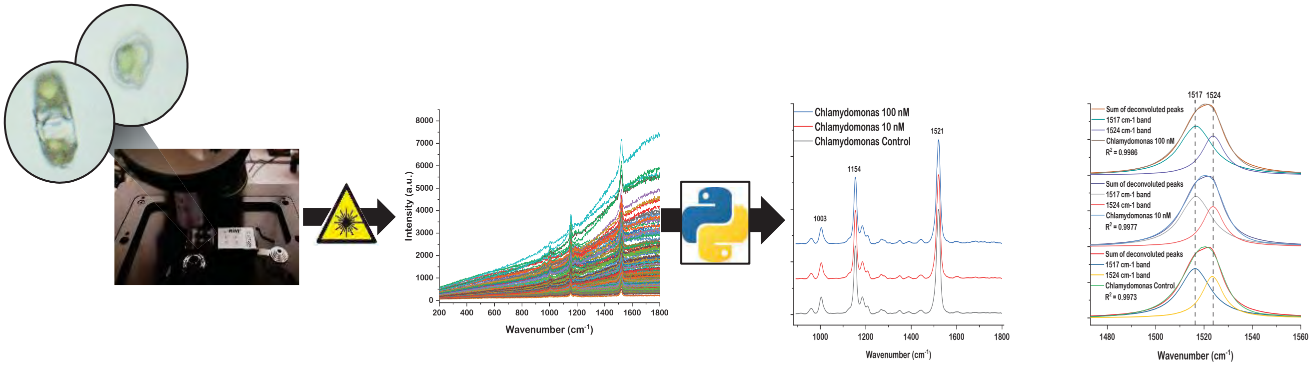

Figure 1: Followed steps during the study, from obtaining Raman spectra to data processing.

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The wide applicability of SERS-tags in environmental science, bionanotech and agritech

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The advent of Surface Enhanced Raman Spectroscopy made possible to achieve Raman signals at the ultimate sensitivity of a single molecule. Nevertheless, sometimes the analytical targets of interest are counterintuitively more challenging than that. The discrimination between healthy and malignant cells is based on the antigens expressed at the cells membrane, very difficult to achieve by a label-free SERS approach. The so-called SERS-tags are sophisticated architectures in which a core, plasmonic, nanostructure is functionalized with a targeting agent and reporter species (dyes, for instance), serving as indirect monitor for the presence of the target under investigation. Our recent best results are herein presented about the application of this concept in three, apparently, different fields. Environmental nano- and microplastics are identified in offshore ocean surface waters thanks to the selective labelling of Au nanostars functionalized with MercaptoBenzoic Acid with tuneable hydrophobicity.[1] Secondly, the immune checkpoint programmed death ligand proteins in cancer cells are targeted by clusters of Au nanospheres functionalized with an *in-silico* engineered peptide.[2] At last, confocal 3D Raman imaging of terpenoids containing microcapsules are obtained thanks to the introduction of SERS-tags in their formulation, allowing to elucidate the role of chitosan as outer capsule layer.[3]

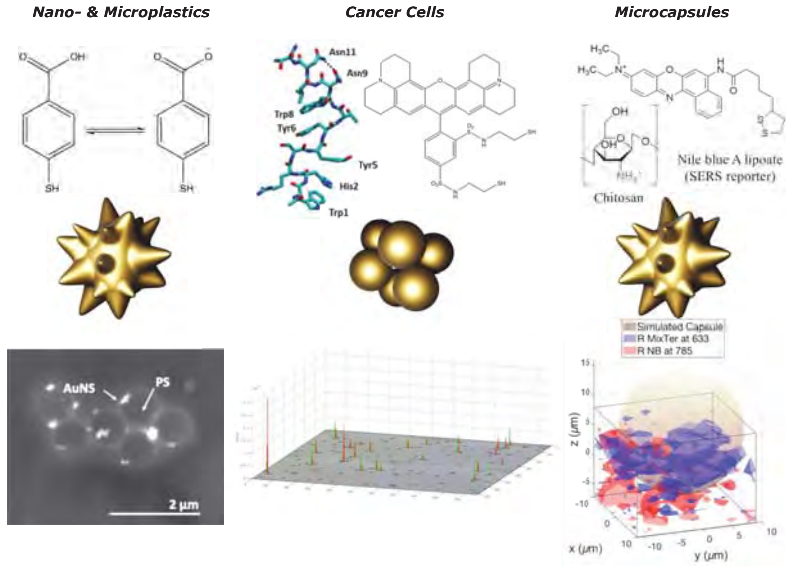
Nano- & Microplastics
Cancer Cells
Microcapsules

Figure 1: the concept of SERS-tags is herein applied in environmental science, bionanotech and agritech.

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Vibrational spectroscopic techniques as diagnostic tools for cardiovascular deseases

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According to the World Heart Federation's 2023 World Heart Report, cardiovascular diseases (CVD) resulted in 20.5 million deaths in 2021 and impact over half a billion individuals globally. Despite a decreasing trend in CVD-related mortality over the past three decades, there exists a plateau in global advancements against cardiovascular diseases, indicating a potential risk of trend reversal without coordinated efforts. Therefore, innovative methodologies for investigating CVD are imperative to enhance their diagnosis and provide valuable insights that could facilitate the discovery and development of novel therapeutic strategies.

While the recent work by Yamamoto et al. [1] successfully explored the assessment of myocardial infarction in cardiac surgery patients using Raman spectroscopy, paving the way for the use of vibrational spectroscopy to monitor the effects of heart failure [2,3], the combined application of FTIR and Raman spectroscopies in the field of cardiovascular research remains poorly investigated.

The present study inspects the application of Raman scattering and FTIR imaging, in conjunction with multivariate statistics, to identify unique spectroscopic features for understanding pathophysiological mechanisms leading to CVD and to develop innovative approaches for diagnosis and pharmacological treatment.

To this end, various experimental murine models of heart failure (HF) were employed [4-6]. By concentrating on the cardiac ventricles and kidney tissues, primary organs affected by pathological dysfunctions, the research enabled the extraction of pertinent biomedical information from complex spectra, facilitating both qualitative and quantitative analyses of the substrates under investigation. Overall, this comprehensive approach suggested vibrational spectroscopy to stand as a promising avenue in HF diagnostics, holding potential for applications in clinical settings.

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Brillouin and Raman microspectroscopy and ATR-FTIR spectroscopy to investigate *Staphylococcus aureus* – induced osteomyelitis and periprosthetic joint infections.

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Osteomyelitis and periprosthetic joint infections (PJIs) are significant public health issues in Western countries due to the increasing life expectancy. These infections often result from bacterial spread through fractures, implants, or blood-borne transmission. The presence of pathogens leads to excessive inflammatory responses, which reduces the regenerative capacity of bone tissue. Surgical intervention is required for treatment as bone tissue is not easily permeable to drug administration. This involves the precise removal of infected tissue, thorough wound cleansing, and direct on-site administration of antibiotics. Systemic treatment is often necessary when an infected prosthesis is involved[1]. Staphylococcus aureus (SA) is the most frequently isolated etiologic agent of infection-induced osteomyelitis and PJIs. SA is a common bacterium that can form a multilayered antimicrobial-resistant biofilm. This biofilm is often found in nosocomial environments[2]. A methodology for investigating SA infection's impact on bone tissue structure, chemical composition, and mechanical properties is discussed. This methodology aims to improve our understanding of the effects of SA infection on bone tissue and to identify specific markers for detecting damaged tissue or pathogen presence with micrometric resolution. The analysis of bone tissue biopsies is carried out using Raman-Brillouin micro-spectroscopy (BRamS) and AFT-FTIR spectroscopy. BRamS and ATR-FTIR are non-destructive, non-contact scattering techniques that do not require labeling and can be used for in vivo applications[3,4], whereas ATR-FTIR's rapid measurement speed can be utilized for the analysis of bone tissue biopsies.

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Live stimulated Raman histology.

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Stimulated Raman histology (SRH) is a novel emerging technique to perform fast histology that mimics classical histology using haematoxylin and eosin (H&E). SRH images are acquired using stimulated Raman scattering (SRS) for two Raman shifts 2845 cm^{-1} and 2930 cm^{-1} corresponding to CH_2 and CH_3 chemical bonds. After acquisition, tile images are processed, stitched and mapped to an H&E-like colour code to generate an image suitable for diagnosis [1]. In principle, any microscope system that performs SRS imaging can generate SRH images. However, there are a number of requirements that need to be fulfil to generate informative and reliable SRH images and they have not been clearly addressed into the literature. In this contribution we wish to fill this gap and we present a SRH technology and imaging pipeline to generate informative SRH images. Our approach uses a shot noise limited dual-colour SRS laser source that can acquire simultaneously the CH_2 and CH_3 images together with a novel lockin detection unit that has been optimized to get the best SNR. We emphasize how beam alignment, laser power, pixel dwell time, lockin optimization and calibration on test samples enable to generate the best SRS images. Next, we present a general virtual colouring scheme that enable to change the CH_2 and CH_3 images into H&E images. With this our system can generate live SRH images over 300µm x 300µm at ~1 frame/s and mm^2 areas SRH images in few minutes. Next, by developing an automatic wavenumber tuning across the full [400-3000 cm^{-1}] we generate images of brain tumour into the fingerprint spectral region and discuss their relevance to complement CH_2 and CH_3 images. Finally, we explore the possibility to perform Epi SRS detection and Epi SRH imaging.

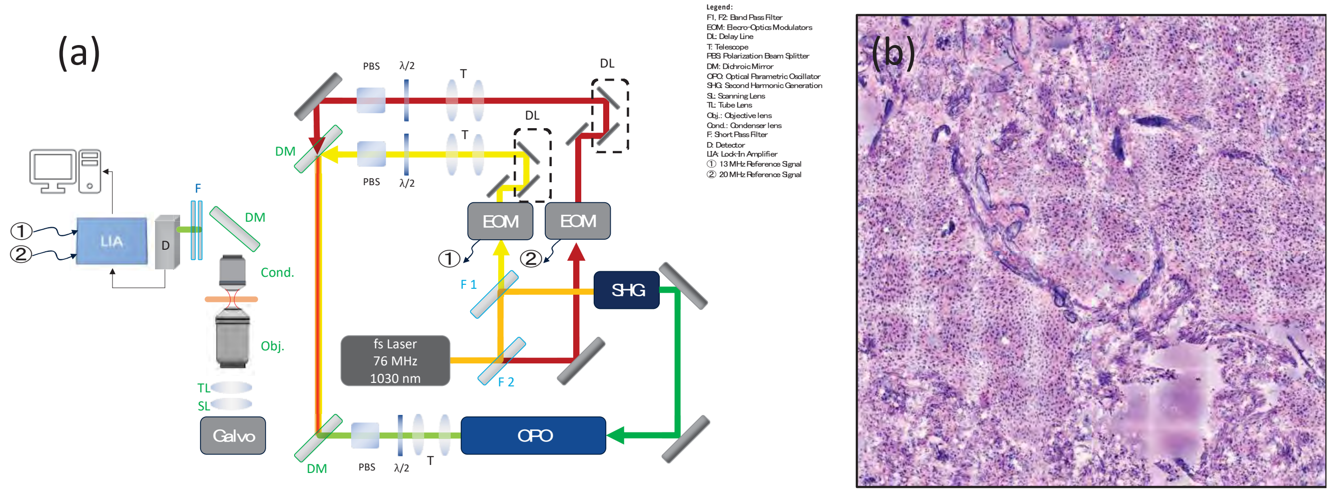

Figure 1: Stimulated Raman histology. (a) Dual wavenumbers ps SRS laser source based on a fs Ytterbium laser, **(b)** Example of brain SRH image across, FoV= 1.6mm x 1.6mm.

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Hydration of sugars as probed by Extended Depolarized Light Scattering (EDLS), Raman and IR spectroscopies

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The hydration of sugars has been extensively investigated due to the relevance of this class of compounds in various fields encompassing, life and medical sciences, biology, pharmaceutics, and food technology.

Despite being a classical topic in solvation science, understanding how and to what extent sugars affect the H-bonding network of water remains elusive, as evidenced by the presence of very contradictory views found in the recent literature. Often different spectroscopic approaches led to different pictures concerning both *H-bonding structure and dynamics* of water, such that a molecular-level understanding of the sugars' hydration is still incomplete.

In the last decade, the Extended frequency range Depolarized Light Scattering (EDLS) technique has been developed in Perugia, to study the fast molecular dynamics in aqueous media by probing the low-frequency (GHz to THz) Raman spectrum [1-3]. EDLS has proven suitable to disentangle the dynamics of solute from that of the solvent and, in turn, to separate the contribution of hydration water from the bulk for several biorelevant systems, including peptides, proteins and sugars [1-3]. More recently, novel structural information has been obtained on glucose and trehalose solutions by using vibrational Raman spectroscopy in conjunction with Mid-IR and Near-IR spectroscopies. Other than being classic hydrophilic model systems, glucose and trehalose are relevant in biopreservation methods, often explained based on water-mediated effects [4,5]. To gain more specific insights on the H-bonding features of hydration water, the so-called solute-correlated (SC) spectra [6] have been extracted from both Raman and IR data.

The results of this comparative analysis will be discussed, presenting a coherent view on the impact of glucose and trehalose on both *hydrogen bonding structure and dynamics* of water, addressing various inconsistencies present in the existing literature.

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Investigating Self-Association and Structural Properties of Mononucleotide G-quadruplexes at Low pH via Raman Optical Activity

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G-quadruplexes (G4) are secondary structures of nucleic acids that frequently form in guanine-rich sequences. Beyond their significant role in genomics, they hold potential applications in bionanotechnology and pharmaceutics. The fundamental unit is guanosine-5'-monophosphate (5'-GMP), capable of self-associating to form mononucleotide G-quadruplexes (mG4) at higher concentrations. These assemblies are held together by weak intermolecular interactions, such as hydrogen bonds and stacking, etc. Despite 60 years of research, various dynamic and structural properties remain undiscovered, including structural variations connected to different stabilizing cations, interconnection of hydrogen bonds in the outer part of mG4, as well as the regularity, helicity, and ordering of G-quartets. All these factors significantly influence the behavior of mG4.

We investigated the agglomeration process of 5'-GMP at low pH (~ 5) using Raman scattering and its chiral variant, Raman optical activity (ROA). Under these acidic conditions, the formation of mG4 is significantly enhanced, leading to the formation of a highly viscous gel [1]. Well-resolved Raman and ROA spectra were obtained in the entire range of fundamental vibrations, proving that ROA exhibits much greater sensitivity compared to Raman scattering towards mG4 formation. Notably, vibrational bands exhibited sharpening and increased intensity (especially in the THz region < 200 cm^{-1}), as illustrated in **Fig. 1**. While ROA spectra of monomeric 5'-GMP units are almost identical at both pH 8.5 and 5, the spectra of mG4 agglomerates are highly distinctive and reflect the higher-order structure. The influence of external conditions, such as temperature and ions, were also investigated.

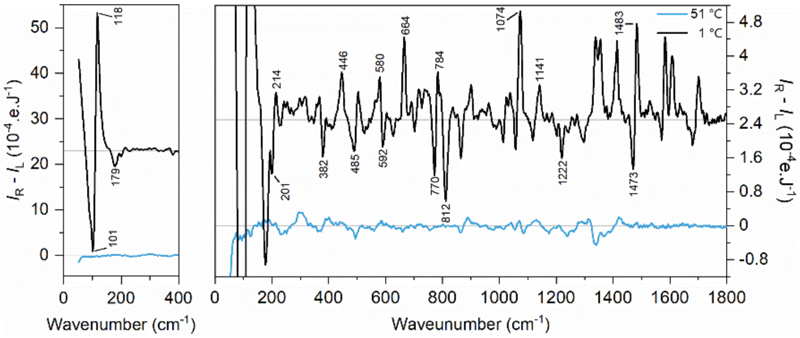

Figure 1: ROA spectra of Na(5'-GMP) in aqueous solution at pH 5.0 – monomer 5'-GMP (black, 51 °C) and mG4 (blue, 1 °C)

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Raman spectroscopy unveils nanostructure and phase engineering of manganese oxide thin films

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Manganese can exhibit different stable oxidation states, giving rise to a variety of oxides (MnO_x). In particular, nanostructured MnO_x thin films with controlled properties show promising application in critical technological fields such as energy conversion and storage (e.g. as cathodes in post-Li batteries and supercapacitors), catalysis, sensing, and environmental engineering. Raman spectroscopy is potentially very powerful in providing useful information on the nanoscale structure of MnO_x thin films, however the chemical complexity and structural richness of MnO_x, also involving mixed-valence, cation-inserted and metastable species, make correct identification via Raman spectroscopy challenging; in addition, interpretation of MnO_x Raman spectra is further complicated by a conflicting literature scenario and by severe laser sensitivity.

Here, we explore the capability of pulsed laser deposition (PLD) - followed by post-deposition thermal treatments - to successfully grow engineered and pure phase MnO_x thin films, whose crystalline phase and nanoscale morphology can be completely decoupled and independently optimized. The detailed Raman characterization of these films enables clear identification of specific MnOx phases and lays the foundations for the unambiguous phase assignment of MnO_x Raman spectra. In detail, we obtained different MnO_x phases (MnO, Mn_3O_4, Mn_2O_3, amorphous MnO_2, and α-MnO_2) with tailored and tunable degrees of porosity and crystallinity by modulating process parameters such as O_2 deposition partial pressure, substrate type, and annealing temperature and atmosphere (air/vacuum).

The reliability of Raman spectroscopy in MnO_x phase assignment is thoroughly investigated and further validated by X-ray diffraction, with the support of other characterizations providing additional insights into composition and crystalline structure [1].

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Micro- and Nano-scale Raman Characterization of VO_2 Thin Films for Smart Window Coatings

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Vanadium dioxide (VO_2) thin films are widely recognized for their reversible thermochromic metal (Rutile) to insulator (Monoclinic M1) phase transition at 68°C, resulting in unique electrical, magnetic, and optical properties. [1] Buildings with glass facades typically have high energy consumption due to heating, ventilation, and air conditioning. The thermochromic phase transition of VO_2 smart window coatings offers a promising solution to reduce energy consumption in such buildings. [2] However, it is challenging to obtain VO_2 thin films of pure monoclinic M1 phase with a lower transition temperature while maintaining good solar transmittance and electrical resistivity using pulsed laser deposition (PLD). [3]

Herein, we applied hyperspectral micro- and nano-Raman imaging for chemical characterisation of layered VO_2 /TiO_2 thin films fabricated using PLD, with a lower VO_2 transition temperature and improved solar transmittance and electrical resistivity. First, the purity of pristine VO_2 thin films was assessed using confocal Raman mapping across the cross-section of a VO_2 thin film, as shown in Figure 1. Confocal Raman spectrum of the VO_2 thin film exhibited the characteristic bands of the monoclinic M1 phase, which confirmed its purity at the sub-micron scale (Figure 1a-c). Next, the reversibility of the thermochromic phase transition of VO_2 thin films was verified using confocal Raman spectroscopy via irradiation with different laser powers. Finally, tip-enhanced Raman spectroscopy (TERS) imaging was performed to investigate the inter-diffusion of VO_2 and TiO_2 within bi- and multi-layer systems, which revealed chemical heterogeneity of the layer interfaces with a nanoscale resolution.

Overall, this study demonstrates the potential of confocal Raman and TERS imaging for micro- and nano-scale chemical characterization of thermochromic VO_2 thin films for smart window coatings.

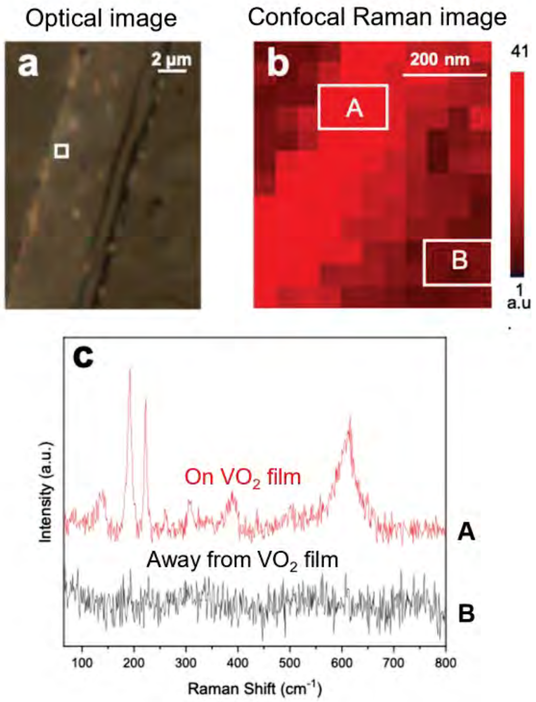

Figure 1. (a) Optical image of the cross-section of a VO_2 thin film. (b) Confocal Raman map of the VO_2 Raman signal measured in the area marked in Panel a. (c) Average confocal Raman spectra measured in the regions A (on VO_2 film) and B (away from VO_2 film) marked in Panel b.

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Unravelling the structure-property correlation in some novel candidate organic ferroelectrics

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The exceptional characteristics of organic ferroelectrics, including their lightweight, flexibility, and non-toxicity, make them highly promising options for sensors and Fe-RAM applications. They are potential replacement for the lead-based ferroelectric materials [1,2]. The investigation of croconic acid's above-room-temperature ferroelectricity has generated considerable enthusiasm in the quest for novel organic ferroelectric materials [3]. Hexamethylene-based co-crystals (*HMX*) were synthesized and analyzed using single-crystal XRD characterization. In-depth analysis of the structural properties included the implementation of powder X-ray diffraction (PXRD), Raman spectroscopy, and differential scanning calorimetry (DSC). Ferroelectric and dielectric measurements demonstrated that *HMX* exhibits room-temperature ferroelectric characteristics with a remarkably high polarization value. Anomalies in dielectric behaviour around 315 K (T_1) hinted at a potential transition from ferroelectric to paraelectric phase, supported by DSC revealing an exothermic shift around T_1. Analysis of temperature-dependent PXRD data through Rietveld refinement revealed a deviation in lattice parameters corresponding to the dielectric anomaly at T_1. Additionally, temperature-dependent Raman phonon frequencies (FWHM) exhibited anomalous behaviour at T_1, providing further confirmation of structural distortions at T_1 = 315 K in *HMX*. The mode assignment by DFT calculation for the compound, threw light into the mechanism of origin of ferroelectricity in *HMX*. This study emphasizes the combined use of various structural characterization techniques, each probing distinct physical properties, to unveil the intricate structure-property relationships in organic ferroelectric materials.

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Anomalous Coupling of Phonon Modes to Specific Electronic States in bulk $PdSe_2$

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The distinctive atomic structure of Palladium diselenide ($PdSe_2$) fosters significant interlayer interactions, leading to layer-dependent electronic properties [1] and anisotropic features [2,3]. In this work, we present a comprehensive analysis of the phonon behaviour in $PdSe_2$ using Raman spectroscopy across a wide temperature range spanning from 5 K up to 300 K. At 120 K, a striking modification in the Raman spectrum is evident: a significant amplification of the out-of-plane phonon A^1_g mode Raman response, along with a suppression of the intensity of the in-plane A^2_g mode. This intriguing observation is attributed to an effect of temperature dependent resonant Raman excitation. The results are corroborated by density functional theory (DFT) calculations, revealing temperature-dependent variations in electron-phonon coupling, strongly linked to the character of the electronic states. Furthermore, we identify nonlinear frequency shift in all phonon modes, indicative of the decay of the optical phonons into multiple acoustic phonons [4]. This investigation, complemented by linear optical spectroscopy, unveils the details of electron-phonon interactions in $PdSe_2$.

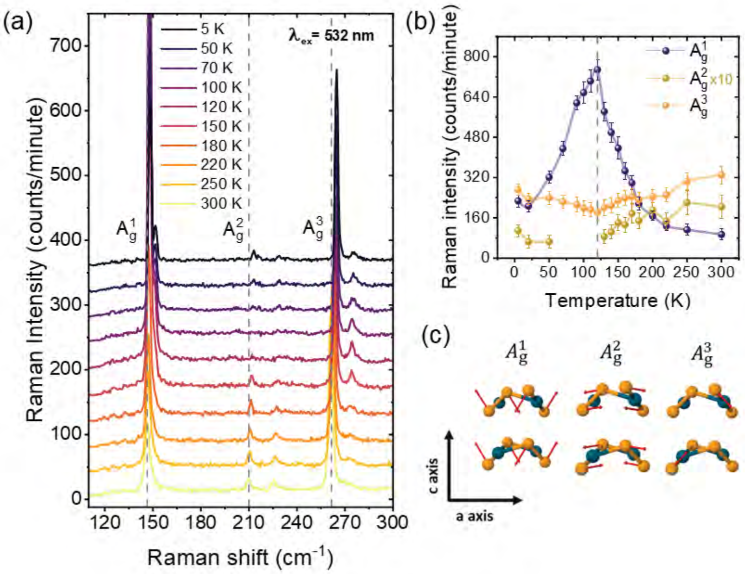

Figure 1: (a) Temperature-dependent Raman spectra of bulk $PdSe_2$ in parallel configuration. (b) The Raman spectral intensities of the A^1_g, A^2_g, and A^3_g modes, as a function of temperature. (c) Atomic displacements corresponding to the A_g phonon modes of $PdSe_2$ projected along the b-axis.

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Raman Spectroscopic Investigation of Nitrogen-containing highly-hydrogenated Amorphous Carbon Nanofoam

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Raman spectroscopy [1] is a versatile technique to probe the insights of carbon-based materials and other nanostructures. In the present work, we control the photoluminescence background and hydrogen content of the nitrogen-containing highly hydrogenated amorphous carbon. These properties are identified by Raman spectroscopy using visible wavelength laser excitation. The nitrogen-containing highly hydrogenated graphite-like amorphous carbon nanofoam is prepared using Pulsed laser deposition at room temperature and 300 Pa background pressure under N2-H2 gas. Photoluminescence background is estimated from the ratio of slope of Raman spectra in the range of 1000 to 1900 cm^{-1} and height of G-peak. We demarcate the region of the carbon nanofoam with varied hydrogen content in the Ferrari-Robertson three-stage model[2] and Casiraghi's model of hydrogenated amorphous carbon[3]. Moreover, the variation in photoluminescence background, hydrogen content, and structural quality of nitrogen-containing highly hydrogenated amorphous carbon is found to be deciding factors on the wettability and hence the electrochemical charge-storage properties while implemented as an energy storage electrode in aqueous electrolyte.

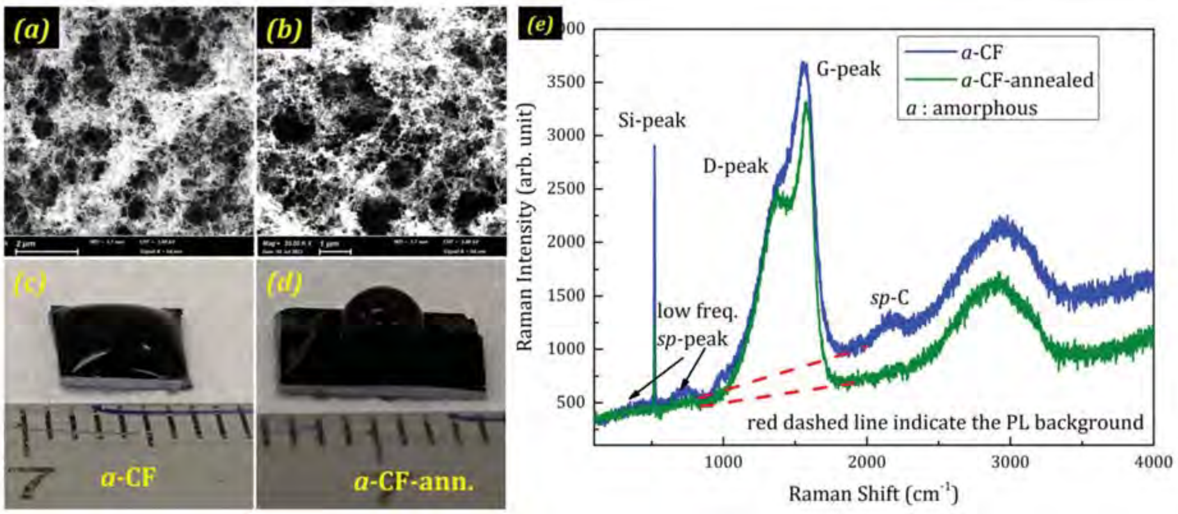

Figure 1: Scanning electron micrograph of (a) amorphous carbon nanofoam, a-CF, and (b) annealed a-CF. (c-d) water wettability and Raman spectra of studied carbon nanofoams.

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Composite Analysis of Natural and Biogenic Minerals by Low-frequency Raman, Resonance Raman and ATR Far-Infrared Spectroscopy

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Since Raman spectroscopy and infrared spectroscopy are complementary techniques, composite analysis using those has widely been used. Low frequency region is also not exception, and has been employed for various composite analysis such as the analysis of crystal polymorphism of pharmaceutical and the analysis of inorganic substances. On the other hand, there have been relatively few cases where such composite analysis has been applied to natural and biogenic minerals such as shellfish.

We focused our attention on this point and analyzed natural minerals such as quartz and pumice and biogenic minerals such as shellfish made of calcium carbonate using Raman spectroscopy, including low frequency region (3750-10 cm^{-1}) and attenuated total reflection mid- and far-infrared spectroscopy (ATR-IR and ATR-FIR, 4000-50 cm^{-1}) [1]. In this study, we demonstrated that ATR-FIR is quite useful for exploring polymorphism and differences in the crystal structure among aragonite biogenic minerals. Using ultra-low frequency Raman spectroscopy, we found that a boson peak appears in the spectra for obsidian and pumice and the difference in the average atomic volume of these minerals by comparing the Raman shift of boson peak (Figure 1). Through these results, the possibility for analysis of both crystal and amorphous minerals by low-frequency Raman spectroscopy and ATR-FIR was demonstrated [1]. Outside of the low frequency region, we also found that we can analyze fluids and bound water by ATR-IR and detect quite small amounts of various carotenoids in shellfishes by resonance Raman effect (Figure 2). In order to advance our research further, we have been working on Raman and IR analyses of shellfishes from different production areas, including the ultra-low frequency region of ATR-FIR. This theme is an important matter because falsification of the origin of shellfishes has become a social issue in Japan recently.

In this presentation, we will report the results of finding differences crystal structures in the FIR region and the results of the comparison of in the carotenoids contained in each shellfish by resonance Raman spectroscopy.

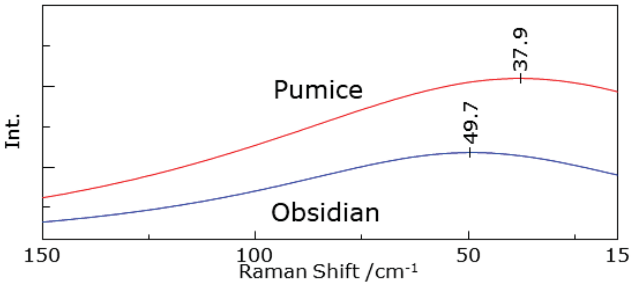

Figure 1: Boson peaks in the low-frequency region of Raman spectra of amorphous SiO_2 minerals (fitted band with baseline corrected)

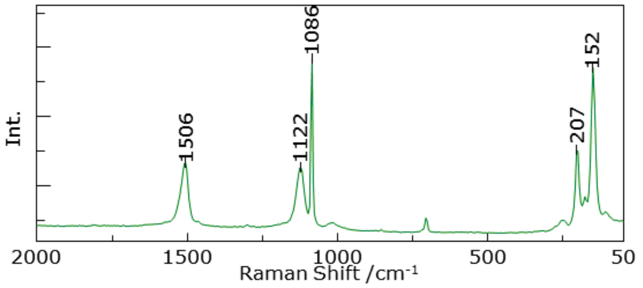

Figure 2: A Raman spectrum of shellfish (*Corbicula japonica*, made of $CaCO_3$) with bands of carotenoids (1506, 1122 cm^{-1})

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Cryo-Raman imaging:
capturing the moment of biological activities

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Raman microscopy has become a powerful tool for visualizing both the morphological and chemical details within biological samples. Despite its potential, traditional Raman imaging has been hindered by low detection sensitivities due to the inherently small cross-section of Raman scattering, making it challenging to image molecules at low concentrations. Additionally, the requirement for long exposure times has often introduced ambiguities in capturing the dynamic spatial and temporal information about sample morphology, biological and chemical states, and their distribution.

Addressing these challenges, we have developed a Raman microscope for imaging cryofixed biological samples maintained at low temperature [1]. The enhanced physical and chemical stability of samples at low temperature enables prolonged exposure times, realizing high sensitivity Raman microscopy of molecules while preventing motion artifacts in the imaging process. Cryofixation also serves to preserve the physiological state of samples, including the redox state of molecules and ion distributions that are not maintained through chemical fixation methods. Utilizing thes advantages, we have successfully achieved Raman imaging of cryofixed cells and tissues, demonstrating significant improvements in spatial and spectral resolution and sensitivity.

Furthermore, we have innovated a technique for the rapid freezing of samples directly on the microscope stage [2]. This technique allows the immediate suspension of cellular activities under optical observation, facilitating the precise determination of the timing for cryofixation and thus enabling a "Raman snapshot" of cellular activity at specific moments. This approach unveils chemical information during physiological processes within the cell that is inaccessible by conventional techniques.

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Implementation of the Hammett Acidity functions in Raman spectroscopy for determining the acidity level accessible in ionic liquids

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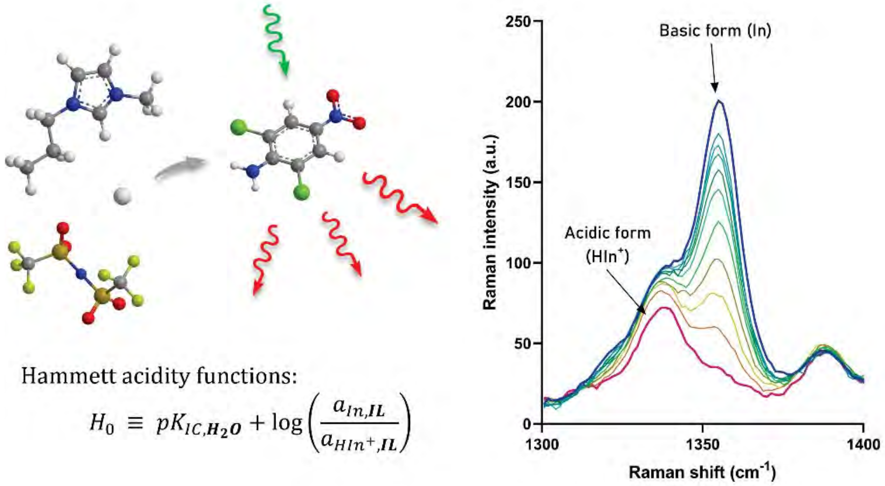
Ionic liquids (ILs) possess outstanding properties, such as specific solvation abilities and a super-acidic feature, explaining their use as solvents for acid-catalysed reactions, for instance.[1] In that context, understanding and determining the levels of acidity accessible in ILs is essential to better apprehend their use as super-acidic media for novel chemical processes. The fundamental level of acidity achievable in ILs (and in all solvents generally) is related to the solvation properties of the solvent on the proton.[2] However, data on solvation energies for protons in non-aqueous solvents, and ILs especially, are still missing. Indeed, energy of solvation of protons in such solvents can only be determined experimentally through extra-thermodynamic hypotheses. One of the methods relying on such extra-thermodynamic hypotheses is the Hammett method, based on the protonation equilibrium of nitroanilines, monitored by UV-Visible spectroscopy (UVS).[3] However, the transfer of the Hammett method from molecular solvents, as originally proposed, to ILs might not be straightforward due to the possible interactions between the nitroanilines and the ions of the ILs (π-staking) as well as the suggested non-dissociating behaviour of ILs. In this work, we propose to implement, for the first time, the determination of the Hammett acidity function using Raman spectroscopy (RS). Using RS instead of UVS would enable the use of new, non-coloured, more spherical and non-aromatic pH-reporters, better meeting the fundamental hypothesis of Hammett. In this work, the level of acidity of three 1-alkyl-3-methylimidazolium bis(trifluoromethane)sulfonimide ILs (alkyl = ethyl, butyl, octyl) were determined by the method of Hammett, to study the influence of the alkyl chain length on the acidity the solvents, either by UVS and RS to confirm the appropriate implementation of the approach in RS. The coordination number of the protons in such mediums was also determined by RS, giving insights into the super-acidic feature of ILs compared to water. Since RS has a lower sensitivity than UVS, the influence of the pH-reporter concentration on the observed acidity level (hence the solvation energy) was also assessed. Nitroanilines are intrinsically basic and could decreasing the level of acidity accessible in the ILs. Finally, other constituting cations were studied, selected to study the influence of specific interactions between the IL and the pH-probe: 1-Butyl-1-methylpyrrolidinium bistriflimide and 1-*tert*-butyl-3-methylimidazolium bistriflimide were chosen to study the consequence of aromaticity and steric hindrance on the acidity, respectively.



Hammett acidity functions:

$$H_0 \equiv pK_{IC,\mathbf{H_2O}} + \log\left(\frac{a_{In,\mathbf{IL}}}{a_{HIn^+,\mathbf{IL}}}\right)$$

Figure 1. Left: Scheme of the protonation of 2,6-dichloro-4-nitraniline (IC) in the 1-butyl-3-methylimidazolium bistriflimide ionic liquid (IL) measured by Raman spectroscopy, and equation of the Hammett acidity functions. Right: Evolution of the 1300-1400 cm^{-1} Raman spectrum of the IC with increasing amounts of bistriflimidic acid in the same IL.

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Determining the Geographic Origin of Palm Oil using Handheld Raman Spectroscopy

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- **Objective of work**

  Field-deployable Raman spectroscopy has been effectively applied for the geographic discrimination of palm oil originating in West Africa. The strong vibrant red and orange colours of palm oil are largely due to its richness in beta-carotene. Palm oil is often vulnerable to economically motivated adulteration with various synthetic dyes which illegally enhance the colour of the oil. In addition, Amnesty International have reported links between palm oil production and child and forced labour. Therefore, there is a need for analytical methods which can give a high level of traceability back to a plantation of origin where fraudulent or inappropriate profiteering activity may take place.
- **Methodological detail**

  A portable CBEx handheld Raman spectrometer operating at 1064nm was used to collect data with an acquisition time of 10 s per sample.
- **Main results of study**

  A total of 172 palm oil samples from across West Africa were analysed, and Principal Component Analysis (PCA) was employed as a dimensionality reduction method to discriminate between samples from their countries and regions of origin. Subsequent PCA loadings plots indicated that the beta-carotene content of the oils had a large influence on the clustering of the scores plot which was associated with the farm/location of the origin of the oil. This demonstrated the usefulness of beta carotene as an excellent source of chemometric variance. Further analysis also indicated the presence of palm kernel oil instead of palm oil from some plantations. Although derived from the same plant, the two oils show vast chemical and nutritional differences.
- **Implications**

  Current analysis techniques used for food analysis are often laboratory-based and require extensive sample preparation as well as the added time and cost associated with transporting samples; possibly adding weeks or months onto a project's timeline. Portable Raman spectrometers allow for rapid on-site measurements along with the added benefit of being a non-invasive technique with very little sample preparation.
- **Meanings**

  Portable Raman spectrometers can therefore be used as an effective field-deployable technique which can save both time and costs.
- **Conclusions**

  Portable Raman spectroscopy coupled with PCA was able to discriminate between the palm oils from various plantations across West Africa, demonstrating the potential of these devices for food analysis.

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Looking beyond noble metals: Quest for inexpensive but efficient SERS substrates

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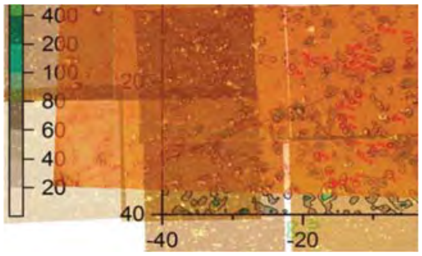
Typically, substrate for SERS are made of gold or silver films or/and nanostructures, however, those may suffer from contamination by S-containing compounds and corrosion Substrate, which is far less expensive and much more universally available in comparison to gold is Al foil. Also it is passivated with several nm thick layer of Al_2O_3 and thus may be more stable and less susceptible to contamination. Therefore, Al foil may make SERS more attractive for variety of real life applications in chemical analysis. Our group demonstrated that application of commercial gold nanoparticles on untreated Al foil as SERS substrate in detection of various analytes (urea. paracetamol, 4-NBT, CV, melamine) can be efficient (e.g. EF up to 10^7) and sensitive (LOD as low as 0.1-0.2 nM) [1, 2] . Since dimerization/association of gold nanoparticles modified with Raman active thiols should potentially create additional hot spot between those nanoparticles (NPs), which would make signal in SERS immuno or other assays effected by NP association we are interested in studding this kind of effects on several substrates (gold, silver, Al film, silicon) . We do this by combining Raman and AFM maps or Raman and SEM maps, using a new algorithm, that we have not seen yet reported in literature, making a significant improvement in combination procedure reported in Sergiienko et. al.[3, 4] The third direction of our group is SERS sandwich assays of biomarkers. Using this method, we could detect about the same amount of human immunoglobulin in 24- hour comparative assay on silicon and in the assay on gold; while the assays on Al foil gold and on gold obtained the similar LOD for tuberculosis biomarker MPT64 around 2 ng/mL [5,6]. The selectivity for human IgG (vs rabbit and rat IgG) in the simultaneous assays on Al foil and on silicon was significantly higher in comparison with the selectivity in the assay on gold (by ~1.7-2 on Al and 8-10 on Si). [6] In this talk we present the development of all those directions.



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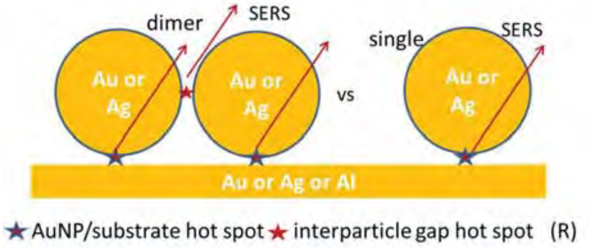

(R)

Figure 1 Extract of Raman/AFM map combination (L), scheme of NPs modified with SAM of a thiol (R)

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Structural and Optical properties of Yb^{3+}, Er^{3+}co-doped WO_3 upconversion nanoparticles

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Recently, rare-earth doped upconversion nanomaterials have attracted great interest due to their low toxicity, deep light penetration, low autofluorescence, and photostability. Because of their excellent physicochemical properties, upconversion nanomaterials are extensively applied in the detection of various analytes, bioimaging, therapies, energy conversion, and security [1]. In this study, we report the structural and optical properties of Yb^{3+}, Er^{3+} co-doped WO_3 nanoparticles (WO_3:Yb^{3+}, Er^{3+} NPs) as a function of annealing temperature. To investigate the effect of annealing temperature on the crystal structures of the WO_3:Yb^{3+}, Er^{3+} NPs, a Raman scattering study has been conducted. The Raman spectra of the samples are very comparable to WO_3, indicating that WO_3:Yb^{3+}, Er^{3+} NPs do not change the crystalline phase with Yb^{3+}, Er^{3+} co-doping or annealing temperatures. The intense Raman peaks centered at 807 cm^{-1} and 714 cm^{-1} are the characteristic for crystalline monoclinic WO_3 (m-phase), which correspond to the stretching vibrations of the bridging oxygen ν(O-W-O) and ν(W-O), respectively [2]. The intensity of the Raman peak increased with increasing annealing temperature, as did the photoluminescence emission intensity and the intensity of the XRD peak. These results indicate that the crystallinity of the WO_3:Yb^{3+}, Er^{3+} NPs improved with increasing annealing temperatures.

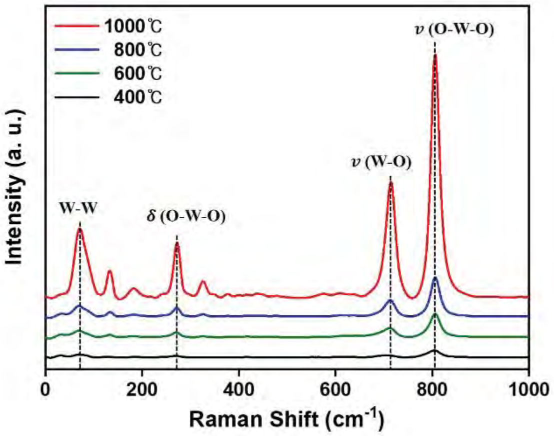

Figure 1: The Raman spectra of the WO_3:Yb^{3+}, Er^{3+} NPs as a function of annealing temperature.

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Mechanistic and Theoretical studies on Molecular Molybdenum Sulfide Hydrogen Evolution Reaction Catalysts

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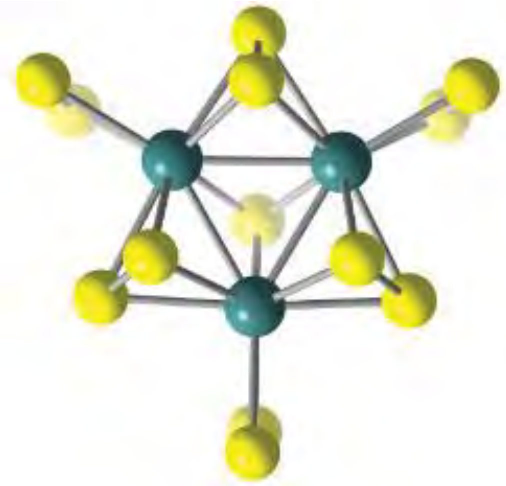

Molecular metal sulfides, or thiomolybdates, are promising molecular catalysts for the photocatalytic or electrocatalytic hydrogen evolution reaction (HER).[1] In particular, the prototype cluster $[Mo_3S_{13}]^{2-}$ (left) has been used under a variety of homogeneous and heterogenized conditions for HER catalysis. However, this reaction is complex and not yet fully understood. In order to improve the HER performance and stability of the clusters, in-depth understanding of the catalytic mechanisms, as well as processes leading to structural changes and degradation of the clusters is necessary.[2] This study aims to identify the different steps of the mechanism using different spectroscopic and computational analysis methods. We will study the structural changes of thiomolybdates under catalytic conditions using in-operando Raman-spectroscopy.

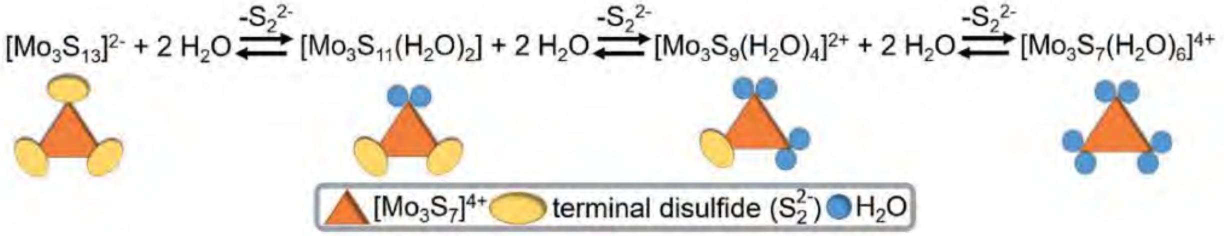

Figure 1: Top : Illustration of the $[Mo_3S_{13}]^{2-}$ prototype thiomolybdate. Bottom: proposed ligand exchange, leading to the introduction of terminal aquo ligands on the molybdenum sulfide $[Mo_3S_7]^{4+}$ core.

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Graphene mediated SERS detection of DNA Hybridization

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Surfaced Enhanced Raman Spectroscopy (SERS) stands in the foremost of modern spectroscopic analytical techniques, offering remarkable ultrasensitivity and specificity in the detection and identification of target molecules. In this work, graphene-mediated SERS (G-SERS) ultrasensitive detection technique was efficiently used for DNA hybridization. Specifically, our detection strategy involved designing a hybrid plasmonic nanosensor based on directly anchored gold nanospheres (AuNPs) (~35 nm diameter, proved by Transmission Electron Microscopy) onto the graphene sheets via an adopted *in situ* reaction [1]. Then, this nanoplatform was used to graft polyadenine of various lengths (i.e. 5, 10, 15 and 20 bases) by broking the disulfide bonds using tris(2-carboxyethyl) phosphine (TCEP) followed by the addition of the polyadenine solution to graphene oxide AuNPs (GO-AuNPs) solution [2]. Finally, the DNA hybridization was evaluated using polythimine as complementary single strand DNA of various length (i.e. 5, 10, 15 and 20 bases) at different concentrations, ranging from 10^{-7} to 10^{-4} M, using 633 and 785 nm excitation laser lines. Our findings not only contribute to the fundamental understanding of DNA-DNA hybridization, but also offer valuable insights for the development of ultrasensitive biosensors and diagnostic platforms.

Acknowledgements: This work was supported by the project PNRR-C9-I8-CF-199/28.11.2023. V. Cucuiet acknowledges the financial support from the Special Scholarship for Scientific Activity, grant awarded by STAR-UBB (Babes-Bolyai University) contract number 36263/24.11.2023.

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Machine Learning-Assisted Raman Spectroscopy for the Classification of 21 Strains of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa (PA) is a multi-drug resistant bacterial pathogen and the leading cause of ventilator-associated pneumonia (VAP). In point-of-care settings, conventional culture-based methods for bacterial-detection diagnosis are too time- and labour-intensive to be highly effective, often resulting in the inappropriate prescription of broad-spectrum antibiotics [1]. Alternative methods for the rapid identification of PA strains and their associated drug-sensitivity are therefore of vital importance to enable targeted treatment of VAP and mitigating the rise of anti-microbial resistance (AMR).

Recently, Multi-excitation Raman (MX-Raman) spectroscopy has shown to provide highly accurate results for species, resistance, and strain-level characterisation of bacteria [2]. Combined with machine learning (ML) techniques such as support vector machines, MX-Raman promises to be a highly effective strategy for rapid pathogen detection [3].

This poster will present a performance comparison of multiple ML models for the rapid and accurate characterisation of 21 strains of PA, forming part of an automated spectral analysis workflow for strain-level bacterial identification with Raman spectroscopy. Additionally, the poster will compare ML model performance with spectral data acquired with both single and multiple excitation methods.

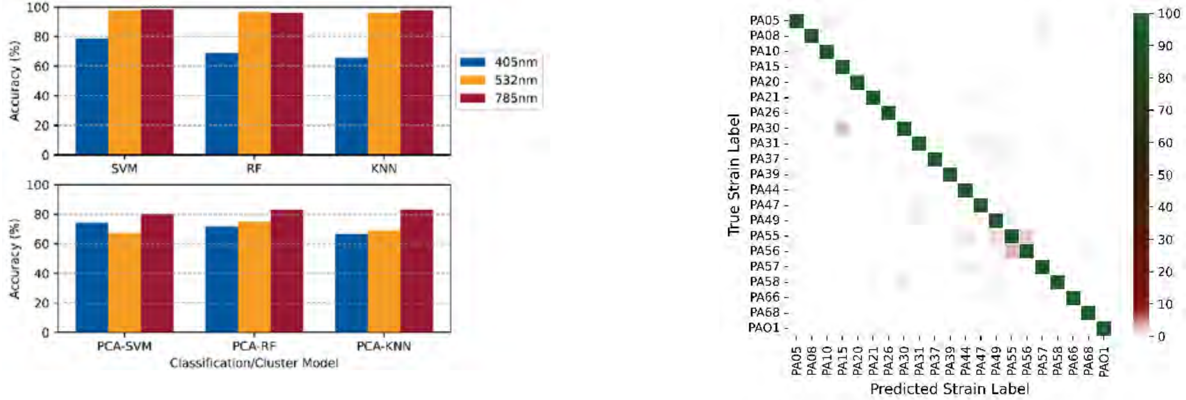

Figure 1 (**LEFT**) Mean classification accuracy for a support vector machine (SVM), random forest (RF), and k-nearest neighbours (kNN) classifier trained with a labelled 21 PA strain dataset acquired using a 405nm, 532nm, and 785nm excitation laser with and without principal component analysis (PCA) applied prior to training. (**RIGHT**) SVM mean confusion matrix trained using a 785nm excitation laser without PCA.

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Exploring the Structural and Electronic Dynamics of Self-Healing Materials for Energy Conversion and Storage: A Raman Spectroscopy Investigation

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Delving into the cutting-edge realm of using linear and non-linear Raman spectroscopy techniques[1] to thoroughly explore the structural and electronic properties of functionalised self-healing polymers. These polymers have emerged as a promising path for robust and resilient systems in energy conversion and storage applications. Although these polymers exhibit two self-healing mechanisms, that is, intrinsic and extrinsic, our focus lies on intrinsic self-healing which is rooted in the reversibility of polymer matrix bonding [2, 3]. It incorporates the integration of reversible cross-linking capabilities through bonds such as Diels-Alder reactions [4], Disulfide bonds [5], Acylhydrazone [6], metal-ligand interactions [7], and more.

Raman techniques are employed to unravel the complex molecular mechanisms that underlie self-healing processes. They are non-destructive with high specificity and are also highly effective analytical tools for identifying and quantifying compounds (chemical bonding and structural changes), elucidating reaction mechanisms, and monitoring dynamic processes in real time. In addition to these techniques, 2D correlation analysis will be utilized to untangle the intricate correlations between different spectral features, Density Functional Theory (DFT) to offer a theoretical foundation for the experimental observations, while multicomponent analysis will be applied to disentangle complex mixtures of compounds. These techniques serve as powerful allies, providing complementary insights into the molecular dynamics at play.

For instance, temperature-dependent Raman spectroscopy was employed to explore the reversibility of a functionalized polymer whose self-healing properties are linked via the acylhydrazone bonds within its matrix. 2D correlation analysis was further used to reveal intricate relationships among spectral features and to provide deeper insights into the dynamic processes underlying the polymer's functionality enriching the understanding of its unique properties.

Acknowledgements: The authors would like to thank Deutsche Forschungsgemeinschaft (DFG) for their support and funding under project number 455748945.

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Role of solvated lithium-ions on non-aqueous Li-CO_2 battery

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It is debatable how the performance of Li-CO_2 batteries is affected by the interaction between the catalyst surface and the interfacial microenvironment. The shell-isolated nanoparticles-enhanced Raman spectroscopy (SHINERS) technique allows us to acquire direct spectral information on species adsorbed on the surface of single crystals utilizing in-situ Raman spectroscopy[1,2]. Here, we investigate the impact of changes in the microenvironment of the three fundamental crystal planes of Au single crystals and solvent molecules on the CO_2 reduction reaction (CO_2RR) process route using electrochemical in-situ Raman spectroscopy in conjunction with theoretical calculations. In-situ SHINERS and ab initio molecular dynamics (AIMD) simulations prove that in the presence of organic solvents, CO_2 was adsorbed to the surface of the Au single crystal through carbon-oxygen bidentate adsorption to form CO_2^-. The pathway of CO_2 reduction then changes according to any changes in the microenvironment at the interface. In addition, after combining the electrochemical data and the observed CO_2^- Stark slope, it was discovered that Au(110) had the best activity. These findings suggest that by adjusting the solvent molecules at the interface to improve the lithium-ion solvation rate, the formation state of the insulating lithium salt can be optimized, thereby greatly improving the reaction rate and cycle stability of the catalyst.

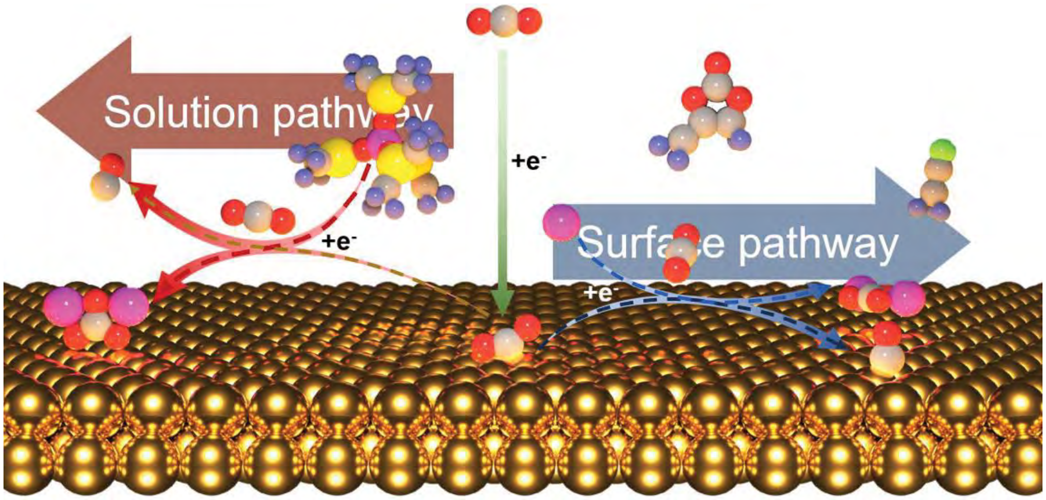

Figure 1: Schematic illustration of the CO_2 reduction mechanism

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In situ Raman study of photocatalytic water oxidation on $BiVO_4$

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[b] *College of Materials, Xiamen University.*

Photocatalytic water splitting is one of the promising solutions towards the world's sever energy and environmental crisis, which is hampered by the sluggish oxygen evolution half-reaction. This can be traced back to the limited mechanistic insight about this bottleneck reaction, especially for the interfacial water information over metal oxides, which is of huge significance but seldom mentioned in related research. Herein, taking the advantages of shell-isolated nanoparticles enhanced Raman spectroscopy (SHINERS) [1], the spectroscopic evidence of reaction intermediates and interfacial water over highly (010) facet exposed $BiVO_4$ during photocatalytic water oxidation reaction are simultaneously obtained. Combined with ab initio molecular dynamics (AIMD) and density functional theory (DFT), under photocatalytic conditions, 'O'-side down and 'H'-side down interfacial water coexist on $BiVO_4$ surface, resulted from the localized hole on surface Bi atom and hydrogen bond interaction with surface O atom, respectively. This implies that the characters of interfacial water on metal oxides significantly differs from that on metal. Besides, highly (110) facet exposed $BiVO_4$ show inferior photocatalytic performance, and exhibited different intermediate and interfacial water spectroscopic signature, which is the molecular origin of semiconductor photocatalysis crystal facet effect [2].

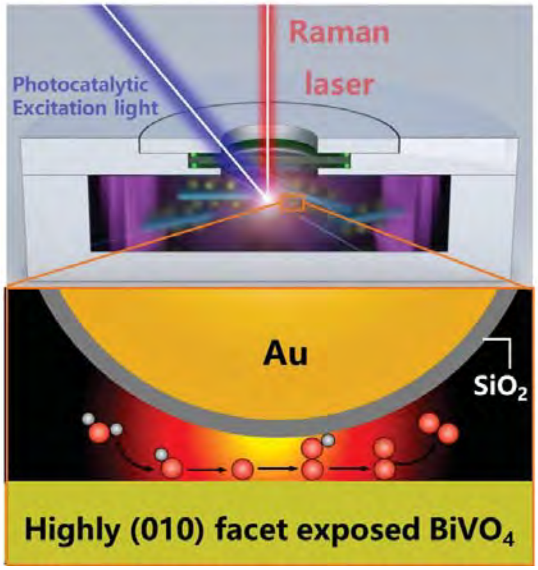

Figure 1: Schematic illustration of in situ Raman experiment of photocatalytic water oxidation on $BiVO_4$

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Probing the Mesomeric Effect and its Influence on the Molecular Electron Density via Surface-Enhanced Raman Spectroscopy

Marc Bröckel, Kai Braun and Alfred J. Meixner
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Understanding the molecular electron density, a fundamental property governing molecular interactions, plays a vital role in determining molecular structures, bonding patterns, and overall chemical reactivity. This knowledge is crucial for e.g. designing molecules with tailored properties in fields such as pharmacology, materials science, or organic optoelectronics. The most common way to taylor the molecular electron density is to introduce substituents with a mesomeric (M-) or inductive (I-) effect. Recently, we have shown via tip-enhanced Raman spectroscopy that a positive bias voltage applied to a gold tip in proximity to a molecule on a gold surface leads to a reversible electron density shift from the Au-substrate to the molecule observed as intensity increase in the corresponding TERS-spectra. [1] This positive bias voltage can be viewed as an external I-effect.

In this study, we employ surface-enhanced Raman spectroscopy (SERS) to probe the electron density of molecules from the 2-mercaptobenzothiazole (MBT) family. Introducing an ethoxy group (EMBT) or a chlorine atom (CMBT) as +M substituents drastically changes the molecular electron density which can be directly observed in the SERS intensity. We employ the Natural Bond Orbital (NBO) theory to provide a comprehensive insight into the processes leading to the experimentally observed shift in electron density.

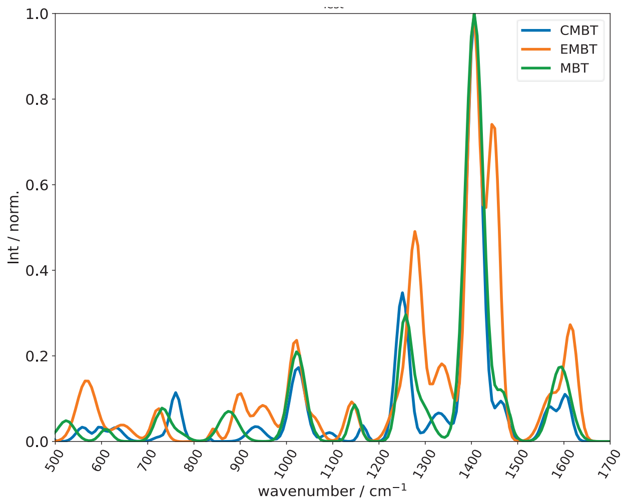

Figure 2: SERS Spectra of CMBT, EMBT and MBT. The peak intensities differ due to the different substituents

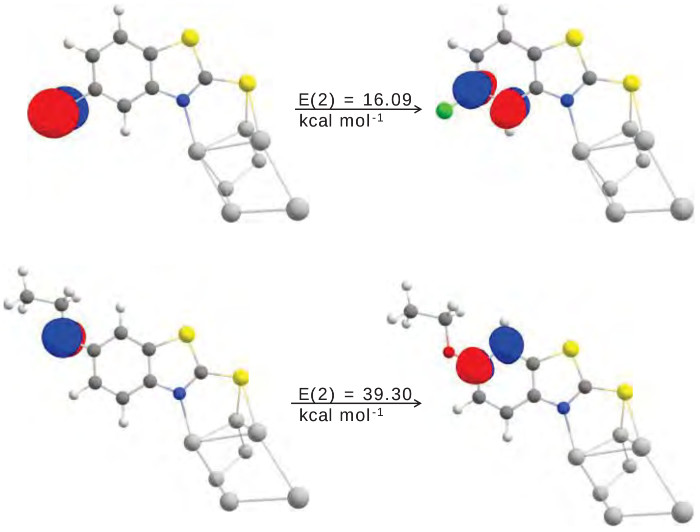

Figure 1: NBOs with corresponding E(2) energies for the substituents' mesomeric effect

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Metal-nanoparticle-topped plasmonic tip structures for tip-enhanced Raman spectroscopy

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Tip enhanced Raman spectroscopy (TERS) was experimentally demonstrated by combining scanning probe microscopy (SPM) and surface enhanced Raman spectroscopy in 2000 [1–4]. TERS is a powerful tool to meet growing needs for nanoscale structural and chemical analysis in various fields. Although the potential scope of TERS covers all materials to which both SPM and Raman spectroscopy are applicable, its current applications are biased toward a few types of nanomaterials, such as carbon nanomaterials. One of the reasons for this situation is that in most cases, sufficient electromagnetic field enhancement can only be obtained by using the “gap-mode” configuration. The gap-mode configuration utilizes the plasmonic coupling between a metallic SPM tip apex and a metal substrate surface and thus severely limits sample sizes. To extend TERS application, it is crucial to design tip structures that can provide strong electromagnetic field enhancement without substrate support.

Here, we introduce a plasmonic tip structure in which a single Ag nanoparticle (NP) is placed at the tip of an Ag-coated atomic force microscope (AFM) probe. Our theoretical calculation using a finite difference time domain (FDTD) method revealed that the plasmonic coupling between the Ag NP and the Ag tip enhances electromagnetic field at the apex of the tip structure more effectively than the tip alone (Fig. 1). We experimentally tested TERS performance of the tip structure not by fabricating the Ag-NP-topped structure but by bringing commercially available Ag-coated Si AFM tips into contact with Ag NPs dispersed on the surface of a sample. Graphene sheets and Si substrates were employed as samples to validate the Raman enhancement effect of the tip structure for nanomaterial and bulk material. The TERS contrast when the tip was in contact with the nanoparticle was more than 20-fold and 2-fold higher for graphene and silicon, respectively, than for the tip alone. These results indicate that the NP-topped tip structure can be a promising candidate to improve TERS sensitivity without the gap-mode configuration.

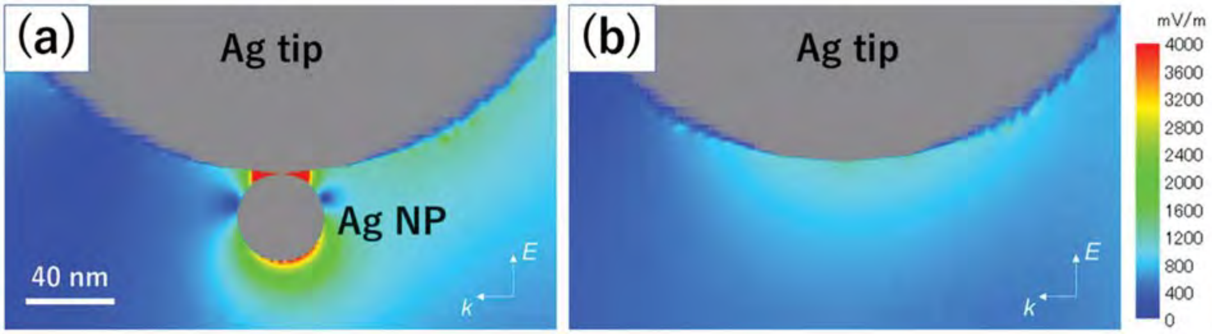

Figure 1: Electromagnetic field distributions simulated by using an FDTD method for (a) the Ag-nanoparticle-topped tip and (b) the Ag tip alone.

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Probing Plasmon-Driven Chemistry with a Transition Metal Photocatalyst

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Iron phthalocyanine (FePc) is a versatile catalyst that can facilitate reactions such as C-C bond formation, CO_2 reduction, oxygen reduction, and oxidation reactions[1]. Optimizing the energy efficiency of these processes is crucial for applications in photodynamic therapy and clean fuel synthesis. Recently, plasmonic confinement of catalysts has been shown to enable access to alternative reaction pathways that enhance catalytic activity and selectivity[2–4]. Irradiating noble metal nanoparticles with visible or near-IR light induces a collective oscillation of surface conduction electrons known as a localized surface plasmon resonance (LSPR), which generates “hot spots” of electric field enhancement. This LSPR then decays, either radiatively via light scattering, or non-radiatively by forming electron-hole pairs. The interaction between plasmon and catalyst has been comprehensively studied for the prototypical photocatalyst $[Ru(bpy)_3]^{2+}$, but there has yet to be any study of the photochemical and photophysical properties of FePc under plasmonic confinement[5]. Hence, this work builds upon our lab's prior investigations of $[Ru(bpy)_3]^{2+}$ under plasmonic confinement, but with a focus on substituting the precious metal catalyst with iron, a more cost-effective and abundant transition metal. This work investigates the plasmon-induced heating and energy transfer pathways at the interface between FePc catalysts and gold plasmon resonances with varying electronic properties to enhance its innate heating and catalytic properties. Elucidating interaction mechanisms as well as the physical orientation of the photocatalyst with respect to the plasmonic nanoparticle can inform further catalyst design to improve reaction efficiency[6]. These reaction dynamics can be probed by a range of linear and nonlinear optical techniques, including in situ SERS and ultrafast transient absorption spectroscopy.

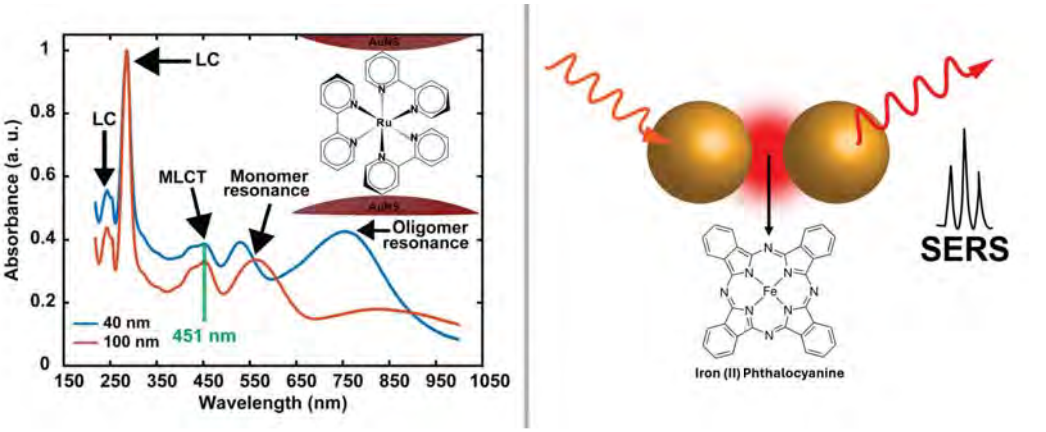

Figure 1: ***(left)*** **Absorption spectra of $[Ru(bpy)_3]^{2+}$ under plasmonic confinement.** ***(right)*** **FePc under plasmonic confinement for SERS.**

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Modified graphene oxide-gold nanoparticles composites as sensitive rhodamine 6G and folic acid detection substrates

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Improving methods for detecting various substances has become an important issue nowadays. The development of science require the used methods to be as sensitive and easy to prepare as possible. Surface-enhanced Raman spectroscopy is ideal for this purpose. In recent years, there has been interest in graphene oxide in the context of SERS substrates. It has been proven that placing an analyte on its surface enhance the Raman signal [1]. The combination of graphene oxide with noble metal nanoparticles results in additional amplification of the SERS signal, the enhancement results of the synergistic effect of two mechanisms: electromagnetic and chemical. It has been shown that conditioning graphene oxide in various solutions affects the SERS enhancement [2].

To prepare substrates, we used graphene oxide modified in solutions with pH = 1, pH = 5.6, pH = 13, as well as gold nanoparticles in the shape of nanobowls, which in previous studies showed very good SERS enhancement [3]. The tested compounds were rhodamine 6G and folic acid in concentrations ranging from 10^{-5} M to 10^{-11} M.

It has been proven that a composite consisting of graphene oxide modified at acidic pH and gold nanoparticles enhances the spectra of both rhodamine 6G and folic acid better than composites with graphene oxide conditioned at a higher pH. It was also shown that folic acid adsorbs on the composite surface in a different way - it depends on the modification of graphene oxide.

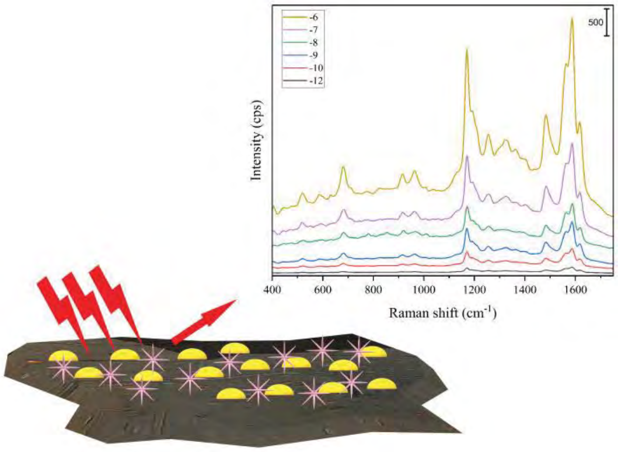

Figure 1: Scheme of the substrate and Raman spectra of folic acid with concentrations from 10^{-6} to 10^{-11} M.

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Fully Atomistic Multiscale Modeling of Surface-Enhanced Raman Scattering for Biosensing Applications

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Surface-enhanced spectroscopies are revolutionizing the field of biosensing devices with their remarkable sensitivity and selectivity towards (bio)molecules, enabling detection even down to the single-molecule level, by harnessing localized surface plasmons [1].

From a modeling perspective, multiscale approaches can be exploited to predict the spectroscopic response of target molecules near plasmonic substrates, such as in Surface-Enhanced Raman Scattering [2]. Recently, a Quantum Mechanics/Molecular Mechanics approach for calculating SERS spectra has been proposed by some of the authors [3], utilizing the cost-effective frequency-dependent force fields ωFQ [4] and ωFQFμ [5] to describe the linear electronic response of noble metals and graphene-based nanostructures to external radiation. These classical fully atomistic force fields enable the tuning of the plasmon resonance frequency, thereby enhancing spectroscopic signals of the target molecule.

This contribution adds depth to the previous model by introducing a fully atomistic three-layer model, which incorporates solvent electronic polarization and specific molecule-solvent-plasmonic substrate interactions. Within this model, the molecule is described at the TDDFT level, the plasmonic substrate is characterized using the ωFQ/ωFQFμ models and the solvent is represented by employing the polarizable Fluctuating Charges/Fluctuating Charges and Fluctuating Dipoles FQ/FQFμ force fields [6].

The proposed multiscale QM/ωFQ(Fμ)/FQ(Fμ) approach, combined with sampling of the configurational phase space of the system through classical molecular dynamics simulations [7], is challenged to predict the surface-enhanced Raman signals of molecule/plasmonic nanostructure/solvent interfaces, comparing the performance of the model against experimental data.

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Acknowledgements

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SERS Efficiency in Detecting G-Quadruplex: Citrate- vs. Spermine-Stabilized Silver Nanospheres

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G-quadruplexes (G4) are unique secondary structures formed by certain DNA or RNA sequences rich in guanine (G) bases. These structures arise from the stacking of G-rich sequences into planar arrangements called G-quartets. In a G-quartet, four guanine bases interact through Hoogsteen hydrogen bonding, forming a square-like structure. Multiple G-quartets can stack on top of each other, resulting in the formation of a G4. G-quadruplexes have attracted considerable attention owing to their distinctive structural features and various biological roles, including their relevance in drug design [1]. Surface-enhanced Raman scattering (SERS) spectroscopy has arisen as a potent method for investigating the structural dynamics and stability of G-quadruplexes. By leveraging the amplification of scattered radiation upon the adsorption or chemical binding of molecules to a nanostructured metal surface, SERS enables detailed probing of the intricate features of G-quadruplex structures [2].

In this study, the G-quadruplex formed by the human telomeric sequence Tel24 stabilized by Na^+ ions was investigated using SERS. Two different SERS-active substrates, silver nanoparticles stabilized by citrate and spermine surface layers, were employed, and laser excitations at both visible (532 nm) and near-infrared wavelengths (785 nm) were applied. The distinctive SERS signatures, particularly the bands corresponding to breathing vibrations of adenine (~730 cm^{-1}) and guanine (~660 cm^{-1}), allowed the detection of the molecule at concentration of 4×10^{-5} mol/L. Monitoring the relative intensity of these bands enabled the observation of structural changes from the unfolded oligonucleotide to the G-quadruplex form [3]. The spectral differences, characteristic of the structural forms, were analyzed, providing insights into the efficiency of the SERS substrates in capturing distinct G-quadruplex conformations. This research contributes to the understanding of G-quadruplex structural dynamics and highlights the importance of choosing the right SERS-active substrate to monitor such changes.

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The role of Raman spectroscopy in the identification of cadmium yellow pigments

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Raman spectroscopy is a fundamental method that is increasingly included in research on paintings to obtain information about material composition. Different yellow pigments were used by artists since the 19th to 20th century. The research focused on yellow pigments on the base of cadmium in the paintings by prominent Czech artist František Kaván (1866–1941). Besides cadmium yellow (CdS), he used other yellow pigments such as Naples yellow ($Pb_2Sb_2O_7$, $PbSb_2O_6$), chrome yellow ($PbCrO_4$), barium yellow ($BaCrO_4$), zinc yellow ($K_2O{\cdot}4ZnCrO_4{\cdot}3H_2O$), and the rare pigment cadmium chrome yellow ($2CdCrO_4{\cdot}KOH{\cdot}H_2O$) [1]. The discrimination of the cadmium based yellow pigments is not possible by elemental analysis. Raman spectra show considerable difference in the position of the bands characteristic for cadmium yellow and cadmium chrome yellow. Cadmium yellow is characterized by Raman bands at ~ 304 and 607 cm^{-1}. Cadmium chrome yellow has the most intensive characteristic band at ~857 cm^{-1} and less intensive bands at ~338, 353, 393, 764, 879, and 935 cm^{-1}. So far, cadmium chrome yellow was identified only in several paintings by Kaván from the 1890s and in a paint tube labelled J. de Cadmium Citrone produced by Vilhelm Pacht in Copenhagen, Denmark [2]. The research showed the important role of Raman spectroscopy in the identification of yellow pigments in the painting. The obtained information serves as comparative data within the process of identification of artwork's counterfeits.

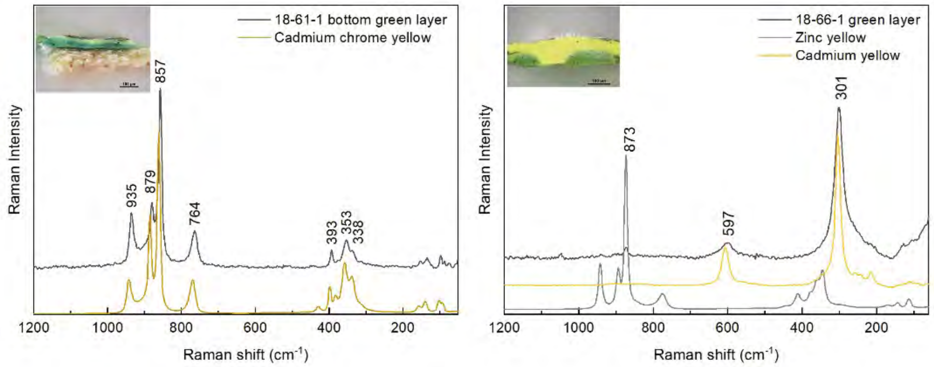

Figure 1: Raman spectra and cross-sections of the samples from the paintings by František Kaván: *Near a pond* with cadmium chrome yellow and *The end of June* with cadmium yellow

The work was supported by a project run by the Ministry of the Interior of the Czech Republic: VJ01010004.

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Gold Nanoparticles with Semi-Wrapped Prussian Blue for Highly Reliable and Sensitive Surface-Enhanced Raman Scattering Detection

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Researchers have struggled to develop highly reliable and sensitive surface-enhanced Raman scattering (SERS) substrates for detecting compounds in complicated systems. In this work, a novel strategy by constructing Au cores with incompletely wrapped Prussian blue (PB) for highly reliable and sensitive SERS substrate is proposed. The wrapped PB layers can provide the internal standard (IS) to calibrate the SERS signal floatation, whereas the exposed surface of Au cores remains the enhancement effect. The balance between the signal self-calibration and enhancement (hence the trade-off between SERS reliability and sensitivity) is obtained by the approximate semi-wrapping configuration of PB layers on Au cores (SW-Au@PB). The proposed SW-Au@PB nanoparticles (NPs) exhibit the similar enhancement factor as the pristine Au NPs and contribute to the ultralow RSD (8.55%) of calibrated SERS signals using R6G as probe molecules. The simultaneously realized reliability and sensitivity of SW-Au@PB NPs also enables the detection of hazardous pesticide residuals such as paraquat and thiram in herbal plants, with the average detection accuracy up to 92%. Overall, this work mainly provides a controllable synthetic strategy for incompletely wrapped NPs, and most importantly, explores the potential with a proof-of-concept practical application in accurate and sensitive Raman detection of hazardous substances with varying polarities.

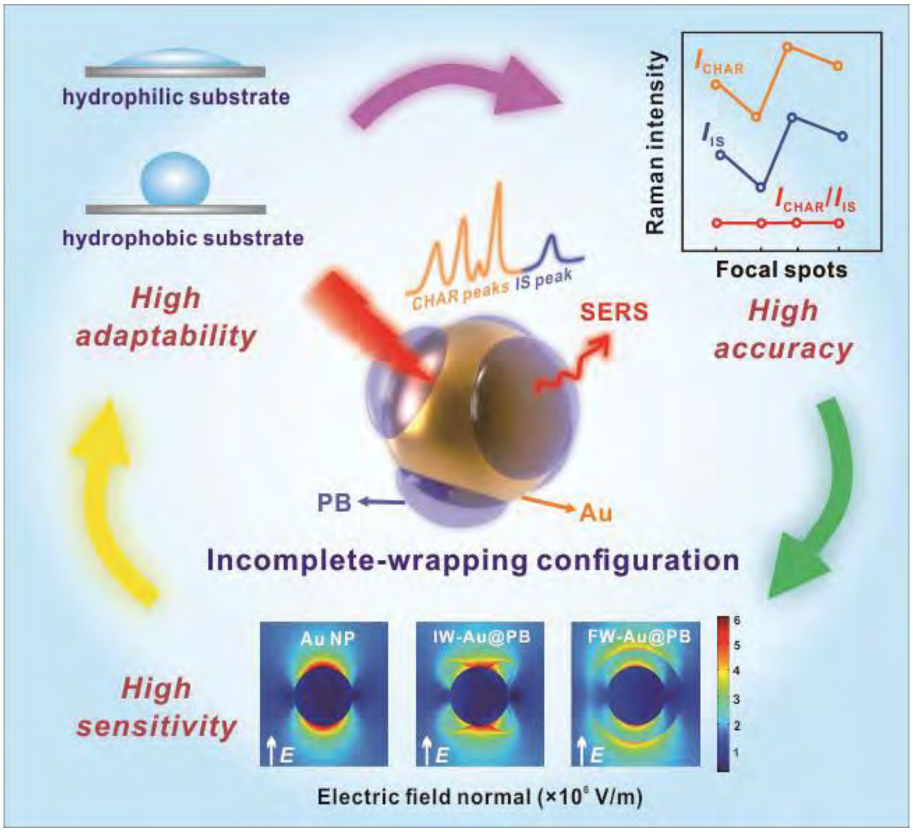

Figure 1: illustration of the character and working principle of the dual-functional SW-Au@PB NPs.

Unveiling Photochemical Reactions of Acetaminophen on Plasmonic Substrates using advanced spectroscopic methods

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Surface-enhanced Raman scattering (SERS), a technique that has been in existence for fifty years, offers numerous advantages. Nevertheless, accurately interpreting SERS spectra remains a challenging task, especially when dealing with exceptional cases. This challenge originates from the diverse impacts of chemical enhancement, potentially resulting from changes in chemical species induced by radiation interaction. Despite numerous reports documenting such occurrences, the scope of molecules undergoing such transformations appears almost limitless and includes compounds of significant medicinal importance. [1,2]

In this study, we present our observations of the photochemical reactions of acetaminophen adsorbed on Ag, Au, and Cu plasmonic substrates. By employing various excitation wavelengths across the visible (457, 532, 633 nm) and near-infrared (785 and 1064 nm) spectral regions, we thoroughly investigated this phenomenon, revealing acetaminophen's notable tendency to undergo transformation because of incident radiation. Through sequential measurements, we identified multiple potential pathways undertaken by the adsorbed acetaminophen molecules. These findings were further supported by Density Functional Theory (DFT) calculations, which played a crucial role in clarifying some of the observed effects. The behaviour of molecules on the surface can be further investigated using an advanced technique known as Tip-enhanced Raman scattering, which achieves spatial resolution at the nanoscale level.

Given acetaminophen's medical importance, we believe that our findings offer promising results for its SERS-based spectrochemical analysis. Furthermore, our results contribute to addressing inquiries within the broader domain of plasmon-assisted photochemistry.

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Development of a multimodal analysis framework for a comprehensive investigation of bacterial vesicles

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Bacterial membrane vesicles are involved in many essential cellular functions, such as communication or defense mechanisms, by transporting a variety of different cargoes across biological membranes [1]. Although they constitute exciting research objects for basic biology as well as translational disciplines (e.g. drug delivery), conventional analytical tools are labor-intensive, time-consuming and often lack chemical information. In this study, we combined Raman spectroscopy for the first time with biochemical assays for elucidating the chemical composition of outer membrane vesicles (OMV) secreted by the clinically relevant pathogen *Pseudomonas aeruginosa*. We investigated OMVs during the different growth phases (see figure 1A) by employing atomic force microscopy (AFM) and dynamic laser scattering (DLS) alongside Raman spectroscopy to complement the analysis of physical properties (size, charge) with a detailed chemical analysis (figure 1B). The acquired Raman spectra indicated higher lipid levels in OMVs after reaching the stationary phase with a lower degree of unsaturation over time. Moreover, while proteins were found in samples over the whole bacteria lifecycle, higher nucleic acid abundance was detected in the mid-to-late log phase, revealing a time-dependent change in OMV cargo. These results corroborate the value of Raman spectroscopy as a novel, fast and chemically-selective approach to form a comprehensive analysis framework for bacterial vesicles.

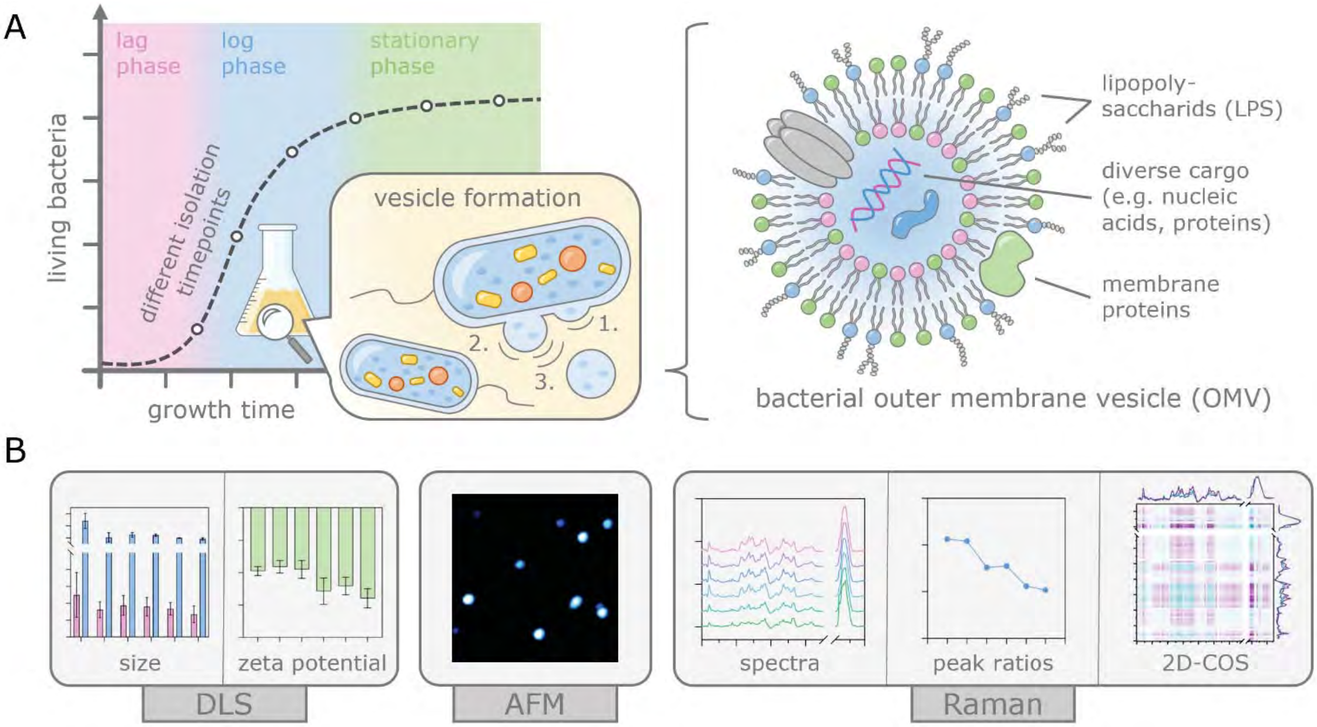

Figure 1: A. Schematic overview of bacteria growth phases and OMV formation and composition. **B**. Employed analysis methods for characterization, namely DLS, AFM and Raman spectroscopy.

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Scrutinizing the Consequences of Surface Complex Formation on Surface-enhanced Raman Spectra

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The formation of surface complexes is even after 50 years one of the most challenging aspects of surface-enhanced Raman scattering (SERS) spectroscopy. Although it contributes minorly to the overall enhancement, it may significantly affect the resulting appearance of the SERS spectra [1]. Aside from this, the formation of molecular complexes on the enhancing substrates' surface may lead to modification of molecular absorption spectra, making chemisorbed molecules more susceptible to undergo light-induced transformations. Additionally, there are several types of molecules reacting simultaneously (**Fig. 1**), which manifests itself, for example, in different reaction kinetics. [1, 2].

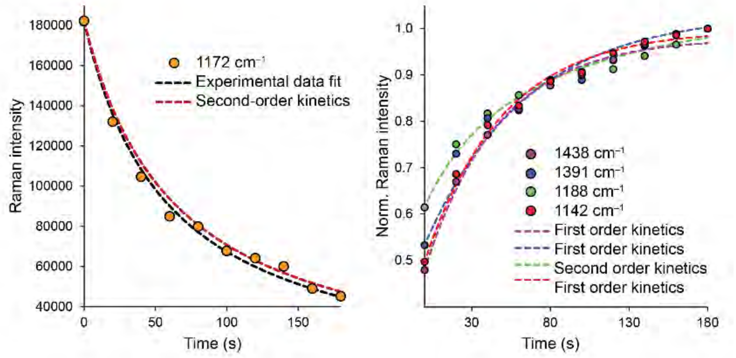

Figure 1: Kinetic behaviour of the selected SERS bands of 4-aminobenzenethiol and its products

Even if the formation of metal-molecule complexes does not lead to photochemical transformation, it may still remarkably enhance the intensity of inelastically scattered photons either in narrow [3] or wide [4] spectral regions. Here, we are presenting several examples of the above-mentioned cases, while focusing not only on the physico-chemical aspects but also on the fields such as chemical/plasmon catalysis and analytical chemistry.

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Study on the Effect of Structure-mediated Plasmon Resonance on Charge Transfer in MoS_2 based on SERS spectroscopy

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Electron migration in semiconductors has always been a hot topic in the field of optoelectronic devices. Understanding and addressing the limitations of electron migration can more effectively improve the utilization efficiency of optoelectronic devices. Surface-enhanced Raman scattering (SERS) is a very efficient analytical method that can be used to study electron migration in semiconductors. In this study, based on the excellent SERS activity of the noble metal/semiconductor composite structure, we investigated the electron transfer mode between semiconductor-probe molecules mediated by electric field strength. First, the changes in electric field intensity in the system are adjusted by regulating the size of metal particles, and theoretical simulations are used to calculate the intensity changes and distribution of the electric field in the system. Then, density functional theory was used to analyze the effects of different field strengths on the molecular orbitals and electronic structures of the semiconductor-probe molecules. Finally, the charge transfer induced by the electric field effect in the composite system was analyzed based on SERS spectral data and simulation calculation results. This study can effectively improve the electron migration efficiency in semiconductors and thereby improve its photoelectric utilization efficiency. In addition, we can provide certain guidance for the development of high-sensitivity SERS substrates. The detailed mechanism will be discussed in this presentation.

CRISPR/Cas13a-mediated SERS-based dual-flow assay paper chip for point-of-care testing of SARS-CoV-2

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The gold standard method for diagnosing SARS-CoV-2 target gene is RT-PCR. However, amplifying the target gene requires a reverse transcription and thermocycling process, taking approximately 3-4 h. Due to the rapid spread of the virus, the lateral flow assay (LFA) strip is also employed as a diagnostic method. This approach is quick and simple but exhibits a high false-negative rate due to limitations of detection sensitivity. The trans-cleavage activity of the CRISPR/Cas system has recently demonstrated excellent potential for sensitive and rapid detection of the target gene [1]. In this study, we developed a CRISPR/Cas13a-mediated SERS-based dual-flow assay paper chip to detect SARS-CoV-2 RNA directly [2]. Initially, the CRISPR/Cas13a reaction occurred through the first channel flow, followed by the sequential flow of SERS nanotag solution through the second channel flow. This sequential flow arrangement allows for both the CRISPR/Cas reaction and the SERS assay within a single chip, enabling the sensitive detection of SARS-CoV-2 RNA. Furthermore, by utilizing the CRISPR/Cas13a system, direct detection of SARS-CoV-2 RNA is possible without a reverse transcription process. Consequently, our SERS-based dual-flow paper chip holds significant potential for point-of-care testing (POCT) by enabling the direct detection of target RNA through two distinct reactions within a single chip.

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SERS-based microdroplet sensor for the rapid classification of SARS-CoV-2 and influenza A infections

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We developed a dual-mode surface-enhanced Raman scattering (SERS)-based microfluidic device capable of simultaneously diagnosing and distinguishing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza A. In this device, separate SARS-CoV-2 and Influenza A assays were performed in alternate droplets. Magnetic immunocomplexes were aligned on one side of the channel using a permanent magnet embedded in the microfluidic device, and parent microdroplets containing magnetic immunocomplexes and supernatant solutions were split into two smaller daughter droplets at the Y-shaped junction of the channel. Subsequently, Raman signals of sequential droplets, including supernatant solutions, were measured for the quantitative analysis of the SARS-CoV-2 and Influenza A. Additionally, an internal standard was employed in the chip to improve the accuracy of the measurement process [1]. The entire analysis process, from droplet generation to SERS detection, took less than 10 min because all experimental conditions were controlled within the exquisitely designed microfluidic channel. We anticipate that this SERS-based immunosensing platform will serve as a new point-of-care diagnostic tool for detecting SARS-CoV-2 and Influenza A infections.

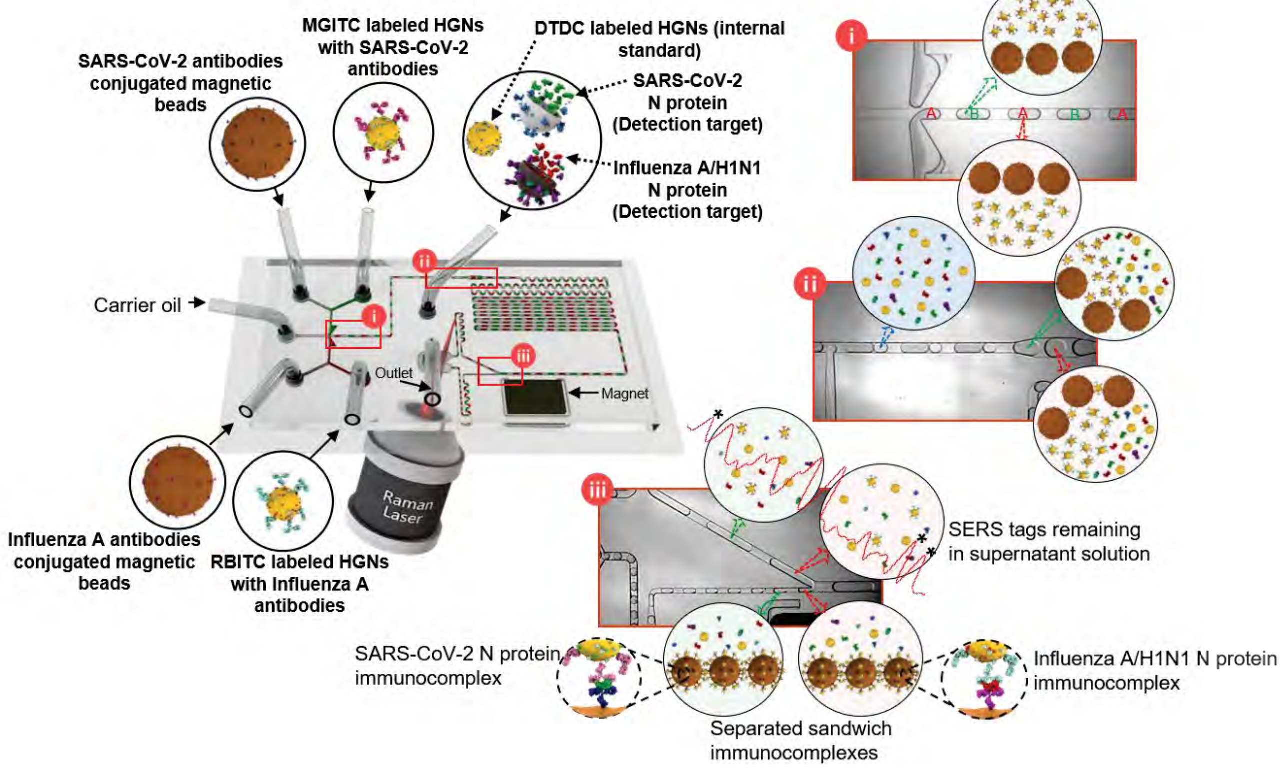

Figure 1: Schematic design of the integrated SERS-based microfluidic channel

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SERS Activity of Electromagnetic and Charge Transfer Effects on PS/Ag/MoO$_3$ Film by RF Magnetron Sputtering

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In this study, a radio frequency (RF) magnetron sputtering system was used to fabricate PS/Ag/MoO$_3$ films. The films were fabricated by depositing layers Ag and MoO$_3$ on a polystyrene substrate [1]. Our study focused on the electromagnetic (EM) effect and charge transfer (CT) in semiconductor as a function of the film thickness. The thickness variation was achieved by varying the sputtering time of Ag to adjust the EM effect. The metal-semiconductor molecular interface was investigated by studying surface-enhanced Raman scattering (SERS) spectra using 4-mercaptobenzoic acid (4-MBA) as a probe molecule. Based on the relative intensity changes of the 4-MBA band, we analyze the CT process in composite systems. The findings contribute to the research and development of metal/semiconductor-based SERS substrates through the exploration of EM and CT effects. This has the potential to pave the way for customized applications in the field of SERS. In this presentation, the details of the results will be discussed.

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RNA Release During Aberrant Phase Transition of Stress Granule: A Raman/Brillouin Imaging and Machine Learning Study

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Stress granules (SGs) are transient membraneless organelles formed via intracellular liquid-liquid phase separation (LLPS) in response to various cellular stresses[1]. SGs serve to protect cells from harmful stresses; however, a chronic stress can trigger an abnormal phase transition of SGs, resulting in the formation of aggregates associated with a variety of diseases[2]. To understand the mechanism of the formation of disease-related aggregates, it is necessary to observe both the macroscopic physical changes and microscopic molecular behavior within SGs during the formation of aggregates. In this study, we tracked the phase transition of SGs in living cells under a long-term oxidative stress using near-IR fluorescence and Raman/Brillouin imaging. Labeling G3BP1, a scaffolding protein of SGs, with near-IR fluorescent protein iRFP, enables the identification of the location of SG aggregates. Raman imaging provides information about the chemical concentration and molecular structure inside SG aggregates, while Brillouin imaging provides information about the stiffness of SG aggregates.

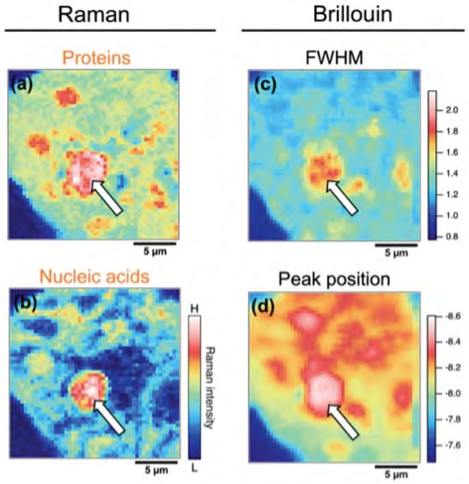

Figure 1: (a, b) Raman and (c, d) Brillouin images of stress granule aggregates induced by long-term oxidative stress. White arrows indicate the location of aggregates.

Figure 1 shows Raman and Brillouin images of SG aggregates obtained after 6 hour oxidative stress. The Brillouin images showed that the SG aggregates exhibit a broader band at higher frequency, suggesting SG aggregates were harder than the surroundings. Raman images revealed that SG aggregates contain proteins and RNA. The peak position in the Brillouin spectra and the intensity ratio between proteins and RNA in the Raman spectra of SG aggregates varied from one to another, suggesting the physical and chemical properties of SG aggregates were different for each other (Fig. 2). We applied machine learning to classify the Raman and Brillouin spectra and elucidate the difference among aggregates. The result shows that the lower the RNA concentration inside the SG aggregates, the higher the protein concentration, the larger the amount of β-sheet structure, and the harder the aggregates, suggesting that the release of RNA is a key to the aggregation of SGs.

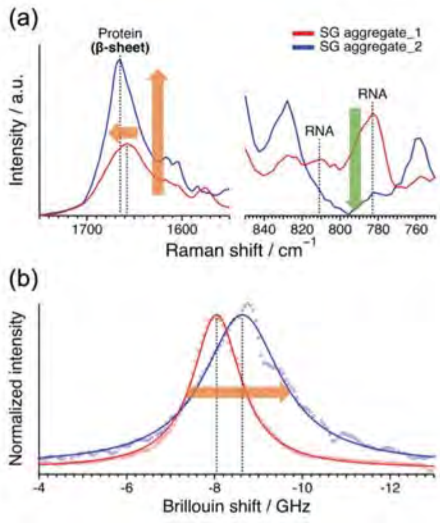

Figure 2: (a) Raman and (b) Brillouin spectra of two different SG aggregates.

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Fabrication of Flexible Nanoporous Biosilica-Based SERS Active Platforms Using Layer-by-Layer Technique

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Surface-enhanced Raman scattering (SERS) is an emerging analytical technique used for the characterization of biological and non-biological structures. The plasmonic properties of nanostructures are the main factors influencing SERS performance. Thus, the fabrication of plasmonic nanostructures having different plasmonic properties is of significant research interest. Recently, guided-mode resonances (GMRs) in nanoporous biosilica (natural diatomite) have received significant attention due to their potential contribution to SERS enhancement. Furthermore, there is also evidence showing that nanoporous biosilica can be utilized to improve SERS enhancement by optically coupling the GMRs of the nanoporous biosilica with the localized surface plasmon resonance (LSPR) of the nanostructures.[1,2] In this study, layer-by-layer assembly is used to fabricate nanoporous biosilica-based SERS active platforms by coating the nanoporous biosilica with silver nanoparticles (AgNPs). The fabricated SERS platforms are characterized using UV-Vis spectroscopy and scanning electron microscopy (SEM). The SERS performance of the platforms was evaluated using 4-aminothiophenol (4-ATP) and rhodamine-6G. The results demonstrate that the SERS performance of the platforms is dependent on the number of layers in the structures. The SERS platform having the highest SERS activity can be used for the characterization of any molecules of interest.

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Insights into the Vibrational Dynamics and Phase Transitions of IM_3PbBr_5 Perovskite: Temperature and Pressure-Dependent Studies

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We present an extensive investigation of the vibrational properties of the lead bromide perovskite compound, IM_3PbBr_5, focusing on its temperature- and pressure-dependent behavior. IM_3PbBr_5 displays a unique crystal structure featuring $PbBr_6$ octahedra arranged in 1D single-chains, with imidazolium cations (Im) located within the cavities between these octahedral chains [1,2].

Using Raman and infrared (IR) spectroscopy, we conducted vibrational measurements, observing distinct shifts in vibrational modes during phase transitions associated with the ordering of imidazolium cations. These transitions resulted in significant changes in peak positions, intensities, and bandwidths, revealing a coupled relationship between the ordering of Im cations and the inorganic sublattice. Crystallographic structures were determined for each phase, offering detailed insights into the rearrangement and ordering of imidazolium cations within the IM3PbBr5 crystal structure. Additionally, we explored the impact of pressure on the phase transitions and vibrational properties of IM_3PbBr_5, uncovering transitions and their effects on the crystal structure and vibrational modes.

The interplay between external pressure and structural dynamics in IM_3PbBr_5 was revealed, enhancing our understanding of its behavior under varying environmental conditions. This comprehensive study contributes valuable insights into the temperature and phonon properties, mechanisms of phase transitions, and crystallographic structures of this perovskite. The observed coupling between the ordering of imidazolium cations and lattice dynamics is crucial for the development and optimization of lead bromide perovskite-based devices. These findings have implications for advancing optoelectronics, photovoltaics, and related fields.

In summary, our study provides a thorough examination of the temperature- and pressure-dependent vibrational characteristics, phase transitions, and crystallographic structures of IM_3PbBr_5. The identified coupling between the ordering of imidazolium cations and lattice dynamics contributes valuable insights for the development of lead bromide perovskite-based devices.

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Translating Raman-Based Characterization of Cefazolin's Interaction with *Staphylococcus aureus*: From Extracellular Bacteria to an Intracellular Infection Model

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Staphylococcus aureus is a notorious pathogen capable of causing severe infections which can potentially progress into sepsis [1]. Therapeutic challenges and recurrent infections are partly attributed to its immune escape strategy to invade and persist within eukaryotic host cells [2]. Current approaches to study the response of bacteria to antimicrobial treatment in the intracellular environment are restricted.

In this study, Raman spectroscopy was used to characterize metabolic changes in the *S. aureus* strain 6850 after interaction for 2, 4 and 6 hrs with cefazolin at the minimal inhibitory concentration (MIC) of 0.5 mg/L. Raman spectra were acquired in suspension using a combined dielectrophoresis-Raman setup established in earlier studies [3]. Principal component analysis and linear discriminant analysis (PCA-LDA) identified spectral differences upon treatment, consistent with the mode of action of cefazolin. With these features, a classification model was established to differentiate treated and untreated bacteria.

In an *S. aureus* intracellular infection model within human endothelial EA.hy 926 cells, antibiotic treatment with 10xMIC was administered one day post-infection for 6 hours. Raman spectra of intracellular bacteria were extracted from false color Raman images using the N-FINDR unmixing algorithm directly from intact host cells. The classification model trained with the spectral features of extracellular bacteria was used to predict the treatment status of the intracellular bacteria. An excellent agreement could be achieved with classical plating methods to assess treatment success.

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Electrochemically Fabricated Gold-Coated TERS Tips for Applications in Air and in Aqueous Medium

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Nanoscale analysis remains an essential challenge for areas such as molecular electronics, materials science and biology. Tip-enhanced Raman spectroscopy (TERS) has gained considerable interest due to its nanoscale spatial resolution, high sensitivity, and ability for simultaneous topographic and spectroscopic imaging [1]. By using a sharp metallic nanotip to confine and enhance the light field near the tip apex, a localized hot-spot is excited and nanoscale chemical imaging is achieved [2]. Several physicochemical methods have been developed to fabricate TERS tips such as vacuum evaporation, chemical deposition, nanoparticle attachment, and microfabrication methods [3,4].

Here, TERS active tips were fabricated via an electrochemical method, controlling the applied potential and the time duration to vary the curvature radius of their apex and their plasmonic properties. These tips could enhance Raman signals of carbon nanomaterials in air and biomolecules like Aβ(1-42) amyloid fibrils in liquid environments. In particular, TERS imaging of Aβ(1-42) amyloid fibrils was performed in liquid water using our bottom-illumination TERS system in total internal reflection, thus providing chemical, structural, and morphological mapping with nanoscale spatial resolution. In the future, our metal-coated tips will be further optimized, and tested on other biological samples such as membranes and cells.

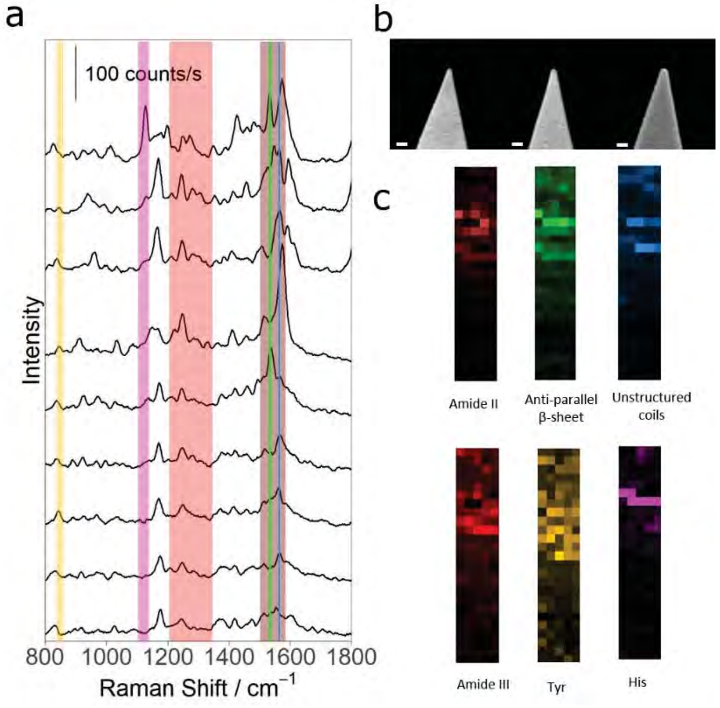

Figure 1: (a) Selected TERS spectra of Amyloid Beta. (b) SEM images of gold-coated TERS tip with different radii of curvature. Scale bar: 500 nm. (c) TERS maps of amide bands and amino acid residues. Experiment performed in aqueous medium.

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Analysis of interactions between multiple pathogens using Raman spectroscopy

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Biofilms dominate acute and chronic lung infections [1]. Understanding the internal structure, formation processes, and other characteristics of biofilms composed of multiple pathogens will aid in the development of targeted therapies. Interactions between bacterial species within biofilms can strongly influence the potential for cooperation in spatially structured microbiomes [2]. Therefore, it is critical to clarify the interactions between common pathogens in the respiratory tract. Raman spectroscopy in microbiology has many advantages including high resolution, low sample demand, and is non-destructive [3]. Here, we used a non-contact co-culture device to cultivate several common pathogens and then collected and compared their Raman spectra under mono-culture and co-culture conditions. The PCA-LDA analysis results demonstrate the effectiveness, which lay the foundation for studying mixed biofilms.

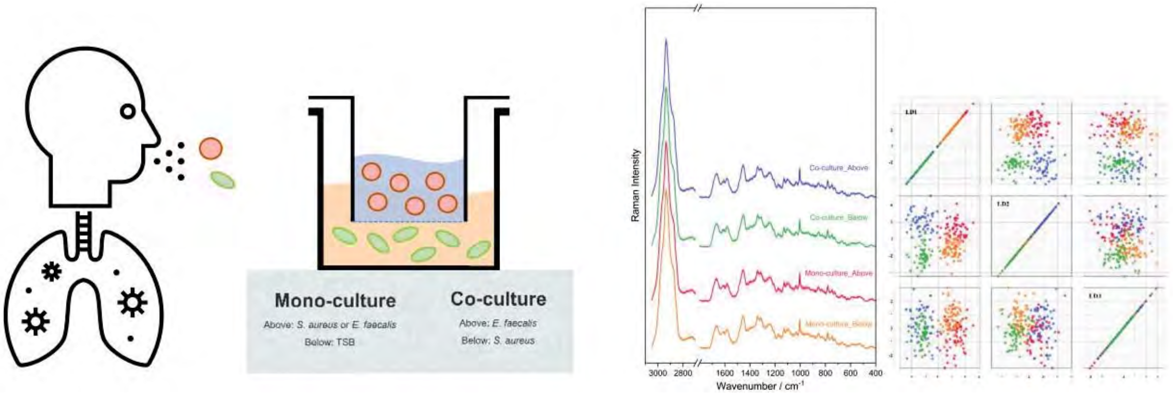

Figure 1: Analysis of interactions between multiple pathogens using Raman spectroscopy and non-contact co-culture devices under mono-culture and co-culture conditions

Acknowledgement

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Sofosbuvir polymorphs distinguished by polarized Raman microscopy

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Most drugs are produced in the solid state, where the poor bioavailability of an active pharmaceutical ingredient may be optimized by the preparation of different polymorphs, co-crystals, solvates, or salts. It is thus desirable to develop efficient analytical tools to monitor such crystalline forms in the pharmaceutical production.

To this end, we investigated the potential of linearly polarized Raman microscopy for discrimination of three polymorphs of sofosbuvir, an antiviral drug used to treat hepatitis C. The polarized Raman spectra were recorded on a modified Raman microscope, including the low-frequency region (<200 cm^{-1}). By simulating the spectra, we tried to understand the main spectral features. Due to the size of the molecules, we used a fragmentation and transfer approach that is based on calculating important intermolecular interactions in the crystals. [1]

The results indicate that depolarization ratios of the fingerprint bands originating from the functional groups strongly involved in the H-bonding are convenient to discriminate between the three polymorphic forms. The low-frequency Raman bands are sensitive to the crystal density and symmetry, and high signal intensity in this region is most likely due to the presence of an aromatic residue. [2]

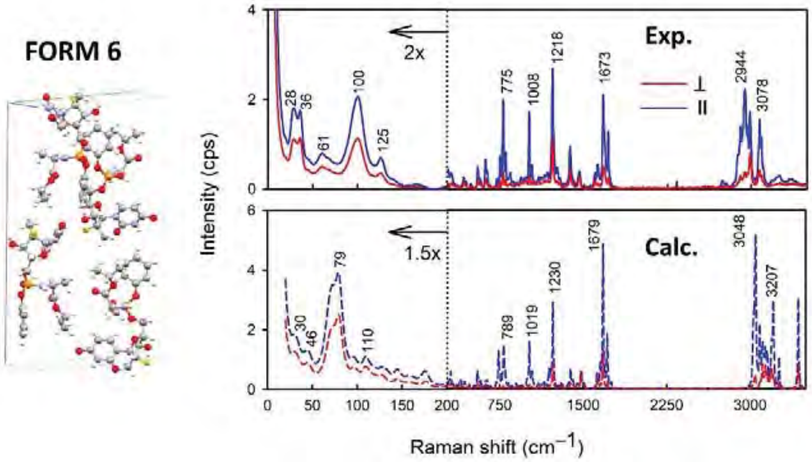

Figure 1: Experimental and calculated linearly polarized Raman spectra of sofosbuvir form 6.

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Electronic and Vibrational Study of Plasmon Driven Charge Transfer Dynamics in Tris(2,2'-bipyridine) Ruthenium (II) on Gold Nanospheres

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Ruthenium (II) complexes containing polypyridine ligands have been widely studied owing to their light-harvesting properties.[1] Particular attention has been given to dye molecules adsorbed on semiconductors such as TiO_2. After absorption of light, the photo excited molecule can transfer an electron to the semiconductor substrate, making it an excellent choice for applications such as solar cells and photocatalysis.[2] While the light harvesting properties of the Ru complex have been extensively studied, their adsorption on noble metal nanostructures have received less attention. The adsorption of this complex on a metal surface affects its charge transfer and Raman scattering properties.[3] Understanding the interaction between these complexes and noble metal surfaces is crucial, as it significantly influences electron transfer processes from the excited molecule to the substrate.[4] Studies have shown that nano particle aggregates show increased plasmonic effects compared to isolated nano particles.[5] Hence, we perform transient absorption (TA) studies with tris(2,2'-bipyridibe)ruthenium(II) dye adsorbed on gold nanospheres oligomers. The study helps us understand the effect of surface plasmons on the charge transfer dynamics of the molecule. Furthermore, plasmonic environments enhance the Raman signals of Ru-based dyes, which are otherwise challenging to obtain due to background fluorescence.[6] Surface enhanced femtosecond stimulated Raman spectroscopy (SE-FSRS) study of Ru dyes adsorbed on gold nanosphere allows us to probe the coupling between the vibrational modes of the dye and plasmon waves at the nanoparticles surface, providing valuable insight into dye-substrate interaction. Overall, the study contributes to deeper understanding of the mechanism of interfacial electron transfer, providing further insight into applications like light harvesting and photocatalysis.

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InAs-InP superlattice nanowires with tunable phonon frequencies

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The control of heat transport through the manipulation of phonons in solids is of fundamental interest and can be achieved by nanostructuring [1], as in superlattice (SL) nanowires (NWs) [2]. SLs are superstructures consisting of a periodic arrangement of two materials. On the other hand, semiconductor NWs offer many possibilities to engineer systems at the nanoscale by controlling the size, the crystal structure, the chemical composition, and by combining different materials or crystal phases in heterostructures which cannot be realized in bulk and 2D materials [3]. In our work, we studied the effect of varying the periodicity of the SL on the tunability of the phonon frequencies in InAs/InP SL NWs. In figure 1 (a) a scanning electron microscope (SEM) image of a NW with the SL embedded between two InAs segments is shown. Figures 1 (b-d) show the Raman spectra of the reference InAs and InP NWs, as well as the Raman spectrum of a 4.8 nm period SL NW, where phonon modes close to the ones of the InAs and InP reference NWs (indicated with the vertical lines) can be seen. Figures 1 (e-g) show the Raman spectra of different SL NWs (4.8 nm, 7.1 nm and 11.5 nm period), as well as the scanning transmission electron microscopy (STEM) images of the respective SL NW. As it can be seen, the number of phonon modes increases with the period of the SL. This is due to the reduction of the first Brillouin zone (BZ) because of the longer lattice parameter, with the resulting “folding” of the phonon bands to fit inside the first BZ. Furthermore, since in SLs with longer period there are more atoms in the unit cell, the number of phonon modes increases proportionally [4].

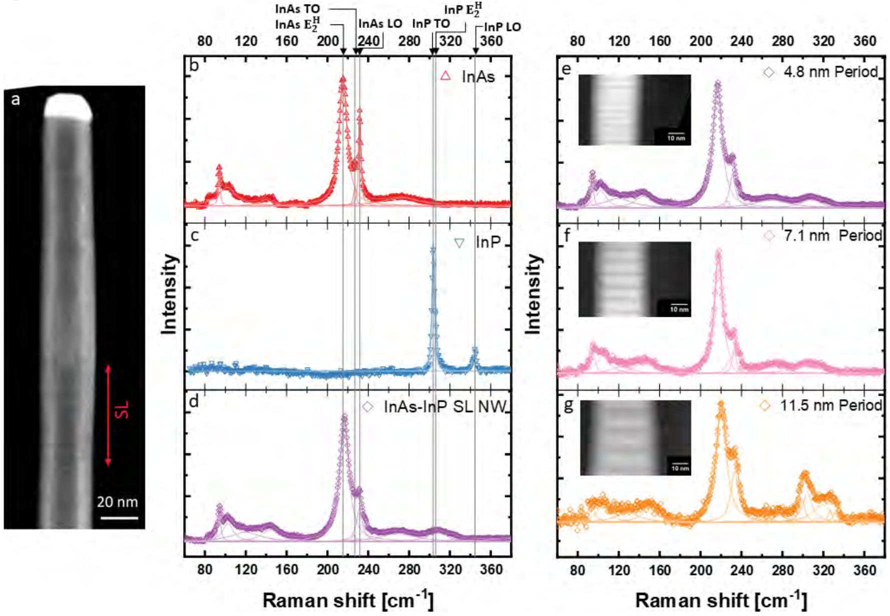

Figure 1: (a) SEM image of an individual SL NW. In the SL portion (red arrow), the InP segments appear with darker contrast. (b-d) Measured Raman spectra of (b) an InAs reference NW, (c) an InP reference NW and (d) a 4.8nm period InAs-InP SL NW. (e-g) Raman spectra of SL NWs with periodicity of (e) 4.8 nm, (f) 7.1 nm, and (g) 11.5 nm, along with the STEM images of the SL portion of the respective NW as inset.

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Porphyrin as chemoreceptor for dissolved carbon dioxide detection by Surface-enhanced Raman scattering (SERS)

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The capture and monitoring of carbon dioxide (CO_2) in water systems have become a critical scientific task in the past years [1]. Surface-enhanced Raman Scattering (SERS) is a promising analytical tool for this purpose, owing to its high selectivity and sensibility. It also has other advantages that hold particular significance in sensor development, as reduced time requirements, cost-effectiveness and the potential for in-situ measurements [2]. However, the direct SERS detection of CO_2 is limited due to the low Raman cross-section and lack of affinity for plasmonic nobel metal nanostructure [3]. Therefore, a chemoreceptor is needed to trap CO_2 close to the SERS generator metal nanoparticles surface allowing to monitor the presence of this small molecule by varying in the chemoreceptor conformation. In addition, the selection of this indirect SERS strategy reduces the potential matrix interference and enhances the sensitivity of the analytical method. Thus, we designed and produced a SERS substrate based on gold nanostars as an optical enhancer and porphyrins as the chemoreceptor. Gold nanostars were specifically chosen due to their ability to concentrate the electromagnetic field at their tips, generating high density of hot spots in a single particle [4]. Porphyrins are basic pyrroles that contain an aminated macrocyclic cavity that present excellent physicochemical properties such as high internal surface areas and void volume. Porphyrins are also very versatile since there is wide catalogue of porphyrins with different functional groups, which may affect their interaction with dissolved CO_2 [5].

In this work, we investigated the behaviour of three porphyrins with different functional groups, $-SO_3^-$, -COOH and $-NH_2$, functionalized with gold nanostars in aqueous solutions, at different pH conditions, by UV-vis-NIR spectroscopy and SERS. Notably, NH_2-contained porphyrin displayed an absorbance band centred at ca. 750 nm in neutral to acidic pH conditions, which was in resonance with the 785 nm laser line used, leading to the phenomena of surface-enhanced resonance Raman scattering (SERRS). Interestingly, the highest variation of the SERS spectra in presence of CO_2 was identified with COOH-contained porphyrin. The SERS fingerprint of amine-contained porphyrin was modified also in presence of carbon dioxide but less pronouncedly. The quantification of dissolved CO_2 by SERS was studied with COOH- and NH_2-cotained porphyrins using the portable Raman system. The obtained results demonstrate the promising application of porphyrin as chemoreceptor for SERS-based sensing platform for carbon dioxide monitoring in water.

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Variations in surface enhanced Raman scattering (SERS) of proteins

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Surface enhanced Raman scattering (SERS) of protein molecules is extremely sensitive to minuscule changes in their interaction with a plasmonic substrate and therefore can vary even with small changes in experimental conditions, such as altered molecular concentration[1]. It is compatible with aqueous environments and can be performed in the absence of additional labels and at high enhancement. This makes SERS an excellent technique to probe nanoscale events in biological and biomimetic systems.

Proteins are structurally diverse biomolecules whose interaction with nanoparticles is important in many applications and need to be understood. SERS spectra of single proteins/protein mixtures can show great variations[2]. Thus, the high sensitivity makes SERS ideal to investigate protein structure and composition.

To obtain the structural information contained in SERS spectra of proteins, it is necessary to compare spectra measured under different experimental conditions, such as varying protein concentration, different types of proteins and different plasmonic substrates. Here, we probed different proteins using gold and silver nanoparticles as SERS substrates. Prior to treatment with nanoparticles, the proteins were dissolved in phosphate-buffered saline (PBS) which can provide an environment for the proteins similar to biological systems. Single protein solutions as well as protein mixtures were studied using this methodology.

With the large number of spectra obtained, we performed a statistical analysis combined with qualitative comparison of spectral features to relate the data to protein size, amino acid content and sequence, as well as type of protein-nanoparticle interaction. The results can provide insight into protein structure and protein-nanoparticle interactions in biological and/or biomimetic systems. This can pave the way for the fabrication of specific nanostructures as well as better understanding of protein SERS data.

Acknowledgements

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The Influence of Corroded Copper Surface on the Enhancement of Raman Signal

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Advantages and drawbacks of the surface enhanced Raman Scattering (SERS) technique are studied, monitored, and developed for more than 40 years since the first observation of this phenomenon. One of the common materials for the preparation of enhancing surfaces is copper, but it is challenging to utilize it like a reliable substrate for its proclivity to corrode. The corrosion of the copper surface changes not only the morphology of the substrate, but also the reactivity/active sites and dielectric properties, which are affecting the final enhancement.

In our work, we have investigated copper substrates prepared by electroless deposition [1] and exposed them to a corrosive atmosphere (humid air) to study the relationship between morphological and chemical changes and final enhancement factor. The corrosion process was examined in-situ by infrared reflection/absorption spectroscopy (IRRAS) in a chamber where the relative humidity was controlled, and afterwards the surfaces possessing different degrees of corrosion were investigated by various vibrational spectroscopic techniques from the macro-, to the nanoscale, as well as with other microscopic and spectroscopic techniques (e.g., electron microscopy). The SERS experiments to verify the signal enhancing ability of the corroded surfaces were performed using riboflavin as the model analyte.

Funding

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Aluminium nanoparticles for UV-SERRS detection of biomolecules

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Surface-enhanced Raman scattering (SERS) is an excellent tool for detection of molecules adsorbed or near the metal (usually Au, Ag, or Cu) nanostructures. Many important biomolecules (aromatic amino acids, bases, etc.) usually absorb in UV spectral region providing an additional resonance Raman enhancement up to 8 orders of magnitude [1]. Thus, working with UV surface-enhanced resonance Raman spectroscopy (UV-SERRS) allows to expect ultrasensitive detection of biomolecules with low interference from impurities. For this method, nanostructures prepared from copper, cobalt or aluminium are proposed. Aluminium nanostructures emerge as new and highly-promising material in plasmon-related applications [2]. Current advancements concern the fabrication of UV-range plasmonic NPs of various sizes and morphologies and nanostructured surfaces allowing to tune localized surface plasmon resonance frequency to match the SERS requirements [3]. In biomolecular studies using UV-SERRS, the Al NPs have several advantages: (i) Raman sensitivity increases towards UV according to the v^4 rule, (ii) aluminium has higher SERS quality factor in the UV than in the rest of spectrum, and (iii) biomolecules undergo an electronic transition in UV range, which enables additional resonance enhancement (UV-SERRS effect). Deep-UV-SERRS has already showed remarkable limit-of-detection for biomolecule adenine up to 10 amol concentration [1].

In this work Al nanoparticles were synthesized, characterised using SEM, DLS, UV-VIS and other methods and suggested to apply for UV-SERRS detection of low-molecular-weight aromatic biomarkers, such as adenine. The UV-range plasmonic NPs will lay ground for development of cheap, non-invasive Raman sensor that is tunable and highly specific to designated biomarker. The development will also advance the fields of nanotechnology, plasmonics, and metamaterials.

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Thermal Phase Transition Behavior of 2D Perovskite Microcrystals as Studied by Low-Frequency Raman Microspectroscopy

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Two-dimensional (2D) perovskites are expected to have various applications such as solar cells and LEDs. Thermally induced phase transitions resulting from the disordering of organic cation chains have a significant impact on the chemical and physical properties of 2D perovskites, but a microscopic understanding of the process is insufficient. Here, we studied the orthorhombic *Pbca* to *Acam* phase transition behavior of microcrystals of the 2D perovskite $(C_8H_{17}NH_3)_2PbI_4$ (see Fig. 1a for the optical image of a typical microcrystal) near room temperature in a spatially resolved manner using low-frequency Raman microspectroscopy (excitation wavelength: 632.8 nm) while the temperature was varied with a high-precision (±0.1 °C) temperature control stage.

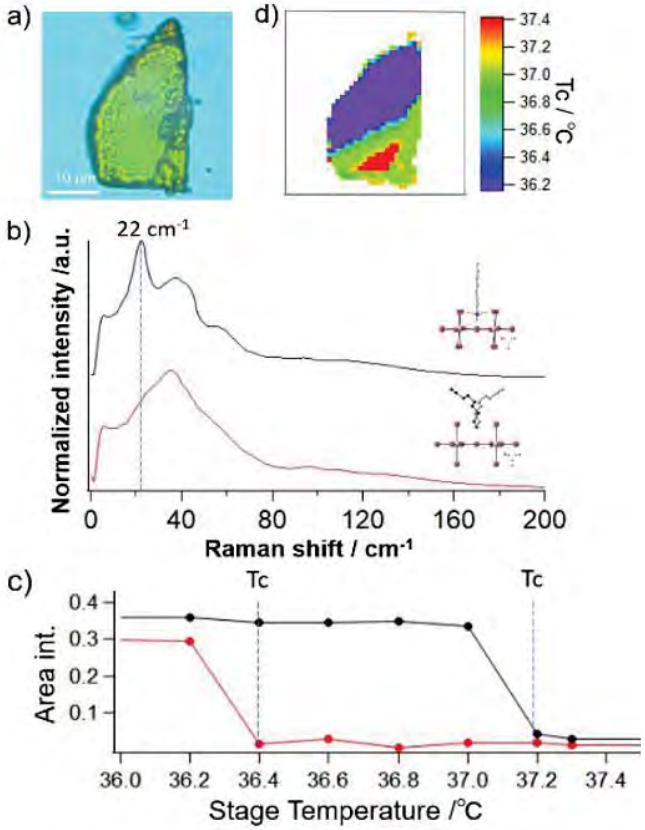

Figure 1. (a) Optical image of a $(C_8H_{17}NH_3)_2PbI_4$ microcrystal. (b) Low-frequency Raman spectra of the *Pbca* (black) and *Acam* (red) structures of $(C_8H_{17}NH_3)_2PbI_4$. (c) Temperature dependence of the area intensity of the 22 cm^{-1} band. (d) Image of T_c determined from temperature dependences like c for the $(C_8H_{17}NH_3)_2PbI_4$ microcrystal shown in a.

Figure 1b shows typical low-frequency Raman spectra of $(C_8H_{17}NH_3)_2PbI_4$ in the *Pbca* (black) and *Acam* (red) structures [1]. The phase transition temperature T_c was evaluated from the sudden change in the area intensity of the 22 cm^{-1} lattice Raman band characteristic of the *Pbca* structure (Fig. 1c). The T_c image so obtained (Fig. 1d) for the microcrystal shown in Fig. 1a shows that T_c varies from location to location within the crystal, with differences of up to 1.2 °C. This inhomogeneity of T_c within the same crystal may be due to structural non-uniformity of the crystal, which could be evaluated by polarization measurements of low-frequency Raman microspectroscopy [2].

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Cetyltrimethylammonium chloride-mediated strategy for one-step SERS detection of copper ions

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Copper ions, as heavy metals, pose significant hazards to both human health and environmental ecosystems. In this work, a novel strategy for the detection of copper ions by Surface-Enhanced Raman Scattering (SERS), using cetyltrimethylammonium chloride-mediated Au-4 Mercaptobenzoic acid @Ag nanocubes (Au-4MBA@Ag NCs) without additional modifications was proposed. CTAC services as a surfactant to assist nanoparticles form a cube configuration to boost the sensitivity. On parallel, CTAC of the NCs surface acts as a source of chloride ions, thus triggering the chemical reaction of Ag atoms to Ag ions. The SERS signal of 4MBA deposited between the Au core and the Ag shell gradually decreases as the Ag shell is etched, allowing for the quantitative detection of copper ions content. The proposed Au-4MBA@Ag NCs exhibit an enhancement factor up to 10^{10}, which facilitates the detection of ultra-low concentrations of copper ions. In addition, this method can also detect copper ions residues in herbal plants, with an average detection accuracy of 95%. In summary, this work mainly provides a controllable synthesis strategy for the one-step SERS detection of copper ions and demonstrates the potential for accurate and sensitive Raman detection of harmful substances in complex systems through proof-of-concept.

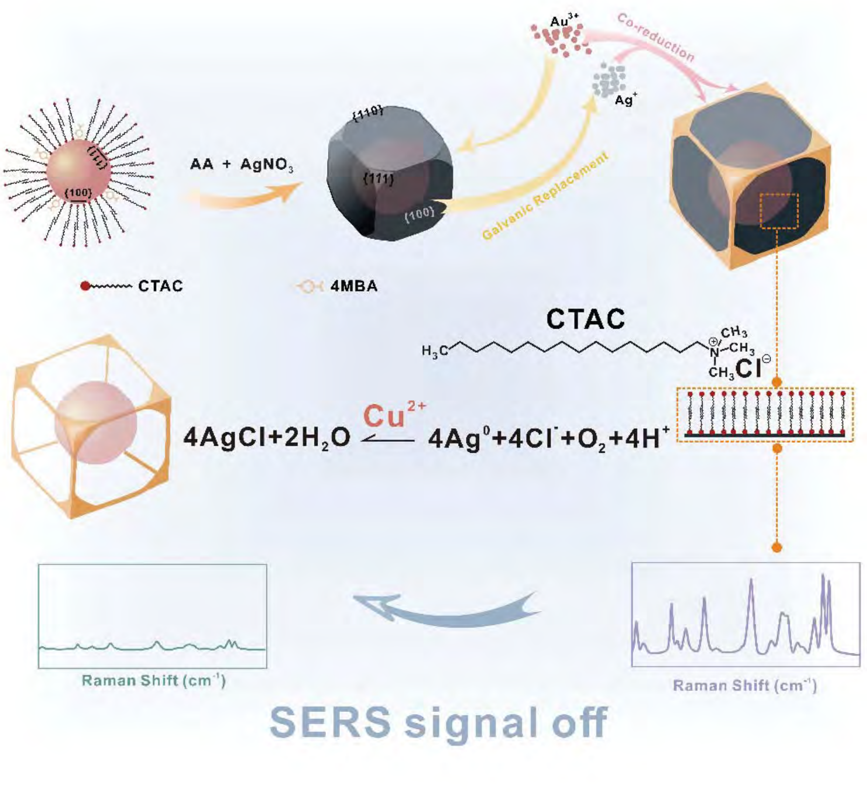

Figure 1: illustration of the working principle of the Au-4MBA@Ag NCs.

Temporal Evolution of Single-Molecule SERS Spectra in Plasmonic Nanocavity

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Raman spectra provide insights into a molecule’s structure and its interaction with the surrounding environment. Although the Raman scattering process is very inefficient, the use of properly fabricated noble metal nanoparticles can enhance the Raman signal by more than ten orders of magnitude. Such a significant enhancement enables the registration of SERS spectra from single molecules [1]. Even though more than twenty years have passed since the first report of single-molecule SERS (SM-SERS) spectra [6], some unexplained experimental results [5] make their interpretation and understanding difficult. These observations include fluctuations in the SM-SERS signal. Here, we will present a study of the time evolution of SM-SERS spectra in plasmonic nanocavities. Currently, these fluctuations are attributed to the movement and rotation of molecules within plasmonic nanocavities, atomic reorganization of nanocavity, chemical reaction, desorption, alteration in the molecule’s electronic properties, and even decomposition of the molecule.[1-4]

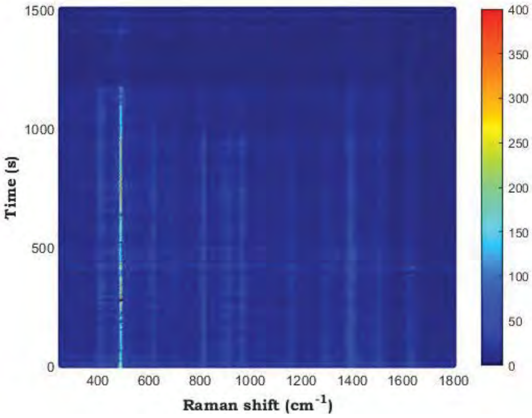

Figure 1: The time evolution of the SERS spectra of thionine recorded with consecutive exposure times of 0.5 s.

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Raman Spectroscopy and Artificial Intelligence: a synergistic combination for liver cancer diagnosis

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Hepatocellular carcinoma (HCC) is a primary cancer of the liver. The diagnosis of this disease is complicated by the limitations of current diagnostic techniques and its clinical and biological complexity. To respond to this global emergency, we explored the combination of Raman spectroscopy (RS) and artificial intelligence (AI) to improve the diagnosis of liver cancer (**Figure 1**). Actually, RS is an innovative vibrational spectroscopy technique that enables label-free, non-invasive and non-destructive examination of biological samples to detect biochemical differences between healthy and malignant conditions [1]. In this study, we performed morphological and spectroscopic characterization of uncultured primary tumor and non-tumor single cells from a patient with HCC. In this context, RS enabled the identification of their unique Raman signatures, which revealed a significant variation in their biomolecular composition, as evidenced by the significant nucleic acid content in tumor cells in contrast to healthy cells. RS confirmed the correlation between HCC and altered nuclear DNA content [2]. In the context of tumor diagnosis, several studies have pointed out the important contribution that AI makes to automate and accelerate the detection and classification of spectral data [3]. On this basis, we have developed two different machine learning (ML) methods based on linear discriminant analysis (LDA). In particular, the first ML model, namely Hyper-parameters tuned LDA, was developed based on hyperparameter tuning; whereas, the second model is based on the combination with principal component analysis (PCA), namely PCA-LDA. Both LDA-based models achieved high accuracy in classifying tumor cell spectra, close to 90%. To evaluate the effectiveness of the developed ML algorithms, we examined two cell samples with different proportions of tumor and non-tumor cells, namely MIX1 and MIX2. We found that the prediction results at the single cell spectrum level were about 80% and 60% for MIX1 and MIX2, respectively. The obtained results correspond to the real percentage of tumor cells in each sample. In this way, we have shown that the strategy based on the combination of RS and AI is a powerful tool to support medical decisions in the case of HCC.

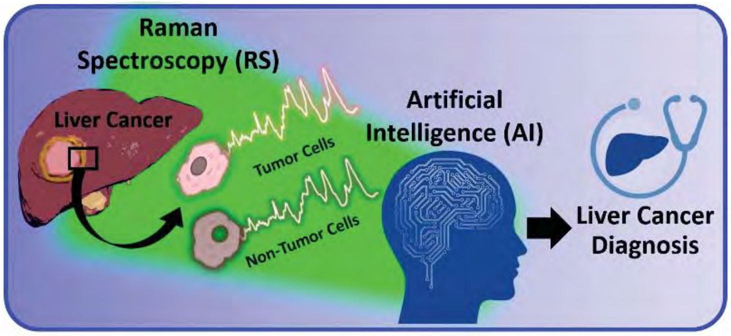

Figure 1: RS and AI-based strategy to support liver cancer diagnosis.

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Chiral Sensing of Monosaccharides by Surface-Enhanced Raman Spectroscopy

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Chiral sensing has attracted considerable attention in the fields of pharmaceutics, biomedicine, *etc.* due to the chiral-selective molecular interactions in numerous biological processes. Circular dichroism (CD) spectroscopy is commonly used for chiral sensing, but the applications are limited by high sample concentrations and the absence of molecular-level information. Surface-enhanced Raman spectroscopy (SERS) has been regarded as a promising technique of chiral sensing with superb sensitivity and molecular-specific vibrational information. The chirality of target molecules is often probed by the spectral changes of reporter molecules induced by chiral-specific interactions between the target and reporter molecules [1]. However, the detailed understanding of SERS spectral changes used for chiral sensing, such as those arising from the adsorption geometry changes of reporter molecules by chiral-specific interactions with target molecules, is not yet reported. In this study, the chiral sensing of monosaccharides such as glucose (Glu) and fructose (Fru) will be explored by SERS of the reporter, phenylalanine (Phe). Figure 1 shows the SERS spectral changes of Phe obtained with the addition of Glu or Fru of the same chirality as Phe. The changes in the relative intensities of δ(COO^-), ν_s(COO^-), and ring modes of Phe are used for the quantitative chiral analysis of target molecules in the concentration range of 2×10^{-5} ~ 2×10^{-9} M. The spectral changes of Phe upon the chiral interactions with targets are well understood as the bidentate to monodentate adsorption changes of carboxylate [2].

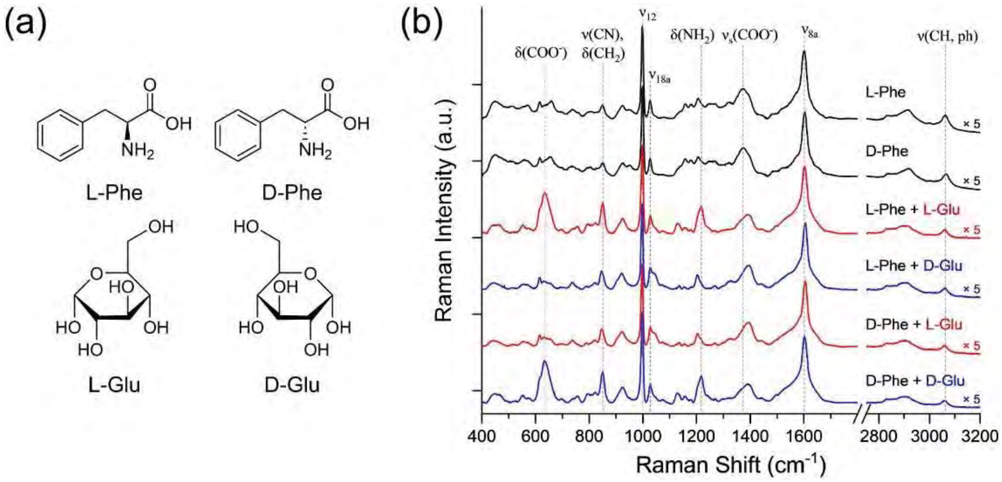

Figure 1. (a) Molecular structures of L- and D-phenylalanine (Phe) and L- and D-glucose (Glu), (b) SERS of Phe on gold colloidal nanoparticles depending on the chirality of surface-adsorbed Phe and added Glu.

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Raman Spectroscopic Study on Microstructure of B_2O_3-CaO-SiO_2 Glasses Jointly with Molecular Dynamics Simulation

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Investigating the microstructure of borosilicate glass holds significant importance for expanding its application range and enhancing its properties[1]. For this purpose, molecular dynamics and Raman spectroscopy methods were employed to study B_2O_3-CaO-SiO_2 glasses (molar ratio of CaO:SiO_2 was 1.25:1, and the addition of B_2O_3 at contents of 6, 8, and 10 mol%, respectively).

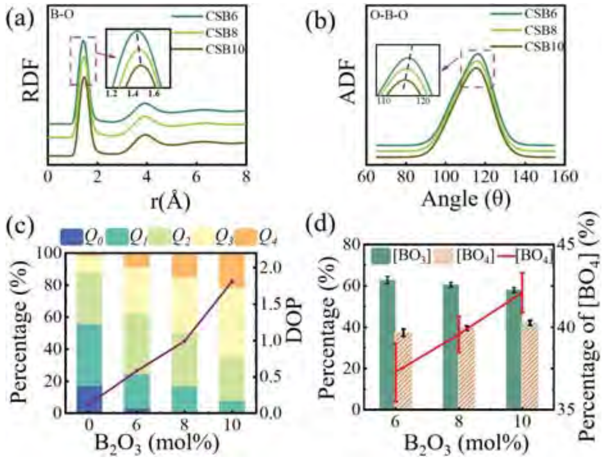

Figure 1: (a) Radial distribution function of B-O (b) Angular distribution function of O-B-O (c~d) Effect of B_2O_3 content on Q_n, [BO_3] and [BO_4]

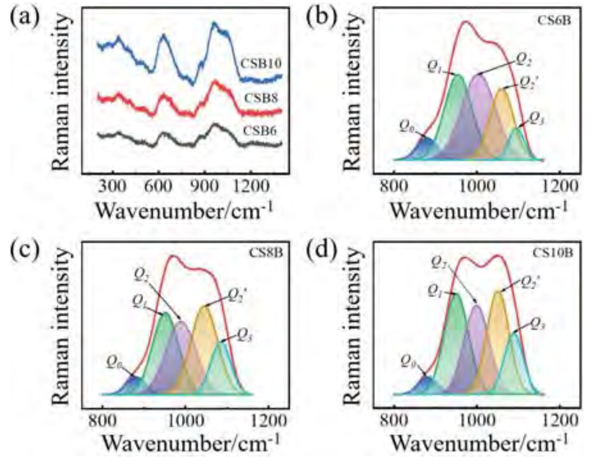

Figure 2: (a)~(d) Raman Spectroscopy of B_2O_3-CaO-SiO_2 glasses and its deconvolution with different B_2O_3 contents of 6, 8, and 10 mol%

In Fig.1(a & b), it presented the results of the radial distribution function (RDF) and the bond angle distribution function (ADF) from MD simulation calculations, further research revealed that with the increase of B_2O_3, the main peak position of the B-O bond gradually shifts towards larger values, while the O-B-O bond angle slightly decreased. This trend was closely related to the content of [BO_4] structural units, consistent with the results shown in Fig.1(d). In Fig.1(c), the quantities of Q_0 and Q_1 units gradually decreased, the quantity of Q_2 slightly declined, whereas the contents of Q_3 and Q_4 units increased with rising B_2O_3 content. The increasing ratio of Q_3 and Q_4 to Q_0, Q_1, and Q_2 fundamentally underpined the gradual increase in the system's degree of polymerization (DOP). In Fig.2, by correcting the experimental Raman spectra with theoretically calculated Raman scattering cross-sections and conducting peak fitting, the microstructural content in the glasses was quantitatively analyzed[2].

It was found that the quantity of Q_2 remained essentially unchanged and the quantity of Q_3 significantly increased with an increase of B_2O_3 content，which indicated the formation of more bridging oxygens, thus a higher degree of polymerization, and effectively strengthening the mechanical properties of the glass.

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Raman Spectroscopy and Molecular Dynamics to Shed Light on Process of Tau Aggregation in Alzheimer’s Disease

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Neurodegenerative diseases are a heterogenous group of diseases manifesting in progressive cognitive decline. Despite their heterogeneity, a frequently shared hallmark is tau accumulation. However, the precise mechanisms by which tau aggregates and forms pathogenic oligomers and fibrils remains poorly defined. The pathology-associated conformational changes which lead to this accumulation are disease specific[1] and so understanding relevance of these conformational changes could aid drug design to combat the aggregation. Tau aggregation in AD is primarily driven by association of two 6 amino acid sequences[2], PHF6 and PHF6* and these motifs are the focus of our work.

Using Raman spectroscopy in range of parameterised solvents, we hope to categorise the vibrational frequency shift of a vibrational probe in response to a range of dielectric conditions, thus allowing calculation of the electric field experienced by the probe.[3] The electric field experienced by a molecule can be thought of as the sum of all the interactions experienced on the molecule, including solvent interactions, hydrogen bonding, dipole interactions etc. and gives a profile of the environment experienced by the molecule at a given time.

The calculation of electric fields can then be compared to those predicted by Molecular Dynamics (MD) simulations to validate and calibrate the results of MD simulations.

Choice of the probe of choice has been critical, and we have been able to isolate the probe’s Raman spectra from that of the solvent. Extensive work has gone into choice of probe and experimental set-up and conditions, allowing probe Raman signal to be extracted from solution at modest concentrations.

Through this technique we hope to validate the results of tau aggregation *in-silico* MD experiments and thus potentially provide a framework to apply to other protein aggregates and a platform to test potential therapeutics.

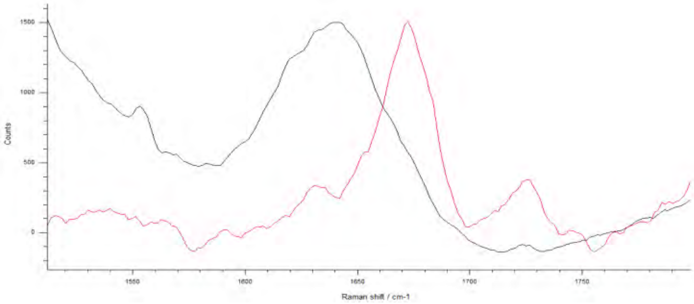

Figure 1a (left) shows the red shift in vibrational frequency of the probe carbonyl from H2O (black) and DMSO (red)

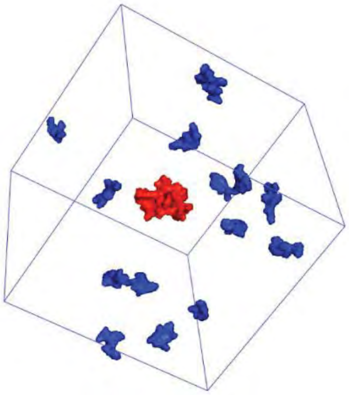

Figure 1b (left) shows the preliminary results from MD simulations of PHF6 aggregation

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Raman Mapping in Anoxic Conditions for Multimodal Analysis of Iron Archaeological Artifacts

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Studying the degradation of iron-based materials within porous, non-transparent media is usually challenging due to the impossibility to directly observe transient corrosion processes. However, recent advances in nuclear imaging techniques, such as bimodal neutron and X-ray computed tomography (NX-CT), now allow accessing these previously invisible structures for in-depth study. The CORINT project's [1] partners are collaborating to investigate transient and hidden corrosion phenomena using the NX-CT framework developed at the Paul Scherrer Institut (PSI). This advanced imaging method, enhanced by Machine Learning (ML) algorithms, enables precise segmentation and quantification of corrosion phases, both on the geometric and chemical level [2], offering valuable insights into their deterioration mechanisms [3].

The researchers from the HE-Arc conservation-restoration in Neuchâtel (CH) are focusing their effort on the in-situ corrosion of Iron Archaeological Artifacts (IAA) and their evolution post-excavation due to the contact with the atmosphere. In order to develop the use of NX-CT to its full potential, it is necessary to train the ML algorithm specifically on IAA corrosion products. As part of this calibration step, Roman nails were sampled from an archaeological site in Avenches (canton Vaud, Switzerland) with their surrounding soil and imaged. Working in anoxic conditions, the nails were then embedded and cut into cross-sections. µ-Raman analyses were performed through the glass of an anoxic set-up using the RENISHAW Virsa Raman analyzer. In parallel, overviews of the cross-sections were created by stitching multiple bright field optical microscope images (OM). Large maps were acquired over the cross-sections and were processed to provide single-component pixel masks. These masks we used to train a U-net ML model to correlate these data with the OM images and NX-CT corresponding slice (2D-2D fusion) and later to extrapolate them to the NX-CT volume (2D-3D fusion).

This poster will first show the sample preparation and its mounting. Then, the experimental set up is detailed. At this stage, Laser power, acquisition time, spectral resolution, map size and step size between points were parameters that had to be optimized to acquire a sufficiently large amount of data in a reasonable amount of time. Lastly, the data processing that allows to product pixel masks that would be used as Labels for the ML training will be detailed.

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Spectroscopic Techniques for Characterization of Clean Energy Based Nanomaterials

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In the pursuit of more sustainable and clean energy technologies, nanomaterials have emerged as promising alternatives, displaying enhanced capabilities in industries such as solar cells and energy storage.[1–3] With the increasing demand for clean energy sources, it is imperative for the scientific community to maintain a deep understanding of these materials to effectively apply them. This study aims to explore the physical and chemical properties of clean energy based nanomaterials. We study the behavior of the tris(2,2'-bipyridine)ruthenium(II) photoredox catalyst-gold nanosphere oligomer complex using different spectroscopic techniques. We employ ultrafast transient absorption (TA) spectroscopy and surface-enhanced Raman spectroscopy (SERS) to probe the unique interaction between the Ru dye and plasmonic gold nanoparticle upon photoexcitation with visible light. The study reveals important information about the charge transfer dynamics between the dye and the substrate.[4] We also study a highly conductive copper-carbon composite material using two dimensional x-ray absorption near-edge spectroscopy (XANES).[5] Elemental characterization provided by XANES is pivotal in understanding the cause of the enhanced conduction and shows the temperature dependent behavior of the hybrid material. Overall, this study aims to characterize two clean energy based nanomaterial systems using different spectroscopic techniques and highlights the importance of maintaining an extensive understanding of these materials in order to advance their design, and implementation in sustainable and clean energy technologies.

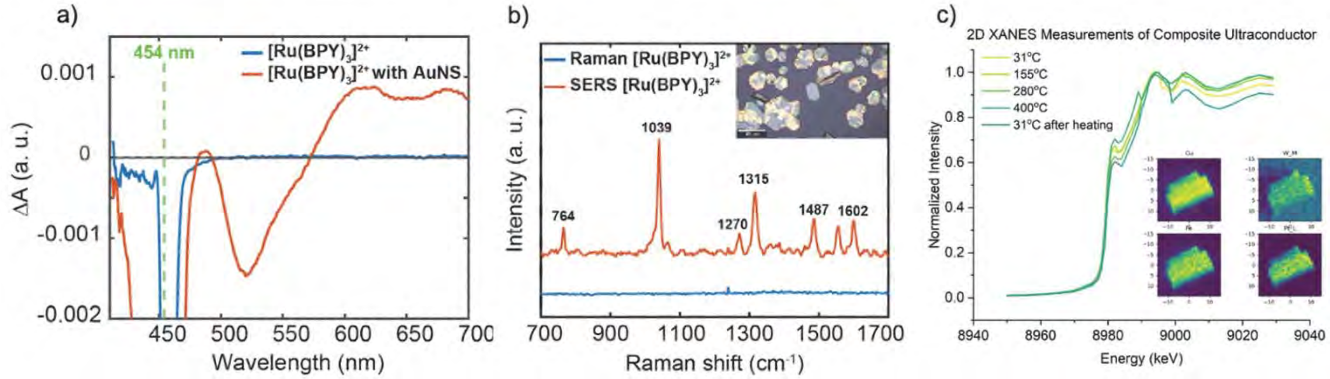

Figure 1: a) Ultrafast Transient Absorption (TA) spectra of $[Ru(BPY)_3]^{2+}$ without AuNS (blue) and with AuNS (red) showing the stabilization of excited state species. b) Raman spectrum of $[Ru(BPY)_3]^{2+}$ without AuNS (blue) and surface-enhanced Raman spectrum with AuNS (red) c) Two-dimensional X-ray absorption near-edge spectra (2D XANES) of composite ultraconductor under varying temperature conditions.

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Performance Evaluation of Multimodal Coherent Raman Microscope Part of a New qCSI Imaging Infrastructure

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Quantitative Chemically-Specifc Imaging Infrastructure for Material and Life Sciences (qCSI) is an open-access imaging infrastructure developed by a consortium of three Finnish universities and funded by the Research Council of Finland. It started its operation in the beginning of 2023 with two new spectroscopic imaging platforms: multimodal coherent Raman microscope mainly focused on stimulated Raman scattering (SRS), and a scattering-type near-field optical microscopy (SNOM) system. Here, we present the construction of the coherent Raman microscope, general performance evaluation and demonstrated its capabilities.

The microscope is built in-house by adding non-descanned detectors and a fs dual-output laser (InSight X3+, Spectra Physics) to an Olympus FV3000 laser scanning microscope while preserving all the original fluorescence imaging capabilities. The add-ons routinely used are SRS and second-harmonic/sum frequency microscopy while multiphoton and coherent anti-Stokes Raman scattering modalities are also available. The Raman spectroscopies employ a spectral focusing technique that is a feature of Spectral Focusing Timing and Recombination Unit (SF-TRU) by Newport [1] included in the microscope customization. A LabView-based graphical user interface provides an automated and calibrated access to all the additional imaging features.

The presented performance evaluation includes comparisons of different imaging modalities, determination of the spatial and spectral resolutions as well as accuracy of spectral focusing Raman shift calibrations. To demonstrate application of the instrument, we show preliminary results of in-situ imaging the dissolution behaviour of PVPVA-ritonavir film (amorphous solid dispersion prepared by solvent evaporation). The data consists of SRS hyperspectral images which were analysed with a classical least squares algorithm to produce false colour images shown in Figure 1. The results show that the performance is among the state-of-the-art and makes a valuable contribution to the Nordic scientific imaging infrastructure.

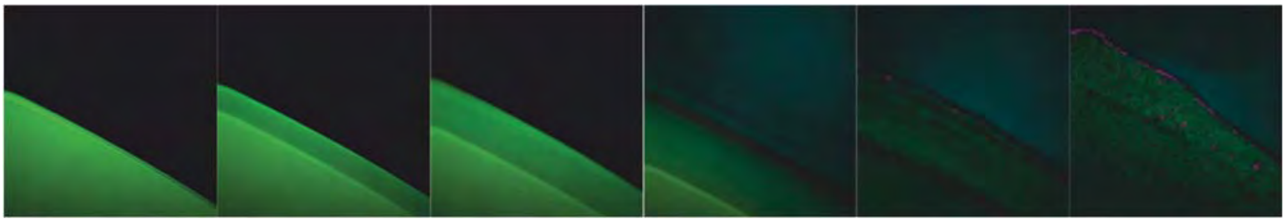

Figure 1: Dissolution of PVPVA (polymer, green) – ritonavir (drug, magenta) film at 20% drug loading. Dissolved product shown in cyan. Time points 1-2-5-10-15 minutes after adding the phosphate buffer solution (pH 6.8). First, the film wets and then forms a gel layer in which the drug separates. Image size 212x212 µm.

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Au@Ag Core-Shell Nanoparticles for SERS

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Plasmonic metallic nanoparticles, particularly gold (Au) and silver (Ag), are widely used nanomaterials in different applications due to their unique properties. One significant application is surface enhanced Raman spectroscopy (SERS), an exceptionally sensitive spectroscopic technique for identification of unique signature (fingerprint) information of target molecules. SERS is widely acknowledged for its ultrasensitive label-free detection capabilities and finds applications in diverse fields[1-3]. It can be used to detect and study a broad range of chemical and biological analytes. Because of unique gold and silver localized surface plasmon resonance (LSPR) properties, method uses plasmonic metal surfaces to enhance Raman signal[4]. Combining gold and silver nanoparticles into a core-shell systems, leads to a hybrid LSPR band that can be tuned continuously[5]. Core shell nanoparticles have an inner material core and an outer material shell and due this construction, properties can be modified by changing many parameters including the type of core or shell materials, the thickness layer, and the core to shell ratio[6]. Studies have shown that bimetallic core-shell nanoparticles produce broader range of plasmonic effects and demonstrate higher SERS enhancement, making them effective and attractive in SERS application, comparing to single Au or Ag nanoparticles[2,7].

In this study, gold nanoparticles were synthesized by the chemical reduction method. Following that, a seed mediated growth method was applied to coat AuNPs with Ag shell. The adjustable shell thickness was achieved using various (0.5, 1, 2.5, 5 mM) concentrations of $AgNO_3$ solution. The obtained nanoparticles were evaluated by transmission electron microscopy (TEM), UV-Vis, and Raman spectroscopy methods. Furthermore, the selected Au@Ag NPs were employed for adenine SERS imaging.

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Structural analysis of chiral phosphorus-containing compounds

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Phosphorus is an essential element of many chemical and biological systems. However, stereochemical analysis of P-containing compounds can be sometimes rather challenging. It has been already shown that compounds with a stereogenic center on the phosphorus atom (known as P-chirogenic molecules) can be advantageously studied by the advanced methods of nuclear magnetic resonance (NMR)[1]. Here we demonstrate that the results of NMR analyses can be further enhanced by employing the methods of molecular spectroscopy. A combined use of Raman and infrared spectroscopy and their chiral variants (Raman optical activity - ROA and vibrational circular dichroism - VCD) may help us to gain insight into the stereochemistry of the phosphorus chiral center. For this purpose, we have utilized rigid model compounds – phosphorylated derivatives of isopinocampheol (Figure 1)[2]. Experimental data are correlated with the results of *ab initio* calculations.

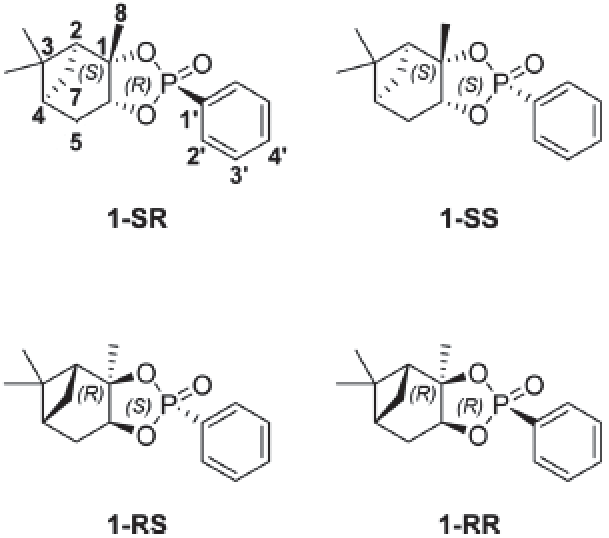

Figure 1: Chemical structures of the studied compounds[2]

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Porous frameworks for low-concentration methane trapping and SEIRA sensing

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In light of the urgent need to mitigate unwanted methane emissions, e.g. leakages from faulty pipelines, the development of compact sensors for detecting low concentrations of methane takes on heightened significance. The physical phenomenon of surface-enhanced infrared absorption (SEIRA) offers a method for monitoring a distinctive peak in the methane infrared absorption spectrum, enabling detection at concentrations below a few parts per million (ppm). Successful signal enhancement requires confining methane molecules within regions that demonstrate significant electromagnetic field amplification near intricately engineered metallic nano and micro antennae. Previous research has documented various trapping environments [1]. This study introduces a novel class of hyper cross-linked porous polymers known for their remarkable gas storage capabilities [2]. The development of SEIRA platform with integrated trapping environment is presented, coupled with simulated results and benchmarking strategy for SERS and SEIRA platforms alike.

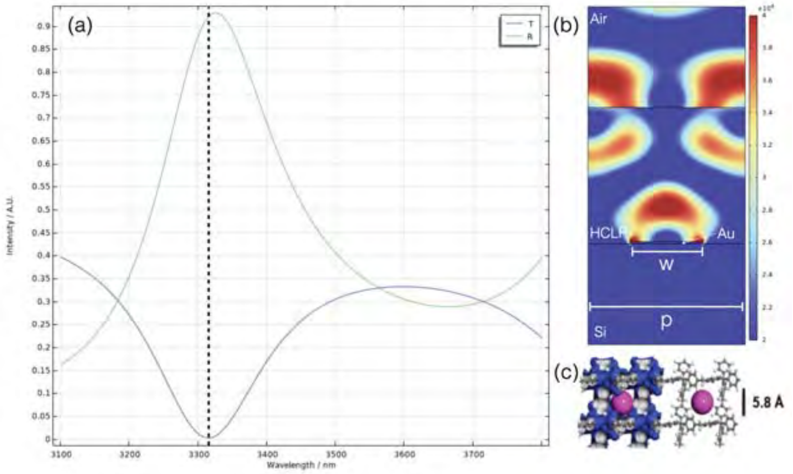

Figure 1: (a) Reflectance and transmittance spectrum corresponding to an array of gold antennae with width w of 1400 nm and pitch p of 3100 nm. (b) Corresponding electromagnetic field arising above the metallic stripes within the hyper cross-linked polymer (HCLP) environment synthesize on top of the substrate. (c) Scheme of the HCLP with microporous structures compatible with gas uptake at low-pressure [2].

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Uncertainty Quantification in AI using Monte Carlo Dropout for Raman Spectra Classification

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Artificial Intelligence (AI) has become an indispensable tool for classification tasks. However, the opacity of its decision-making processes remains a significant challenge, often observed in models based on Raman spectra due to the high variability between biological samples and measurement setups. Our methodology combines the Monte Carlo Dropout (MCD) method [1,2] with convolutional neural networks (CNNs). MCD integrates a dropout mechanism, typically used during CNN training, into the inference phase. This allows for the generation of diverse predictions, thus quantifying model uncertainty. Our analysis of the prediction distributions assesses the model's confidence and reliability, emphasizing a balance between decision accuracy and outcome confidence. The proposed methodology clusters unseen data based on prediction certainty using established thresholds, highlighting the importance of uncertainty quantification. The spectral data characterized by low uncertainty show a much higher balanced accuracy than those with higher uncertainty values, as shown in Figure 1. Our results highlight the distinction between predicted confidence levels (certainty) and traditional probability scores. Significantly, we observed an overall accuracy of 83.1%, which escalates to 94.2% within the high-certainty subset.

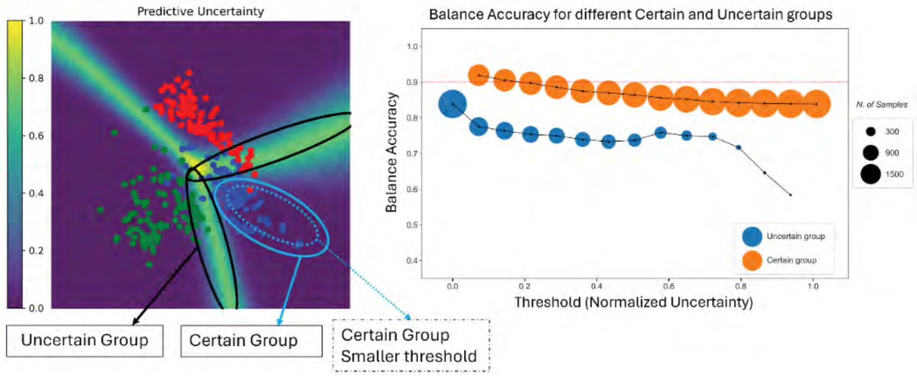

Figure 1: (left) Predictive uncertainty for three bacteria classes. (right) Relationship between various thresholds and the Balance Accuracy of unseen data segmented as Certain and Uncertain.

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Synthesis of Magnetite Nanoparticles and Decoration with Silver Nanoparticles

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The synthesis of magnetic particles triggers the interest of many scientists due to their relevant properties and wide range of applications in starting from MRI contrast agents, drug delivery systems, magnetic separators and hyperthermia agents and begging to the environmental, catalysis and biosensing fields. These nanoparticles capped with a plasmonic silver or gold layer could also be applied in surface-enhanced Raman spectroscopy due to signal enhancement for two reasons: the concentration of the sample using magnet and surface plasmons. Magnetite particles are suitable for such applications due to their stability, biocompatibility, simple synthesis, low price and great magnetic response [1]. Silver nanoparticles have been proven to be very good candidates for applications in catalysis, photocatalysis and various plasmonics devices, for example in sensors utilizing surface-enhanced Raman scattering (SERS) effect [2].

In this work, we present the magnetite nanoparticles (FeNP), obtained via microwave assisted synthesis route and decorated by different shape silver nanoparticles (AgNP) synthesized different synthesis time and temperature. Obtained nanoparticles were investigated using X-Ray spectroscopy, scanning electron microscopy, transmission electron microscopy (TEM) (Fig. 1), ultraviolet–visible spectroscopy, and applied for surface-enhanced Raman spectroscopy (SERS)

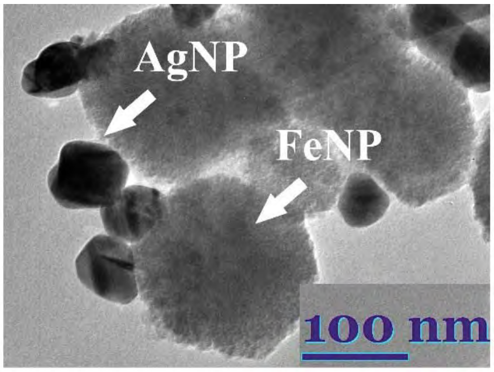

Figure 1: TEM of silver decorated magnetic nanoparticles. Magnetic iron oxide nanoparticles (light grey), silver nanoparticles (dark grey)

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Improvement of 5-halouracils detection driven by Watson & Crick pairing recognition. A SERS-DFT study.

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Taking advantage of Watson & Crick (W&C) interactions in DNA has become an emerging strategy for developing SERS-based methodologies with improved specificity and detectability, crossing the research frontier from genetics toward plasmonic materials [1]. Substituted uracils (5-fluor-, 5-bromo, and 5-chloro-uracil) have antimetabolic and antineoplastic activity or can be biomarkers. Besides, they easily pair with adenine through hydrogen bonding following the W&C rule. As adenine has a well-known high affinity for noble metal nanoparticles and a very intense SERS spectrum, contrary to the observed behavior for uracil and its derivatives, pairing them is a way to specifically drive a favorable orientation of halouracils improving their interaction with silver nanoparticles (AgNPs). This work combines experimental SERS and DFT calculations to elucidate the specificity of the W&C pairs – AgNPs interactions and their impact on the SERS response. Here, it is shown that the W&C pairs favor the orientation of the halouracils, acting as an additional signal enhancer other than the AgNP surface itself. Also, each pair shows a particular induced orientation on the metallic surface, matching the more intensified vibrational modes observed in the experimental SERS spectra (Fig. 1). The formation of hydrogen bond interactions was verified through the intensification and frequency shifts of the N-H modes, indicating that the W&C pair formation plays a main role in the signal enhancement observed. More importantly, it was also evidenced that the enhancement is directly influenced by the halogen's electronegative character, which strongly determines the larger affinity observed for the W&C 5-BrU. The results shown here are on the path to optimizing biosensing methodologies based on plasmonic materials.

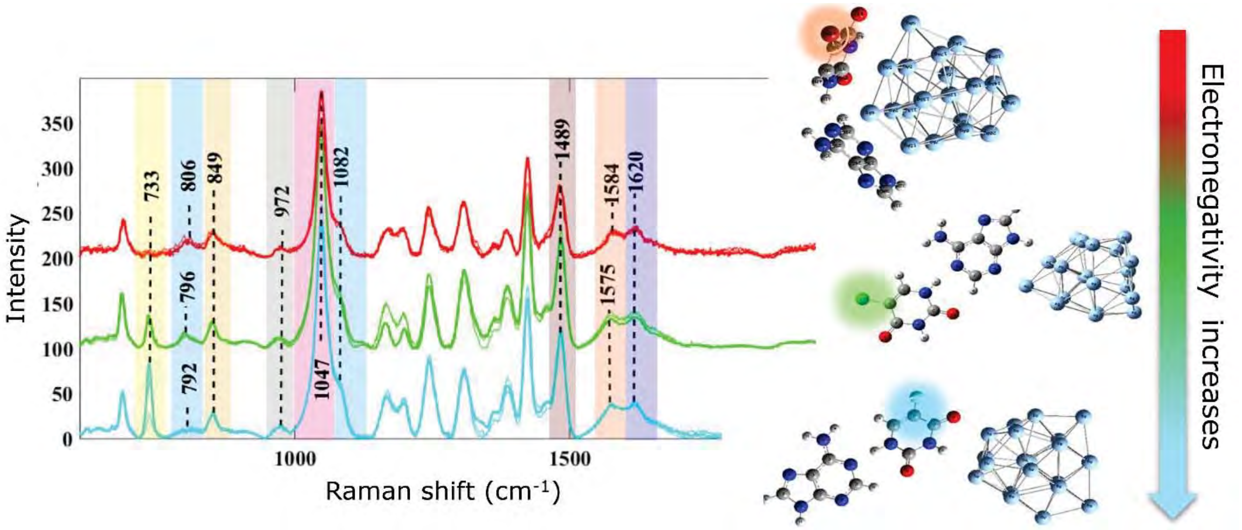

Figure 1: SERS spectra of W&C halouracil pairs (red:5-BrU; green: 5-ClU; turquoise: 5-FU) on AgNPs and their DFT-optimized structures on Ag_{20} clusters.

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Investigation of data fusion pipelines for different cell subtypes analysis

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Data fusion enables integration of diverse and complementary data sources, enhancing the accuracy and reliability of classification [1]. We analyzed data fusion strategies for multiclass (B cells, CD4+, CD8+, neutrophils) classification of cell subtypes in mice using five different datasets generated from Raman imaging, fluorescence lifetime imaging (FLIM), multimodal images (CARS, TPEF, SHG), optical photothermal infrared (O-PTIR) spectra, and high-throughput (HT) Raman spectra. Each imaging technique applied offers unique insights into different aspects of the study, collectively providing a comprehensive perspective for analysis and interpretation. Our research focuses on the integration of image and spectral information for improved classification performance. In the initial stage of the analysis, we intend to employ a combination of Raman spectroscopy and FLIM data following the local-level data fusion approach. We extract features from the reconstructed FLIM images using a pre-trained deep learning model and select the feature vectors related to the desired cell area as the segmentation task before the classification (see Figure 1). In the investigation, we perform the single-mode data analyses and implement combined Raman and FLIM analysis. We utilize the nested group k-fold cross-validation to assess the performance of the model tests in the outer loop [2]. In the single-mode studies, three distinct models were used (LDA, PCA-LDA, SVM) with balanced accuracy (mean sensitivity over all the classes) ranging from 50% to 95% for the analysed data types. In the following step, we compare the single-mode analyses with the results obtained using data fusion strategies.

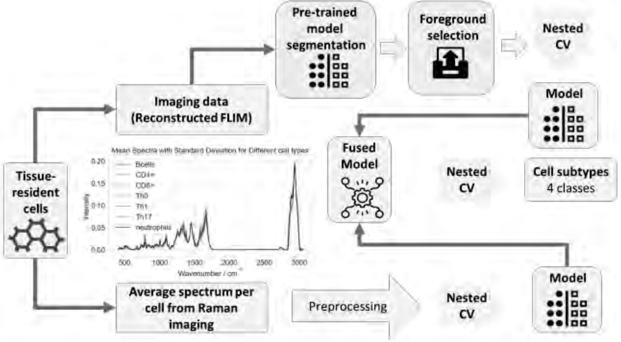

Figure 1: A workflow to integrate Raman spectroscopy and FLIM data for cell subtype analysis.

Acknowledgments

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Formation of Molecule/Metal Surface Complexes and their Effect on SERS-spectra in the Systems of Amphetamine-Based Drugs and Colloidal Nanoparticles

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Surface-enhanced Raman scattering (SERS) spectroscopy emerges as a promising technique for addictive substances analysis. It overcomes limitations of standard Raman spectroscopy, albeit with potential signal variations caused by molecule-surface interactions [1]. This study explores the potential of SERS for the detection of amphetamine-based addictive stimulants, with a particular focus on possible surface-complex formation and its effect on the resulting SERS spectra's intensity and profile [2]. Prime attention was paid to the correlation between SERS and extinction spectra. Spectra obtained with two excitation wavelengths highlights the importance of selected excitation, as excitation of 785 nm emerges as the optimal choice in terms of bands' intensities and formation of molecular complexes.

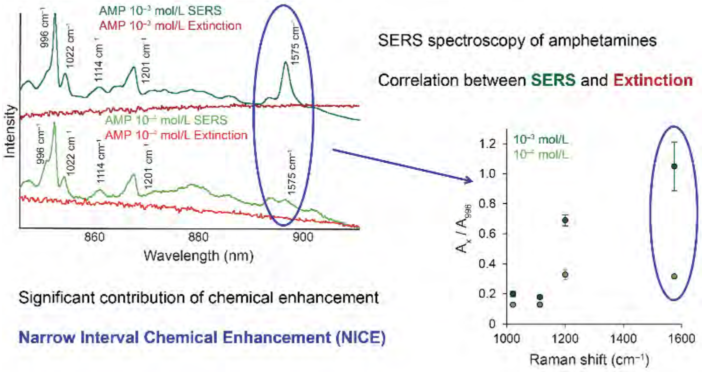

Figure 1: Narrow Interval Chemical Enhancement in the SERS spectra of amphetamine

To extract the maximum amount of information from the obtained spectra, we used density functional theory computations. Our results show that in cases where molecule-metal complexes are formed, the SERS signal is much higher and more specific than in the opposite cases. We believe that the presented findings could prove useful when considering SERS spectroscopy as a tool for amphetamine-based drug analysis and from a physico-chemical perspective, where insight into the chemical enhancement mechanism is provided [3].

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Tin Disulfide as an ultrasensitive SERS substrate for the detection of anionic molecule

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Raman spectroscopy is a powerful analytical tool for providing important information about the chemical structure of target analytes. However, Raman scattering events are inherently weak (~1 of 1 million incident photons). Therefore, very low detection sensitivity is a developmental bottleneck of Raman spectroscopy. In the 1970s, this scenario began to change with the discovery of the SERS effect, an intensification effect observed on nanostructured surfaces. The enhancement of the Raman signal is attributed to electromagnetic field enhancement resulting from localized surface plasmon resonance (LSPR) at a nanostructured metal surface and chemical enhancement due to charge transfer between analytes and metal surfaces [1]. Various materials, from noble metals to nanostructured materials, have been employed as SERS substrates. Metal (Au, Ag) plasmonic materials are the most common substrates for SERS applications. However, the cost of noble metal precursors, moderate stability, poor reproducibility, and low affinity for anionic molecules are challenges for this type of substrate. In recent years, 2D inorganic materials have been widely used in developing SERS-based chemical sensors owing to their unique thickness-dependent physico-chemical properties with enhanced chemical-based charge-transfer processes. Graphene was the first 2D material that demonstrated enhancement of the Raman signal [2]. Since then, several 2D materials have been applied to date. This work explored tin disulfide (SnS_2) as a SERS substrate for detecting anionic molecules (Congo Red dye). The substrate generated chemical enhancements and excellent molecule adsorption, achieving detection limits to picomolar and reproducible detection. The SnS_2 substrate performed similarly to the graphene substrate for the molecule studied. We observed that the enhancement factor depends on the number of layers of 2D materials. The substrate with six layers showed a more significant signal intensification of the molecule of interest. The ease of preparation by mechanical exfoliation, in combination with stability, relatively low cost, easy surface modification, and environmentally friendly material, justifies further SERS studies of SnS_2 layered materials.

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Strain Analysis of SiGe Layers on SOI: A Tip-Enhanced Raman Spectroscopy Approach for Accurate Germanium and Strain Percentage Determination

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In recent years, strained silicon technologies have emerged as a key avenue in semiconductor research, offering a promising way to improve device performance. Among the key players in this field is the integration of silicon-germanium (SiGe) layers onto silicon substrates, which are strategically designed to induce strain and subsequently increase the overall efficiency of strained silicon devices [1].

In this work, through a weighted comparison of Raman spectroscopy and high-resolution X-ray diffraction (HR-XRD) measurements with Tip-Enhanced Raman Spectroscopy (TERS) [2] measurements obtained on SiGe changes on silicon-on-insulator (SOI) at different concentrations and thicknesses, a model was extracted that allows the simultaneous measurement of the percentage of Ge and strain present in nanometric volumes [3]. Furthermore, also the innovative titanium nitride (TiN) AFM probe [4], which allows clean room implementation for in-line characterization, was used.

The results highlight TERS as a powerful tool for monitoring the quality of semiconductor production lines, with unprecedented resolution and speed.

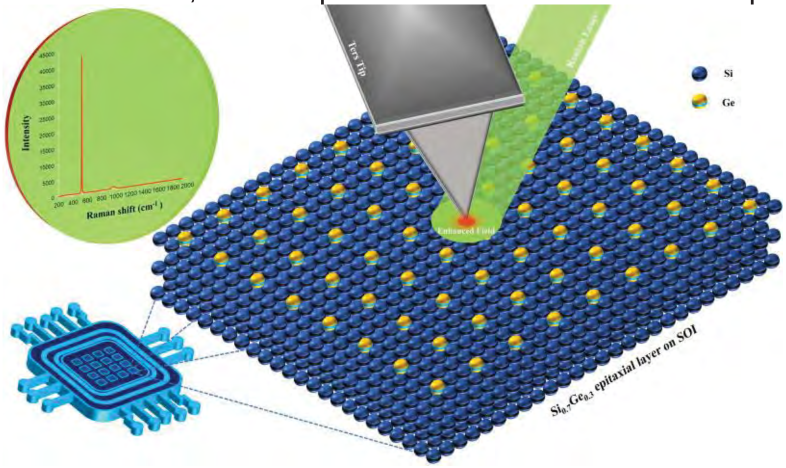

Figure 1: Graphical abstract of the presented work

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Theoretical DFT and experimental Raman characterization of octocrylene for its detection in sunscreen cosmetic products and in saltwater bodies from balneary resorts

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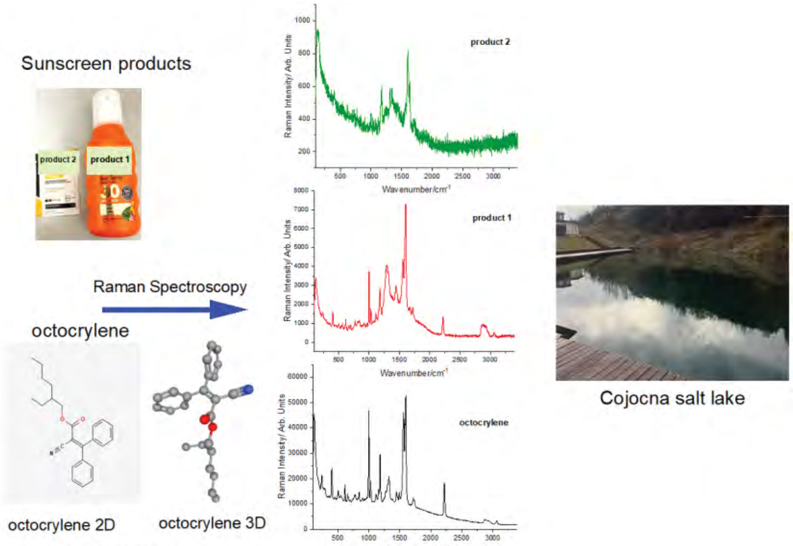
Octocrylene ($C_{24}H_{27}NO_2$, 2-Ethylhexyl 2-cyano-3,3-diphenylacrylate,OCT) a widely used ingredient in sunscreen products, pose dangerous effects on the water microorganisms and ecosystems [1] comprising water bodies intensively used for recreational or balneary treatment activity. Recent results [1,2] demonstrated adverse effects on corals, zebra fish, and marine algae [2].

To develop a reliable detection method for effective monitoring this compound or its decomposition by-products, we firstly characterized either theoretically or experimental Raman spectra of OCT in solid state and completely assigned the Raman data. Secondly, we investigated several cosmetic products commercially available to establish marker bands suitable in formulations. Further, we developed a detection algorithm based on surface–enhanced Raman scattering with Ag nanoparticles for trace detection of OCT in spiked (sprayed salt water bodies). We further investigated the saltwater bodies from a balneary resort spiked with product 1 (spray) and turned that the SERS method is effective in unambiguous deterction the intact OCT in the presence of AgNPs.



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Coherent Phonon Spectroscopy as an emerging technique for space exploration

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Raman spectroscopy has shown to be a suitable method for identifying minerals and their compositions for in situ planetary research. With the development of space-ready femtosecond lasers [1, 2], utilization of alternative approaches based on time-domain techniques has become a relevant question. Time-domain techniques have the advantage of being compact and chip-integrable. In this contribution, we investigate the potential of coherent phonon spectroscopy (CPS) as an alternative to Raman-spectroscopy [3]. The applicability of CPS as an in situ spectroscopic technique to identify matter by their characteristic spectra of Raman-active phonons is evaluated. It is shown that a spectral bandwidth of beyond 30THz (~ 1000cm^{-1}) and a resolution of 100 GHz (~ 4cm^{-1}) can be easily achieved, while any fluorescence background is avoided.

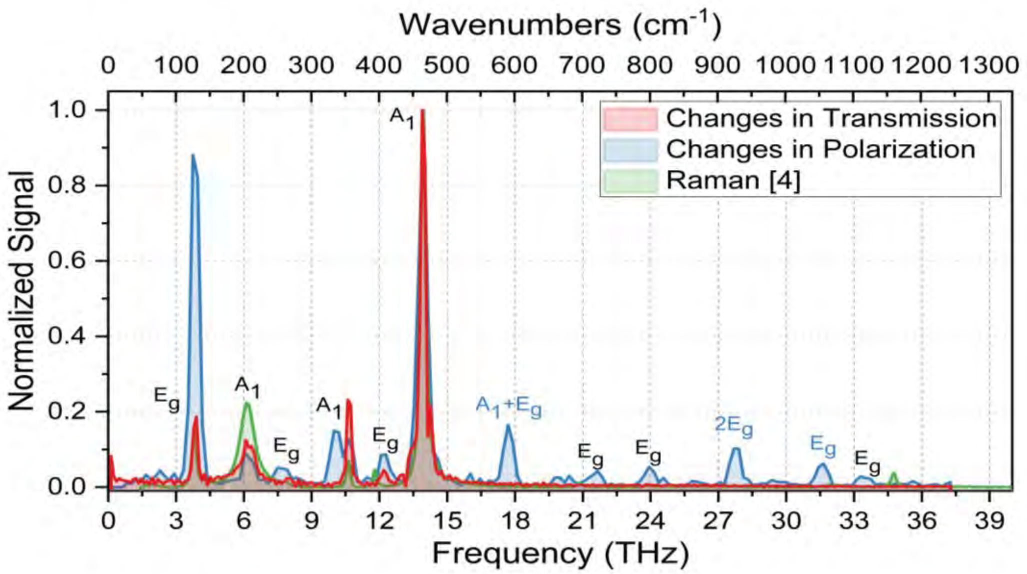

Figure 1: Comparison of the Raman spectrum of Quartz taken from the RRUFF database [4] with the CPS spectrum. All expected Raman-active modes are observed with both techniques. The polarization-sensitive CPS measurement furthermore reveals even additional expected (combination) modes.

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Raman-based Detection of Natural Products in Microbial Communication

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The critical role of the interaction between prokaryotic and eukaryotic microorganisms in ecosystem functionality is well recognized, yet our understanding of the mechanisms underlying these microbial community interactions is limited. Polyketides derived from arginine, known as arginoketides, are produced by *Streptomyces* species and play a key role in mediating cross-kingdom microbial interactions with *Aspergillus* fungi, leading to the production of natural products. These arginoketides can either be cyclic, such as monazomycin and desertomycin A, or linear, like lydicamycin and linearmycin A, all of which produced by *Streptomyces iranensis* and have been found to stimulate the orsellinic acid gene cluster in *Aspergillus nidulans* [1]. The characterization and detection of natural products by surface-enhanced Raman spectroscopy (SERS) is expected to shed light on how they are released and transported through the environment, and how they trigger responses in other microorganisms [2].

SERS measurements were carried out using a dendritic silver substrate fabricated on a silicon wafer fabricated by means of galvanic replacement of silver and sulfur ions. At the nanoscale, the branches of the tree-like structure form sharp angles and narrow gaps that are supposed to turn into “hot spots” where the Raman signal of the target molecules should be enhanced. Raman and SERS spectra of the bacterial secondary metabolites monazomycin, desertomycin A, lydicamycin, and linearmycin A and the fungal products orsellinic and lecanoric acids were characterized in terms of assigning marker modes, thus enabling their detection and identification in the microbial culture.

Acknowledgement

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Optimizing Raman Signals in customized spectrometers: Understanding the Tradeoff between Core Size, Numerical Aperture, and Slit Width

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In recent years, an increasing number of laboratories entrust their Raman analysis to the use of customized spectrometers. The sensitivity and spectral resolution of these Raman setups can be significantly affected by variations in the optical configuration and design of spectrometers and other elements in the optical chain. By carefully considering these factors, it is possible to optimize Raman signal analysis and enhance the sensitivity and spectral resolution of measurements. The present work aims at experimentally demonstrating the tradeoffs between collection-fiber core size, numerical aperture (N.A.), and spectrometer entrance slit size to maximize the collected light in a Raman-based gas-measurement application as described in [1], where the limit of detection (LOD) is favored against other parameters such as spectral resolution.

For this work, we have used an *Avantes* spectrometer *SensLine, model AvaSpec-ULS2048x64-TEC-EVO* with a 160mW, 532nm laser excitation by RGB LaserSystems, model Lambda Beam. Different collection slits were used, ranging from 500 to 100 µm. In addition, several different optical fibers were employed, with different combinations of core sizes (between 100 and 400 microns) and N.A. (from 0.1 to 0.39). The goodness of all the combined configurations were evaluated in terms of Signal-to-noise ratio (SNR) of the N_2 Raman peaks acquired from air samples in laboratory conditions.

The work presented here provides significant correlations regarding these factors, such as the decreasing SNR with N.A., or increased SNR with core size (see Figure 1). This kind of experimental approach for the configuration of optical setups can be of help to scientists and technologists in taking informed decisions regarding their experimental setup configuration to maximize the collected light on the collection-fiber to spectrometer-slit interface.

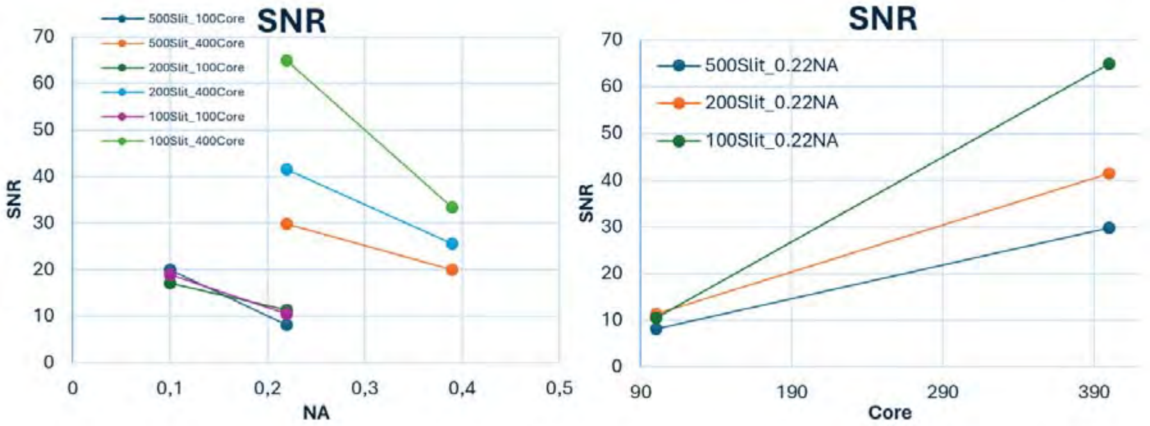

Figure 1: SNR comparisson with different collection configurations: fiber N.A., core size and input slits

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FT-Raman Spectra, tautomerism and some Molecular Properties of 5-methylcytosine

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The study of properties of molecules of biological importance continues to attract interest in these days because they help in the understanding of many biological processes. The vibrational spectra of some of the nucleic acid bases, like uracil and cytosine and their derivatives have been studied in detail [1,2]. The increasing interest in the study of the derivatives of cytosine is due mainly to their biological and pharmacological importance. The inclusion of a methyl group at the fifth position of the cytosine molecule leads to 5-methyl cytosine (5-MeC), which has a slight variation of charge distribution in the structure but with different and important properties [3]. However, its tautomerization, its structure and spectra appear little studied [4-6]. DFT studies on tautomerism of 5-MeC has been reported previously [5,7], at low MP2 *ab initio* level [8]. In the present study, we have remarkably improved these results optimizing the structures and energies of all possible tautomer forms using MP2 *ab initio* methods at high level, and the results compared with those of cytosine molecule.

We have also accurately assigned the IR and Raman spectra of 5-MeC molecule, which have not been reported yet completely. Raman spectroscopy and quantum-mechanical analysis of tautomeric forms in cytosine and 5-MeC have been only reported on gold surfaces [6]. For this reason, the interpretation of its structure and vibrational spectra appear necessary. For this task and to improve the predicted vibrational spectra in the solid state, it was simulated by a tetramer form and compared with the experimental ones in the solid state. The FT-Raman spectrum in the range 0-3500 cm^{-1} was experimentally recorded and accurately assigned by using DFT calculations together with the linear scaling procedure (LSE) [9]. The effect of the methyl group on the geometry and vibrational wavenumbers was analyzed by comparing with those of cytosine molecule. The $C\text{-}CH_3$ and $C\text{-}NH_2$ stretching vibrations have been identified at 1200 and 1280 cm^{-1}, respectively in agreement with literature values in related molecules.

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Raman and XRD characterization of PVP-Stabilized All-Inorganic Perovskites, with applications for Ozone gas detection

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The gas sensing falls into one of several categories. These include electro-chemical/resistive/impedance, photonic, and acoustic platforms. When gas molecules adsorb to a semiconducting layer of the right bandgap and electrical/chemical properties, such films can provide state-of-the-art performance for detecting gases via changes in electrical resistance and/or impedance. However, more research is required to identify sustainable materials with comparable manufacturing capabilities.

Recently, metal-halide perovskite materials, which have been studied for photovoltaics, have drawn interest as sensing components due to the ability of their optoelectronic properties to change reversibly when exposed to gases like ozone, CH_4, H_2, and other gases [1]. Numerous studies have demonstrated the use of metal halide and oxide perovskites in detecting various air contaminants [2]. Despite different research, the stability and quality of the films still inhibit the commercialization of perovskite-based sensors. Here, we report the fabrication of thin films of $CsPbI_xBr_{3-x}$ (x=3,2,1.5,1,0) perovskites for gas-sensing applications. To make them stable, homogenous, and smooth films, we used a polyvinylpyrrolidone (PVP) surfactant in the precursor solution. The surfactant helps to grow homogeneous films and it stabilizes the photoluminescent phase of $CsPbI_xBr_{3-x}$ in ambient conditions. The effect of PVP on the structure and vibrational mode has been studied using PXRD and Raman. We studied the effect of ozone gas on the optical and vibrational modes of all-inorganic perovskites. The as-fabricated thin film of the perovskites also shows remarkable sensitivity to ozone. Future gas sensing devices based on the PL phase of $CsPbI_xBr_{3-x}$ stabilized with surfactant could be made easy and cost-effective with high sensitivity because of all these appealing qualities and the straightforward fabrication procedure.

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Effects of intense freeze-thaw cycles on Arctic biological soil crusts as studied by Raman microspectroscopy

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Soil microbial metabolism is extremely important to large scale processes such as nutrient cycling and climate change. At the same time, the changing climate influences soil structure and function, especially in the Arctic region which has been experiencing faster and more intense warming compared to anywhere else in the world [1]. To better understand the microscale processes and how they are affected by changing temperatures and extreme weather events, we use soil microchips, mimicking the soil structure, and incubate them with microorganisms from Arctic biological soil crusts. The more frequent freeze-thaw cycles are recreated both in the field, and in the laboratory environment through soil microchip treatments at different temperatures.

Raman microspectroscopy is employed to study the chemical composition and metabolic processes of individual live microorganisms in near real time [2]. The microbial metabolic activity is monitored using SIP (stable isotope probing) Raman microspectroscopy. We inject SIP labelled substrates into the soil microchips and follow the intensity of SIP related spectral bands as microorganisms incorporate the labelled substances. Raman microspectroscopy and optical microscopy measurements are also combined with bulk analyses of microbial respiration to identify parameters connecting single-cell level processes with macroscale responses.

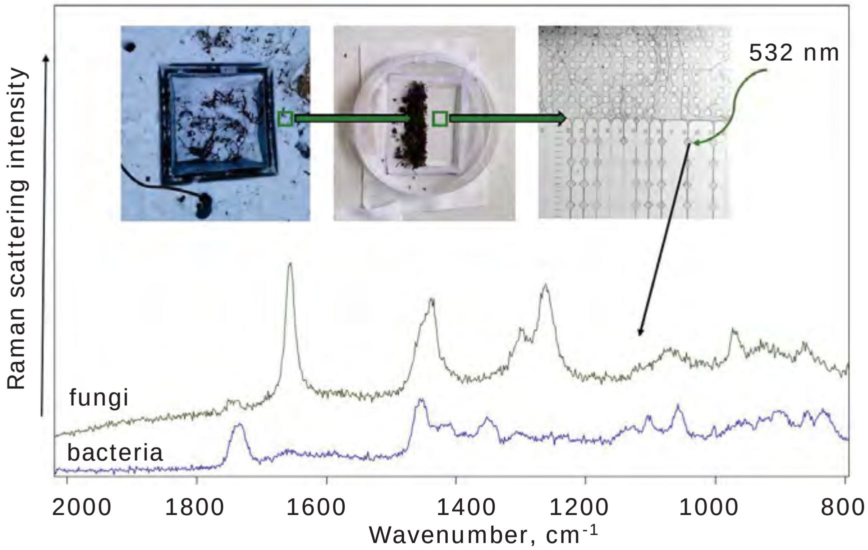

Figure 1: Averaged Raman scattering spectra of microorganisms (fungi and bacteria) in Arctic soil microchips. Pictures show the sample location, soil microchip and its microscopic image.

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Raman technology for process control: transparent, chitin-based polymer foils produced from crustacean waste

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The blue bioeconomy stands out as one of the fastest-growing research fields, with a major goal of exploiting aquatic waste to obtain new and valuable products.

Here we report an eco-friendly demineralization process of crustacean waste (*Callinectes sapidus*, *Squilla mantis*, and *Maja squinado)*, resembling the real-world management scenario of mixed shell species valorization, without conventional powdering, heating, or stirring of the demineralization bath. Crustacean shells represent a significant resource of chitin [1], an abundant biopolymer renowned for its various applications [2]. We aimed to employ Raman technology for process control, decision-making and characterization of the final products. Species-specific demineralization times were demonstrated by the decrease in carbonate Raman bands and the increase in chitin signal, up to the complete formation of transparent, flexible chitin-based foils. Raman analysis of the leftover solution revealed an astaxanthin-based supernatant and calcium acetate solution, subsequently purified into fine calcium acetate hydrate crystals. The yielded calcium acetate by-product exhibited different hydration states compared to equivalent forms obtained from shells of the invasive sea snail *Rapana venosa* immersed in a 9% acetic acid solution, or from the geogenic calcium carbonate. Thus, the Raman spectroscopy-assisted steps demonstrate efficacy as a process control tool, facilitating informed decisions (whether to continue, change or stop) regarding the acetic acid demineralization process. In situ evaluation was performed using a hand-held flexible TacticID Raman system, while the final products underwent cross-validation through FT-IR, XRD, and SEM-EDX techniques [3].

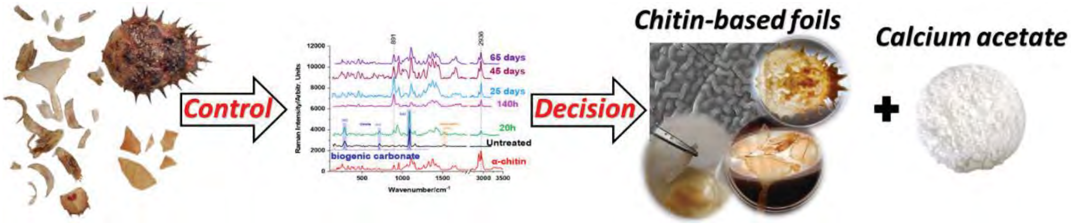

Figure 1: Process control of acetic acid demineralization of crustacean shells through Raman techniques

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Raman technology for the development of a novel biogenic calcite based bone substitute material

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Regenerative medicine is at the forefront of scientific innovation as it holds the promise to repair and heal damaged organs and tissues by stimulating the body's own repair mechanisms. Among the numerous fields of regenerative medicine, bone tissue engineering, seems to attract significant scientific attention [1]. The present proposes the development of a novel bone replacement tissue biomaterial based on wasted crab shells. Raman Spectroscopy, assisted by Scanning Electron Microscopy and X-Ray Diffraction were used to monitor and control the process.

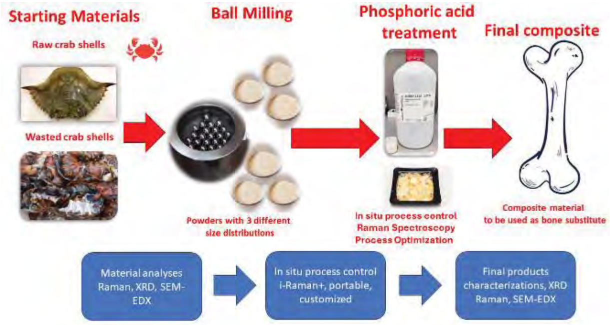

Figure 1: Graphical sketch of the experiment

The biogenic material, the shell from crustacean C. Sapidus (blue crab), in 2 forms: raw and post consumption, (thermal treated), was powdered using a calibrated ball milling procedure. The powders were subsequently subjected to phosphoric acid treatment. Process control was achieved using Raman Spectroscopy, by optimizing the powdering procedure (milling time, frequency), as well as the phosphoric acid concentration, in order to obtain a novel formulation of calcium phosphate from biogenic calcium carbonate. The main advantage of this formulation is the preservation of carotenoid content of the biogenic calcite conferring the formulation antioxidant properties. The complete reaction was instantly read out by observing the disappearance of the phosphate band at 894 cm^{-1} in phosphoric acid (P=O symmetric stretch) and the appearance of the specific calcium phosphate raman band at 960 cm^{-1} (P-O symmetric stretch). The quality of the final product was investigated using Raman spectroscopic techniques and XRD in conjunction with morphology and semi quantitative elemental analysis using SEM-EDX.

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Microplastics influence on human lung cells - subcellular identification of particles with the use of Raman spectroscopy

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One of the priority challenges facing humanity nowadays is plastic pollution of the natural environment. The greatest danger to human health is the unnoticeable microplastics forming, among others, as a result of the degradation of larger plastics fragments under the influence of environmental factors [1]. Microplastics can enter the body in 3 ways: by inhalation, with food, and by skin as a result of the use of plastic objects or directly from the environment [2].

The purpose of this study was to evaluate the potential toxic effects of micro- and nanoplastics on the lung fibroblasts. To achieve this goal human C-12360 fibroblasts line was exposed to polystyrene particles with the diameters of 1 and 5 μm as well as 100 nm. The viability, cytotoxicity and apoptosis of the cells were studied at different particles doses (0.1, 0.05, 0.01, 0.005, 0.001, and 0.0005 mg/ml) and exposure times (6, 24 and 48 h). Raman microspectroscopy was used to follow internalization/distribution of polystyrene particles in cells as well as to assess potential changes in the accumulation and distribution of biomolecules induced by them. Typically, the cells were subjected to the raster scanning with a 532 nm laser beam (step size of 0.5 μm) in a plane parallel to the sample carrier, and then the chemical mapping of selected Raman bands was carried to visualize proteins, lipids, organic matter and polystyrene.

The cellular biology tests showed decreased viability of the human lung fibroblasts exposed to nano-/micro-plastics, and the exposure time-dependence of the observed toxic effect. The obtained Raman data confirmed great utility of the technique in the detection and identification of plastics particles within cells. Raman microscopy made it possible to confirm the internalization of polystyrene particles by lung fibroblasts and determination of the changes in the accumulation of organic matter in cells resulting from the exposure to them. The present study shed new light on the *in vitro* toxicity of the microplastics and provide a basis for further regular research in this area.

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OPTIR is a method to monitor active substances for cartilage regeneration in 3D spheroid cultures of stem cells

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The use of stem cells in various therapies raises great hopes because these cells can differentiate into any cell type. In the era of an aging society, therapies for senescence-related cartilage loss due to overuse and osteoarthritis are still sought. Our motivation for obtaining new polymeric-based carriers is to reduce the need for surgical interventions. Designed systems enable the controlled release of active substances with chondroprotective and chondrogenic effects. As a gold standard TGFβ1 is used. In this study 3D spheroid cultures of stem cells were exposed to modified hyaluronic acid as carrier of kartogenin [1] - an active substance that has proven chondroprotective and chondrogenic effects. Kartogenin is a hydrophobic compound with limited bioavailability and is often administered in DMSO solution, thus to increase its bioavailability we are working on kartogenin delivery systems. The initial characterization of kartogenin and TGFβ1 influence on cells was performed using viability tests, the expression level of relevant chondrogenic genes (SOX9, COL2A1, ACAN), osteogenic marker (COL1A1 as negative control) by RT-PCR, as well as selected histological staining.

Testing novel materials on cells requires using time-consuming methods like RT-PCR to evaluate the expression level of relevant chondrogenic genes and/or Western Blot to determine the amount of the selected proteins' expression.

Optical Photothermal Infrared microspectroscopy (OPTIR) was used here to speed up the general evaluation of biochemical changes under the influence of tested polymeric-based systems for kartogenin delivery in a label-free manner in cells. OPTIR besides shortening the imaging time, improves spatial resolution compared to FT-IR. Before measurement samples were fixed and cut with a microtome and mounted on CaF_2 glass slides to obtain slices thickened ~5μm.

We evaluated the content of selected protein levels i.a. collagen, lipids, and macromolecule orientation in 3D spheroid cultures of stem cells, cultured in chondrogenic medium.

In this work, we propose a complex methodological approach combining novel techniques in vibrational spectroscopy and biological assays focused together on a better understanding of kartogenin and TGFβ1 action in cells. Moreover, we indicated the most effective polymer as a carrier of kartogenin for cartilage regeneration purposes.

Acknowledgments

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Raman Spectroscopic Properties of Pharmaceuticals in Aqueous Solutions: Interplay of Theory and Experiment

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The design of new pharmaceutics or the improvement of existing ones requires a fundamental understanding of their bioactivity and bioavailability, starting from their structural properties.

This is typically done through vibrational spectroscopy techniques, whose resolutions is high enough to provide detailed information on structural changes even in complex environments, and can be used to monitor reaction pathways and identify weak intermolecular interactions. Among them, Raman spectroscopy, together with infrared spectroscopy, which provides complementary information to Raman, is a suitable tool for these kinds of studies. Raman spectroscopy has the advantage of avoiding water signal interference, naturally present in physiological environments.

In this contribution, we present a combined experimental and theoretical approach aimed at establishing a robust computational framework for the spectroscopic study of drugs in aqueous solutions. Raman spectroscopic properties of commonly used drugs, chosen as model compounds, were measured experimentally and simultaneously calculated using classical theoretical methods such as Molecular Dynamics (MD) and quantum methods such as Density Functional Theory (DFT). Multiscale quantum approaches often lack the description of the atomistic and dynamic nature of the solvation shell [1]; the use of MD techniques, in conjunction with statistical clustering methods, allows going beyond the static approach in modeling the environment. [2,3] Furthermore, structural analysis of the solvation shell facilitates the computational treatment of the vibrational problem at the anharmonic level, a notoriously challenging task for flexible systems, especially for solute-solvent molecular systems with weak hydrogen-bond interactions. [4]

This deep understanding of the Raman phenomena in a complex medium and their molecular nature is a valuable pathway for the application of advanced enhancement-based experimental techniques such as Surface enhanced Raman spectroscopy (SERS) and Resonance Raman (RR): this progress is essential to accurately interpret the complex but accurate chemical information these techniques can provide. [5]

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Quantitative Analysis of Intracellular Distribution and Metabolic Processes of Supersulfides Using Raman Microscopy

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[Introduction] Supersulfides are compounds in which sulfur atoms are excessively linked through S-S bonds in a molecule. In recent years, it has become evident that supersulfides are abundantly present in mammalian cells and organisms. Supersulfides exhibit higher reactivity than conventional sulfur compounds, and their strong redox properties are involved in maintaining various biological functions [1]. However, the subcellular localization and metabolism of supersulfides remain unclear due to the lack of direct observation of supersulfides in a living cell. We have previously succeeded in establishing a protocol that can efficiently introduce poorly soluble compounds into cells and quantify the concentration of intracellular molecules using the O-H stretching Raman band of water as an intensity standard [2]. This study successfully applied this method to supersulfide molecules to directly observe the concentration distribution and metabolic processes of intracellularly introduced supersulfides in a label-free manner.

[Experiment] In this study, we compared the intracellular introduction of dimethyl trisulfide (Me_2S_3), a supersulfide molecule, and dimethyl disulfide (Me_2S_2). In addition, dimethyl tetrasulfide (Me_2S_4) was introduced into HeLa cells. These molecules were solubilized and introduced into cells using an amphiphilic phospholipid polymer (PMB). An aqueous solution of Me_2S_n solubilized in PMB was mixed with the culture medium of the cells, and Raman imaging was conducted.

[Results and discussion] We succeeded in observing Raman spectra of Me_2S_3 in a HeLa cell. Me_2S_3-introduced HeLa cells showed the S-S stretching band due to Me_2S_3 at 485 cm^{-1} in the cytoplasm and lipid droplets. This band was prominently observed in the lipid droplets, indicating the preferential localization of Me_2S_3 in the lipid droplets. The concentration of Me_2S_3 in the lipid droplets was found to be highly concentrated over 500 mM. The lipid droplets showed the S-S bands at 439 and 459 cm^{-1}, which are absent in Me_2S_3, indicating the direct observation of the metabolic reaction inducing the sulfur extension of the S-S bond in the lipid droplets (Fig.1a). Me_2S_2 was also detected in the lipid droplets; however, its Raman bands were much weaker than those of Me_2S_3 and no metabolic bands were observed (Fig.1b). Furthermore, upon introduction Me_2S_4 having an S-S-S-S structure into the cells, further extension of the S-S bond in the lipid droplets was observed, resulting in the generation of S_8. In conclusion, we succeeded in directly observing the metabolic process of supersulfides in a single cell.

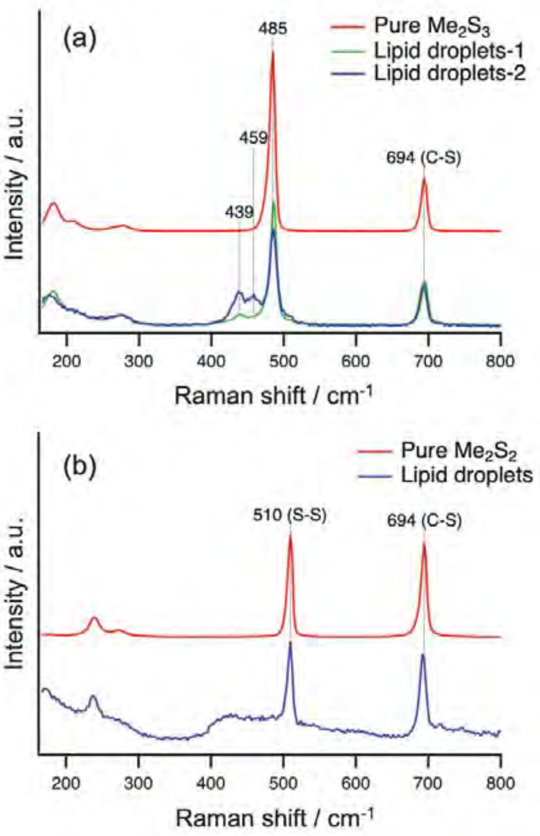

Figure 1: Raman spectra of lipid droplets in HeLa cells incubated with Me_2S_3 (a) and Me_2S_2 (b).

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Unraveling the Impact of Salinity on Ancient Paper: A Raman Spectroscopy-Centric Approach with Optical Complementarity

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Nowadays, most books and documents preserved in archives present numerous critical issues. The causes of risk to archival assets can stem from chemical, physical, and biological phenomena, as well as human factors such as the lack of preventive conservation-restoration measures. In recent decades, the frequency of extreme natural events like torrential rains, flash floods, and floods has increased due to climate change, exacerbating the vulnerability of these materials.

This study investigates the effects of direct immersion in various water solutions on different types of paper and its consequent degradation due to salinity. We synthesized various saline solutions to replicate different salinity levels associated with common hydrogeological phenomena. Specifically, our focus was on understanding how salt affects the crystalline structure of paper. Typically, archival items subjected to hydrogeological phenomena undergo processes to remove salt. Thus, our goal is to determine the necessity of such washing processes and assess if samples can be spared further stress.

The analysis primarily relied on Raman spectroscopy, supplemented by optical techniques including luminescence and reflectivity. These non-destructive methods offer valuable insights into the molecular structure and composition of paper samples. Moreover, their portability facilitates in situ measurements, making them applicable in the field of cultural heritage.

This research solely addresses paper; however, future investigations aim to assess the impact of salt while considering the presence of inks and pigments.

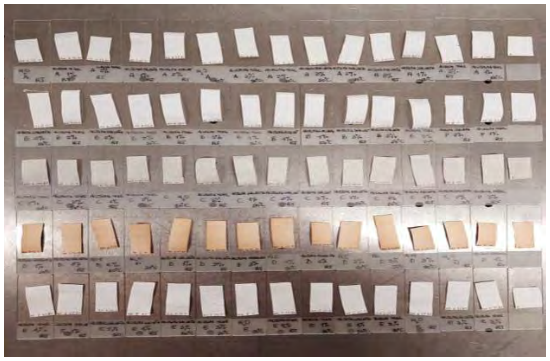

Figure 1: Analyzed samples

Spectrally resolved Bell test exploiting Stokes-Anti Stokes photon correlation

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[1]*EPFL- École Polytechnique Fédérale de Lausanne*

Two-photon coincidence measurements under pulsed excitation offer various ways to study the non-linear response of materials, including Raman scattering. Emission of correlated Stokes – anti-Stokes photon pairs can be mediated by the exchange of real [1] or virtual [2] phonons, a process described by a resonant term in the third-order $\chi^{(3)}$ tensor of the material. When the non-resonant, electronic contribution to $\chi^{(3)}$ is added, the quantum state of photon pairs emitted by spontaneous four wave mixing can become polarisation entangled at some Raman shifts. [3]
Here, we perform a spectrally-resolved Bell test on single-crystal diamond pumped by ~1ps laser pulses, using fiber dispersion and two-photon coincidence measurements.
Our results show a statistically significant violation of the CHSH Bell inequality in a large range of Raman shifts around the pump, in agreement with the theoretical predictions.

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In vivo Real-time Multiplex Detection of Plant Signalling Molecules Using Surface-Enhanced Raman Scattering Nanosensor

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To understand the biological and chemical dynamics of plants is essential part in next-generation agriculture, and various sensor technologies are studied and applied. The chemicals as known as 'signalling molecules' released against adverse stimuli to activate defence system of plants are drawing attention as a key to understand plant's biological status under stressors. As one of the analytical approaches to detect signalling molecules, SERS-based optical nanosensor has shown strong potential for its non-invasiveness, water-transparency, and capability of real-time detection of chemical dynamics.

In our research, we fabricated PDDA-capped Ag bumpy nanoshell (AgNS@PDDA) and introduced it into plants by infiltrating through stoma [1]. Infiltrated AgNS@PDDA were found to be localized in intercellular space. Thanks to the plasmonic properties of AgNS@PDDA in near-IR window, it was possible to evade chlorophyll's strong autofluorescence and obtain SERS signals. PDDA polymer effectively attracted signalling molecules most of which are negative conjugate base form by coulombic interaction. Since the interaction between the polymer chain and analytes was reversible, AgNS@PDDA was found to be effective to monitor the change of the concentration of signalling molecules in real time. Also, for quantitative analysis in complicate mixture, we made mathematical models to calculate the concentration of each component from the intensities of SERS signals.

Then, we studied the plant's reaction against biotic and abiotic stress with SERS signal monitoring. When plants leaf was physically wounded, nasturlexin B, one of the phytoalexin, and eATP were detected and those molecules were successfully to monitor the change of concentration in real time for 1 hour after the wound occurred. In addition, glutathione could be detected in watercress exposed to non-freezing cold stress. Meanwhile, biotic stress was studied for the plants infected with fungal disease. *F. Graminearum* was cultured and injected into the plant, and the emerging of signaling molecules in the plant was monitored according to the elapsed days after injection. Even only 2 hours after the fungi injection, when no lesion was detectable, SERS signals of eATP and salicylic acid related to systemic acquired resistance (SAR) were successfully detected.

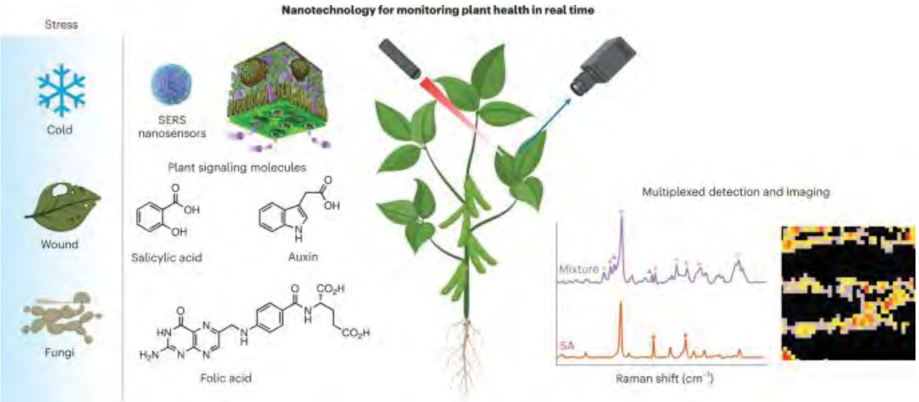

Figure 1: Illustration of SERS nanosensor for plant monitoring

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In operando Raman spectroscopy correlation analysis of electronic and structural changes during homogeneous and heterogeneous catalytic activity

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Renewable energy continues to be a futuristic and sustainable option amidst the high demand for energy resources. In this study we are investigating in operando photo-catalytic activity for homo- and heterogeneous systems via Raman spectroscopy. The first system in our investigation builds upon prior research on dynamic irradiation's impact on water oxidation catalysis, aiming to uncover barriers in artificial photosynthesis and mitigate deactivation pathways [1]. Presently, we delve deeper into the influences of dynamic irradiation and its effects on photosensitizer degradation, environmental influences, system stability, and optimization. In our second system we are probing into the matrix of electro-spun fibers that can be used for HER (Hydrogen evolution reaction). Since Raman spectroscopy is non-invasive and requires little to no sample preparation, we were able to study the composition of the fibers in their original state. Together these findings are presented, and the capability of both systems as photocatalytic systems discussed.

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Polydopamine functional surfaces for SERS monitoring of organic pollutants and food contamination

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According to World Health Organization (WHO) unsafe food causes more than 200 different diseases. Food contamination can result from pollution in water, soil or air, as well as unsafe food storage and processing [1].

Today, food safety is mainly assessed by liquid chromatography and microbiology methods [2] but most of these methods require pre-treatment steps and long times of analysis. Recently, surface-enhanced Raman scattering (SERS) has gained importance because it is fast, reliable, and simple to perform, and, in most cases, requires minimum sample pre-treatment. However, rigid substrates based on deposited noble metal nanoparticles (NPs) on glass or silicon are not suitable for sampling and detecting contaminants and pollutants on solid matrices. On the other hand, the development of flexible SERS substrates can be a promising strategy: they can adhere to curved surfaces providing *in-situ* detection and can be combined with portable Raman devices to provide a reliable tool for on-field food safety monitoring [3, 4].

Here we will present the preliminary results of a project [5] aimed at developing a new class of flexible SERS substrates for a rapid and ultrasensitive detection of different types of pollutants and pesticides. All these SERS-active materials are based on polydopamine (PDA), an emerging nature-inspired biopolymer that possesses outstanding properties, including self-assembly and universal adhesion [6]. PDA has been combined with both plasmonic and non-plasmonic SERS substrates, in order to develop optimized flexible SERS substrates, which could extend their application to other analytical targets, like emerging pollutants, micro- and nano-plastics and endocrine interferents.

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Evaluation of Osteogenic Differentiation Using Extended-focus Raman Imaging

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In periodontitis treatments, periodontal ligament stem cells (PDLSCs)-based therapy has emerged as a promising alternative to regenerate damaged periodontal tissue completely [1]. However, the challenges including the heterogeneity of cells and the time-consuming, destructive evaluation method of cell osteogenic ability hinder the application of this method [2-4]. To investigate potential discrepancy between the cells with different osteogenic abilities, we used non-invasive Raman microscopy to monitor the osteogenic differentiation of PDLSCs with low and high osteogenic abilities and applied multivariate analysis including principal component analysis (PCA) and K-means clustering analysis (KCA) to the acquired Raman spectra. PCA and KCA results show that the spectra could be clustered into different groups that highly correlate with the osteogenic differentiation time. This indicates that each group may correspond to a different stage of differentiation and the cells with two osteogenic phenotypes may have a similar differentiation progress. Nevertheless, some separation trends were observed in the PCA results for each measurement day, indicating the presence of identifiable spectral differences between two phenotypes in the early differentiation process. These findings highlighted the potentiality of Raman spectroscopy combined with multivariate analysis methods for non-destructive cell screening at an early stage of osteogenic differentiation.

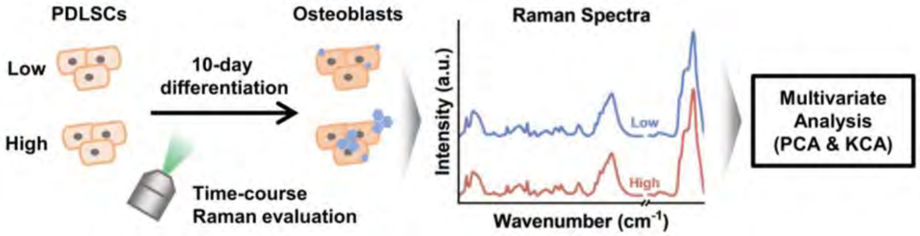

Figure 1: Evaluation of osteogenic differentiation of mice-derived PDLSCs with low and high osteogenic abilities using Raman spectroscopic imaging coupled with multivariate analysis methods.

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Metal-organic frameworks as sensors for CO_2 from water

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Metal-organic frameworks (MOFs) are a versatile class of crystalline materials formed by inorganic building units that are linked to organic molecules via strong bonds. Their large structural variety leads to widespread applications [1], including gas separation, and storage leveraging their remarkable porosity and tunable pore size. While the influence of the crystal structure of MOF crystals is well understood, the role of particle morphology in shaping their properties remains challenging to elucidate. Conventional characterization methods provide bulk-information, omitting the single-particle level. High-resolution imaging and diffraction techniques like SEM, provide single-particle resolution but are limited in throughput and are often invasive, hindering in situ studies.

To address this gap, we developed a multimodal optical spectroscopy and imaging correlation analysis called MOSAIC [2] for studying the heterogeneity of the framework. MOSAIC enables correlative, label-free analysis of MOF in situ, and combines nonlinear microscopy with fluorescence lifetime microscopy (FLIM) and spontaneous Raman scattering spectroscopy in one imaging system to investigate the topologies, morphology and local defects in particles. Spontaneous Raman spectroscopy, in combination with Four-wave mixing and coherent Raman imaging localize, quantify, and monitor the uptake and distribution of guest molecules inside the framework. In this contribution, we present our developed imaging system MOSAIC and its application [3] for CO_2 sensing and removal from water by various MOFs, including UiO-66, and JUK-8.

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Mode optimized Tip-enhanced Raman Scattering

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The quest for increased sensitivity and resolution in nanoscale spectroscopy has driven the development of tip-enhanced Raman spectroscopy (TERS). One potential limitation for the signal enhancement is suboptimal tip-near-field coupling and local environmental perturbations. We address these issues applying adaptive modal optimization (MO), loosely following the idea of Lee et al. [1]. Our integrated system combines scattering-type scanning near-field optical microscopy (s-SNOM) [2] with vectorial adaptive optics [3] and computer-generated holography to optimize the longitudinal electric field (E_z) at the apex of a TERS tip. This results in a significant increase in signal intensity. Both s-SNOM and TERS signals are enhanced considerably. Notably, the optimization can be achieved with our setup in ~5 minutes. The improved speed and signal strength promise broader applicability under ambient conditions, opening new avenues for nanoscale materials characterization [4].

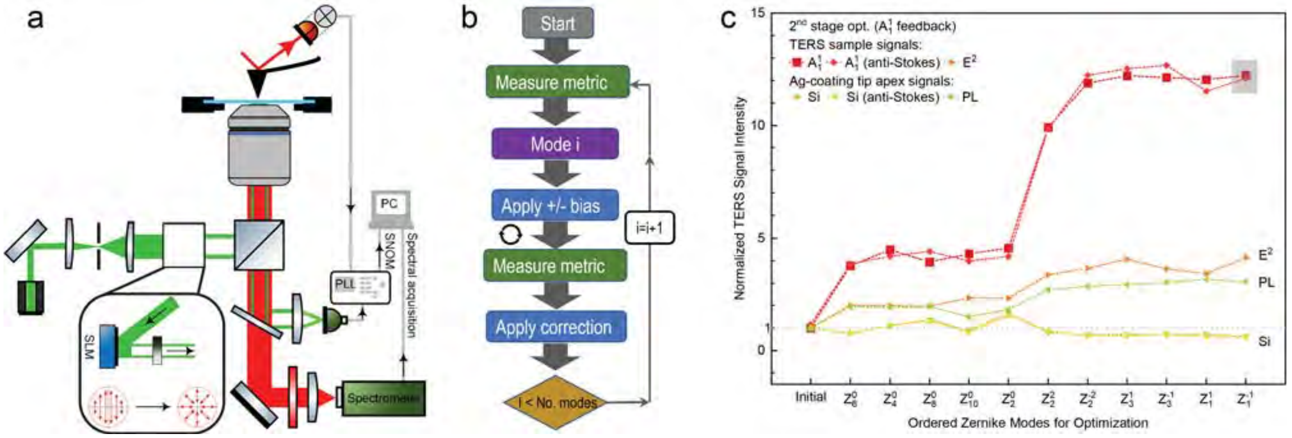

Figure 1: Adaptive MO-TERS System Designed to Optimize Near-field E_z Intensity and Maximize TERS Signal. (a) MO-TERS system. (b) Adaptive mode optimization procedure (c) Corresponding TERS signal evolution.

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Controlling the Surface in Surface-enhanced Raman Spectroscopy

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Within surface-enhanced Raman spectroscopy considerable emphasis is typically placed on measuring, understanding and optimising the plasmonic properties of the enhancing substrate. However, even simple metal colloids typically provide excellent plasmonic enhancement and in experiments where SERS signals are weak or undetectable it is normally the interaction between the molecular target and the surface of the substrate which has failed. For this reason, we have used SERS to understand the chemical nature of the active surface and then used that to inform and guide the development of methods for creating enhancing substrates with high affinity for target molecules.[1] Typically these are based on metal nanoparticles which have been assembled into larger structures using methods which avoid fouling the active surface. This creates materials with exposed surfaces which are free to interact with molecular targets and can be utilised both for SERS sensing and as plasmonic catalysts.[2] This presentation will discuss both the preparation of these materials and examples of how surface chemistry is key to detection of compounds such as PAHs which we have shown bind strongly to appropriate substrates despite the widespread belief that they have poor adsorption onto coinage metals under ambient conditions.[3]

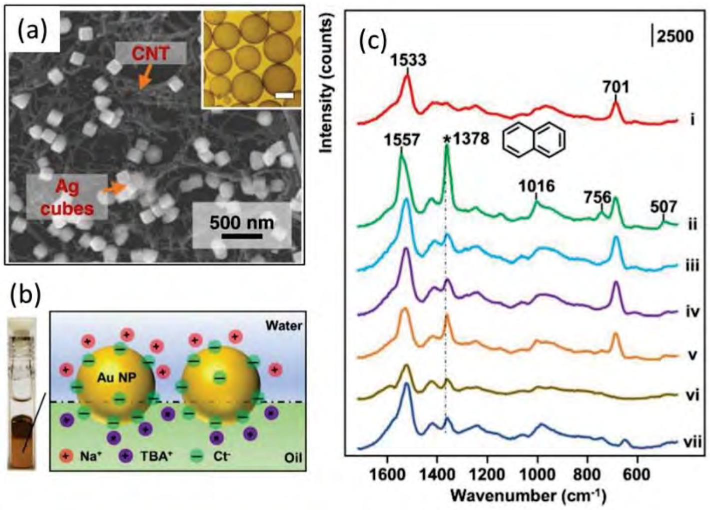

Figure 1: (a) SEM of a Pickering emulsion (optical image inset) stabilised with carbon nanotubes and functionalised with exposed Ag nanocubes.[1] (b) Illustration of the formation of a metal-like liquid film (MeLLF) with exposed nanoparticles at an oil/water interface.[3] (c) SERS spectra of naphthalene recorded at a range of concentrations using a Au MeLLF.[3]

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CHEMICAL ANALYSIS OF ROCK PIGMENTS FROM SITE PONTA DA SERRA NEGRA IN THE SETE CIDADES NATIONAL PARK, PIAUÍ

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1: Federal University of Maranhão, Brazil; 2: Federal University of Piaui, Brazil

The archaeological site Ponta da Serra Negra is an important cultural heritage of rock art located in the Sete Cidades National Park (Piauí - Brazil)[1]. The present work aims at the chemical analysis of clays used as pigments in rock arts using analytical techniques such as portable X-Ray Fluorescence (pXRF)[2] and Raman spectroscopy[3]. The results revealed that the main substances responsible for color in the pigments were: hematite (red in different shades), goethite (yellow) and amorphous carbon (black). Also, the presence of saline efflorescence and substances from the metabolism of lichens and bacteria show that some paintings exhibit traces of natural degradation. Portable X-Ray fluorescence, in combination with Raman spectroscopy, proved to be important non-destructive analytical tools in the study and conservation of rock art, as they allow for the identification of pigment materials and degradation processes without causing any damage to these invaluable cultural heritage items..

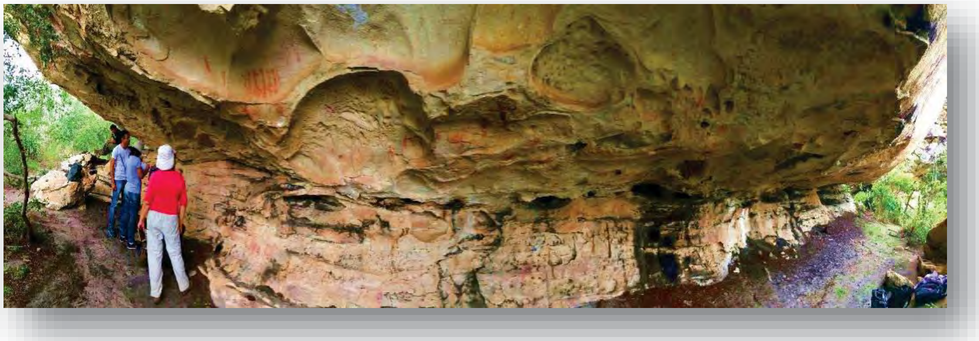

Figure 1: Pinturas Ruprestes no sob em rochas de constituição arenítica no Sítio da Serra Negra, Piauí, Brasil

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High Throughput Multimodal System for Rapid Cell Analysis

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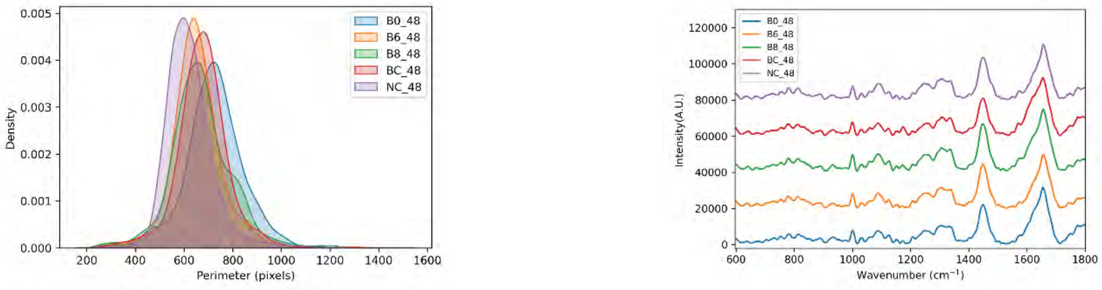
The investigation of infectious diseases at the cellular level is inherently complex, necessitating innovative approaches for comprehensive analysis [1]. We present the development of a fully automated multimodal optical system integrating phase-contrast, fluorescence and brightfield microscopy with Raman spectroscopy, which offers a versatile platform for single-cell analysis. Controlled by a custom LabVIEW program, it enables efficient imaging and spectroscopic data collection through advanced image processing techniques and save acquisition time compared to traditional Raman systems.

The integration of multiple modalities facilitates non-invasive examination and real-time tracking of cellular physiological processes. Fluorescence microscopy provides molecular-specific information, while phase-contrast microscopy enhances contrast for internal cellular structures. Raman spectroscopy offers compositional insights, with the potential to detect specific molecular changes through changes in refractility [2].

Initial experiments with Baculovirus-infected cells reveal distinct differences in cellular features between infected and non-infected cells (figure 1). Moreover, production of fluorescent proteins during the infection process correlates with changes in the intensity of protein bands in Raman spectra.

In conclusion, this multimodal high-throughput system holds significant promise for advancing research and clinical studies in infectious diseases by providing a holistic understanding of cellular processes at the single-cell level.



(a)

(b)

Figure 1: (a) Comparative analysis of cell batches based on perimeters reveals increase in cell sizes following infection. (b) Mean Raman spectra unveil molecular changes pre- and post-infection across all batches.

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Dyed Hair and Swimming Pools: The Influence of Chlorinated Agitated Water on Surface-Enhanced Raman Spectroscopic Analysis of Dyes on Hair

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Chlorine, commonly found in pools and tap water, presents an intriguing concern in forensic hair analysis due to its sources and composition. Current forensic analysis involves optical microscopy which is subjected to advanced training where even multiple experts can deliver opposing conclusions for the same hair sample. Despite challenges in traditional analysis methods, emerging techniques like surface-enhanced Raman spectroscopy (SERS) offer promising solutions, showcasing success even in harsh environments like prolonged sunlight or stagnant water immersion. We used four different dyes, two permanent and two semi-permanent, to dye hair from the same individual and place it into chlorinated and non-chlorinated agitated water simulating regulated swimming pool conditions for up to 8 weeks. A small amount (5 μL) of gold nanoparticles were applied to one inch of hair collected after sampling and scanned using a TE-2000U Nikon inverted confocal microscope, equipped with a 20x objective. Scattered light was collected using the same magnification and directed using a 50/50 beam splitter into an IsoPlane-320 spectrometer (Princeton Instruments) equipped with a 600 groove/mm grating. All spectra were baseline-corrected and normalized before processing using partial least squares discriminant analysis.

Integrating AFM and Raman Spectroscopy to study facet specific properties: the case of quercetin-dimethylformamide crystals

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The application of Raman spectroscopy in the identification and evaluation of flavonoids has attracted considerable interest in a variety of fields, such as medicinal chemistry, food science, and pharmaceutics[1]. However, accurate spectral interpretation necessitates a thorough comprehension of distinctive vibrational peaks and how intermolecular interactions modify them. In this work, the Raman spectra of quercetin-dimethylformamide (QDMF), a solvate of quercetin, are investigated using a comparative analysis of spectra obtained from distinct facets of QDMF single crystals. The current approach combines computational and experimental techniques, to investigate the relationship between crystallographic structure and surface properties of crystals. The primary aim was to validate the surface analysis in CSD-Particle (Mercury[2] software from CCDC) with the Atomic Force Microscopy (AFM), thereby providing insights into topographical features, hydrophilicity/hydrophobicity, and roughness. Then, the integration of AFM with Raman spectroscopy is used because it facilitates a detailed examination of the chemical properties exhibited by various facets, particularly elucidating the dynamics of the time-dependent desolvation process[3] inherent to QDMF.

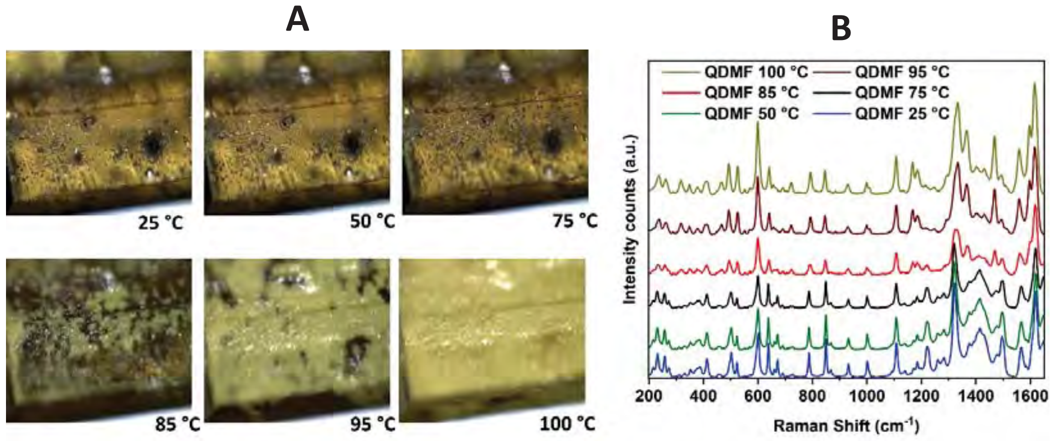

Figure 1: Desolvation process of quercetin in dimethylformamide. (A) Optical images from 25°C to 100°C. (B) Raman spectra at different temperatures.

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Raman Spectroscopic Investigation of Dormancy in *Myxococcus xanthus*

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The ability of bacteria to withstand harsh conditions has become a focal point of extensive research across healthcare, food safety, and biotechnology **[1]**. We have used Raman spectroscopy to study the biochemistry of spores, which is a highly resistant microbial state **[2]**. We exposed the vegetative cells of Myxococcus xanthus, a gram-negative bacterium found in soil, to a nutrient-deficient environment to induce starvation **[3]**. The Raman spectra obtained from cells/spores at various stages of sporulation process exhibited distinctive Raman spectral features. Univariate analysis revealed a decrease in nucleic acids and proteins, indicating a slowdown in bacterial metabolism, while lipids showed an initial increase up to 24 hours, followed by a subsequent reduction in intensity. The germination efficiencies of the spores displayed a robust correlation with specific Raman spectral peaks, and the values predicted through Partial Least Square Regression strongly matched the measured values. Using Raman spectroscopy, we were able to quickly and non-invasively detect bacterial spores, and quantify specific spectral markers associated with sporulation. Additionally, we showcased the capability of Raman spectroscopy in assessing the germination potential of these spores.

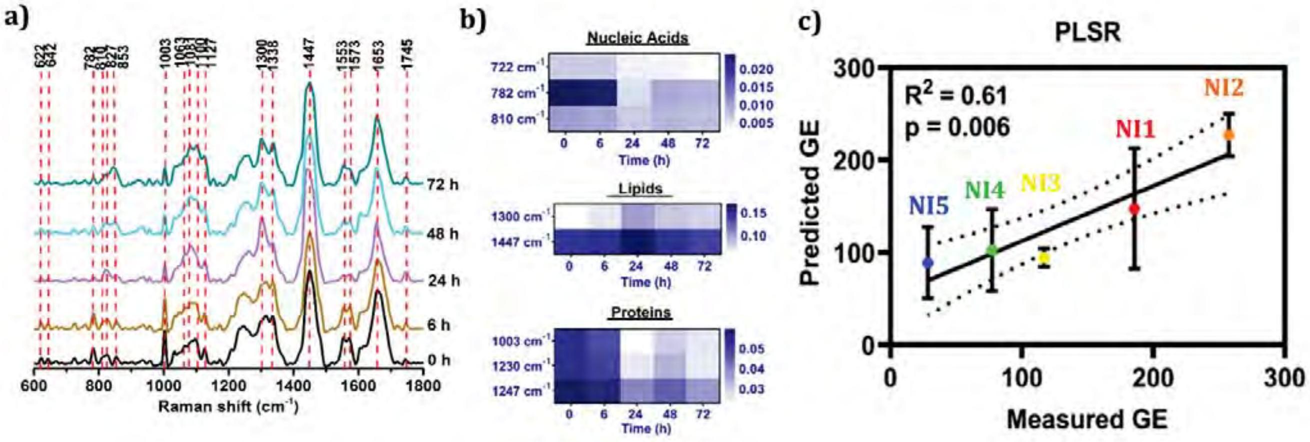

Figure 1: a) Mean ± S.D Raman spectra from cells and spores at 0, 6, 24, 48, and 72 h during sporulation b) Heat map showing changes in nucleic acids, lipids and proteins during sporulation c) Correlation between predicted and measured germination efficiencies of natural isolates

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Temperature-dependent Resonance Raman spectroscopy of polymeric semiconductors

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Temperature-dependent absorption measurements of thin films of organic semiconductors universally show a slight red shift as the temperature is decreased towards cryogenic values, usually interpreted as increase in polymer planarity and consequently conjugation length. However, structural data do not usually accompany such measurements. Here, we employ temperature-dependent Resonance Raman spectroscopy (RR) to develop a basic understanding of how temperature affects the conformation of polythiophene films and thus its photophysical properties. We look at the effect of temperature on thin films of P3HT (a) with differences in solvent processing, producing either more H- or more J-like photophysical aggregates, (b) when blending with high dielectric insulating polymers, or (c) when the alkyl side chains are replaced by hydrogen-bonding ones. We find, in all cases, contrary to the interpretation of the temperature-dependent absorption spectra, increase in the polymer backbone torsional disorder when cooling, attested by upshifts in the thiophene C=C symmetric stretch and decrease in the ratio between the ordered and disordered population in the film. Association of the upshift of the vibrational bands with reduced anharmonicity at lower temperatures is discounted from observation of the C=C overtone bands with temperature. Interestingly, following the frequency shift and the relative intensities as a function of temperature we observe a step change in the trends at specific temperatures that could be interpreted as a phase transition. This could be associated with a structural change due to side chain mobility that leads to modification of the excitonic coupling. These results suggest that interpretation of the optical spectra is not as simple as described in the literature so far, but a combination of the structural information obtained by RR and excitonic models for photophysical aggregates are necessary to understand the optical properties.

Investigation of Tablet Coating Discoloration using Raman Spectroscopy and Chemometrics

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Coating of tablets plays a pivotal role in the pharmaceutical industry, elevating the quality of solid dosage forms, especially those intended for oral administration [1]. This process not only enables controlled drug release but also shields active pharmaceutical ingredients from environmental elements, preserving their stability during storage and transportation [2]. However, tablet discoloration remains a significant challenge, impacting visual appeal, patient confidence, and medication effectiveness. In this study, we investigate tablet discoloration during drug product stability testing of a tablet using vibrational spectroscopy and chemometrics. Here, we have shown that the degradation of PEG polymer, in the coating material of drug product which occurred during stability was responsible for tablet surface discoloration. Analysis of the tablet's surface by Raman microscopy with subsequent chemometrics helped to extract valuable insights from the spectroscopic data, linking spectral information from tablet composition to tablet discoloration. This integrated approach holds promise for understanding the root causes of tablet discoloration and provides a foundation for developing targeted strategies to prevent or alleviate this issue in future pharmaceutical formulations.

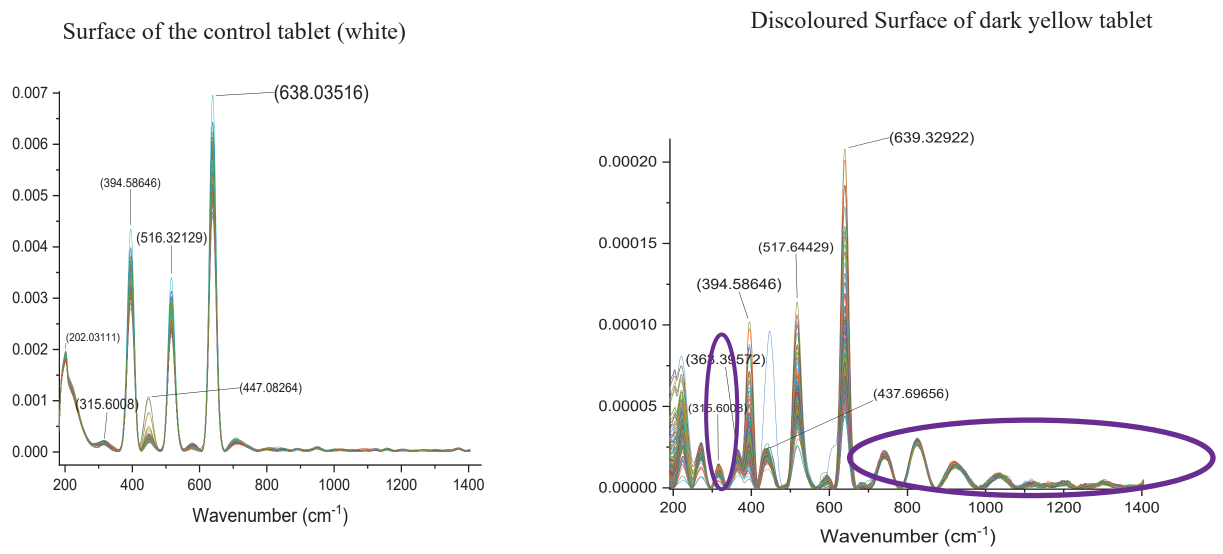

Figure 1: Raman spectra extracted from the PCA map for the control region in comparison to the dark yellow region. The primary distinctions in the spectra are indicated by encircled regions, highlighting the key differences between the two datasets.

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Simultaneous combination of electrochemistry and SERS effect for the fast detection of fentanyl

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Fentanyl is a synthetic opioid 50 times stronger than heroin and 100 times stronger than morphine. Although pharmaceutical fentanyl is prescribed by doctors to treat severe pain, especially after surgery and for advanced-stage cancer, the illicit fentanyl and its analogues drive overdose deaths to record highs in recent years. For that reason, the development of new and fast methods for the detection of this drug is a challenging topic in the scientific community [1].

Over the last years, combination of electrochemistry (EC) and surface-enhanced Raman scattering (SERS) has received growing attention due to the enhancement of Raman intensity associated with EC-SERS effect. However, the generation of reproducible SERS substrates is a traditional limitation of this effect. In that way, activation of metallic screen-printed electrodes (SPEs) by electrochemical routes allows to obtain reproducible nanostructures with excellent SERS properties [2], opening new gates for analytical applications.

In this work, the development of a fentanyl detection method based on SERS effect is achieved. Spectroelectrochemical screening of this drug with metallic SPEs consists of two consecutive steps performed in a single experiment: (1) electrochemical generation of metallic NPs with SERS effect and (2) Raman detection of fentanyl present in solution (Figure 1). Metallic SPEs provide interesting results being not only useful in the characterization of fentanyl, but also for analytical purposes. Calibration curve obtained with gold shows linear behaviour from 0.33 ug/ml to 3.37 ug/ml fentanyl, demonstrating the potentiality of this method.

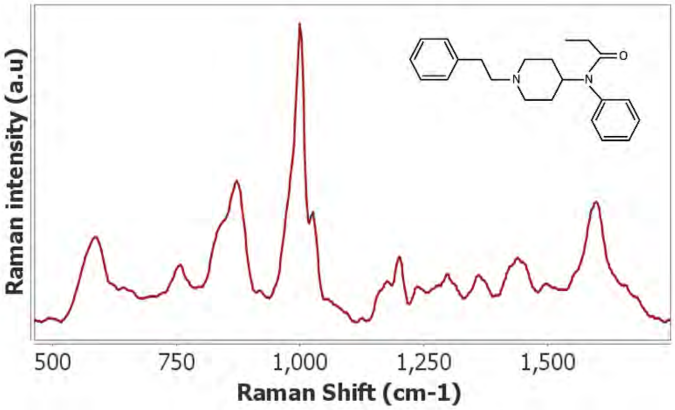

Figure 1: Chemical structure (inset) and SERS spectrum of fentanyl obtained with gold SPEs.

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Multimodal Raman, Photoluminescence & SHG Imaging of 2D Materials

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Transition metal dichalcogenides (TMDCs) are exciting next-generation 2D semiconductors with versatile layer-dependent optoelectronic properties. The spatial characterisation of lab-grown TMDCs is crucial to understanding their unique optoelectronic properties and optimising film growth conditions. In this work, we present a multimodal imaging platform that combines optical widefield, Raman, photoluminescence and second harmonic generation imaging for the characterisation of 2D materials.

The imaging platform was used to characterise CVD grown WSe2 crystals. Using Raman imaging, the presence of both monolayer and multilayer WSe2 could be identified through a change in the intensity and peak position of the E12g / A1g phonon modes. Photoluminescence imaging confirmed the presence of monolayer WSe2 with exciton emission at 780 nm, and identified two distinct multilayer regions in the centre of the crystal through changes in the photoluminescence wavelength and intensity. Finally, second harmonic generation imaging with femtosecond laser excitation was used to obtain the relative layer orientation of the multilayer domains in the WSe2 flake.

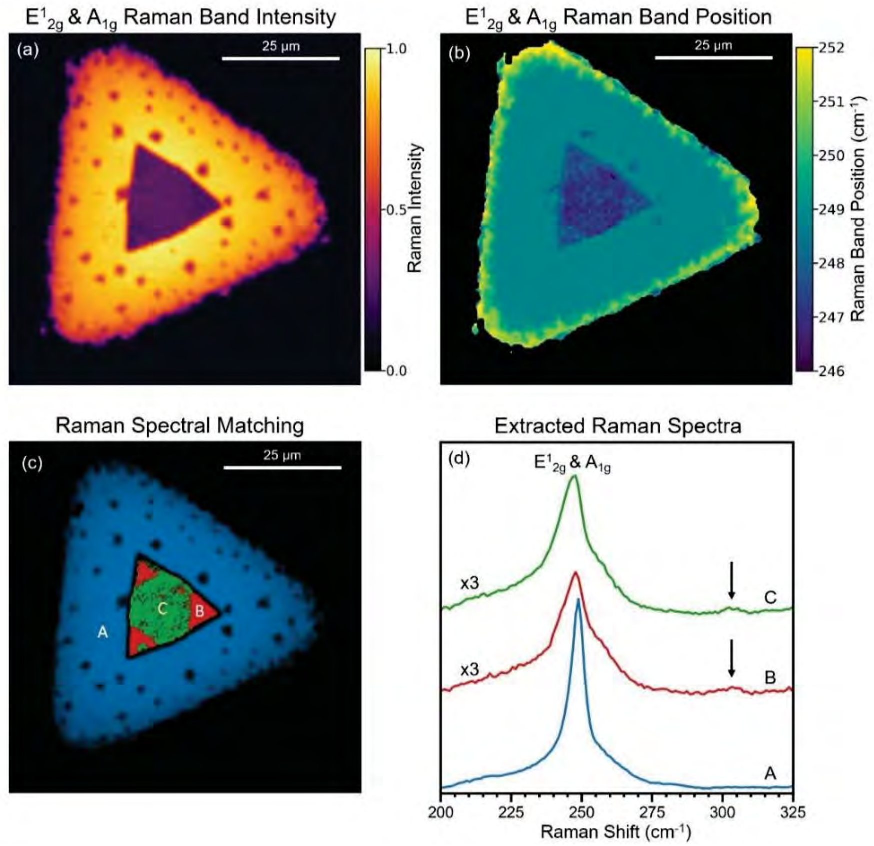

Figure 1: Raman imaging of WSe2. (a) Intensity of the E12g / A1g (250 cm-1) Raman band, (b) Peak position of the E12g / A1g (250 cm-1) Raman band, (c) least squares spectral matching revealing three distinct Raman spectral areas, (d) Averaged Raman spectra from areas A, B and C.

Monitoring cell dynamics via 3D SERS imaging using biocompatible SERS tags

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Cell-cell communication via extracellular vesicles (EVs), such as exosomes, plays an important role in normal biological functions and those associated with disease such as metastatic cancers.[1] Whilst much focus has been given to developing analytical methods to separate and analyse their molecular cargo, we know relatively little about doner - recipient cell EV dynamics. In this context, to better understand the role of exosomes in tumor cell dynamics, we explored to use of surface-enhanced Raman scattering (SERS) imaging using gold nanoparticles labelled with Raman-active molecules to develop a library of different SERS tags.[2,3] In order to achieve cell-specific labelling, we developed antibody labelled SERS tags (AB-tags), capable of recognizing cell surface molecules, also present in EVs, thus allowing us to follow cell dynamics and EV-based cell communication.

Our initial findings suggested that whilst live cell labelling and imaging using SERS mapping was indeed possible, observation of EVs using SERS was impossible due to the large volume of media surrounding the cells, effectively diluting out any AB-tagged EVs. To overcome this, we explored the use of microdroplets to encapsulate cells, thus confining cells, EVs and AB-tags in a small volume. Using human breast cancer cells (MCF-7) and human fibroblasts (HDF) as our model cell-cell communication system, we report on the potential of microdroplets to study cell communication using 3D SERS mapping in real-time and in a non-invasive manner. This strategy offers potential to study the effects of cell heterogeneity in disease models, including therapeutic cell targeting strategies.

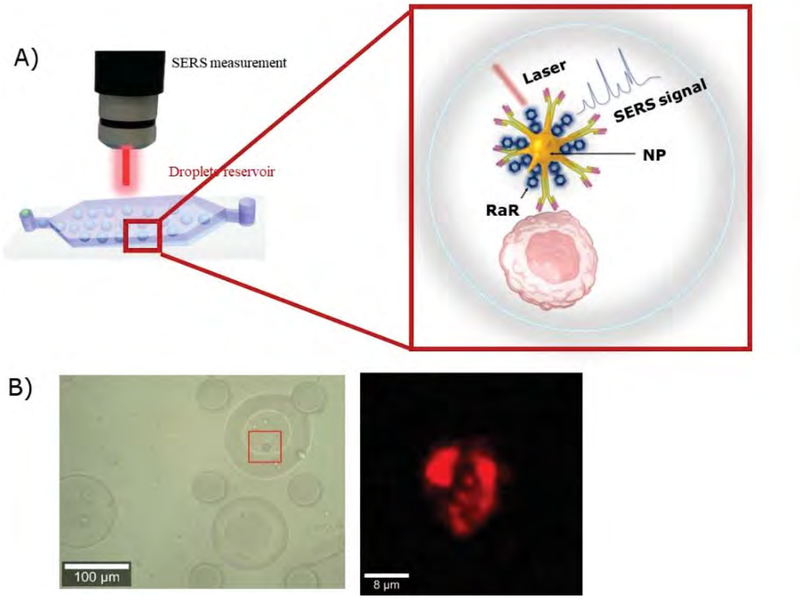

Figure 1: A) Schematic representation of the SERS-based microdroplets system to monitor cell communication in real time. B) Optical image (left) and SERS map (right) of a single cell inside microdroplets.

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Fuel cell characterization by Raman spectroscopy: from research to manufacturing.

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With the global energetic transition and the move from thermic to electric cars, the improvement of battery becomes crucial. One of the energetic vectors highlighted as key for the future of mobility is H_2. Consequently, the investments in H_2 and battery improvements become essentials for key players and governments. Fuell cells, i.e. batteries working with H_2, require so adapted analytical techniques to understand the physical and chemical processes occurring at the electrodes and electrolyte levels. Moreover, H_2 is a costly fuel. Its consumption must be optimized and controlled.

During this talk, we will present why Raman spectroscopy is an ideal method for Fuel cells and H_2 as it provides physical and chemical properties of materials in a non-invasive approach. More specifically we will demonstrate why Raman is the perfect method for process optimization and H_2 gas consumption during Fuel cell stack characterization, especially thanks to its ability to work in a wet environment.

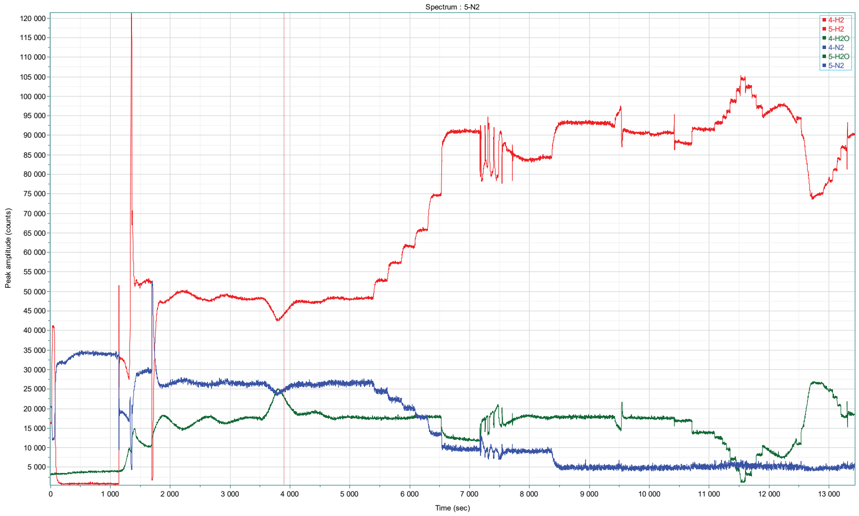

Figure 1: Fuel Cell activation process - Gas concentration monitoring – Red: H_2, Blue: N_2, Green: H_2O

Transmission Raman Spectroscopy: A New Alternative to Enhance Process Raman for Biopharmaceuticals

Jeppe Hagedorn[a,b], Lars Poulsen[c], Pernille Kjærgaard Qwist[a], Erik Skibsted[a] & Martin A.B. Hedegaard[b]
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In this work we present our efforts into developing a novel Raman collection setup, capable of operating in both backscatter and transmission modes. We utilize this to assess the viability of Transmission Raman Spectroscopy (TRS) as an alternative to the current commercial Process Raman Systems for the analysis of upstream samples from various biopharmaceutical processes, such as yeast and CHO cells [1]. TRS offers certain advantages over traditional Raman spectroscopy for the analysis of turbid liquid samples, like those encountered in cell cultures. Effects such as reducing background effects and probing a much larger sample area are the major selling points of TRS. Moreover, we assess the spectrometer's capability to minimize fluorescence, background noise and enhance the effective signal-to-noise ratio — key factors in improving measurement accuracy.

With this novel collection design we can test and compare the merits of both modes in monitoring critical quality attributes (CQAs) of biopharmaceutical samples in several process points. We will present examples of how both modes perform when faced with increasing fluorescence backgrounds found in high cell density cultivations for CQAs such as titer, cell density, glucose, and lactate. Additionally, we delve into data analysis strategies incorporating advanced machine learning algorithms, aiming to refine the predictive accuracy of CQAs in upstream bioprocessing.

Overall, our work highlights the potential for new modes of measurement which can enable next generation process monitoring of highly efficient cell cultures in biopharmaceutical manufacturing.

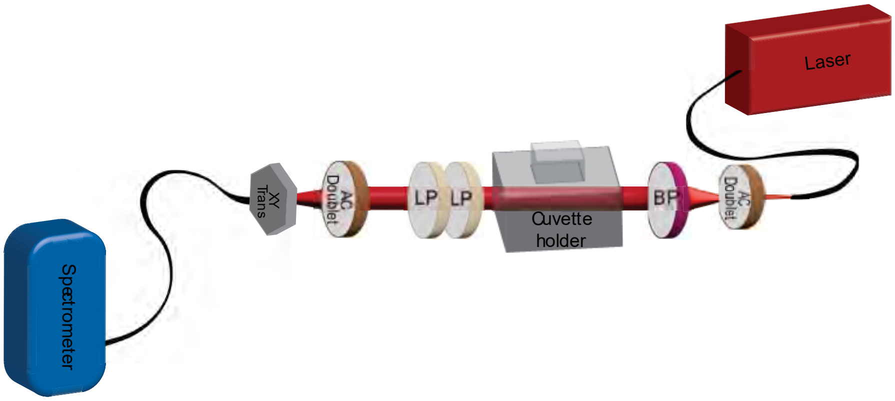

Figure 1: Transmission Raman setup schematized.

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Raman Imaging System for 2D Materials Characterization

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Two-dimensional materials (graphene, transition metal dichalcogenides, etc.) are undoubtedly hot topics in the scientific community. Such materials have applications in photovoltaics, electrodes, transparent sensors, etc. Confocal Raman microscopy is one of the most powerful analytical technique that can reveal a large amount of information on the physical characteristics of 2D materials and their quality. A new SOL instruments Raman microscopy approach [1] (Figure 1) enables easy-of-use solution for 2D materials analysis. The Raman imaging system (Confotec® MR200) is very compact, fully integrated with an optical microscope, has sufficient spectral resolution.

Specially designed Raman confocal system has been applied for micro spectroscopic measurements of various 2D materials.

Figure 1: Confocal Raman microscope Confotec® MR200 (SOL instruments)

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Moving samples: adjusting to the speed of the process line.

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[a]*ELODIZ Ltd*

As a non-contact, non-destructive analytical technique, Raman spectroscopy is increasingly used in process control and analytics. Probe based Raman instruments are a prime design for online, real-time monitoring of processes. Material production processes can employ these systems to perform quality control on different stages of the process line, backed up with chemometric modelling to have accept/reject criteria for their products. However, what may be easily distinguished in the lab on a stationary sample, may become more convoluted when measuring a moving sample online. A demonstration of this issue, and guidance in adjusting for changes in sample speed, will be presented here.

Instant Histology of Fresh Tissue Samples Using a Clinical-Compatible Stimulated Raman Imaging Device

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Histological examination plays a crucial role in cancer diagnosis, traditionally relying on hematoxylin and eosin (H&E) staining. Staining, requiring several labor-intensive steps for tissue preparation, prevents a quick diagnosis and leads to long waiting times in the operating room or the need to repeat surgery. To overcome this problem, we have developed a fully integrated, clinical-compatible stimulated Raman scattering (SRS) imaging device giving access to the complete Raman spectrum during tumor surgeries. Leveraging the advantages of a compact and robust fiber laser, we have integrated the entire microscopy system into a clinical cart, facilitating deployment in diverse clinical environments. The laser provides rapid tunability within milliseconds across a broad spectral range of 700 to 3300 1/cm, covering biomedically relevant resonances in CH-stretch and fingerprint region.

By detecting two Raman vibrations at 2845 and 2930 1/cm, we show how SRS imaging can differentiate cell nuclei and cell bodies, two key features of conventional H&E-based histopathology. We demonstrate excellent agreement between acquired SRS images and classic H&E-stained images of fresh human and mouse samples. Furthermore, our device enables visualization of Raman bands in the SRS fingerprint region, including nuclei acid, phenylalanine, amide, and collagen. As a proof-of-principle Fig. 1 shows a virtually stained SRS image of mouse stomach tissue. The virtual H&E image (left) clearly shows the distribution of the cell nuclei, their size and shape as well as the cellular environment. The complete fingerprint spectrum (right), acquired across 100 µm^2, gives detailed insights into the chemical composition of the sample. This capability offers valuable image contrast for accurate pathological assessment and for discovering new biomarkers.

For a comprehensive examination of larger tissue samples, we have incorporated a high-speed, low-resolution imaging mode to quickly identify cancerous hot-spots and examine general tissue architecture in a field-of-view up to 11 times 7.5 cm, followed by a high-resolution imaging mode for assessing details on cell level.

The clinical compatibility of our integrated SRS imaging device marks a significant advancement, overcoming limitations of bulky, less versatile and slow SRS systems. With real-time histological assessment, our device has the potential to improve the efficiency and accuracy of tumor surgeries, enabling faster decision-making and enhancing surgical outcomes.

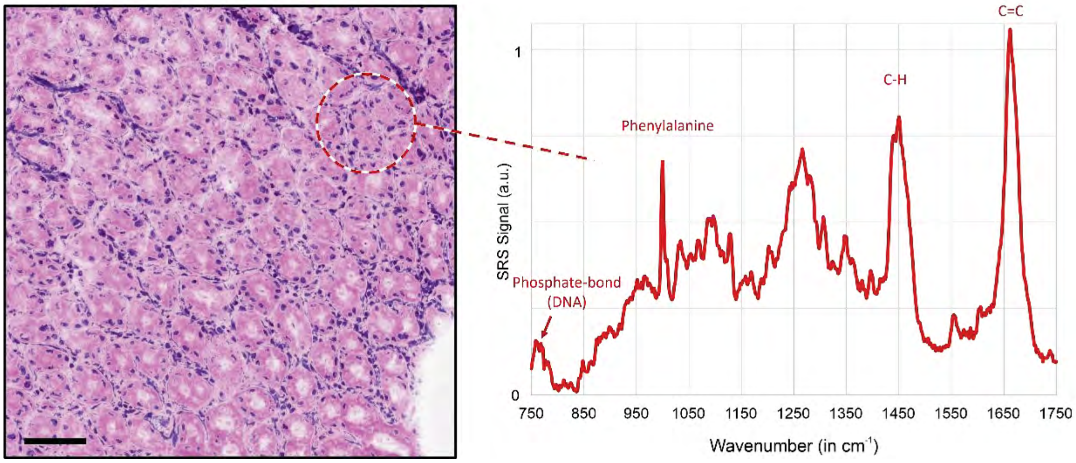

Figure 1: Virtual H&E image of a mouse stomach section, generated by SRS imaging at 2845 and 2930 1/cm. The virtual H&E image (left) shows cell nuclei, their size and shape as well as the cellular environment. The acquired fingerprint spectrum (right) gives detailed insight into the chemical composition. Scale bar: 100µm.

Enhanced Drug Enforcement with Compact Spectrometer Solutions in Forensic Technology

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Narcotics pose a profound challenge for law enforcement and public health sectors worldwide, necessitating advancements in methods to reduce the availability of drugs on the market. Consequently, there is a need for improved forensic technologies. The miniRaman Spectrometer (mR), is an innovative, compact, handheld Raman device, utilizes a patented internal reference channel for constant recalibration. This spectrometer ensures unparalleled accuracy and operational stability with each acquisition. The elimination of manual calibration processes results in a device that is not only compact and light but also significantly more affordable, making it more accessible to the government sector. The spectrometer's ground-breaking approach to optical optimization, including the novel use of real-time spectral deblurring, effectively addresses laser wavelength drifts, enhancing resolution without the need for energy-intensive cooling systems [1]. Additionally, a compact confocal microscope has been developed into which the mR can be inserted. This microscope attachment measures 120 mm x 130 mm x 280 mm (LxWxH) and weighs only 7 kg, making it easily portable for customs and police officers to conduct in-situ trace detection where tiny particles of illegal substances can be traced. The goal is to automate the search for points of interest via the analysis of visible images obtained from the field camera integrated into our microscope. This can be achieved with some AI/ML algorithm, or even classic image-processing approaches. In collaboration with NIRLAB, we are developing a versatile software platform and an extensive spectral library to process spectral data for the identification and minute trace detection of illegal substances.

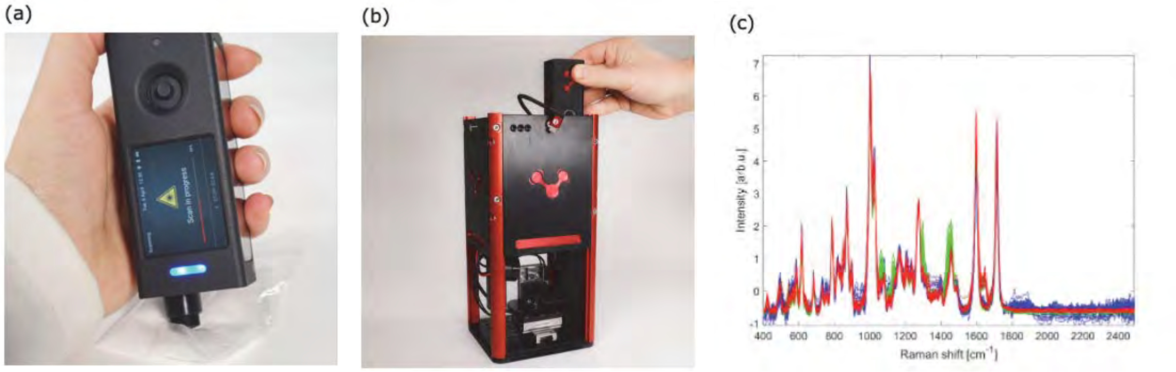

Figure 1: (a) miniRaman in hand measuring powder through plastic bag; (b) miniRaman Microscope; (c) Plastic-subtracted cocaine spectra.

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Towards the Analysis of Nanoplastics with Optical-Photothermal Infrared Spectroscopy

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The continual use of plastic materials has led to a globally widespread occurrence of micro- (1 µm to 1 mm) and nanoplastics (<1 µm) in the environment and consumer products. The risk posed by these particles depends on plastic type, morphology, and especially size. Nanoplastics are, therefore, expected to be more dangerous because of their ability to breach biological barriers and enhanced capacity to transport absorbed toxic compounds due to their smaller size and extensive surface-to-volume ratio [1]. This threat highlights the necessity for advanced nanoplastic detection methods. While traditional (FT)IR spectroscopy is limited to particles larger than ~10 µm, Raman Microspectroscopy can identify particles down to 1 µm (or even 200-300 nm) but often suffers from (strong) interference of fluorescence [2]. Optical-Photothermal Infrared Spectroscopy (O-PTIR) represents a novel analytical technique with excellent capability to further advance the spectroscopic analysis of nanoplastics. By merging the higher resolution of typical Raman laser wavelengths (e.g., 532 nm) with the chemical specificity of IR wavelengths, O-PTIR can achieve IR spectra at submicron spatial resolution, particularly benefiting the analysis of nanoplastics affected by substantial fluorescence interference. Additionally, this method allows for the simultaneous collection of IR and Raman spectra, offering complementary information and cross-validation between these two techniques. An illustrative example of the simultaneous measurement technique on a ~880 nm polystyrene (PS) particle is depicted in **Figure 1**, confirming O-PTIR´s high potential for the detailed analysis of nanoplastics.

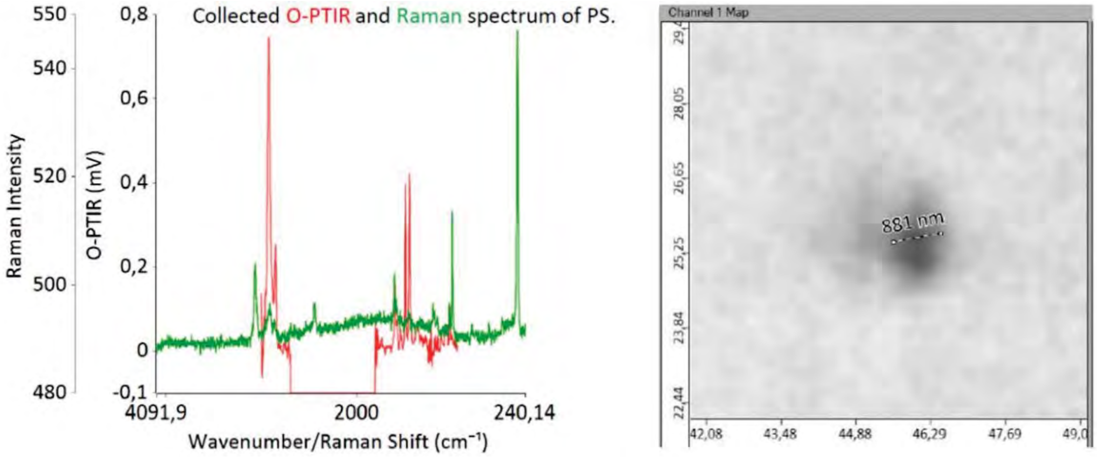

Figure 1: Complementary O-PTIR and Raman spectral overview of a ~880 nm PS particle (left); Corresponding optical image of the analyzed PS particle (right)

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Photon-Counting Raman at MHz Spectral Rate for Biochemical Imaging of Entire Organism

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Raman spectroscopy, strumming the tunes of fine molecular vibrations, holds a crucial position to interpret covalent bonds, chemical compositions, and other molecular dynamics in mixture by reading their vibrational fingerprint signatures. However, over the past decades, Raman encounters longstanding barriers in both sensitivity and speed, limiting its territory extending to broader biochemical applications. Here, we introduce an all-round analytical workhorse, fiber-array Raman engine (termed FIRE). In FIRE, a distinctive fiber array bundle delays the Raman shifts in scale of 3-960 ns, and a highly dynamic single-channel photon-counting detector fulfils spectral measurement outperforming the best commercial confocal Raman. Crucially, FIRE features a major advantage in non-repetitive single-shot spectra measurement at MHz repetition rate with full Raman span (-300-4300 $cm^{-1)}$ covering fingerprint, silent, C-H, and O-H regions, and therefore takes the major step to break the tradeoffs among sensitivity, speed, and spectral span. We demonstrate full Raman spectral imaging of metabolic activity of intact Caenorhabditis elegans. FIRE exhibits superior performances in all aspects of Raman, including fluorescence suppression, and will shine light on a variety of biochemical applications.

Novel approach for biofilm characterization using Raman spectroscopy

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[a]*Ben-Gurion University of the Negev*

Bacterial biofilms are ubiquitous communities in nature, consisting of cells and extracellular matrix. The biofilm matrix is often referred to as the “dark matter” in microbiology. Therefore, it is necessary to design novel approaches for studying it. Here, we developed a new method for characterizing the biofilm matrix using Spontaneous Raman spectroscopy. It allowed for structural study of polysaccharides, lipids, and proteins of biofilm matrix produced by *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Klebsiella pneumoniae*. We found that matrix from each of the bacteria has a unique composition, and Raman spectroscopy is highly effective in discriminating between them. Moreover, we were able to characterize complex multi-species biofilms based on the composition of the biofilm matrix and predict with high accuracy the species composition using ratiometric analysis. By utilizing high-resolution population dynamics tracking with recently developed Tn7 DNA barcoding technique, we will be able to correlate the molecular and species composition in the complex biofilm during its development using Raman spectroscopy.

Dual-mode nanothermometers exploiting Raman scattering and emission in the optical region

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Measuring temperature at the nanoscale is nowadays a hot topic in various technological fields, such as microelectronics and nanomedicine [1]. For instance, the temperature of a living cell can monitor its health, since pathological cells are warmer than normal ones due to their enhanced metabolic activity [2]. Therefore, thermometry represents a powerful diagnostic tool, also useful during hyperthermia treatments to localize heat release and avoid unwanted damage to surrounding healthy tissues [3].

Optical thermometry typically includes fluorescence methods, often based on the temperature dependence of lanthanide emission, but also on Raman methods, in which the sample temperature is determined from the Stokes-antiStokes Raman band ratio.[3]

Two different types of materials have been investigated as potential thermometers:

a) Commercial titania (anatase) as a Raman thermometer.

b) A core-shell system formed by a CaF_2 core doped with Yb^{3+} and Er^{3+}, covered with a titania shell, in which the core acts as an upconversion fluorescence thermometer and the shell as a Raman thermometer (dual mode optical thermometer).

Structural characterizations and the measurement of typical figures of merit for thermometry, i.e., thermal relative sensitivity, have been investigated in both systems above. The thermometer performance has been evaluated in the near-infrared region (NIR), in correspondence of the first biological transparency window, both for fluorescence (owing to the upconversion of the Yb^{3+}/Er^{3+} doped CaF_2 core part) and for Raman scattering. Titania was chosen for its biocompatibility and rather low energy Raman bands. The synthesis and study of hybrid plasmonic – inorganic systems is currently underway, in view of the implementation of surface enhanced Raman scattering (SERS), rather than simple Raman, as a method for temperature measurements [4].

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Forward and Epi-Detected Stimulated Raman Spectra and Images of Two-Dimensional Dielectric Crystals

Hwansoo Jeon and Sunmin Ryu
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Spontaneous Raman spectroscopy of two-dimensional (2D) dielectric crystals is often challenged by weak signals, because they have small Raman cross sections and thicknesses. In this work, we report on a micro stimulated Raman spectroscopy (SRS) and imaging setup for 2D dielectric crystals.[1] The spatial and temporal overlap of ps pump and Stokes beams were optimized by using transient reflectance signals of graphene. The Raman loss in the pump beam was lock-in-detected with a fast photodiode while the Stokes beam was modulated at 20 MHz. SRS spectra were verified with respect to the spontaneous Raman spectra of hexagonal BN and further optimized to improve on the detection limit (~6L of hexagonal BN). SRS signals in a forward-detection mode were ~5 times higher than those for an epi detection. The SRS spectra of 2D hexagonal BN and $Ca(OH)_2$ revealed prominent vibrations at 1366 and 3652 cm^{-1}, respectively. In the epi-detected SRS, unexpected intensity modulations occurred because of optical Kerr effects (OKE). The forward-detected SRS exhibited greatly reduced OKE, because of an order of magnitude higher transmission that enabled the use of lower-power pump and Stokes beam. Raster-scan imaging was also optimized for calcium hydroxide and compared with topographic and optical imaging. We will discuss possible origins of the spectral artifacts and instrumental refinements required to reach the single-layer limit.

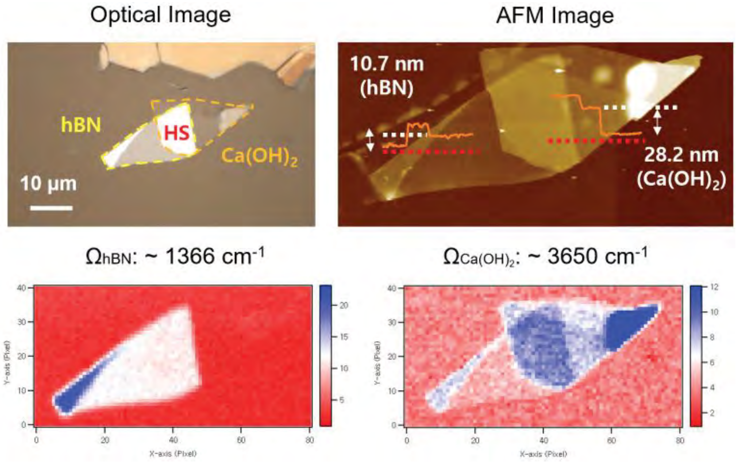

Figure 1: Chemical imaging of hBN/$Ca(OH)_2$ 2D heterostructure using SRS

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Synthesis, characterization and usage of PANI-coated gold nanoparticles as SERS redox potential sensor

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Accurate determination and monitoring of redox potential is a very important issue, especially in biological samples [1]. The combination of electroactive molecules with plasmonic nanostructures allows the redox state of the sample to be determined using electrochemical SERS. This is possible by measuring the ratio of the intensities of the spectral bands of oxidized and reduced forms. Conductive polymers can be good redox markers in such systems due to their high electroactivity and strong Raman spectrum resulting from the presence of aromatic rings.

We used polyaniline-coated gold nanoparticles for this purpose. The ratio of gold nanoparticles to polymer was optimized to avoid aggregation caused by excess PANI suspension. Using cyclic voltammetry, the electrochemical properties of the obtained structures were determined in the pH range 3-8. Electrochemical SERS measurements were carried out in a special three-electrode device.

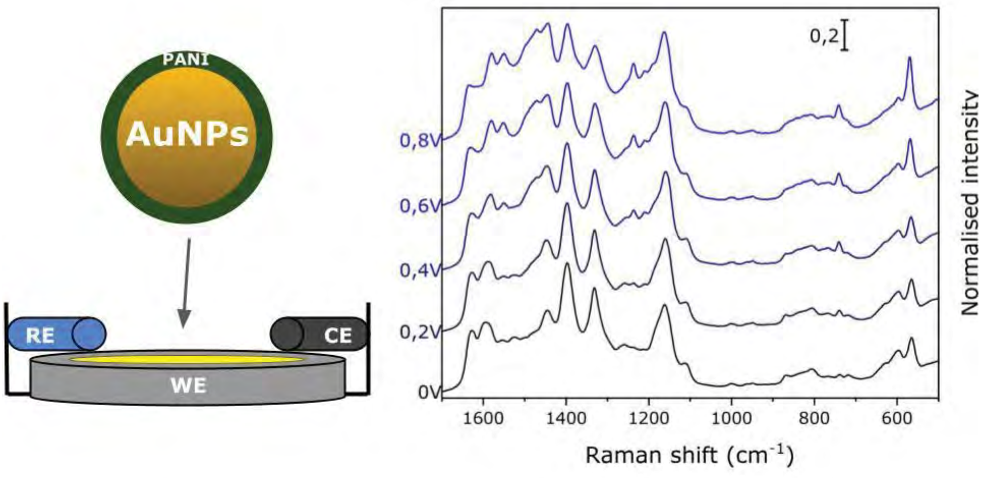

Figure 1: Electrochemical SERS measurement system with collected spectra

We obtained nanostructures with a very strong and repeatable SERS spectrum, the changes of which allow the estimation of the redox potential in the range from 300 to 800 mV. We did not observe a significant impact of pH on the form of the spectrum and calibration curves. Based on Raman spectra, we assume that mainly PANI chains with relatively low molecular weight, which contain phenazines in their structure, were attached to the nanoparticles. Additionally, thanks to the presence of citrates as stabilizers of gold nanoparticles, the pH range in which the sensor operates increases (normally polyaniline is electroactive only in highly acidic pH).

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Raman Spectroscopy and Optoelectronic Properties of Green Dye Doped Nanoparticles Embedded Polymer for Biomedical Applications

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Recently, significant scientific and technological interest has focused on dye-sensitized optical sensor based on polymer-inorganic nanoparticles. Dye-doped polymer nanoparticles are a new category of materials which exhibit good optoelectronic properties. These materials are interesting because of their flexible and transparent nature, and they are easy to fabricate. Therefore, the search alternative materials are needed for building construction applications [1-3]. In this work, nanocomposite thin films developed using green dye extracted from Ficus Carica (FC) doped ZnO nanoparticles embedded into Polyvinyl alcohol (PVA) with varying weight percentage (wt%) of ZnO were prepared. The optoelectronic parameters of the prepared nanocomposite films are presented. The Raman spectroscopy and characterizations of the nanocomposite films were performed and found to be stable in structure. Finally, we show new results regarding the incorporation of nanopartcles into green dye, as well as the performance can be optimized by adjusting the parameters for the nanocomposite films according to different working conditions to meet the requirements. The obtained results showed that the nanocomposite makes it attractive for biomedical applications and could open the way for further and future prospective studies among green dyes with nanomaterial or other dyes in developing optical devices.

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Designing better solar cell materials through experiment and theory

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Our research focuses on the use of experiment and theory to better understand the processes that occur in key components of solar cells, namely the dyes that capture the light. Upon excitation, the dyes having donor and acceptor unit exhibit electronic charge transfer (CT) transitions. Thus, the energy of the CT transitions can be tuned by altering the donor and acceptor moieties.

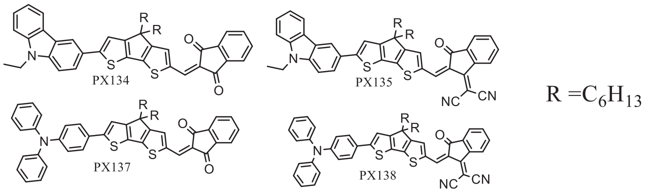

Figure 1: Chemical structure of PX134, PX135, PX137 and PX138.

To understand the effect of donor-acceptor interaction, we are aimed to direct our attention towards a collection of four donor-π-acceptor dyes that have been modified in a systematic manner. The dyes incorporated a triphenylamine (TPA) and carbazole unit as donor connected to a 4,4-dihexyl-4H-cyclopenta[2,1-b:3,4-b'] dithiophene (DCTP) linker. The degree of interaction between the donor and acceptor by selectively attaching them either at the meta- position or the para position of the carbazole to the dithienothiophene π-linker. Tuning of the indane-based acceptor unit can also be achieved by using two variations of electron acceptors, 2-(3-oxo-2-vinyl-2,3-dihydro-1H-inden-1-ylidene)malononitrile (InOCN), and 2-vinyl-1H-indene-1,3(2H)-dione (IndO), altered at both the C1 and C3 positions. These acceptor variations have been investigated previously and used in other dyes and have good electron accepting ability, allowing for fluorescence, solvatochromic behavior, and other interesting photophysical properties.

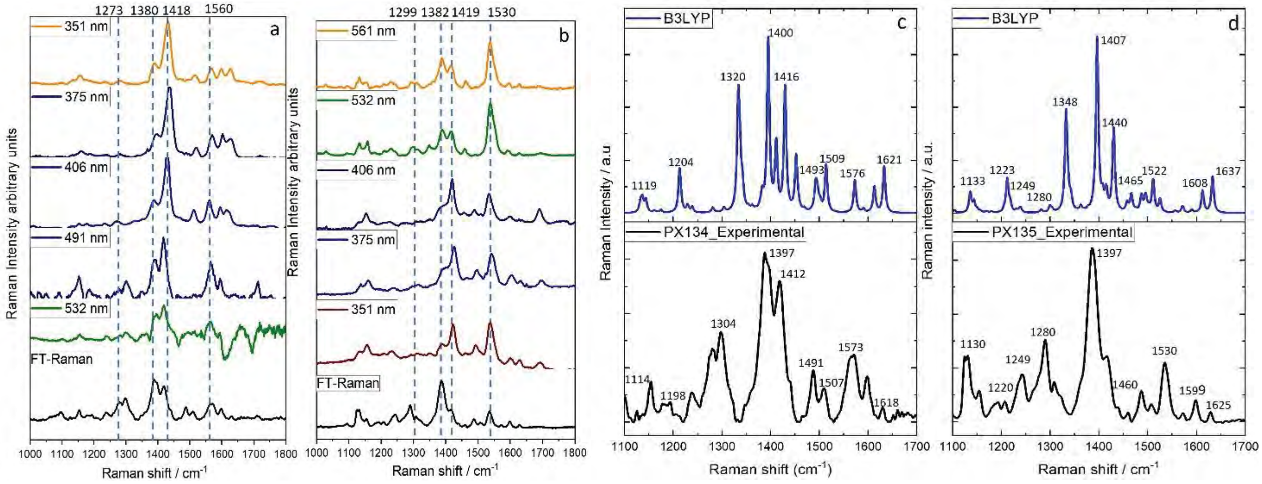

Figure 2: (a)FT Raman (b) resonance Raman (c) and (d) computational and experimental FT Raman of PX134 and PX135. B3LYP WITH 6-31g(d) basis set is used for computational FT Raman calculation.

Raman spectroscopy investigations of electric field-induced structural changes in Lithium Niobate crystals.

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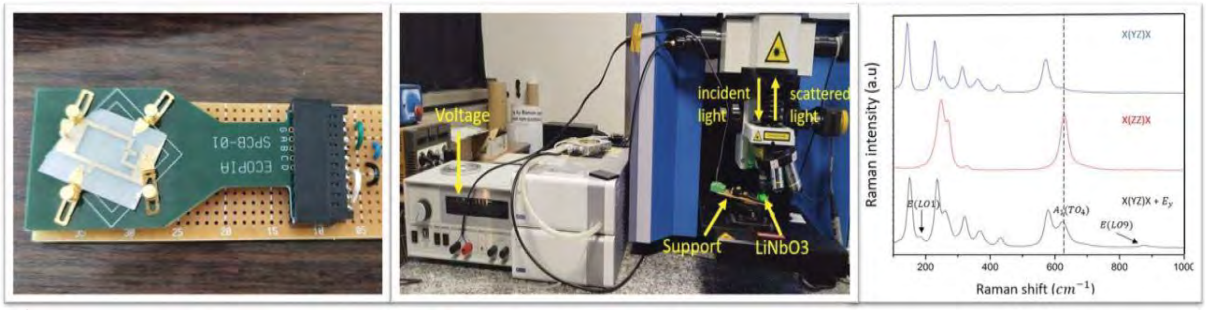
Communications are omnipresent in our world, with ever-increasing flows and data rates. In this context, technologies such as optical fibers and electro-optic modulators based on lithium niobate (LN) are particularly popular, given LN's particular non-linear optical properties.
Raman spectroscopy has been widely used for the structural, mechanical and electrical characterization of LN [1] [2]. Mach-Zehnder type modulators made from LN experience aging over time due to the application of a high electric fields, so a "drift" is introduced (a direct current measurement chain, high maintenance, and cost...) to compensate for ageing. The idea of this study is to measure the variation in the non-linear optical properties of LN as a function of the applied electric field, and to predict its ageing using Raman micro-spectroscopy [3]. Additionally, we investigate the influence of the applied electric field on the Raman modes [4], aiming to deduce the pressure induced by the application of an electric field parallel and perpendicular to the optical axis.



a) b) c)

figure 1: a) the experimental set-up designed and implemented to apply an electric field to an X-cut lithium niobate slide parallel and perpendicularly to the optical Z axis, b) experimental set-up to measure the Raman response and map it as a function of the applied electric field, and c) examples of obtained Raman spectra showing the appearance of new quasi modes due to the presence of external electric field.

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Using surface-enhanced Raman scattering and multivariate analysis for the simultaneous multiplex detection and quantification of malodorous thiols

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Odour is widely known to be due to the microbial conversion of odourless molecules produced from the apocrine glands to volatile organic compounds (VOCs), which includes thiols.[1,2] These thiols strongly contribute to the odour but are only found at low detectable quantities. Additionally, these molecules are highly volatile and small, making them difficult to sample and analyse, including by gas chromatography-mass spectrometry.[3] This leads to the loss of information which is needed for understanding the formation of odour. In this study, colloidal surface-enhanced Raman scattering (SERS) combined with chemometrics has been utilised to simultaneously detect and quantify these thiols individually and in multiplex solutions containing target thiols. To calculate the limit of detection (LoD), both univariate and multivariate methods were used to compare between these methods. Partial least squares regression (PLS-R) was used as the multivariate technique. The LoD values calculated appeared to agree between the two methods, with LoDs calculated to be between 0.0227 ppm and 0.0153 ppm using PLS-R for the target thiols. The target thiols were then examined and quantified simultaneously in 120 mixtures using PLS-R. These models showed high linearity (Q^2 values between 0.9712 and 0.9827 for both PLS-1 and PLS-2) and low values of root mean squared error of predictions (RMSEP) (RMSEP values between 0.0359 ppm and 0.0459 ppm for PLS-1 and PLS-2). To prove that these models worked and SERS could detect unknown concentrations, these models were then applied to 15 new blind test samples. These experiments were performed two weeks after the initial 120 samples. The results proved that these PLS-R models and SERS could be used to predict the unknown concentrations of these thiols in a mixture. In conclusion, these results display the usability of SERS for the simultaneous multiplex detection of analytes and its potential for future development for use in detecting gaseous thiols produced from the apocrine glands.

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Novel OF2i®-Raman sensor for dynamic single particle analysis with high throughput for nano- and microparticles in liquids

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As part of the Nano-Vision project, a novel technique is being developed to characterize and chemically analyse nano- and microparticles in an aqueous environment with high throughput and single-particle sensitivity. The technique combines the methods of opto-fluidic-force-induction (OF2i®) and Raman spectroscopy. OF2i® uses fluidic and optical forces to exert momentum on single particles inside a microfluidic measurement cell. A weakly focused 2-watt laser beam traps particles inside the measurement cell in the transverse direction and accelerates them in the longitudinal direction. The laser-induced velocity change of the particles enables size and concentration determination by analysing the elastically scattered light with an ultramicroscope setup [1].

In the OF2i®-Raman setup, a Raman spectrometer is added to additionally analyse the inelastically scattered light, thereby allowing chemical analysis of the trapped particles. To take full advantage of the OF2i®-Raman-coupling, a new measurement mode is used in which the laser is directed in the opposite direction of the particle flow (see figure), thus slowing down the particles near the laser focus. This way, both an increased recording time of the Raman spectrum and a constant flow of particles through the measurement cell (up to 100 particles per minute) is possible. For small particles or particles with weak Raman signals, the optical and fluidic forces can be readily adjusted to completely stop individual particles, enabling measurement times of several minutes per particle.

In this work, we show a Proof-of-Concept of this new high-throughput single-particle Raman method for both micro- and nanoparticles and elaborate on its potential.

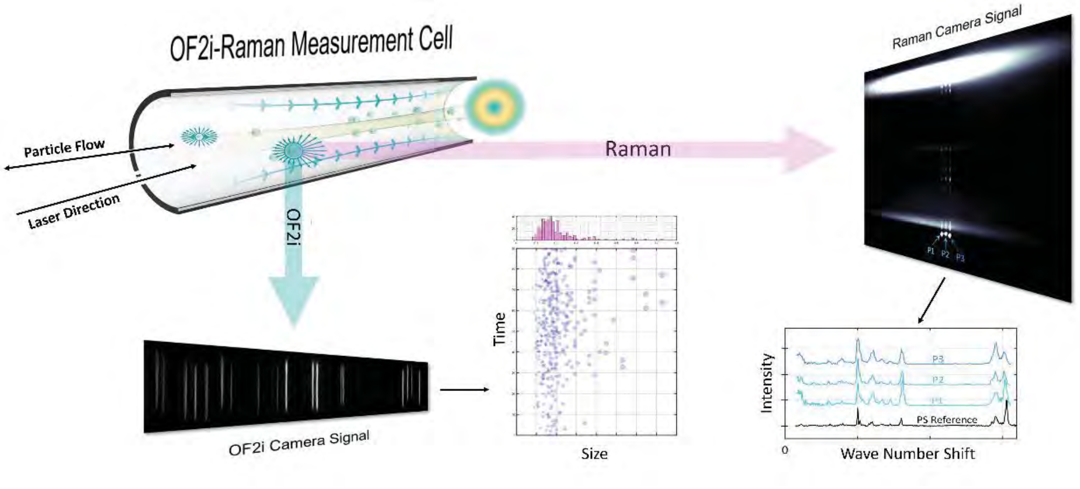

Figure: Illustration of the new OF2i®-Raman operation mode. The OF2i path is used for particle characterization; the Raman path is used for chemical analysis (demonstrated with polystyrene particles).

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Miniaturised Raman spectrometer with real-time spectrum deblurring

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Handheld Raman spectrometers are widely used for identification of unknown substances and raw materials control. Small size, attractive price and ease of operation make a handheld device an attractive candidate also for applications in different fields, such as health care, medical and biological studies. However, handheld Raman devices are still rather large and require a big battery for autonomous operation. Reducing size, weight and power consumption creates even more application opportunities for Raman spectroscopy, for example when devices are installed on drones as a remote sensor, or even on nanosatellites as a part of a microgravity laboratory. Here we present a miniaturised Raman spectrometer, where reduction of size and power requirements is achieved by using a non-stabilized laser source with no active thermo-electrical cooling. An in-build reference sample is used to compensate for the laser wavelength drift. Also, additional optical optimization techniques were implemented to avoid cooling of the spectroscopic sensor, reducing power requirements even more [1]. Continuous laser wavelength drift requires performing real-time calibration during each acquisition, which is done in embedded software. The same software corrects resulting spectra for the device's optical transmittance profile and performs intensity normalisation. One step forward to improve quality of output spectra, which we reveal in the current presentation, is an attempt to perform spectral deblurring in real time. Due to mode hops of the laser, some of the measured spectra may be blurred, which leads to reduced resolution. The internal reference sample, which is used for on-the-go calibration of the device, is also used as a master reference to obtain spectral blurring function of the laser, and subsequently to reconstruct deblurred spectra of the measured sample. This approach allows the use of all measured data, even when the laser is unstable.

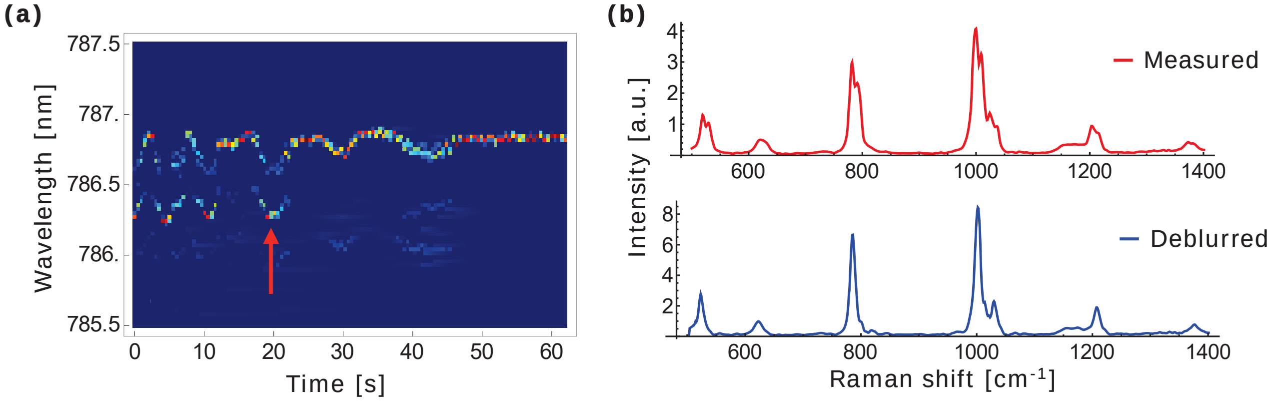

Figure 1: (a) Laser wavelength during mode hopping; (b) Measured and deblurred Raman spectra

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Optics miniaturization strategy without performance compromise for demanding Raman spectroscopy and microscopy applications

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We present a novel miniaturization strategy that allows us to create versatile compact Raman spectrometers and microscopes based on cheap non-stabilized laser diodes, densely-packed optics, and non-cooled small pixel size sensors. We demonstrate that the achieved performance is comparable with expensive and bulky research-grade Raman systems. Our miniaturization concept is based on real-time calibration of Raman shift and Raman intensity using a built-in reference channel that is independent of the main optical path [1]. We have demonstrated the miniaturization of the whole device dimensions down to several centimeters and achieved excellent sensitivity, low power consumption, perfect wavenumber and intensity calibration combined with high spectral resolution of around 7 cm-1 within the spectral range of 400-4000 cm-1.

The high performance and vast versatility offered by our strategy facilitate simple integration into various applications. As examples, we show the quantification of methanol in alcoholic beverages through a glass bottle, in-vivo Raman measurements of human skin, quantification of p-coumaric acid and Serine during fermentation by E. coli bacteria, high resolution Raman mapping, quantitative SERS mapping of the anti-cancer drug methotrexate and in-vitro bacteria identification by Raman mapping. We foresee that the proposed miniaturization strategy will allow realization of super-compact Raman spectrometers for integration in e.g. smartphones and medical devices.

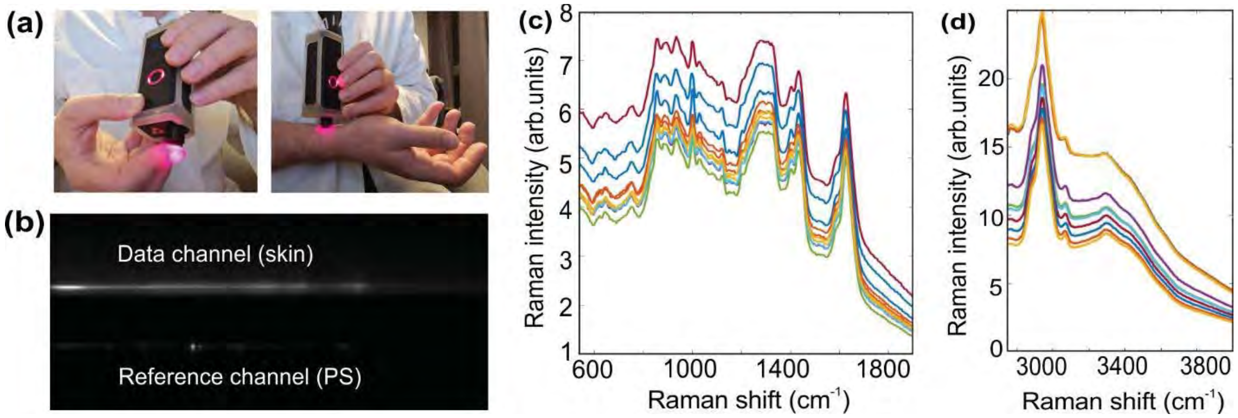

Figure 1: (a) Photograph of compact Raman device, (b) sensor image with data and reference channels, (c,d) typical Raman spectra of human skin obtained by compact Raman device.

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Raman spectroscopy: a rapid microbiological tool for the food and pharmaceutical industries?

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The translation of science into business is challenging. We want to share in this case study our journey of the translation of Raman spectroscopy of microbes from lab results to commercial, dedicated instrument and finally to the application in qc microbiological laboratory for routine work.

There are many areas in the food and pharmaceutical industries that could benefit from detection and identification of microbiological contamination within 1-3 hours:

- o Rapid sterility testing - especially in the field as cell therapies
- o Bioburden testing
- o Manufacturing support and root cause analysis - when things go wrong

All of these areas can benefit from a rapid, non-growth microbiology technique.

Fluorescence/Hyperspectral imaging[1] is a promising label-free tool for the detection of microorganisms at the single cell level and could therefore be of great importance as a culture-free method for sterility testing in the pharmaceutical industry. However, the detection of false positive counts limits the application of these technique. Raman spectroscopy[2] has the ability to identify individual bacteria and is able to reduce the number of false positive counts significantly and opens up the possibility of a cultivation-free sterility and bioburden tests allowing rapid identification and risk assessment of any pathogens that may be present. Combining both techniques into one automated instrument gives the potential of a fast, accurate detection and identification of microorganisms. Applications cover early spoilage detection and process monitoring.

In a detailed study for cell therapy sterility testing we explain the challenges of the introduction of a new technology. With the were able to detect a contamination level of less than 10 cells/ml. CHO cell suspensions were spiked with different concentrations of bacteria or fungi. The samples were then lysed and filtered. Image analysis of microscopic images of the filter provided the coordinates of potential pathogens. The microorganisms were identified by Raman spectroscopy. A PLS-LDA model was used to identify and discriminate between live and dead microorganisms. The results were correlated with the corresponding plate counts.

The case study demonstrates the importance of proper sample preparation as well as the technological limitations of this spectroscopic approach. And finally, we would like to share the insight that regulatory requirements are more important for the commercial success of a technique than the excellence of the spectroscopic method.

Our results show the great potential of this fast and cost-effective approach for quality control in the food and pharmaceutical industry.

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Expanding Space R&D Frontiers with Compact, Energy-Efficient Raman Spectrometer in NanoSats, CubeSats, and on Rovers

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The space research market is experiencing growth as satellite launches become more affordable. Nano and CubeSat satellites offer cost-effective alternatives, significantly increasing accessibility to space-related research and development (R&D). This market can be segmented into three main categories: government-owned space agencies, academic institutions, and corporate R&D departments, each with distinct R&D needs. The unique space environment—characterized by microgravity, vacuum, and radiation—opens new research avenues. The miniRaman Spectrometer (mR), an innovative compact, handheld Raman device, incorporates a patented internal reference channel for continuous recalibration, ensuring unmatched accuracy and operational stability [1]. By eliminating manual calibration, the mR becomes more compact, lighter, and consumes less power, making it ideal for space applications. Lightnovo, in collaboration with the European Space Agency (ESA), is developing project ELPINA (Efficient Low-Power Spectrometer for Integrated Nano/Cube Satellite Analysis) to further miniaturize the miniRaman and enhance its energy efficiency for integration into nano- and CubeSat satellites. This integration aims to equip satellites with a miniaturized laboratory featuring the mR, providing various R&D capabilities for analyzing organic materials like rare cells, proteins, and bacteria, identifying recyclable materials among on-orbit debris, pharmaceutical applications, and more. Furthermore, the transferability of ELPINA technology to Rover and CubeRovers holds potential for planetary exploration, including the search for signs of life, highlighting the mR's versatility in space research applications [2].

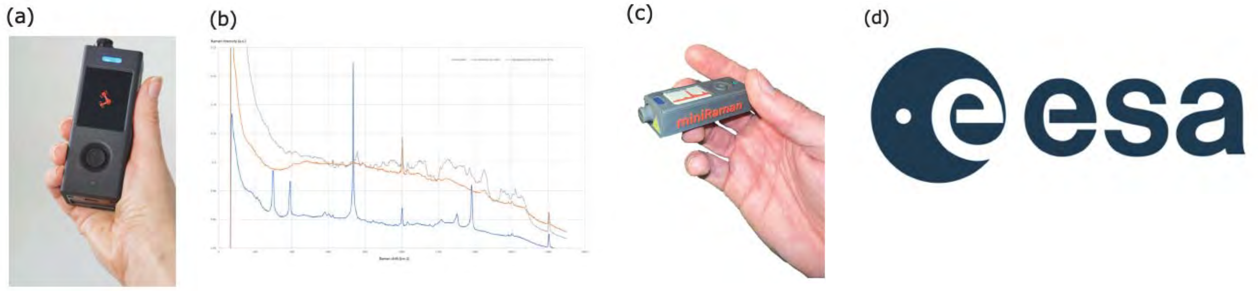

Figure 1: (a) Current mR design in hand; (b) Raman spectra of Teflon film (blue), bacteria in cell B4 on teflon film (orange) and staphylococcus aureus bacteria measured directly on Petry dish (grey); (c) mR concept model exemplifying the compactness target for space-ready technology; (d) ESA logo.

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Inline Analysis of Multicomponent Bioprocesses Using Raman Spectroscopy and RAMANMETRIX™

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In the field of bioprocess analysis, accuracy is of paramount importance. These complex processes that depend on living organisms require fast and powerful analytical tools for immediate control and response to irregularities. Our study highlights the effectiveness of combining RAMANMETRIX™, an AI-driven tool, with Raman spectroscopy for real-time monitoring of complicated multicomponent bioprocesses.

In our experiment, we grew a genetically engineered E. coli strain in a fermenter. We took samples at regular intervals for analysis, using a Raman spectroscope from Wasatch Photonics to collect spectral data. These samples were also analyzed by HPLC. RAMANMETRIX™ is characterized by easy data import, calibration, pre-processing and model generation. This methodology enables the rapid evaluation of new samples and avoids the delay of laboratory analysis. The developed model enables immediate online tracking of the carbon source, products or by-products, facilitating timely process management.

Recognizing the need for rapid analysis, we have further developed our system to support online capabilities in accordance with Process Analytical Technology (PAT). By integrating a probe into a viewport, we have achieved continuous spectra acquisition, enabling live sample analysis without interrupting the bioprocess. This online technique, complemented by traditional HPLC assessments, forms the basis of our reference system.

In summary, the integration of AI-powered software with Raman spectroscopy provides an easy-to-use method for real-time analysis of complex bioprocesses. This approach not only promotes efficient production in the pharmaceutical and food industries, but also emphasizes the importance of fast, non-invasive monitoring and control of these

processes. A particular focus of our research has been the introduction of direct inline measurements through a Raman probe integrated via a viewport as an interface to the bioprocess. This approach greatly facilitates the transfer of direct measurements into AI models and offers a perspective for accelerated model building and increased robustness of models through a new approach to experimental design.

1. D. Storozhuk, O. Ryabchykov, J. Popp, T. Bocklitz, RAMANMETRIX: a delightful way to analyze Raman spectra, 2022, arXiv:2201.07586v1

Quantitative evaluation of active ingredient skin penetration using confocal Raman spectroscopy: comparison of univariate and multivariate analysis

Active ingredients in topical skincare and pharmaceutical products are expected to penetrate human skin to deliver their efficacy. Their kinetic absorption (e.g. how much, how fast, and how deep they permeate into human skin) is of prime interest. However, the quantitative evaluation has been hampered by the lack of accurate and reliable experimentation and analysis methodologies.

Confocal Raman spectroscopy is a label-free, non-destructive, and in situ technique and is ideal for evaluating the permeating kinetics of active ingredients in the skin. Nicotinamide formulation was topically applied on human skin models, and the Raman spectra was measured in a fingerprint region (500-1900 cm^{-1}) along different depths of skin. For the quantitative evaluation of nicotinamide concentration in the skin, both univariate and multivariate spectral analyses were implemented, and their results were compared with the conventional analytical approach (LC/MS/MS). For the univariate analysis, series of skin phantoms with different nicotinamide concentration were constructed, and Raman peaks were calibrated from the skin phantom. The height of corresponding peak of the Raman spectra from the skin was calculated, and by comparing it with the skin phantom reference, the quantitative value of nicotinamide concentration was evaluated. For multivariate analysis, a spectral library of 11 different human skin molecules and nicotinamide (80mg/mL) was constructed. Non-negative least squares (NNLS) analysis was performed on measured Raman spectra of the skin, and nicotinamide concentration was calculated from the NNLS coefficients. The direct comparison of these univariate and multivariate analysis results with the LC/MS/MS results demonstrated that both analyses provided a reasonable range of quantification, but multivariate analysis is more robust and accurate than univariate analysis.

In summary, our Raman spectroscopy experimentation and NNLS multivariate analysis can advance topical formulation development by the quantitative measurement of skin penetration of active ingredient.

Tracking the Poisoning Tolerance of $(Pt_{0.9}Rh_{0.1})_3V$ Intermetallics by In Situ Raman Spectra

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Hydrogen-oxygen fuel cells are promising energy conversion devices but suffer from poisoning by the carbon monoxide (CO) impurity in industrial hydrogen fuels. Thus, it is vital to in situ track the adsorption of CO and then give a guidance for designing highly effective electrocatalysts.[1] Here in situ Raman spectra were employed to exploring the relationship between the poisoning tolerance and structures of Pt-based alloys. As the compressive strain increases, the Raman shift of Pt also increases, which indicating the weakened binding of CO intermediate (CO*). The doping of oxytropic Rh into Pt-based alloys further facilitated the removal of CO*, as confirmed by the change of Raman signals of CO* with potential increasing. The electrochemical tests and theory calculations further support the results with limited degradation under CO/H_2 mixed fuels and reduced binding strength of CO* by inducing compressive strain and dual-functional effect when forming $(Pt_{0.9}Rh_{0.1})_3V$ Intermetallics.

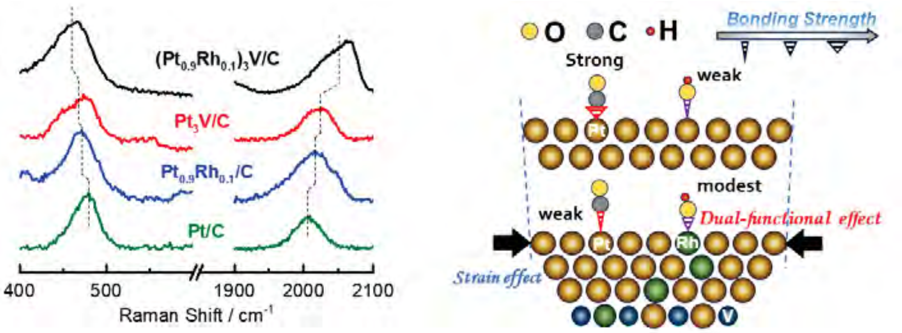

Figure 1: Raman spectra and schematic illustration of CO* on Pt-based catalysts

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Revealing the role of interfacial water and key intermediates at ruthenium surfaces in alkaline hydrogen evolution reaction

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The detailed structures of interfacial water and key intermediates regulated by catalyst surfaces significantly affect alkaline hydrogen evolution reaction (HER) activity. However, the underlying mechanisms are not fully understood. We therefore for the first time simultaneously captured dynamic spectral evidence of the interfacial water, *H and *OH key intermediates by core-shell nanoparticle-enhanced Raman spectroscopy, and their interactions with Ru catalyst surfaces with different valence states (RuOx) during alkaline HER. Significantly, we observed three different structures of interfacial water, two distinct *H and *OH intermediates [1]. They interact with different valence states Ru catalyst surfaces resulting in different HER activity. The combination of direct spectral evidence with density functional theory (DFT) calculations allows us to reveal the role of interfacial water and key intermediates as follows: 1. The local cation tuning effect of hydrated Na+ ion water promotes interfacial water dissociation at large work function high-valence Ru(n+) surfaces. 2. Compared to low-valence Ru(0) surfaces, the high-valence Ru(n+) surfaces have more moderate adsorption energies for interfacial water, *H, and *OH, resulting in superior catalytic activity. 3. OH_{ad} acting as an electron-favorable proton acceptor or geometrically favorable proton donor promotes the interfacial water dissociation and Volmer-step kinetics involving Ru(n+)-H on RuOx surfaces. They therefore facilitate the HER activity.

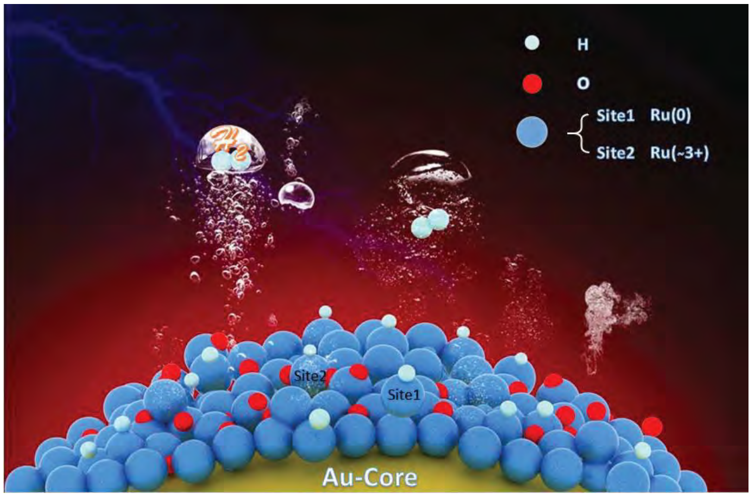

Figure 1: Model of Au@Ru core-shell NPs and the mechanism of the alkaline HER process revealed by core-shell nanoparticle-enhanced Raman spectroscopy.

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Cyano-Hydrol Green derivatives: novel chromophores for expanding 9-cyanopyronin-based vibrational palette

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Raman microscopy, which detects molecular vibrations, has been developed as an imaging technique with superior capability for multiplexed detection compared to fluorescence microscopy. Moreover, recent development of electronic pre-resonance (epr) - stimulated Raman scattering (SRS) detection system has overcome the low sensitivity of conventional Raman imaging [1]. Especially, 9-cyanopyronin [2] is a promising scaffold that exploits resonance Raman enhancement to enable sensitive, highly multiplexed biological imaging. Here, we developed cyano-Hydrol Green (CN-HG) derivatives as novel resonance Raman scaffolds to expand the vibrational palette of 9-cyanopyronins. CN-HG derivatives exhibit sufficiently long wavelength absorption to produce strong resonance Raman enhancement for near-infrared (NIR) excitation, and their nitrile peaks are shifted to a lower frequency than those of 9-cyanopyronins. Moreover, the fluorescence of CN-HG derivatives is strongly quenched due to the lack of the 10th atom, unlike pyronin derivatives, and this enabled us to detect spontaneous Raman spectra with high signal-to-noise ratios. In spite of the attractive feature of CN-HG derivatives as resonance Raman probe, a firstly prepared analogue, CN-HG with tetramethyl moiety as *N*-substitution was too unstable under physiological conditions. Then we synthesized ring-extended CN-HG derivatives to improve the stability. As a result, the ring-extension not only improved stabilities under physiological conditions, but also increased Raman intensities along with the redshift of the absorption maxima. We believe these CN-HG derivatives are powerful candidates for high performance vibrational imaging.

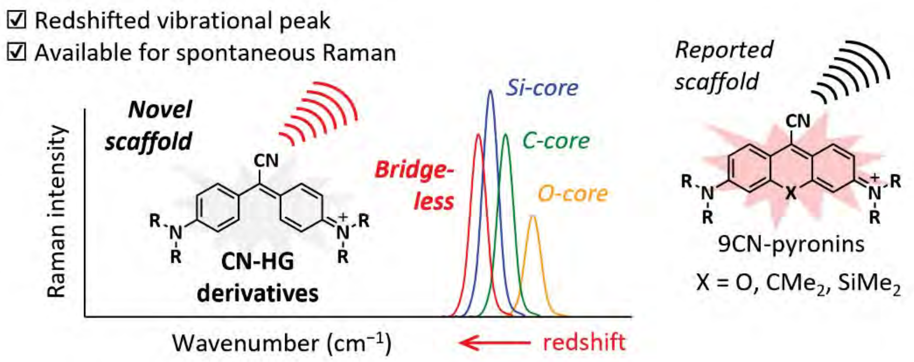

Figure 1: Chemical structures and vibrational illustrations of cyano-Hydrol Green (CN-HG) derivatives and 9CN-pyronins.

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Investigating the influence of particulate matter $PM_{2.5}$ on human bronchial epithelial cells by Confocal Raman microscopy and imaging

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Air pollution is one of our era's greatest problems, leading in a worst-case scenario to death. Many air pollutants are accountable for this premature death, including $PM_{2.5}$ (particulate matter with an aerodynamic diameter of 2.5 microns or less). They can easily penetrate in the respiratory tract therefore causing an inflammatory response (secretion of IL-6, IL-8, TNF-α …) that triggers and exacerbates several respiratory diseases including asthma[1,2]. The goal of our studies is to detect the effects of particles on lung cell models. To achieve this goal, human bronchial epithelial cells (BEAS-2B) were prepared under normal conditions and under stress conditions which was induced by 24 hr. of exposure to $PM_{2.5}$ to induce inflammation. For this purpose, confocal Raman microscopy was used to identify particulate matter in cells and to evaluate potential changes induced by them. Raman images and spectra were acquired for the healthy and PM exposed cells. The outcomes of this research revealed striking morphological and spectra changes in Raman after exposure to $PM_{2.5}$ compared to the control. We are using various computational methods to extract the response of the cell to these particles. The results confirm that Raman spectroscopy is undoubtedly a powerful technique for identifying changes within the cell.

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The challenge of tracking plastic fragments in the environment: Micro-RAMAN for the analysis of biodegradable plastics in compost

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Micro-RAMAN spectroscopy is an established method for the detection of microplastics in various matrices. However, it requires extensive sample preparation to observe and characterize microplastics. We are working on the optimization of the extraction and analysis of microplastics in compost samples: Removal of organic matter with a Fenton reagent followed by density separation are the two main steps for the extraction.[1] Usually, the Fenton reaction is carried out at temperatures of 70-80 °C,[2] for fast and efficient removal of organic matter. However, given the properties of biodegradable plastics, such temperatures cannot be applied.[3] At elevated temperatures, the physicochemical properties of the polymers will suffer changes, with possible degradation of the particles before analysis. For this reason, milder conditions are required, extending the required treatment time and potentially leaving unwanted compost particles.[4] As a consequence, it requires intensive filtration following organic matter removal and density separation. These additional steps must ensure a suitable sample for a fast and accurate characterization of all plastic particles by micro-RAMAN spectroscopy. Following the work of Wohlleben et al (2023)[5] we focus on optimizing the extraction procedure to reduce the overall sample analysis time, optimizing the spectroscopy observations, to avoid fluorescence interference, and achieving a high polymer recovery at the same time. For spectroscopy, we are using a Horiba Jobin Yvon LabRAM-HR spectrometer with particle scout software to detect the microplastics in our sample; determining their size, number concentration, and polymer type. Our results indicate that, using the optimized extraction procedure, the analysis of microplastics from compost is possible, including the ones from biodegradable polymers. Future work will focus on the analysis of samples from laboratory-simulated composting conditions and industrial composting samples. The presented methodology might be used for quality control monitoring in industrial composting plants, focusing on micro-RAMAN spectroscopy for the analysis of microplastics.

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The unbiased interpretation of Raman spectra for NCM cathode materials based on two-dimensional correlation analysis

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As civilization becomes more sophisticated, mankind needs more energy. Especially in modern times, securing stable and abundant energy is essential for industrial development. Since the global declaration of carbon neutrality by 2050, many countries around the world are making efforts to reduce carbon usage in various fields. Among them, in the automobile sector, the EU has confirmed a ban on internal combustion engine production after 2035, accelerating the transition to electric vehicles. For this, many countries efforts to improve battery performance (long driving range, fast charging time) are reminiscent of war.

The capacity, therefore performance, of lithium-ion batteries decreases depending on the number of charging and discharging cycles, which is directly related to the deteriorated structure of the cathode materials. One commonly used cathode material is lithium nickel cobalt manganese oxide, abbreviated NCM. A single NCM particle usually has a size of about 10 ㎛, which is aggregates of primary particles sized between 100 and 200 nm, and a polycrystalline structure. In Raman spectroscopy, the NCMs spectral patterns are clearly distinguished according to the composition ratio, but accurate understanding of the spectra is difficult due to the lack of theoretical studies on the polycrystalline structure with the short-range order. Here, we have performed two-dimensional correlation analysis, which is a mathematical technique to discriminate altered Raman spectra for the following NCM series, NCM 111, 433, 5 2.5 2.5, 622, 7 1.5 1.5, 811, and 9 0.5 0.5. This tool can improve the understanding and analytical reliability of Raman spectra. With this approach, the subtle structural changes can be predicted in cathode materials during charging and discharging. This knowledge helps design structures that improve battery life and increase capacity.

Using time-resolved Raman spectroscopy to probe phonon dynamics and out-of-plane heat transport in layered quantum materials.

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The high thermal conduction anisotropy of layered quantum materials makes them a strong candidate for the development of integrated devices with improved thermal management [1]. Some of these materials, such as graphene and hBN present excellent in-plane thermal conductivities, that ensures a good dissipation of heat through in-plane diffusion, combined with out-of-plane thermal conductivities up to several orders of magnitude smaller, offering the possibility of out-of-plane thermal insulation [2].

While in-plane heat transport can be observed efficiently by established methods, such as Raman thermometry, and a new technique developed in our group, based on spatiotemporal pump-probe microscopy [3, 4], new experimental methods need to be developed in order to properly assess the out-of-plane transport of heat in layered quantum materials and van der Waals stacks. We present our work towards the development of such a new technique, which is based on time-resolved Raman spectroscopy (TRRS) [5]. This technique can be used to either probe the phonon dynamics of one specific layer [6, 7] or track the out-of-plane transport of heat with a layer by layer precision by monitoring the changes in the Raman modes features as the heat flows through the sample.

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Probing Deuteration-Induced Phase Separation in Supported Lipid Monolayers using Tip-Enhanced Raman Spectroscopy

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Deuterium labelling is a common strategy to track the distribution of molecules in biological samples.[1][2] However, the alteration of biophysical properties caused by deuteration is not well understood yet. Conventional analytical tool lacks the sensitivity and spatial resolution to investigate this at the nanoscale. Herein, we demonstrate that hyperspectral tip-enhanced Raman spectroscopy (TERS) imaging is an effective tool to visualize deuteration-induced phase separation in a mixed dipalmitoylphosphatidylcholine (DPPC):d_{62}-DPPC (1:1) monolayer supported on Au(111) surface. The DPPC:d_{62}-DPPC monolayer was transferred onto the Au(111) surface via the Langmuir Blodgett technique. Successful monolayer transfer was confirmed via topography measurements using atomic force microscopy. Since d_{62}-DPPC has a strong C-D stretching signal (I_{C-D}) in the silent region of the Raman spectrum around 2100 cm^{-1}, and DPPC has a strong C-H stretching signal (I_{C-H}) around 2900 cm^{-1}, the I_{C-D} / I_{C-H} ratio was used to visualize the phase separation. In TERS images, there were distinct DPPC-rich and d_{62}-DPPC-rich domains, which can be attributed to the differences in the molecular size and polar/non-polar interaction between the deuterated and non-deuterated chains of the d_{62}-DPPC and DPPC molecules. Finally, the size and polarity of d_{62}-DPPC and DPPC were quantitatively examined using LC-MS and correlated with their phase separation behavior. The novel insights gained in this work expand our biophysical understanding of supported lipid membranes.

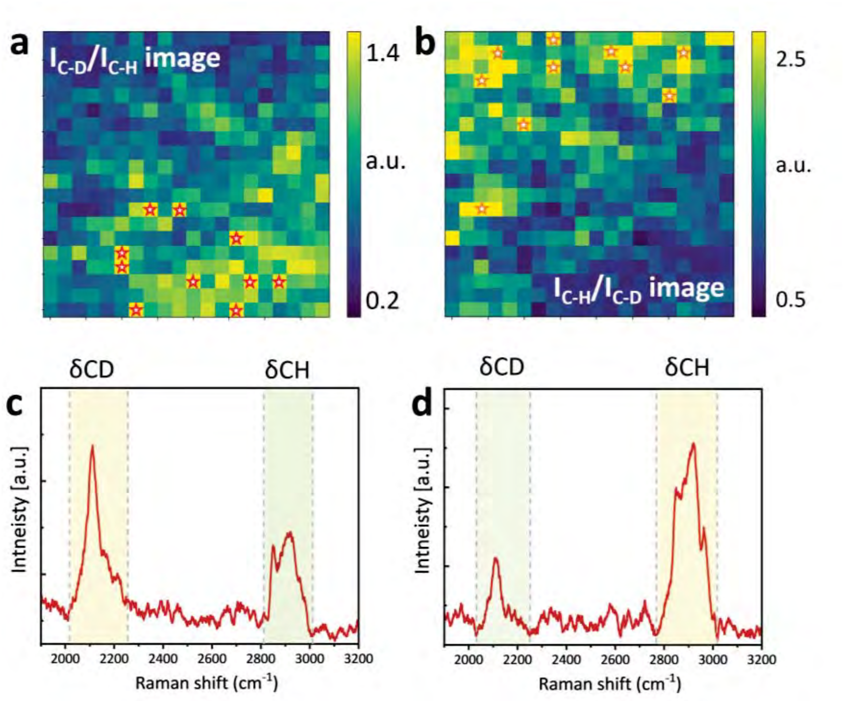

Figure 1. TERS images of the (a) I_{C-D}/I_{C-H} and (b) I_{C-H}/I_{C-D} ratio showing phase separation of the DPPC:d_{62}-DPPC domains. Averaged TERS spectra at the locations marked in Panels (c) a and (d) b.

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Surface Enhanced Raman Spectroscopy for Soil Fungal Systems: Possibilities and Limitations

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The discovery of surface-enhanced Raman spectroscopy has finally made it possible to use Raman scattering for analytical purposes, which require the method to be not only selective, but also capable of working with small amounts of analytes [1]. There are many reports on SERS substrates especially for biosensing [2], demonstrating its growing popularity. However, the intimate contact between enhancing surface and the analyte may initiate photo-induced reactions, leading to the formation of new compounds [3]. This can be a serious problem in biological samples, where searching for specific analytes is already difficult due to the presence of a complex matrix.

An example of a biological sample where SERS can be utilized as an analytical tool are soil fungal systems. Using microfluidic chips that mimic the natural environment, but in a structurally and chemically controlled manner, it is possible to investigate the processes that occur between individual fungal cells [4,5], for instance, during interspecific interactions. Possibility to characterize diverse secondary metabolites produced by the fungi in such systems [6] could aid in understanding fungal competition and its role in larger scale nutrient cycling. Raman microscopy is one of the very few techniques that provides the opportunity to study them at the microscale [7].

In this work we present the effect of SERS substrate structure on the outcome of the Raman results of various biologically active compounds. A better understanding of how the structure of an analyte can be altered upon contact with the enhancing nanostructure and how the substrate type affects the detection limit is essential for biosensing of secondary metabolites. We also discuss strategies for developing SERS substrates for this purpose.

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Real-time integrated diagnosis and antimicrobial resistance infection profiling by Raman spectroscopy and machine learning

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Ventilator Associated Pneumonia (VAP) is typically acquired in hospital settings following 48 hours of endotracheal intubation with mechanical ventilation. VAP infections represent one in four infections in critically ill patients, posing a worldwide health risk.[1] Biofilm formation in endotracheal tubes is associated with the development of VAP by acting as a reservoir for pathogens, resulting in relapses of VAP following antibiotic treatments.[2]

Delayed diagnosis is a key challenge to effective treatment, often resulting in overuse of broad-spectrum antibiotics and incorrect diagnosis.[3] The absence of real time diagnostics for VAP and antibiotic susceptibility testing hinders efforts aimed at preventing antibiotic resistance proliferation. Current methodologies are time consuming and often diagnostics follow the administration of broad-spectrum antibiotics. [4]

Bacterial species and strain can be identified using their unique Raman spectroscopic fingerprint, which reflects differing genotypic and phenotypic characteristics of different species and their growth conditions. Raman spectroscopy has clear advantages over traditional diagnostics as it is non-destructive and requires minimal sample preparation.[5]

This poster will outline the development of a Raman spectral database of bacteria using Raman mapping which can be used for real-time identification and differentiation of VAP pathogens. The continued development of this Raman spectral database in combination with machine learning is a key step in enabling real-time integrated diagnosis of infections, helping restrict future antibiotic resistance proliferation. In this study I have used multi-excitation Raman spectroscopy to characterise pathogens present in VAP on type strains. The data collected demonstrates the ability to differentiate and identify the bacteria present in patient samples which will form the basis for a Raman database.

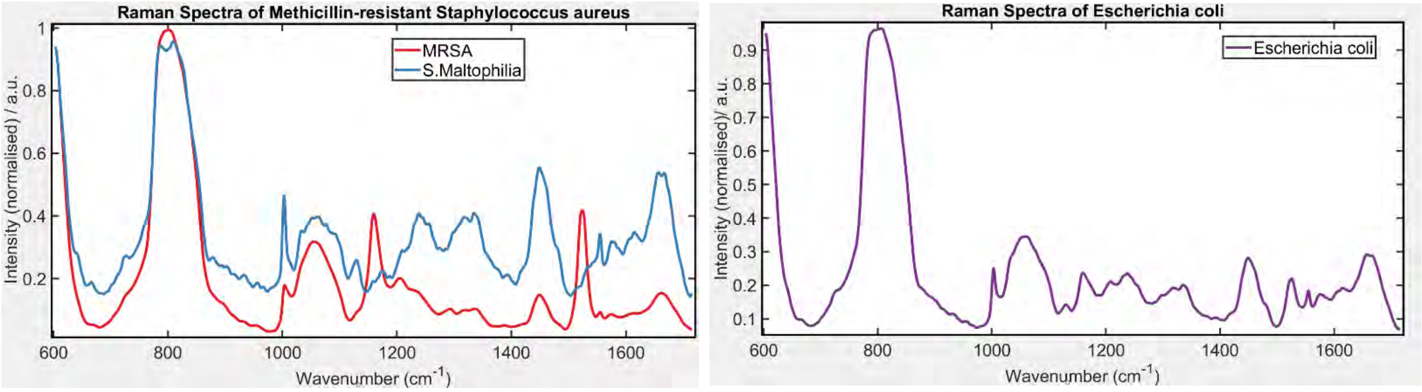

Figure 1: (A) Raman spectra taken from Raman mapping of MRSA and S. Maltophilia taken at 532nm. **(B)** Raman spectra from mapping of Escherichia Coli at 785nm.

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Shaped-induced enhanced Raman scattering to enhance-SERS effect in manufacturing ultra-sensitive sensors

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Since of discovered surface-enhanced Raman scattering (SERS) several strategies have been developed to achieve more efficient and highly sensitive substrates. In this sense, silicon substrates with cavities or microchannels obtained from the lithography have been more exploited due to the robustness of these platforms[1], whose cavities could be given additional contributions to SERS signal intensification (enhanced-SERS), which we called shaped-induced enhanced Raman scattering (SIERS) [2,3]. Scattered electric field simulations reveal that bare V-shaped Si substrates exhibit spatially distributed interference patterns from the incident radiation used in the Raman experiment, resulting in constructive interference for an enhanced Raman signal. Experimental data show a 5-fold increase in Raman signal intensity for bare V-shaped Si microchannels when compared with flat Si substrates. Briefly, SIERS effect is a valuable tool to improve the effect of metallic nanoparticles in SERS substrates, providing reproducible SERS substrates with a high sensitivity to SERS-active platforms. Herein, we demonstrated how the synergistic SERS@SIERS effect contributes to detecting ultralow concentrations, more specifically, the silicon V-shaped microchannel (Si-V) evaluated in the presence of gold nanorods (AuNRs) was able to detect until 10^{-10} mol L^{-1} of Thiram, 10^{-12} mol $L^{-1}$of atrazine and 10^{-18} mol L^{-1} (attomolar sensitivity) of of rhodamine 6G molecules. The spectra were collected for different regions of the Si-V substrates, i.e., in the inside, edge, between (flat top), and far from Si-V (coffee-ring AuNRs aggregation) to assess the performance of Si-V microchannels obtained. The IDMAP statistical projection reveals a higher silhouette coefficient of 0.91 for the inside of Si-V, indicating a more excellent spectral reproducibility with closer relative intensities. Our findings show that the sinergism between the V-shaped Si microchannel array and AuNRs (or others plasmonic structures) may provide very intensity patterned SERS (vip-SERS) substrates. The device platform used in this study stands out as a commercial robust option to reproducible (and recycle) ultra-sensitive SERS sensors, demonstrating exceptional sensitivity in detecting a diverse range of molecules at ultralow concentrations.

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Quantitative Chemical and Physical Analysis of Heterochromatin in a Living Cell by Simultaneous Raman-Brillouin Imaging

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[Introduction] Chromatin plays a crucial role in the epigenetic regulation of gene expression. The degree of chromatin condensation is a key factor in regulating the activity of gene transcription[1]. Chromatin is classified into relaxed euchromatin (ECh) and condensed heterochromatin (HCh) based on its degree of condensation; However, there are few reports on which biomolecules are involved and how much are concentrated in HCh and ECh. The physical properties of intracellular chromatin also remain unknown, including whether it exists in a liquid or solid phase. In this study, we performed label-free quantification of chromatin in living NIH3T3 cells by simultaneous Raman-Brillouin imaging. Chemical properties, such as concentration and composition, as well as physical properties, such as viscosity and elasticity, were quantitatively visualized using Raman and Brillouin imaging, respectively.

[Result and Discussion] Raman and Brillouin images of an NIH3T3 cell were simultaneously acquired, and 3600 Raman spectra in a Raman image were decomposed into 10 component spectra by applying multivariate curve resolution (MCR) analysis. Four derived component spectra were assigned to proteins, lipids, RNA, and DNA based on their spectral shapes (Fig. 1a). Quantitative concentration images were achieved by utilizing the O-H stretching band of extracellular water as an intensity standard (Fig. 1b, c) [2]. In the concentration images, we succeeded in the visualization of DNA-rich HCh in a label-free manner. The DNA concentration image also showed quantitatively that the DNA concentration in HCh was four times higher than in ECh. Furthermore, the lipid concentration was found to be higher in HCh than in other regions within the nucleus. The Brillouin image showed that the Brillouin shift was higher in HCh, showing that HCh was stiffer than ECh (Fig. 1d).

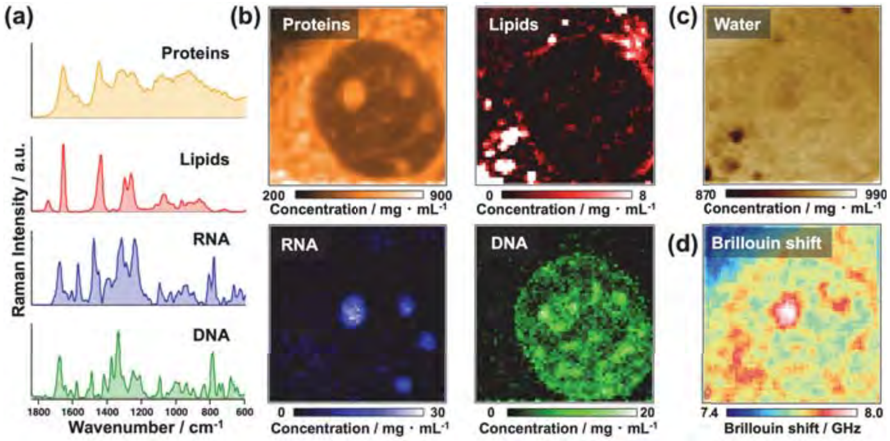

Fig. 1 (a) MCR Raman spectra. (b) Quantitative MCR images obtained by utilizing the O-H stretching band as an internal intensity standard. (c) The corresponding water density image obtained by mapping Raman intensity of the O-H stretching band (3500-3600 cm^{-1}) relative to that of extracellular regions. (d) Brillouin image obtained by mapping the peak position.

Next, NIH3T3 cells were treated with Trichostatin A (TSA, HDAC inhibitor) to relax HCh, and changes in chemical composition and physical stiffness were quantified from Raman and Brillouin images. The DNA concentration was shown to decrease by 30%, whereas lipid decreased by 60% after the TSA treatment. Brillouin images show that HCh became softer than TSA-untreated HCh, directly confirming that the HCh was relaxed. From these results, we conclude that the relaxation and softening of HCh are not due to changes in DNA concentration but rather due to a decrease in lipid-molecule concentration. In other words, lipids play an important role in the chromatin condensation.

[Conclusion] By combining Raman-Brillouin imaging and MCR analysis, we achieved a comprehensive visualization of chemical and physical properties inside a living cell. We quantitatively showed that the DNA concentration in HCh is four times higher than in ECh, and HCh is stiffer than ECh. We also found that lipids are concentrated in HCh compared to other regions in the nucleus. Furthermore, both DNA and lipid concentrations in HCh decreased after TSA treatment and HCh became softer, suggesting that lipids are involved in the chromatin condensation.

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Cellulose-based Silver Nanoparticle Composite as Promising Flexible SERS Substrate

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Preparation and optimization of nanostructured surfaces with incorporated metal nanoparticles (Ag, Au) are crucial for effective qualitative and/or quantitative analysis using surface-enhanced Raman spectroscopy (SERS). Cellulose-based substrates are an interesting platform for label-free SERS detection, as cellulose represents a matrix for silver nanoparticle immobilization and its flexibility makes it convenient and possibly superior to rigid and fragile substrates (glass) in practical applications. Using cellulose for SERS substrate is also a step towards sustainable and green chemistry. [1, 2]

We propose a two-step method for preparation of easy-to-handle SERS substrates, which are based on silver nanoparticles (AgNPs) immobilized on chromatography paper. In the first step, a set of anisotropic (shapes such as prisms are highly efficient for SERS enhancements) Ag dispersions with tuneable optical properties were synthesised using trisodium citrate as a stabilizer and H_2O_2 as an etching agent. In our work, stable AgNPs were prepared without the need for an additional stabilizer (polyvinylpyrrolidone, PVP), that is otherwise typically used. [3, 4] Those AgNPs were then immobilized on chromatography paper using two adsorption strategies – AgNPs were adsorbed directly on the paper surface, and immobilization on paper and branched poly(ethyleneimine) (PEI) composite through its amino groups. $AgNP_S$ dispersions and cellulose-AgNPs substrates were characterized by UV-Vis, DLS, TEM, AAS, SEM, and EDS. To evaluate SERS effectiveness, SERS detection of 10^{-5} M adenine solution was performed using excitation lasers of 455 nm, 532 nm, 633 nm, and 780 nm wavelengths, then analytical enhancement factors were calculated with values reaching up to 10^7 for substrates where PEI was used. Results show that rather than AgNPs shapes, the level of their aggregation and NP concentration on the cellulose surface is a key factor for successful analysis. In conclusion, presented cellulose-based substrates that use highly branched PEI as a linker for AgNPs immobilization are promising platforms with high application potential as SERS substrates.

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Towards a SERS optical nose for VOC and gas sensing

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Optical noses are not yet able to replicate the multiplexed continuous sensing of volatile organic compounds (VOCs) that biological noses are capable of. Current sensing technologies such as GCMS are highly sensitive, but have high complexity, size, and cost. Here we show that surface-enhanced Raman spectroscopy (SERS) could deliver an in-situ inexpensive sensing methodology with high sensitivity and specificity for trace VOC concentrations, by repeated removal of all organics from plasmonic hotspots then precision re-scaffolding.[1-3]

We have developed a reproducible SERS substrate that exploitings self-assembly of gold nanoparticles (AuNPs) into 2D close-packed aggregate arrays with precision (0.9nm) plasmonic nanogaps defined by cucurbit[n]uril molecular spacers.[2-3] Immobilization of the AuNP aggregates on a substrate provides direct analyte access to the nanogaps, but also access to the gap scaffolding molecules for further treatment. These SERS substrates can be regenerated by stripping organic molecules and oxidizing the nanoparticle surfaces, providing a clean metastable substrate that can be re-scaffolded with a wide variety of molecules. This allows for tuning of the hotspot chemistry and reuse of substrates while retaining high SERS signals. We demonstrate the static sensing of a range of VOCs and gases using such SERS substrates with a number of surface functionalizations, and their integration into gas flow cells. We monitor the binding and unbinding of small gas molecules such as acetone, CO_2 and ammonia to demonstrate their capability for sensing gases and VOCs in real time.

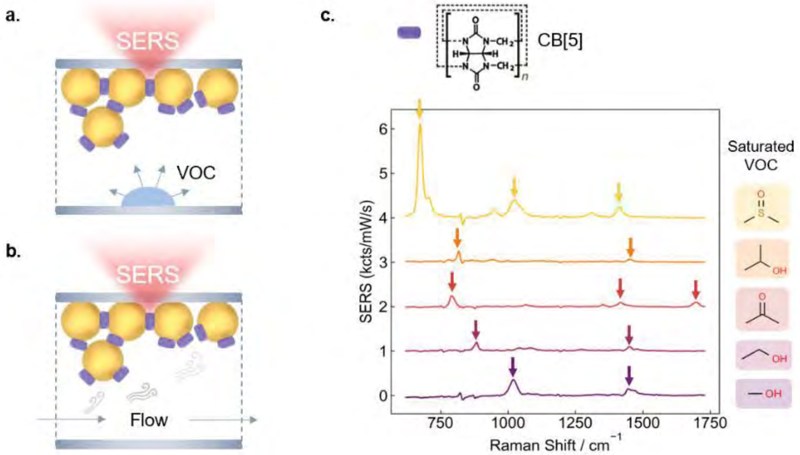

Figure 1: Schematic of immobilised AuNP monolayer aggregate for (a) saturated VOC or (b) in-flow VOC and gas sensing. (c) Background subtracted SERS spectra for sensing of a number of VOCs at saturation, with cucurbituril molecular scaffold. Arrows indicate characteristic peaks of the VOC present.

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Confocal polarized Raman microscopy to obtain orientation information on the alignment layer liquid crystal interfaces

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The phase adopted by liquid crystals, in applications such as displays, is controlled by alignment layers. Typically this phase originates from a layer of unidirectionally rubbed polymer on a solid support. The extent of alignment of the liquid crystal by the alignment layer can be measured with transmission spectroscopy, which quantifies the net effect of the alignment over the entire depth of the LC phase present. The polarization dependence of the Raman scattering of liquid crystal molecules, e.g., 5CB, makes it possible to determine molecular anisotropic ordering (alignment) directly by polarised Raman microspectroscopy[1]. However, the optical properties of the oriented LC phases and ITO on glass electrodes has a potentially large impact on the spatial resolution achieved, in particular with regard to depth along the microscope axis. Here we introduce a resonance Raman active component, i.e. $[Fe(bpy)_3](BArF)_2$, into a PMMA alignment layer as an isotropic reference for Raman scattering and to estimate depth confocality in complete liquid crystal cells (Figure 1). The Raman scattering of $[Fe(bpy)_3](BArF)_2$ in the PMMA allows for changes in LC orientation near the interface to be followed and compared with changes to the orientation of molecules in the bulk of the liquid crystal. This approach provides depth spatial resolution of the realignment of LC molecules upon application of electric fields.

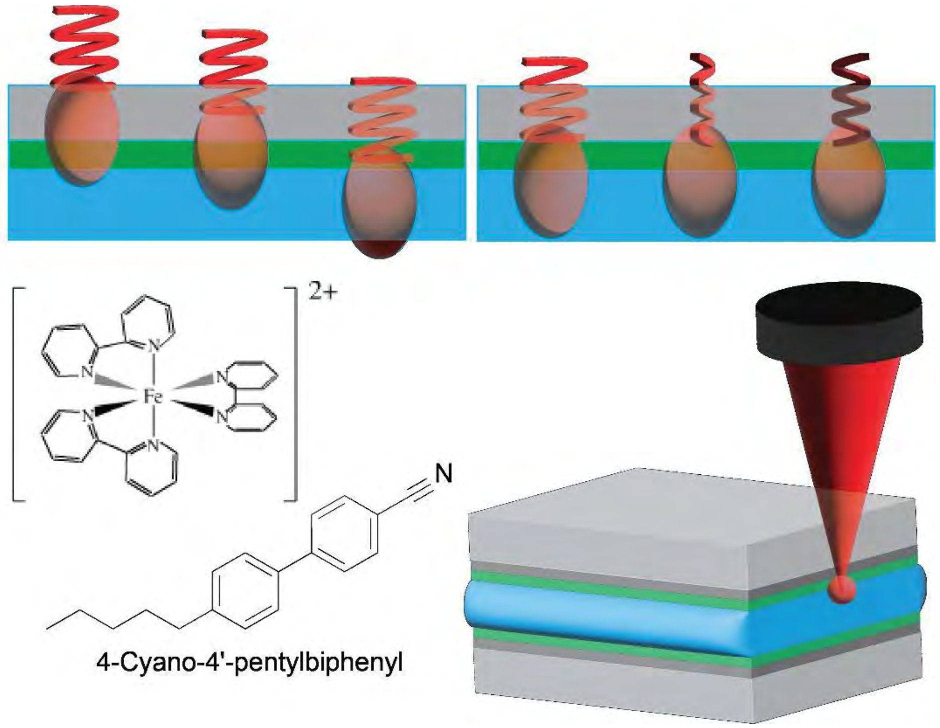

Figure 1: Confocal polarized Raman microscopy on thin layer of polymer with $[Fe(bpy)_3](BArF)_2$ (green) and liquid crystal (blue).

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Molecular structure and film morphology studies in neat films of organic polymeric semiconductors and blends with non-fullerene acceptors

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In recent years, organic semiconductors have attracted significant attention for application in organic photovoltaics (OPVs), due to their attractive properties, such as their light weight, low-cost production, low temperature processing, mechanical flexibility, and abundant availability. Among them, donor - acceptor (D-A) polymers stand out due to their unique advantages [1]. Specifically, PTB7-Th (also known as PCE10), has been one of the most promising donor - acceptor polymers for such applications because of its low bandgap, its favorable HOMO energy level, and its broad absorption spanning 300-800 nm [2]. As the photoactive layer nanoscale morphology plays a critical role in determining the power conversion efficiency, this work takes a holistic approach to correlate the intrinsic molecular structure of each component in a ternary blend of this donor - acceptor polymer (PTB7-Th) with two non-fullerene acceptors (NFAs), to the nanoscale morphology, and together to the phototophysical processes in these blend systems, to gain deeper insights as to the requirements for a more efficient device. We employed Resonance Raman (RR) spectroscopy as a sensitive probe of molecular structure to (a) identify the effect of blending, doping and temperature on the conformation of each component, (b) to probe the effect of NFA side chain substitution, and (c) to assess the effect of thermal annealing treatment. Grazing-incidence wide-angle X-ray scattering (GIWAXS) experiments revealed the extent of crystallinity of the material and the impact of blending and thermal annealing on the film macromolecular structure, complemented by atomic force microscopy (AFM) imaging. We find that blending leads to loss of crystallinity of all the components in the films, which is, however, recovered with thermal annealing, but only for the polymer, leading to phase separation, and affecting the photoexcited species formed as probed by ultrafast transient absorption spectroscopy.

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Deciphering the wavepacket dynamics in TADF molecule using Transient absorption and ultrafast Raman Loss spectroscopy

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Photoinduced charge transfer reactions are ubiquitous in nature [1]. Charge transfer molecules are important for making Organic Light-Emitting Diodes (OLEDs). Recently, the strategy of incorporating heteroatoms such as Boron (B) and Nitrogen (N) in a conjugated carbon-based system (non-polar) can be used to make TADF materials. These charge transfer processes in a molecular system can affect structural relaxation in the excited state. Finding and understanding these non-radiative structural decay pathways are crucial to improving the quantum efficiency of TADF materials. In the present work, we investigate the coherent nuclear dynamics of the excited state of 10-dixyleylboryl phenoxazine (BNO) and the effect of solvent parameters such as polarity, viscosity and polarizability on excited state by femtosecond transient absorption spectroscopy and ultrafast Raman loss spectroscopy (URLS).

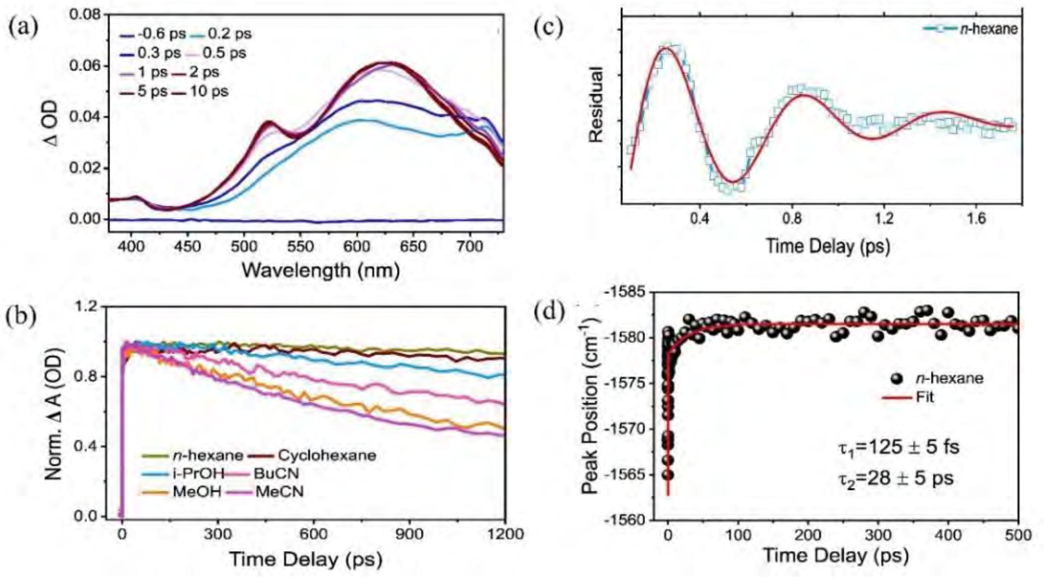

Figure 1: (a) Transient absorption spectrum at 320 nm photoexcitation in n-hexane (b)Normalized kinetics decay in solvents with different polarity (c) Coherent vibrational wave packet and (d) Raman peak position fitting at ~1582 cm^{-1} in *n*-hexane.

The coherent vibrational wave packet is observed in the kinetics trace of the transient absorption signal up to 1.5 ps as shown in Figure 1(c) which shows the real-time structural planarisation of the phenoxazine ring in the BNO molecule. Fourier Transformation of the observed oscillating signal results in low-frequency butterfly vibrations of ~60 cm^{-1} which is responsible for the Planarization of the phenoxazine ring. This work will provide an important reference for interpreting excited state dynamics in phenoxazine and BN-based charge transfer systems.

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Screening and differentiation of Virus-like-particles using Raman spectroscopy

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The COVID-19 pandemic has presented the most significant global health crisis in the past century, resulting in rapid development of vaccines ahead of the anticipated timelines (7-8 years). Viruses exhibit multiple mutations, thereby lowering the potency of vaccines. Virus-like particles (VLPs) that are structurally very similar to viruses but lacking genomic material, have been exploited as candidates for vaccine development. The characterization of these VLPs is crucial to ensure similarity with the original virus and that no transfer of genomic material occurs during immunisation. Different techniques such as PCR, RP-HPLC, SDS-PAGE, and microscopy are extensively used to characterize the virus-like particles, which are laborious, time-consuming, and resource intensive[1].

Raman spectroscopy is a high-precision, label-free analytical technique that work in the chemical fingerprint region of molecules. Vibrational spectroscopy-based studies for VLPs are underexplored. In this work we used Raman spectroscopy to study the chemical composition of virus-like particles in combination with multivariate data analysis methods to improve the differentiation and classification of VLPs. The Raman spectra of Beta and Omicron strain SARS-CoV VLPs when compared with Raman spectra of SARS-CoV 2 virions[2] revealed the missing peaks for RNA at 1242, 1110, 782, 723 and 670 cm-1 missing confirming the formation of VLPs without any genetic material. These studies will result in the quick characterization of VLPs during the production ensuring the development and optimization of safe and effective vaccines in the future.

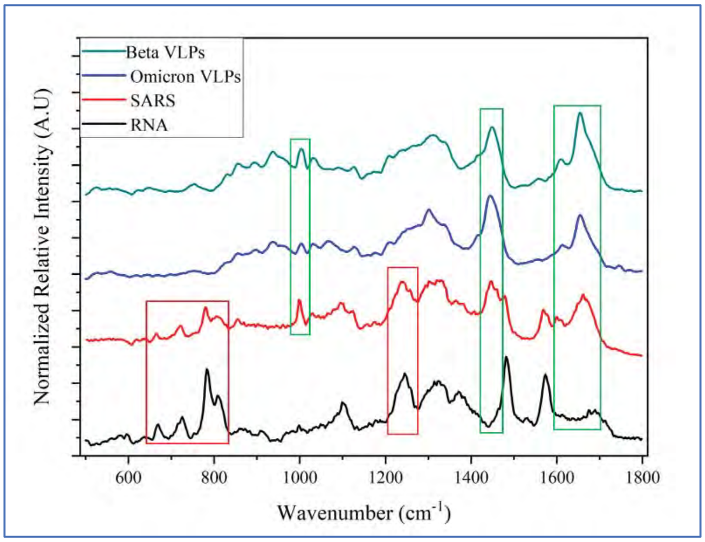

Figure 1: Raman spectra of Beta and Omicron VLPs in comparison with SARS CoV II virus and RNA

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In vivo and in vitro studies of efficient mephedrone adsorption over zirconium-based metal-organic frameworks

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Mephedrone (4-MMC) prevalence, coupled with its low price, makes it one of the stimulants leading to strong psychological and physical addiction, overdose risks, and severe withdrawal symptoms [1]. This work focuses on an investigation of zirconium-based metal-organic frameworks (MOFs) for efficient removal of 4-MMC. Several methods including FTIR, µRaman, 1H NMR, and UV–Vis were used to characterize the 4-MMC adsorption on MOFs. The study showed that the highest 4-MMC adsorption was achieved by NU-1000. The adsorption on this material reaching approximately 45 wt.% from 1000 µM aqueous solution. Moreover, a modulated synthesis of UiO-66 with hydrochloric acid increased the 4-MMC adsorption efficiency from 24 wt% to 35 wt%. The in vitro and in vivo experiments have confirmed that the addition of MOF to the 4-MMC solutions decreases the 4-MMC concentration allowing proper Danio rerio embryo development.

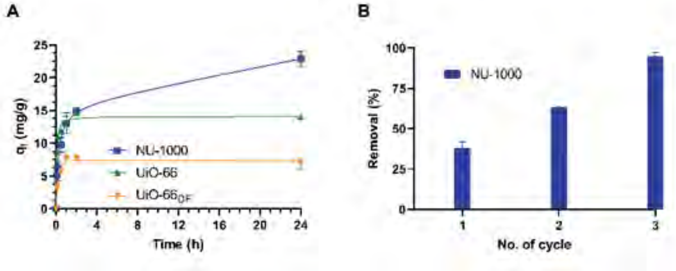

Figure 1: (A) 4-MMC removal efficiency on prepared Zr-MOFs, (B) 4-MMC cascade removal efficiency on NU-1000 sample

Acknowledgement

The work was supported by the National Science Centre, Poland, under the research project “MOF-antidote”: Novel detoxification materials based on metal-organic frameworks for drugs of abuse removal – synthesis, chemical characterization, toxicity and efficacy in in vivo and in vitro studies”, no. UMO-2021/43/B/NZ7/00827.

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Auger-assisted secondary hot carrier transfer in a type I MoS_2 / $PtSe_2$ heterostructure

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Based on the mature understanding of the carrier dynamics in type II heterostructures, many functional materials with excellent electronic and optoelectronic properties have been constructed by stacking two-dimensional materials. However, the dynamics and mechanism of hot carrier transfer in type I heterostructure remains elusive. In this work, we utilized transient absorption spectroscopy to study the carrier dynamics of a type I heterostructure constructed with 1L MoS_2 and 2L $PtSe_2$ (Figure 1). By exclusively exciting the small bandgap $PtSe_2$, we observed clear exciton peaks of MoS_2 that directly probes the primary hot carrier transfer from $PtSe_2$ to MoS_2 within 70 fs, with a quantum efficiency of 34%. Importantly, we also found a secondary charge transfer process at delays of several tens of picosecond, which we attribute to hot carriers that are regenerated in the $PtSe_2$ by intralayer Auger scattering. Moreover, we observed a continuous blue shift of the exciton peaks within 100 ps, which probes the dynamic buildup of an internal electric field across the heterostructure interface. These results are rationalized by coupled rate equation calculations and transient absorption spectra simulations. Our work uncovers two-step (primary and secondary) charge transfer processes and reveals Auger-assisted hot carrier transfer mechanism in type I heterostructures. This finding suggests the possibility for designing optoelectronic and photocatalytic devices by optical sub-bandgap excitation in type I heterostructures made of two-dimensional materials, largely expanding the scope of their applications.

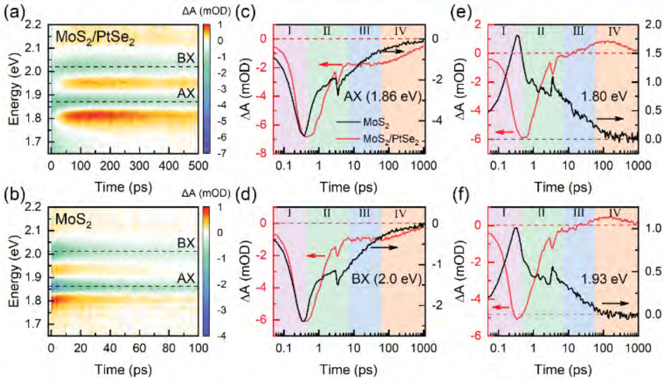

Figure 1: Secondary charge transfer in a type I heterostructure. (a-b) Comparison of TA spectra of the MoS_2/$PtSe_2$ heterostructure with 800 nm pump excitation (a) and of 1L MoS_2 with 400 nm pump excitation (b); (c-d) Dynamics of negative exciton bleaching peaks in MoS_2 (solid black lines) and the MoS_2/$PtSe_2$ heterostructure (solid red lines) probed at 1.86 eV (A exciton) and 2.0 eV (B exciton), respectively; (e-f) Dynamics of the peaks at the low energy sides of the A and B exciton resonances in MoS_2 and MoS_2/$PtSe_2$ probed at 1.80 eV and 1.93 eV, respectively.

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Early screening of oral cancer from human saliva using surface-enhanced Raman plasmonic droplet assay platform

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The emergence of personalized healthcare approaches has motivated the exploration of photonics techniques for analysing body fluids. Optical spectroscopic methods, like Surface-Enhanced Raman Spectroscopy (SERS), stand out for their sensitivity and compatibility with lab-on-a-chip devices [1]. Integration with droplet assay platforms, which compartmentalize samples for individual analysis, is particularly promising for biomarker detection at single-molecule sensitivity even with minimal sample volumes [2]. Herein, a plasmonic droplet assay platform (PDAP) is fabricated by incorporating plasmonic gold nanoparticles selectively onto a non-adhesive quartz substrate prepared via silicone oil grafting. The difference in wettability and adhesive nature of the gold nanoparticle attached region and the silicon oil grafted region facilitate the splitting of a microliter droplet of fluids such as water and saliva into sub-microliter droplets, depending on the dimension of the hydrophilic zone. The SERS performance of the PDAP was demonstrated with standard Raman dyes Rhodamine-6G and Crystal Violet and obtained an impressive limit of detection of 134 pM and 10.1 nM. Further, by using the PDAP, we carried out the SERS analysis of human saliva from over 20 individuals in distinct categories of oral cancer including normal, premalignant, and malignant cases by splitting it across the hydrophilic regions in the wettability contrast. The analysis of saliva samples was based on the distinctive SERS signature primarily associated with amino acids and proteins, facilitating the classification of spectroscopic data into their respective cancer classes. By employing an advanced ML with four types of classifiers such as Nu-Support Vector Classifier (nu-SVC), Random Forest (RF), Adaboost, and k-Nearest Neighbour (kNN), the spectroscopic data was effectively categorized into distinct oral cancer classes. Among the four classifiers, nu-SVC demonstrated exceptional classification performance with accuracy, sensitivity, and specificity values of 93.05%, 93.35%, and 96.48%, respectively. This research reinforces the significance of Raman spectroscopy in investigating the molecular fingerprint of saliva, thereby facilitating its translation for liquid biopsy cancer diagnostics.

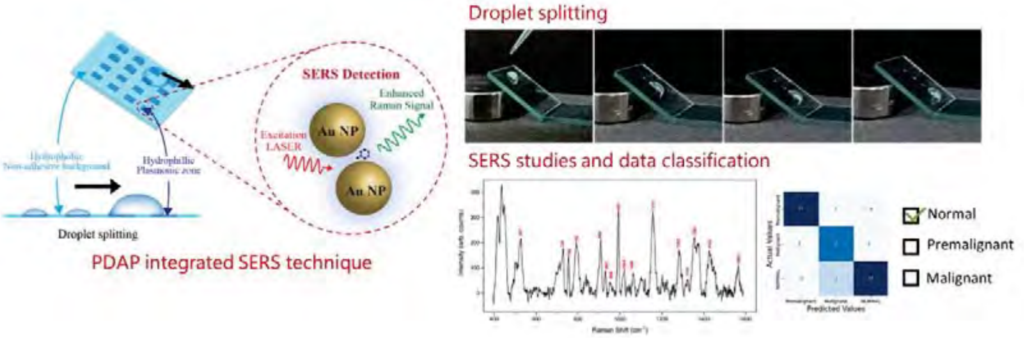

Figure 1: PDAP integrated SERS technique for early screening of oral cancer

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Cobalt-Based Electrochemical UV-SERRS of Interfacial Adenine Structure

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Surface-enhanced Raman spectroscopy (SERS) provides high-sensitivity fingerprint information of molecules adsorbed or near metal (usually Au, Ag, or Cu) nanostructures. Furthermore, many important biomolecules (aromatic amino acids, bases, etc.) have electronic transitions in the UV range, providing an additional resonance Raman enhancement up to 6 orders of magnitude. By integrating UV resonance Raman spectroscopy with SERS, this approach holds the potential for ultra-sensitive biomolecule detection. Such capability minimizes interference from impurities and is particularly advantageous for elucidating the structural and functional characteristics of proteins.

In this study, we explore the structure of adenine at the electrochemical interface of cobalt electrode-buffer solution, employing UV-SERRS with an excitation wavelength of 325 nm. The interaction between adenine and cobalt surfaces under UV excitation results in distinct spectral features that identify adenine's adsorption mechanism and structural dynamics. The study highlights how electrode potential and solution pH influence the interfacial structure of adenine. Shift in the electrode potential from –0.90 to –0.70 V led to a twofold increase in the band intensity at 1336 cm^{-1}, indicating a higher degree of adenine molecules adsorbing onto the cobalt surface. Furthermore, the spectral shifts with varying pH suggest that adenine maintains its interaction with the cobalt surface across a wide pH range, predominantly in its neutral form. This investigation not only broadens our understanding of the UV-SERRS effect but also contributes to the development of novel biosensing platforms with enhanced sensitivity and specificity for nucleic acid detection.

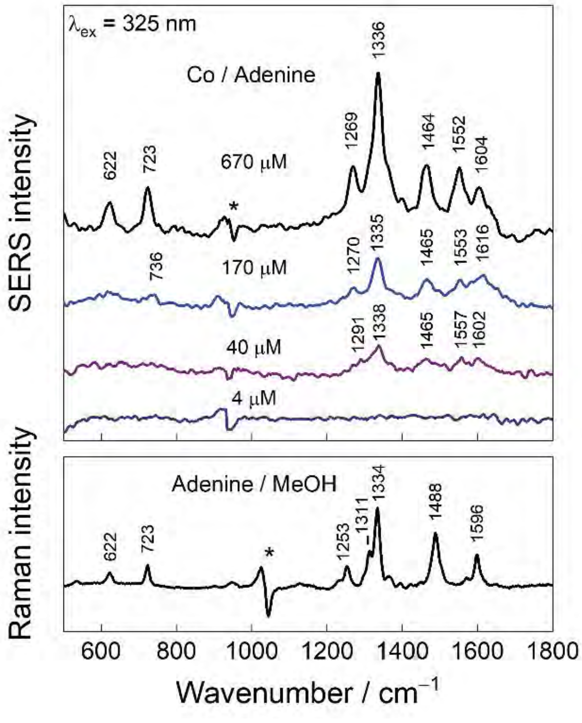

Figure 1: Adenine concentration-dependent UV-SERRS spectra obtained from cobalt electrode (top panel) and Raman spectrum of adenine dissolved in methanol (bottom panel). The excitation wavelength was 325 nm.

This work received funding from the Research Council of Lithuania. The project number is S-MIP-23-30 "Magneto-plasmonic nanoparticles for UV-SERRS detection of low-molecular-weight biomarkers (MAG-UV-SERRS)".

Use of Electrochemical SERS to Investigate Changes of Analgesic-Metal Surface Complexes

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Surface-enhanced Raman scattering (SERS) spectroscopy is a powerful technique for probing molecules adsorbed on plasmonic nano/microstructures. Over several decades, it has transcended its role as merely an analytical tool, offering additional insights into micro-surface chemistry. It has been demonstrated that SERS enhancement is a complex phenomenon influenced by numerous factors. While the primary contributions is caused by electromagnetic mechanism, this study places equal emphasis on the crucial chemical mechanism. In this study, the combination of SERS and electrochemistry (EC-SERS) is utilized to examine the molecular layer. By applying electrode potential, the layer in close proximity to the surface and then the surface complexes are affected. Using EC-SERS, adsorption processes can be investigated *in-situ* and potentially reveal new species. It was demonstrated that this technique is able to study various kinds of molecules adsorbed on the surfaces [1] and affect the creation of the surface complexes [2, 3].

One example of a compound under investigation is acetaminophen, a member of the analgesic drug family. Given that some of these drugs are relatively small molecules found in low concentrations in nature, SERS emerges as a valuable tool for their analysis. This study is aimed on surface complexes formed by acetaminophen and copper. It was found that the molecules do not create only one type of the complex with the metal but applying potential the new products of surface reactions were observed. We believe that this research holds promise for advancing SERS sensors development and deepening our understanding of surface reactions.

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Monitoring Plasmon-Catalyzed Radical C-C Coupling Reaction on Immobilized Noble Metal Nanoparticles

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By the decay of localized surface plasmon resonances (LSPR) on metal nanoparticle (NP) surfaces, hot charge carriers can be generated that can lead to photochemical reactions. In addition to driving the reaction through the generation of hot carriers, the NPs provide high local fields for surface-enhanced Raman scattering (SERS).[1],[2],[3] While a few important model reactions were key to establish the potential of plasmonic catalysis, plasmon driven reactions of molecules that can generate new functionalized and hybrid materials have become an important aspect of recent efforts.
Here, we present the advantage of immobilization of silver- and gold-NP to investigate the radical C-C-coupling reaction of 2-propene-1-thiol (allyl mercaptan) with SERS. Specifically, the plasmonic character of the reaction is evidenced by a dependency of reaction product formation on excitation wavelength (or LSPR) and laser intensity. The formation of the reaction product occurs on silver and gold nanostructures at different excitation wavelengths, in agreement with the LSPR of the respective materials. The rate of the reaction decreases upon lowering laser intensity, due to the production of fewer hot charge carriers. This confirms that the reaction is mediated by localized surface plasmons. From the spectral data, we can infer on the structure of the reaction product and suggest a reaction mechanism that involves hot electrons activating the double bonds of the molecule.

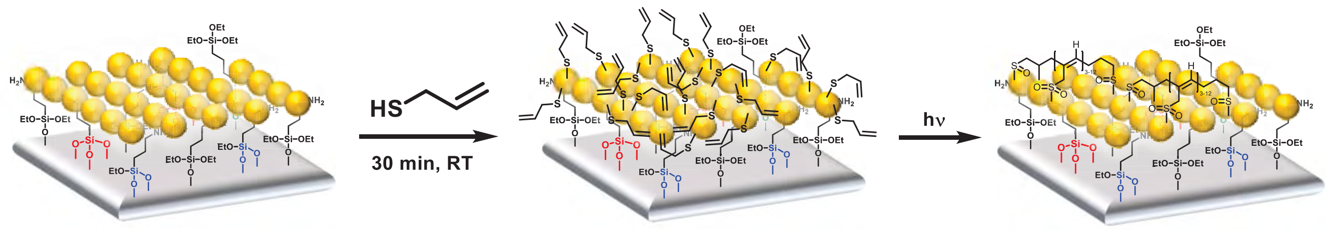

Figure 1: SERS substrate with immobilized AuNP functionalized with 2-propene-1-thiol.
The proposed reaction product on the substrate could be identified from its SERS spectra.

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Exploring the Effects of Labeled Ceramide on the Intracellular Response through Surface Enhanced Raman Scattering

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Ceramide is a lipid involved in several crucial aspects of cellular metabolism, as substrate as well as product, and suggests to be a promising probe to study lipid metabolic enzyme activity and function. We have recently reported that surface-enhanced Roman scattering (SERS) can probe lipid-nanostructure interactions in lipid molecular models containing ceramide and other phospholipids [1, 2]. Labeled by the fluorescent dye NBD (4-Chlor-7-nitrobenzo-2-oxa-1,3-diazol), ceramide can be employed to follow sphingolipid metabolism and localize Golgi apparatus, membranes and vesicles by fluorescence [3].

In this study, we investigated the effect of NBD labeled ceramide in living cells through SERS and machine learning. Fibroblast cells were incubated with gold nanoparticles for different times, followed by exposure to NBD-ceramide or pure NBD. SERS spectra were obtained from living cells, revealing both common spectral patterns and subtle variations among different incubation conditions. Our analysis suggests that the uptake of labeled ceramide or NBD may induce notable cellular changes, potentially affecting the interactions of intracellular molecules with gold nanoparticles. Machine learning techniques are employed to further analyse the SERS data and uncover spectral variations associated with cellular changes prompted by NBD-ceramide or NBD.

The results demonstrate the potential of SERS with gold nanoparticles to probe the intracellular physiological processing of labeled ceramide, which is expected to benefit the further investigation of ceramide in cells. Integrating spectroscopic and computational approaches offers valuable insights into understanding cellular responses and enzyme functions.

Acknowledgments

We thank EC2/BIG-NSE, Germany's Excellence Strategy – EXC 2008 – 390540038 – UniSysCat, and DFG (projects Kn557/16-1 and SE 3319/4-1) for financial support.

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Study of the Matrix Phase Evolution in Nitride Bonded Silicon Carbide Refractories using Raman Imaging

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Optimizing the production of nitride bonded silicon carbide (NBSC) refractories relies on a thorough understanding of the matrix phase composition. These refractories are highly sought-after in high-temperature industrial environments due to their superior thermal and mechanical properties. [1,2] However, the specific composition and distribution of the matrix phases play a critical role in determining their performance and service life.

This work investigates the potential of Raman spectroscopy for identifying and differentiating the individual matrix phases such as alpha (α) and beta (β) silicon nitride (Si_3N_4) or silicon oxynitride (Si_2N_2O). By analyzing Raman images of different parts of nitrided samples, we aim to establish a non-destructive technique to monitor and optimize the production process of NBSC refractories. Additionally, we explore the influence of processing parameters on the relative abundance and distribution of these phases, providing valuable insights for tailoring their properties for specific applications.

This work presents Raman spectroscopy as a powerful tool for understanding and controlling the matrix phase composition in NBSC refractories, ultimately leading to improved production efficiency and the development of next-generation high-performance refractories.

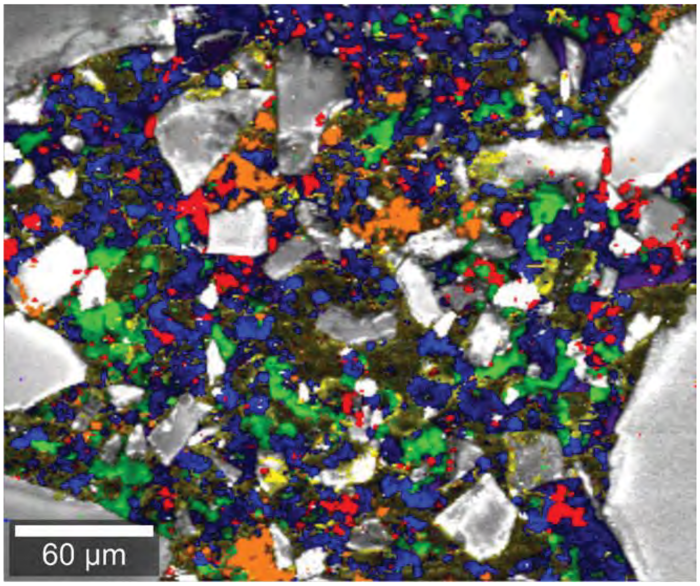

Figure 1: Raman image of the matrix of a NBSC shape, taken on a polished surface. It is possible to clearly distinguish between the different phases. Red: Silicon, blue: α-Si_3N_4, green: β-Si_3N_4, orange: Si_2N_2O, grey: SiC, yellow: epoxy resin

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Development of coherent anti-Stokes hyper-Raman scattering (CAHRS) spectroscopy

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Hyper-Raman (HR) spectroscopy has attracted much attention because it can provide information on molecular vibrations distinct from conventional spectroscopic methods. For example, HR spectroscopy can detect both infrared- and Raman-inactive modes, so-called "silent modes." Despite its potential application, HR studies have been very limited because of its extremely weak signals. HR intensities are about 10^{-5} times weaker than spontaneous Raman under typical experimental conditions. As a result, it takes a long time to obtain spontaneous HR spectra with high signal-to-noise ratios, at least several hours. To overcome this difficulty, previous studies proposed a coherent anti-Stokes hyper-Raman scattering (CAHRS) process that combines the HR process with a coherent Raman process [1,2]. In this study, we successfully detected CAHRS signals for the first time [3].

Figure 1 shows diagrams of the spontaneous HR and CAHRS processes. In the CAHRS process, electric fields with frequencies of ω_1 and ω_2 induce a vibrational coherence with a frequency of $\Omega=2\omega_1-\omega_2$. Then, an additional electric field with a frequency of ω_1 generates fifth-order nonlinear optical polarization with a frequency of $4\omega_1-\omega_2$ that emits a CAHRS signal.

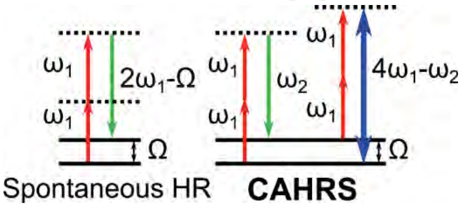

Figure 1: Diagrams of spontaneous HR and CAHRS processes.

Figure 2 shows our CAHRS spectrometer. A femtosecond Yb: KGW laser was used as a light source to generate a narrowband ω_1 beam (wavelength: 1030 nm, pulse width: 1 ps, power: 60 mW) and a tunable ω_2 beam (530~560 nm, 200 fs, 5 mW). Two beams were focused into samples at angles satisfying the phase matching conditions.

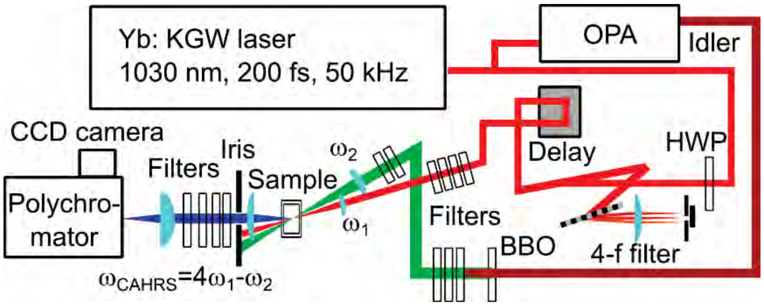

Figure 2: Schematics for our CAHRS spectrometer.

Figure 3a shows raw spectra of para-nitroaniline (PNA) in $CHCl_3$ and neat $CHCl_3$ from 1200 to 1400 cm^{-1}. Figure 3b is the normalized spectrum with a non-resonant background signal from neat $CHCl_3$, showing an asymmetric and dispersive lineshape around 1330 cm^{-1} due to the NO_2 stretching modes of PNA. Our result suggests that CAHRS spectroscopy provides information equivalent to spontaneous HR spectroscopy.

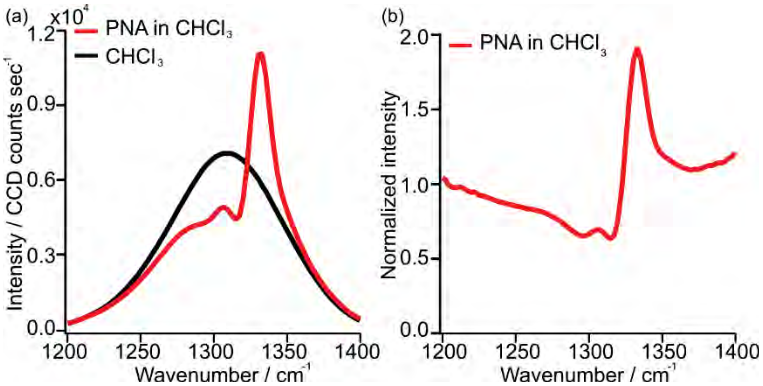

Figure 3: (a) Raw spectra of PNA solution (red) and neat $CHCl_3$ (black). (b) Normalized spectrum of PNA solution.

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In situ monitoring of cell surface proteins using SERS platforms

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Cell surface proteins (CSPs) are key indicators of cellular properties and play a pivotal role in numerous biological functions. Some of them are used as important biomarkers for disease diagnosis. However, existing CSP detection methods often lack selectivity and lack in situ analysis capabilities, despite preserving spatial information between cells. We will present the development of nanoprobes for surface-enhanced Raman scattering (SERS) immunoassays. SERS nanoprobes are designed to conjugate specific antibodies to silica-coated gold nanoparticles containing individual Raman reporters. By applying our SERS immunoassay to multiple HEK293 cell lines expressing different levels of the ACE2, we succeeded in statistically distinguishing ACE2 expression levels. In addition, our system accurately determined the levels of epithelial CSPs (EpCAM and E-cadherin) in both fixed and living cells without cytotoxicity. Our findings provide valuable insights into the development of a versatile biosensing platform that can be applied to various biomedical fields.

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SERS-based diagnosis of a urinary tract infection using magnetic beads immobilized with gold nanoparticles

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The current gold standard method for diagnosing urinary tract infection (UTI) is urine culture. However, this method takes at least two days to obtain assay results. Usually, it requires additional complicated steps, such as biochemical tests, polymerase chain reaction (PCR), or mass spectrometry, to confirm whether the isolated strain is the pathogen of interest [1,2]. We developed magnetic beads immobilized with gold nanoparticles (MB-AuNPs) as a surface-enhanced Raman scattering (SERS) substrate to address these issues. The MB-AuNPs offer several advantages, including excellent magnetic properties that simplify the assay procedures and good SERS properties resulting from densely packed AuNPs. Using these MB-AuNPs, we performed a sensitive and rapid SERS-based assay to detect the most common bacteria causing UTI, *E. coli*.

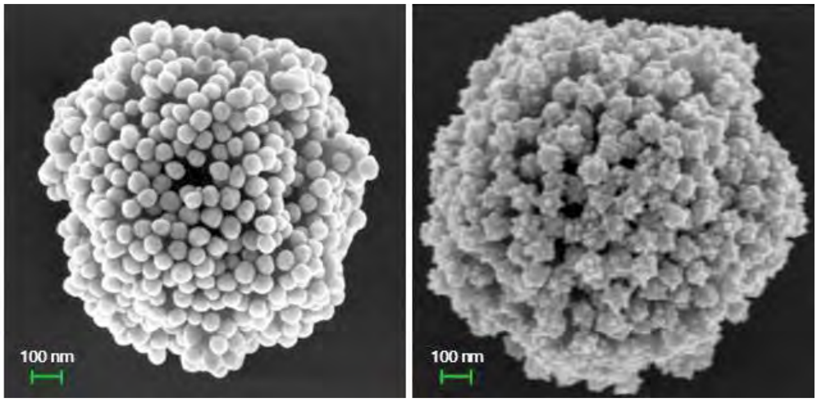

Figure 1: SEM images of magnetic bead immobilized with (a) gold nanospheres and (b) gold nanoflowers

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SERS-based acoustofluidic chip for rapid and sensitive detection of UTI-causing bacteria

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Urinary tract infection (UTI) is a bacterial infection in the urinary tract that triggers an inflammatory response, affecting 50-80% of all women at least once in their lifetime. Proper treatment of UTI requires promptly identifying the causative bacteria and prescribing appropriate early-stage antibiotics. Diagnosis methods for UTI encompass urine culture and molecular diagnosis. In the urine culture test, a small amount of bacteria in a patient's urine sample is cultured over several days to ensure a sufficient bacterial concentration. UTI is diagnosed when more than 10^5 CFU/mL of bacteria grow, determined through colony counting. Molecular diagnosis involves obtaining an adequate bacterial concentration through liquid culture. The bacteria undergo lysis, and the extracted genes are amplified to identify the bacteria and determine their concentration. However, urine culture tests require several days for culturing, and molecular diagnostic methods require a cumbersome cell lysis process and extended gene amplification time. Therefore, we have developed a fast and sensitive SERS-based acoustofluidic chip that eliminates the need for bacterial culture and lysis. Applying ultrasonic waves to the fluid inside the microfluidic channel allows for the separation of particles based on size [1]. Specific receptor-conjugated SERS nanotags and non-lysed bacteria react in a microtube and are then injected into the microfluidic channel for UTI-related bacteria detection. When ultrasonic waves are applied to this channel, bacteria densely flow to the center, and upon laser irradiation at this position, a strong SERS signal can be detected from the SERS nanotags attached to the surface of the bacteria. This acoustofluidic chip enables the detection of UTI-related bacteria within a few minutes, eliminating the need for complicated culturing and lysis processes.

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Electrophoresis-coupled SERS-LFA strips for the accurate separation and sensitive detection of sample matrix

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Lateral flow strips (LFA) often encounter either false-positive or false-negative issues, due to the complexity of the clinical sample matrix and the limited detection sensitivity of the colorimetric assay. Consequently, they cannot provide reliable results for early clinical diagnosis [1]. Therefore, we developed an accurate and sensitive electrophoresis-coupled surface-enhanced Raman scattering (SERS)-LFA strip to address these inherent issues. Size- and charge-dependent electrophoresis can effectively separate our targets (human immunoglobulin G and bovine serum albumin) from the complex matrix. Besides, by controlling the migration of the sample, targets can accumulate to be test zones on our constructed LFA strip without any receptor. The coupling of the electrophoresis function also helps mitigate the false-positive problem (non-specific binding or cross-reaction). Furthermore, electromagnetic hotspots caused by localized surface plasmon resonance (LSPR) are generated in the SERS nanotags, providing highly sensitive SERS detection capability and yielding precise results. We believe that electrophoresis-coupled SERS-LFA strips are poised to become a new LFA strategy that can accurately and sensitively detect trace amounts of targets in complex clinical samples.

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Development of a wash-free detection of DNA using Marangoni force-induced three-liquid-phase gold nanoparticles self-assembly

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Since the COVID-19 pandemic, the need to develop fast and accurate diagnostic methods has emerged. Many existing diagnostic methods require washing steps, which has the disadvantages of requiring time to perform and decreasing the accuracy of the diagnosis. We have developed a wash-free detection method that can separate unreacted DNA without washing steps. A very dense gold nanoparticle monolayer substrate is created using the Marangoni force-induced three-liquid-phase self-assembly method by the difference in surface tension of the three liquids[1]. During the formation of the gold nanoparticle monolayer, capture DNA is attached to the gold nanoparticles, and a Raman reporter is placed between the gold nanoparticles using the modified detection DNA. In the three-liquid self-assembly method, the unreacted DNA is not placed between the gold nanoparticles, and the reacted DNA is located between the gold nanoparticles. This method is expected to enable highly precise quantitative analysis by obtaining strong SERS signals from robust hotspots formed in very narrow nanogaps.

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Analysis of Separators for Li-S Batteries Using PHBHx

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The increasing demand for batteries due to the growth of the electric vehicle market has led to the need for research on batteries that improve battery performance and reduce environmental pollution caused by waste batteries after use. Among them, lithium-sulfur batteries (LSBs) are attracting attention as a next generation of battery due to their theoretical high specific capacity and energy density, low cost, and abundant resources. However, it is crucial to improve them due to the permanent capacity reduction caused by the elution of lithium polysulfide that occurs during the cycle.[1]

In this study, to reduce environmental pollution caused by waste batteries, we fabricated separators using poly(hydroxybutyrate-*co*-hydroxyhexanoate) (PHBHx), an eco-friendly polymer that biodegrades in soil and seawater, and investigated their applicability to LSBs. The separators were prepared by electrospinning two types of PHBHx with different ratios of Hx. Their chemical properties at the molecular level were investigated using IR and Raman spectroscopy. The details of the results will be discussed in this presentation.

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Raman spectroscopic analysis of aerobic *Bacillus* and anaerobic *Clostridium* species

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The Gram-positive endospore-forming Bacilli and Clostridia are globally distributed and either thrive in an aerobic or an anaerobic environment. Most *Bacillus* species are innocuous, exceptions such as *B. anthracis* (causative agent of Anthrax) and *B. cereus* (responsible for food poisoning) merit attention. In contrast, the *Clostridium* genus displays remarkable diversity, featuring pathogenic strains including *C. perfringens* (linked to gas gangrene), and *C. difficile* (associated with antibiotic-associated diarrhoea and pseudomembranous colitis)[1, 2]. In the medical industry, the imperative for swift and dependable detection methods targeting pathogenic *Clostridium* species. In this context, Raman spectroscopy is applied to delineate classification based on phylogenetic relatedness, growth conditions, and their capacity to produce endospores[3]. The focal point lies in the systematic compilation of an extensive Raman spectra library derived from both vegetative cells and endospores of pathogenic and non-pathogenic strains within the *Bacillus* and *Clostridium* genera.

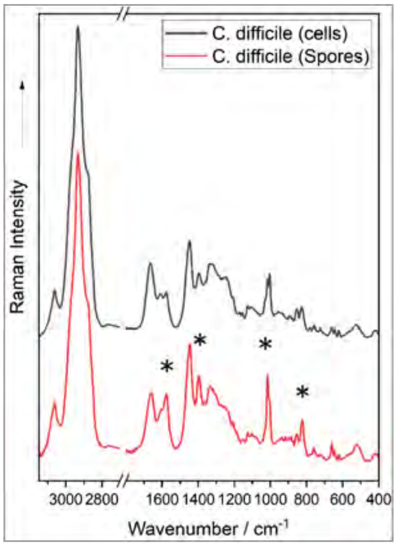

Figure 1: Mean Raman spectra of vegetative cells and endospores of *C. difficile*. *CaDPA

Acknowledgments

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Understanding Equilibrium of Cysteamine on Metal surface with Surface-enhanced Raman Scattering in Various Solvent

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Nanomaterials can be dispersed in various solvents depending on the properties of their components or surfaces, with water and ethanol being among the most used solvents. There are primarily two methods for modifying the surface of nanomaterials: vapor-phase and solution-phase modifications, both of which can rapidly alter the surface properties of nanomaterials. The solution-phase approach, in particular, is known for its ability to create self-assembled monolayers (SAMs) on surfaces where the orientation or structure of the adsorbed molecules can vary depending on the solvent used. While there are many research focusing on the structural changes of adsorbed molecules, the majority of these studies have been conducted in single solvents like water, with a noticeable lack of study into the behavior of molecules in various organic solvents.

This study investigates the structural changes of cysteamine molecules adsorbed on nanoparticle surfaces in different solvents using Surface-Enhanced Raman Scattering (SERS). Cysteamine is known to adsorb onto metal surfaces in two configurations: gauche and trans. A significant difference in the distribution of these structures was observed between acetonitrile and water, with the gauche configuration being more stabilized in water due to hydrogen bonding interactions. The SERS signals of cysteamine in four different solvents-acetonitrile (ACN), isopropyl alcohol (IPA), ethanol (EtOH), and water (H_2O)-were examined, revealing that nanoparticles with high enhancement factors could exceed the Raman cross-sections of solvents. The study aimed to explain the variation in cysteamine's structural ratio across different solvents in terms of hydrogen bonding. It was found that structural changes in mixed solvents occurred over a significantly longer timescale compared to adsorption alone. This research not only elucidates the behavior of short-chain molecules in a range of organic and mixed solvents but also demonstrates the feasibility of SERS measurements in non-aqueous environments.

Effect of halogen substitution on the mechanisms of phase transitions probed by vibrational spectroscopy

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Organic-inorganic halide hybrids based on bismuth ions have drawn ongoing attention in the scientific community because of their ferroelectric, optoelectronic, luminescent, semiconducting, and dielectric properties [1-3]. There is a variety of halide hybrids composed of halogenobismuthate anionic 3D-1D subnetworks, but recently, a novel class of 0D materials with the general formula $[A]_3BiX_6$ (A^+ = organic cation, X^- = halide ion) has been described as a group capable of creating polar structures [4,5].

Furthermore, it has been demonstrated that the selective fluorination of the ethylammonium cation (EA^+) at position 2 has a significant effect on the properties, including the onset of polar order, an increase in dielectric response, and the rise in phase transition temperature [5]. The effect has been attributed to the increased dipole moment of the 2-fluoroethylammonium cation (FEA^+). As the mechanism of halogenation appears much more complex, we decided to undertake a systematic comparative study of the complete set of halogenated analogues of chlorides and bromides.

In this study, we investigated the phonon properties of a zero-dimensional (0D) hybrid organic-inorganic bismuth chlorides and bromides templated by ethylammonium cation and their fluorinated, chlorinated (CEA^+), and brominated (BEA^+) counterparts. The detailed analysis of the thermal evolution of the spectra was used to identify the primary driving forces responsible for the nature and temperatures of the phase transformations that occurred. Because of the existence of ordering processes in the studied crystals, Raman and IR spectroscopy are excellent techniques for evaluating changes in the molecular dynamics of organic cations, hydrogen bond networks, and anionic sublattice distortion.

A better knowledge and deeper understanding of the structural properties that determine whether polar order may exist in this class of halides is especially important for the purposes of possible future applications.

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Differentiation of antimicrobial resistance groups via label-free Raman spectroscopy

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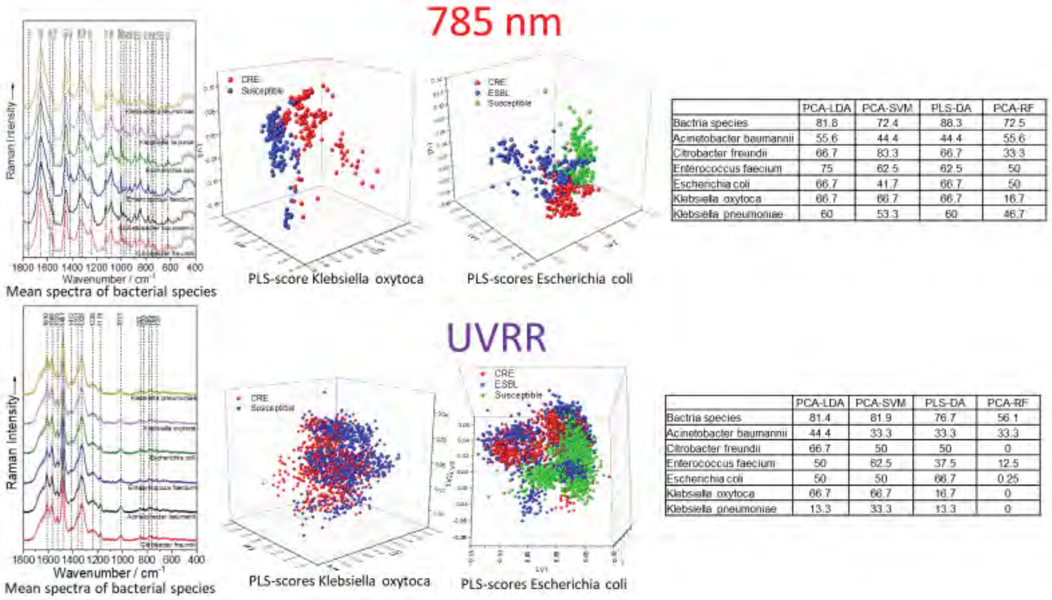
Over the past decades, antimicrobial resistance (AMR) has developed into a major health issue. A need exists for fast and inexpensive clinical laboratory methods that can provide strain-specific AMR-information as rapid as possible [1]. Here it is attempted for the first time to determine bacterial resistance groups of clinical isolates using Raman spectroscopy without previous exposure to antibiotics. Two Raman methods were used, UV-resonance Raman spectroscopy (UVRR) using 244 nm excitation and Raman fibre probe directly on the culture dish using 785 nm excitation. A total of 56 clinical isolates of the species *Acinetobacter baumanii*, *Escherichia coli*, *Citrobacter freundii*, *Enterococcus faecium*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* were analysed. In the Gram-negative isolates, 3 resistance groups were discriminated, namely susceptible, Extended-spectrum beta-lactamases producers (ESBL) and carbapenem-resistant Enterobacterales (CRE). The *Enterococcus faecium* isolates were vancomycin susceptible (VSE) and vancomycin resistant (VRE).
In the data analysis, first a classification of the species was performed, followed by classification of the resistance group in each species separately, using different chemometrics methods. Results are shown in Figure 1. These first results show that Raman spectroscopy has the potential to discriminate resistance groups of pathogens in a label-free way, providing results fast and without the need for expensive consumables.



	PCA-LDA	PCA-SVM	PLS-DA	PCA-RF
Bactria species	81.8	72.4	88.3	72.5
Acinetobacter baumannii	55.6	44.4	44.4	55.6
Citrobacter freundii	66.7	83.3	66.7	33.3
Enterococcus faecium	75	62.5	62.5	50
Escherichia coli	66.7	41.7	66.7	50
Klebsiella oxytoca	66.7	66.7	66.7	16.7
Klebsiella pneumoniae	60	53.3	60	46.7

	PCA-LDA	PCA-SVM	PLS-DA	PCA-RF
Bactria species	81.4	81.9	76.7	56.1
Acinetobacter baumannii	44.4	33.3	33.3	33.3
Citrobacter freundii	66.7	50	50	0
Enterococcus faecium	50	62.5	37.5	12.5
Escherichia coli	50	50	66.7	0.25
Klebsiella oxytoca	66.7	66.7	16.7	0
Klebsiella pneumoniae	13.3	33.3	13.3	0

Figure 1: Classification results of resistance groups

Acknowledgments

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Gold nanoparticles prepared in HEPES buffer as SERS-active substrates

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Surface-enhanced Raman scattering (SERS) is a vibrational technique that provides information about the structure of molecules located very close to a rough metal surface [1]. The most commonly used metal substrates are nanoparticles dispersed in a colloidal suspension or immobilized on a solid support. Colloidal suspensions are usually prepared by reducing metal salts with analytes, which can act not just as reducing agents but also as stabilizers of metal nanoparticles, preventing the oxidation and aggregation of colloidal particles [2].

In this research, SERS substrates were prepared by reduction of tetrachloroauric(III) acid with 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES buffer) [3], with and without adding silver nitrate, which acts as a shape-directing agent in gold nanoparticle synthesis. Stability and SERS efficiency of prepared colloids were tested using rhodamine 6G (R6G) and (E)-1,2-di(pyridin-4-yl)ethene (BPE) as model molecules. Also, for both prepared colloidal solutions, the influence of the addition of different polymer molecules, such as polyvinylpyrrolidone, cetyltrimethylammonium bromide, and poly-L-lysine, on the stability of the metal nanoparticles and the enhancement of light scattering were investigated.

In all prepared metal substrates, the strongest SERS effect was found for the BPE molecules, most likely due to their lack of charge and optimal structural features. Unlike BPE, positively charged rhodamine 6G molecules were electrostatically repelled from the gold nanoparticles by HEPES molecules since the buffer molecules were located on the metal surface in the form of zwitterions, stabilizing the nanoparticles. Adding the silver nitrate during colloid synthesis resulted in a more efficient SERS substrate, probably due to the formation of nanoparticles with different morphologies that scattered radiation more efficiently compared to the colloid obtained only from metal salt and buffer. By introducing the polymers into the system, a steric barrier was created between the nanoparticles and the investigated molecules, so the obtained spectra of the model molecules showed very weak bands. However, such modified colloidal nanoparticles were more stable over time compared to gold colloids prepared without polymer molecules.

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Illuminating drug delivery: Localization and characterization of polymer-based nanoparticles in fibrotic liver cells

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A promising way to address liver fibrosis is to utilize functionalized polymer-based nanoparticles (NPs) to target the natural vitamin A receptor of liver cells, STRA6 (stimulated by retinoic acid 6). Those NPs are designed to encapsulate drugs in the necessary concentration to inhibit the signalling pathway inside the hepatic stellate cells that are responsible for inflammation.

To investigate the NPs within cells and tissue, molecular selective microspectroscopic techniques are well suited. Here, we report about the application of Raman microscopy (Fig.1a), confocal fluorescence microscopy (Fig.1b), and coherent anti-Stokes Raman scattering (CARS) microscopy (Fig.1c) to study the localization of NPs in liver cells and tissue. Furthermore, Raman spectroscopy in combination with two-dimensional correlation analysis (2DCOS) is utilized for the characterization of polymers, NPs, and drugs prior to their analysis in biological environments. Such a detailed Raman spectroscopic analysis of the polymers and the corresponding NPs allows for the identification of characteristic vibrations that enables a label-free localization of the NPs by CARS microscopy within cells. Fluorescence-lifetime imaging microscopy (FLIM) providing insights into the release of the encapsulated drugs out of the NPs has also been applied (Fig.1d).

These microspectroscopic approaches are aimed for a spatiotemporal localization of the NPs and is supported by tailor-made image analysis routines to further characterize the NP uptake processes and induced metabolic influence.

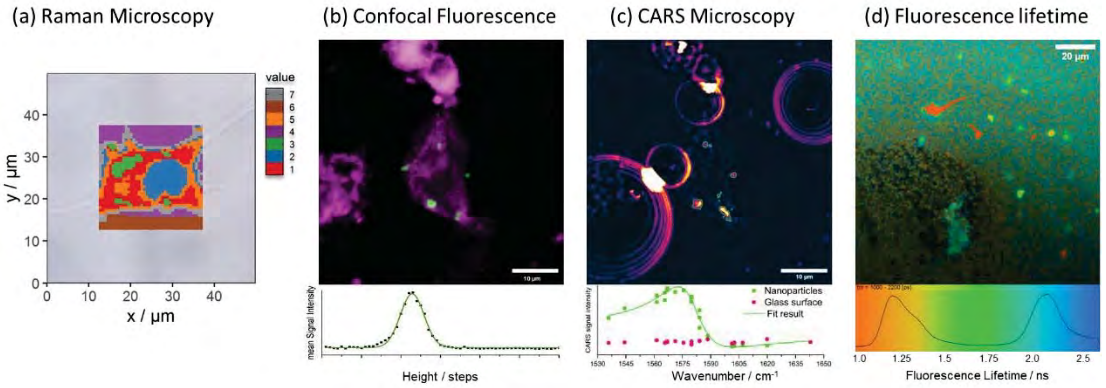

Figure 1: A variety of linear and non-linear modalities can be utilized to localize and characterize polymer-based nanoparticles inside of cells and tissue.

ACKNOWLEDGMENTS

This study was funded by the German Research Foundation (DFG) through the Collaborative Research Centre PolyTarget 1278 “Polymer-based nanoparticle libraries for targeted anti-inflammatory strategies” (project C01) under DFG project number 316213987.

Aptamer-based detection schemes in SERS for the detection of pollutants in water

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Water pollution has become a major global environmental concern and due to the toxicity of pollutants they seriously harm both the natural environment and public health. SERS is known as powerful tool to address the analytical need in water and environmental analysis.[1] We aim the development of an innovative detection method for pollutants in water using SERS in combination with aptameric microfluidic sensors. Due to optical, electrochemical features aptamer-conjugated AuNPs gained huge attention for designing of biosensing systems.[2] Three standard chemicals are under investigation, i.e. carbamazepine, diclofenac and benzotriazole, which represent contaminations in wastewater. In order to target these chemicals, SERS-active surfaces are modified with aptamers (single-stranded DNA molecules) which are specific toward their respected target, as shown in Figure 1(a). Thus, these molecules can be identified in low concentrations accompanied by a high specificity. Within this presentation, we will introduce our SERS platform to be applied in water analytics, which is a microfluidic platform with incorporated gold nanoparticle arrays, as illustrated in Figure 1(b). The recorded SERS spectra are dominated by the contribution of the aptamer molecules, i.e. the DNA bases, and it is expected that their SERS response is altered upon interaction with their target due to a changed orientation towards the metallic surface.

Figure 1 (a) Aptamer-modified SERS substrate. **(b)**Microfluidic chip.

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Microwave-Assisted Fabrication of Ag-NP Decorated TERS Tips with Enhanced mechanical Stability for Investigations in liquid environments

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There is a demand for efficient TERS tip fabrication as due to the delicate nature TERS tips are prone to damage or contamination during experiments. A microwave-assisted (MW) procedure, initially developed for stable silver nanoparticle (Ag-NP) SERS substrates,[1] is adapted to create Ag-NP decorated TERS tips. Briefly, commercial Si cantilever tips are coated with a thin layer of Ag salt, which is reduced by controlled EtOH evaporation through MW heating. The resulting TERS tips yield a dense layer of Ag-NPs with diameters ranging from 20 to 40 nm. Verification of the mechanical stability of these fabricated TERS tips is conducted through Scanning Electron Microscopy (SEM), confirming both reproducibility and sharpness. Subsequent evaluation of TERS performance involves the application of a monolayer of 4-nitrothiophenol (4-NTP) on gold (Au) nanoflakes. MW tips manifest strong enhancement capabilities and a prolonged tip lifetime in liquid environment compared to Ag-NP decorated tips produced by physical vapor deposition (PVD). These compelling findings underscore the potential for advancing the reliability of liquid TERS investigations, and addresses current limitations of rapid TERS tip degradation, such as AgNP peeling off due to the low adhesion.

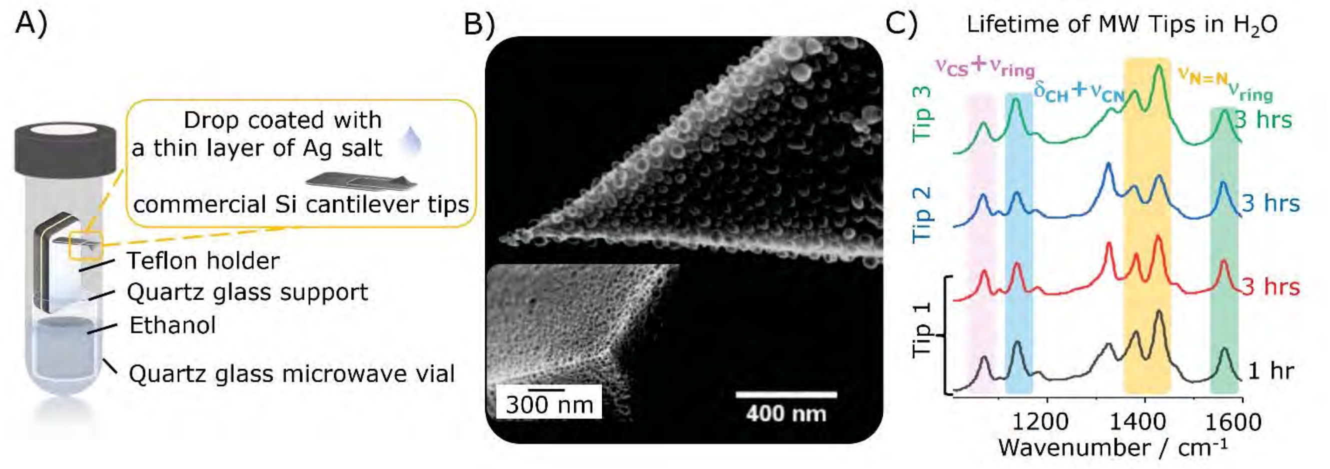

Figure 1: A) Schematic illustration of microwave (MW) based TERS tip fabrication; B) SEM images of a MW TERS tip; C) TERS spectra of 4-nitrothiophenol (4-NTP) on gold (Au) nanoflakes confirmed the prolonged lifetime of MW-tips in H_2O

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Discrimination of K1 and K2 serotypes of *Klebsiella pneumoniae* by Raman spectroscopy

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K1 and K2 serotypes of *Klebsiella pneumoniae* (Kp) are predominant in invasive infections considered highly virulent to humans, exhibiting elevated mortality rates worldwide [1]. Ensuring timely antibiotic treatment is crucial to minimize mortality and morbidity. However, traditional methods require time for sample preparation, lack routine serotyping, and may yield subjective results. In recent years, Raman spectroscopy has emerged as a promising tool for the identification of microorganisms, reducing the reliance on traditional methods that require a culture step. This work aims to explore the potential of Raman spectroscopy in combination with machine learning tools to differentiate between K1 and K2 serotypes of Kp.

Sixteen Kp isolates were examined: K1 (n=7) and K2 (n=9). Bacteria were plated on Columbia blood agar and incubated overnight. The bacterial colonies were suspended in NaCl 0.9%, and three 30 µL drops of the suspension were placed on a microscope slide wrapped in aluminium foil and left to dry for 30 minutes. We collected between 60 and 150 Raman spectra of each isolate using a JASCO NRS-4500 confocal microscope (532 nm).

The quality of the Raman spectra was improved by a preprocessing step (Fig. 1a). A 1-D convolutional neural network (1-D CNN) was trained using the preprocessed spectra, focusing on the model's capability to generalize to new unknown data. This resulted in an accuracy of over 95% (Fig. 1b). Finally, we identified the essential molecular information that the model uses to differentiate between K1 and K2 serotypes.

Raman spectroscopy combined with deep learning was able to correctly distinguish between the K1 and K2 serotypes of *K. pneumoniae*.

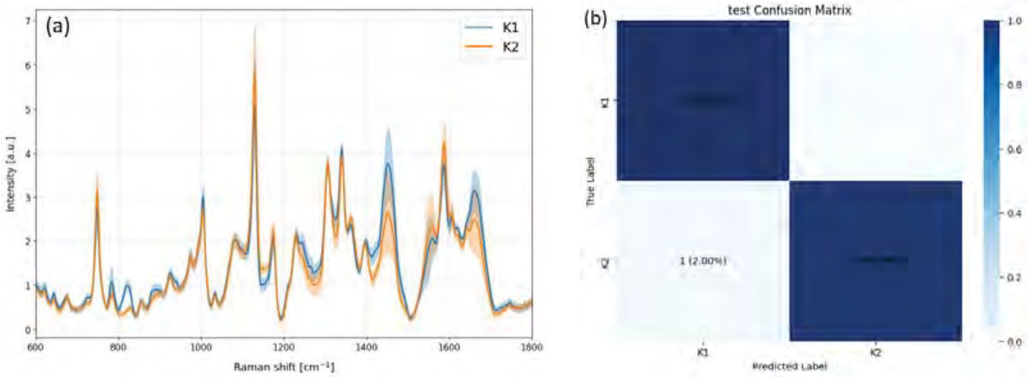

Figure 1: Average preprocessed Raman spectra for K1/K2 serotypes of *K. pneumoniae* with their variance bands (a) and confusion matrix obtained from a 1-D CNN trained with the preprocessed Raman spectra of K1/K2 serotypes of *K. pneumoniae*. An accuracy of 98% is achieved (b).

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Towards plasmon-enhanced Raman spectroscopy of single molecular adsorbates with defined surface orientation

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Surface- and tip-enhanced Raman scattering (SERS/TERS) are capable of detecting single molecules via very high local electric fields that are generated by optical excitation of metal nanostructure supporting localized surface plasmon resonances. The observation of single molecules over extended periods of time necessitates their immobilization on substrates to minimize Brownian motion. Triphenyl (trityl)-substituted carbon centers, also termed molecular tripods, fulfill this requirement: i) The three phenyl substituents contain sulfur atoms for strong multivalent binding to gold surfaces and ii) the fourth ligand attached to sp^3-hybridized central carbon atom, also termed tail group, has a well-defined perpendicular orientation to the gold surface.[1]

To this end, we have synthesized and characterized a molecular tripod with three acetyl-protected sulfur atoms in *meta* position to the central carbon center and an alkyne tail for spectroscopic analysis. The terminal alkyne moiety exhibits a strong and isolated Raman peak in the Raman-silent region around 2100-2300 cm^{-1}.[2] Furthermore, for the analysis of other tail groups, the alkynes can be converted into other moieties via click chemistry.

We present preliminary Raman-spectroscopic results of this molecular tripod adsorbed on gold surfaces. We compare conventional Raman scattering of the tripod in solution with nanogold-based SERS in order to confirm chemisorption through vibrational spectroscopic differences. Monolayers of the tripod on Au(111) single crystals were prepared for spectroscopically probing the surface orientation of the alkyne tail with both conventional Raman scattering and TERS.

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Integrated SERS-based microdroplet platform for marine biotoxin detection in water

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Marine biotoxins are molecules naturally produced during harmful algal blooms (HABs). These biotoxins can accumulate in marine organisms, e.g. shellfishes, and migrate along the food chain [1]. For that, HABs pose a challenge regarding food safety and one of the best strategies to reduce the risk in human health is through establishing methodologies for their in situ monitoring [2].

To address this challenge, we propose to develop a microdroplet-sensing platform combined with a high sensible surface-enhanced Raman scattering (SERS) detection. This integrated SERS-based microdroplet platform presents several advantages. On one hand, SERS measurements can be carried out in nanoliter volume in an automatic and reproducible manner; on the other hand, SERS integrated with microdroplet device allows an ultra-fast multiplexing detection in small volumes [3]. Thus, this platform can dramatically improve the sensitivity and selectivity for marine biotoxin detection.

SERS substrates based on covalent organic polymers (COPs) and/or covalent organic frameworks (COFs) with gold nanoparticles (GNPs) were selected according with their capacity to adsorb a specific biotoxin family (i.e. PSP, DSP). We successfully encapsulated these SERS substrates into microdroplets generated using a T-junction generator device. A long mixer after microdroplet generation was integrated to facilitate the interaction between the biotoxins and the SERS substrate. Finally, we optimized SERS measurements parameters, such as time acquisition, laser power and detection module, using Raman reporting molecules with similar physicochemical properties of biotoxins, but higher Raman cross-section. Initial results have proven encouraging in encapsulation and detection (1μM), paving the way for efficient and accurate detection of biotoxin trace levels in sea water.

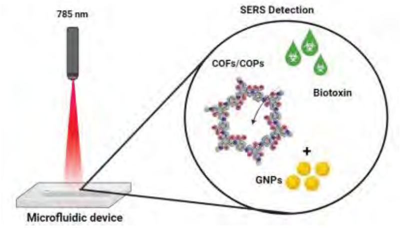

Figure 1: Schematic representation of marine biotoxins detection through a SERS-based microdroplet system

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Raman spectroscopy – a valuable tool in studies of soft lead halide perovskites

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Hybrid organic-inorganic lead halide perovskites have attracted great interest in recent years due to their excellent optoelectronic properties that make them attractive for solar-cell, light-emitting, nonlinear optical, switchable dielectric and ferroelectric applications [1-3]. Properties of these compounds are modified through electron-phonon coupling and change in the lattice dynamics [4,5]. Furthermore, some of these properties, like ferroelectricity or second-harmonic generation, are induced by specific ordering of the organic moieties [2,3]. It is therefore of great interest to study phonon properties, monitor lattice dynamics and understand mechanism of structural phase transitions in these soft perovskites. We will show that temperature-dependent Raman studies of $BA_2MA_2Pb_3I_{10}$ (BA=butylammonium; MA = methylammonium), which undergoes two structural phase transitions, provide information that ordering of BA^+ (MA^+) cations triggers the structural phase transition observed at higher (lower) temperature. Analysis of the low-wavenumber range also shows that this ordering is associated with significant deformation of the inorganic sublattice. We will also show that Raman spectroscopy gives deep insight into mechanism of the phase transitions observed in ethanolammonium lead iodide, which cannot be derived using single-crystal X-ray diffraction due to reconstructive character of the high-temperature transition from the red phase into the yellow metastable phase, which has its own phase transition at low temperatures.

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Raman hyperspectral imaging of calcium carbonate polymorphs in fish otoliths

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Within the inner ear of bony fishes is a calcareous structure called an otolith, which normally consists of aragonite (a polymorph of calcium carbonate).[1] The presence of vaterite, which is a metastable and a rare polymorph of calcium carbonate, is thought to be the product of environmental stress, and has been shown to cause hearing impairment in fish, as well as disorientation and abnormal behaviour, which can be detrimental to their survival.[2] Additionally, slight changes can occur in the crystal structure that also influence the shape and size of the otolith, with vaterite formation resulting in morphological deformation of the otolith.

Scanning electron microscope imaging of a group of otoliths collected from the *Engraulis australis* species retrieved from the food of the Australasian gannet *Morus serrator in* the Hauraki Gulf, New Zealand, showed some abnormal morphological features on the surface. Raman hyperspectral imaging of cross sections of these otoliths was able to identify zones of vaterite (Figure 1) in some of these otoliths.

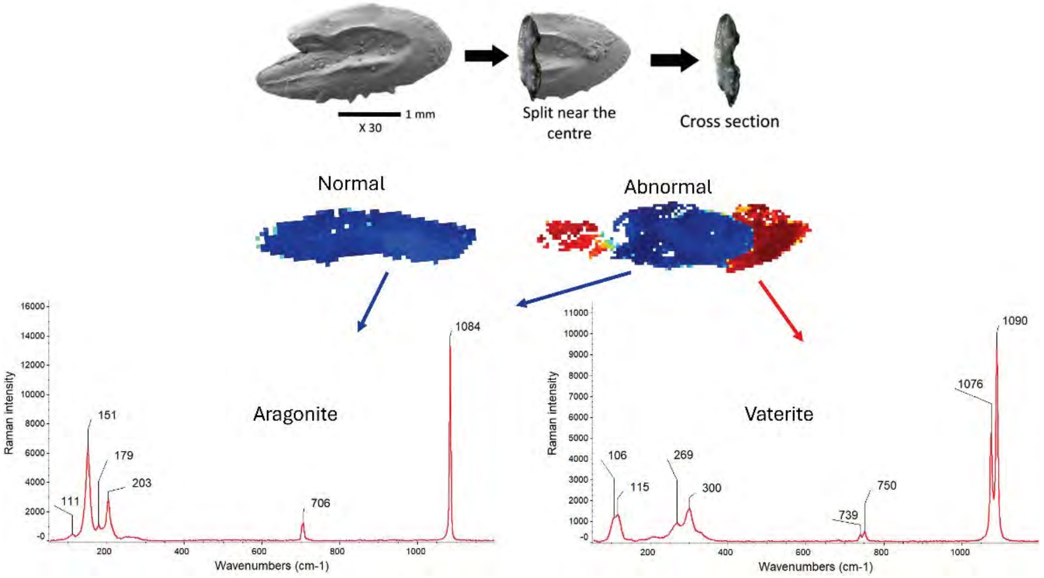

Figure 1. Raman hyperspectral imaging of the inner cross section of fish otoliths.

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Raman spectroscopy and associated techniques reveals abnormalities in RBC membranes in diabetic mice.

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Health complications induced by diabetes stem from dysfunction of the endothelium of blood vessels (ED) [1]. Additionally, it has been demonstrated that one of the key reasons of pathology in diabetis mellitus (DM) is the interaction between red blood cells (RBC) and endothelial cells of blood vessels [1]. Recent studies indicate that DM leads to excessive adhesion of RBC to ED. This may be associated with changes in the deformability of red blood cells and alterations in their membrane composition induced by DM [2-7].

In this research, we present results obtained using Raman spectroscopy, FTIR microspectroscopy, atomic force microscopy and blood morphology analysis from mice at different developmental stages, both with the Db/Db DM model and C57 control mice.

Raman spectroscopy employed two lasers with wavelengths of 488nm and 532nm. Analysis of Raman spectroscopy spectra revealed statistically significant changes related to the lipid structure in RBC membranes. As a complimentary technique to Raman spectroscopy, FTIR microspectroscopy was applied. Blood morphology exhibited changes in parameters such as cell volume, cell count, and haemoglobin content within cells. Atomic force microscopy was utilized to support observed changes in blood morphology through measurements of fixed red blood cells.

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Photoswitching of Triptycene-Based Molecular Machines Followed by Raman Spectroscopy

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Light-powered molecular machines, particularly when organized into regular 2D structures, are attracting great scientific interest due to their potential applications ranging from information processing, nanoelectromechanical systems, energy, biology, to medicine. The photoswitches are a type of molecules that can change their structural geometry and chemical properties upon irradiation with electromagnetic radiation.

Recently we characterized triptycene-based molecular rods prepared using Langmuir−Blodgett (LB) technique on a gold (111) surface [1]. As the next step, photoswitchable molecular machines were added on the surface of these molecular rods. Here we compare their isotropic Raman spectra in solution with anisotropic spectra of LB monolayers as well as their ability to photoswitch in both states. The experimental results were in a good agreement with extensive Density-functional theory (DFT) calculations.

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Unearthing algal sea-crets by Raman microspectroscopy

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Green algae are ubiquitous primary producers in nature and, therefore, not only play a pivotal role in global carbon fixation but also have a decisive influence on geochemical processes in soil, ice and aqueous habitats. Beyond their ecological importance, they find manifold applications in biotechnology, such as in the pharmaceutic, food or fuel industries.[1] Understanding the algae's intricate biochemical processes, the dependence of artificial or nature-like culture conditions on algal growth and the influence of their bacterial communities with mutualistic or antagonistic effects is thus of crucial importance, not only for life on earth but also for industrial advancements.

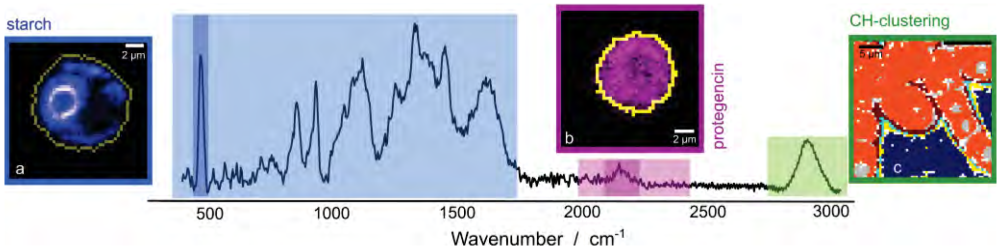

Figure 1: Raman-algal analysis in the fingerprint (a), wavenumber silent (b) and CH_x-stretching region (c).

We here present how Raman microspectroscopy can complement the gold-standard TEM and conventional developmental biology to gain profound insights into the chemical composition of micro- and macroalgae *in situ* in a non-inversive manner, thereby providing valuable insights from the whole wavenumber range between 400 cm^{-1} and 3100 cm^{-1} (Fig. 1). We outline, how the CH_x-stretching wavenumber region (2800 cm^{-1}-3100 cm^{-1}) of the Raman spectrum allows for the analysis of cell differentiation, cellular malformations, and biofilm formation in the green macroalgae *Ulva* spp. and guides subsequent analyses in the fingerprint region as a vibrational beacon. The fingerprint region (400 cm^{-1}-1800 cm^{-1}) delivers insights into cellular composition, such as the appearance of ulvan or cellulose as membrane constituents in *Ulva* spp. or the starch distribution patterns of the microalga *C. reinhardtii* that can be essentially varied in cultures exposed to structured 3D-environments. The typically underestimated silent wavenumber range (2000 cm^{-1} – 2300 cm^{-1}) completes the Raman algal analysis, e.g., for the detection of the polyyne toxin protegencin, which causes the lysis of *C. reinhardtii* when interacting with *P. protegens*[2]

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The study of microenvironment on SERS substrate

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The presence of microenvironments or reactions occurring at the interfaces of nanomaterials has a direct and significant impact on their physical and chemical properties, interfacial material transformation mechanisms, and analytical processes. However, analyzing and researching these aspects pose considerable challenges. Surface-enhanced Raman spectroscopy (SERS) is a highly surface-sensitive fingerprint spectroscopy technique. The enhanced signal in SERS is influenced by various factors, including the microenvironment at the interface of the enhanced substrate surface. Interferences from impurities in reagents or protective agents on the enhanced substrate surface can greatly disrupt the reliable qualitative and quantitative analysis using SERS.[1-4]

Through the integration of SERS technology with chromatography and mass spectrometry, our research has revealed that establishing a standard SERS detection method for standard substances using sol-gel SERS substrates can be complicated by the presence of ultra-trace impurities that significantly impact the qualitative analysis of SERS. Furthermore, we have observed that surfactants like citrate at the substrate interface are not inert; rather, they can undergo reactions catalyzed by Au/Ag NPs to generate new surface species, thereby altering the interface environment. Our study not only provides novel research directions for identifying unknown SERS spectral signals but also offers insights into mitigating interfering factors at the SERS substrate interface.

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FABRICATION OF UNIQUE SERS ACTIVE SUBSTRATES THROUGH INTEGRATION OF SILVER NANOCOLLOIDS AND MBBA LIQUID CRYSTAL MOLECULES ORGANIZED IN LANGMUIR-BLODGETT FILMS

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Efficient SERS active substrates have been fabricated through interactions of silver nanocolloids (AgNcs) with N-(4-Methoxybenzylidene)-4-butylaniline (MBBA) nematic liquid crystal molecules organized in Langmuir Reverse Schaefer (L-R Sh) film. Two sets of AgNc1 and AgNc2 of average diameters ~30 and 63 nm respectively were prepared following the recipe as prescribed by Lee and Meisel [1] and the L-R Sh films of MBBA were dipped in the colloids for 48 hours. The resultant films so attained after incubation in AgNc1 and AgNc2 for 48 hours are named as Substrate-A (S_A) and Substrate-B (S_B) respectively. While FESEM image of the S_A shows “fingering pattern”, S_B on the other hand exhibits agglomerated clusters of AgNcs on the L-R Sh film matrix of MBBA. The reason behind such textures of S_A and S_B has been envisaged. The SERS efficacy of the S_A and S_B has been tested to detect Rhodamine 6G molecule and 1, 10-phenanthroline molecules at ultrasensitive concentrations down to ~10^{-12} M and ~10^{-10} M. The two substrates show the catalytic conversion of 4-ATP to p,p’-Dimercaptoazobenzene (DMAB). The as prepared substrates will be used as “lab on chips” for chemical and biochemical sensing at trace concentrations [2].

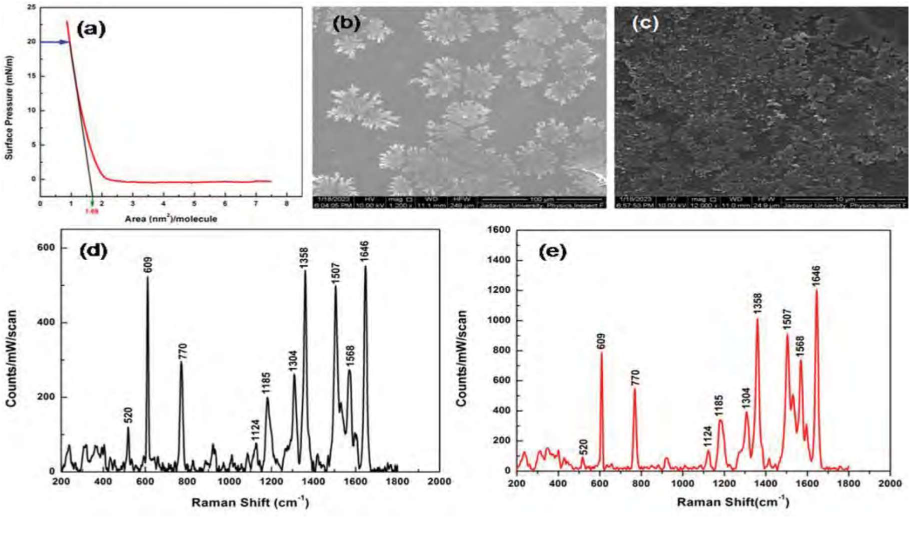

Fig. 1. (a): Room temperature surface pressure (π)-area (A) compression isotherm of Langmuir monolayer of MBBA molecule in water subspace (pH-6.8). FESEM images of **(b):** Substrate-A and **(c)**: Substrate-B. SERS spectra of 10^{-12} M aqueous solution of R6G molecule adsorbed on **(e):** Substrate-A **(f):**Substrate-B (λ_{ext}=532nm)

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Correlative TERS and nano-FTIR spectroscopy on virions

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Understanding the intricate surface and molecular architecture of SARS-CoV-2 is pivotal for elucidating its pathogenic mechanisms. The surface of the virus, comprising lipid bilayers, structural proteins and glycoproteins, plays a crucial role in viral infectivity.

We combined Tip-Enhanced Raman Spectroscopy (TERS) and nano-Fourier Transform Infrared (nano-FTIR) spectroscopy, to comprehensively characterize the SARS-CoV-2 virions. TERS provides exceptional spatial resolution, enabling precise identification of nanoscale features, while nano-FTIR offers complementary biochemical specificity.

The comparison between TERS and nano-FTIR results facilitates the elucidation of lipid-protein interactions, different lipids (fig.1), and structural differences in viral proteins [2]. The amide I region was observed in both TERS and nano-FTIR spectra in the range of ~1610-1687 cm^{-1}. TERS indicates two lipid peaks in the ranges of 1750-1755 cm^{-1} and 1765-1775 cm^{-1}. Similar lipid features were also detected in nano-FTIR spectra in the regions: 1715-1730 cm^{-1} and 1750-1770 cm^{-1}. The lipid peaks exhibited a slight variance in frequency within the TERS spectra, typically differing by 5-10 cm^{-1}. In the nano-FTIR spectra, this variance was in the range of 10-20 cm^{-1}.

Moreover, to enable a direct comparison of spectral features and chemical bonds between TERS and nano-FTIR data, a phase subtraction ((φ_4) -(φ_3) has been performed, providing spectra that highlight information from the first layers of surface of virions [1].

By correlating the findings from TERS and nano-FTIR analyses, this study provides robust evidence supporting the structural characteristics of SARS-CoV-2 surface components, thus advancing our understanding of virus-host interactions. Overall, the techniques aim to synergistically exploit the strengths of both techniques, allowing for a more comprehensive understanding of SARS-CoV-2 architectures.

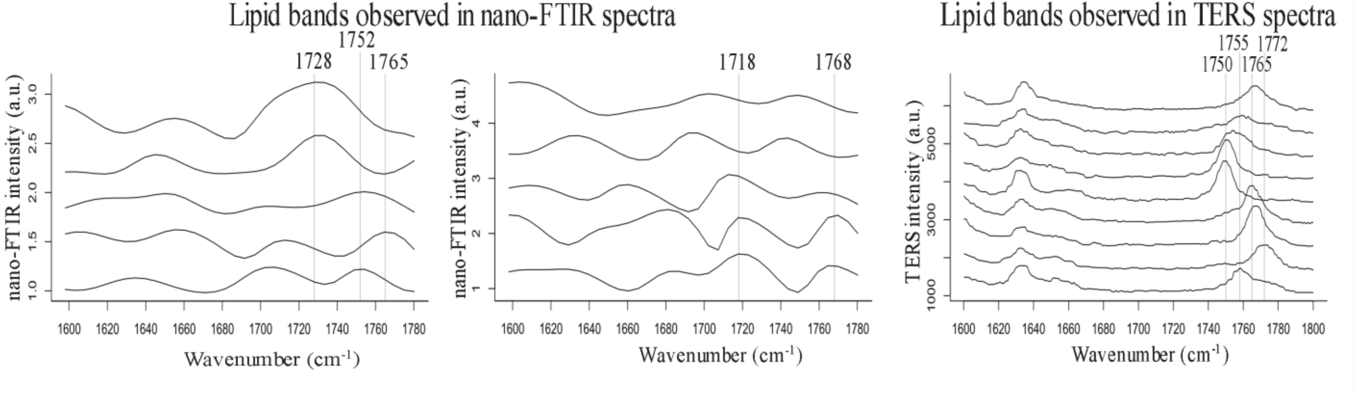

Figure 1: The nano-FTIR spectra (left) and TERS spectra (right) of the SARS-CoV-2 virus

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Development of Computational Models to Decipher Raman Optical Activity Spectra of G-quadruplexes

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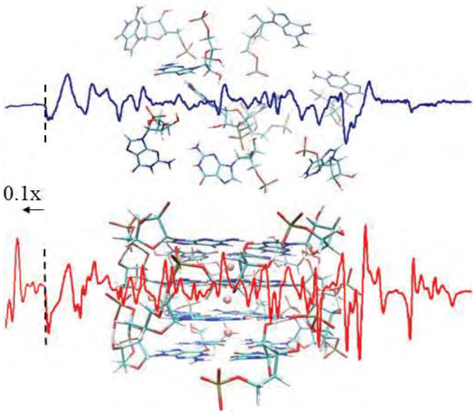
G-quadruplexes are good models of DNA structures involved in the metabolism of living cells and are frequently adopted by guanine-rich nucleic acids. They play crucial roles in genome functions and stability, the pathogenesis of several neurodegenerative diseases, and cancer biology. The guanine nucleotide (G) and its derivatives are well known for their property to self-associate into various complexes through H-bonding and stacking interactions. G-quadruplexes are formed at higher concentrations as nanoscale cylindrical structures consisting of G-quartet disks stacked one above the other[1]. These supramolecular assemblies are also potential candidates for nanotechnology and chemical biology applications.

Raman optical activity (ROA), which measures differential Raman scattering of right and left circularly polarized light, is promising for studying nucleic acid structures and their dynamics because of its sensitivity to subtle changes in geometry[2]. Recently, characteristic Raman and ROA spectral changes upon G-association were observed. A combination of molecular dynamics (MD) and quantum-chemical computational techniques have been used to model and interpret the observed Raman and ROA spectral features of G-quadruplexes under various experimental conditions. The fragment-based cartesian coordinate-based tensor transfer (CCT)[3] method is employed in the spectra calculations to embrace the enormous size of the G-quadruplexes.



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Detecting Folate Receptor α on single cancer cells: influence of the SERS nanotags design on the analytical performances

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Surface-Enhanced Raman Scattering (SERS)-active nanotags are an emerging kind of optical labels that possess a huge potential for biological applications owing to their superior characteristics compared to existing labels [1]. Notably, SERS nanotags have found plenty of applications for detecting membrane receptors at the surface of single cancer cells [1]. Analytical techniques with the ability to phenotype cancer cells at the single cell level are increasingly required due to the importance of cell-to-cell heterogeneity in the overall behaviour of cancers [2]. However, the structure of the SERS nanotags and the analytical methodology used to extract the signal and compare the samples have a major influence on the ability of the technique to properly evaluate the expression of membrane receptors of interest.

In the frame of our research [3], [4], we designed and compared three different kinds of SERS nanotags, all aimed at Folate Receptor α (FRα), and we compared their ability to distinguish between FRα-overexpressing and non-overexpressing cancer cells. We also compared different methods to extract the SERS signal, using either the signal intensity or active-pixels. We synthesised nanotags based on Au@Ag core@shell nanoparticles as plasmonic core, and two kinds of polymers (poly(allylamine)-PAH or polyethylene glycol-PEG) were used for surface protection and linking of folic acid (used to target FRα). Briefly, we observed that despite a higher loading of folic acid molecules on the PAH coating, the nanotags suffered from more non-specific interactions than nanotags with the PEG coating, limiting the discrimination capability of the method. We also evaluated the introduction of a “non-specific” PAH-nanotag through a ratiometric approach to try to improve the discrimination between cells. Ultimately, the best discrimination was obtained with the PEG-coated nanotags, and cells expressing different amount of FRα could be distinguished with a high contrast (4-fold higher SERS response) while the level of non-specific interactions was strongly reduced.

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Ultrasensitive SERS detection of cytokines through specific binding and multiple reporter molecules

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Surface-enhanced Raman spectroscopy (SERS) has attracted much attention in the field of biosensing, and indirect detection methods of antigen-antibody interactions are more commonly used in some fields. A SERS immunobiosensor based on gold nanoparticles modified silver nanospace folded rod (AuNPs@AgNF) is designed to achieve high sensitivity detection of IL-6 and IL-1. Silver nanospace folding rod (AgNF) array is prepared by OAD technology, and 3-MPA probe molecules are modified on the surface, and antibodies are connected as SERS immune base. AuNPs are prepared by chemical synthesis method, 4-MBA and DTNB act as Raman reporter molecules linking IL-6 and IL-1 antibodies to synthesize SERS immune tags. When target antigen is present on SERS immune substrate, it specifically combined with SERS immune tag to form immune sandwich structure. Detection limits of IL-6 and IL-1 are 1 pg/mL and 0.93 pg/mL, respectively, by reading Raman reporter molecules on the label. In addition, hybrid detection of two cytokines has been realized, which can selectively and sensitively locate the relevant antigens in tissue samples.

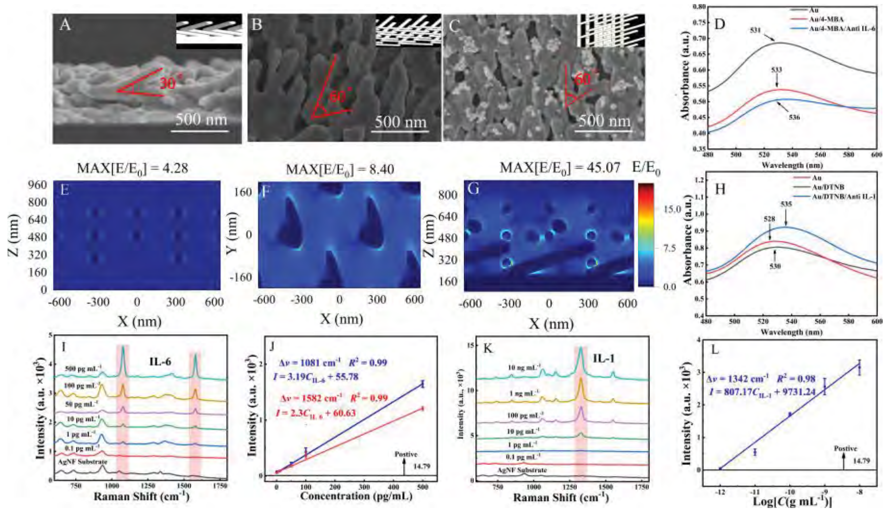

Figure 1: (A) SEM cross-section of AgNF; (B) Top SEM view of AgNF and (C) AuNPs@AgNF structure; (E) electric field distribution of AuNPs and (G)AuNPs@AgNF substrate in *x-z* plane, and (F) electric field distribution of AgNF in the *x-y* plane; (D) UV-VIS spectra of Au/4-MBA/Anti-IL-6 and (H) Au/DTNB/Anti-IL-1. (I) and (K) are SERS spectra for detecting different concentrations of IL-6 and IL-1; IL-6 (J) and IL-1 (L) fit logarithm of signal intensity and concentration at 1081 cm^{-1}, 1582 cm^{-1} and 1342 cm^{-1}, respectively

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Temperature Measurement and Imaging of Fission Yeast Spores Using Stokes/anti-Stokes Raman Microspectroscopy

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Spores are dormant cells that are formed when nutrient sources (especially nitrogen sources) are depleted. They show higher thermotolerance than vegetative cells, but the mechanisms of their thermotolerance are not fully understood. To gain new insight into the thermal response of the spore, we used the anti-Stokes to Stokes Raman intensity ratio (I_{AS}/I_S) to measure the temperature inside spores of the fission yeast *Schizosaccharomyces pombe* in a space-resolved manner without any temperature probe and to visualize the temperature distribution inside the spore.

Vegetative cells of *S. pombe* were cultured in YES liquid medium for 2 days. For sporulation, *S. pombe* cells were pre-cultured on YES agar medium, cultured on PM liquid medium for 1 day, and then cultured on PM-N liquid medium (PM medium without NH_4Cl) for 3 days. During Raman measurements, 100 μL of the sample was heated from room temperature to 50 °C with a step of 5 °C using a precise temperature control stage (VAHEAT, Interherence). The Raman spectra in the low-frequency (both Stokes and anti-Stokes parts) and fingerprint regions of single *S. pombe* vegetative cells and spores were measured using a laboratory-built, 532-nm-excited confocal Raman microscope.

Figure 1(a) shows the measured Raman spectra of vegetative cells and spores of *S. pombe*. The fingerprint region of the spore spectrum is in good agreement with the literature [1]. I_{AS}/I_S was calculated for the region of 20–200 cm^{-1} and calibrated for the sensitivity of the apparatus using the low-frequency Raman spectrum of water [2]. The temperatures of spores, vegetative cells, and medium were determined by fitting the calibrated I_{AS}/I_S curve with a theoretical equation based on the Boltzmann distribution (see the inset of Fig. 1(a)). The calculated temperature of spores is found to be close to the stage temperature, whereas those of oil droplets (lipid-rich) and the cytoplasm (protein-rich) exhibit temperatures 15–20 °C above the stage temperature throughout the temperature range studied (Fig. 1(b)). This discrepancy could be due to differences in the level of autofluorescence (resulting in systematic errors) and the response to local laser heating between the spore and the vegetative cell. We also mapped the temperature distribution inside the spore (Fig. 1(c)) at room temperature. As can be seen from Fig. 1(d), the polysaccharide-dominated region has temperatures similar to the environment, but the other lipid-dominated region has higher temperatures.

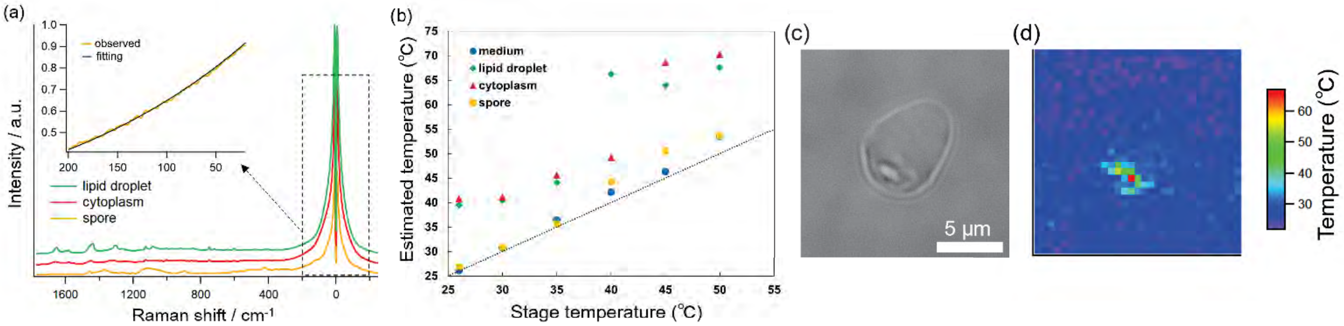

Figure 1 (a) Representative Raman spectra of a spore and a vegetative cell of *S. pombe*. The inset shows the calibrated I_{AS}/I_S curve and its best fit. (b) Temperatures of spores, vegetative cells (oil droplets and the cytoplasm), and medium calculated from the intensity ratio I_{AS}/I_S at different stage temperatures. (c,d) Optical image of the spore (c) and temperature distribution calculated from I_{AS}/I_S (d).

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Nonlinear multimodal imaging towards endoscopic applications

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Since its inception in the 1980s, CARS microscopy has undergone significant advancements, enabling label-free imaging of molecular distributions and integrating ultra-short pulse lasers to support modalities such as SHG and TPEF. Translating these nonlinear imaging techniques for clinical diagnostics via endoscopy presents challenges, including fiber background, miniaturization of probes, optimization of light transmission, and enhancement of data processing. Addressing these challenges, we present two innovative nonlinear endoscopic systems: a multicore fiber-based probe [1] featuring proximal remote scanning and a customized reconstruction algorithm for improved image clarity, and a Double-Core Double-Clad fiber (DCDC) based endoscope [2] enabling non-resonant FWM background-free laser delivery and sub-cellular imaging. These systems hold promise for imaging performance in various application scenarios.

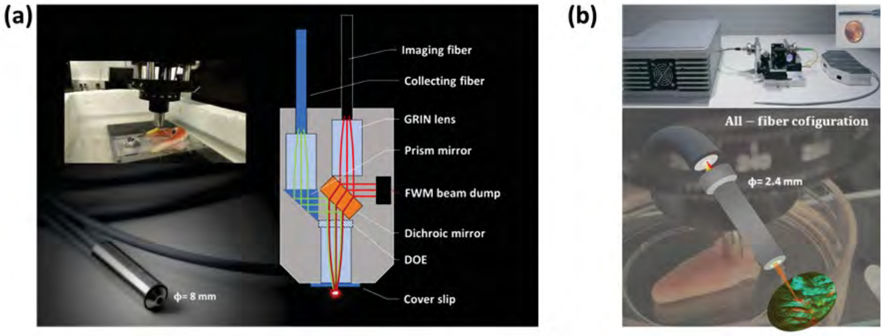

Figure 1: Nonlinear multimodal endoscopes with different conceptual designs resulting from different optical fiber applications. (a) Multicore fiber endoscope: Remote scanning without electronically and mechanically moving parts in probehead (b) DCDC fiber endoscope: Laser delivery without non-resonant FWM background from the fiber, in all-fiber-connected system.

Acknowledgements

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Resonance Raman studies of triphenylamine-based acceptor-donor dyes

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Three acceptor-donor dyes were investigated to study the effect of changing the acceptor's functional group from ketone to malononitrile on the photochemical properties of donor-acceptor triphenyl-based dyes. To characterize the dyes, we used various methods such as absorbance, emission, Raman spectroscopy, and theoretical calculations. We utilized density functional theory (DFT) with the B3LYP method and 6-31g(d) basis sets to gain further insights into the nature of the electronic transitions and the molecular vibrations. The dyes consist of triphenylamine, allyl, and indane as the donor-π-linker-acceptor moieties. The only variation in the dyes was the functional group in the acceptor moiety. The first dye had two malononitrile groups (TPA-π-InCN$_2$), the second had one malononitrile and one ketone group (TPA-π-InOCN), and the third had two ketone groups (TPA-π-InO$_2$). The results showed that the addition of the ketone functional group resulted in a significant red shift in the intramolecular charge transfer (CT) from 521 nm to 647 nm. According to the computational calculations, the presence of the malononitrile functional group increases the bending of the ground state structure. In addition, the malononitrile group increases the stability of the LUMO orbital; resulting in a stronger acceptor. As a result, the gap between the HOMO and LUMO orbitals is decreased, which causes the blue shift of the CT.

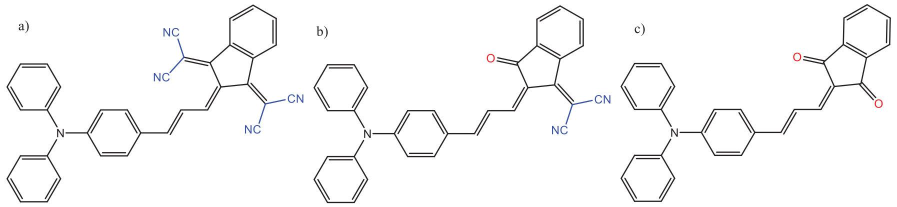

Figure 1: The structure of a) TPA-π-InCN$_2$, b) TPA-π-InOCN, c) and TPA-π-InO$_2$.

The solvatochromic behavior of was studied by analyzing them in two solvents: dichloromethane (DCM) and toluene. The fluorescence spectra showed an increase in the Stokes shift in the polar solvent. TPA-π-InO$_2$ showed the highest Stokes shift, indicating a more polar excited state compared to the other two dyes. However, the TPA-π-InOCN dye had two higher energy CT transitions (HOMO-LUMO, and HOMO-L^{+1}) according to the computational results. Furthermore, the patterns of the Resonance Raman spectra of TPA-π-InOCN in DCM suggested the presence of two different CT transitions in the range between 591 nm and 491 nm, which further supports the computational results. The quantum yield, lifetime, and decay rates were calculated in DCM. The results showed the highest quantum yield for TPA-π-InOCN of 0.25. However, the nonradiative decay of all three dyes was about 10 times higher than the radiative decay. Moreover, the lifetime of all three dyes was approximately 5 ns, with TPA-π-InO$_2$ having the highest lifetime.

Unveiling surface properties of barium titanate through high-resolution tip-enhanced Raman spectroscopy

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Abstract:

As the applications of barium titanate ($BaTiO_3$) within the electronics and photocatalysis sectors have advanced to the nanoscale, the influence of surfaces and interfaces on the material properties has become predominant. Conventional phonon Raman spectroscopy is a powerful experimental technique for studying the $BaTiO_3$ structure, enabling the exploration of adsorption, phase transition and domain formations at length scales down to ~1 µm [1-2]. For a comprehensive understanding of the surface phenomena and size effect, it is important to provide information on the phonon vibration modes within the nanometer region. Tip-enhanced Raman spectroscopy (TERS) combines plasmon-enhanced Raman spectroscopy with scanning probe microscopy, allowing for exploring phonon modes beyond the diffraction limit of light.

Leveraging highly efficient gold tips, our research achieved high-resolution Raman imaging on the $BaTiO_3$ nanoparticles and revealed the presence of a carbonate monolayer on their surface, as shown in figures 1 (a) and 1 (b). The size effect on the individual $BaTiO_3$ nanoparticles was also observed through the diminished intensity of phonon modes indicative of the tetragonal phase, as illustrated in figure (c). Beyond this discovery, we successfully activated forbidden Raman modes in the TERS spectrum, providing deeper insights into surface vibration modes.

These contributions present significant advances in the direct experimental investigation of adsorption monolayers and the identification of forbidden Raman modes in individual polar perovskite nanoparticles. Furthermore, this work sheds light on the fundamental size effect in $BaTiO_3$ and paves the way for novel methodologies in probing the material's dielectric permittivity at the scale of a single-unit cell.

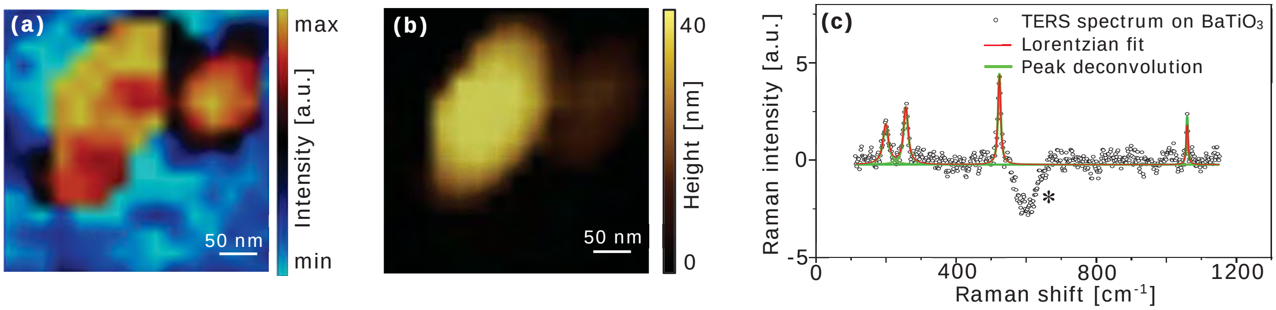

Figure 1. (a): 15 px × 15 px TERS imaging of the $BaTiO_3$ nanoparticle was acquired over the carbonate peak that indicates monolayer CO_2^{3-} adsorption on the surface. (b): simultaneously recorded topography scan. (c): TERS spectrum averaged across 10 pixels on $BaTiO_3$ nanoparticle, central negative peak (indicated by an asterisk) ascribed to a spectrometer mirror artifact.

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Structure Study on Binary Na_2O-TiO_2 Melts by *in-situ* High Temperature Raman Spectroscopy

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Functional materials of sodium titanates have excellent physical and chemical properties, which have been widely used in the field of semiconductor, optical component, catalyst, metallurgy, and aerospace, etc. [1]. It is preparation process involved the crystallization process from the melt. Observing the microstructure of sodium titanate melt is of great scientific significance to prepare traditional titanates material and to exploit novel functional titanate-based materials. *In-situ* Raman spectra of (1-x) $Na_2O \cdot xTiO_2$ (x=33, 50, 56, 60, 65, 71.5, 75, 80 mol%) melts were measured with high SN (signal to noise ratio) at 1473 K. As shown in Figure 1, as the molar ratio of TiO_2 content increases from 33 to 80 mol%, the characteristic peaks in the mid and high frequency ranges undergo major change, and the microstructure of the melts inside have changed radically.

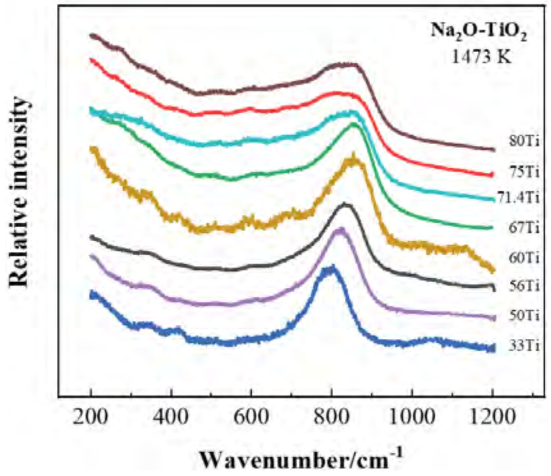

Figure 1: *In-situ* high temperature Raman spectra of (1-x) $Na_2O \cdot xTiO_2$ (x=33, 50, 56, 60, 65, 71.5, 75, 80 mol%) melt at 1473 K

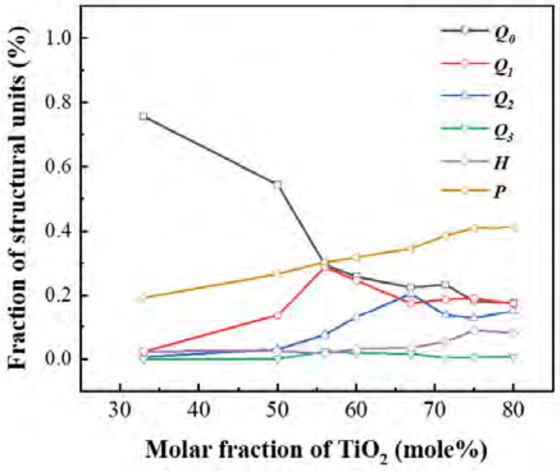

Figure 2: Abundance of different species varying with different TiO_2 content in binary Na_2O-TiO_2 melts at 1473 K

A series of model clusters assumed in melt were constructed and simulated by quantum chemistry *ab initio* calculation method. The characteristic Raman active vibration wavenumbers and their scattering cross section were obtained [2]. As shown in Figure 2, the distribution of cluster structures in the melt was analyzed and preliminarily quantified through spectral analysis. The accurate quantitative distribution of the molar percentage content of Q_i、P and H species were thus described quantitatively with the change of TiO_2 composition. The results show that there are H、P、Q_0、Q_1、Q_2 and Q_3 species in binary Na_2O-TiO_2 system melt. With the increase of TiO_2 concentration, the tetrahedron of four-coordinated TiO_4 in the melt gradually transforms into five-coordinated TiO_5 pentahedron and six-coordinated TiO_6 octahedron. The main component is $TiO_4(Q_i)$ species, followed by $TiO_5(P)$ species, and the least is $TiO_6(H)$.

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Microplastics Monitoring in Dietary Supplements using Raman Spectrocopy: Omega-3 Polyunsaturated Fatty

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Omega-3 polyunsaturated fatty acids (PUFAs) are widely recognized for their role in reducing diseases such as cardiovascular disorders, inflammation, developmental issues, and depression. This has made them immensely popular in the health supplement industry. However, it's important to note that most omega-3 PUFAs are derived from sources like fish or algae, which have been reported to be contaminated with microplastics. In this study, various omega-3 dietary supplements were purchased for monitoring microplastic contamination. Sample amounts were based on the daily intake indicated on product labels. A 30% hydrogen peroxide solution was used for pre-treatment, and samples were filtered through silicone filters with a 1 μm pore size. As a result of analysis of 1-20 μm microplastics using a micro Raman spectroscopy, an average of 13.1 microplastics were found per sample, and a maximum of 22 and a minimum of 3 microplastics were found depending on the sample. The most frequently detected types were Polypropylene (44%), followed by Polyethylene (25%) and Polyvinyl chloride (10%). Since this study couldn't determine whether the microplastic contamination originated from packaging or the omega-3 source materials, further research is necessary to investigate the source of microplastics in omega-3 products.

Raman and Tip-Enhanced Raman spectroscopy characterization of milk-derived extracellular vesicles: Toward nanoscale single vesicle analysis

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[c]*Research Center for Nanotechnology applied to Engineering of Sapienza University of Rome (CNIS);*
[*]*These two authors equally contributed to the present work*

Tip-enhanced Raman Spectroscopy (TERS) is an advanced technique to perform local chemical analysis of the surface of a sample combining Raman spectroscopy and atomic force microscopy (AFM) [1]. In this work, TERS is demonstrated to represent an innovative and powerful approach to study extracellular vesicles, in particular bovine milk-derived extracellular vesicles (mEVs), which are nanostructures with considerable potential in drug delivery and therapeutic applications [2]. Raman spectroscopy has been used to analyze mEVs to obtain a detailed spectrum to identify the 'signature' of mEVs in terms of their characteristic molecular vibrations and, therefore, their chemical composition. TERS has been used to study individual mEVs [3], demonstrating the possibility to investigated a single mEV selected on the surface of the sample and, moreover, to analyze specific locations on the selected mEV with nanometer lateral resolution. Thanks to the property of TERS to acquire the signal from only the first few nanometers of the surface, chemical investigation of the lipidic membrane at the various locations of the selected mEV could be performed by analyzing the Raman shift peaks in the relevant range of the spectrum (2800-3000 cm^{-1}). Despite limited to mEVs, this work demonstrates the potential of TERS in analysis of extracellular vesicles.

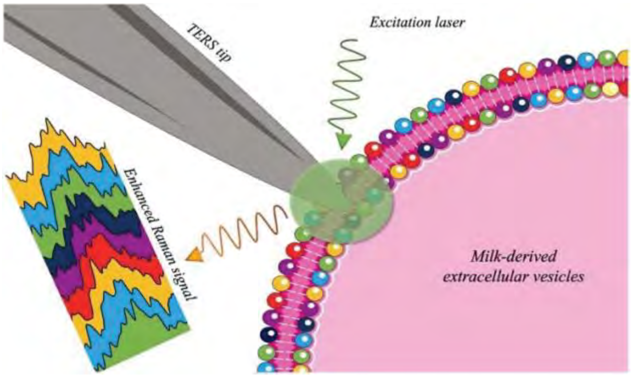

Figure 1: Graphical abstract of the presented work

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Cocrystal quantification in tablets using transmission Raman spectroscopy with partial least squares-discriminant analysis

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Polymorphs, salts, cocrystals, and amorphous materials have been used to improve the solubility, storage stability, and processability of active pharmaceutical ingredients (APIs). A cocrystal has a crystalline structure composed of an API and a coformer. Cocrystals can be used when salts are difficult to form. Techniques for quantifying cocrystals in pharmaceuticals are important for quality control. However, high-performance liquid chromatography (HPLC) cannot be used to detect cocrystals because they dissociate during the mobile phase. Transmission Raman spectroscopy (TRS) is useful for quantifying an API in formulations without destruction, including co-crystals, and can be included in the manufacturing process as an in-line process analytical technology tool. In our previous study, we reported the effectiveness of TRS in quantifying co-crystals composed of carbamazepine and succinic acid and their dissociation in tablets using partial least squares regression (PLSR) [1]. However, PLSR typically requires the preparation of formulations for models with compositions that are assigned, and the PLSR model has to be maintained. In the present study, we used PLS type 2-discriminant analysis (PLS-DA) as an easier method than PLSR. Although PLS-DA is inferior to PLSR in terms of quantification, it requires fewer model formulations and only requires pure spectra of each component.

Indomethacin (IND), nicotinamide (NIC), its cocrystal (IND/NIC), and granulated D-mannitol (MAN) as fillers, were used for the model. The 0–20% cocrystal model tablets spiked 0–5% IND and NIC were prepared. TRS was performed using a TRS100 instrument (Agilent Technologies, Inc.). PLS-DA models were constructed using the Raman spectra of the IND/NIC cocrystal, IND, NIC, and MAN.

PLS-DA models preprocessed using the 1st derivative and standard normal variate (SNV) correlated with the contents of the cocrystal (Fig.1) and distinguished the 1% levels of IND and NIC. These methods are feasible for quantifying the cocrystals and detecting their dissociation. Because there are few precedents, the pharmaceutical industry tends to avoid using chemometrics, except for PLSR, in pharmaceutical assays. PLS-DA has been considered to be inferior to PLSR in quantification. However, in this study, the PLS-DA model using only the TRS of each pure component showed good linearity in score plots and preferable prediction performance for the quantification of the cocrystal and detection of its dissociation. Cocrystals are relatively stable, but may dissociate over time. Therefore, TRS with PLS-DA is a promising option for the simple and effortless evaluation of cocrystal stability in tablets.

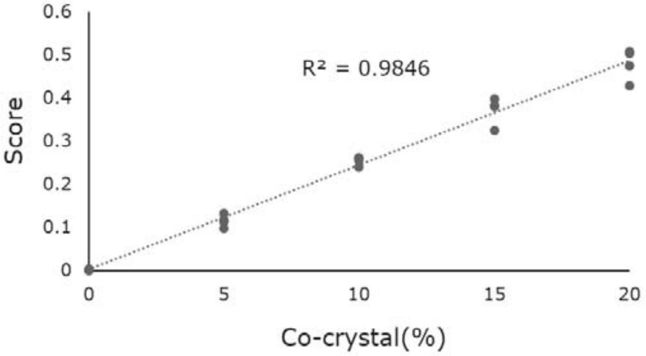

Fig.1 Relationship between cocrystal and PLS score

References

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SERS spectroscopic study of cross-coupling reaction

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The cross-coupling reactions catalyzed by transition metals have been intensively studied for over the last few decades. The importance of these reactions is widely recognized. Its authors, R. Heck, E. Negishi and A. Suzuki, were awarded by the Chemistry Nobel Prize in 2010. These catalyzed reactions have introduced a novel synthetic pathway for the production of compounds that are challenging to synthesize by conventional means. Understanding the mechanism of these reactions is crucial for further advancements in the field [1].

The primary objective of this study is to monitor the Suzuki-Miyaura cross-coupling reaction (SMCR) between aryl halide and arylboronic acid in the presence of a base on the surface of the plasmonic nanoparticles [2]. The 4-position substituted aryl halides (mainly aryl iodides) and aryl boronic acids were used as the substrates. The resulting products were derivates of biphenyl exhibiting a characteristic band in Raman spectra around 1288 cm^{-1} (inter-ring CC vibration). However, the exact wavenumber varied depending on the substituents in 4,4´-positions of the biphenyl. The reactants and products were studied by normal Raman scattering spectroscopy in solid state, with an excitation wavelength of 780 nm. Surface-enhanced Raman spectroscopy (SERS) was employed when the reactants were adsorbed on the Ag nanoparticles surface and the reaction progress was monitored with 532 nm excitation. Notably, in the SERS spectra following the addition of palladium catalyst (K_2PdCl_4) a band at approximately 1288 cm^{-1} appeared (Figure 1), which confirmed the success of the SMCR.

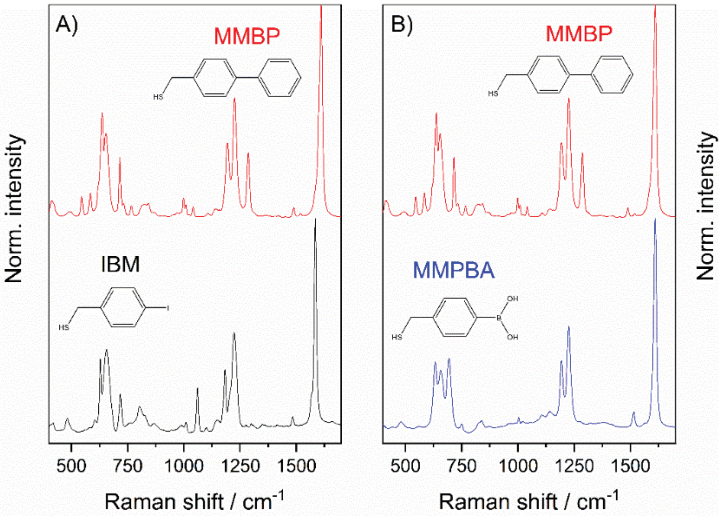

Figure 1: Two different ways of preparing [1,1′-biphenyl]-4-methanethiol by SMCR from different reactants (4-iodobenzenemethanethiol or (4-(mercaptomethyl)phenyl)boronic acid).

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Acknowledgement

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Decoding the Continuous Emission from Gold Nanoparticles in SERS Experiments

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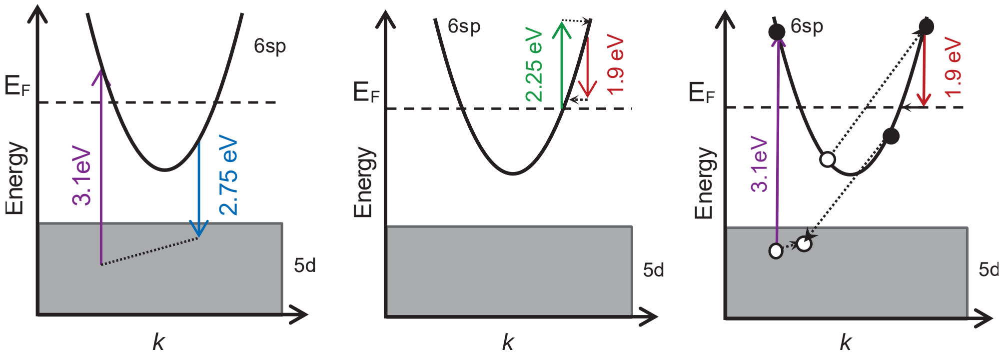
[a]*University of Potsdam*

SERS substrates based on nanostructured gold are notorious for their large background emission originating from the substrate itself [1]. Traditionally, efforts have been directed to minimize the background. The emission contains however also valuable information on the distribution of excited electrons in the substrate, which is highly relevant in the investigation of photocatalytic processes [2]. While the discussion regarding the underlying processes of this nanoparticle response is ongoing, it is most often attributed to photoluminescence (PL) or electronic Raman scattering (ERS) and thus to intraband processes. Especially for gold nanoparticles, the contribution of interband transitions must be considered.

Here, we present a microscopic study on the emission from a typical SERS substrate, consisting of unordered gold nanoflowers. Utilizing photoluminescence excitation (PLE) spectroscopy, we show the strong dependence on interband excitation, while the emission spectrum continues to be determined by plasmon-enhanced intraband processes. We hence propose that the main emission pathway proceeds through an Auger-like excitation of 6*sp*-band electrons with subsequent interband recombination. This observation indicates that the Auger-process is an additional efficient pathway to generate hot electrons in plasmonic nanostructures.



Interband Recombintation

Intraband Recombintation

Auger Recombintation

Figure 1: Different mechanisms with potential contributions to the SERS background: interband transitions form d-bands (left), intraband transitions within the conduction band and Auger excitation involving both, absorption from the d-bands and emission within the conduction band.

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Study of phase transitions in SbSI-based thin films by temperature dependent micro-Raman spectroscopy.

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Advancements in green energy and optoelectronics require technological breakthroughs to surpass current methodologies and performance standards. Therefore, material science researchers are exploring new promising roads to create innovative materials and devices with superior performance.

One of the most challenging fields in today's research is the one aimed to the realization of solar cells based on ferroelectric materials. Ferroelectric compounds, indeed, have an intrinsic internal polarization and field that can induce photovoltaic effects and possibly charge separation without the need of a p-n junction. In this context, antimony sulphoiodide (SbSI) is one of the most promising materials, not only because of its ferroelectric properties but also because of a proper band gap value, that usually cannot be find in most of ferroelectric materials. Hence a deeper study of the properties of this material is becoming more and more important.

Several experiments on bulk materials have shown that SbSI undergoes two phase transitions. The first transition occurs around 20°C, causing the material to shift from a paraelectric to a ferroelectric phase. The second transition, which is a second-order transition, occurs at about -40°C and has been studied less extensively.

The objective of this study is to examine phase transitions of SbSI samples in thin-film form (the one which is optimal for photovoltaic application) by means of micro-Raman analysis across critical temperatures.

The studied SbSI thin-films have been deposited for the first time using the innovative Pulsed Electron Deposition (PED) technique and micro-Raman spectroscopy proved to be effective in the analysis of both phase transitions as a function of temperature.

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Electrostatic Interactions by Bacteria in Hospital Wastewater: A Raman Spectroscopy Odyssey

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Healthcare wastewater, a reservoir for pathogenic bacteria like *E. coli* and *P. aeruginosa*, requires meticulous monitoring to prevent outbreaks [1-2]. Conventional bacterial identification methods are time-consuming and susceptible to variations in microbial species composition due to sampling methods or location [3]. Moreover, the cultivability of only a fraction of the bacterial diversity in laboratory settings poses limitations [4,5], prompting the exploration of Raman spectroscopy as rapid, label-free alternative. Nevertheless, the complexity of wastewater poses challenges, affecting bacterial adaptations and Raman spectra accuracy. By investigating factors such as environmental pH and bacterial physiological and metabolic states, our aim is to refine culture-free identification methods, enriching our comprehension of electrostatic interactions governing bacterial behaviour and ultimately improving our management of wastewater pathogens.

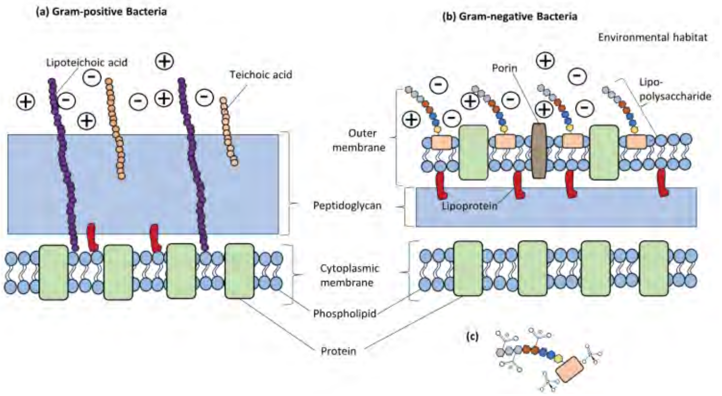

Figure 1: Schematic depiction of surface properties of (a) Gram-positive bacteria, (b) Gram-negative bacteria, (c) ionization of molecules ⊕ cation in solution, ⊖ anion in solution

Acknowledgements

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Calcium acetate drug produced from Rapana venosa invasive gastropod shell: green process control assisted by Raman technology

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Calcium acetate is a widely used medication as phosphate binder in kidney failure disease [1] while in nutraceuticals and food additives is increasingly demanded. However, the current synthesis method relies on unsustainable sources, contributing to environmental issues and impurities in the final product.

Here, a green and sustainable approach to produce calcium acetate is reported, using the increasingly abundant *Rapana venosa* shells as a rich source of biogenic calcium carbonate, comprising both aragonite and calcite polymorphs [2]. Doing so, Raman spectroscopy involving both lab-based and hand-held instruments is employed for every step of the process control, providing valuable information on the raw material as well as during the demineralization control and final product development and quality control. X-Ray Diffraction (XRD), Scanning Electron Microscopy coupled with Energy Dispersive X-Ray Spectroscopy (SEM-EDX) and FT-IR validated the final product identity, hydration status, crystalline morphology, and elemental composition. 48,6 g of gastropod shell was processed to produce 45,67 g of $C_4H_6CaO_4$ with a yield of 126,86 mg/ml of acetate solution.

This perspective, in the context of blue bioeconomy, offers a cleaner and efficient method of calcium acetate production with important biomedical applications.

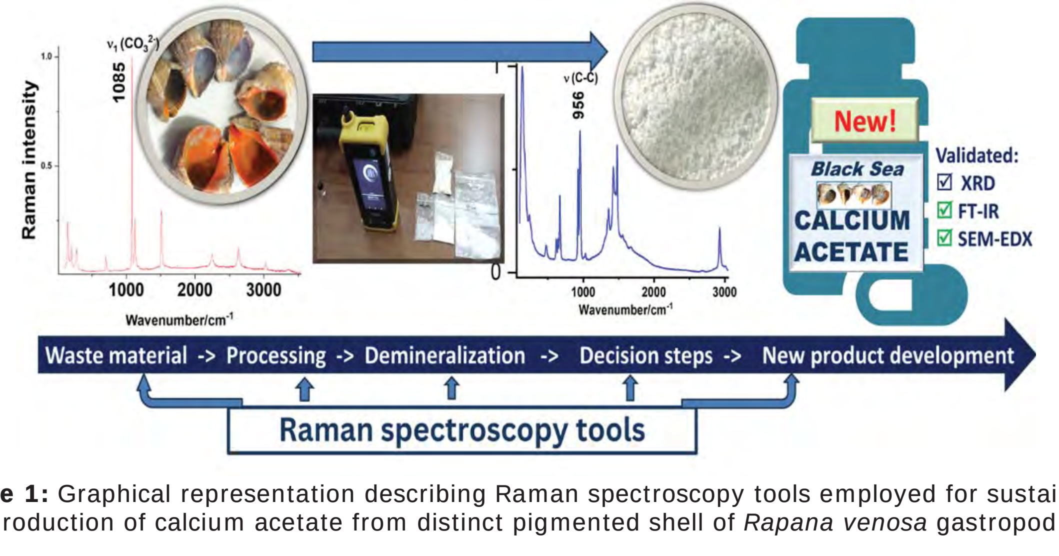

Figure 1: Graphical representation describing Raman spectroscopy tools employed for sustainable production of calcium acetate from distinct pigmented shell of *Rapana venosa* gastropod.

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Rapid antimicrobial susceptibility testing using the RamanBioAssay platform

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Antibiotic resistances are a major public health concern, complicating infectious disease treatment and has severe medical and financial implications. To overcome these problems, there is an urgent need for faster antibiotic susceptibility testing. One approach is to use Raman spectroscopy to rapidly investigate the phenotypic response of pathogens to antibiotic treatment. The RamanBioAssay™ (RBA) platform will be employed as a rapid antimicrobial susceptibility test (AST) for clinical isolates of *Escherichia coli* and *Staphylococcus aureus*. The information obtained from the Raman data of both resistant and sensitive strains will be matched with the findings from microdilution tests. The Raman-based AST results focus on reliable, growth-independent analysis aiming for potential diagnostic applications. *E. coli* and *S. aureus* strains are exposed to appropriate concentrations of respectively Ciprofloxacin and Oxacillin as part of the RBA. In a dielectrophoresis-chip [1], bacteria are collected in a micrometre range for high-quality Raman measurements directly from bacterial suspensions. The readout of phenotypic molecular changes during antibiotic treatment is possible after short duration of bacteria-antibiotic interaction, ensuring the entire test is completed in ≤ 3 hours.
Common bacteria for bloodstream infections, were effectively treated with appropriate antibiotic concentrations and tested successfully using on-chip Raman spectroscopy. An interaction time of only 90 min between bacteria and antibiotics is enough to detect the antibiotic effects by Raman spectroscopy. The results are consistent with those of the gold standard and current routine diagnostics. The method enables precise detection of phenotypic susceptibility to antimicrobial agents and represents therefore an improvement over both the gold standard and current routine diagnostics.

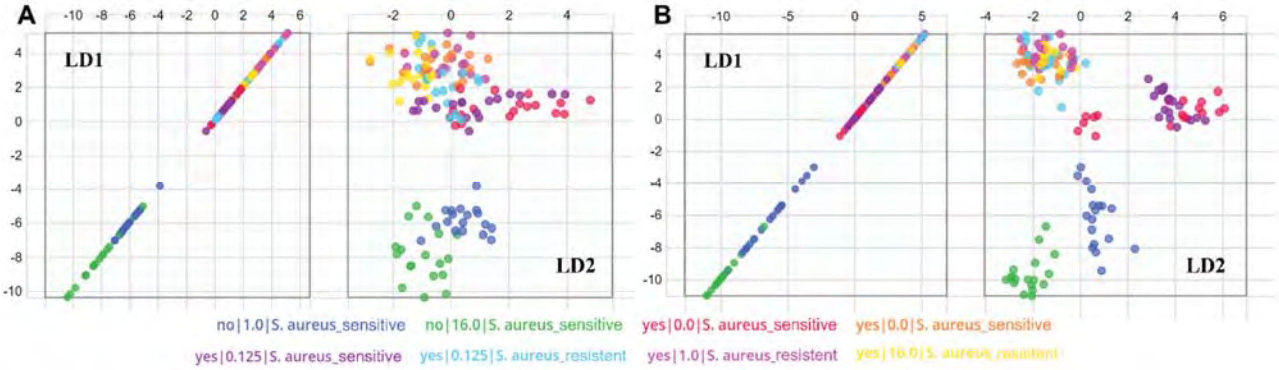

Figure 1: After 90 minutes of cell-drug interaction, Raman data can be utilised to predict bacterial growth at a given antibiotic dose, indicating resistance or sensitivity. PCA-LDA analysis was performed on Raman data acquired from *S. aureus* measured at 532 nm (**A**) and 785 nm (**B**).

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Acknowledgements

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Unveiling the Time-dependent Mineralogical Evolution of Calcium Aluminate Cement Hydration by *In Situ* Raman Imaging

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Calcium aluminate cement (CAC) is a high-performance binder in refractory applications due to its exceptional high temperature resistance. Thus, understanding its hydration process is crucial for predicting its long-term performance. This study uses *in situ* Raman imaging to track the real-time mineralogical evolution during CAC hydration, as well as the onset and completion of the hydration process. This powerful technique provides spatial and chemical information on key hydration products within the cement.

In this study, we observed the transformation of initial CAC phases into calcium aluminate hydrates (CAHs) such as CAH_{10}, C_2AH_8, and the more stable C_3AH_6. In addition, we were able to detect the formation of gibbsite (AH_3), a significant by-product of hydration. Raman imaging provided a unique visualization of the spatial distribution of these CAHs and gibbsite, revealing the formation of distinct hydration zones. This combined approach provides insight into the reaction sequence and evolution of these zones.

The results of this study contribute to a deeper understanding of the complex CAC hydration process and to the optimization of the performance and durability of CAC-based refractory materials.

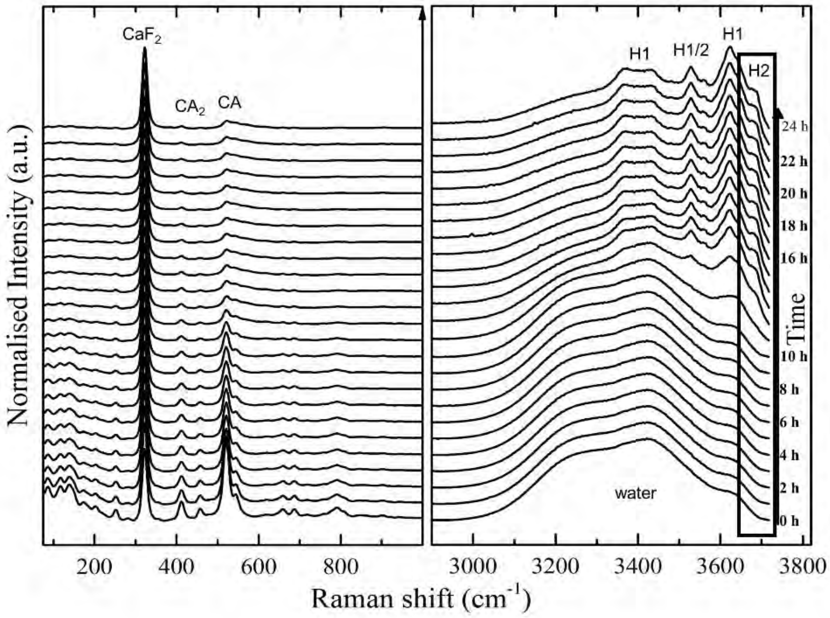

Figure 1: In-situ measurements of the hydration of a commercially available CAC. The Raman spectra shown are averages of one 60x60 µm² Raman image each, measured over a total period of 24 hours. One image was measured for 1 hour. The formation of two CAHs H1 (C_2AH_8) and H2 (CAH_{10}) can be observed. The CaF_2 in the Raman spectra is the signal from the window of the experimental cell through which the measurements were obtained.

A SERS-LFIA Approach for Multiplex Detection of High-Risk Pathogenic Bacteria in Point of Care Settings

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The ability of pathogenic bacteria to persist and cause infections in high-risk settings, such as hospitals, poses a threat to public health and their detection before host infection is key to limiting the potential deadly impacts of pathogenesis.[1] Numerous detection methods are available for bacteria detection, but these are not optimal when considering cost, time and sensitivity. Paper-based lateral flow immunoassays (LFIA) present a simple, low-cost platform which can be integrated with surface-enhanced Raman scattering (SERS) tags combining classic lateral flow colorimetry with SERS quantification.[2] SERS tags can be encoded with different highly Raman active molecules to yield a library of tags individual to target strains through surface functionalisation with antibodies. The immuno-chromatographic approach works by capturing SERS tag-bacteria complexes in a region of immobilised capture antibodies and as the concentration of complexes captured at the line increases, a colorimetric and concurrent SERS response is observed. By encoding SERS tags, multiple targets can be detected and differentiated through SERS spectral analysis of distinct Raman active vibrational modes for each reporter. With the advances in technology and the development of hand-held spectrometers, SERS analysis can move away from the laboratory and into point of care (POC) settings allowing this sensing platform to be used and analysed in a short time period. This labelled SERS approach provides a fast, specific and sensitive multiplexing route for detecting multiple bacterial species in POC settings.

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Raman microspectroscopy in micro-structured chips for tracing single-cell metabolic stress responses in soil microbes

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Large scale ecosystem processes, and especially many of those governing terrestrial nutrient cycles, are driven by soil microbes via their metabolism, e.g. organic matter decomposition or nitrogen fixation. Small changes in the microbial functioning can thus have large effects on the resulting net CO_2 fluxes released from soil as well as soil fertility. Climate change and human activity-induced changes of environmental conditions affect the microbial communities, and better understanding of their metabolic processes can help to determine which direction the nutrient cycles will be affected in.

Because organisms in a community differ in their function and activity levels, and individually respond to the conditions in their immediate microenvironment that trigger specific metabolic reactions, microbial metabolic processes can be better understood if we (also) study them at microscale. Emergent applications of microfluidic technology in soil microbial ecology have opened up possibilities for studying growth and behaviour of living soil microbes, including studies of communities from real soil inocula[1]. Such microstructured *soil chips* serve as habitats for microbes inoculated within and can be set up at a structural and chemical complexity level which allows mimicking real soil.

We further proposed that Raman microspectroscopy can be applied in soil chips to analyse the chemistry of soil microbial processes *in vitro*[2]. For instance, their metabolic activities can be studied via stable-isotope probing (SIP), and microbial identification and phenotypic characterization can be performed via signatures of their intrinsic chemistry. We used confocal Raman microspectroscopy to analyse uptake rates of deuterium-labelled glucose by the hyphae of laboratory grown soil fungus *P. subviscida* as it is exposed to copper. The results demonstrate the potential of the approach to monitor fungal responses in terms of metabolic activity and phenotypical (e.g. change in protein production) changes to abiotic stressors. We are further exploring a similar approach using deuterium-labelled alanine to investigate organismal fitness under varying frequencies of freeze-thaw cycles in natural microbial community from arctic biological soil crusts. In order to trace microbial metabolic responses in the extracellular space, surface-enhanced Raman microspectroscopy and plasmonic effects at the surface of noble metal nano-structures introduced (e.g., via liquid colloids) into the devices will further be employed. Ultimately, clarification of metabolic processes by tracing single-cell microbial chemistry will be crucial for pin-pointing key factors affecting their functioning under changing environmental conditions and their feedbacks on global nutrient cycles.

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Application Perspectives of Nonlinear Spectroscopic Imaging with Broadband CARS

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Nonlinear spectroscopic imaging techniques, such as Coherent Anti-Stokes Raman Scattering (CARS), have emerged as powerful tools for biomedical research, supporting medical diagnostics and therapy. Broadband CARS (BCARS) plays a promising role in the field since it enables label-free and chemically specific imaging at subcellular resolution of a broad vibrational spectrum at high speed [1].

We present a BCARS setup based on a chirped pulse amplification laser and white light generation in a bulk crystal or a fiber for BCARS spectroscopy. We showcase measurements of various samples, e.g. subcellular components, cell cultures, solvents, and complex human tissue samples. The imaginary part of the BCARS spectrum corresponds to the spontaneous Raman spectrum. It is reconstructed through a phase retrieval algorithm based on the Kramers-Kronig relations. Machine learning algorithms are employed to further explore the information content of the extensive spectral data cubes.

With that, we are able to access the characteristics of the vibrational spectra of the (bio-) chemical compositions, allowing the differentiation of tissue types, the observation of differences in metabolism, the tracking of lipids or the monitoring of processes such as drug delivery. The achievable imaging speed due to the coherent process sets the stage for time-resolved imaging *in situ* and live imaging *in vivo* making specific contrast, e.g. digital staining, accessible in the clinical environment.

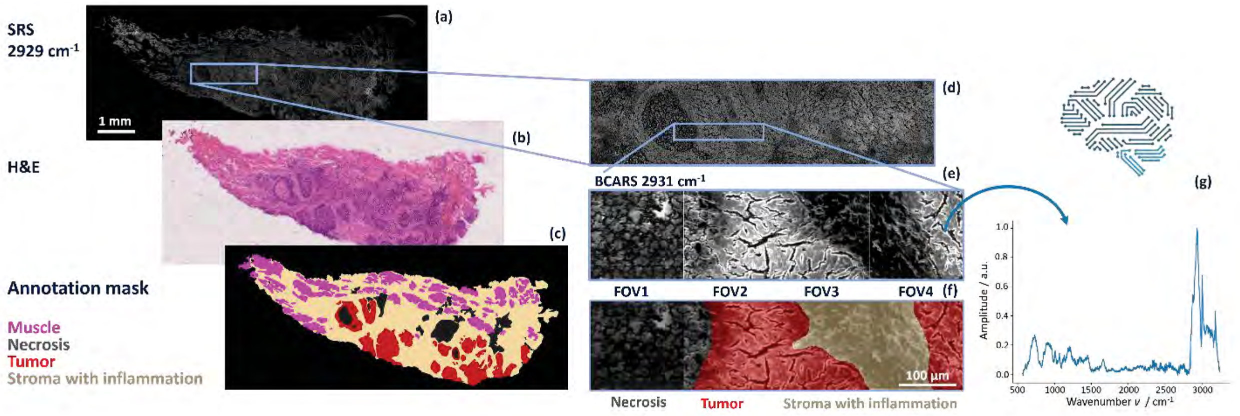

Figure 1: Example of the segmentation and registration of SRS measurements (a) and H&E images (b) combined with histo-pathological annotation masks (c) to spectral BCARS data cubes (e,g) of head and neck tissue samples as part of the ground truth validation for the AI-based tissue differentiation. (g) represents a reconstructed vibrational broadband CARS spectrum.

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Rapid Fabrication and Bioapplication of Novel Planar SERS Substrates: Bridging the Gap Between High Sensitivity and Cost-Effectiveness

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The development of cost-effective yet highly sensitive substrates is crucial for broadening the applicability of Surface-Enhanced Raman Spectroscopy (SERS), particularly in rapid screening and bioanalytical applications. We introduce two novel methods for the fabrication of planar SERS substrates: **Spark Discharge (SD)** and **Interelectrode Material Transfer (IMT)**. These approaches yield substrates with moderate to high sensitivity, achieving a detection limit of approximately 10^{-10} M for adenine, which serves as a benchmark molecule in the context. Additionally, these methods substantially reduce both the cost and preparation time compared to more sophisticated techniques like nanoimprint lithography [1] or self-assembly approach [2].

The SD method enables production of SERS chips within minutes, suitable for preliminary research and high-throughput applications [3]. Concurrently, the IMT technique ensures homogeneous substrate creation, well-suited for extensive mapping experiments. Our optimization and characterization detail the substrates' efficacy for qualitative and quantitative biomolecule analysis, highlighting their potential for diverse bioapplications.

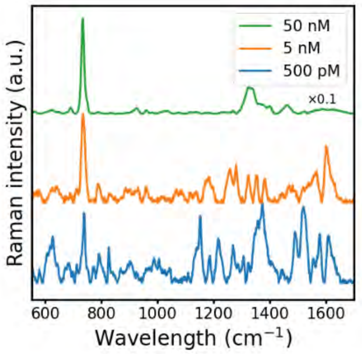

Figure 1: Raman spectra of adenine at low concentrations obtained using a SD fabricated SERS substrates.

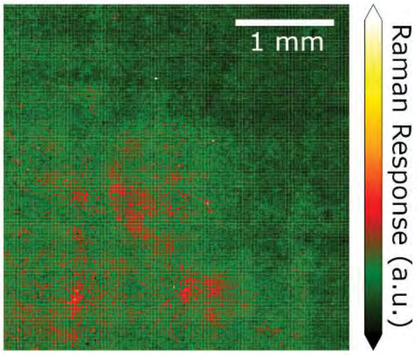

Figure 2: Large-area Raman mapping of a 3x3 mm segment on an IMT SERS substrate. The image comprises approximately 43,000 automatically evaluated spectra [4], with the colour scale indicating the Raman response (area of the dominant adenine signal).

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Raman spectroscopy and structure of selected secondary metabolites in living *Streptomyces* cultures

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We used Raman spectroscopy to analyze a class of poly-unsaturated compounds from bacteria of genus *Streptomyces* known as polyenic antibiotics.

Streptomyces are filamentous bacteria that produce a wide range of bioactive compounds. They are exploited for production of various antibiotics, antifungals and other drugs, and include promising candidates for biotechnological production of new antibiotics and other natural products with pharmaceutical applications.

We explored the relationship between the molecular structure, vibrational modes and Raman spectra of the polyenic antifungal compounds. We took advantage of the near-resonant Raman signals of the polyene chains under excitation by 532 nm laser. The analysis of Raman spectra from reference samples of various polyenes accompanied by computer simulations based on Density Functional Theory (DFT) calculations allowed us to quantitatively link the key structural features of polyenes, such as their length and configuration changes, with shifting positions of the vibrational modes and their spectral signatures.

We concurrently acquired Raman spectra of several live *Streptomyces* strains producing polyenic compounds and analyzed their production *in vivo* under various culture conditions. Our analysis revealed distinct spectral signatures of several polyenes, as well as other secondary metabolites in the living mycelia of the *Streptomyces* samples. Our results underscore the enormous potential of Raman spectroscopy *in vivo* for biotechnology applications involving the monitoring of antibiotic production and screening for new antibiotics.

Aknowlegment

Electron microscopy and Raman spectroscopy analysis provided at Core Facility Electron microscopy and Raman spectroscopy, ISI CAS, Brno, CR, is supported by MEYS CR (LM2023050 Czech-BioImaging)

The Culture Collection of Actinomycetes (www.actinomycetes.bcco.cz, BCCO, Biology Centre Collection of Organisms) was supported from the programme Strategy AV21 Land Conservation and Restoration (2020 - 2024) of the Czech Academy of Sciences.

Raman Stable isotope probing of single bacterial cells

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Raman-stable isotope probing is a technique that is attracting attention to identify and understand various bacteria and their metabolic pathways. The basic building blocks of any living organism are biomolecules namely, carbohydrates, lipids, proteins and nucleic acids. Such macromolecules inside bacterial cells can be analysed in detail using Raman microspectroscopy[1,2]. Previously, there have been studies comparing Raman spectra of different stable isotope labelled bacteria during its growth period. Different genera of bacteria when cultured and studied in same medium showed that the isotope incorporation took different routes [3]. However, now it would be fascinating to know how these metabolic pathways are chosen when the same bacteria are cultured in media containing different carbon sources (with isotopes).

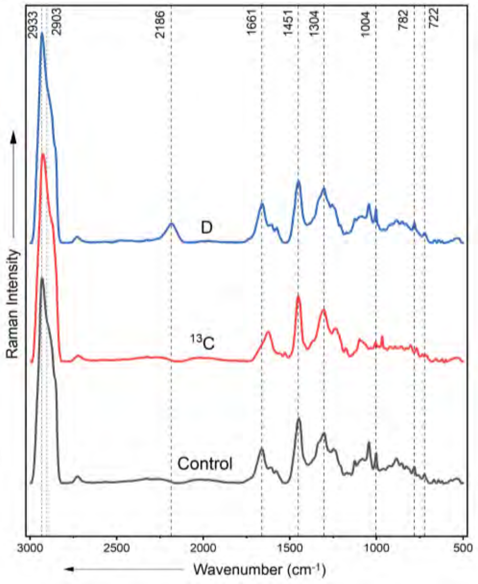

Figure 1: Raman spectra of single bacterial cells control (black), with ^{13}C isotope(red) and with deuterium (blue)

Acknowledgements

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SERS Characterization of anti-biotics drug molecule by using modified silver nanoparticles as substrate

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Abstract

The synthesis of nanostructured materials with tuned photoelectric properties is a crucial step in the successful development of SERS sensing platforms. The success of SERS is highly dependent on the interaction between adsorbed molecules and the surface of plasmonic nanostructures. In general, Au and Ag are most often used as SERS substrates because they are air stable materials.

The abuse of synthetic antibiotic ciprofloxacin (CFC) is on the increase and caused the diffuse of drug resistant. It called for a rapid and powerful technique to detect the CFC residue level in food products and environment. Highly sensitive surface enhanced Raman spectroscopy (SERS) is a robust tool offering intrinsic vibrational information of molecule could be developed to become a fast approach for detecting trace targeted species.

Graphene Oxide, GO has been demonstrated to be an effective component for breaking the barrier of the weak binding between target molecules and "hot spots" of noble metals due to its superior ability of molecular enrichment, which is called chemical enhancement. Moreover, GO serves as a known fluorescence quencher, has the ability of reducing the fluorescence interference and enhancing SERS signal intensity. Therefore, we consider combining the GO membrane and noble metal nanoparticles as SERS substrate to improve detection performance.

Herein, we decorate silver nanoparticles on GO as a SERS substrate for direct trace detection of CFC. The developed analytical approach posed an unprecedented limit of detection of 10^{-12} M with a wide dynamic range of 10^{-4} -10^{-14} M.

Exploring Chiral Surface-Enhanced Raman Scattering through Analyte-Capped Colloidal Systems

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The detection of molecular chirality in surface-enhanced Raman scattering (SERS) experiments has gained significant interest due to the possibility to identify and examine chiral molecules even at minimal concentrations. However, routine application of this technique encounters numerous obstacles, such as low sensitivity, time-dependency of the chiral signal, limited understanding of interactions between chiral molecules and metal surfaces, variations in nanoparticle size and shape, and sample instability. Previously, investigations into surface-enhanced Raman optical activity (SEROA) were conducted to identify chiral analytes using mediator molecules attached to silver surfaces [1]. In the present study, we expand upon the promising outcomes of the prior investigation by highlighting that the preparation of silver colloids in the presence of the analyte i.e. colloidal nanoparticles capped with the chiral analyte can further amplify the SEROA signal. The circular dichroism (CD) observed in these colloids suggests that both polarized Raman scattering and CD contribute to the recorded SEROA spectra, sometimes exhibiting bisignate characteristics. It is crucial to maintain well-dispersed, non-aggregated colloids to observe genuine molecular vibrational SEROA spectra, as polarized Raman scattering and circular dichroism may dominate the measured signal in the case of aggregated colloids.

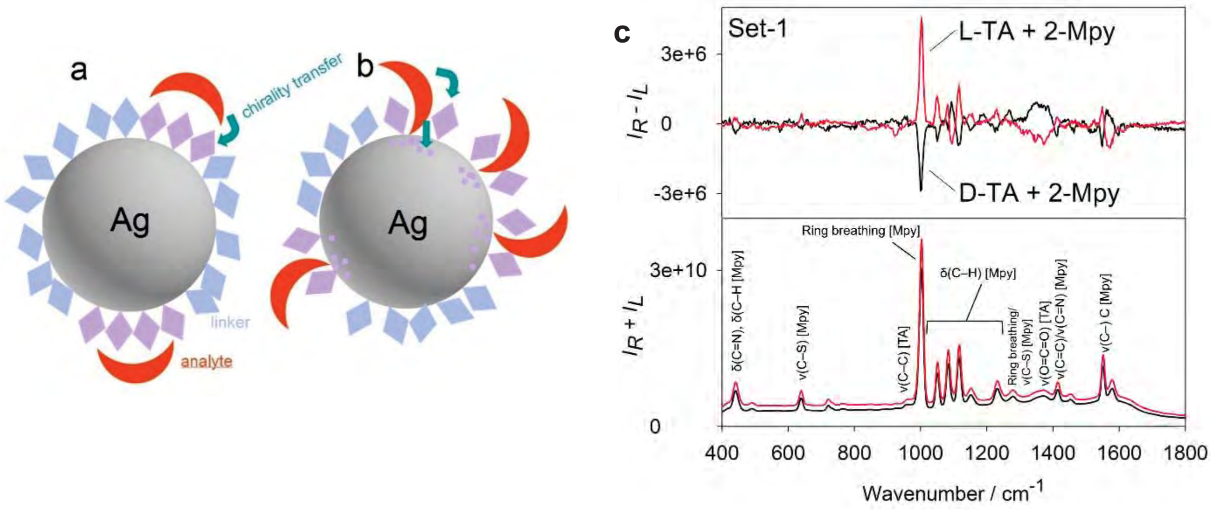

Figure 1: (a) The ‘sergeant and soldiers principle’ experiment where chirality is induced in the linker by the analyte which acts as a chiral modifier on the surface and (b) the present study, where the surface is “capped” with both the analyte and the linker. (c) SERS and ‘mirror-image’ bisignate SEROA spectra of tartaric acid (TA) and 2-mercaptopyridine (2-Mpy) capped silver nanoparticles along with assigned vibrations.

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Multiplexed SERS for wastewater treatment utilizing highly absorbent biogenic powders to eliminate environmentally realistic mixtures comprising inorganic heavy metals, antibiotics, and dyes

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Wastewater poses a significant threat to the public health and environment. Contaminated water can spread diseases, harm aquatic ecosystems, and compromise drinking water sources. Addressing wastewater issues is crucial for safeguarding both human well-being and the delicate balance of our ecosystems. The intricately arranged 3D nanoarchitecture of pores and channels in biogenic carbonate from crustaceans is optimal for the effective adsorption of solutions. Therefore this powdered material has succeeded to expand its applications as a drug carrier [1], an upgrade in pharmaceutical formulation [1], [2], [3] , an ecological biostimulant for soil amendments [4] and as wastewater remediation for removal of antibiotics and dyes [5]. Here we propose SERS-based methodology to control copper sulfate, doxycycline hyclate and methylene blue in waste water treatment with highly adsorbent biogenic carbonate, which has a preserved nanoporosity suitable for efficient adsorption, in a pellet formulation upon exposure to water. SERS analysis of aqueous solutions: examining raw water with three added components for comparison, initially conducted has been used to track the marker bands evolution in a time-dependent manner, to asses the powder's adsorption capabilities for multiple molecular species. Thus, real-time monitoring of the SERS signal is a sensitive option for pollutants removal control. Biogenic carbonate loaded with doxycycline hyclate and methylene blue has previously demonstrated efficacy, showcasing its ability to adsorb the pollutants effectively.[5] Doxycycline hyclate's SERS is concentration-dependent, exhibiting major bands at 1333 and 1578 cm^{-1} and methylene blue's SERS peaks at 1623 cm^{-1} . Understanding the intricacies of SERS in this methodology has showed innovation in broadening the environmental adaptability of biogenic waste materials and reinforcing SERS as an effective instrument for managing wastewater.

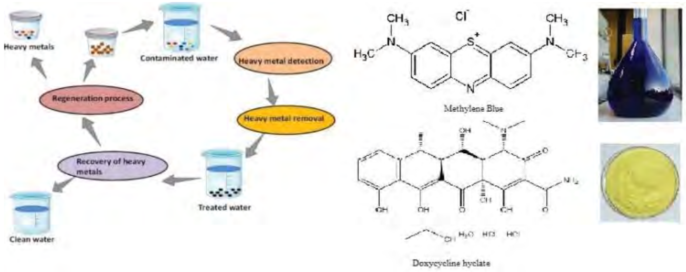

Figure 1: The design of utilizing SERS as a proficient tool for wastewater management and regeneration through the application of biogenic carbonate powders and molecular structure of methylene blue and doxycycline hyclate

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Impact of wet to dry transition in Surface-Enhanced Raman Scattering signal based on electromagnetic interaction by dynamic analyte behaviour

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The impact of wet to dry transition is essential to better comprehend the electromagnetic interaction at hot spots by the dynamic behaviour of molecule interaction based on near-field effects of complex electromagnetic interaction that profoundly affect the SERS spectrum shape[1,2]. In this sense, a systematic investigation of R6G solution drying process on SERS substrates prepared by self-assembled gold nanorods (AuNRs) nanostructures. The experimental data were interpreted based on changes in the surrounding dielectric environment investigation combined with statistical analysis highlighted the potential to produce efficient and extended to other more for (bio)sensing applications and extend to other more scientifically challenging molecules. More specifically, multidimensional analysis ensured the distinction of the study's requirements applied to the SERS response for wet, dry, and water addition after dry molecule on SERS substrate, exhibiting a silhouette coefficient of 0.92 in the least-squares projection technique. Changes in the SERS spectra profile from wet to dry state condition of R6G dye solution until 10^{-8} mol L^{-1} can be interpreted as the dynamic behavior of R6G molecules correlated to distinct molecular adsorption and(or) surface distribution of the R6G molecules proving different plasmonic resonances. Simulations obtained from BEM calculation in experimental data corroborate that the SERS enhancement is strongly dependent on the nanoparticle coupling in the nanoscale and the dielectric environment.

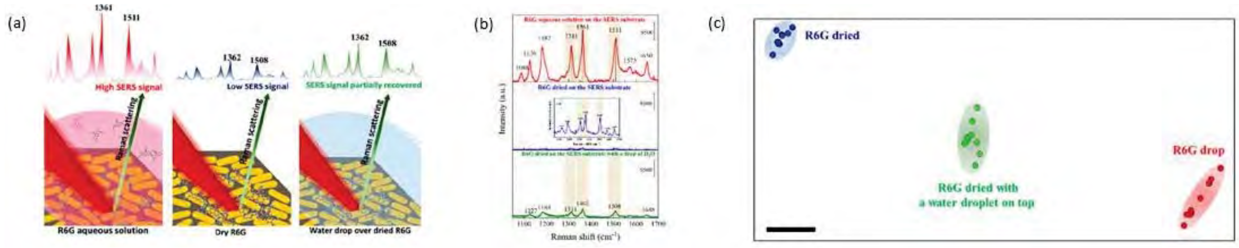

Figure 1: (a) Schematic illustration SERS spectra in different analysis conditions. (b) SERS spectrum of 5 μL of R6G aqueous solution on the SERS substrate (red line), spectrum after R6G dry on the SERS substrate (blue line), and a drop of water (5 μL) on the top of SERS substrate with R6G already dried. (c) LSP plot of the whole of the SERS spectra for R6G aqueous solution (red dots), R6G dried on the SERS substrate (blue dots), and a drop of water on the top of SERS substrate after R6G drying (green dots).

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Towards CARS super-resolution in opaque media

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Microscopy using focused light and chemically selective excitation is an important tool in chemical, (bio)physical and biomedical research, particularly with respect to the study of living organisms. Unfortunately, the resolution of the optical microscope is limited: the diffraction of light imposes limitations on the spatial resolution achievable. Consequently, the fine details of a specimen of a size below this diffraction-limit cannot be imaged. To tackle this issue, our team has developed a microscope based on stimulated Raman scattering, combined with randomly structured light illumination technique, called Blind-S3[1]. The technique enabled fast super-resolution in stimulated Raman scattering microscopy at low power levels and in opaque tissues. We are now developing a new super resolution microscope based on the Coherent Anti-Stokes Raman Scattering in order to further increase the super-resolution gain. Yet, the presence of a non-resonant background complicates reconstruction of super-resolved CARS images. In this contribution, we develop a new methodology to demonstrate super-resolution in CARS microscopy in opaque materials. We establish a frequency modulation approach to extract coherent artifacts in the CARS images: an amplitude frequency modulation of the CARS signal $I(\Delta\delta) = I(\omega_1) - I(\omega_2)$ can be extracted by using lock-in detection in a high-speed manner [2]. We present a mathematical formulation of the image formation and further analyze the imaging acquisition process to demonstrate super-resolution by a factor of 1.27 .

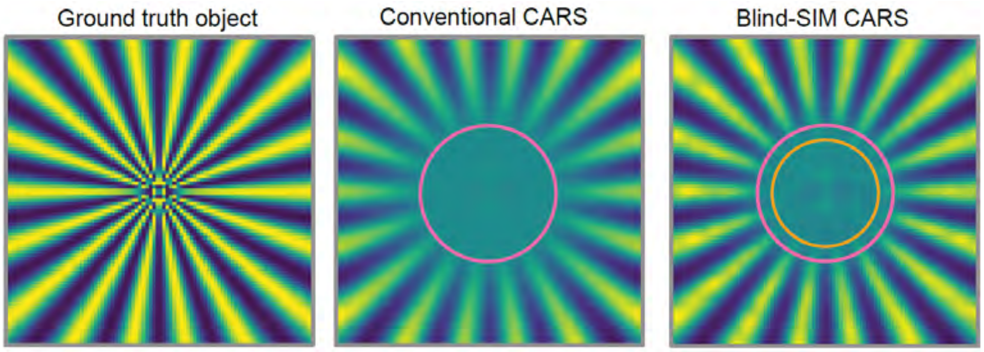

Figure 1: Resolution comparison between conventional CARS and blind-S3 in simulation. On the left, the Siemens star object used for the simulation. In the middle, conventional CARS image and on the right blind-SIM technique applied to a Siemens star object. The pink circle represents the diffraction limit for conventional CARS and the orange circle represent the achieved resolution for the blind-SIM new method developed.

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Investigation of plasmon-mediated thiolate surface reactions

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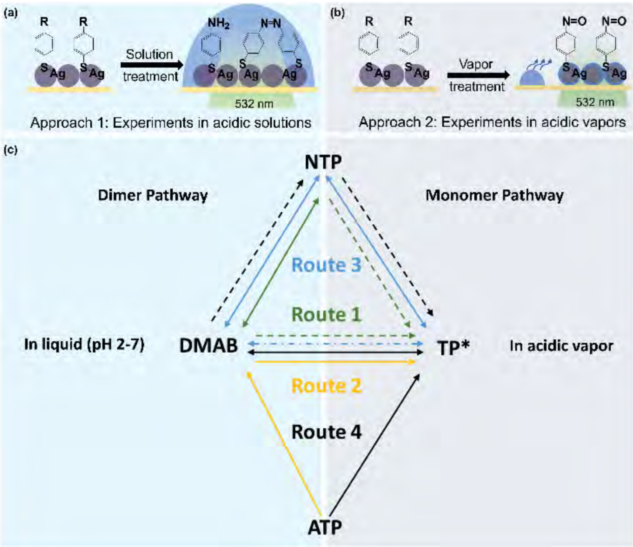
In the study of plasmon-mediated catalysis, 4-aminothiophenol (ATP) and 4-nitrothiophenol (NTP) are widely used. The current discussion focuses on two reaction pathways: the dimeric pathway, which involves the formation of 4,4′-dimercaptoazobenzene (DMAB), and the monomeric pathway, which proposes the reduction of NTP to ATP in an acidic solution containing Cl^-. We recognized a stable compound 4-nitrosothiophenol (TP*) in our experimental study by carefully controlling the reaction conditions in acidic solutions and vapor. The plasmon-mediated reaction mechanisms were identified and correlated with computational modelling of the plasmonic hybrid systems. Time-dependent SERS measurements tracked the reaction dynamics, establishing a clear link between the dimer- and monomer-based pathways and suggesting possible reaction routes under different environmental conditions (Scheme 1). The thermodynamics of thiolate reactions induced by plasmon-mediated catalysis at the molecular level were critically analyzed, complementing the spectroscopic results.



Scheme 1. Sketch of different experimental approaches among NTP, ATP, DMAB and TP*.[1]

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We acknowledge supports from the Leibniz Science Campus “InfectoOptics”, DFG SFB 1278 “Polytarget” (B04), DFG Project 44866627, QUEM-CHEM (ERC, grant no. 772676) and DFG SFB 1375 “NOA”–(A04 and C02).

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Machine Learning for detection of dissolved CO_2 using porphyrins by surface-enhanced Raman scattering (SERS)

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Recently, carbon dioxide (CO_2) monitoring in water systems has become an important scientific field [1]. For this purpose, Surface-Enhanced Raman Scattering (SERS), which offers high selectivity and sensitivity, has emerged as a promising analytical technique. It is especially useful for sensor development due to its affordability and practicability. However, because of its low Raman cross-section and lack of affinity for plasmonic noble metal nanostructures, direct SERS detection of CO_2 is limited. Therefore, SERS substrates based on porphyrins, which act as chemoreceptors, and gold nanostars, which act as optical enhancers, were created. Thus, the identification of dissolved CO_2 was performed by spectral variation of SERS fingerprints of porphyrins when the interaction between CO_2 and porphyrins occurs [2]. As this field has become progressively data-driven, it can now benefit from comprehensive Machine Learning (ML) approaches [3].

In our study, we developed a pipeline that preprocesses in-house portable SERS spectra to enhance data quality, employ data augmentation to enrich the training data and utilize unsupervised and supervised ML algorithms for the automated detection of CO_2 in aqueous environments. By extracting relevant features and with feature engineering, from SERS spectra, we were able to achieve a 96.3% accuracy model with an Extra Trees classifier after hyperparameter tuning and feature selection. This multidisciplinary integrated approach enhances CO_2 monitoring in water systems, providing promising advancements in environmental sensing and monitoring.

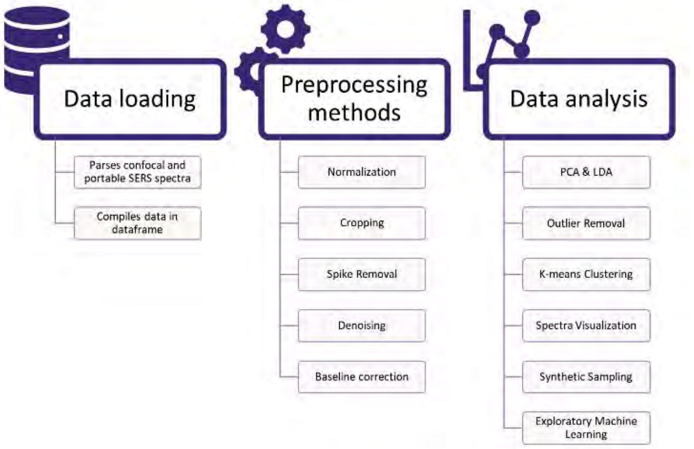

Figure 1: Graphic representation of the pipeline for preprocessing and analysing SERS data.

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Raman Spectroscopy for Real-Time Nutrient Monitoring in Bioreactor Growth Media: Feasibility and Limitations

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Raman spectroscopy, a well-known sensing technique for molecular identification, finds extensive application in analysing aqueous solutions due to its ability to precisely determine concentrations of mixtures using cost-effective lasers and spectrometers, making it suitable for use in laboratories or industrial settings [1] [2]. Its capability to offer real-time, in situ monitoring without the need for complex sample preparation makes it a promising tool for facilitating the optimization of culture conditions in bioreactors. Specifically, Raman spectroscopy offers critical information for factors such as nutrient availability, pH, temperature, and oxygen levels directly within the bioreactor environment [3] [4].

In this work, we are focusing on the detection and monitoring of nutrients concentrations in growth media of cell cultures using Raman spectroscopy. Real samples provided by bioreactors were extensively analysed using a portable MarqMetrix All-in-One Raman. The Raman system is equipped with a 785 nm solid state laser, with spectral resolution averaging at 6.5 cm^{-1} across a spectral band range from 100 to 3200 cm^{-1}. To focus the laser beam, a fibre optic Probe equipped with a sapphire spherical lens was in contact with the liquid surface contained in a quartz cuvette. Key substances in two different growth media (K3 and van Niel) such as glucose, nitrate, sulphate, and phosphate, were targeted for detection and concentration determination. Raman spectra were acquired and analysed for media with varying nutrient concentrations at different time points during culture growth. Prototype samples, including single nutrients and mixtures, were also measured to evaluate the reliability and accuracy of the methodology.

The results of this work demonstrate the feasibility and effectiveness of Raman spectroscopy for the real-time monitoring of nutrients in complex growth, while also revealing its inherent limitations. The outcome of this study will be later on tested with Raman on chip sensors developed within an EU project named LIBRA towards the development of a benchtop smart multisensing system for the in-line automatable screening of cultivation processes in bioreactors with the use of PIC sensors.

Acknowledgment: LIBRA Project. This project has received funding from the European Union's Horizon research and Innovation programme under grant agreement. No 101093150.

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Moiré induced thermal conductivity in twisted-bilayer $MoSe_2$

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Twisted bilayer (t-BL) transition metal dichalcogenides (TMDCs) attracted considerable attention in recent years due to their distinctive electronic properties, which arise due to the moiré superlattices [1,2] that lead to the emergence of flat bands and correlated electron phenomena. These materials also exhibit intriguing thermal properties, including a reduction in thermal conductivity. In this study, we investigate the thermal conductivity of monolayer (1L) and t-BL $MoSe_2$ at specific twist angles around symmetric stacking AB (0°) and AB' (60°), as well as one intermediate angle (31°), utilizing the optothermal Raman technique [3]. The observed thermal conductivity values are 13 ± 1, 23 ± 3, and 30 ± 4 W $m^{-1}K^{-1}$ for twist angles of 58°, 31°, and 3°, respectively, findings which are consistent with our first-principles calculation results. The decrease in thermal conductivity in t-BL $MoSe_2$ compared to monolayer (38 ± 4 W $m^{-1}K^{-1}$) can be attributed to phonon scattering induced by moiré superlattice formation. The moiré superlattices within this twisted configuration contribute extra phonon scattering, which decreases the mean free path of phonon and, consequently, the thermal conductivity. Apparently, when adjacent $MoSe_2$ layers are twisted, the number of atoms in the unit cell increases, which leads to an increment in the number of phonon vibration modes. This increment can reduce the phonon lifetime, as well as more phonons to scatter. In addition, the reduction in thermal conductivity found in t-BL $MoSe_2$ might be attributable to Brillouin zone changes [4]. These modifications are caused by in-plane rotation, which results in the formation of many folded phonon branches. Theoretical phonon lifetime studies and electron localization function (ELF) analyses further elucidate the origin of angle-dependent thermal conductivity in t-BL $MoSe_2$. This research paves the way towards tuning twist-angle dependent thermal conductivity in bilayer TMDC systems.

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In situ Raman characterization of the conversion of preceramic polymer structures prepared by DLP 3D printing to silicon oxycarbide ceramics

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Digital Light Processing (DLP) is one of the 3D printing methods which could be used to process photocurable polymers. With the appropriate selection of specific polymers, it is possible to obtain 3D samples that could then be converted to ceramic materials by thermal processing. One example of such preceramic polymers are polysilsesquioxanes modified by methacrylate groups, which may be used to obtain 3D silicon oxycarbide (SiOC) structures. The physicochemical properties of the SiOC materials are related to their characteristic amorphous silica structure with carbon introduced as a substituent of some oxygen atoms as well as a separate phase [1].

The application of DLP as one of the 3D printing methods offers the possibility to design the desired product microstructure. However, in order to prepare a 3D SiOC structure, it is necessary to perform a high-temperature processing of the 3D-printed polysilsesquioxanes sample which not only provide the polymer-to-ceramic conversion, but also does not deform the designed geometry. Therefore, it is important to perform a detailed characterization of the structural changes in the function of temperature, which could be related to the results of microstructural investigations in order to optimize the thermal conditions of this process.

As it was mentioned, the properties of SiOC materials are linked to the form of carbon in their structure. In the present study, we propose to apply Raman spectroscopy for the in situ characterization of polymer-to-ceramic conversion, using an alpha300M+ Raman spectrometer (WITec, Germany) with TS1500 attachment (Linkam Scientific, UK). The measurements were carried out in argon flow to ensure an inert atmosphere to prevent carbon oxidation. As the temperature increased, degradation of the polymer and formation of the ceramic material with a characteristic carbon phase were observed. Based on this results, we propose a complete procedure for the preparation of 3D SiOC structures from the polysilsesquioxanes preceramic polymers.

Acknowledgments

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Development of a Handheld Combined Raman-XRF System for Future Lunar Astronauts

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Human missions to the Moon and Mars are the next step in both ESA and NASA planetary exploration roadmaps. During such missions, astronauts will be required to perform geological analyses as part of surface characterisation and resource utilisation mapping.

PANGAEA is ESA's astronaut training programme which aims to provide astronauts with the fundamental knowledge and training to become field scientists during future lunar (and Mars) exploration missions [1]. To aid with training (and ultimately surface operations), two Portable Handheld cOmbinEd RamaN-LIBS-XRF (PHOENIX) spectrometers have been developed as fast and real-time tools for in situ geological and mineral analysis [2].

We present the design and development of the PHOENIX Raman-XRF prototype handheld system and discuss the performance of the instrument in the context of lunar analogue sample testing.

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Unveiling Insights with Interpretable Models: Explainable AI in Raman Spectroscopy

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Raman spectroscopy is a widely used technique for analysing the chemical composition of materials and biological systems. The complex data obtained by Raman spectroscopy, on the other hand, might be difficult to understand, limiting the development of robust and reliable analytical models. Explainable AI techniques have emerged as a powerful tool for providing insights into the inner workings of complex models, enhancing their transparency and trustworthiness[1]. However, it's important to note that complete interpretability may not always be achievable, and the effectiveness of explainable AI methods can vary across different types of models and tasks[2,3]. In this work, we present a novel explainable AI method specifically designed for interpreting various complex models used in spectroscopic data analysis. The proposed method is evaluated through two case studies: pathogen detection and sepsis detection. In the pathogen detection case study, our inhouse developed method successfully identifies the Raman spectral features that are most critical for distinguishing between different types of pathogens. In the sepsis detection case study, our method effectively pinpoints the sepsis-specific biomarkers that are detected by a generative AI (GAN) model. These case studies demonstrate the significant utility of the proposed method for providing valuable insights into the interpretation of Raman spectroscopy models. We also compared our explainable method with existing methods, including LIME and SHAP, which show superior performance and agreement with PCA analysis loadings.

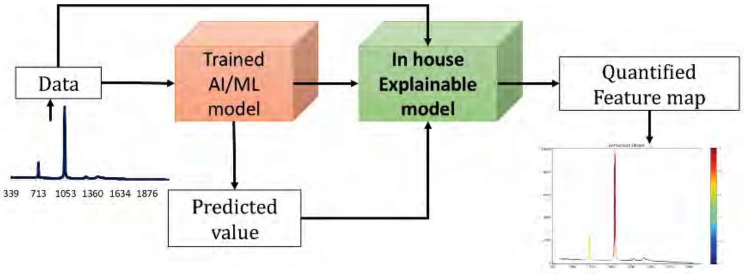

Figure 1. Interpretation model employed to get quantified feature map

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Improved plasmonic detection of Resazurin on a solid copper patterned platform

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Resazurin is a fluorescent dye which reduces in the presence of cell metabolites like NADH and converts into resorufin. This reaction is beneficial in sensitive detection of microbial activity leading to a wide range of applications like cytotoxic analysis in food and water samples, development of metabolic rate assays [1]. Current technologies like colorimetric detection, fluorescence-based sensing have demerits like variable time detection, preparation of different assays for different strains of bacteria associated with them [2].

In order to overcome these problems, we show Raman enhanced detection of resazurin on nanostructured patterned copper surface. We present rapid micro molar detection of resazurin. The merits of this platform include improved control over hotspot distribution and signal uniformity. We present Raman imaging results to demonstrate spatial distribution of hotspots and intensity correlation. Further, we apply principal component analysis techniques to distinguish enhanced spectra. This technique helps in overcoming current problems associated with majority of plasmonic enhancement platforms.

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Lizardite's Crystallographic Orientation Elucidated by Raman Spectroscopy

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Serpentinites are a common rock type that are primarily composed of serpentine minerals ($Mg_3Si_2O_5(OH)_4$). There exist four common forms of serpentine: lizardite, chrysotile, antigorite and polygonal serpentine. Each form has a distinct structure that is a means of accommodating a slight lateral mismatch between the tetrahedral silicate sheet and octahedral magnesium sheet that make up a T-O layer.

Over the course of several Raman measurements of serpentinite thin sections, it was observed that there were significant shifts in the main O-H stretching frequency of lizardite - this was also observed occasionally throughout the literature.[1] While initially attributed to metal cation substitution distorting the lattice, it became apparent from scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) that there was insufficient compositional differences between crystals within most samples to account for such shifts (ca. 14.5 cm^{-1}).

A summary of results from our recent publication are presented whereby it was determined that the O-H peak shifts were dependent on the inclination of lizardite's *c*-axis relative to the impinging laser.[2] This was determined through Raman measurements on polycrystalline thin sections whose grains had their orientations independently determined by electron backscatter diffraction (EBSD). Additionally, Raman measurements were taken on a single crystal mounted on a spindle stage that permitted the inclination of the *c*-axis to be accurately altered.

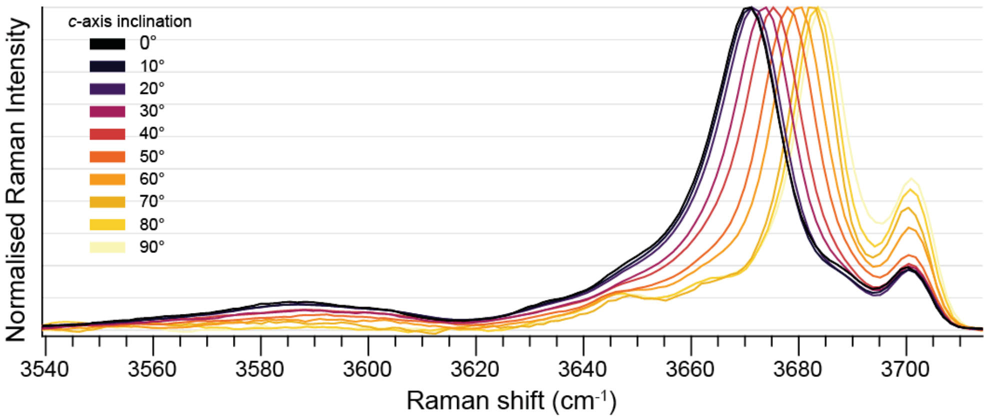

Figure 1: Hydroxyl blue shift based on incline.

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Time-resolved Raman Spectroscopy using a CMOS SPAD array to separate Raman and fluorescent signals

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Many biological samples have a stronger fluorescent signal than Raman signal. Time-resolved Raman spectroscopy is able to separate Raman signals and fluorescent signals in the time-domain due to fluorescence occurring at longer time scales than Raman scattering [1].

We demonstrate the advantages for this application by using a time resolved single-photon avalanche diode (SPAD) line array with 512 pixels operating in time-correlated single photon counting (TCSPC) modality [2], configured as a spectrometer [3]. The Raman spectra of the sample is obtained by focussing a pulsed laser onto the sample. The backscattered photons are detected by the SPAD array, resulting in 512 histograms of photon arrival times, which are dispersed by wavelength across the array. The arrival time of the photons are then used in post processing to separate Raman and fluorescence.

SPAD line arrays with integrated timing electronics allows rapid and multiplexed single photon counting where numerous detectors measure the Raman and fluorescent signal. This increases possible count rate, therefore decreasing measurement time for practical application. In this work we typically use a measurement time of ~30 s.

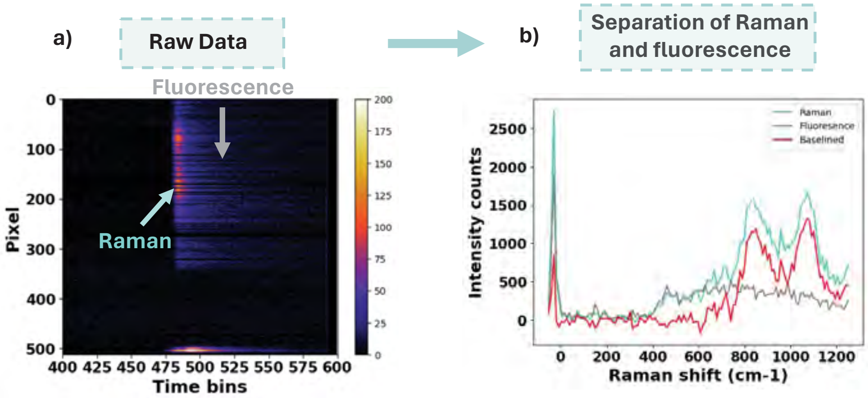

Figure 1: a) TCSPC measurement showing the Raman and fluorescent signal of olive oil. b) Separation of fluorescence and Raman signal.

In Figure 1. we show the separation of Raman and fluorescence scattering in olive oil. The Raman scattering occurs at earlier time bins compared to the fluorescent signal which occurs at later time bins.

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Simultaneous SERS & SEIRA with Single Molecule Detection – Characterization of Plasmonically Resonant Structures with Optical Photothermal Infrared and Raman spectroscopy

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Optical Photothermal Infrared (O-PTIR) spectroscopy has established itself as a breakthrough vibrational microspectroscopy tool, offering significant advantages over the traditional FTIR/QCL & Raman spectroscopy, providing submicron simultaneous IR+Raman.

In a first, the combined and correlative IR+Raman microspectroscopic approach was used to explore Simultaneous Surface Enhanced Infrared Absorption (SEIRA) and Surface Enhanced Raman Spectroscopy (SERS). Both SERS and SEIRA were simultaneously measured from the same location, at the same time at the same resolution on plasmonically active substrates. The sensitivity of this approach enabled very small quantities of molecules, even to the single molecule level to be interrogated while providing complementary information from both infrared and Raman spectroscopy. This arrangement provides additional improvement of the SEIRA sensitivity through the enhancement of both the optical photothermal detector signal and the infrared absorption. The plasmonic substrates tested were silver nanospheres and a gold coated atomic force microscope tip. The concurrent acquisition of SEIRA and SERS is further demonstrated by nano-sampling material onto an atomic force microscope tip. The analytes, Buckminsterfullerene and 1,2-bis(4-pyridyl) ethylene, were analyzed individually and as mixtures. The concurrent acquisition of SERIA and SERS is a unique approach. It has general applications in trace surface analysis and for the analysis of returned planetary samples.

In a further application of the ultra-high far-field IR spatial resolution properties (<500nm), the newly engineered, counter-propagating mode was utilized to characterize, both spatially via single frequency IR imaging and spectrally, plasmonic structures consisting of patterned gold on 500micron thick CaF2 windows. Exceptional IR images utilizing a 100x, 0.9NA glass objective were collected showing unprecedented spatial detail of structures <500nm at different IR wavelengths.

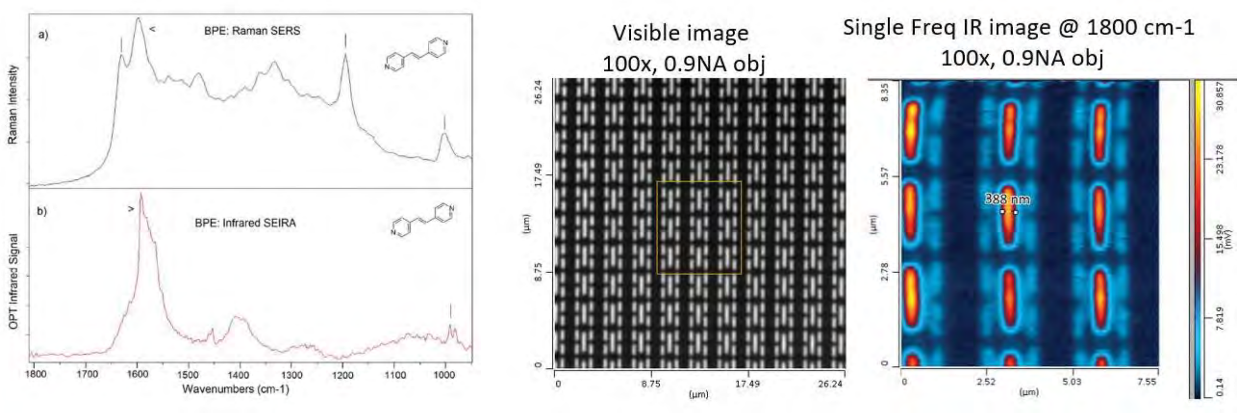

Figure 1: (a) Raman (SERS) and (b) infrared (SEIRA) spectra from a single molecule of BPE. Centre & Right: Visible image (centre) and IR image at 1800cm-1 (right) of patterned gold on CaF2 structures

Organ-specific probing of mitochondrial and lipid properties in *Caenorhabditis elegans* with Raman microspectroscopy

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We employed Raman microspectroscopy to bioimage different organs of immobilized *C. elegans*, a transparent round worm with fixed number of cells and a lifespan around 2 weeks. Using this approach, we simultaneously analyzed the relative quantity, composition, and conformation of lipids and hemes of mitochondrial cytochromes type C and B. We demonstrated the sensitivity of Raman microscopy to the activity of the mitochondrial ETC using strains of *C. elegans* bearing mitochondrial mutations. Combining fluorescent and Raman microscopy, we analyzed the protein and lipid features of single neurons *in situ* (Fig.1) and showed similar properties to human samples [1], which makes *C. elegans* a perfect model of neurodegenerative disease development. We investigate how mitochondria redox properties, protein distribution, and lipid content and composition may be affected by stress and aging.

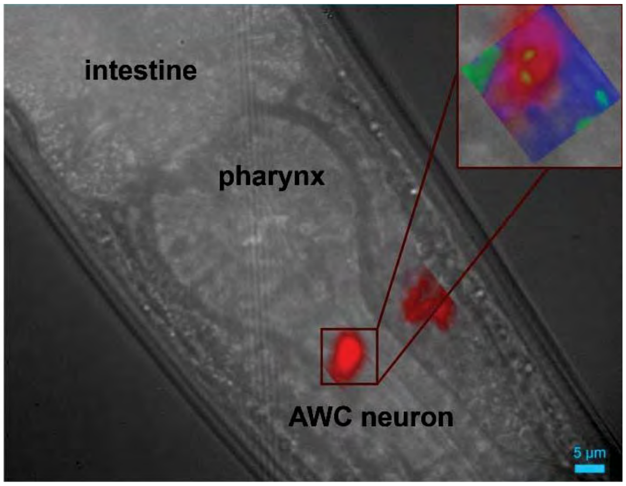

Figure 1: Combined fluorescent (**red**) and Raman (**proteins**, **lipids**) imaging of a single neuron *in situ* in the worm's head.

References

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