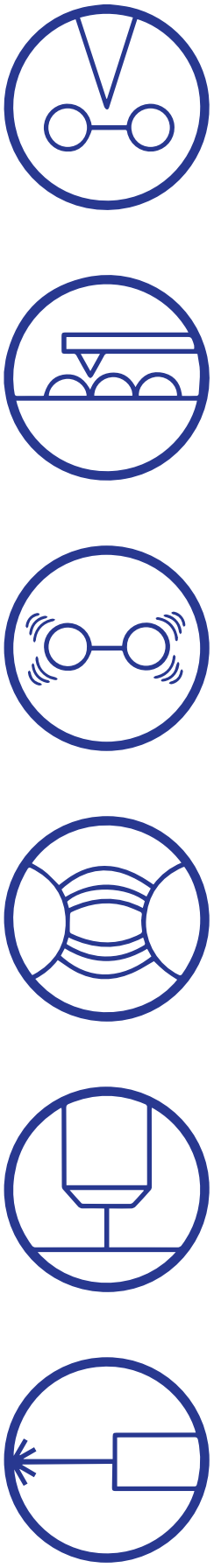
XXVII ICORS

PLENARY PRESENTATIONS



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.

Here we will review the latest innovations in SRS microscopy. In particular, we will highlight three exciting areas in our group: (1) single-molecule vibrational imaging; (2) metabolic imaging in animals; (3) super-multicolor tissue imaging.

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Atomic limit in microscopy & photon confinement

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Optical microscopy on the Å-scale in lateral resolution is attained through Tip-enhanced Raman scattering (TERS) carried out in the atomistic near-field (ANF). To the extent that we see objects by the light they scatter, it is possible to see atoms; albeit, using a plasmonic lens to replace the magnifying glass. In turn, TERS images of isolated atoms establish that on atomically terminated silver needles (asperities) the photon is atomically confined by converting into time-harmonic charge and acquiring mass. As in the limit of optical image resolution, confinement of the photon is fundamentally determined by the atomic granularity of matter. These experimental findings can be put on rigorous footing through a quantum treatment of plasmonics that govern the ANF, where the distinction between light and matter becomes moot, where optics and electronics, and physics and chemistry, merge. This is captured by quantization of the dielectric, which is implicit in quantization of the electromagnetic density required to describe a photon in matter (plasmon or polariton). Experimental illustrations and more general implications will be presented under this unusual light.

Multiplexed and Sensitive Bioanalysis using SERS and SESORS

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Surface enhanced Raman scattering (SERS) is a technique with several advantages over competitive approaches in terms of improved sensitivity and multiplexing. However, the lack of quantitative data relating to real samples has prevented more widespread adoption of the technique. Detection of specific biomolecules is central to modern biology and to medical diagnostics where identification of a particular disease is based on biomarker identification. Many methods exist and fluorescence spectroscopy dominates the optical detection technologies employed with different assay formats. We have made great progress in the development of SERS as a quantitative analytical method, in particular for the detection of biomolecules. Another advantage of SERS over existing detection techniques is that of the ability to multiplex which is limited when using techniques such as fluorescence. A focus of our research is developing multiplexed bioassays using SERS to allow the simultaneous measurement of multiple species in one measurement.

During this presentation we will 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.[1] 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. [2] Also presented will be our recently published work on the use of nanoparticles functionalised with resonant Raman reporter molecule for the visualisation of a 3D breast cancer tumour models using Spatially Offset Raman (SORS) combined with SERRS (SESORRS). [3]

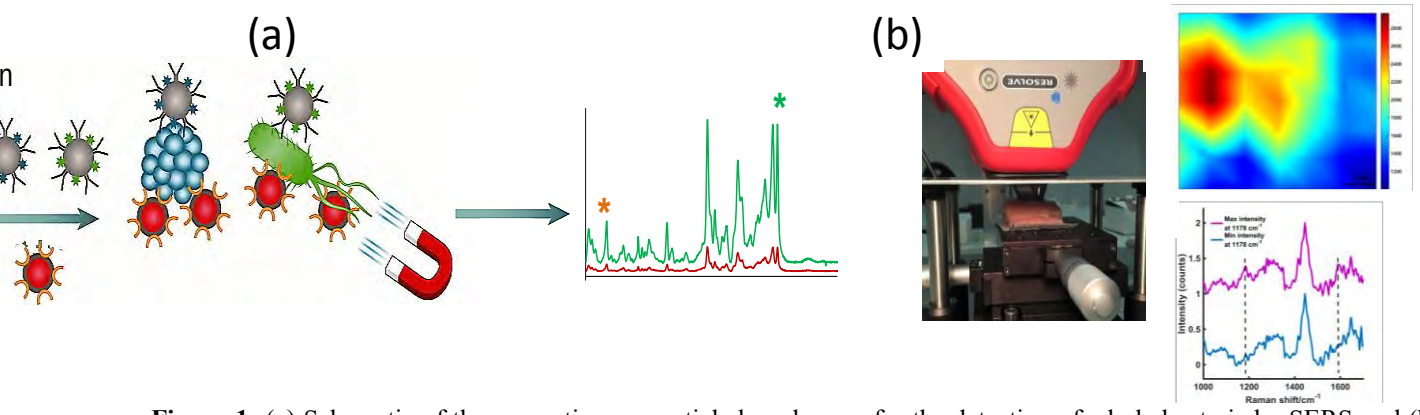

Figure 1: (a) Schematic of the magnetic nanoparticle based assay for the detection of whole bacteria by SERS and (b) the set up used for SORS imaging of tissue mimic using a hand held SORS including a typical SESORRS image of nanoparticles at depth and SERRS spectra of nanoparticles at depth.

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Spatially Offset Raman Spectroscopy (SORS)

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The presentation will focus on the development of Spatially Offset Raman Spectroscopy (SORS) and its variants. SORS is a concept permitting noninvasive interrogation of diffusely scattering media such as biological tissues, opaque containers (paper/plastics/glass) and pharmaceutical powder formulations (tablets/capsules) at depths exceeding those accessible by conventional Raman approaches [1,2,3]. The technique permits rich chemical information on the composition of sublayers or the contents of containers to be retrieved through barriers, as well as to characterise their physical nature (eg temperature).

The covered topics will include introduction into underpinning physical phenomena, discussion of SORS variants and emerging SORS applications comprising disease diagnosis, airport security, quantitative pharmaceutical analysis and characterisation of paint sublayers in cultural heritage.

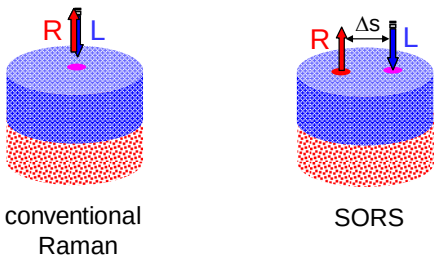

Figure 1: Schematics of conventional Raman and SORS concepts.

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Low-wavenumber Raman spectroscopy in multilayer graphene and related van der Waals heterostructures

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When studying Raman scattering from a material, some valuable Raman signal can be found at very low wavenumber (LW) (below 50 cm^{-1}). The traditional approach to performing such Raman signal involves the use of a triple spectrometer. However, this greatly reduces the signal intensity compared with the combination of a single monochromator and a notch filter/edge filter, although the latter arrangement usually does not allow one to detect modes below ~50 cm^{-1}. Recent exciting progress in Volume Bragg Grating technology enables the design of notch filters with low scattering, high optical density and narrow bandwidth of 5~10 cm^{-1}, which make the LW Raman measurement be available in an easy, compact, and accessible single stage spectrometer, no longer restricted to large research systems or complex and expensive instruments. The corresponding set-up is simple, relies on commercial components, and enables one to obtain good signals with low excitation power and short acquisition time. This technique has the potential to explore the application of Raman spectroscopy in material science, physics and chemistry research.

In this talk, we will first introduce the challenge and some key developments in LW measurement technique. We then focus on our recent efforts on low-wavenumber Raman spectroscopy in multilayer graphene and related van der Waals heterostructures. The research progress on the rigid-layer vibrations both for shear and layer breathing modes in various two-dimensional materials (2DMs) from graphenes to transition metal dichalcogenides are reviewed. Their scaling rule with number of layers can be modeled by an atomic linear chain model, with general applicability to any layered material, allowing a reliable diagnostic of their thickness. This can be further applied to identify the stacking order of multilayer graphene grown by chemical vapor deposition. The corresponding shear modes in multilayer graphenes can be well-fitted with a Breit-Wagner-Fano line shape, which arises as quantum interference between the shear mode and a continuum of optically-active electronic transitions. The shear and layer breathing modes can also be used to probe the interfacial coupling in van der Waals heterostructures (vdWHs). Furthermore, the strong cross-dimensional coupling between the layer-breathing phonons well extended over tens to hundreds of layer thick vdWH and the electrons localized within the few-layer 2DM constituent is briefly discussed.

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Making Raman spectroscopy ultrafast

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One of the unique properties of molecular vibrations is its extreme sensitivity to molecular structure. Miniscule changes to bond lengths and angles, or the slightest changes in interaction with the molecular environment can have a dramatic effect on the associated vibrational frequencies, making vibrational spectroscopy extremely sensitive to molecular structure. Recording vibrational spectra as a function of time thus offers the prospect of following structural dynamics with high structural sensitivity and time-resolution, given sufficient sensitivity and optical pulses of appropriate duration using traditional pump-probe approaches.

A key challenge herein is the fact that the structural sensitivity, which largely stems from being able to measure vibrational frequencies with high accuracy, is at odds with achieving high-temporal resolution, which leads to energy broadening through the time-energy uncertainty relation. This difficulty is exacerbated when attempting to reach the ultimate goal of monitoring molecular dynamics in real time, given that molecular vibrations define the respective time scales of molecular vibrations in the first place.

I will discuss efforts in ultrafast vibrational spectroscopy with a focus on Raman scattering over the past 20 years aimed at reaching the very limits in terms of obtaining structural information from vibrational spectra with femtosecond temporal resolution. Beginning with femtosecond stimulated Raman spectroscopy (FSRS), I will illustrate how structural information beyond the traditional time-energy uncertainty limits can be obtained, under the assumption that the underlying signal generation process is well-understood. This improvement in temporal resolution is analogous to, for example, improvements in the resolution of optical microscopes through single molecule localisation. I will conclude by demonstrating the (dis)advantages of translating the same measurement into the time-domain, in the form of impulsive vibrational spectroscopy (IVS).

Structural and optical properties of 2D van-der-Waals materials

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Two-dimensional (2D) van-der-Waals materials are atomically thin crystalline layers with out-of-plane van-der-Waals (vdW) bonds. They can be almost arbitrarily combined into vdW homo- or hetero-stacks with their physical properties depending sensitively on the material combination, twist angle, and dielectric environment. Despite the large anisotropy between strong in-plane and weak out-of-plane bonds, the out-of-plane contributions of electron wave functions play a crucial role for the physical properties of the entire structure. For example, they determine the direct-to-indirect band gap transition from single-layer to two-layer materials in the semiconducting transition-metal dichalcogenides (TMDCs). In this context, Raman spectroscopy is a versatile tool not just to determine the structural and optical properties of such 2D vdW materials, but to obtain valuable insight into the spatial extent of electron wave functions and into the interlayer coupling. This can be achieved by combining the optical resonances with the symmetry of vibrational modes. In this talk I will discuss the current state of optical and Raman spectroscopy in 2D vdW materials and present some of our recent results [1-3] from combined theoretical and experimental studies on excitons and phonons in 2D van-der-Waals stacks.

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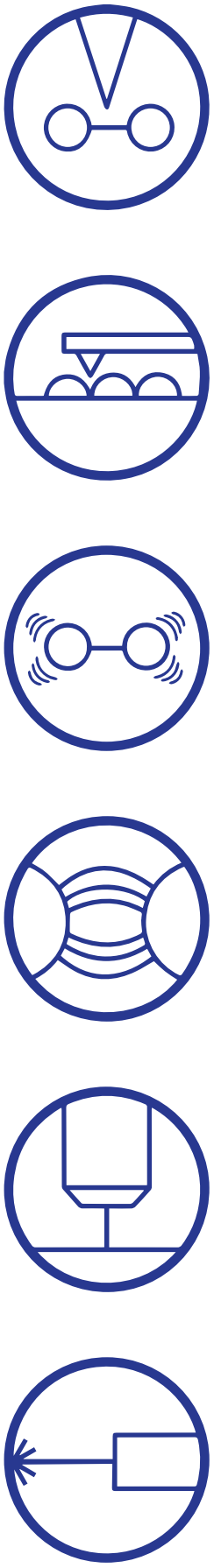

Raman Scattering in the Quantum Domain: Entangling Light and Matter

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Large-scale quantum systems often reveal new phenomena that cannot be studied using classical simulation. Further, such systems enable modes of computing (or indeed other information processing tasks) that cannot be tackled at all using conventional machines, based on classical design principles. Photonics has proven to be an effective means to engineer quantum systems of this kind, and for the investigation of such effects. The challenge is to build a quantum state of sufficient complexity, both in the number of photons and the number of modes.

A promising approach to this task is the concept of hybrid light-matter quantum networks,[1] in which the channels use light as a communication medium and the nodes use matter for processing operations. These have emerged as a particularly important class of architectures for constructing and utilizing complex quantum states. A quantum network, consisting of simple nodes with some quantum dynamics, interconnected by coherent channels, inherits the features of robustness and scalability from its classical network counterparts, but it also exhibits functionality that goes beyond what is possible with conventional technologies.[2]

Raman scattering provides an important means to effect the interface of quantum light and matter.[3] Raman and related controllable two-photon interactions [4] therefore enable a route to preparing quantum states that have applications in quantum information processing. The design, operation and applications [5] of such photonic quantum memories in ambient conditions will be discussed.

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Vibrational Spectroscopic Imaging to Unveil Hidden Signatures in Living Systems

Ji-Xin Cheng

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Chemical microscopy utilizing fingerprint vibrational spectroscopic signals is able to map the chemical contents temporally and spatially. Such capacity opens a new window to visualize the orchestra of molecules and/or biological structures inside living systems. Meanwhile, it is important to emphasize that label-free chemical imaging is not simply adding a spectrometer to an existing microscope. Because the intrinsic signal is much weaker than fluorescence from a dye, integrated innovations in both instrumentation and data science are needed to enable high-sensitivity, high-resolution chemical imaging of a living system. Cheng and his research team have been dedicated to pushing the boundary of vibrational microscopy [1], discovering molecular signatures in diseases, and translating the label-free techniques to clinic for molecule-based precision diagnosis or treatment. Most recent advances include single-molecule plasmon-enhanced stimulated Raman scattering [2], wide-field infrared photothermal imaging [3], bond-selective phase microscopy [4], and discovery of metabolic signatures defining bacterium susceptibility to drugs [5] and cancer cells under stress [6].

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Ballistic of photoisomerization in 13-cis, 15-syn microbial rhodopsins: finally a predictive structure / photodynamic correlation?

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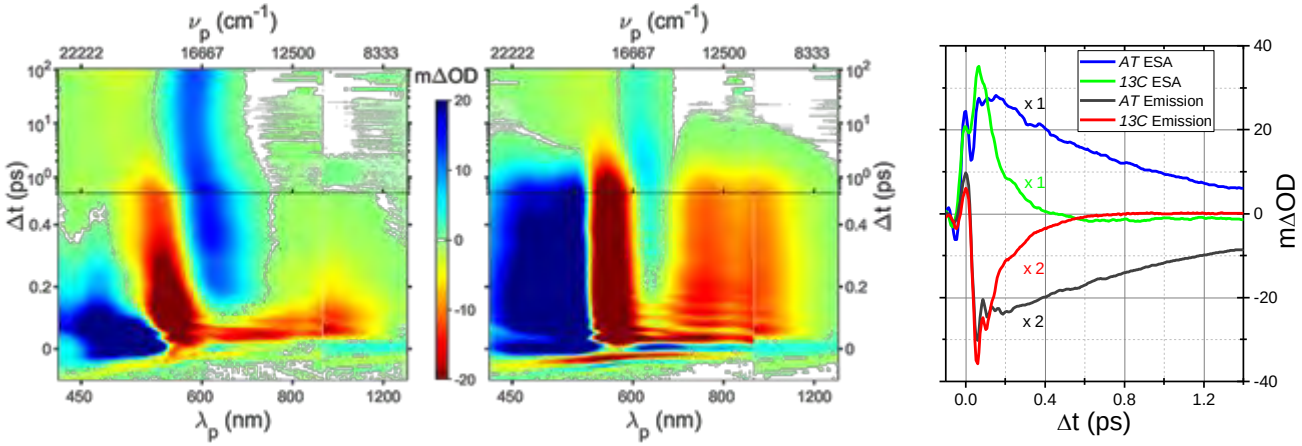
The decades long ultrafast examination of nearly a dozen microbial retinal proteins (MRPs), ion pumps and sensory photoreceptors, has not identified structure / function indicators which broadly predict photo-isomerization dynamics. Whether it will be sub–picosecond and ballistic, or drawn out with complex curve crossing kinetics. Here we report the emergence of such an indicator. Using pH control over retinal isomer ratios, photoinduced transient absorption is recorded in an inward proton pumping Antarctic microbial rhodopsin (AntR) for *13-cis* and *all-trans* (AT) retinal resting states.[i] As shown in the frames of figure 1, the fluorescent state of AT AntR decays with 1 ps essentially exponential kinetics. In contrast in 13-cis it decays within ~300 fs accompanied by continuous spectral evolution indicating ballistic internal conversion. The coherent-wavepacket nature of *13-cis* isomerization in AntR matches published results for bacteriorhodopsin (BR) [ii] and Anabaena sensory rhodopsin (ASR) [iii] which also accommodate both all-trans and *13-cis* retinal resting states, marking a structure – photodynamics indicator which holds for all three tested pigments. Possible mechanisms for this consistent trend will be



discussed.

Strong spectral modulations due to coherent vibrations are observed after excitation of all-trans AntR which are assigned to Raman-induced wave packets in the ground state. Aside from serving to verify the isomer ratio of the reactants, impulsive Raman signatures from S_0 are employed for determining the quantum efficiency for AT AntR, proving it is very high at 70±15%. Along with these, equally strong spectral modulations are also observed due to excited state vibrational coherence, primarily in low frequency torsions of the retinal backbone and in the range of hydrogen out of the plane (HOOP) motions but not for C=C. The latter two observations go against predictions from near resonance Raman spectra. These observations, akin to previously recorded impulsive spectra for BR as well, extend this enigma to the whole family of MRPs, stressing importance of its clarification.

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Magneto-Raman Spectroscopy to Identify Spin Structure in Low-Dimensional Quantum Materials

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Raman spectroscopy, imaging, and mapping are powerful non-contact, non-destructive optical probes of fundamental physics in graphene and other related two-dimensional (2D) materials, including layered, quantum materials that are candidates for use in the next quantum revolution. 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 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 temperature, laser energy, and polarization. Our unique magneto-Raman spectroscopic capabilities will be detailed, enabling diffraction-limited, spatially-resolved Raman measurements while simultaneously varying the temperature (1.6 K to 400 K), laser wavelength (tunability from visible to near infrared), and magnetic field (up to 9 T) to study the photo-physics of nanomaterials. Additionally, coupling to a triple grating spectrometer provides access to low-frequency (down to 6 cm^{-1}, or 0.75 meV) phonon and magnon modes, which are sensitive to coupling. By utilizing electrical feedthroughs, studying the strain-dependent effects on magnetic materials utilizing MEMs devices is also a novel opportunity.

Current results on intriguing quantum materials will be presented to highlight our capabilities and research directions. One example leverages the Raman spectra from α-$RuCl_3$ to probe this Kitaev magnet and possible quantum spin liquid[1]. Within a single layer, the honeycomb lattice exhibits a small distortion, reducing the symmetry from hexagonal to orthorhombic. We utilize polarization-dependent Raman spectroscopy to study this distortion, including polarizations both parallel and perpendicular to the c-axis. Coupling of the phonons to a continuum is also investigated.

Using Raman spectroscopy to probe magnetic phenomena in the antiferromagnetic metal phosphorus trichalcogenide family[2], we highlight $FePS_3$ and $MnPSe_3$. Using magneto-Raman spectroscopy as an optical probe of magnetic structure, we show that in $FePS_3$ one of the Raman-active modes in the magnetically ordered state is actually a magnon with a frequency of ≈3.7 THz (122 cm^{-1}). In addition, the surprising symmetry behavior of the magnon is studied by polarization-dependent Raman spectroscopy and explained using the magnetic point group of $FePS_3$. Using resonant Raman scattering, we studied the Neel-type antiferromagnet $MnPSe_3$ through its ordering temperature and also as a function of applied external magnetic field. Surprisingly, the previously assigned one-magnon scattering peak showed no change in frequency with an increasing in-plane magnetic field. Instead, its temperature dependence revealed a more surprising story. Combined with first-principle calculations, the potential origin of this Raman scattering will be disucssed.

Finally, the magnetic field- and temperature-dependence of an exciting ferromagnetic 2D material, CrI_3, will be presented[3]. We report a magneto-Raman spectroscopy study on multilayered CrI_3, focusing on two new features in the spectra which appear below the magnetic ordering temperature and were previously assigned to high frequency magnons. Instead, we conclude these modes are actually zone-folded phonons. We observe a striking evolution of the Raman spectra with increasing magnetic field

applied perpendicular to the atomic layers in which clear, sudden changes in intensities of the modes are attributed to the interlayer ordering changing from antiferromagnetic to ferromagnetic at a critical magnetic field. Our work highlights the sensitivity of the Raman modes to weak interlayer spin ordering in CrI_3.

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Ultrafast dynamics at the water interfaces revealed by femtosecond phase-sensitive nonlinear vibrational spectroscopy

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The liquid interface provides a unique environment where various important chemical/physical processes take place. However, our understanding of interfacial molecules is insufficient, compared to the plenty of accumulated knowledge about molecules in the bulk. It is because of the difficulty of selectively investigating interfacial molecules by separating them from the vast number of the same molecules present in the bulk liquid phase. Heterodyne-detected vibrational sum-frequency generation (HD-VSFG) spectroscopy is a powerful technique to study liquid interfaces [1]. The advantage of HD-VSFG over conventional VSFG spectroscopy is that it enables us to directly measure the phase and amplitude of the 2nd order nonlinear optical signal generated only from the interface region with a few monolayer thickness, providing interface-selective vibrational spectra that can directly be compared with infrared and Raman spectra in the bulk. The spectra obtained with HD-VSFG are linear to the 2nd order susceptibility of the interfacial molecules and hence they are the simple sum of the constituent spectral components. This characteristic is critically important for time-resolved measurements because time-resolved HD-VSFG provides interface-selective time-resolved vibrational spectra that can be interpreted simply as the spectral change induced by photoexcitation, as in the case of time-resolved infrared or Raman spectra [2].

We realized femtosecond time-resolved HD-VSFG for direct observation of ultrafast processes proceeding at the water interfaces. Recently, using IR-excited time-resolved HD-VSFG and 2D HD-VSFG, we clarified the vibrational relaxation mechanism of free OH at the air/water interface [3] and disclosed the hidden isolated OH at the hydrophobic water interface [4]. Furthermore, with UV-excited time-resolved HD-VSFG, we succeeded in tracking a photochemical reaction of phenol that occurs at the water surface [5]. Our data revealed that the reaction of phenol is drastically accelerated at the interface, compared to the reaction in solution. The marked difference in the reaction dynamics was considered to arise from the unique hydration environment at the interface, and it has been confirmed by quantum chemical calculation combined with MD simulation [6]. These studies on phenol photochemistry also indicated the generality of the significant difference in reaction dynamics between the interface and bulk.

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XXVII International Conference on Raman Spectroscopy
Novel SERS and PIERS substrates for designing bioanalytical platforms

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One of the Raman techniques which brings enormous potential in the detection of subtle molecular changes is surface-enhanced Raman spectroscopy (SERS). This method exhibits ultra-high sensitivity, fingerprint specificity, and surface selectivity, and for that reason, SERS spectroscopy has found numerous applications in bioanalytical chemistry. In our work, we designed SERS label-nanotags and analytical platforms to investigate specific chemical responses in cells and to detect cells in tissues being inflammation markers. For instance, we successfully employed 4-mercaptobenzoic acid conjugated with Au nanoparticles to follow changes in intracellular pH of endothelial cells stressed by inflammatory factors [1,2]. While conjugates of SERS nanotags and antibodies against smooth muscle cells (SMCs) were designed to quantify SMCs within atherosclerotic plaque by using immuno-surface-enhanced Raman scattering imaging (iSERS), see Fig. 1 [2]. The inflammatory state appears at an early stage of most diseases and its detection in a clinically applicable timeframe would have an invaluable diagnostic potential.

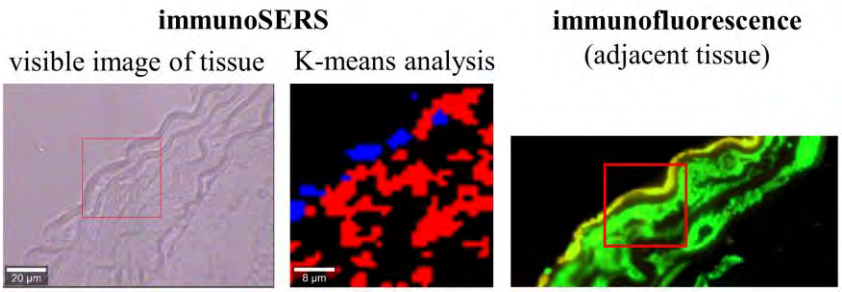

Figure 1: Localisation of SMCs (red) and PECAM (blue) in the BCA cross-section by iSERS imaging(A) and by IF (B). In both cases, secondary antibodies were labeled by SERS nanotags (indirect iSERS) and fluorophores.

Nanomaterial advances have been progressively developed and are intrinsically linked with the fabrication of ultrasensitive SERS sensors. For this purpose, we designed plasmonic nanostructures on porous aluminia and TiO_2 coatings coupled with noble metals [3]. Based on them we constructed SERS-based sandwich immunoassays to capture inflammatory interleukin 6 at a level of ca. 20 pg/mL from fluids [4]. In addition, the Ag@TiO_2 hybrid systems were designed to generate the photo-induced enhancement of the Raman signal (PIERS). The UV photo-treatment of these nanoplatforms induced electron migration from the TiO_2 conduction band to the silver energy levels causing long-lasting (up to 4 h) and superior amplification of the Raman signal (almost 20-fold greater than in SERS). The combination of nano-oval Ag structures and a thin TiO_2 film can open new perspectives SERS applications in analytics [4].

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Micro- versus nano-Raman spectroscopy in two-dimensional systems

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The major limitation of micro-Raman spectroscopy when addressing nanoscience is the spatial resolution, kept higher than hundreds of nanometers due to the restriction imposed by light diffraction. This resolution limitation has been overcome with the achievement of tip-enhanced Raman spectroscopy (TERS), which has already reached the sub-Angstrom resolution and enabled the observation of single atoms or single vacancies in low dimensional (both 1D and 2D) lattices.

The protocols to quantify and qualify defects, strain or doping by Raman scattering have been built, however, based on experimental calibration procedures in the micro-Raman limit. For nano-Raman (TERS), the presence of a nanoantenna, besides allowing materials characterization with spatial resolutions beyond the diffraction limit, it changes the light-matter interaction.

In this work we show that the characterization of defects in graphene-based structures, which is a solid, well-established research field, has its own regime in the nanoscale, where interference related effects affects the expected outcome of measurements. We provide experimental examples of the impacts brought by interference in the characterization of graphene nanoflakes (see figure 1), showing that a reparameterization must be performed in order to apply existing and already validated protocols from the far-field to the near-field. Our results are of special interest from a metrological standpoint, especially for materials sciences and industry related endeavours, with significant impacts that must be accounted for when using nano-Raman spectroscopy to explore materials science.

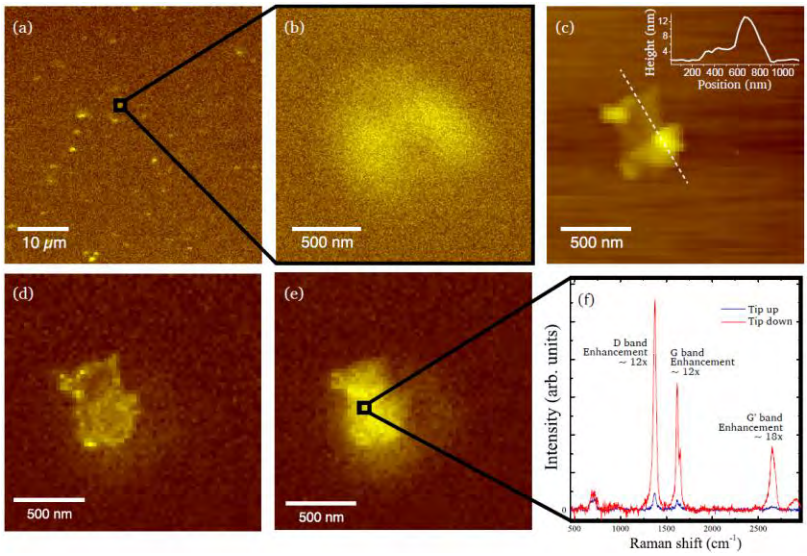

Figure 1: (a,b) micro-Raman images for the G band of a graphene nanoflake displayed in (c) using AFM. (d) and (e) are nano-Ramna (TERS) images generated from the D and G Raman bands respectively. (f) Shows is the Raman spectra obtained at the point marked in (e) with (red spectrum) and without (blue spectrum) the presence of the tip [1].

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Machine learning and chemometrics as tools for bio-medical Raman data analysis

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Raman spectroscopy and Raman based imaging techniques feature properties allow a non-destructive and label-free measurement of the (bio)-chemical composition of samples even in aqueous media. Due to these features Raman based measurement techniques are ideally suited for bio-medical diagnostics or characterization. Nevertheless, the Raman data is untargeted, because most often the acquisition is performed without a label. To tackle this issue and to generate an interpretable contrast in the Raman data, machine learning methods and chemometrics are needed to translate the spectral measurements into useful information. If these analysis techniques should be applied to small Raman data sets, typically encountered in Raman based studies the Raman data needs to be standardized and corrected for disturbing artefacts. Finally machine learning methods and chemometrics are combined into complex data analysis pipelines to fully automate the analysis of the Raman data. Here, we present two data analysis pipelines: for the analysis of Raman spectra and Raman related image data. In the present contribution a summary of our recent studies will be given and pitfalls leading to overestimated, unrealistic model performances will be highlighted.

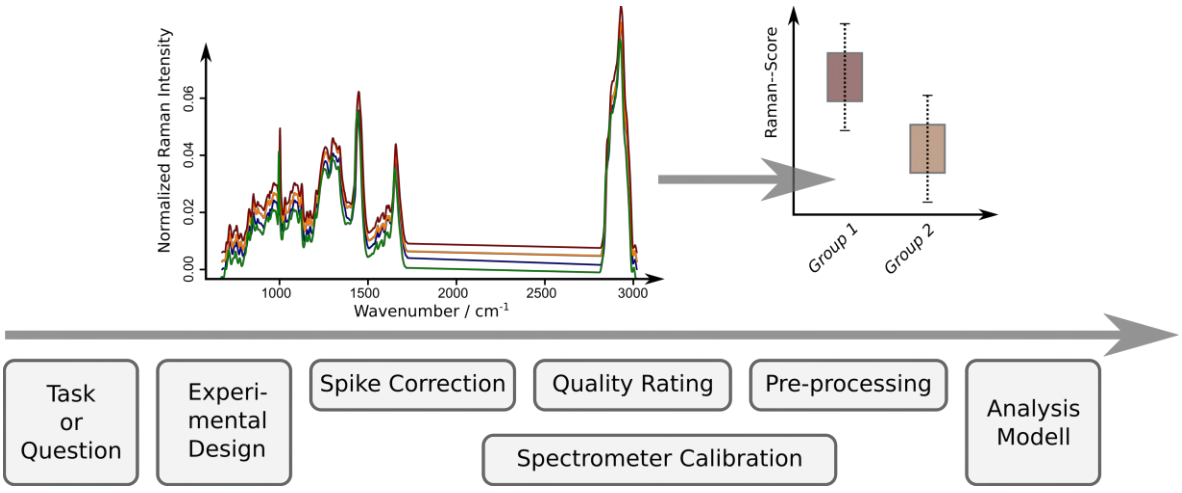

Figure 1: A Raman spectroscopic data analysis pipelines to fully automate the analysis is shown.

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 Foundation are greatly acknowledged.

Structural analysis of complex biomolecules using Raman optical activity (ROA)

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Raman optical activity (ROA) is now a mature chiroptical technique, in which we measure the small difference in Raman scattering from chiral molecules using right- and left-circularly polarized light. ROA combines high sensitivity to stereochemistry and molecular composition and, as a result, ROA has become a highly informative tool for investigating the conformation and behaviour of all classes of biological molecules. After a short introduction to the ROA technique, this talk will present results from a number of studies that illustrate the ability of ROA to provide detailed and novel information on complex biological systems. These include both experimental and quantum mechanics/molecular dynamics investigations into:

i) protein secondary and tertiary structure,

ii) carbohydrate structure and the role of solvation in the conformational dynamics of sugars,

iii) glycoprotein and virus organisation.

Chirality is critically important in biology, and the techniques developed during these studies have opened new opportunities for ROA spectroscopy in understanding the behaviour of diverse complex biomolecules. As new challenges arise in biology, it is likely that even more applications of this structurally sensitive technique will be presented.

Plasmonic core-shell nanostructures for in-situ probing surface reactions

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Surface-enhanced Raman spectroscopy (SERS) can provide fingerprint structural information of molecules with ultrahigh surface sensitivity. However, only a few metals (like Au, Ag, and Cu) with particular nanostructures can generate strong SERS effects. Such material and morphology limitations have greatly hindered the applications of SERS. To overcome the long-standing material limitation of SERS, we developed the "borrowing" strategy using plasmonic Au/Ag core-transition metal shell nanoparticles as the SERS substrate.[1] Ultra-thin layers (usually less than 5 atomic monolayers) of catalytically active materials (like Pt, Pd, Ru, etc.) were coated onto SERS active Au/Ag nanoparticles. Thus, Raman signals of the species adsorbed on the thin films were greatly enhanced by the EM field generated on the Au or Ag substrate.

Though the "borrowing" strategy has partially solved the material limitation of SERS, it is very difficult, if not impossible, to coat all the materials on the Au or Ag nanoparticles. Furthermore, the "borrowing" strategy cannot be used at the single crystal surfaces that are the most important model systems in surface catalysis and electrochemistry. Such a significant challenge was further addressed by the invention of shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS).[2] In SHINERS, Au/Ag nanoparticles are coated with ultrathin, pinhole-free, silica shells, forming shell-isolated nanoparticles (SHINs). The Au/Ag-cores work as Raman amplifiers that enhance the Raman signals of probing targets nearby, while the pinhole-free silica shells eliminate the influence of the Au-cores on the Raman signals by preventing molecules from directly contacting to them. SHINERS overcomes the material and morphological limitations of SERS and, in principle, can be used on substrates with any material or morphology by manipulating the structure of the SHINs. Using SHINERS, we have in-situ studied the surface electro-oxidation, CO electro-oxidation, CO_2 reduction, and oxygen reduction reaction at Au(hkl), Cu(hkl), Pd(hkl) or Pt(hkl) surfaces. Hydroxyl, peroxide, and superoxide were directly observed as intermediates, which proved the long-standing speculation in surface catalysis and electrochemistry.[3-5] Furthermore, a SHINERS-satellites strategy was established by assembling nanocatalysts on SHINs to achieve the in situ study of catalytic processes on practical nanocatalysts.[6]

The concept of shell-isolated nanoparticle-enhancement is being applied to other spectroscopies [5] such as fluorescence, IR, SFG, and tip-enhanced spectroscopies to improve the sensitivity or spatial resolution. Such advanced techniques also have great potentials for the in-situ study of reactions/catalysis, at single atoms or a single molecule.

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Computational resonant Raman spectroscopy of 2D materials: Exciton-phonon coupling and non-adiabatic effects

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2D materials and its layered bulk compounds are known to exhibit very pronounced excitonic effects due to the confinement of electrons and holes in a layer and due to the weak dielectric screening of the electron-hole interaction. In vibrational spectroscopy, the exciton-phonon coupling must therefore be included in order to obtain a qualitative understanding of the spectra and in order to obtain quantitative results. We present our methods for the calculation of exciton-phonon coupling via a finite displacement [1] and via a diagrammatic approach [2], both using many-body perturbation theory.

We present ab-initio calculations of resonant Raman intensities with the combined inclusion of both excitonic and non-adiabatic effects. In bulk hBN, which has high phonon-frequencies due to the light atoms, we demonstrate the emergence of strong quantum interference between different excitonic resonances due to non-adiabatic effects. In MoS_2 and $MoTe_2$, our calculations explain the observed different intensity dependences of the A_1' and E' modes on the energy of the exciting laser [3].

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Surface enhanced coherent Raman scattering: blessing or curse?

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Coherent Raman scattering (CRS) techniques are recognized for their ability to induce and detect vibrational coherences in molecular samples. The generation of coherent light fields in CRS produces much stronger signals than what is common in incoherent Raman spectroscopy, while also enabling direct views of evolving molecular vibrations. Despite the attractive attributes of CRS spectroscopy, the technique's sensitivity is insufficient for performing measurements on single molecules, thus precluding the ability to coherently drive, manipulate and observe individual vibrational quantum oscillators with light. The single-molecule sensitivity that has been achieved in surface-enhanced Raman scattering (SERS) with the aid of plasmonic antennas suggests that a similar approach may be used to push CRS techniques to the single-molecule detection limit. Compared with SERS, however, experimental successes in surface-enhanced coherent Raman scattering (SE-CRS) are few, and a theoretical understanding of surface-enhancement in CRS is still incomplete. In this lecture, we discuss some of the principles and challenges in SE-CRS and summarize the latest advances in the quest of performing routine CRS experiments on single molecules.

Clinical Raman spectroscopy - a potential panacea (or just a pretty good compliment to current clinical diagnostics)?

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Raman spectroscopy has been demonstrated by numerous groups over 20+ years to be able to provide sufficient biochemical information from biofluids, cells and tissues to provide a pathological measure of disease in a vast array of conditions [1,2]. Raman can provide a rapid, non-destructive measure of the molecular fingerprint of biological materials[3]. It has been shown to be reproducible and can exceed the performance of independent pathologists[4]. Furthermore, there is building evidence that the rich biochemical signatures can provide a prognostic indicator of likely patient outcomes in a number of conditions[5].

Raman spectroscopic tools can be utilised in the pathology lab to analyse cells, tissues and fluids. It can be applied at the point of care for rapid analysis, providing real-time intraoperative information able to direct clinicians[6]. Finally, it can be applied to open surgical fields[7], the lining of hollow organs[8] or bulk tissues using smart Raman needles[9] or deep Raman approaches, relying on photon migration for the Raman biochemical signals to diffuse from the sample[10].

Recent work in translating Raman to the clinic will be presented and its utility as a clinical tool discussed.

Will Raman become the new gold standard for diagnosis or be a complimentary technique for those difficult cases?

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Phonons engineering and phonon transport in low dimensional systems

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The recently growing research field called "Nanophononics" deals with the investigation and control of vibrations in solids at the nanoscale. Phonon engineering leads to a controlled modification of phonon dispersion, phonon interactions, and transport [1,2]. However, engineering and probing phonons and phonon transport at the nanoscale is a non-trivial problem.
In this talk, we discuss how phononic properties can be engineered in nanowires (NWs) and the challenges and progresses in the measurement of the thermal conductivity of nanostructures and low dimensional systems.
We demonstrate the versatility of Raman spectroscopy and show that it can be used to determine the main crystalline, phononic, and electronic properties of the most challenging type of heterostructure: a nanoscale system with constant material composition, but different crystal phases (cubic and hexagonal) [3]. The general procedure that we establish can be applied to several types of heterostructures. The concept of phonon engineering in NWs is exploited in superlattice (SL) NWs. We experimentally show that a controlled design of the NW phononic properties can be decided à la carte by tuning the SL period [4].
Finally, Raman thermometry is used to probe the temperature profile along the NWs upon application of a thermal gradient, enabling the differentiation between ballistic and diffusive flow regimes [5].

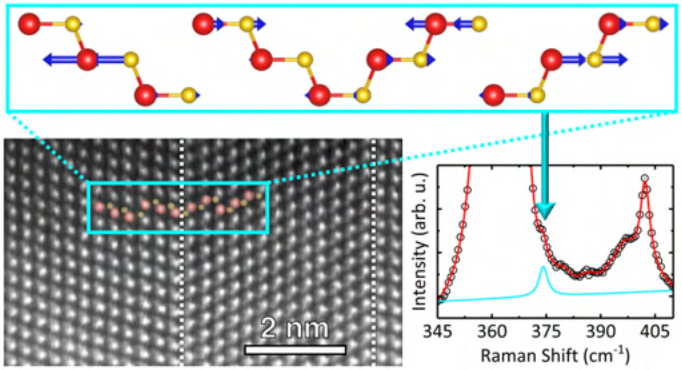

Figure 1: Cartoon illustrating one of the twinning superlattice phonon modes

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Towards Simple, Real-Time Spectroscopic Coherent Raman Imaging of Biology

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Spectroscopic coherent Raman imaging (CRI) methods allow label-free, chemically specific imaging of materials and biological systems, and are opening up many exciting possibilities for understanding phenomena in these systems. When we first introduced broadband spectroscopic coherent anti-Stokes Raman scattering (BCARS) microscopy in 2004 [1], we could acquire spectra only from bulk polymers and liquids. Now, after many years of development, we easily acquire label-free micrographs of weakly scattering biological cells and tissues at 3.5 ms for each spectral image pixel [2]. Figure 1 provides an example of a murine pancreas section, showing major structural features, although much more spectral image information is available. Initially it was not clear that acquiring such spectra would be possible, but a recognition that an intrinsic and strong nonresonant signal could be used to enhance weak resonant signals [3,4] made it possible to obtain these signals above detector noise, allowing for highly robust and rapid spectral imaging. I will discuss key aspects of BCARS operation and biological insights that we have recently obtained from BCARS imaging of *C. elegans* and virally infected human cells. We are now working to transform the sensitivity of Fourier-transform BCARS as we did with spectral-domain BCARS. I will introduce FT-BCARS innovations that portend further discovery of new biology through rapid, in-depth spectroscopic characterization of highly complex biological systems.

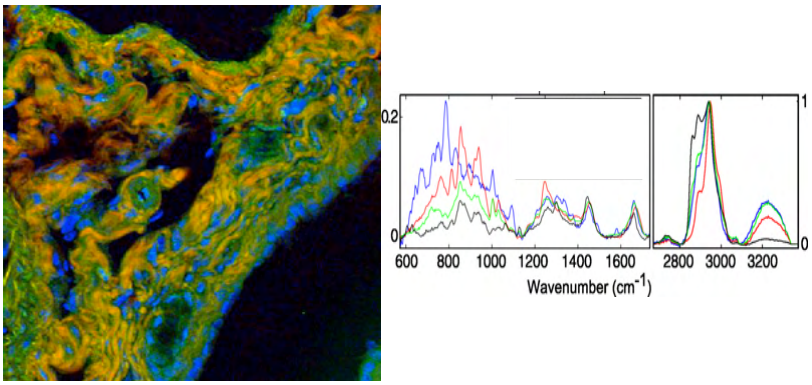

Figure 1: Murine pancreas with contrast from Raman spectra (e.g., shown at right) obtained at each pixel in 3.5 ms. Contrast: Cell nuclei (blue), collagen (red), and arterial wall (green).

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Tip-enhanced Raman Spectroscopy for Nanoscale Characterization of Two-Dimensional Materials

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Two dimensional materials hold great promises in electrical, optical, and chemical application due to their extraordinary optical and electronic properties. These properties are significantly influenced by the local defects usually on the nanometer scale or less [1]. However, the understanding of these unique optical and electronic properties and their correlation with defects is still very limited. The main reason is that they are so small that their signals are usually submerged in the strong signals of surrounding pristine materials. Tip-enhanced Raman Spectroscopy (TERS) can obtain not only the topological but also vibrational information of a sample at the sub-10 nm or even sub-molecule spatial resolution[2]. Recently, we demonstrated that TERS can spatially observe the different Raman features of edges of mono- and bi-layer MoS_2 and investigate the edge related modified lattice and electronic length at 10 nm resolution in ambient[3]. We found a new defect-induced Raman peak (396 cm^{-1}) in multilayer MoS_2 which is enhanced by double resonance Raman scattering (DRRS). It showed a unique electron-phonon interaction of defects. We further developed a method to determine the edge types (zigzag and armchair) by performing Raman imaging over the edges of 2D materials. Then we revealed the evolution of the active sites of MoS_2 during the hydrogen evolution reaction by EC-TERS, which can rationally control the reaction by the electrode potential and characterize the reaction at the nanometer spatial resolution[4] on AFM–based EC-TERS. We observed totally different spectral evolutions of the inactive (basal plane) and active edge sites during the electrocatalytic process. The power of TERS demonstrated in MoS_2 can be extended to other 2D materials [5], which may guide the defect engineering for desired properties.

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Versatile Applications of Two-Dimensional Correlation Analysis in Raman Spectroscopy

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2D correlation spectroscopy (2D-COS) is one of the popular techniques applicable to the in-depth analysis of spectral data obtained under the influence of external perturbations in various spectroscopic experiments. [1-5]. From the 2D correlation spectra, we can explore the understanding of the inter- or intra-molecular interactions, obtain better resolved spectral information, and determine the sequential order of the events. Therefore, it has been well-accepted as a powerful analytical technique to sort out important information in many fields of spectroscopic studies, which can provide new insights at the molecular level into the system understanding. It can often provide very interesting results, which is sometimes hardly detected in 1D spectral analysis.

A variety of analytical techniques, such as Raman, IR, fluorescence, Raman optical activity (ROA), vibrational circular dichroism (VCD), UV-Vis, and NMR spectroscopies, as well as those from chromatography or microscopy studies, have been successfully applied in conjunction with 2D-COS scheme in the sample system of interest to induce spectral changes, which can be analyzed by complex cross correlation analysis. Among many analytical techniques, Raman spectroscopy is one of the most popularly used in 2D-COS.

In this presentation, the brief background of 2D-COS, its new and noteworthy developments and versatile applications in Raman spectroscopy will be introduced in detail.

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Probing elementary molecular events by stimulated X -ray Raman spectroscopy

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Ultrafast nonlinear x-ray spectroscopy is made possible by newly developed free electron laser and high harmonic generation sources. The attosecond duration of X-ray pulses and the atomic selectivity of core X-ray excitations offer a uniquely high spatial and temporal resolution. A particularly promising application of novel X-ray probes is the direct observation of Conical Intersections (CoIns) by stimulated Raman type spectroscopies. CoIns are energetically degenerate regions on molecular potential energy surfaces causing a breakdown of the Born-Oppenheimer approximation and opening ultrafast non-radiative relaxation channels. They thereby dominate the pathways and outcomes of virtually all photophysical and photochemical molecular processes. Short X-ray pulses can directly detect the passage through a CoIn with the adequate temporal and spectral sensitivity. The technique is based on a coherent Raman process that employs a composite femtosecond/attosecond X-ray pulse to directly detect the vibronic coherences (rather than populations) that are generated as the system passes through the CoIn.

The existence of CoIns as fundamental features is widely accepted, yet their observation is usually based on ultrafast internal conversion rates via electronic state populations. X-ray stimulated Raman probes offer more direct access to CoIns by being sensitive only to coherences emerging here. We demonstrate this unique sensitivity on the example of uracil photorelaxation [1]. We further discuss how the stochastic phases in SASE-generated XFEL pulses, so far prohibiting multidimensional coherent spectroscopy, can be overcome in a covariance-based approach. By recording the frequency-dispersed spectrum in coincidence with the stochastic X-ray pulse generating it, the coherent X-ray stimulated Raman signal can be completely recovered and is thereby accessible with current technology [2].

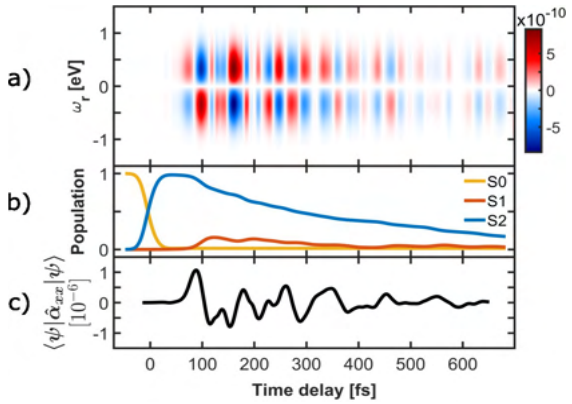

Figure 1: **X-Ray stimulated Raman signal** for the ultrafast photorelaxation of uracil through a CoIn. **a)** Frequency-dispersed TRUECARS signal **b)** Population dynamics **c)** Transition polarizability

Coherence-based signatures stemming from CoIns are intrinsically weak and must compete with significant loss channels like ionization or Auger-Meitner decay. We introduce quantum optimal control as a tool to selectively amplify these signals and thereby to potentially bring them over the detection threshold. Control strategies that maximize the coherence include shaping of the optical pump [3] or application of an additional infrared field in resonance with the CoIn [4]. In addition to stimulated Raman, these signatures also exist – and can be amplified – in diffraction-type measurements [4,5]

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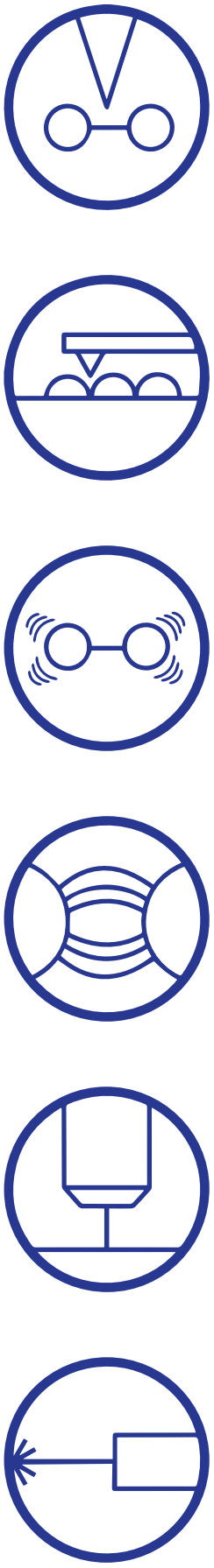
XXVII ICORS

INVITED PRESENTATIONS



Ultrafast Raman Spectroscopy in the Single Phonon Regime: Entangling Light and Vibration

Santiago Tarrago Velez[a], Anna Pogrebna[a], Nicolas Sangouard[b], Vivishek Sudhir[c], Christophe Galland[a]
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Mechanical oscillators have been identified as new resources for quantum optics and its applications in metrology, sensing and information processing. Developing new techniques to prepare non-classical states of mechanical oscillators and engineer their quantum correlations with light fields also promises new insights into the dynamics and decoherence of vibrations in the quantum regime.

This talk describes a new ultrafast pump-probe Raman spectroscopy technique that harness time-correlated single-photon counting to prepare and characterise non-classical states of Raman-active vibrations in crystals and molecules [1]. To illustrate the new possibilities offered by this technique, I will show how we achieved for the first time the preparation and measurement of a single phonon Fock state with a THz-frequency vibrational mode at ambient conditions in a diamond crystal [2]. I will then present the first demonstration of Bell correlations between light and vibration at room temperature [3]. Our technique enables to watch the decoherence of a vibrational qubit with 200 fs resolution. This result paves the way toward more general exploration of quantum superpositions in mesoscopic molecular ensembles, and it constitutes a stepping stone for future research with ultra-high frequency nano-fabricated oscillators and hybrid qubit - optomechanical systems.

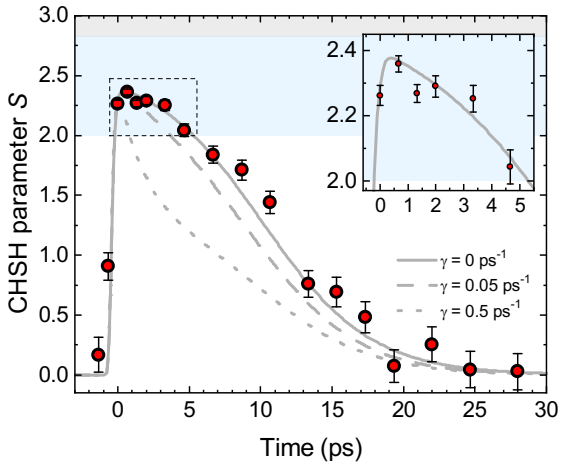

Figure 1: Evolution of the CHSH parameter as a function of delay between Stokes and anti-Stokes scattering. Bell correlations are mediated by a vibrational qubit in a superposition of two temporal ("time bin") modes. Data are consistent with a zero pure dephasing (gamma)

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Accessing excited potential energy surfaces by Raman excitation profiles measured via time-domain Raman spectroscopy

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Spontaneous Raman spectra depend on the displacement along the normal coordinates between ground and excited potential energy surfaces, and hence the relative intensities of the measured Raman bands encode information on the PESs relative displacement. Critically, to extract such molecular information, several spectra must be recorded scanning the pump wavelength across the absorption profile, with the detection of the experimental signals that is typically hampered by the overwhelming fluorescent background. Most importantly, spontaneous Raman spectroscopy cannot be applied to monitor ultrafast chemical reactions on electronically excited states. Herein, I will discuss how to circumvent these limitations building on a time-domain impulsive Raman scattering experimental scheme: a femtosecond pulse impulsively launches nuclear wave packet motions in the system under investigation and then their couplings with an arbitrary excited state potential are measured by a resonant Raman process enabled by a delayed probe pulse. A perturbative treatment of the scattering process, validated by time-dependent density functional theory, reveals that the signal is generated by the interference between multiple quantum pathways resonant with the excited state manifold. The relative phase of such components is experimentally tuned by varying the probe chirp and we demonstrate how to decode the nuclear displacements along the different normal modes from the experimentally detected impulsive Raman excitation profiles, thus revealing the multidimensional potential energy surfaces.

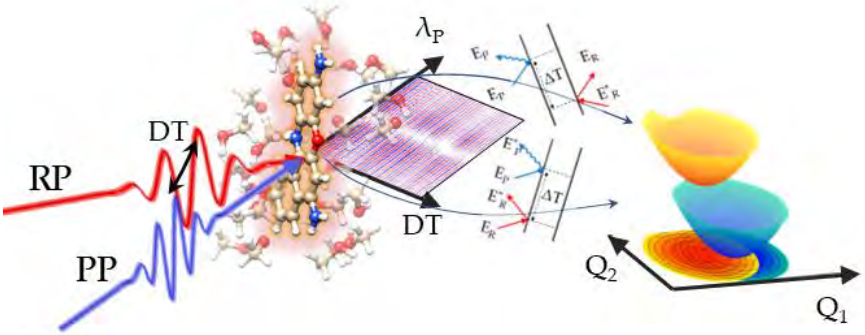

Figure 1: Concept of the impulsive vibrational spectroscopy experiment used to record Raman excitation profiles.

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High Pressure Raman study of Novel Carbon Materials

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Design and synthesis of new carbon materials have been attracting intensive attention due to their application in various fields. The study of carbon nano materials under high pressure provides us a very effective method to the creation of new carbon material which is hardly discovered at ambient condition because of the appearance of nanometer scale size effect and the novel high pressure behavior. Deeply understanding those unusual high pressure structures and physical phenomena also unveils new aspects of the intrinsic physics of nanomaterials. Fullerene and nanotube are representative zero and one dimensional nano material in carbon family which have high bulk modulus, providing us ideal building blocks to study novel phase and design new carbon materials induced by high pressure. In this presentation, several examples on high pressure induced novel structures in recently studied typical nano-confined fullerene and their Raman spectroscopy will be outlined, including unique long range ordered crystal with amorphous nano clusters as building blocks (OACC) in solvated fullerene crystals as well as in energetic molecular cubane doped fullerene co-crystals which brings new physical insight to understand order and disorder concept and new approach to design novel carbon materials with excellent properties, such as super incompressibility and anomalous negative volume compressibility. We will also present a new carbon allotrope with a fully sp3 bonded monoclinic structure (termed V carbon) from compressed C_{70} inside of single wall nanotubes (C_{70} peapod). These findings present a new strategy for constructing new carbon material with application of high pressure by tuning the structure of building blocks and their long- ranged order.

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Development of novel techniques for the analysis of microplastics using Raman imaging

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Microplastic analysis represents itself as a challenging task requiring both spectral and microscopic information. The development of novel techniques, such as extraction, detection and quantification for reliable analysis of microplastics in various matrices therefore is of high interest.

Development of a novel filtration system enabling a fractionation of particles in the µm- down to the nm-range enables the efficient analysis in cosmetics, human (tissue, blood [1], arteriosclerotic plaques) and environmental samples (river water, snow from the Alps (Fig. 1) and Antarctica [2]).

The performance of the newly established technique is discussed and compared to infrared spectroscopy methodologies.

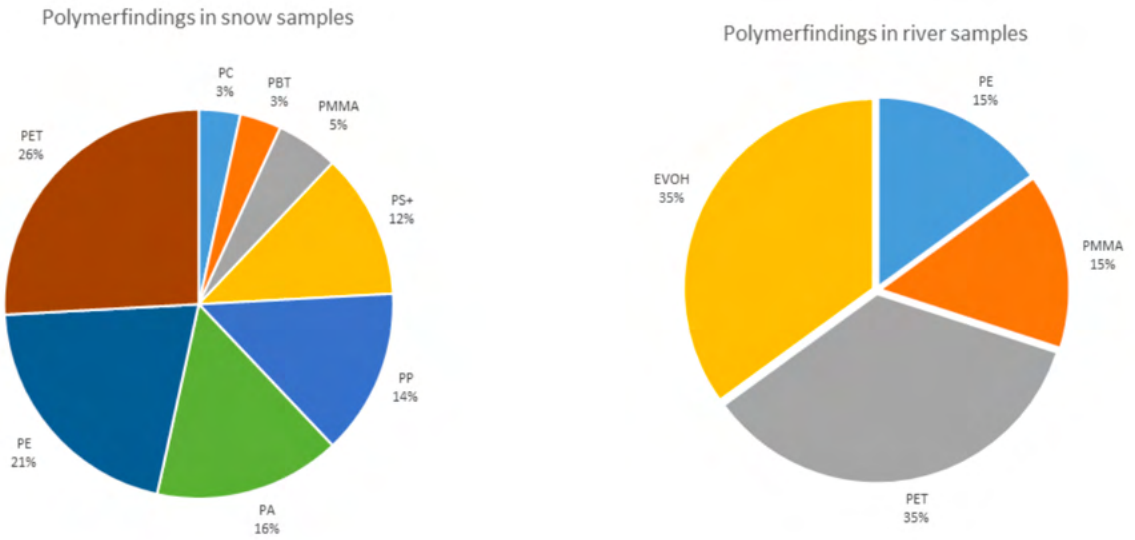

Fig. 1 Distribution of microplastics found in snow and river water (Innsbruck, Austria)

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Single-shot femtosecond stimulated Raman histology of gastroscopic biopsy

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Abstract:

Gastroscopic biopsy provides the only effective way for gastric cancer diagnosis, but the gold standard histopathology is time-consuming and incompatible with gastroscopy. Conventional stimulated Raman scattering (SRS) microscopy has shown promise in label-free diagnosis on human tissues, yet it requires the tuning of picosecond lasers to achieve chemical specificity at the cost of time and complexity. Here, we demonstrate that single-shot femtosecond SRS (femto-SRS) reaches the maximum speed and sensitivity with preserved chemical resolution by integrating with U-Net. Fresh gastroscopic biopsy is imaged in < 60 seconds, revealing essential histoarchitectural hallmarks perfectly agreed with standard histopathology. Moreover, a diagnostic neural network (CNN) is constructed based on images from 279 patients that predicts gastric cancer with accuracy > 96%. We further demonstrate semantic segmentation of intratumor heterogeneity and evaluation of resection margins of endoscopic submucosal dissection (ESD) tissues to simulate rapid and automated intraoperative diagnosis. Our method holds potential for synchronizing gastroscopy and histopathological diagnosis.

Arrayed Nanoplasmonic Sensors and Actuators

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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 objects with unprecedented precision. In this paper, we will explore nanoplasmonics of these "patterned" structures on a substrate from three aspects: substrate dependence, material choices, and arrayed far-field coupling. Specifically, we have employed advanced nanofabrication to precisely control the removal of a portion of the substrate dielectrics to optimize the ensemble performance. We have also demonstrated the use of gold, gold-silver alloy, and nanoporous gold for tailored nanoplasmonics. We have further investigated arrayed behavior of far-field plasmonic coupling in various designs. We will show potential applications of these arrayed nanoplasmonic platforms in the context of sensing and actuation.

Quantum advantage of seeded, squeezed light in stimulated Brillouin Spectroscopy and Imaging*

Girish S Agarwal**
Texas A&M University, USA

Abstract Absorption and gain measurements are routinely used in science and engineering. For such measurements using laser beams, the sensitivity is theoretically limited by the shot noise due to the fundamental Poisson distribution of photon number in laser radiation. Here, we use bright squeezed light to demonstrate that direct absorption and gain measurements can be performed with sensitivity beyond the shot-noise limit. We report direct sub-shot-noise measurement of absorption and gain that require neither homodyne/lock-in nor logic coincidence detection schemes. Previously we reported [1] more than 1 dB quantum advantage [3 dB when corrected losses in optical paths] for the measurement sensitivity at faint absorption levels. We present our detailed results on definite quantum advantage of about 3dB in measurements of Brillouin gain. The figure below is a typical representation of our data on quantum advantage. We would present applications of this advantage to Brillouin imaging.

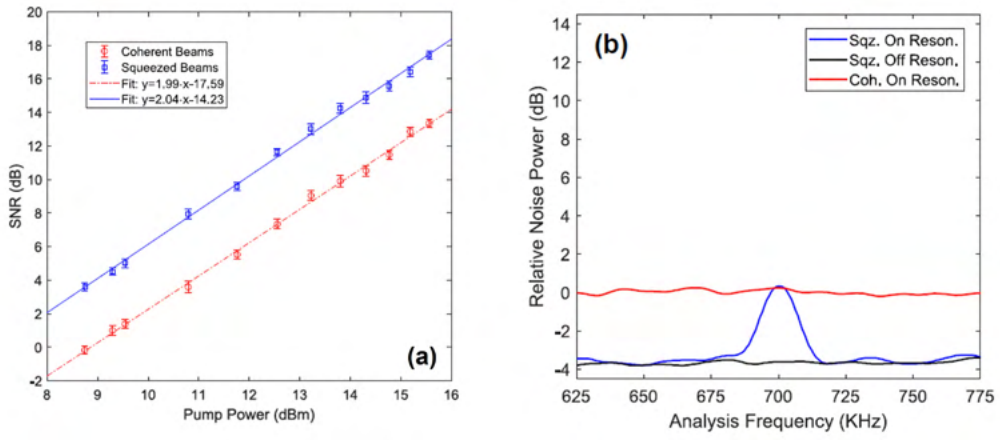

Fig. (a) The signal to noise ratio (SNR) in decibel as a function of pump power in dBm. The blue squares and red circles are measurements for the squeezed and coherent light respectively. The blue solid and red dotted lines are linear fits, which yield a 3.36 dB quantum advantage calculated from the fitting parameters. (b) A typical set of SBS measurements with a spectrum analyzer when pump power is 7.5 mW, corresponding to the lowest point in (a). Red curve is obtained with coherent light, blue curve is obtained with squeezed light, black curve is also obtained with squeezed light but with lasers locked away from the water SBS resonance.

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*Collaborators: T. Li; F. Li; X. Liu and V. Yakovlev

** girish.agarwal@tamu.edu

Entangled light in Raman excitation, two-photon absorption and black hole radiation

Marlan Scully

Texas A&M, Princeton and Baylor Universities

We find that stimulated Raman excitation of an atom or molecule by a two-photon pulse can be enhanced by orders of magnitude if the photons are simultaneously frequency correlated and spatially anticorrelated [1]. This is opposite to two-photon absorption by a three-level atom, in which the enhancement occurs if photons in the pulse are frequency anticorrelated and spatially correlated. There is a relation between these effects of entanglement and similar pairing correlations between photons above and below the black hole horizon [2,3].

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Resonant Inelastic X-Ray Scattering of condensed matter

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Local probes of the electronic ground and valence excited states are essential for understanding hydrogen bonding in aqueous environments. Vibrational infra-red (IR) spectroscopy is an established technique for investigations of hydrogen bonding. High-resolution X-ray absorption spectroscopy [1] and resonant inelastic X-ray scattering (RIXS) [2] offers a complement to IR vibrational spectroscopy. The propagation of the nuclear wave packet in dissociative core-excited state results in the long vibrational progression seen in both theory and experiment. This gives great advantage of RIXS in comparison with IR spectroscopy which probes mainly the first OH excitation. We show how different resonant inelastic X-ray scattering (RIXS) channels deliver separate information; about the local structure via long-range dynamics in quasi-elastic RIXS and about short-range dynamics, which is much less sensitive to the structure, in the electronically inelastic $1b_1$ and 4a'' channel in water and methanol, respectively. Our theoretical framework is composed of classical ab initio molecular dynamics (MD) simulations, calculation of local potential energy surfaces from the sampled configurations, and quantum wave packet modeling of the nuclear motion in relevant degrees of freedom. Thereby, we reach insights into the variations in the local HB environment, which strongly affects the long-range part of the OH potential energy curves (PEC). For enhanced insight, we derive the distribution of PECs of OH bonds with intact and broken HBs as reconstructed from experimental RIXS data of liquid water. In contrast by analysis of the dynamic mechanisms, we show that the splitting, emerging for pre-edge core-excitation, has a purely dynamical origin and is primarily sensitive to the short-range part of the PEC since the splitting of the $1b_1$ and 4a'' peaks is formed at short time-scales before fragmentation. We overview recent theoretical and experimental results devoted to liquid water, methanol, acetic acid and correlated materials.

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Controlling Plasmonic Nanogap Chemistry to Tune Analyte Interactions

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Surface enhanced Raman scattering (SERS) spectra from surfaces with chemically defined nanogaps, when analyzed with machine learning algorithms (SERS + ML), are demonstrated to fingerprint metabolic responses associated with complex biochemical processes in cells.

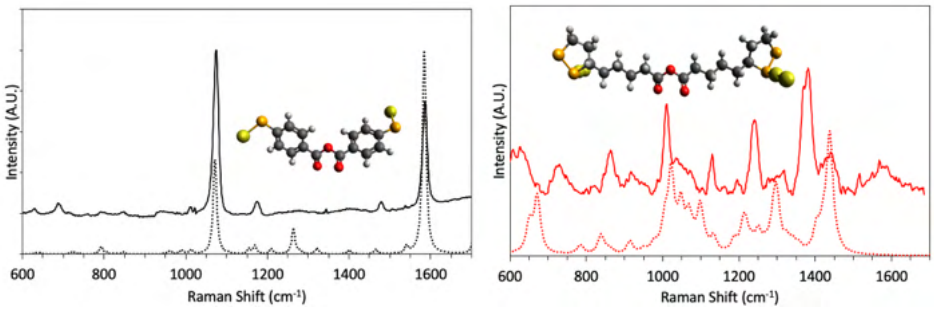

Figure 1: Experimental SERS spectra of 4-mercaptobenzoic acid (left) and lipoic acid (right) functionalized nanoparticles deposited via EHD flow (solid curve) plotted with calculated Raman spectra (dashed curve) using density functional theory confirming anhydride bond formation. Inset shows calculated relaxed structure of molecules.

Just as one can smell the difference between coffee and chocolate amongst multiple odors in olfactory systems, the combinatorial information in complex spectral data from cell lysate is used to differentiate metabolic response of bacteria to stress. While SERS spectra of bacterial metabolites, are not readily interpretable on their own, in combination with ML analysis, these spectra can be easily categorized and distinguished.SERS surfaces composed of gold nanoparticles (NP) are fabricated in microfluidic channels in a capacitator architecture.[1] Electrohydrodynamic flow induces NP-NP interactions on electrode surfaces for control of nanogap chemistry and spacing, achieving single molecule limits of detection.[2] Examples of simulated and experimental SERS spectra are shown in Figure 1. Control of nanogap chemistry provides tunable *affinity* between hots spots and metabolites. The performance in metabolomic analysis relies on *weak specificity*.[3] Specific detection would require knowing what metabolites to look for, which is not feasible in, for example, clinical samples of unknown bacterial content. Consider that olfactory systems do not recognize the chemical structure of menthol, nor does menthol bind to one receptor. Rather, the olfactory bulb *interprets* the activation levels of receptors as a complex signal characteristic of menthol. Similarly, the *combination* of sensitive and robust SERS surfaces and ML analysis imparts detection to our platform. SERS+ML whole cell metabolic sensors are able to provide early detection of bacterial biofilms,[4] monitor nutrient sources as quality control during biomanufacturing, as diagnostics for antibiotic therapy,[5] water quality sensors for heavy metal toxins and other threats to human health when training the ML algorithms on alternate feature selection.

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Exciton-Phonon Coupling in CdSe Nanoplatelets from Resonance Raman Intensity Analysis

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Nanoplatelets (NPLs) are a class of semiconductor nanostructures that have a precisely defined and small thickness in one dimension, typically just a few atomic layers, and are much larger in the other two dimensions. Because the thickness in the quantum confined dimension is constant over most of the NPL, there is very little inhomogeneous broadening of the lowest excitonic transitions and their absorption and emission spectra are much sharper than those of quantum dots (QDs). The resonance Raman (RR) spectra of CdSe NPLs, like CdSe nanocrystals of other shapes, are dominated by the longitudinal optical (LO) phonon mode near 202 cm^{-1}. However, while the RR spectra of CdSe QDs depend only slightly on excitation wavelength, the RR spectra of NPLs show an overtone to fundamental intensity ratio that varies strongly with excitation wavelength (Figures 1 and 2).

We have measured the RR spectra, including absolute cross-sections and depolarization ratios, for 4.5 monolayer thick CdSe NPLs dispersed in chloroform. Five excitation wavelengths between 514.5 and 476.5 nm were employed. The RR excitation profiles and absorption spectra were numerically simulated using standard resonance Raman intensity theory.[1] The strong wavelength dependence of the LO fundamental and overtone intensities is reproduced well as a direct consequence of the vibronic structure of the electronic transition and the small amount of inhomogeneous broadening. The absolute Raman cross-sections are best fit using a Huang-Rhys parameter for the LO phonon of about 0.08 on resonance with the lowest heavy-hole transition, a factor of 2-3 smaller than found previously for CdSe QDs.[2] That an overtone to fundamental intensity ratio greater than one is obtained at certain excitation wavelengths despite the small Huang-Rhys parameter demonstrates the danger in estimating electron-phonon coupling strengths from overtone intensities alone.

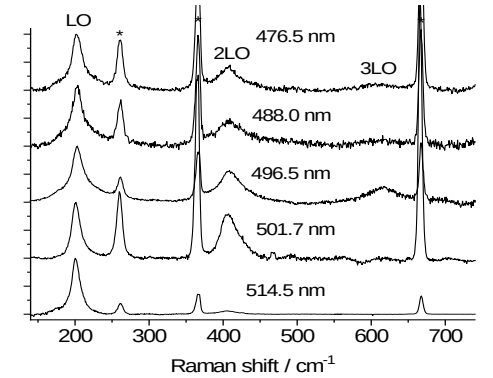

Figure 1. RR spectra of 4.5 monolayer thick CdSe NPLs at the indicated excitation wavelengths.

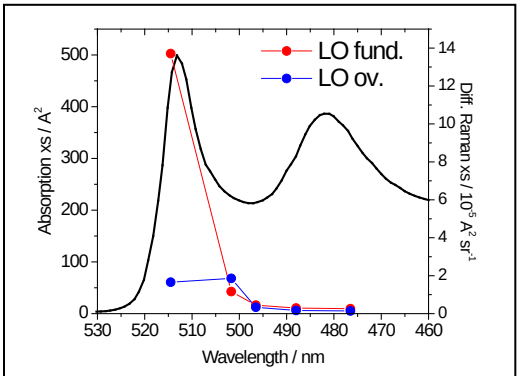

Figure 2. Comparison of experimental absorption spectrum (black) and LO fundamental (red) and overtone (blue) RR cross sections for CdSe NPLs.

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XXVII International Conference on Raman Spectroscopy
(Please do not exceed 20 words for the title)

James Cryan [a,b],
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[b] *Linac Coherent Light Source, SLAC National Accelerator Laboratory*

Electron motion is a key ingredient of all chemical reactions. The natural timescale for such electronic motion is typically in the range of tens to hundreds of attoseconds in small molecular systems. Consequently, the study of ultrafast electronic phenomena requires light pulses that can access this extreme timescale. We present recent experimental results using attosecond x-ray free electron laser (XFEL) pulses which enables further exploration of this extreme timescale [1]. XFELs offer continuous wavelength tunability across the soft x-ray (SXR) region allowing for atomic-site specific probes of the electron density in a molecular system. The high pulse energies possible with XFEL sources enable higher-order interactions enabling non-linear spectroscopies. We will discuss our demonstration of a coherent electronic wavepackets created by stimulated X-ray Raman scattering using attosecond SXR pulses [2]. I will discuss our first observation of this process, and our plans for resolving ultrafast electronic with attosecond temporal resolution and atomic site specificity.

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Compact Fiber Lasers for Coherent Raman Scattering Microscopy and Spectroscopy

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The widespread use of coherent Raman scattering (CRS) microscopy outside of research laboratories has been hindered by the lack of suitable low cost, compact and robust laser sources. It is thus important to develop these laser sources. We present an all-fiber laser source for CRS microscopy and spectroscopy based on spectral focusing [1, 2]. The laser covers from ~2800 cm^{-1} to 3100 cm^{-1} which is an important resonant frequency window for studying biological tissues.

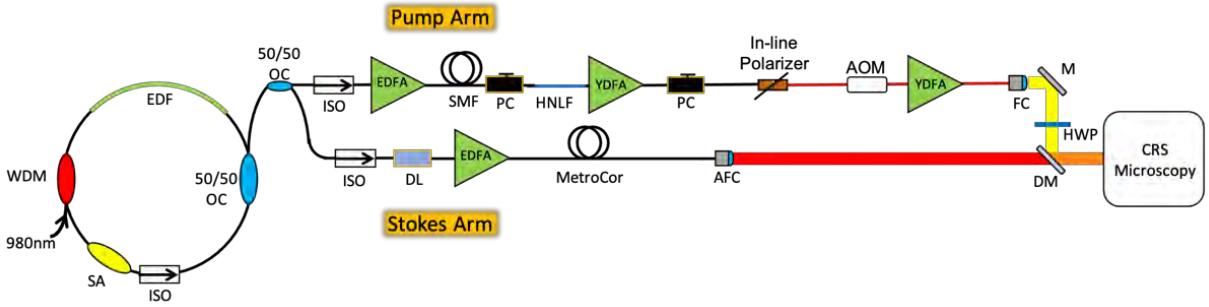

Figure 1: All-fiber laser system for coherent Raman scattering microscopy. WDM: wavelength division multiplexer; SA: saturable absorber; PC: polarization controller; EDF: Er-doped fiber; OC: output coupler; ISO: isolator; DL: fiber-pigtailed delay line; EDFA: Er-doped fiber amplifier; SMF: single mode fiber; HNLF: highly nonlinear fiber; AOM: acoustic-optic modulator; YDFA: Yb-doped fiber amplifier; FC: fiber collimator; AFC: adjustable fiber collimator; M: mirror; HWP: half-wave-plate; DM dichroic mirror. Red and black lines represent polarization-maintaining fibers and non-polarization-maintaining fibers, respectively.

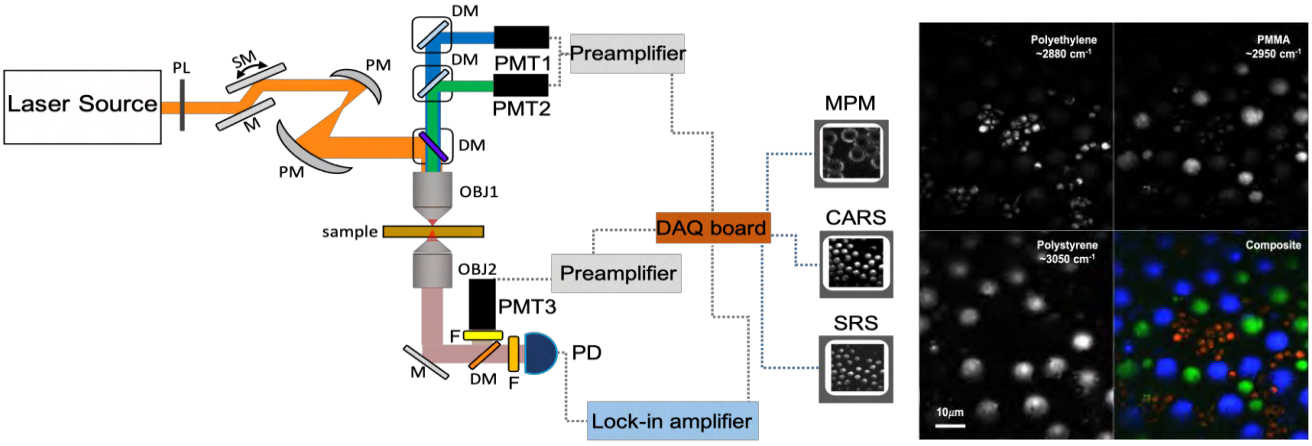

Figure 2: The schematic diagram of the microscope system for coherent Raman imaging. PL: polarizer; M: mirrors; SM: scanning mirrors; PM: parabolic mirror; OBJ1, OBJ2: microscope objectives; DM: dichroic mirror; F: filter; PMT1, PMT2: photomultiplier tube; PD: photodiode; MPM: multiphoton channel; CARS: coherent anti-Stokes Raman channel; SRS: stimulated Raman channel. Right panel: CRS images of various samples.

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Dependence of Vibrational Energy Transfer on Distance in a Four-helix Bundle Protein

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Vibrational energy exchanges between various degrees of freedom are critical to barrier-crossing processes in proteins. Heme proteins are highly suitable for studies of the vibrational energy exchanges in proteins. The migration of excess energy released by heme in a protein moiety can be observed using time-resolved anti-Stokes ultraviolet resonance Raman spectroscopy. The anti-Stokes resonance Raman intensity of a tryptophan residue is an excellent probe for the excess energy and the spatial resolution of a single amino acid residue can be achieved. In the present study, we used cytochrome b_{562} to investigate the dependence of the energy transfer in the protein on the distance.[1] Figure 1A shows the X-ray crystal structure of cytochrome b_{562} from *Escherichia coli* (PDB ID: 256B).[2] The protein is in a four-helix bundle structural motif composed of 106 amino acids and a heme prosthetic group. By taking advantage of the periodic character of α helices, it is possible to change the position of the residue to probe the excess energy in equidistant increments along the helices without changing the orientation of the residue to heme. Moreover, the wild type cytochrome b_{562} contains no Trp residue; therefore, it is convenient to prepare mutants that contain a single Trp residue, which serves as the probe of the excess energy without destabilization of the protein structure. The vibrational energy transfer from the heme group to a single tryptophan residue introduced by site-directed mutagenesis was examined for different heme-tryptophan distances by a quasi-constant length with the periodicity of α helices. Taken together with structural data obtained by molecular dynamics simulations, the energy transfer could be well described by the model of classical thermal diffusion, which suggests that continuum media provide a good approximation of the protein interior, of which the atomic packing density is very high.

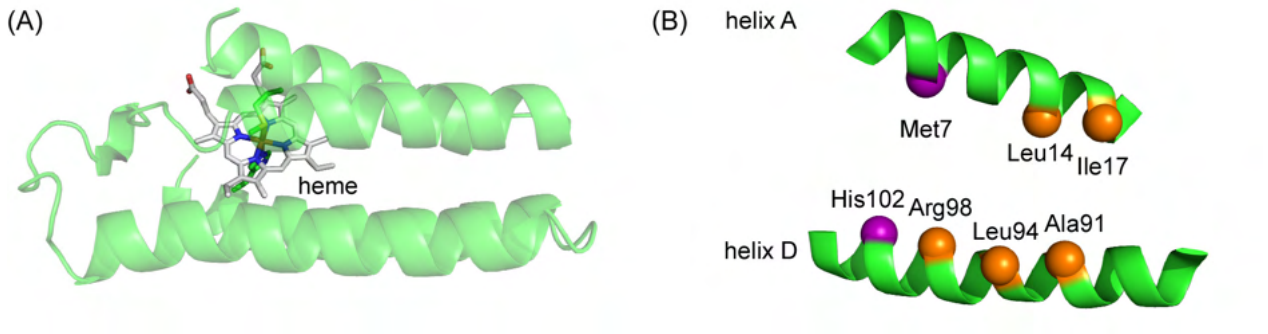

Figure 1: X-ray crystal structure of cytochrome b_{562} (PDB ID: 256B). (A) Overall structure. Heme and coordinating residues (Met7 and His102) are shown by the stick representation. (B) Positions of Trp and the coordinating residues in the A and D helices of the mutants. The α carbons of Trp and the coordinating residues are shown as purple and orange spheres, respectively.

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Raman signature of SARS-CoV-2

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in an unprecedented need for diagnostic testing that is critical in controlling the spread of COVID-19. We, purified virion particles were characterized with Raman spectroscopy, synchrotron infrared (IR) and AFM-IR.[1] The SARS-CoV-2 virions were purified from the supernatant of infected Vero cells deactivated by fixation with 4% formalin. [20] Aliquots from the purified stock solution were deposited and dried onto a BaF_2 window. The presence of virions was confirmed by means of Transmission Electron Microscopy (TEM). TEM images clearly show spherical particles approximately 120 nm in diameter with multiple spikes forming the solar crown structure, characteristic of coronaviruses (**Fig. 1 A, B**). Atomic Force Microscopy (AFM) confirmed the presence of spherical particles of approximately 120 nm in diameter, notably aggregated together in large clusters (**Fig. 1 C, D**). Synchrotron-FTIR spectra were collected from the individual clusters and the mean and second derivative spectrum calculated (**Fig. 1E)**. Raman spectroscopy confirms the purity of the virion extraction and shows the very strong RNA bands that characterize this RNA rich virus. The bands between 800-700 cm^{-1} are unique identifiers for RNA as evidenced by the corresponding RNA spectrum, which matches perfectly to many bands in the virion particle spectrum **(Fig. 1F)**. The lack of bands from media or fixative confirms the purity of the virions. The relative contribution of RNA bands to the Raman spectrum is very high, likely reflecting the very high content of RNA in SARS-CoV-2, which contains the largest genome RNA-containing viruses.

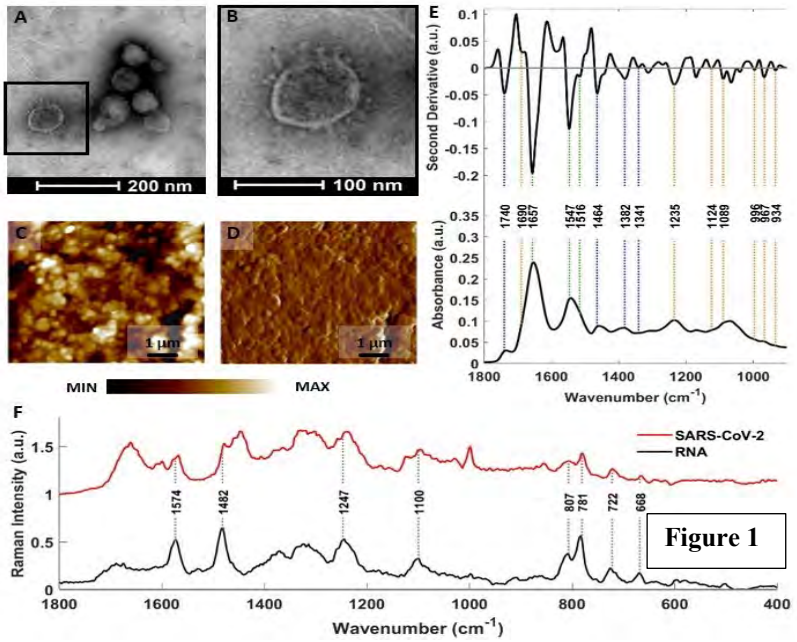

Figure 1

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Label-free identification of human T cells activation using Raman spectroscopy

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The activation of leukocytes is an important step in inflammation, which in turn causes a cascade of molecular and biochemical changes within cells. Inflammatory conditions can lead to different molecular responses regarding the type of leukocytes. T cells are white blood cells of the immune system that play a crucial role in the adaptive immune response. T cells develop from hematopoietic stem cells in the lymphopoiesis process and to become fully functional effector cells their activation is required. T cell activation involves a series of biochemical intracellular events that lead to the production of subtype-specific effector proteins and accelerated proliferation.

In our studies we applied a label-free spontaneous Raman imaging for molecular characterization and discrimination of activated states in T cells. Naïve lymphocytes were isolated from healthy donors and then activated with the use of magnetic beads. Based on Raman images, it was possible to evaluate the biochemical changes in the morphology of T cells upon activation process, as well as detailed analysis of the biochemical alterations in activated T cells. We have defined spectral biomarkers that can be used for reliable Raman-based differentiation between normal and activated cells. The accumulation of carotenoids found exclusively in naïve T cells, but not in activated cells, was clearly evidenced in the Raman spectra. For comprehensive spectroscopic analysis, we applied a detailed analysis of the average spectra with application of principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). Our results prove the potential of Raman spectroscopy in the identification of activated T cells and in studying metabolic and morphological changes related to the activation process.

Acknowledges: The „Label-free and rapid optical imaging, detection and sorting of leukemia cells" project is carried out within the Team-Net programme of the Foundation for Polish Science co-financed by the EU.

Tracking Structural Evolutions during Charge Separation Processes with Time-Resolved Impulsive Stimulated Raman Spectroscopy

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Photoinduced charge transfer (CT) dynamics is the most fundamental chemical reaction, which is of great interest to many experimental and theoretical chemists because it is an essential process for producing electrochemical energy from light in natural and artificial photosynthetic systems. In general, the CT dynamics in the excited state induces significant changes of electronic structures (absorption and/or fluorescence) of the systems and thereby time-resolved absorption and fluorescence methods have been widely used to investigate the mechanism, rate, and efficiency of the CT processes. In particular, nuclear rearrangements are also accompanied in intramolecular CT (ICT) reaction and the geometrical changes are a key factor in many cases. However, by using time-resolved electronic spectroscopies, it is difficult to identify whether molecular structures change or not during the CT processes, since those techniques do not directly provide structural information. For this reason, many researchers have utilized time-resolved vibrational (infrared or Raman) spectroscopies, providing a rich seam of structural information, to overcome this limitation.

Here, we discuss the structural dynamics during charge separation (CS) processes with time-resolved impulsive stimulated Raman spectroscopy (TR-ISRS), which is a multidimensional time-domain Raman method that enables us to record excited-state vibrational snapshots by combining actinic excitation and ISRS probing with femtosecond time-resolution [1]. First, we will present CS dynamics in a donor-acceptor-donor type perylene bisimide (PBI). We clearly observed drastic frequency shifts for a large number of Raman bands with their population kinetics, signifying that symmetry breaking CS accompanies significant structural changes in the PBI core. Furthermore, a direct comparison between time-resolved Raman spectra of the neutral S_1 state and the radical anion species demonstrates that the spectral signatures especially in high frequency (1200-1950 cm^{-1}) region provide important clues to bond length alternation patterns in the PBI core [2]. Second, we will present two-step CS dynamics in a diketopyrrolopyrrole-pyrrolopyrrole (DPP-PP) dyad (AD). After photoexcitation, AD starts from the initial bright exciton and goes through a partial CT state before finally reaching the CS state. The transient Raman spectra measured in different solvents give quantitative insights into the CT characters of the bright state (0.1 e) and intermediate partial CT state (0.5 e), as determined by the linear relationship that exists between the vibrational frequency of the marker modes and the CT character [3].

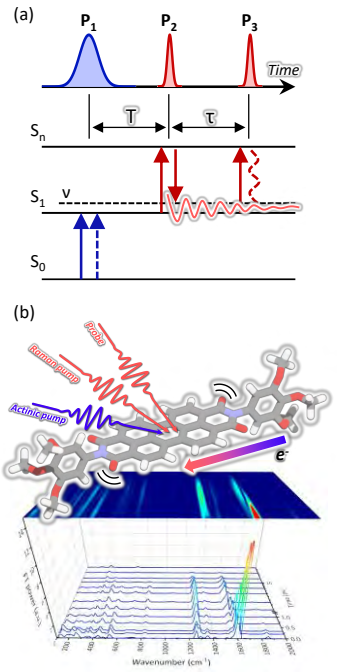

Figure 1. Experimental scheme of TR-ISRS (top) and time-resolved Raman spectra during the CS dynamics in PBI (bottom)

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Raman Vibrational Coherence Spectroscopy and Proton Tunneling in Green Fluorescent Protein

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Studies of the Green Fluorescent Protein (GFP) are presented that probe both the ground and excited state proton transport processes following ultrafast optical excitation of the native chromophore. In the excited state we observe two picosecond kinetic phases with isotopically dependent time-constants. In the ground state "reset" reaction, the rate is nearly exponential, and its temperature and isotope dependence indicate that "deep tunneling" of a single proton is rate limiting [1]. At room temperature, the ground state tunnelling rate in GFP is unexpectedly fast, $(400\ ps)^{-1}$, suggesting that proton tunnelling can play a more general functional role in kinetic trapping and regulating transport along water wires in proteins [1]. The possibility of proton tunnelling in the excited state GFP reaction is discussed in the context of predicted differences in the tunnelling distances expected for the ground and excited state reactions. The role of vibrational promoting modes is also explored for excited state proton transport and the results from various independent experiments, all of which detect vibrationally coherent motions (e.g. Figure 1), are compared and discussed.

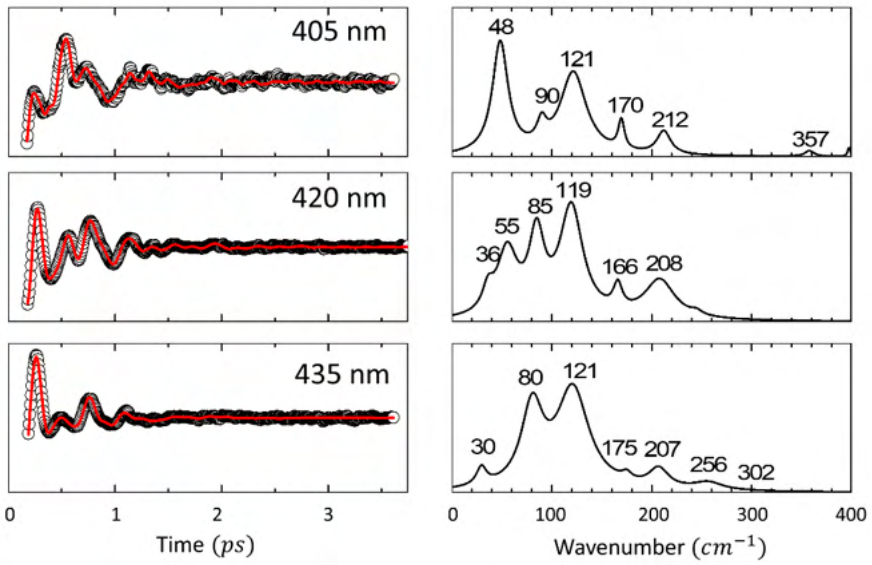

Figure 1: Coherent response (left) and spectral content (right) of GFP at 295 K with pump-probe excitation at 405 nm, 420 nm and 435 nm, respectively. The solid red lines are the linear predictive singular value decomposition (LPSVD) fits to the oscillatory content. The power spectrum amplitudes of these oscillations are plotted in the right panels.

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SERS detection schemes in complex biological matrices

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As powerful detection scheme in bioanalytics and biosensing allowing for molecular specific and highly sensitive analysis, surface enhanced Raman spectroscopy (SERS) gained much attention in the last years. [1, 2] In the case of label-free SERS approaches, the target analytes showing a high affinity toward the metallic surface are dominating the spectral response. Thus, the SERS-based detection even in complex matrices such as body fluids becomes feasible.

Within this contribution, we will introduce label-free or direct SERS detection schemes dealing with complex biological matrices. Due to the combination of SERS with microfluidics, reproducible measurement conditions and high-throughput analysis are achieved. The broad spectrum antibiotic levofloxacin is characterized in simulated urine, mimicking a complex biological composition. [3] First, different parameters such as matrix complexity, aggregation time and matrix dilution on the overall SERS signal is investigated. Moreover, levofloxacin is spiked in human urine and the quantitative analysis is achieved down to a root means square error of prediction (RMSEP) between 0.058 and 0.16 mM for the different investigated urine samples. As a further example, the metabolite pyocyanin, associated with an infection with *Pseudomonas aeruginosa*, was successfully detected via label-free SERS within the medical relevant range. [4] The potential for real application scenarios was furthermore demonstrated by investigating the metabolite in the matrix of artificial sputum combined with a simplified sample preparation protocol. [5] Finally, we will present recent results on detecting co-factors in bacterial membranes as a result from different cultivation conditions.

ACKNOWLEDGEMENT

We gratefully acknowledge the Federal Ministry of Education and Research, Germany (BMBF) for supporting the InfectoGnostics (13GW0096F) and EXASENS (13N13856) project grants as well as BMBF and German Aerospace Center (DLR) for supporting the Myco-NET^2 (01DN15028) project grant.

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Probing Reaction Dynamics in Higher-Lying States using Transient Stimulated Raman Spectroscopy

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Probing the dynamics of molecules in higher-lying electronically excited states (*i.e.* states above S_1) presents many experimental and theoretical challenges. While ultrafast techniques routinely measure the dynamics of molecules in the ground and first-excited states, probing the dynamics in higher-lying states requires new approaches to overcome the limitations of very short lifetimes and poorly resolved electronic transitions. Beyond scientific curiosity, higher-lying states present an opportunity for controlling the outcomes of photochemical reactions by opening new reaction channels. For example, recent experiments have shown that sequential excitation offers a new level of control over chemical reactions by accessing new regions of the higher-lying potential energy surfaces that are not accessible directly from the equilibrium geometry of the ground state [1,2]. Selectively varying the time-delay and wavelength of the secondary excitation allows the molecule to sample different reaction channels upon promotion to the higher-lying state.

We use femtosecond stimulated Raman scattering (FSRS) to probe the ultrafast dynamics following sequential excitation of a molecule. Analogous to ground-state resonance Raman spectroscopy, the resonance condition in the FSRS measurement probes the topology of the upper-state potential energy surface, and therefore provides an insightful window on the dynamics in the higher-lying state and the ability to control reactions via sequential excitation. Specifically, we compare resonance enhancements in the experimental spectra with calculated gradients of the potential energy surfaces for the upper electronic states. On one hand, the experimental spectra provide benchmarks for comparison with the calculations, and on the other hand the calculations help interpret the experimental results. This work highlights important challenges in assigning excited-state resonance Raman spectra due to the resonance condition that is typical of FSRS measurements [3]. Simulations of the resonance-enhancement effects pave the way for making more accurate assignments of FSRS spectra, while also showing that resonance-enhanced FSRS spectra can reveal novel information about the dynamics in higher-lying excited states [4,5].

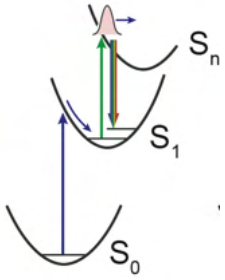

Figure 1: FSRS probes dynamics in the higher-lying state S_n

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Detecting drugs in cells and tissues by Raman/SERS microscopy

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Visualization of drug molecules in live cells is a challenging task since labeling drugs by using fluorescent molecules alters the chemical property of the drugs. Recently Raman tag such as alkyne, which provides distinct Raman peaks at the silent region, was proposed to detect small molecules in intracellular molecular crowding [1,2]. However, the small cross-section of Raman scattering does not provide a sensitivity high enough to detect the Raman signal from alkyne-tagged drugs. Here, we propose the use of SERS to detect the alkyne-tagged drug in live cells. Gold nanoparticles were used as agents that provide the strong SERS signal from alkyne conjugated with drugs [3]. We utilized slit-scanning Raman microscopy with z objective scanning in order to visualize alkyne-tagged molecules taken into a cell volume. The time-lapse observation at a temporal resolution of 20 sec/volume clearly visualized the uptake of the drugs by introducing the drugs into the medium surrounding the live macrophage cells. We also demonstrated the combination of slit-scanning Raman microscopy and a SERS substrate to detect alkyne-tagged drugs taken into a rat brain. By combining fluorescence microscopy, we successfully detected serotonin reuptake inhibitor S-citalopram at the neuronal membrane near a brain vessel [4]. We also used SERS from the alkyne tag for finding a binding site of small molecules in a protein [5]. In order to understand the mechanism of the effect of a drug, it is important to investigate drug binding sites in target proteins. However, it is a time-consuming process to find a binding site from many different proteins and peptides. We propose the use of SERS signal from alkyne for prescreening the peptides that contain drugs. Following with the SERS detection, mass spectroscopy is performed to find out the biding site. An inhibitor of lysosomal cysteine protease, AOMK, was tagged by alkyne and bound to cathepsin B. SERS prescreening of peptide fragments of cathepsin B visualized the alkyne containing fragments. The binding site of AOMK in cathepsin B was successfully identified by mass spectroscopy of the fragments with an alkyne.

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Raman Spectroscopy Studies of 1D Systems: Carbon and Sulfur Chains

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Resonance Raman spectroscopy-based techniques are widely used to characterize a variety of different nanostructures. In particular, Raman-based techniques are trendy on the studies of nanocarbon materials such as graphene (2D systems), carbon nanotubes (quasi 1D systems), and linear carbon chains (truly 1D systems) [1,2]. These low dimensional materials are excellent model systems for studying phonons and electrons because of their strong coupling to each other. Thus, resonance Raman spectroscopy is suitable to provide detailed information about the structure and electronic properties of nanocarbon systems, thus allowing one to probe in the phonon spectra both structural characteristics and their interactions with the environment as well. This concept does apply to other nanomaterials beyond carbon.

In this talk, we discuss the recent results of resonance Raman Spectroscopy in linear carbon chains and sulfur chains encapsulated into carbon nanotubes. We investigated the behavior of these hybrid systems (chains encapsulate into nanotubes) by changing diameter, number of the layers of the tubes, and strain conditions. We show that by using different laser energies, it is possible to probe structural changes and charge transfer mechanisms between the atomic (carbon and sulfur) chains and nanotube shells for various strain levels. This late effect explains a Nanotube enhancement Raman spectroscopy effect observed in sulfur chains.

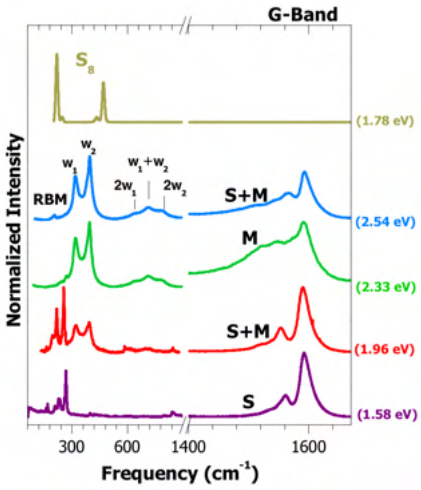

Figure 1: Raman spectra for sulfur chains encapsulated into carbon nanotubes in the region of Radial Breathing Mode (RBM) and G-band measured with different laser excitation energies. W_1 and W_2 stand for modes from sulfur chains. The top trace is for sulfur S_8 polymorph.

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Bringing SERS to the Clinic: A Nanomaterials Chemistry Approach to Plasmonics

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Our planet is populated by an estimated 7.3 billion, highly interacting individuals, who are, at least in the first world countries, enjoying longer, hyperconnected, and comfortable lives. These facts bring substantial strain to the healthcare systems, as people are more exposed to both communicable and non-communicable diseases. In stark contrast, communities in developing and low-income countries have limited access to healthcare and are often exposed to communicable diseases, sometimes of zoonotic origin, in addition to being unable to rely on sufficient screening for other non-communicable diseases, such as cancer. Therefore, there is the need to address healthcare and energy issues for both the rich and the developing countries, taking into account how approaches that could be effective for the rich world have to be made substantially cheaper, rugged, and portable to address the needs of low-income countries.

In my talk, I will discuss how my group has been addressing these needs by leveraging lessons from nanomaterials chemistry, intertwined with inputs from physics, biology, and medicine. I will first present our holistic computational and experimental approach to rationally design novel gold nanoparticles [1, 2], and then describe how these particles can be employed to solve medical problems. For instance, I will show how they can be used, by means of surface enhanced Raman spectroscopy (SERS), to quantify cancer cell phenotype at the single cell level [3], to stratify cancer patients [4], and to understand influenza A virus mutations in single intact cells [5]. I will also show how careful experimental design can be leveraged to detect and quantify opioids, such as fentanyl, in mixtures of recreational drugs, by means of a low-cost, portable, Raman spectrometer. Taken together, all these results can allow us to paint a very optimistic outlook toward the application of SERS in the clinic.

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2D Impulsively Stimulated Resonant Raman Spectroscopy of Molecular Excited States

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The properties of molecules undergoing physical or chemical reactions are determined by interplay of vibrational and electronic degrees of freedom, as dictated by the potential energy surfaces (PESs) over which the dynamics occur. Mapping these surfaces over multiple vibrational dimensions disclose the ultrafast evolution of the system, but is typically hampered by the lack of spectroscopic probes detecting different energy scales with high temporal and frequency resolution.

Here, we present two-dimensional Impulsive Stimulated Raman Spectroscopy (2D-ISRS), which combines the capabilities of multi-dimensional techniques with structural sensitivity at ultrafast timescales to probe vibrational correlations pertaining to targeted electronically excited states [1]. Three temporally delayed femtosecond pulses are tuned to coherently generate and track excited-state vibrational wavepackets, whose evolution reports on the underlying potential energy surface. The pattern of the spectral features in the Fourier transformed 2D maps carries information on the vibronic couplings between the excited state modes, which we decipher by means of a diagrammatic approach.

In particular, we benchmark our approach by addressing the vibronic correlations in the Green Fluorescent Protein during the first steps of its photoinduced dynamics, revealing dark/weak modes as coupling peaks, PES displacements along normal coordinates, and signatures of harmonic mode mixing in the excited states beyond the approximation of linearly displaced potentials.

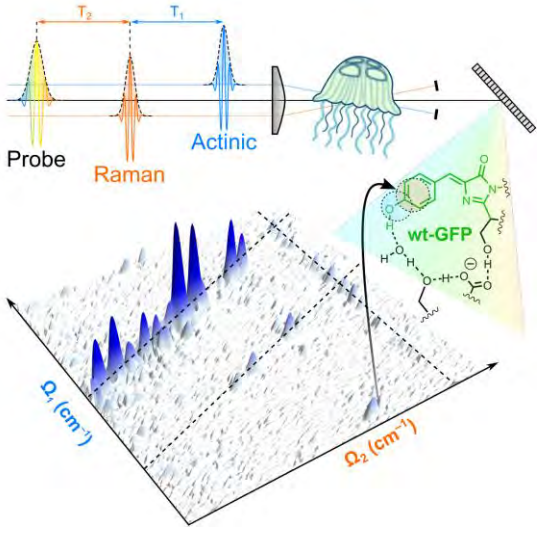

Figure 1: 2D-ISRS reveals vibronic correlations in the excited state of GFP during the first ps of its photocycle.

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Combined Raman-Dielectrophoresis Method for Real Time Study: from Bacteria to Nanoplastic

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Alternating current (AC) dielectrophoresis (DEP) is the electric field-induced motion of micro and nano objects via dielectric polarization under nonuniform electric fields. DEP has been widely used for biotechnology applications in micro/nanoscale environments, and it offers a number of potential advantages over conventional methods for cell/bacteria and micro/nano microparticles manipulation, separation, and concentration [1]. This study presents the application of a combined DEP-Raman spectroscopy and setup to obtain direct, real-time measurements of bacteria or particles from suspension without any labelling or time-consuming sample preparation process [2]. Thanks to the spatial non-uniform DEP fields, bacteria or other suspended micro/nanoparticles can be captured in the focus spot of the Raman microscope, maximizing their signals. By optimizing the setup conditions, the characterization of different bacterial strains, such as *E. coli, S. aureus* and *P. aeruginosa*, with high specificity was carried out. Moreover, the application of the DEP-Raman device on *E. coli* treated with the commonly prescribed second-generation fluoroquinolone ciprofloxacin (1 µg/ml, which is the MIC concentration in our experimental conditions) proved that spectral changes in the bacterial chemical fingerprint due to the mode of action of the antibiotic were detectable after only one hour of treatment. Descriptive and predictive models based on ANOVA-Simultaneous Component Analysis and PLS-Discriminant Analysis were developed to determine cell viability upon different drug treatments, in view of antibiotic resistance and biocide-induced cross-tolerance experiments. These models were validated using independent test-set, proving high specificity and adequate sensitivity. Simultaneously, standard microbiological assays, such as CFU counting and OD_{600} growth curves, were carried out as reference methods. This novel integrated system can be also efficiently used for the chemical identification of suspended polymer micro/nanoparticles and for their direct quantification.

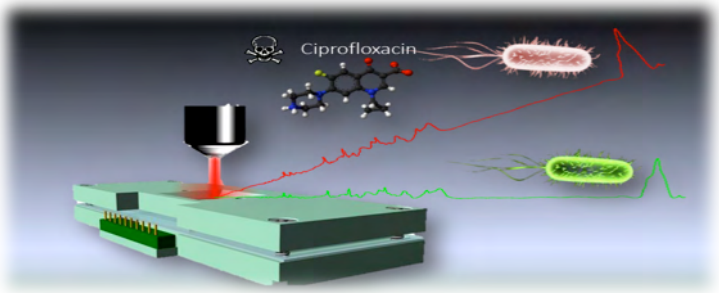

Figure 1: The DEP device for Raman analysis

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High-speed multicolor stimulated Raman microscopy

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Stimulated Raman scattering (SRS) microscopy is a powerful imaging modality that provides molecular vibrational contrast. SRS is advantageous in the sensitive detection of a single vibrational mode, whose vibrational frequency is specified by the optical frequency difference between pump and Stokes laser pulses. This advantage has led to a variety of applications ranging from label-free imaging to imaging with Raman probes. An essential challenge in the original implementation of SRS microscopy was multicolor imaging, where SRS signals at multiple vibrational frequencies are acquired to discriminate different constituents. Typical laser pulse sources such as optical parametric oscillators take seconds to minutes for wavelength tuning, leading to long acquisition time.

We have been developing high-speed multicolor SRS microscopy using a wavelength-tunable pulsed laser system [1,2], which utilizes the spectral filtering of Yb fiber laser pulses with a tuning time of less than a millisecond. This laser was used along with a fixed-wavelength Ti:sapphire laser to realize video-rate SRS microscopy with a frame-by-frame wavelength tunability. Our high-speed multicolor SRS microscopy has enabled the following applications with stringent speed requirements: hyperspectral imaging of skin *in vivo* [1], real-time multicolor visualization of sectioned tissue [3], multicolor imaging of metabolites in moving microalgae [4], and super-multiplex imaging with Raman probes [5]. Recently, the wavelength tuning speed was dramatically reduced to ~50 ns using spectral filters and fiber delay lines to realize multicolor SRS imaging of numerous (>10,000) cells at a high throughput of >100 cells/s in a high-speed flow at 2 cm/s [6]. Not only high-speed vibrational imaging, SRS is finding new applications such as label-free imaging of cellular uptake of boron cluster compounds as a drug for boron neutron capture therapy [7] and three-dimensional analysis of human skin [8].

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Tip-enhanced Raman scattering - high resolution and beyond

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In the recent years Raman based near-field optical spectroscopy demonstrated extreme lateral resolution capabilities. Experiments and theoretical investigations point towards the structural distinction of single bonds. Nevertheless - the examples studied so far with respect to this 'ultimate' resolution were mostly model structures and most frequently studied under cryogenic conditions. The intention of this presentation is to provide an overview of recent technical developments and application examples from the application-oriented point of view based on developments of our laboratory.

The experimental developments will address the use of photoinduced force microscopy and sSNOM measurements as a way to provide a fast alignment and laser mode dependent help that can be easily established in any existing TERS experiment[1] as well as a near-field temperature detection method that provides solely the temperature and plasmonic parameters at the sample site[2].

With respect to applications the focus will be on systems where the required resolution capabilities are on a 1-10 nm scale, but where the intrinsic structural sensitivity of Raman spectroscopy is crucial. Particularly, analytical challenges for two examples will be addressed, namely modern drug delivery polymers[3] and nano-diamond synthesis[4,5].

Furthermore, if time permits a short discussion of our collaborative efforts towards understanding the effects the near-field probe towards the specimen will be briefly addressed.[6]

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785 nm SERS of metalloporphyrins: Chemical enhancement, Herzberg-Teller coupling and forensics

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The study of the vibrational features of metalloporphyrins, especially via resonance Raman, has a long and rich history in the field of Raman Spectroscopy playing a critical role in learning how these molecules perform essential biological tasks and in developing fundamental descriptions of Raman theoretical frameworks.[1] Most prior SERS studies of metalloporphyrins have been accomplished with excitation resonant with the α, β and Soret visible ππ* absorptions, and predominantly due to Ag nanoparticles.[2-4] However, the effects of metal surface interactions are conflated with the resonance Raman effects and photochemical degradation at these excitation frequencies.[3] We show that 785 nm SERS spectra of dried blood on both Au and Ag substrates provide highly sensitive (EF~10^{10}) and specific signatures that can be used for forensic detection and identification.[5,6] However, these SERS spectra, and the SERS of all metalloporphyrins, on

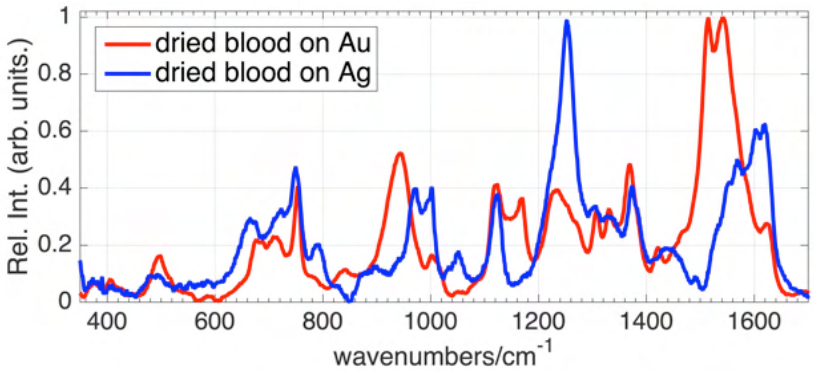

Figure 1: 785 nm SERS spectra of dried blood on Au and Ag substrates.

Au are remarkably different than on Ag (Fig. 1), and their pattern of relative intensities is very different than corresponding normal (non-SERS) Raman spectra. Thus chemical enhancement plays an important role in the 785 nm SERS spectra of these porphyrins. The 785 nm excited SERS spectra of metalloporphyrins, as a function of coordinating metal (Cr, Mn, Fe, Co, Ni, Cu, Zn) and substituents, and the biological heme proteins, on Au and Ag substrates are presented.

DFT calculations of Au and Ag dimer complexed metal protopophyrins complexes reveal that these spectra result from near resonance with weak Au or Ag metal to π* charge transfer states of the physi-adsorbed porphyrin on the nanoparticle surface. The Au and Ag difference largely arises from the different sites of adsorption for the protopophyrin IX species; propionic groups on Ag, heme ring C_β attachment on Au. Furthermore, the observed relative SERS intensities are due to the nuclear coordinate dependence of the near resonant CT state transition moments, i.e. Herzberg-Teller or *B*-term activity dominated. Consequences for SERS spectra of the biological hemes in terms of B or C type heme structures will also be discussed.

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Probing Spectral Fluctuations and SERS Imaging at High Speed

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Surface-enhanced Raman scattering (SERS) is a well-established technique that can be used for trace analysis [1]. Strong fluctuations in SERS intensities are observed from diluted solutions, which complicate quantification. The observation of those fluctuations were earlier assigned as a hallmark for single-molecule detection by SERS [2]. Recently, we have developed experimental methods to monitor those fluctuations at high speed (sub-ms time scale). It is clear that, at those high-speed time scale, the SERS fluctuations are a more general phenomenon that can be observed even from fully-coated nanoparticles [3].

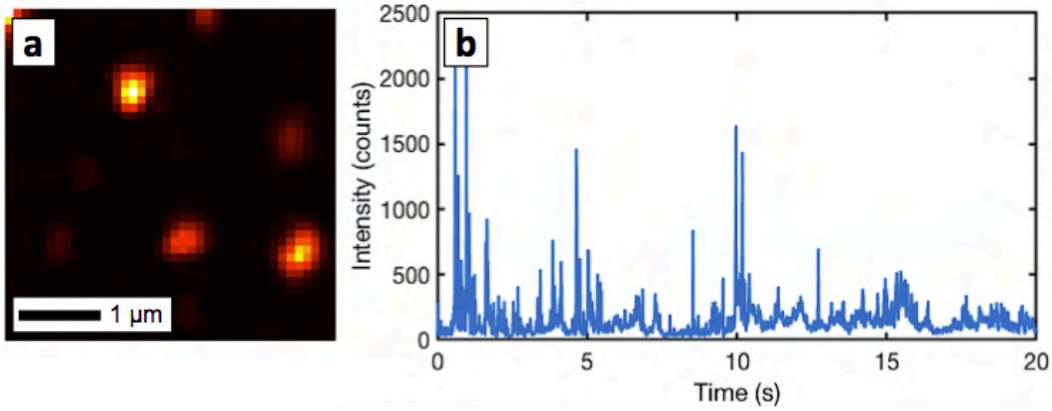

Figure 1: (a) SERS images of individual nanoshells. (b) Time-dependent SERS intensity fluctuations.

Figure 1a shows an example of Raman imaging from a fully-coated (with an organic thiol probe) single nanoparticles excited by a 633 nm laser and recorded within the Raman shift region between 950 cm^{-1} to 1600 cm^{-1}. SERS images of individual nanoparticles, acquired at ~600 fps, are evident in Figure 1a. Figure 1b shows the time trace for the SERS intensity in one of the nanoparticles. The SERS intensity fluctuations observed in Figure 1b were measured for other nanoparticles (nanoshells, nanostars) and substrates prepared by nanosphere lithography and also for other molecular probes, such as organic dyes. The results from Fig.1 allied to super-resolution imaging analysis [4] suggest that single molecule responses from several different hotspots in the same nanoparticle are activated at different time scale. This new high-speed method reveals some important fundamental characteristics of SERS hotspots that can be useful for analytical applications.

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Raman spectroscopy for on-site medical diagnosis and therapy

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Due to an aging society a large increase of cancer or neurodegenerative diseases is observed representing unsolved medical needs with respect to early diagnosis and therapy. In this context an important medical need in pathological diagnostics is a precise intraoperative tumor margin control, to localize the tumor exactly in order to remove it as complete as possible and a reliable tumor typing and grading in order to initiate an individual therapy plan as quickly as possible. In principle, the following applies to all diseases: the earlier treatment begins, the better the chances of cure. Therefore, a great need for new diagnostic methods for an early diagnosis of diseases to start a targeted therapy as early as possible exits. Raman spectroscopy plays a key role in the implementation of these ambitious goals.

Here we will highlight our recent efforts in translating Raman spectroscopy towards routine clinical applications by researching and developing compact clinically usable automated Raman spectroscopic instrumentation and their combination with other spectroscopic / optical modalities, to provide a multimodal approach with high TRL levels, i.e. which can be applied out of specialized labs in a clinical environment. We will start with novel multimodal spectroscopic instrumentation (like e.g. innovative Raman fiber probes [1], clinically usable multimodal microscopes [2,3] or endospectroscopic probes [4,5], etc.) for precise surgical guidance and intraoperative histopathological examination of tissue under in-vivo or near in-vivo conditions. Besides innovative photonic technologies, the presentation will also introduce innovative image evaluation algorithms for the translation of multimodal images into quantitative diagnostic markers [6]. We will show that the presented multimodal approaches can be combined with laser tissue ablation for tissue specific laser surgery [7] and for therapy monitoring [8]. In addition, we report on the application of non-resonant Raman spectroscopy for an early diagnosis of neurodegenerative diseases directly in the fundus of the eye, which can be seen as a window to the brain [9,10].

ACKNOWLEDGEMENT

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, and the Carl-Zeiss Foundation are greatly acknowledged.

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Timing is Everything: A Quest for More Information from Coherent Raman Spectroscopy and Imaging

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Time-delayed coherent anti-Stokes Raman spectroscopy and imaging is explored to evaluate its potential to improve sensitivity of Raman detection in aqueous solutions, to analyze congested Raman bands and to provide additional information about the local nano-environment.

Vibrational spectroscopy based on Raman scattering is a powerful tool for revealing chemical and structural information about molecules in their natural environment without using any additional labels. Traditionally, Raman spectra are analyzed for the position of vibrational bands and the amplitude of Raman peaks. Nonlinear Raman spectroscopy provides a signal enhancement, which can be utilized both for sensing and imaging to allow shorter acquisition times. Raman spectroscopy is often helpful in identifying chemical compounds at the location of the focal spot; however, biological nano-environment, which is essential for productive implementation of myriads of biochemical reactions within a cell, is often hard to digest from overcrowded Raman spectra. We have recently revisited time-resolved coherent anti-Stokes Raman spectroscopy, which is the early days of coherent Raman spectroscopy was proposed as the most distinctive feature of nonlinear Raman spectroscopy capable of providing vibrational relaxation times in molecular systems [1]. We have modified the system for time-resolved coherent anti-Stokes Raman scattering by employing broadband excitation pulses for the pump, Stokes and probe beams (as seen in Figure 1) and improved

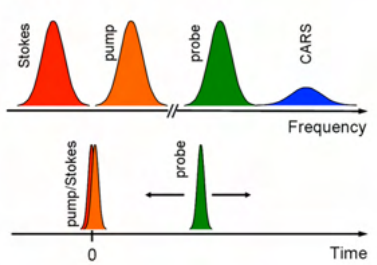

Figure 1. The concept of time-resolved coherent Raman spectroscopy: two ultrashort (in time domain) broadband (in spectral domain) pulses excite vibrational coherence, and the third time-delayed probe pulse interrogates the decay of this coherence.

on the detection system and methodology for signal analysis to allow new capabilities such as ultrasensitive detection of molecular species, improved specificity of detection and assessment of local nano-environment [2-3]. In my talk, I will review the past work on time-resolved coherent Raman scattering and will discuss potential applications for materials' characterization and biomedical sensing and imaging.

This work was partially supported by NIH grants 1R21GM142107, 1R21CA269099 and 1R01GM127696, AFOSR grants FA9550-20-1-0366 and FA9550-20-1-0367, and NSF grant CMMI-1826078.

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A novel photo-induced lattice instability in SnSe observed by femtosecond x-ray scattering

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We demonstrate the generation of a novel above-gap photoexcited lattice instability in SnSe that is distinct from the second-order phase transition associated with its exceptionally good bulk thermoelectric material[1]. In ultrafast x-ray diffraction measurements, we observe three of the four fully symmetric Raman-active modes that undergo displacive excitation following above-gap excitation. Using data from multiple Bragg peaks, we reconstruct quantitatively the quasi-equilibrium positions of the ions and reveal that the lattice is driven towards a new high symmetry structure that is not accessible in thermal equilibrium. First principles calculations help identify how photoexcitation from particular bands drives changes in the orbital hybridization that drives this instability. Preliminary results from femtosecond x-ray diffuse scattering measurements further help us identify concomitant changes in the interlayer bonding associated with this instability. Our work suggest the importance of pump-wavelength for control of structural distortions through orbitally-selective above-gap excitation.

This work was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences through the Division of Materials Sciences and Engineering, including under Contract No. DE-AC02- 76SF00515 and Award No. DE-SC0019978. Use of the LCLS and SSRL is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. DE-AC02-76SF00515 S. Y. acknowledges support by the Fitzpatrick Institute for Photonics through a Chambers Scholarship.

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Electron-phonon processes in twisted bilayer graphene and low symmetry 2D materials investigated by resonance and polarized Raman spectroscopy

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In this presentation, I will discuss the use of Raman spectroscopy to study electron-phonon interactions in 2D materials, focusing on twisted bilayer structures and anisotropic 2D materials. Raman spectroscopy is a fundamental tool to study twisted bilayer graphene (TBG) since the Raman response is hugely enhanced when the photons are in resonance with optical transitions of TBGs. Moreover, new peaks appear in the Raman spectra and are due to phonons within the interior of the Brillouin zone of graphene that are activated by the Moire superlattice by either the intralayer or the interlayer electron-phonon processes. I will first present multiple-excitation Raman results in many different TBG samples with twisting angles and using several different laser excitation energies in the NIR, visible and UV ranges and then explain the results by theoretical calculations of the double-resonance (DR) Raman intensity in graphene by imposing the momentum conservation rules for the intralayer and the interlayer electron-phonon processes. Raman spectroscopy is a powerful tool to study the behavior of phonons in optically anisotropic 2D materials since the intensities of the Raman peaks depend on the polarization of the light with respect to the crystallographic axes. I will present angle-resolved polarized Raman measurements in single-layer (1L) and bulk $ReSe_2$, crystals that exhibit a triclinic symmetry, using two different laser excitation energies, to investigate the effects of dimensionality and excitation energy on the Raman tensors of ReSe2. Phase differences between tensor elements are needed to describe the experimental results for low symmetry 2D crystals since the elements are given by complex numbers. I will then discuss the dependence of the Raman tensors on the excitation laser energy and number of atomic layers and interpret the results considering the quantum model for the Raman intensities. Finally, I show that the wavevector dependence of the electron–phonon interaction is essential for explaining the distinct Raman tensor for each phonon mode.

TWO NEW APPROACHES TO BROADBAND STIMULATED RAMAN SCATTERING MICROSCOPY

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Stimulated Raman scattering (SRS) microscopy is a powerful technique for label-free identification of cells and tissues based on their intrinsic vibrational spectrum. Single-frequency SRS is now working very reliably with high acquisition speeds; however, its information content is not sufficient to distinguish the different components within complex heterogeneous systems. Here we present two broadband SRS microscopy setups combining broad spectral coverage (up to 500 cm^{-1}, covering the entire C-H stretching band) with high frequency resolution (≈ 14 cm^{-1}). Narrowband Stokes pulses at 1040 nm are synchronized with broadband pump pulses in the 715-870 nm range, generated by a tailor-made low-noise optical parametric oscillator at 80-MHz repetition rate. We employed the in-line balanced detection (IBD) approach [1] to suppress laser fluctuations and achieve close to shot-noise-limited sensitivity.

The first solution we propose comprises a hyperspectral SRS detection scheme in which a diffraction grating and a galvanometric mirror after the sample are employed to rapidly scan the Raman frequency using a balanced photodiode and a single-channel commercial lock-in amplifier with 1.8-µs integration time, see Fig. 1 [2]. The second solution involves a multiplex (parallel) detection scheme that employs a recently developed [3] balanced low-noise multichannel lock-in amplifier with 10-µs integration time for collecting the spectrum with up to 32 channels operating in parallel. In both cases, we will present the performances in broadband SRS microscopy, illustrating their advantages and drawbacks.

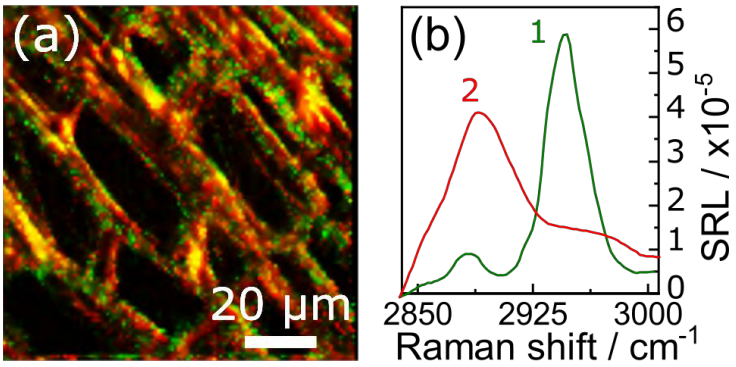

Figure 1: Chemical imaging of an Elodea Canadensis leaf. (a) Concentration distributions of the two main components extracted from multivariate curve resolution chemometric analysis; (b) corresponding spectra.

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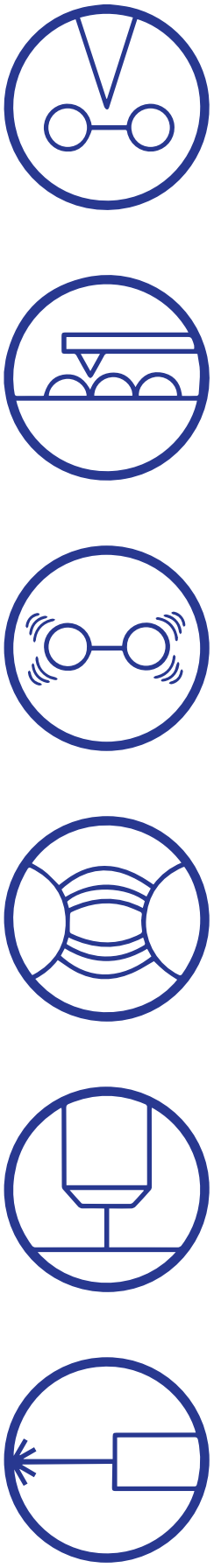
ORAL PRESENTATIONS



Raman Enhancement of Copper Phthalocyanine by Twisted Bilayer Graphenes Promoted by Excited State Charge Transfer

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Few atom thick, twisted bilayer graphene (tBLG) possesses a rotation angle (θ) dependent van Hove singularity (vHs). Fine-tuning vHs serves a potential method to enhance charge transfer (CT) in surface enhanced Raman spectroscopy. In this talk, I show that tBLG having a specific θ promotes as high as a 1.7 times enhancement of the Raman signals of copper phthalocyanine (CuPc) as compared to that caused by single layer graphene (SLG), as shown in Figure 1. The results of a combination of reflection imaging spectroscopy[1] and widefield Raman[2] provide spatial and spectral information about both tBLG with q ranging from 10.9 to 13.7º and the corresponding vHs. Comparison of Raman spectra of CuPc in presence and absence of tBLG demonstrates that a significant enhancement of certain CuPc vibrational modes occurs when the underlying tBLG possesses a $\theta = 12.2$º, showing as high as 6.8 and 1.7 times enhancements of certain vibrational mode as compared to those of CuPc on bare and SLG substrates, respectively. Theoretical calculations indicate that a match between the energies of vHs of tBLG with those of frontier orbitals of CuPc facilitates CT from the distant SLG to CuPc.[3]

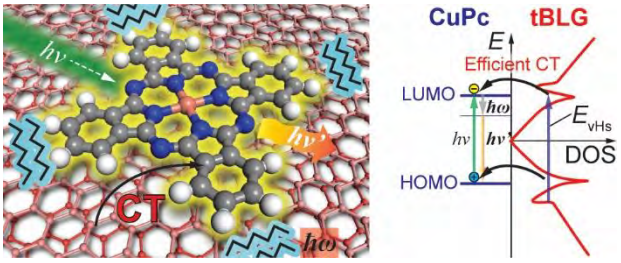

Figure 1. (Left) CuPc on tBLG with specific θ. (Right) Excited charge transfer mechanism from tBLG to CuPc.

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Fluorescence guided photothermal infrared microscopy at single-cell resolution

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Fluorescence imaging is undoubtedly the most widely used optical microscopy technique in biomedical-related research. Numerous studies have been benefitting from the extremely high sensitivity and specificity associated with fluorescence imaging. However, fluorescence labeling is not suitable for metabolites, an essential category of biomolecules. Adding large fluorescent probes to metabolites may perturb the cellular function and undergo the different physiology compared with native molecules.

Vibrational spectroscopic imaging provides an alternative to tackle the limitation by detecting the endogenous chemical bond vibrations from a specific molecule. Optical photothermal infrared (OPTIR) microscopy is a recently developed novel vibrational spectroscopic imaging platform. In OPTIR, a visible probe light is used to sense the IR absorption-induced photothermal effect. Sub-micrometer spatial resolution chemical imaging has been demonstrated for cells and tissues. Coupling stable isotope labeling with OPTIR, the metabolic imaging of newly-synthesized lipid droplets were visualized by targeting at the red-shifted peaks. Despite the rapid technology development and broad applications, the lack of protein specificity restrains its even broader applications in biomedical research.

Here, we improve the protein specificity of OPTIR by incorporating fluorescence as the guidance. OPTIR is highly compatible with fluorescence in terms of instrumentation and signal detection. We demonstrated chemical imaging from IR and fluorescence detection within the same field of view and at same spatial resolution for both point-scanning and widefield OPTIR setup. In addition, since OPTIR detects probe light at the same input wavelength, there is no fluorescence background issue that may occur in Raman measurements.

We used two biology systems to demonstrate the potential enabled by the fluorescence-guided OPTIR system. For the point-scanning system, we studied the role of lipid droplets in Alzheimer's disease. The tau protein distribution within the cells was identified by fluorescence imaging, and the lipid droplet metabolites around the aggregated tau protein were identified by the OPTIR spectroscopy. We further proved the high-throughput imaging capability by implementing the fluorescence guidance on the widefield OPTIR setup. An artificial bacteria mixture from two species under different culture conditions were used as the test bed. We picked out the bacteria species based on fluorescence imaging of fluorescence in-situ hybridized tags that were specific to the bacteria genome. In the meantime, the metabolic genotype of protein synthesis was analyzed by OPTIR.

Collectively, the developed fluorescence-guided OPTIR platform enables protein and species-specific fluorescence imaging and associated metabolic mapping on the same setup, which opens a vast potential for biomedical research.

Application of LC-Raman method to sugar analysis in honey

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Natural products are utilized in various ways such as medicine and nutrients. Quality control based on the identification and quantitation of chemical components is of crucial importance. Raman spectroscopy is one of the most valuable methods for this purpose.

Honey is a popular food. Spectroscopic analysis of sugars in honey has been performed by several approaches. The quantitative analysis of the sugars may not be readily performed because their molecular structures are similar to each other; the molecular structure mainly consists of the single bonds of C, H, O, and solely the chirality at one carbon atom is different (epimer). Consequently, they give similar spectral patterns. A novel experimental method may offer an effective solution.

We have successfully combined the Raman spectrometer with the liquid chromatography (LC) [1,2] with the aid of a vertical flow method [3]. The vertical flow unit improves the signal detection efficiency up to 90 times owing to the total reflection of light, thereby facilitating the collection of the non-resonance Raman signal of the LC eluate online (LC-Raman measurement). This LC-Raman method was applied to the quantitative analysis of the sugars in honey.

Figure (a) shows the two-dimensional LC-Raman data of honey (after subtraction of the solvent bands). Several Raman bands appear at 17-18 min. Singular value decomposition (SVD) analysis deduced three principal components in the observed 2D data (b). MCR-ALS analysis derived three spectral and temporal components (c and d, respectively); judging from the Raman spectral patterns, the detected three components are assigned to fructose eluted at 17.5 min, glucose at 17.2 min, and maltose at 18.4 min. The Raman spectral pattern ensures the separation of the elution peaks in close proximity. Thus, the LC-Raman method offers a novel method to separate and identify the molecules having similar structures and chemical properties to each other in a mixture.

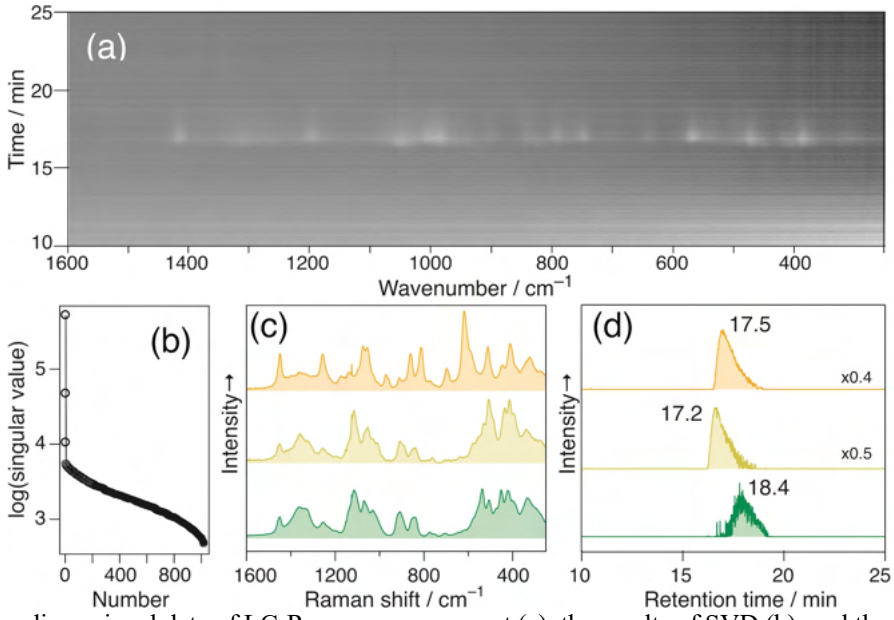

Figure Two-dimensional data of LC-Raman measurement (a), the results of SVD (b), and the spectral (c) and temporal (d) components in the 2D Raman data derived from MCR-ALS analysis.

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Observation of nano-confinement-induced ice nucleation: Ice-vii to ice-i_h transition

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Extensive studies to understand the heterogeneous nucleation have been performed, but only limited to a given specific configuration. Despite many computational studies, experimental probing of heterogeneous nucleation process in nano-confinement still remains unsolved due to it being very challenging. Here, we investigate the confinement-induced ice nucleation of a single water nano-meniscus between Ag coated tip and mica using tip-enhanced Raman spectroscopy (TERS). A new DDAA peak emerges in the OH stretching band as the confinement is stronger. We assign the new DDAA peak as the ice-vii-like structure signal based on the spectroscopic uniqueness of the ice-vii well-known high-pressure ice phase at room temperature. We also observe the exchange between two ice phase peaks, the ice-vii-like peak and the ice-i_h peak, as the confinement is weakened. We believe that the confinement effect may be affected to nucleate the ice-vii-like structure by lowering the pressure threshold. Our result is the experimental demonstration of a new pathway to the ice-i_h by confinement-induced heterogeneous nucleation through the ice-vii. And this result will provide the fundamental understanding of heterogeneous nucleation.

Theory of Abnormal Raman Bands of Wagging Vibrational Modes in Aromatical Amine and Benzyl Radicals

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In the work we present our recent works focusing on the chemical enhancement effect of Raman cross section of the wagging vibrations in aromatic amines and benzyl radical adsorbed on metal surfaces. These molecules can display giant chemical enhancement effect in Raman intensity of the specific wagging vibrational mode[1-4]. First, the wagging vibrational mode of aromatic amine can display large vibrational frequency blue shift and large Raman enhancement effect due to the binding interaction between the lone-paired electron and metal surfaces [1,2,4]. Second, we explored the surface vibrational Raman spectra of benzyl radical and anion binding to the silver electrode [2,3]. The case can be closely associated with the determination of chemical reaction intermediates.

To understand the origin of the Raman intensity enhancement, we calculated the potential energy surfaces along the vibrational mode and analysed the change of polarizability due to the binding interaction. Our results also showed that the change of the polarizability derivatives is closely associated with the binding interaction and the orbital hybridization. Furthermore, we also analysed the anharmonicity of the potential energy surface and the vibrational coupling effect of the wagging vibration contributing to the Raman intensity. The Raman features of these special vibrational modes significantly change in vibrational frequency and the relative Raman signal. Finally, we further predict the contribution of the charge transfer state to the relative Raman intensity based on the density functional theoretical calculations.

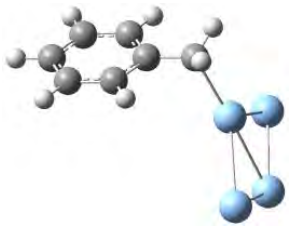

Figure 1: The wagging vibration of the methylene group binding to a Ag_4 cluster

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Coherent Anti-Stokes Raman Scattering-Raman Optical Activity Spectroscopy of a Chiral Organocatalyst in Achiral Solvents

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Coherent Anti-Stokes Raman Scattering-Raman Optical Activity (CARS-ROA) is an enantioselective nonlinear vibrational Raman spectroscopic technique, which combines the advantages of high signal levels of CARS [1] with sensitivity towards chirality; it is theoretically predicted to provide orders of magnitude enhanced chiral signals with respect to spontaneous ROA signals which itself is typically only ~10^{-4}-10^{-5} of the spontaneous Raman signals. Heterodyne-detected CARS-ROA has experimentally demonstrated to have two orders of magnitude higher 'CARS-ROA signal to achiral-CARS background ratio' as compared to 'spontaneous-ROA to Raman signal ratio' obtained in spontaneous Raman spectroscopy [2]. In this work, CARS-ROA spectra of neat liquids like enantiomers of β-pinene were acquired in just 1-minute acquisition time compared to several hours required for conventional ROA. To the best of our knowledge, CARS-ROA has not yet been performed on solutions of chiral molecules in achiral solvents. Here, we present first experimental heterodyne-detected CARS-ROA spectra of 2M solutions of MOM-BINOL molecules (chiral) in DCM (dichloromethane, achiral) also acquired in 1-minute acquisition time [Fig.1a]. MOM-BINOLs ('methoxyl methyl'-protected 1,1′-Bi-2-naphthols) exhibit axial chirality along their aryl-aryl bond; their derivatives belong to a family of widely used organocatalysts in asymmetric synthesis. The acquired CARS-ROA spectra of the two enantiomers of MOM-BINOLs show mostly alternate up and down peaks, a characteristic of chiral-Raman spectra. However, there are some discrepancies in the lower wavenumber region. Nevertheless, the peak positions are in good agreement with the corresponding peaks in the spontaneous Raman spectrum of the same solution [Fig.2, red curve]. In future, CARS-ROA, by virtue of its short acquisition times, will be employed in monitoring asymmetric reactions for simultaneous chemical and chiral identification of the involved chiral molecules.

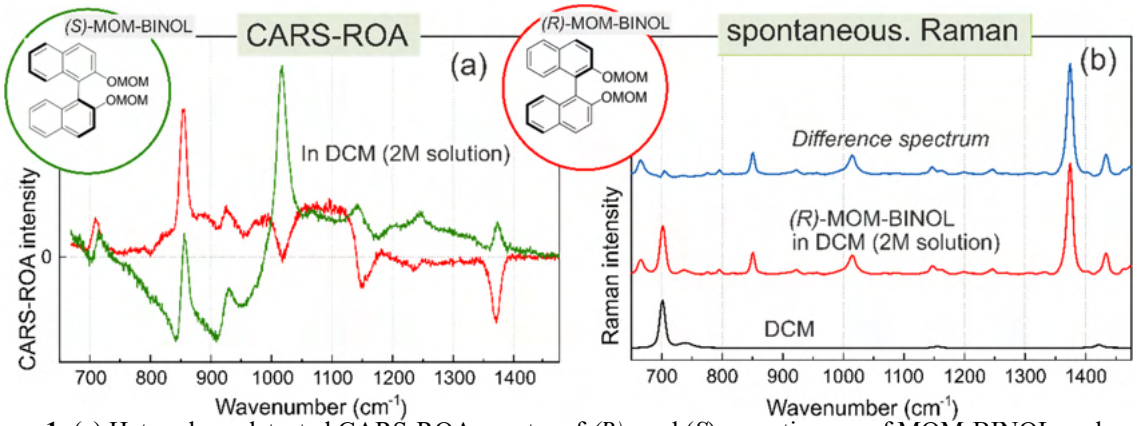

Figure 1: (a) Heterodyne-detected CARS-ROA spectra of *(R)*- and (*S*)- enantiomers of MOM-BINOL molecules in DCM (achiral) solvent. (b) spontaneous Raman spectra of the same solution (in red), solvent DCM (in back) and the solution minus solvent spectra (in blue). It is worth to mention here that due to the enantioselectivity of CARS-ROA, the peaks of the achiral solvent (DCM) are absent in the CARS-ROA spectra.

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Using Raman Spectroscopy to Differentiate Between Various Genospecies of the Lyme Disease Pathogen Using Mouse Blood

Nicolas K. Goff, Tianyi Dou, Artem S. Rogovskyy, D.V.M. Ph.D., Dmitry Kurouski, Ph.D.

Lyme disease (LD) is a tick-borne illness caused by a number of genospecies of the *Borreliella burgdorferi* sensu lato complex. LD is notoriously difficult to diagnose both by serological (the reference standard) and molecular (e.g., PCR) assays because of the LD pathogen is only transiently present in the bloodstream with a follow-up dissemination to collagen-rich and often avascular tissues (e.g., joints). We hypothesized that *B. burgdorferi* can cause substantial changes in blood biochemistry that can be detected and identified by Raman spectroscopy (RS). Our findings showed that RS, in combination with Partial Least-Squares Discriminant Analysis (PLS-DA), can differentiate blood samples taken at various stages of mouse infection. We also found that our innovative approach can differentiate between uninfected (control) and infected mice with 90% accuracy, as well as between the three main pathogenic genospecies, *B. afzelii*, *B. burgdorferi sensu stricto*, and *B. garinii* with 85% accuracy. These results indicate that RS in combination with PLS-DA may transform clinical approaches for pathogen diagnostics in the nearest future enabling inexpensive, non-invasive, non-destructive screening for LD and other bacterial infections.

Exploring new ways of studying photodegradation by means of Raman spectroscopy inside a liquid core waveguide exposure cell

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In many areas, studying the mechanism of photodegradation is of high importance. Conventional methods to do so can be rather time consuming and prone to experimental errors. We have developed an integrated and automated system for the study of light-induced degradation. This so-called 'TooCOLD box' (Figure 1) is coupled online to liquid chromatography (LC) with diode array detection (DAD) for immediate and automated analysis of the composition of the light-exposed samples. A liquid core waveguide (LCW) is used as exposure cell, allowing efficient illumination of the sample over a 12-cm path length. This cell is coupled to a spectrograph allowing in-situ absorbance monitoring of the total sample during irradiation. However, with absorbance spectroscopy alone, the structural information obtained on the photochemical processes during irradiation is of course limited. Therefore, we are now exploring different options to incorporate Raman spectroscopy into the instrumental set-up. The three pathways that are being tested are: a) offline surface-enhanced Raman spectroscopy (SERS) of the light-exposed sample, b) on-line Raman spectroscopy, and c) on-chip SERS inside a microfluidic device. For the first option a), we collect small fractions from the LC separation of the irradiated sample so that SERS analysis can be performed on each degradation product individually. Colloidal silver is added to the fractions and dried before SERS analysis is performed. This is a straightforward approach, but it does not allow monitoring in real-time. For option b) we incorporate a Raman laser and optics to the existing TooCOLD set-up. The irradiation source and excitation laser are alternated so that monitoring with UV-Vis and Raman can both be realized in forward mode. For approach c), a microfluidic device is developed for irradiation in which we incorporate a silver coated leaning pillar chip for SERS analysis. The latter is the most challenging option as memory effects and reproducibility are issues that still have to be improved.

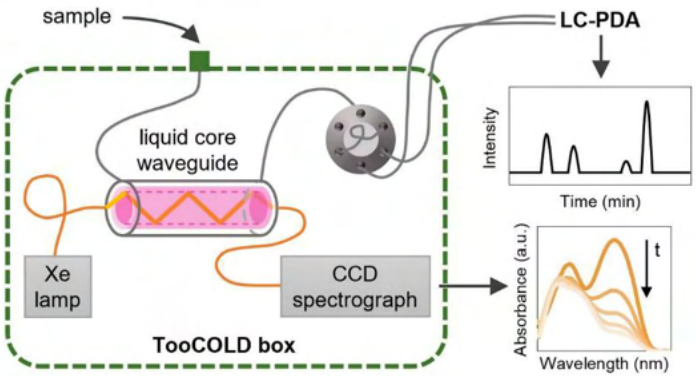

Figure 1. Schematic overview of the TooCOLD box with in-situ absorbance spectroscopy, coupled to LC-DAD.

Interfacial self-assembly and water interactions of model bacterial ice nucleators probed by vibrational sum-frequency generation (SFG)

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Water freezing and ice growth are fundamental for the environment on the planet. Both processes are affected to a great extent by ice active bacteria, which catalyze heterogeneous ice nucleation using specialized proteins. These ice nucleating proteins (INPs) are located at the outer membrane of the bacterial cell and consist of large central domains with repeating threonine motifs that are considered to act as templates for water binding. [1] The INPs can assemble into supramolecular structures on the bacterial membrane, providing large ice nucleation sites that are associated with more efficient ice formation at high sub-zero temperatures. However, the molecular mechanism of INP function and assembly is currently unknown. Probing the interface of INPs on water surfaces is of particular interest for revealing biological ice formation mechanisms. Vibrational sum frequency generation (SFG) spectroscopy, based on nonlinear frequency mixing between infrared and visible laser beams, is a very promising approach for this purpose. Due to its selection rules, SFG probes IR and Raman active modes, and provides molecular structural information exclusively of ordered species at an interface, and thereby allows to follow how INPs and water molecules interact. [2, 3]

Here, we use a synthetic truncated INP at the air-water interface as a model system to study the molecular details of INP self-assembly. We find that the alignment of the INP and its ability to order water strongly depend on the charge state of the protein, which we control in the experiments by varying pH. At neutral pH negatively charged INPs self-assemble into highly ordered structures that can align interfacial water. SFG data in the amide spectral region show that the structural order of the protein at the water surface is disrupted at acidic pH, as the protein net charge approaches zero and electrostatic repulsive interactions become diminished. SFG spectra in the water spectral region provide proof that the ability of the model INP to order interfacial water is severely reduced in an acidic environment. Meanwhile, the secondary structure of the protein in solution remains largely unaltered at acidic pH, as indicated by 2D-IR spectroscopy data. In conclusion, the results suggest that the self-assembled INP layer can be related to efficient class ice nucleators and the data can be used to extend our understanding of the function of ice-nucleating proteins.

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Monitoring the Unusual Deformation and Fracture in Nanoindented Gallium Telluride Multilayers Via Micro-Raman Spectroscopy

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Recent great advances in fabrication of two-dimensional (2D) materials enable considerable interests in 2D materials beyond graphene, including those of low in-plane symmetry whose material properties varying along different in-plane crystal orientations, such as black phosphorus (BP), rhenium disulfide (ReS_2) and gallium telluride (GaTe), with diverse optical and electrical properties shown which strongly depend on their crystal and band structures. Their mechanical properties have also attracted more interests since the extremely high intrinsic in-plane Young's modulus (~1 TPa) and strength (~130 GPa) of graphene was characterized using AFM based nanoindentation.[1] In particular. low symmetry monoclinic phase layered GaTe has attracted much attention recently, not only due to its extremely high photoresponsivity (2×10^{16} A/W) for high performance phototransistors,[2-4] also because of its strong in-plane structural anisotropy. Despite its incomparable advantages in optoelectronic properties, however, notable electrical-mechanical coupling is likely to be resulted in which may change the electronic structure of GaTe during straining process[5] for nanoflexible device application, thus requiring to know their individual mechanical abilities including anisotropy, which is still lacking in GaTe.

In this work, the mechanical properties of both substrates supported and suspended high-quality GaTe multilayers are experimentally characterized and compared for the first time using Berkovich-indenter based nanoindentation, combined with SEM, AFM and micro-Raman stress investigation. Concurrence of multiple pop-ins accompanied with load-drops events are observed, and the role of interlayer sliding as well as the mechanism of layers-by-layers deformation and co-fracture are investigated, which can be sensitively monitored by micro-Raman spectra. These results have provided first-hand mechanical performance and related theoretical mechanism on GaTe multilayers for their potential applications in nanoflexible and strain-modulation optoelectronics.[6,7]

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Analysis of Microplastics in Consumer Goods *via* Femtosecond Stimulated Raman Microscopy

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Microplastics, mostly defined as plastic particles sized 1 µm to 5 mm, are emitted into the environment in large amounts.[1] They originate from degradation of synthetic materials (secondary microplastics) or are produced intentionally (primary microplastics). The latter is often used in household products like cleaners or cosmetics. Raman microscopy is a commonly used method of analysis for microplastic contaminations.[1] It offers the possibility of accessing both quantitative information on the number, shape and size as well as chemical compositions of the contaminating particles. However, conventional Raman microscopy suffers from the usually low Raman scattering cross section of samples, resulting in a long acquisition time, and also from interfering fluorescent background. Therefore, the potential of non-linear Raman techniques such as CARS [2] and SRS [3] for the analysis of microplastics is investigated with great interest. Both techniques show some drawbacks for example in terms of a non-resonant background or incompleteness of spectra, respectively.

Here, Raman imaging of microplastics by femtosecond stimulated Raman Microscopy (FSRM) will be reported for the first time. FSRM [4,5] yields complete Raman spectra with minimal acquisition times as short as 0.1 ms without distortions by fluorescence or a non-resonant background. In our proof-of-concept experiment, a microplastic particle in a commercial facial scrub is imaged (Figure 1). The image reveals the irregular shape of the particle which presumably enhances its exfoliating properties.

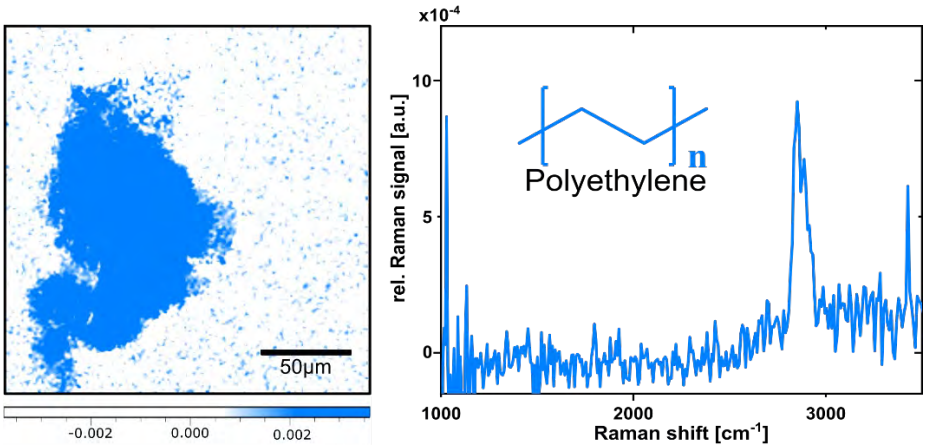

Figure 1: A chemical image of a polyethylene particle in a commercial facial scrub. In the Raman image at the left the FSRM signal at 2849 cm^{-1} is color-coded. Acquisition of the 200x200 µm image with a spatial resolution of 0.5 µm takes approximately 49 minutes. On the right, a single Raman spectrum from the chemical map is shown.

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Selective Enhancement of Peptide Raman Signals explained by Synchrotron Resonance Raman Experiments and Simulations

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Amide spectral signals dominate the spectroscopic response of proteins solvated in their natural environment, i.e. aqueous solution. Since N-acetylglycine-N-methylamide and N-acetylalanine-N-methylamide (also called NAGMA and NALMA, respectively) serve as prototypes for proteins,[1] in this work, we investigate their peculiar Ultraviolet Resonance Raman (UVRR) spectroscopic signatures using a combination of state of the art experiments and simulations. UVRR spectra are recorded by tuning Synchrotron Radiation (SR) at several excitation wavelengths and modeled by using a recently developed multiscale protocol based on a polarizable QM/MM approach.[2]

Our results suggest that the selective enhancement of the amides signals is hydrogen bonding-induced because is intimately linked to the effect that water molecules exert on the C=O and N−H, C−N vibrations. We demonstrated that the inclusion of explicit water molecules concentrates the orbitals involved in the charge transfer in the C−N zones, which ultimately leads to the strong UVRR enhancement of vibrations that have large components of C−N stretching, particularly the AII signal. Thus, quantum effects must be present in any modeling of the solute-solvent interactions of RR spectroscopy for such systems. In addition, due to the constant movement of the solute and its surrounding water molecules, a single snapshot (or cluster composed of the solute and some surrounding water molecules) is not representative of the dynamical nature of the system, and can lead to a heavily biased results if taken to be representative for the ensemble, which is more correctly modelled through an explicit average over a large set of structures.[3]

Acknowledgements

This work has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No. 818064).

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Characterisation of the Cyanate Inhibited State of Cytochrome c Oxidase with surface-enhanced resonance Raman spectroscopy (SERRS)

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Heme-copper oxygen reductases are terminal respiratory enzymes, catalyzing the reduction of dioxygen to water and the translocation of protons across the membrane. Oxygen consumption is inhibited by various substances. Here we tested the relatively unknown inhibition of cytochrome c oxidase (CcO) with isocyanate. In contrast to other more common inhibitors like cyanide, inhibition with cyanate was accompanied with the rise of a metal to ligand charge transfer (MLCT) band around 638 nm. Increasing the cyanate concentration furthermore caused selective reduction of heme a. The presence of the CT band allowed for the first time to directly monitor the nature of the ligand via surface-enhanced resonance Raman (SERR) spectroscopy. Analysis of isotope sensitive SERR spectra in comparison with *Density Functional Theory* (DFT) calculations identified not only the cyanate monomer as an inhibiting ligand but suggested also presence of an uretdion ligand formed upon dimerization of two cyanate ions. It is therefore proposed that under high cyanate concentrations the catalytic site of CcO promotes cyanate dimerization. The two excess electrons that are supplied from the uretdion ligand lead to the observed physiologically inverse electron transfer from heme a_3 to heme a.

Kruse, F., Nguyen, A.D., Dragelj, J. *et al.* Characterisation of the Cyanate Inhibited State of Cytochrome c Oxidase. *Sci Rep* **10,** 3863 (2020). https://doi.org/10.1038/s41598-020-60801-0

Ultrafast spectroscopy of oriented single crystals of [2.2]Paracyclophane: Time resolved springing of a molecular "trap"

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Excited state dynamics following UV photo-absorption of [2,2]-paracyclophane, a great model of aromatic transannular π-interactions, are recorded with broadband femtosecond spectroscopy. As predicted from fluorescence studies [1-3], impulsive excitation to S_1 unleashes violent wave packet motions in this extremely strained molecule due to sudden relief of ground state inter-ring repulsion. Recording this response in oriented single crystals of 2PC proves that the resulting excimer-like state strongly absorbs in the mid- visible with components polarized along all three molecular axes. The component polarized perpendicularly to both benzene planes is deeply modulated by ~ 0.5Å of coherent motion along the symmetric breathing mode coordinate (fig.1a). Accounting for up to 50% of the total transient absorption strength, the extensive motion range along this vital coordinate, reflects on the significant reduction of the inter-ring distance and structural rearrangement which accompany the formation of the 2PC excimer, providing spectral potential energy differences and changes in dipole strengths to higher electronic states on the fly. Analysis suggests that absorption is indeed taking place to several final excited states, and that non-Condon effects are playing a central part in this evolution (fig.1b). Remarkably the underlying periodic reorganization produces only faint ripples in absorption polarized in the rings plane. Pump-probe experiments in THF solution corroborate the existence of the unexpected in-plane absorption of the excimer-like S_1 state and exhibit excited state wave packet signatures which prove that solvation breaks the D_{2h} symmetry of room temperature 2PC and stabilizes a structure with mutual twisting of the aromatic rings (Fig. 1c). These new findings present a refined testing ground for improving electronic structure models for the benzene excimer, an object which continues to intrigue new generations of scientists[4-5].

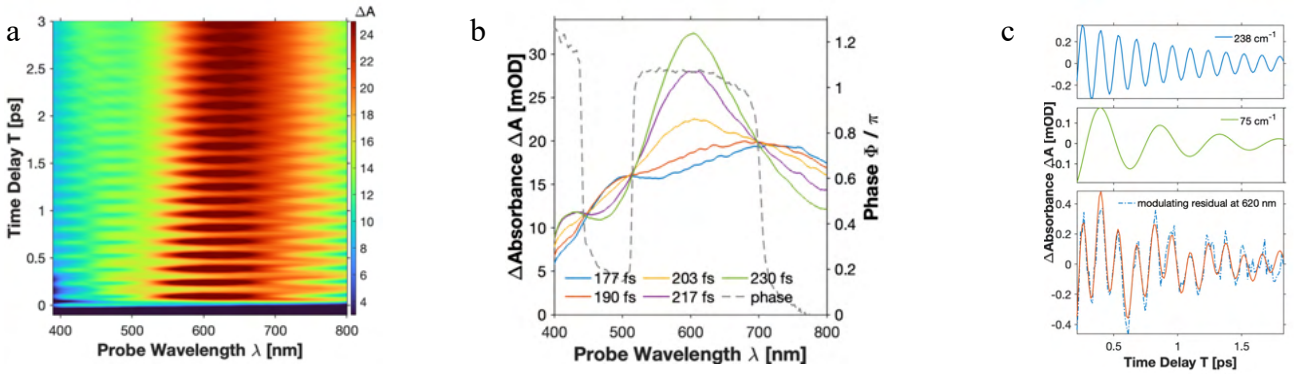

Figure 1 (a) 2D transient absorption map probed along the 2PC inter-ring direction. Deep spectral modulations due to the motion of a coherent wavepacket along the symmetric breathing mode coordinate are observed throuhgout the whole probing range. (b) Selected spectral cuts throughout half a period of the breathing vibration, show that the excimer-like state absorbs to several close excited states. (c)Fourier components of the inter-ring breathing (top) and twisting (middle) modes obtained by fitting the modulating residual (bottom) at 620nm to a series of damped harmonic oscillators..

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Light Sheet Integral Field Raman Microspectroscopy

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Raman scattering provides high molecular specificity of living organisms without labeling or staining. Due to its low cross section and absorption based sample heating, however, traditional imaging approaches such as confocal microscopy fail to acquire fast high quality images. [1]

Current developments based on light sheet illumination avoid unnecessary out of focus excitation, reducing sample heating. A Fourier-transform imaging spectrometer based approach has been demonstrated to be five times faster while providing full hyperspectral information. [2] By choosing a low-noise imaging spectrometer method, further speed improvements can be expected. [3,4]

Here we present a new approach combining light sheet illumination with hyperspectral imaging meeting both optimization criteria: low light load and high signal to noise ratio. [5] Exploiting the astronomical technique integral field spectroscopy [6] we can record 50x50 spectra in parallel at a field of view of 20 µm. The spectral range of our system amounts to the biological fingerprint region from 500 cm^{-1} to 1800 cm^{-1} with a spectral resolution better than 4 cm^{-1} using an excitation wavelength of 578 nm. This system is expected to be 2500 times faster than a comparable confocal one enabling qualitatively new applications in biomedical as well as intracellular clinical research.

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Detection of Diseases Using SERS: Coupling of Magnetic Concentration and Principal Component Analysis for Zika Virus Detection

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Surface-enhanced Raman scattering (SERS) is a powerful spectroscopic technique being able to detect traces of closely adsorbed molecules on plasmonic nanostructures. The association of those nanostructures with biomolecules, such as antibodies with specific affinity for pathogenic agents, is able to provide highly sensitive and selective biomarkers for SERS detection [1]. Considering gold nanoparticles (AuNPs) as an alternative to detect Zika virus by SERS, AuNPs coated with 3 mercaptopropionic acid were used to interact with Zika antibodies – NS1 protein. In order to optimize the nanosensor system, different concentrations of gold nanoparticles in solution, ratios between mols of gold and mols of the capping agent and concentrations of antibodies in solution were systematically investigated UV–vis spectroscopy. The optimal condition involves the coating of nanoparticles with the lower rates of the coating agent, a low concentration of gold nanoparticles as well of NS1 antibodies. In this study, we were able to combine SERS utilizing optimized biofunctionalized gold nanoparticles and the physical preconcentration by superparamagnetic iron nanoparticles (SPIONs) to characterize and detect NS1 Zika virus protein. It was possible to characterize Zika virus antibody and NS1 protein utilizing SERS in concentration of 1 μg L^{-1} (about 10 pM), opening new possibilities for the use of this method to develop a sensor of Zika virus using Raman spectroscopy, directly, label-free. Principal Component Analysis (PCA) of SERS data was successfully used as a chemometric tool to differentiate spectra patters and distinguish samples (Figure 1).

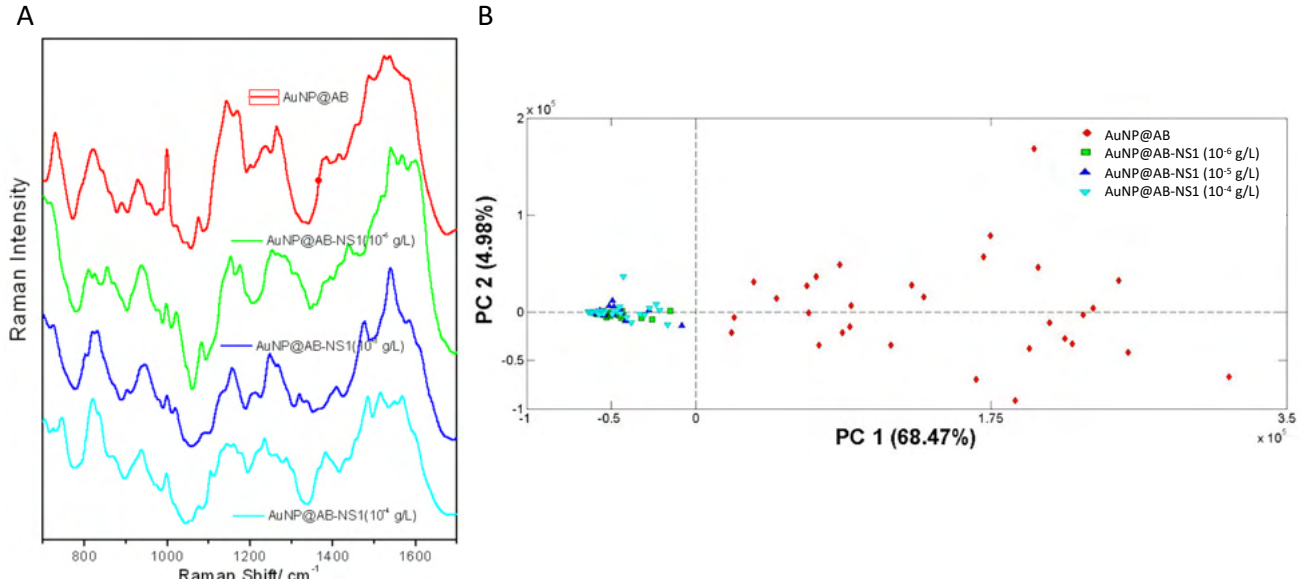

Figure 1: (A) SERS spectra of antibody bound as gold nanoparticles after aggregation with NaCl (aq) (red), and aggregation with NS1 at concentration 10^{-6} g/L (green), 10^{-5} g/L (blue) and 10^{-4} g/L (cyano) and (B) PCA analyses.

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Spatial Separation of Plasmonic Hot Electron Generation and a Hydrodehalogenation Reaction Center using a DNA Wire

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One of the most interesting and promising uses of plasmonic nanoparticles is the possibility to induce chemical reactions at their interface giving rise to the emerging field of plasmon chemistry. The reactions are driven by different processes occurring at the interface between the plasmonic nanoparticle and the molecules, such as the generation of hot-carriers and also the thermalization of these carriers into heat. Even though it is very difficult to distinguish the contribution of the two mechanisms, both are suggested to affect the reaction pathways. Our group recently has shown that brominated nucleobases can undergo a plasmon induced reduction when adsorbed onto gold or silver nanoparticles, and that the reaction can be tracked using SERS. The hot-electrons generated on the nanoparticles are transferred to the brominated nucleobase which is followed by cleavage of the C-Br bond generating the non-brominated base, in a procedure that only requires one electron and one proton *via* a dissociative electron attachment (DEA) mechanism.[1] Here we study this reaction with the brominated nucleobase incorporated in double stranded DNA. We self-assembled 60 nm AgNPs ensembles to probe hot-electron-induced reaction. The nanoparticle ensemble design allowed us to provide electromagnetic enhancement enough to track the reduction of the brominated nucleotide by SERS in a single-point modification scale. Also, due to the addressability offered by DNA, it was possible to insert the modified base at precise positions, allowing us to check the possibility of transferring hot-electrons through DNA. The reaction was observed using SERS, where both the starting bromoadenosine (8BrdA) and the adenosine (dA) peaks can be observed while carrying the reaction. The decrease in intensity of the peak at ~770 cm^{-1} was used to fit the kinetics of the hydrodehalogenation reaction for all the 8BrdA insertion positions. One possible way to check thermal and electronic energy's role is to perform the plasmon-induced reaction with different incident light intensities.[2] To conclude, here we showcased the use of self-assembled nanoparticle ensembles for plasmon-induced reactions. We demonstrate that DNA can transfer hot electrons far from the nanoparticle surface. So far, the reactions were confined to the nanoparticle's surface, which can be troublesome principally due to possible surface contamination and poisoning. Further understanding of the DNA-nanoparticle interaction at the interface and charge injection into DNA needs to be gained in future experiments. We expect that the study presented here serves to understand plasmon-induced reactions that do not require direct contact with the nanoparticle surface.

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Simplified FM CARS and FM SRS with up to 18-fold contrast improvement

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Coherent Raman microscopy is a powerful tool for in vivo diagnostic imaging due to the chemically-selective and label-free nature of the Raman process. Nevertheless, the CARS process suffers from an intrinsic nonresonant background signal that can even overwhelm the CARS signal and leads to a reduced image contrast [1]. This nonresonant signal does not occur within the SRS process, however effects such as cross-phase modulation and two-photon absorption can distort the SRS signal [2].
We substantially simplified frequency modulation (FM) Raman scattering microscopy by using only one single fast and widely tunable light source [3] to implement this scheme [4-5] for CARS and SRS imaging which allows real-time background subtraction and improved image contrast. The principle of FM CARS and FM SRS uses pump pulses alternating in wavelength in combination with Stokes pulses fixed in wavelength. By pulse-to-pulse wavelength-switching the resonant signal as well as nonresonant contributions were probed alternatingly and lock-in detection allowed for direct subtraction, resulting in live imaging with background corrected signals.

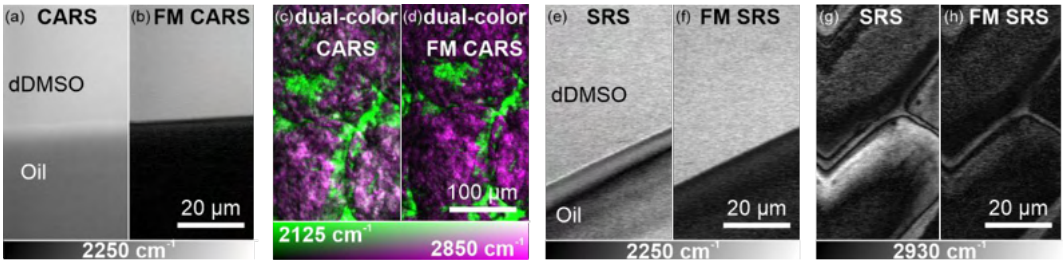

Fig 1.: (a)-(b) Interface of dDMSO and oil, (c)-(d) fat tissue soaked with dDMSO, (e)-(f) interface of dDMSO and cinnamon oil, (g)-(h) red onion cells. All images are 256x512 pixels and were acquired with a pixel dwell time of 10µs.

The contrast improvement of a factor of 18 by using FM CARS is demonstrated in Fig. 1(a)-(b) for a sample consisting of rapeseed oil and dDMSO. FM CARS was also possible in combination with dual-color CARS due to the fast wavelength-switching mechanism of the light source as shown in Fig. 1(c)-(d) for fat tissue soaked with dDMSO. The principle of FM could also successfully be applied to SRS imaging, which is shown in Fig. 1(e)-(f) for an interface of dDMSO and cinnamon oil with an improved contrast by a factor of 3.6. Additionally, the nonresonant signal, arising from pigments in red onion cells, could be suppressed as demonstrated in Fig. 1(g)-(h).
In conclusion, the implemented FM scheme with pulse-to-pulse wavelength-switching of a single compact light source allows for significant background reduction for real-time CARS and SRS imaging, leading to higher contrast values and improved image quality also for out-of-the-lab applications.

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DO-SRS Multiplex Super Resolution Metabolic Imaging in Aging and Diseases

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Abstract

Understanding the dynamics of metabolism in a multicellular organism is essential to unraveling the mechanistic basis of many biological processes in healthy and diseased conditions. There has been an urgent need of high spatial resolution, non-invasive imaging techniques for imaging metabolism of various biomolecules in cells and tissues. Deuterium oxide probed Stimulated Raman scattering (DO-SRS) can generate chemical specific metabolic imaging with high resolution, deep penetration of depth, multiplex, chemical selectivity, 3D volumetric and quantitative capability. In the present work, we developed a new approach that combines super resolution A-SUPPOSe enhanced **DO-SRS** imaging and custom designed clustering methods to visualize mutiplex metabolic activities and subcellular distribution of newly synthesized macromolecules in living organisms. Within the broad vibrational spectra, we can image more than 30 different molecules including lipids subtypes-, protein-, and DNA-specific Raman profiles and develop hyperspectral detection methods to obtain multiplex imaging of various biomolecules. This technology platform is non-invasive, universal applicable, and it can be adapted into a broad range of biological studies such as neurodegeneration, aging, homeostasis, tumor progression, etc. We applied this method to study the diet regulated metabolic dynamics in animals during aging processes, the quantitative lipid and protein turnover rate, the intra-cellular metabolic heterogeneity.

Short abstract:

We developed a new super resolution multiplex optical metabolic imaging platform with A-SUPPOSe enhanced DO-SRS microscopy to detect metabolic dynamics in cells and tissue for studying aging and diseases.

Ultra-narrow lines in Raman spectra of CdTe quantum wells due to effective acoustic phonon selection by in-plane wave vector [1]

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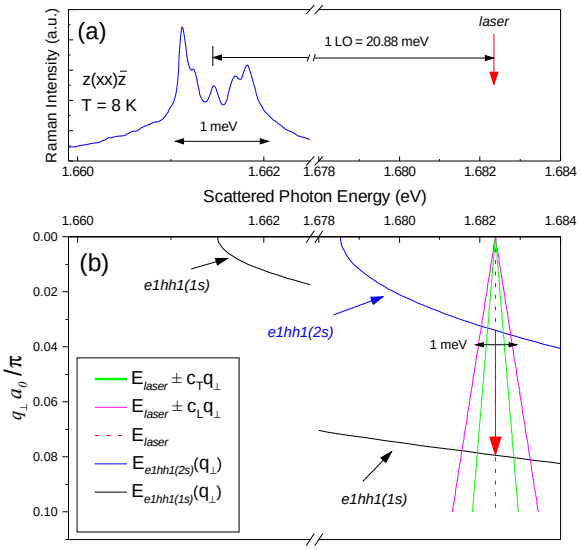
An unusual fine spectrum of very narrow lines (< 1 meV overall) is observed in resonance Raman scattering from a CdTe quantum well (QW) under normal light incidence, when the laser excitation is about one LO phonon energy higher than the QW exciton ground state. The visibility of this fine spectrum is improved by sample doping which reduces the quantum yield of the exciton photoluminescence. The appearance of the four sideway lines of the fine spectrum is explained by the Raman scattering on the combination of LO- and LA-(TA-) phonons with the $e1hh1(2s)$ exciton serving as an intermediate state. The central line is due to elastic scattering of the $2s$-exciton to the hot $1s$-exciton state on a static random potential of the heterostructure followed by emission of a LO-phonon.



(a) Emission spectrum from a 18 ML QW under laser excitation exactly 1 LO phonon above the $1s$-exciton resonance. (b) Calculated energy dispersion curves for $1s$ (black line) and $2s$ (blue line) excitons as functions of the dimensionless in-plane wave vector $q_\perp a_0/\pi$. By magenta (green) lines are shown the dependences $E_{laser} \pm c_S\, q_\perp$ with $S = L(T)$ corresponding to the dispersion of LA(TA) phonons. Intersections of the green and magenta lines with the blue one correspond to the condition of the intermediate resonance leading to the appearance of the side lines on the spectrum in panel (a). Red arrow indicates elastic scattering of the $2s$-exciton to the hot $1s$-exciton state on a static random potential of the heterointerface responsible for the central line on the spectrum in panel (a).

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Gut microbiota and adipose tissue: another pieces in the obesity puzzle

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The gut microbiota is considered as an organ equivalent of the human body and its dysbiosis can lead to autoimmune diseases, obesity and cardiometabolic conditions [1]. The gut microbiota uses non-digestible carbohydrates (e.g., fiber) to produce short-chain fatty acids (SCFAs: acetic, propionic and butyric acid) and through these metabolites plays a regulatory role in metabolism and affects the function of the liver, pancreas, and adipose tissue, and also may prevent weight gain [1]. Therefore, stimulation of the gut microbiota may protect against the development of obesity, related to excessive lipid accumulation and the chronic inflammatory state of the adipose tissue [2]. Feeding a high-fat diet (HFD), even for a short time, contributes to adipocyte hypertrophy as previously observed for one of the most important types of the adipose tissue, i.e. perivascular adipose tissue (PVAT) [3].

The aim of the presented research was to investigate the influence of SCFA on PVAT in a murine model of obesity. 6-week-old mice were fed for 4 weeks a HFD supplemented with sodium butyrate (SCFA, a direct way) or β-glucan (fiber, an indirect way). Chemical changes were tracked using spatially offset Raman spectroscopy (*in vivo*), Raman and fluorescence microscopies (**Fig. 1),** and fiber optic Raman spectroscopy (*ex vivo*). Raman measurements showed a decrease in the degree of lipid unsaturation in all adipose tissue samples due to HFD. However, the effect of SCFA and fiber was different for white and brown adipose tissue. SCFA/fiber supplementation reduced this effect in the white adipose tissue, and reversed in the brown adipose tissue. Both alterations were even more evident for PVAT. Moreover, animals fed a HFD enriched in SCFA/fiber did not result in excess growth of adipocytes nor body weight gain. The obtained results were correlated with Next Generation Sequencing (NGS) confirming the microbiota composition. Overall, the results clearly demonstrate that the gut microbiota and adipose tissue are key players involved in the pathogenesis of obesity.

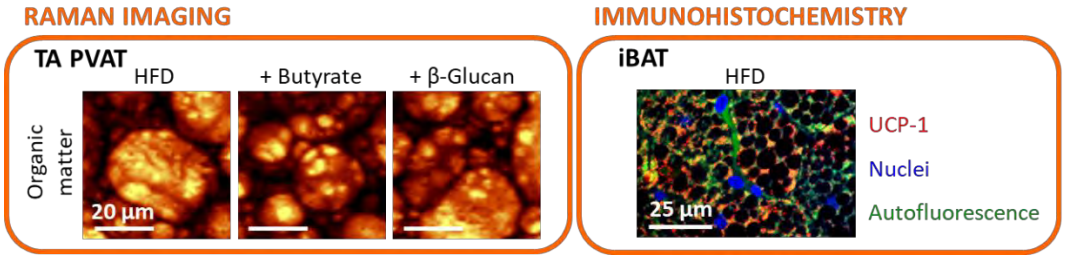

Figure 1: Raman and fluorescence images of adipose tissue fragments. TA PVAT – thoracic aortic perivascular adipose tissue, iBAT – interscapular brown adipose tissue, UCP-1 – uncoupling protein 1.

ACKNOWLEDGMENTS

The work was supported by the National Science Centre, Poland: OPUS17 (no. 2019/33/B/ST4/00878 to A. Kaczor) and PRELUDIUM20 (no. 2021/41/N/ST4/03701 to Z. Majka).

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Graphene Oxide – Silver Nanoparticles Composites for SERS Detection of 4-aminothiophenol

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Ever since the discovery of SERS phenomenon new materials are extensively researched in order to find new SERS-active substrates. Composites of graphene derivatives and noble metal nanoparticles are particularly viable in this case due to their synergy in enhancing Raman spectra [1]. Both electromagnetic and chemical enhancement play important role in improving SERS signal, although the latter one is still a subject of studies because of its complex mechanism.

In metal nanoparticles – graphene composites the main enhancement originates from the surface plasmon resonance that occurs during the interaction between nanoparticles and laser. The role of graphene and its derivatives (graphene oxide, reduced graphene oxide) in SERS spectroscopy is more layered as it not only provides signal increase from the measured compound but also improves the adsorption of both analyte and nanoparticles, suppresses the fluorescence background and enhances the overall stability of the composite containing nanoparticles [2].

In this work we present a simple method of synthesis various composites containing silver nanoparticles (AgNPs) and reduced graphene oxide (rGO). Ascorbic acid was used as a mild reducing agent and different types of graphene oxides were applied during the synthesis. Composites were also modified with diluted ammonia and potassium hydroxide solution to test the influence of base conditions on SERS enhancement. All materials were extensively analyzed using microscopic and spectroscopic techniques. We used 4-aminothiophenol as a model substance to study the ability of obtained substrates to enhance Raman spectra. Our results show that the surface composition and UV-Vis characteristic of composites is correlated with the effectiveness of SERS enhancement [3].

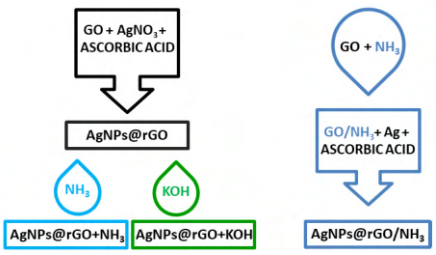

Figure 1: An overview of studied composites.

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Quantitative analysis of the hyperfine structure of binary sodium silicate glasses and their melts by Raman spectroscopy jointly with NMR

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ABSTRACT: Both experimental Nuclear Magnetic Resonance (NMR) and Raman spectroscopic techniques are the most promising quantitative analysis methods applied on various species in the amorphous states of inorganics (including ambient glasses and melts in high temperature). Of course, the adequate and effective quantitative methods should be explored and developed. In this work, quantitative analysis of the cluster structure of binary sodium silicate glasses with different content of Na_2O was carried out by deconvolution of their NMR and Raman spectra with consideration of the concept of the hyperfine structure. With the help of quantum chemistry *ab initio* calculation method, Raman scattering cross section (RSCS) function could be deduced while NMR having the advantage of the simple and direct correlation between intensity and concentration. Although the delicate deconvolution for Raman ($Q^{jklm}_{i(m_1h,m_2q,m_3t)}$) and NMR ($Q_i^{jklm}$) spectra is a little bit different due to NMR is insensitive to the bonding angle of bridging oxygen and Raman wavenumber and intensity are dependent on various ring configurations, both the reduced amount of the primary silicon-oxygen tetrahedron, Q_i species agreed well with each other. And for their melts in high temperature, Raman spectra can also be quantitatively analyzed based on the hyperfine structure by using the same cross section function obtained above for ambient binary silicate glasses.

Keywords Glass, Melt, Silicate, High temperature Raman spectroscopy, Quantitative analysis

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Surported by the National Natural Science Foundation of China (grant number 21773152), Shanghai Committee of Science and Technology Fund, China (grant number 12520709200), National 111 Project (the Programme of Introducing Talents of Discipline to Universities) (grant number D17002), Open Project of State Key Laboratory of Advanced Special Steel, Shanghai University, China (grant numbers SKLASS2015-01, SKLASS2017-02), Special Fund Project of Shanghai Municipality for Science and Technology Development (YDZX20173100001316).

Electrospun Membrane doped with Gold Nanorods for Surface-enhanced Raman Spectroscopy

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Surface-enhanced Raman Spectroscopy (SERS) is a highly sensitive detection that provides abundant information on low concentration analytes from various researching areas. Based on localized surface plasmon resonance, metal nanostructures including gold, silver and copper have been investigated as SERS substrate during recent decades [1]. There has been increasing more attention of exploring good performance, homogenous, repeatable SERS substrates.

Here, we show that electrospinning, which is an inexpensive technique to fabricate large-scale, self-standing and repeatable membranes, can be effectively used for producing SERS substrates. Nanoparticles and nanorods are added to the feed electrospinning solution to collect functionalized polymer fibrous mats. We report stable electrospun membranes as SERS substrate using gold nanorods (AuNRs) [2] and poly(vinyl alcohol). Particularly, a post-processing crosslinking step using glutaraldehyde under acetone environment was carried out to the electrospun membrane [3]. It allows for using the membrane in any liquid environment, including water, which is of interest both for sensing of contaminant in wastewater, as well as for biosensing. This crosslinked AuNRs/PVA membrane has demonstrated excellent performance as SERS substrate for low concentration 10^{-6} M Rhodamine 6G (Rh6G) aqueous solution. This post-processing for fabricating SERS substrate is the first time reported and proved through Raman imaging of excellent stability and outstanding performance. Finally, SERS tests have been applied to several analytes, and the application of AuNRs/PVA membrane is broadened by removing the detected analyte by rinsing. Therefore, this crosslinked AuNRs/PVA membrane is re-usable.

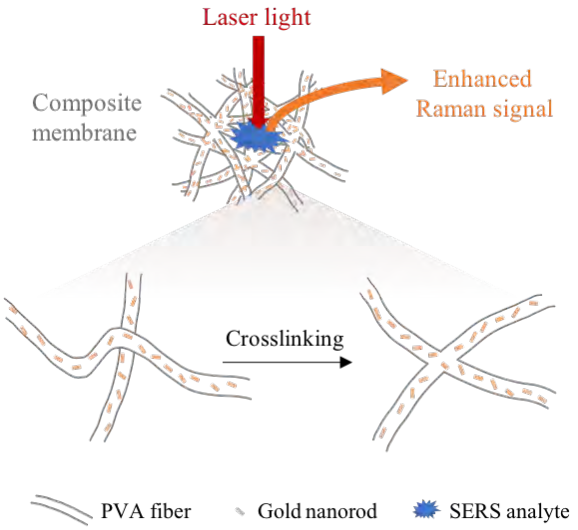

Figure 1: The schematic illustration of a SERS substrate prepared by the electrospun AuNRs/PVA membrane after a post-processing crosslinking step.

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Electronic Raman scattering in layered $NiPS_3$

Xi Ling, Boston University

Raman scattering is often used to probe the vibrations in molecules and materials. In some particular cases, Raman scattering can occur due to the scattering with electrons, thus it can be used to probe the electronic properties of materials. In this work, I will present the electronic Raman scattering phenomenon we observed in layered antiferromagnetic $NiPS_3$. The origin of this phenomenon is investigated and attributed to the unique electronic structure of the material. This study provides insights on the electronic Raman scattering which are not often observed in many materials, and can serve as a powerful tool to understand the physical behaviors of materials.

XXVII International Conference on Raman Spectroscopy

The Biochemical Profile of Breast Cancer - Diagnosing with Raman Spectroscopy

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Abstract:

Breast cancer is the most common type of cancer in women worldwide. Novel diagnostic approaches for a faster and more objective diagnosis are urgent. A promising approach is the analysis based on biochemical differences which are potentially much more specific than morphological characteristics [1-3].

The analysis of three sets of human breast tissue samples collected through breast conserving surgery and classified by control histopathology as containing (1) invasive carcinoma, (2) *in situ* carcinoma and (3) surrounding normal mammary tissue were studied through Raman microspectroscopy. Two contiguous sections were prepared: one with a 10 μm thickness, for probing by Raman spectroscopy aiming at the detection and identification of cancer-related molecular alterations (biomarkers), as well as their spatial distribution; and the other for histopathological analysis and respective correlation.

The tissue sections were chemically dewaxed prior to spectroscopic analysis, according to established protocols [4]. However, as previously concluded, chemical dewaxing was not as efficient as required [5] and digital dewaxing was also necessary [6]. Both the fingerprint and the high wavenumber regions of the Raman spectra were analysed. Data was statistically analysed by Principal Component Analysis (PCA), allowing to obtain a good discrimination between the three sets of samples. The vibrational modes playing a more significant role in the discrimination of the cancer specimens were those ascribed to proteins (mainly collagen), DNA/RNA and lipid CH stretching bands.

Spectral histopathology has real-time molecular imaging capability, high sensitivity and specificity, it is non-invasive (no need for dyes or external probes) providing extremely accurate chemical information, even for highly heterogeneous biospecimens, and allows rapid *in situ* inspection of human tissues, which also renders them tools of excellence for the evaluation of surgical margins which are decisive on the more conservative surgical approach in the context of aesthetical and survival of the patients.

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Tip-Enhanced Raman Spectroscopic Imaging of Phenyl and Benzyne on Cu(100)

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Benzene adsorbed on the Cu(100) surface undergoes dehydrogenation upon exposure to energetic electrons or photons. Despite the low electron (<10 meV) and photon energy (1.96 eV) applied, benzene molecules under the silver scanning tunneling microscope (STM) tip lose hydrogen atoms successively forming phenyl and benzyne single molecules. The exposed carbon chemically binds to the substrate and makes the molecule stand upright. The tip-enhanced Raman spectroscopic (TERS) imaging of those chemisorbed benzene derivatives offers mode-specific vibrational images as shown in Fig. 1. The inhomogeneous field distribution excites individual atoms in the three-dimensional molecule at different depths of focus, posing a challenge to simulating vibrational images. A minimal model using atomically partitioned polarizabilities and a point charge field distribution is employed to simulate the experimental images. The findings from the attempt to match the simulation to experiment will be discussed.

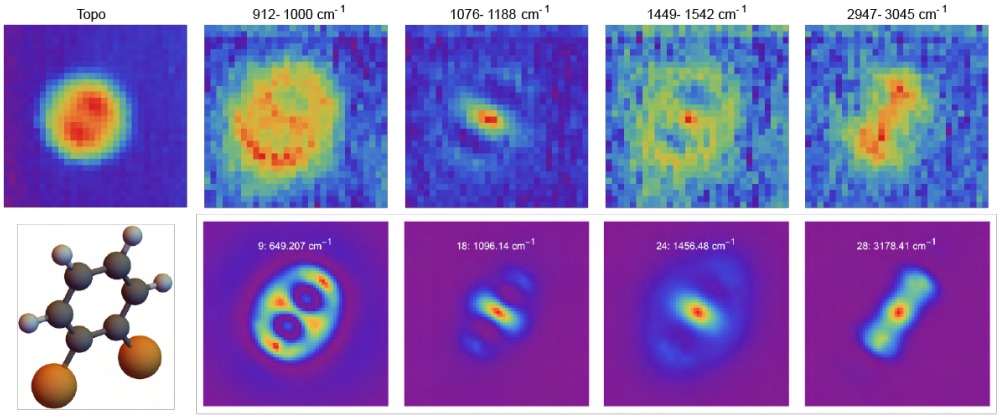

Figure 1: TERS imaging of benzyne on Cu(100). Top: experimental STM topography and Raman images. Bottom: molecular model and simulated Raman images.

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Spontaneous and Stimulated Raman Scattering of amyloid-beta plaques in post-mortem human AD brain tissue

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The accumulation of amyloid-beta (Aβ), commonly known as plaques, is a biological hallmark in the post-mortem Alzheimer's disease (AD) diagnosis. The Aβ deposit is characterized by its accumulation of protein misfoldings (β-sheets) cleaved from the amyloid precursor protein (APP). Therefore, it is expected to detect a Raman shift in the β-sheet region of 1660 to 1670 cm^{-1} using conventional and Stimulated Raman spectroscopy.

Post mortem, freshly frozen AD brain tissue of the CA1 region was cut into a 20 µm section and mounted on CaF_2 microscope slides. Afterward, auto-fluorescent colour images of the section were taken, using a fluorescence microscope with an excitation wavelength of 470 nm. Spectral Raman maps were obtained using a Renishaw inVia spectrometer with a 532 and 785 nm excitation source, usually covering an area of around 0.25 mm^2. Subsequently, stimulated Raman images across the protein peak (1630 to 1690 cm^{-1} in 3 cm^{-1} steps) of the same area were taken using an in-house built SRS system [1]. Hereafter, to confirm and identify Aβ deposits, the tissue was stained with Thioflavin-S. It is worth to emphasize that all measurements were taken from the same tissue section.

Preliminary results show a clear correlation between lipofuscin granule deposits (yellow emission) found in all the auto-fluorescence images and their (non-resonant) Raman maps. Furthermore, the auto-fluorescence images, taken with the 470 nm source, reveal Aβ plaque locations by its bright green emission. When looking at the Raman spectra of Aβ accumulations using 532 nm excitation, peaks at ~1152 cm^{-1} and at ~1522 cm^{-1} could be found, revealing the presence of carotenoids, which are known as anti-inflammatory agents, close or within Aβ-accumulations. Even relatively low levels of carotenoids can give a significant Raman signal because of (pre-) resonance enhancement at 532 nm excitation. These peaks were not found when using the 785 nm source. In none of the conventional Raman measurements, a clear protein peak shift could be found.

In conclusion, the detection of carotenoids in AD tissue, by means of green fluorescence and confirmed by resonance Raman, should be considered as an indicator of plaque locations.

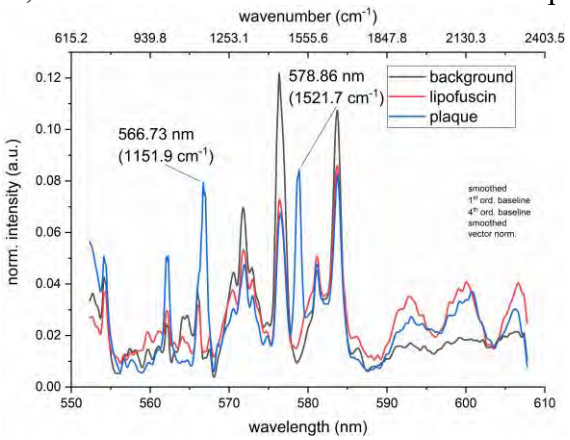

Figure 1: Averaged Raman spectra: background (black), lipofuscin spots (red) and plaques (blue)

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XXVII International Conference on Raman Spectroscopy
Theoretical Design, Synthesis and Characterization of Novel Thermochromic Materials

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In this communication, it will be reported a structural study of a chemical system exhibiting color polymorphism. It includes the design of new chromophore molecules, the prediction of the electronic and vibrational properties by both single molecule and periodic DFT calculations, their synthesis, and the polymorph screening aiming to find diverse crystallographic forms displaying different colors.
Polymorphism refers to the ability of a certain compound to exist in different crystallographic structures, resulting from different packing arrangements of its molecules in the crystal structure, which can present different physical and chemical properties. In the context of this study, the most relevant property that differ in the different polymorphs is the color. [1]
Color polymorphism is a relatively rare phenomenon. One of the few chemical systems presenting this property is the newly synthetized 2,4,6-trinitro-N-(m-tolyl)aniline. So far, we have obtained three different crystalline structures of this molecule, being yellow, light-orange and red. The different colors are attributed to intramolecular electronic effects due to different levels of delocalization of the secondary amino nitrogen lone pair electrons and nitro groups in the aromatic ring in the two polymorphs. [2]
Raman spectroscopy and X-ray crystallography were used to characterize the vibrational response of the different polymorphic forms and their crystalline structures, respectively. The new polymorphs were obtained after recrystallization of the compound from different solvents. In all polymorphs, a strong N-H···O intramolecular bond between the NH group and one of the three NO_2 substituents is found, but the polymorphs differ in the intermolecular H-bonding pattern, which affect the electronic delocalization of the amino and nitro substituents to the aromatic rings. Interestingly, in the light-orange polymorph the H atom of the NH group is shared in a bifurcated H bond.

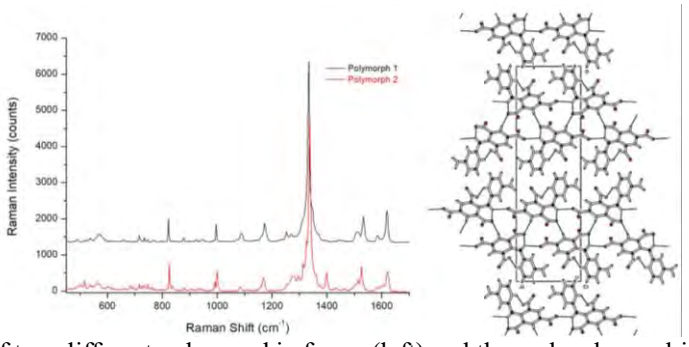

Figure 1: Raman spectra of two different polymorphic forms (left) and the molecular packing of polymorph I (right).

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Multimodal coherent Raman and multiphoton nonlinear optical microscopy to monitor the risk of cancer relapse in human tumors after therapy

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Recent advancements in cancer research revealed that radio- and chemo-therapy can induce tumor cell senescence rather than cell death, which is one of the major causes of cancer relapse [1]. The development of non-invasive, accurate and clinically translatable tools to monitor the presence of such induced senescent cells is thus crucial to evaluate the effectiveness of anticancer treatments and prevent tumor recurrence. Here, combining different coherent Raman and multi-photon ultrafast processes, we demonstrate label-free multimodal nonlinear optical (NLO) microscopy as an effective technique to monitor over time therapy-induced senescent cells, quantitatively and non-invasively. We home-built a multimodal microscope with off-the-shelf components featuring seven different NLO modalities, along with linear transmission light: forward-detected Stimulated Raman Scattering (SRS), forward and epi-detected Coherent Anti-Stokes Raman Scattering (CARS and E-CARS), Two-Photon Excited Fluorescence (TPEF and E-TPEF) and Second-Harmonic Generation (SHG and E-SHG). The laser source delivers 780 nm pump pulses and 950-1050 nm tunable Stokes pulses with 1 picosecond duration, matching the CH-stretching region of the Raman vibrational spectrum (2800-3100 cm^{-1}) [2]. The combination of co-registered microscopy techniques on both deferoxamine (DFO)-induced senescent cells and untreated controls was crucial to unveil label-free quantitative senescence hallmarks (i.e., lipid droplets overexpression and mitochondrial network disaggregation, with p-value < 0,001, along with nuclei irregular spreading), corroborated thoroughly comparing different modalities (Fig. 1A) and monitored over 7 days of therapy (Fig. 1B). These statistically significant results agree with qualitative senescence markers previously reported via standard invasive and/or destructive techniques [3]. We consider that our findings will be extremely valuable resources for the future of clinical anticancer protocols and non-invasive biomedical diagnostics.

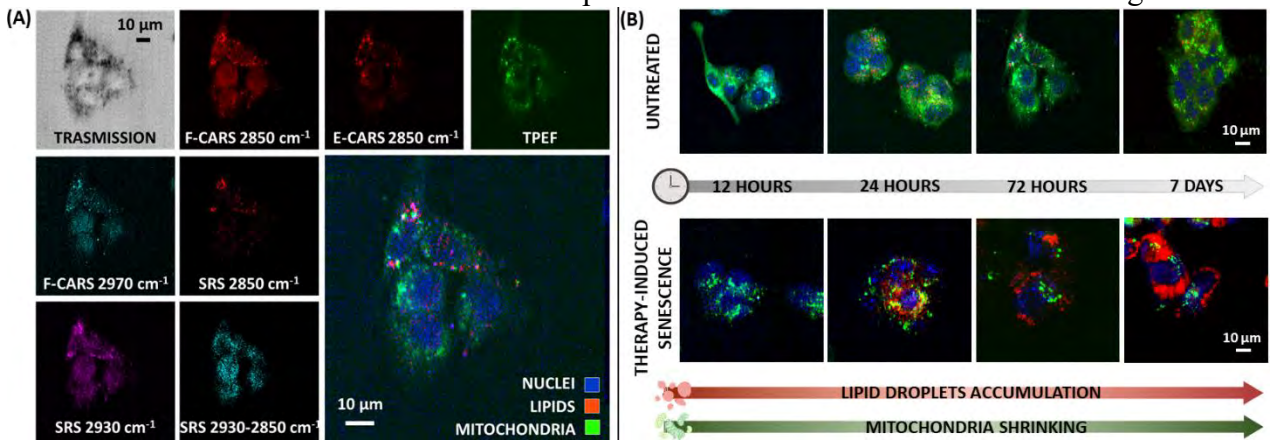

Figure 1. **(A)** Multimodal image of human cancer cells. Pixels: 350 nm dimension, 1.5 ms dwell time. **(B)** Time evolution of senescence hallmarks in label-free therapy-induced senescent cancer cells, versus untreated controls.

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Tip-enhanced Raman Spectroscopy (TERS) of core-shell block copolymer micelles with a cross-linked core

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Block copolymers have received high attention as promising building blocks for the formulation of drug nanocarriers with a core-shell structure [1]. Therefore understanding the property-function relation of such systems is crucial for their design and improvement. In order to address specific properties affecting their functionality, methods are required which provide a high chemical specificity paired with nanoscale resolution. TERS provides these capabilities, and thus, can be applied to address morphological and chemical changes with nanometer precision. However, due to the limited depth information of non-resonant TERS, in principle such investigations may only address the shell properties and functions related to the bio-interface. Recently, we bypassed this limitation by using resin embedded and sliced PS nanoparticles. [2]

Here we investigate micelles formulated from the block copolymer PEO-(t-BGE-co-FGE) with cross-linked core. Access to the internal structure of these core-shell structures is achieved by freeze fracturing of the micelles. The TERS investigations can clearly discriminate the chemical compounds of the shell (PEO) and the core (*bis*-malemide, fufuryl groups and Diels Alder product) (s.f. Figure 1). In particular, the TERS investigations reveal distinct differences of the inner core and the core-shell interface, where the crosslinking efficiency breaks down. The presented study directly demonstrates the high capability of TERS to identify local morphology changes on the nanometer scale.

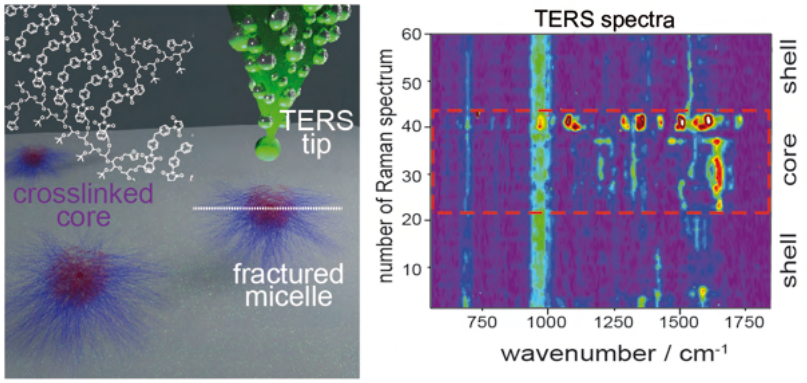

Figure 1: TERS of core cross-linked block copolymer micelles. (A) Scheme of the core-shell structure of a typical block copolymer micelle. (B) TERS waterfall acquired across the micelle diameter.

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Raman Spectroscopy for Blue Bioeconomy

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Blue bioeconomy relies on transforming waste materials of aquatic origin in added value by-products for sustainable environment and society. Current status, local occurrence, properties, diversity and knowledge-based exploitation of biogenic materials or aquatic biomass within the blue bioeconomy concept is very different among EU countries [1, 2].

Here, relying of Raman spectroscopy techniques we demonstrate the wide applicability and usefulness of such approach to track molecular composition and structure from raw, natural waste to new, valuable products. Biogenic materials of aquatic origin mainly resulting from seafood industry focussed on bivalve, molluscs, crustaceans, show intriguing molecular properties when a single Raman technique is applied to characterise their molecular composition. We show how multiple laser lines and different Raman techniques could reveal their complex structure-properties relationship [3]. Furthermore, any industrial processing step applied to biogenic waste could turn or destroy their composition, structure and molecular properties. An experimental train relying on Raman techniques is presented, to propose a new class of bio-stimulants, as new by-products from two ingredients, generally considered as marine waste: biogenic carbonates and seagrass. Moreover, the evaluation of the new bio-stimulants for growing common vegetables such as *Lattuca Sativa* in lab-controlled conditions is described in Raman spectroscopy terms.

Thus, Raman techniques demonstrate powerful translational science tools for blue bioeconomy and circular economy goal.

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Detection of induced chirality by surface-enhanced Raman optical activity

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Sensitive detection of chirality is needed both in enantioselective synthesis and biomolecular applications. One suitable method is the spectroscopy of Raman optical activity (ROA). It was developed into many forms and has been applied to various chiral systems. A big drawback of ROA spectroscopy is the weakness of the signal, e.g., the ROA/Raman intensity ratio, known as circular intensity difference (CID). Combining the surface enhanced Raman scattering (SERS) with ROA into surface enhanced ROA (SEROA) spectroscopy has been a dream of spectroscopists and physical chemists for many years. However, the lack of reproducible experimental procedures has restricted its use for investigations of chiral molecules.

We report a more reproducible method and verify that it is able to detect several chiral enantiomers. We explore molecular SEROA detection through induced chirality of linker molecules attached to a silver colloid. Chiral acids could be detected in concentrations of about 10 μM. We explain the mechanism by binding and self-assembly of the linkers into chiral aggregates on the silver surface. Following the 'sergeants-and-soldiers' principle, the chirality is determined by the relatively minor acidic component. We could detect strong ROA, with a CID up to 10^{-3}, for all linkers and analytes ('chiral modifiers') investigated. The signal could be verified by 'mirror image' SEROA spectra of the enantiomers, measurements on many combinations of the linkers and modifiers, and by reproducing the experiment in two different laboratories. The results were also interpreted on the basis of *ab initio* computations.

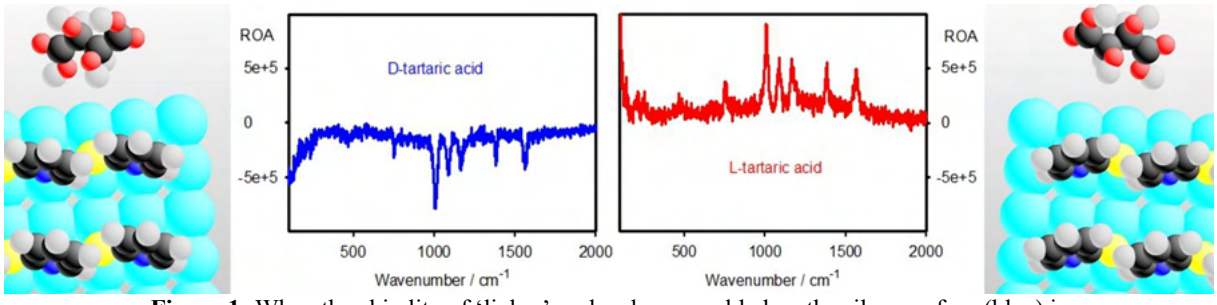

Figure 1: When the chirality of 'linker' molecules assembled on the silver surface (blue) is controlled by chiral molecules (top) in the solution, SEROA spectra of the linker are detected.

Such detection of chiral biomolecules may be useful when other methods, such as electronic circular dichroism, are not sensitive enough. The amplification of chirality through asymmetry induction, resonance and surface effects appears as a promising means for future biomolecular detection and analyses. In future, variations of the chemical structure of the linker or other conditions are needed to provide a more specific signal, allowing one to better discriminate among the optically active molecules.

Decreasing volume, Increasing Impact: A History of Stimulated Raman Scattering Imaging

Richard C. Prince & Eric O. Potma

Stimulated Raman scattering imaging has seen tremendous growth over the last twenty years. Much of that work has focused on demonstrating the utility of SRS to the field of lipid metabolism where it has seen great success. However, it has not managed to get into the hands of as many users as might be desired. SRS is often criticized for a lack of specificity. Here we trace the history of SRS from its first detection to its most recent applications at the bedside and the bench-side. By examining the many use cases demonstrated over the years, we show that SRS is a technique primed for wide adoption in a multitude of fields.

Renewable Hybrid Plasmonic Materials as Platforms for Chemical Reactions

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Plasmonic nanoparticles are extremely promising photocatalysts. They absorb visible light with absorption coefficients that are several orders of magnitude larger than organic dyes. The specific absorption can be tuned by choice of material and the nanoparticle shape and aggregation state. By excitation of the surface plasmon resonance, the photoenergy can be converted into chemical energy through charge transfer or by heat generated around the nanoparticle. It is known that the light may induce a degradation reaction of bromoadenine into adenine, the reduction of nitrothiophenol[1], and the degradation of a series of halogenated thiophenols in the presence of gold and silver nanoparticles.

Looking for future applications, recycling, and upscaling those chemical reactions, and others, it was designed a membrane made of nanocellulose with embebed silver nanoparticles resulting in a renewable hybrid plasmonic material. Nanocellulose, the generic name for cellulose nanoparticles, is a biobased material that originated mainly from different lignocellulosic sources; it has broad applicability since it is possible to tune its chemical and mechanical properties, change its hydrophilicity, add other functional groups, disperse in polymers, ceramics, and others. [2]

Therefore, it was developed one sustainable plasmonic material based on silver nanoparticles dispersed on the surface of nanocellulose membrane similar to Souza et al. [3] that allowed applications in Plasmonically induced chemical reactions. Later, those materials were applied on the degradation reaction of methylene blue and nitrothiophenol. All the experiments were performed using Raman spectroscopy to monitor in real-time the success and possible degradation reaction mechanism. Our results indicate the possibility of reusing the plasmonic membranes several times, increase of their surface area for the degradation reaction and also it will favor the scale up reaction. The degradation reaction certainly will allow the application of these platforms for different classes of molecules that are interesting for the society, like emerging pollutants with severe concerns due to their harmful behavior to avoid new environmental and human problems. This study It will enable another plasmonic applications such as detection, catalysis and enable others chemical reactions.

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Multiplexed Spatial Profiling of Cancer Enabled by SERS Nanoparticles

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Precision medicine and drug discovery seek for advancements in fast and accurate multiplexed imaging with subcellular resolution, which to-date is a task of upmost complexity. Currently, spatial proteomic imaging techniques are limited to either utilizing destructive methods or multiplexing no more than 5 – 6 biomarkers in a single imaging acquisition.

The outstanding sensitivity and unprecedented multiplexing capabilities of surface-enhanced Raman spectroscopy (SERS) makes it a powerful tool for biomedical imaging applications. Local surface plasmon resonance of gold nanoparticles (AuNPs) makes Raman scattering intensities competitive with traditional fluorescence methods while offering the added benefit of unsurpassed multiplexing capabilities. *De novo* synthesized Raman-labeled gold nanoparticles, or SERS NPs, possess a unique fingerprint which originates from a Raman reporter molecule bound to a metallic surface.[1] SERS NPs can be coated with an inert silica shell that offers easy conjugation with bio-targeting species allowing the nanoparticles to be used as sensitive contrast agents for multimarker hyperspectral imaging.

We have created an expansive library of 26 separate nanoparticle batches, each bearing a unique Raman fingerprint.[2] We also demonstrate their potential to specifically target various biomarkers in the hope of gaining a better understanding of the spatial relationships that exist between a multitude of cell types using Raman imaging. Our ability to deconvolve a mixture of all 26 SERS NPs in a single imaging pixel both *in vitro* and *in vivo* opens up entirely new opportunities to efficiently interrogate heterogeneous molecular expression found within and across patient samples. In this study, we have demonstrated that our SERS NPs can effectively target specific biomarkers while providing highplexed subcellular image resolution in biological samples.

Nanoparticles can now serve as guides between the molecular world of biomarkers and the macro-world of tissue architecture, helping to link highplex data to patient outcome in clinical studies, providing insights to guide therapeutic decisions, and uncovering novel therapeutic targets through the discovery of new biomarkers. With these combined capabilities, we anticipate an exciting path forward for the use of highplexed SERS NP Raman imaging to enable the emerging practice of personalized medicine and improve human health.

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Understanding the Mechanism of Formation of Protein Fibrils for Preventing Neurodegenerative Disease

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The formation and build-up of β-sheet rich amyloid fibrils is a complex multi-step process which results in the formation of toxic plaques which are believed to be the cause of various neurodegenerative diseases which include Alzheimer's disease[1]. The size and cellular toxicity of these aggregates is still relatively unclear. Xian et.al [2] explored the protective effect of an oxindole alkaloid known as Isorhycnchophylline, this compound displayed a neuroprotective effect against the neurotoxicity of $A\beta_{25-35}$ in PC12 cells by inhibiting oxidative stress and supressing the mitochondrial pathway of cellular apoptosis. This motivated the current work which involves introducing various synthesised spirooxindole compounds to study their inhibitory effect against the formation of amyloid fibrils. Conventional Raman spectroscopy is a non-destructive method which provide insightful information on the changes in the secondary structure of proteins. Changes that have been observed include in the Amide I and III regions where characteristic changes of the bands take place as the transition from α-helix to β-sheet occurs. Additionally, various bands sensitive to changes in tertiary structure were detected. Fluorescence spectroscopy, Circular Dichroism and TEM were combined with Raman to provide qualitative & quantitative approaches to measuring the formation of fibrils and the effect of each inhibitor compound. The combination of spectroscopic techniques has revealed that the inhibitor compounds which were introduced to the hen egg white lysozyme solution produced off-pathway oligomers which aren't the toxic stacked β-sheet fibril strands. Additional experimentation, involving the study of the effect of these inhibitor compounds against the structure of pre-formed fibrils indicated that these spirooxindole derivatives pull apart the highly ordered structure of β-sheet fibrils.

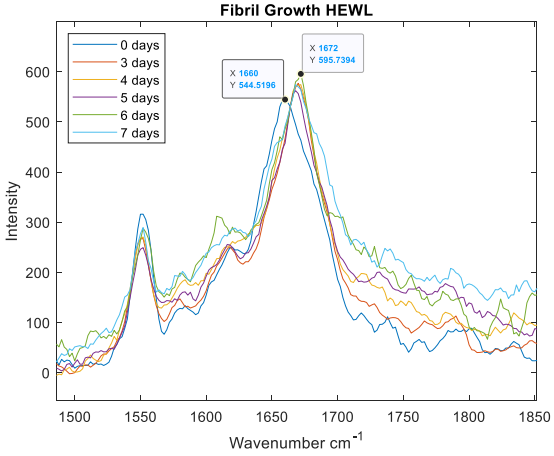

Figure 1: Amide I Region Hen Egg White Lysozyme undergoing Fibril growth

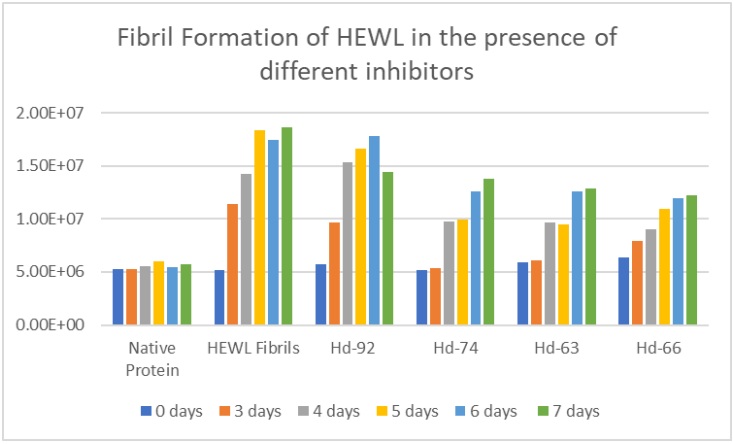

Figure 2: Fluorescence results displaying Fibril Formation of Hen Egg White Lysozyme in the presence of different inhibitors.

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Full Spectrum Raman Excitation Mapping: Rapid Raman Spectroscopy of Nanocarbons from visible to near-IR

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Ordinarily for Raman scattering (RS) spectroscopy, a laser with a given fixed wavelength is chosen as the excitation source, and the wavelength dependence of the scattered light intensity is measured. If many multiple laser wavelength are used on a single band a Raman excitation profile (REP) can be built up. The excitation-emission map that comes from plotting the RS one dimension and the REP in another is the Raman excitation map (REM). When RS is near resonance, the spectral intensities can change a great deal with excitation wavelength and REM is especially information rich.

The idea behind Full Spectrum Raman Excitation Mapping (FS-REM) is to not have to chose the laser wavelengths at all, but rather to use all laser wavelengths at once. We have successfully implemented FS-REM using a supercontinuum white light source in a custom micro-Raman setup. [1] This enables one-shot, essentially instant maps to be generated. Here we describe our technique and recent progress we have made improving it and applying it to ultra-pure single walled carbon nanotubes, an important class of nanocarbons.

The power of REM as an analytical technique is that it captures two distinct types of information both separately and together: vibrational information along one axis and kind of analog of optical absorption along another axis. The usefulness of REM is well-established in the carbon nanotube community, but it is arguably under-used because it is technically challenging and time consuming to implement. Our FS-REM approach makes what would otherwise be a slow and challenging technique rapid, less costly, and more practical. We will demonstrate how full spectrum REM can be used to evaluate ultra-purified carbon nanotubes and related materials intended for semiconductor applications, and why we believe it has the potential for wide applicability.

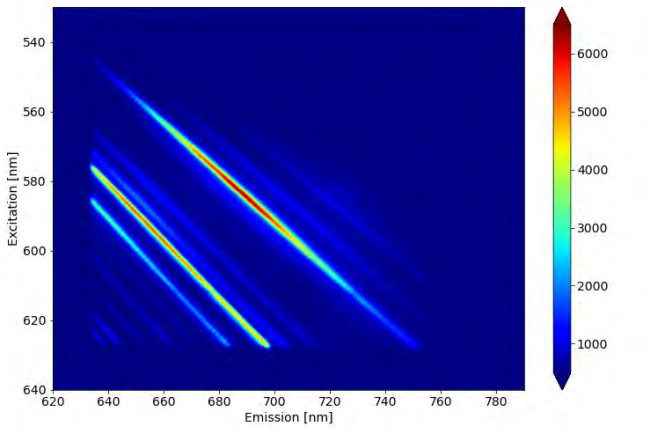

Figure 1 An example of an experimental Raman excitation map of a highly purified nanotube materials obtained in one shot by Full Spectrum Raman Excitation Mapping

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Sub-diffraction nanoscale Raman imaging of the interface in a 2D semiconductor heterostructure

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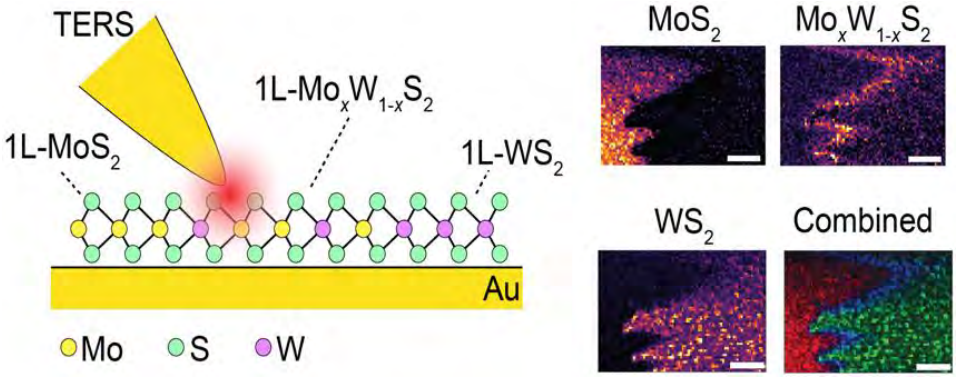
Transition metal dichalcogenide (TMD) semiconductors are solids composed of single sheets of atomic layers that are weakly bonded together via the van der Waals interaction. A single atomic layer yields a 2D single-layer TMD semiconductor with a direct band gap and strong light-matter interactions. This work studies the interface in a single-layer 2D MoS_2/WS_2 lateral heterostructure with a spatial resolution of 50 nm using resonant and non-resonant tip-enhanced Raman scattering (TERS) imaging and spectroscopy. In conventional confocal Raman spectroscopy, a spatial resolution on this scale is not possible because of the diffraction limit. With the sub-diffraction spatial resolution and the vibrational fingerprinting ability of Raman spectroscopy, TERS allows us to probe the composition, size, and heterogeneity of the 2D system on length scales most relevant for nanoscale optoelectronic technologies. We use TERS to reveal that the alloyed transition region varies in size from 50-600 nm within a single crystallite. TERS nanoscale imaging of the transition region allows for tracking of vibrational modes as they evolve across the $MoS_2/Mo_xW_{1-x}S_2/WS_2$ system. By tracing the evolution of the modes across the interface, we are able to deconvolve defect-activation, resonant enhancement, and material composition for several vibrational modes in single-layer MoS_2, $Mo_xW_{1-x}S_2$, and WS_2. This work demonstrates the capabilities of TERS in characterizing monolayer lateral heterostructures on the nanoscale.



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A Raman filter-based system, operating in ambient light through lock-in amplification for real-time, accurate assessment of disease markers

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We developed a novel filter-based Raman medical device, consisting of a 1064 nm laser, a handheld probe, optical filters, photodiodes, and a lock-in amplifier for real-time synchronous signal detection (Fig. 1). As a first demonstration, the weak characteristic Raman scattering peaks of glycerides is identified using the sensitive lock-in amplification technique, which allows for signal detection in ambient light. The system's performance has been evaluated by measuring fat content in lab-made duck fat phantoms and duck liver samples. The signal intensity of the 1550/30-nm channel is linearly correlated with the MRI-calibrated fat content of the phantoms ($\chi = 0.98$) and the duck liver samples ($\chi = 0.964$) and agrees well with the NXR 9650 FT-Raman spectrometer results, with linear correlation coefficients $\chi = 0.986$ (phantoms) and 0.934 (liver samples) (Fig 2). The 1064/3-nm signal intensity is positively correlated with the changes predicted by the Mie theory. The difference in the signals detected in the informative channel under ambient light and in the dark are vanishingly small (0.022% to 0.067%). This is the first report of a Raman-based measurement system operating under normal lighting conditions and is a consequence of the use of a lock-in amplification technique. This medical device is accurate, non-invasive, inexpensive, provides real-time results, easy to use. Early-stage commercialization is at a mature stage at liver transplant centres.

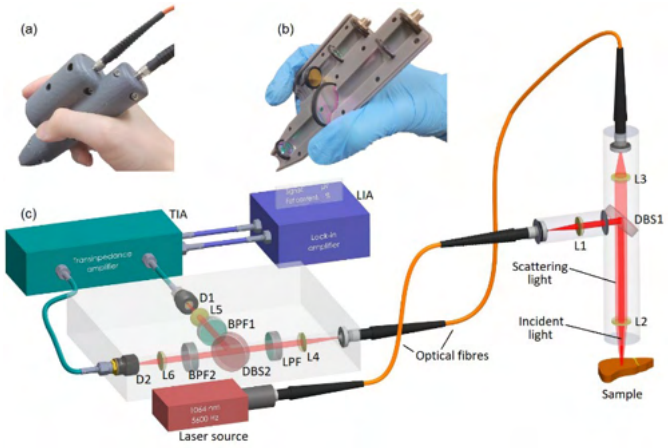

Fig. **1**. (a) 1:1 scale handheld probe held in the author's hand. (b) 1:1 scale handheld probe equipped with optical elements (an Au mirror is used to fold the light path). (c) Schematic of the optical filter-based Raman system. L1-L5: lenses; DBS1: 1180-nm longpass dichroic beam splitter; DBS2: 1500-nm shortpass dichroic beam splitter; LPF: 1400-nm longpass filter; BPF1: 1550/30-nm bandpass filter; BPF2: 1064/3-nm laser line filter; D1-D2: InGaAs photodiodes; TIA: transimpedance amplifier; LIA: lock-in amplifier

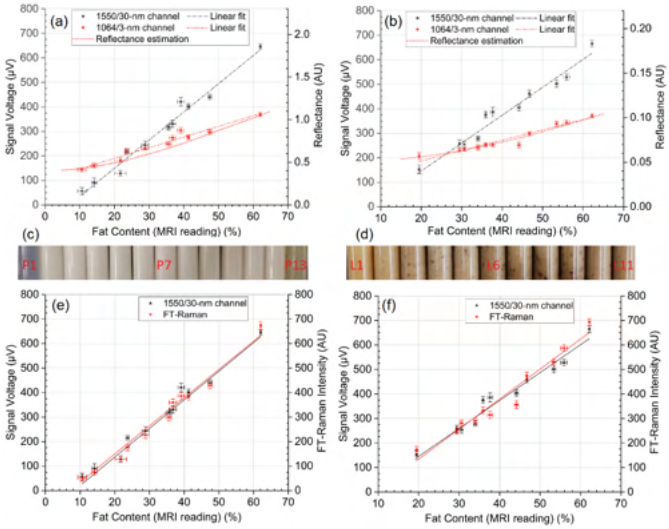

Fig. 2. Signal intensities of the 1550/30-nm channel and the 1064/3-nm channel of (a) duck fat phantoms P2-P12 and (b) duck liver samples L1-L11. (c) Duck fat phantoms P1-P13 (from left to right). (d) Duck liver samples L1-L11 (from left to right). Comparisons of signal intensities of the 1550/30-nm channel with the FT-Raman intensities using (e)) duck fat phantoms P2-P12 and (f) duck liver samples L1-L11. The fat contents were calibrated using the 3T MRI scanner. The error bars are the standard deviations over three sequential repositioning measurements (with a repetition rate of 100 and a time interval of 100 ms). The parameters of optical properties were provided by RefractiveIndex.INFO database.

Porous carbon nanowires for metal-free SERS

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Surface-enhanced Raman spectroscopy (SERS) is a powerful tool for vibrational spectroscopy by virtue of its ability to offer several orders of magnitude higher sensitivity than inherently weak spontaneous Raman scattering. However, the benefit of SERS is somewhat betrayed by its low reproducibility, uniformity, biocompatibility, and durability. This is because it depends on "hot spots" for high signal enhancement via aggregates of metal nanoparticles or engineered metal nanostructures, leading to the generation of large photothermal heat on the metal surface that causes detrimental effects to biomolecules and the oxidization of the metal surface [1]. Despite various efforts, it still has been challenging to develop a SERS substrate that simultaneously achieves high sensitivity, uniformity, reproducibility, and durability since it requires a high density of hot spots with uniform enhancement factors. Here we report our experimental demonstration of a metal-free, topologically tailored nanostructure composed of a two-dimensional array of porous carbon nanowires as a SERS substrate for highly sensitive, biocompatible, and reproducible SERS (Fig. 1) [2]. Specifically, our porous carbon nanowire array (PCNA) substrate provides not only high signal enhancement (~10^6) due to its strong broadband charge-transfer resonance for large chemical enhancement, but also extraordinarily high reproducibility or substrate-to-substrate, spot-to-spot, sample-to-sample, and time-to-time consistency in SERS spectrum due to the absence of hot spots and high compatibility to biomolecules due to its fluorescence quenching capability. We also compare our PCNA substrate with other metal-free SERS substrates [3].

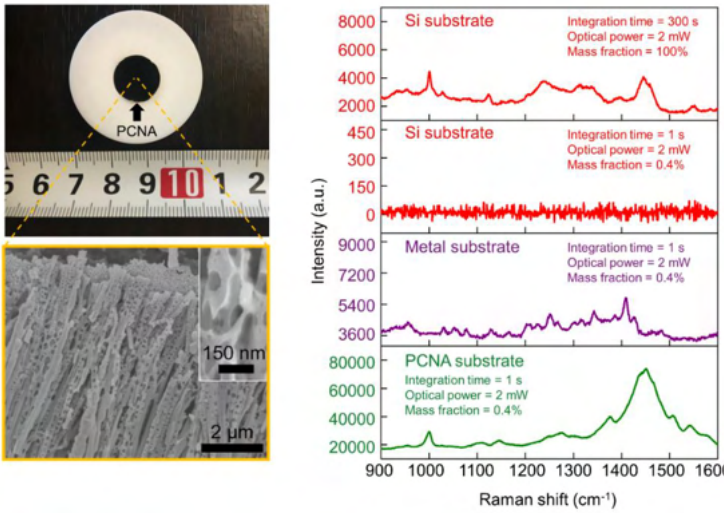

Fig. 1. Experimental demonstration of PCNA-based SERS. Top left: Photo of the PCNA substrate. Bottom left: Scanning electron microscope image of the PCNA substrate. Right: Raman spectrum of β-lactoglobulin (whey protein in bovine milk) on various substrates.

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Hyperspectral and chemically-selective 2D and 3D imaging in mid-infrared

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The vibrational imaging approach comes in two flavors: IR and Raman-based microscopy. From a light-matter point of view, the IR interaction is 10^8-10^{10} times stronger than the Raman effect, yet in practice IR microscopy is not more successful than its Raman counterpart. What could have been a burgeoning technology, MIR imaging currently suffers from several pervasive technical challenges that have held back its broader implementation. One such challenge is found in the detection of MIR light – MIR cameras are affected by thermal noise and there is a trade off between fast acquisition and the pixel density of the camera chip.

Our recent series of works explores, demonstrates and applies the idea of detecting MIR radiation directly on the camera chip by leveraging the intrinsic optical nonlinearity of the semiconductor material[1-3]. Through the process of nondegenerate two-photon absorption (NTA) a MIR photon is detected with the aid of a second photon, in the vis/SWIR range, which is used as a gate. Charge carriers in the camera chip are excited when the total energy of the MIR/gate photon pair exceeds the bandgap energy of the semiconducting material. This strategy has enabled several imaging modalities, including rapid hyperspectral imaging. At the same time, the very nature of the NTA detection principle permits volumetric imaging when femtosecond pulses are used. Optical sectioning of the sample volume naturally follows from the temporal gate provided by the short width of the MIR/gate cross correlation, allowing rapid axial slicing in wide-field imaging mode through a simple scan of the MIR/gate time delay (Figure 1). The corollary is a three-dimensional yet chemical-selective MIR imaging technique with acquisition rates that rival and exceed those of current MIR-based optical coherence tomography methods.

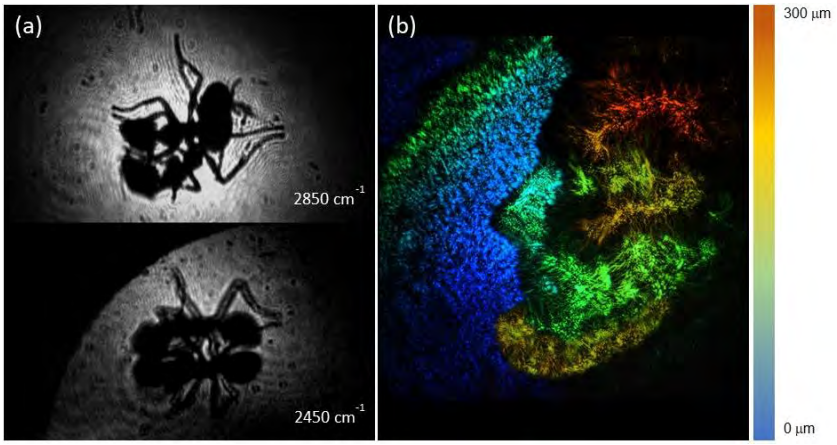

Figure 1. (a) Snapshot from mid-IR video of live pavement ant on (top, 2850 cm-1) and off (bottom, 2450 cm-1) CH-vibrational resonance of the chitin exoskeleton. (b) 3D visualization of Lincoln portrait measured by MIR light reflected/scattered off US penny coin. Acquisition time is 1 second/volume.

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Using a flight-like Raman spectrometer to identify redox couples: a potential energy source for life.

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It is commonly suggested that the habitability, past or present, of a planetary region depends on the presence of three fundamental requirements for life: liquid water, essential elements (such as C, H, N, O, P and S), and a source of energy. The European Space Agency's ExoMars rover mission aims to investigate the habitability of the Martian surface (and sub-surface) in the region of Oxia Planum. The rover will characterise the geology of the region, search for biological signatures and identify mineralogical evidence of processes associated with life [1].

The Raman Laser Spectrometer (RLS) within ExoMars' Analytical Laboratory Drawer will play a key role in assessing the habitability of Oxia Planum: it was specifically designed to identify organic compounds and mineral phases linked with water-rock interactions and biological activity [2]. As part of the mission preparation, it is important to determine and characterise the response of the RLS and its sensitivity limits to key target materials that are associated with habitability.

On Earth, materials containing redox products are considered to be evidence for habitable environments since various microbe species are capable of harnessing the energy from reduction-oxidation (redox) reactions [3]. For example, the oxidation that results in the transformation of magnetite (Fe II), to hematite (Fe III) [4] is often associated with microbial activity; both minerals are abundant on the Mars surface [5]. Another example includes gypsum to pyrite: some terrestrial gypsum ($CaSO_4 \cdot 2H_2O$) samples exhibit transformation into pyrite (FeS_2) as a result of the microbial precipitation [39]; both gypsum and pyrtite have been observed at Gale Crater [6].

Here we present the Raman analysis of two Martian analogue samples containing redox couples:

(i) A weathered basalt containing magnetite and disseminated haematite, and
(ii) Shale samples containing veins of gypsum and precipitates of pyrite.

The analysis was performed using a flight-representative Raman spectrometer and using representative spectral acquisition techniques. We present the full sample characterisation, and highlight the spectral signatures associated with redox couples. We review the level to which such signatures can be identified by a flight representative instrument, and hence infer the performance of the RLS during mission operations.

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Three-Dimensional Scaffolds for Monitoring Drug Diffusion and Cell Death Events by Surface-Enhanced Raman Scattering

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Surface-Enhanced Raman Scattering (SERS) is a promising tool for the study of the tumoral microenvironment in a non-invasive manner [1]. However, most applications involve the use of 2D plasmonic materials and cancer models, their integration being far from perfect [2]. Additionally, 2D cultures involve some limitations, including a lack of the cell-cell and cell-extracellular matrix (ECM) interactions that are required to generate specific 3D microenvironments. For that reason and to better capture dynamic processes in complex cellular environments, the integration of versatile sensors, homogenously distributed within well-defined three-dimensional (3D) networks, would be required to provide precise information in real-time about 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 is introduced as a promising strategy for mimicking a specific cell niche and identifying concentration gradients. We therefore prepared hydrogel-based models containing gold or silver plasmonic nanoparticles for *in situ* biodetection. In addition to being a suitable system for sustaining cell growth, the printed nanocomposite inks can uncover drug and death diffusion profiles by SERS, with a high spatiotemporal resolution [3]. We additionally demonstrate that the acquired information could lay the groundwork for designing novel strategies for drug discovery in cancer therapy, through the study of drug diffusion profiles and the resistance of different cells in the cancerous niche to common chemotherapies.

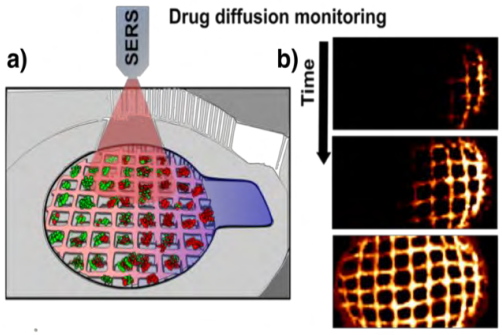

Figure 1: a) Schematic representation of a nanocomposite scaffold within a 3D tumor cell environment for the detection of drug diffusion. **B)** Methylene Blue diffusion patterns in nanocomposite scaffolds over time.

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DNA Origami Directed Plasmonic Hot-Spot for Studying Molecular State and Spin Crossover by Single Molecule SERS

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Determination of molecular state and understanding of a system at single molecule is of importance and rather challenging than their ensemble study. Surface enhanced Raman spectroscopy (SERS) is one of the promising approaches in this regard; however, study of molecular properties and state at single molecular level using the principle of SERS demands precise positioning of the molecule at plasmonic hot-spot which shows maximum field enhancement for highest Raman signal.[1] In this regard, we propose a DNA origami technique to generate higher order DNA nanostructures for assembling plasmonic nanoparticles (in terms of plasmonic nano dimers) for precise positioning of molecules (biomolecules) at the hot-spot for single molecule study.[2] Using the principle of G-quadruplex-hemin complexation chemistry, we investigate the molecular state of single hemin by SERS methodology. The proper choice of laser wavelength allowed us to record SERS spectra of singly folded G-quadruplex and subsequently bound hemin molecule placed at the plasmonic hot-spot owing to high field enhancement. (**Figure 1**) The success of the SERS measurement of single hemin placed at the hot-spot of as-designed DNA origami based plasmonic nanostructures further opened up the possibility of studying external stimuli driven spin crossover of hemin at the hot-spot in single molecule scale proposing a new way to systematically study single molecules and their chemical interaction.[3]

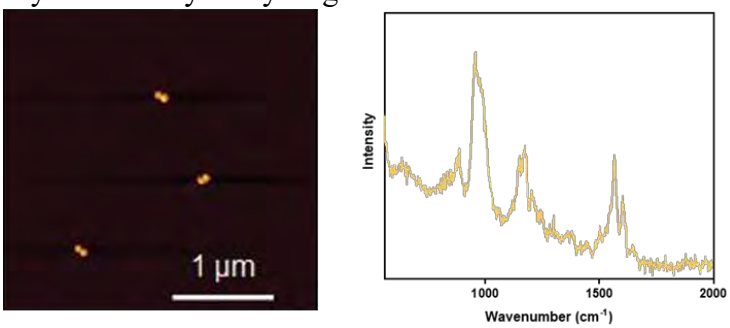

Figure 1: Representative AFM image of Au nano dimers on DNA origami platform and a characteristic single molecule SERS spectrum of hemin trapped at the hot-spot of a randomly chosen Au nano dimer.

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Ultrafast structural changes of large [$Au(CN)_2^-$] oligomers in triplet excited state observed by time-domain Raman spectroscopy

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The rearrangement of chemical bonds is one of the most fundamental phenomena in chemistry, which is essential for chemical reactions. Real-time observation of the bond formation requires triggering a chemical reaction at a desired, well-defined timing, which is still challenging. Recently, the trimer of the [$Au(CN)_2^-$] complex has attracted much attention since photoexcitation induces the formation of the covalent Au-Au bonds in the excited state [1-4]. Even though the exact photo-induced processes of the trimer are still under debate, the insights of larger oligomers can provide another viewpoint to fully understand aurophilic oligomer properties. At high concentrations, the [$Au(CN)_2^-$] complex forms oligomers as large as the tetramer and pentamer [5], which provides a unique opportunity to study the bond formation dynamics and structural changes of large oligomers in the excited state. In this study, we successfully distinguished the Raman bands of the large oligomers (tetramer and pentamer), and their structural changes in the triplet excited state through femtosecond time-resolved impulsive stimulated Raman spectroscopy (TR-ISRS).

Fig 1(A) illustrates time-resolved Raman spectra of $K[Au(CN)_2]$ aqueous solution (685 mM) from 0 to 100 ps after excitation at 343 nm. The excitation wavelength was carefully chosen so that the tetramer and pentamer are selectively excited. In the time-resolved Raman spectra, four distinct peaks are observed at ~60, ~100, ~140, and ~400 cm^{-1}. The temporal profiles of the Fourier transform amplitude shown in Fig 1(B) reveal that the ~100 and ~140 cm^{-1} Raman bands show a ~2.6-ps rise, and an additional ~13-ps rise is also recognized for the ~100 cm^{-1} band. Based on our previous studies, the ~3-ps and ~13-ps time constants are assignable to ISC of the tetramer and the pentamer, respectively [3,7]. Furthermore, the ~100- and ~140-cm^{-1} bands exhibit a significant frequency upshift with a ~4-ps time constant. We attribute this upshift of the Raman bands to a bent-to-linear structural change occurring in the triplet excited state of the tetramer after the Au-Au bond formation. The quantum chemical calculation is in progress to compute the tetramer bent structure associated with its Raman bands in the triplet excited state.

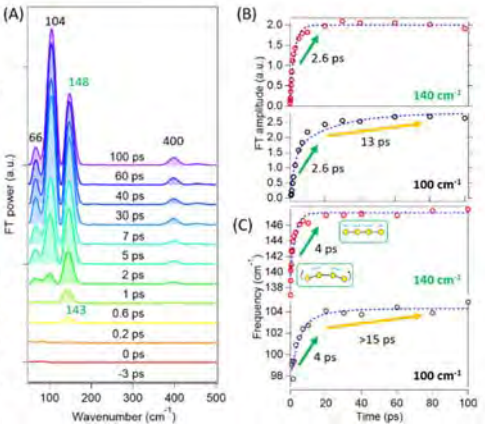

Fig 1. Time-resolved impulsive stimulated Raman data obtained for 685mM $K[Au(CN)_2]$ aqueous solution. (**A**) Fourier transform power spectra at different delay times **(B)** Fourier transform amplitude of the Raman bands at ~100 and ~140 cm^{-1}. **(C)** Frequency shift of the transient Raman bands at ~100 and ~140 cm^{-1}. Inset: plausible structural changes of tetramer in the triplet excited state.

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Resonant Raman Scattering "Suppressed" in MoS_2 Fullerenes: A high Pressure and Low Temperature Study

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Resonance Raman scattering has been widely used to study electronic band structures and to investigate the nature of electron-phonon interactions in semiconductors. A key issue in those studies is the role played by intermediate exciton states. We previously [1, 2] demonstrated how Raman scattering of bulk *2H*-MoS_2 is resonantly tuned by pressure and temperature - shifting of the exciton energies, towards that of the exciting laser. Here, we extend our study to Inorganic Fullerenes (IF) of MoS_2 and find fundamentally different behavior: non-resonant temperature and pressure dependences of IF-MoS_2 Raman scattering for first and second - order Raman transitions.

Limits on the use of excitons as intermediate states in the scattering process concern, among other aspects, their lifetime. We attribute this different behavior to structural effects that reduce the exciton lifetime: the relative abundance of defects and the random nature of the trigonal prismatic MoS_2 layers [4].

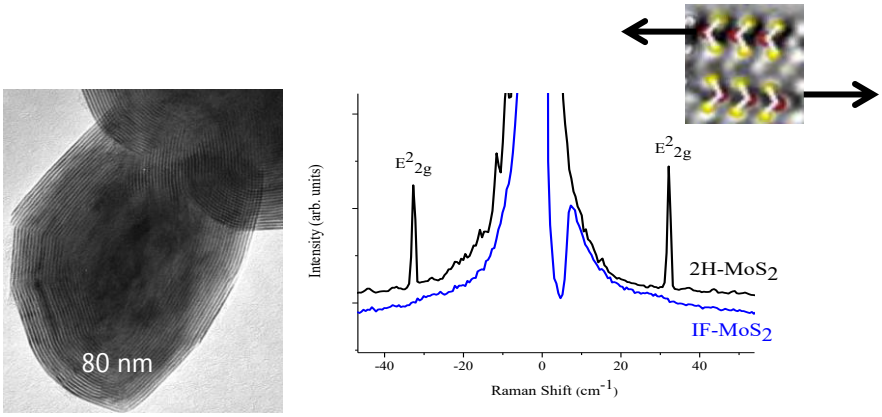

Figure 1: TEM of IF of MoS_2 (left); Shear mode Raman scattering in the bulk and its absence in the IF (right)

The random stacking and incommensurate nature of the IF structure prevents the formation of a pure 2H or 3R symmetry [4] and is argued to be the origin of the hindrance of the shear movement (Fig.1). We will present the pressure and temperature dependent Raman scattering and compare the resonant response of the IF system to that of the bulk of 2H-MoS_2. The effect of structural damage and the IF "interlocked" nature on the Raman spectra, will also be discussed.

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Transient Raman study of excited state dynamics of 1,9'-bianthryl

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Bi-chromophoric molecular homodimers continue to excite interest in photophysics and materials science. The role and consequences of coupling between adjacent chromophores have been studied for many years – going back to Kasha. Recent research has been focused on temporal evolution following excitation of chemically linked homodimers, and a plethora of interesting phenomena have been observed depending on the chromophore geometry and environment. These include excimer formation, symmetry breaking charge separation and singlet fission. The latter is believed to have great potential in overcoming the Shockley and Queisser limit to the efficiency of solar cells. One of the most studied examples of bichromophoric molecules is 9,9'-bi-anthryl, one of the earliest examples of symmetry breaking charge separation in polar solvents. In sharp contrast to other bi-chromophores (e.g. pentacene, tetracene, pyrene and perylene…) there have been rather few studies of the structure dependence of the photophysics of bi-anthracenes. Here we report an investigation of the newly synthesised 1,9'-bianthryl, which has been characterised through steady-state electronic spectroscopy, ultrafast transient absorption and femtosecond stimulated Raman spectroscopy. In Figure 1 we show the evolution of the transient state Raman spectra in polar acetonitrile, in which fast charge separation is characterized though the Raman of both the initial neutral and the rapidly forming charge separated state. These are isolated through exploitation of their distinct resonances at 560 and 650 nm. Further data on the spectroscopy and unusual solvent dependence will be described.

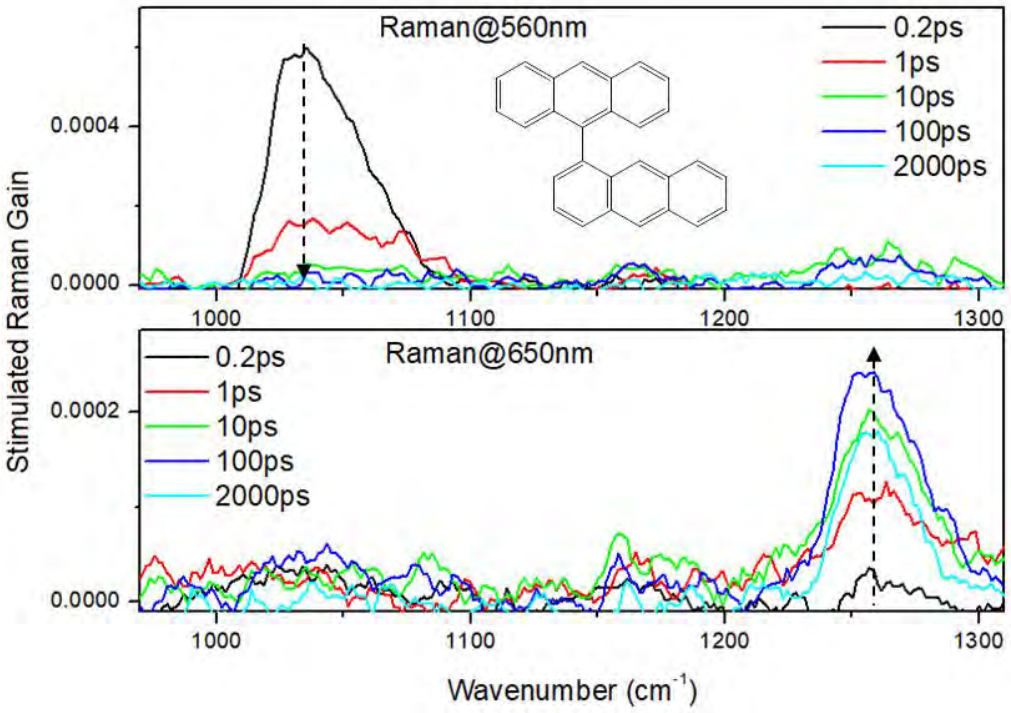

Figure 1: Excited state Raman evolution of the neutral (560 nm) and CT state (650 nm) of 1,9'-bianthryl.

Hydration Water Character on Atomically Dislocated Surfaces Revealed by Surface Enhanced Raman Spectroscopy

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Hydration is ubiquitous in any kind of water–substance interaction such as in various interfacial and biological processes. Despite substantial progress made to date, however, still less explored is the hydration behavior on complex heterogeneous surfaces, such as the water surrounding the protein, which requires a platform that enables systematic investigation at the atomic scale. Here, we realized a heterogeneous self-assembled monolayer system that allows both controllable mixing with hydrophobic or hydrophilic groups and precise distance control of the functional carboxyl groups from the surface by methylene spacer groups. Using surface-enhanced Raman spectroscopy (SERS), we first demonstrated the hydrophobic (or hydrophilic) mixing ratio-dependent pK_a variation of the carboxyl group. Interestingly, we observed a counterintuitive, non-monotonic behavior that a fractionally mixed hydrophobic group can induce significant enhancement of dielectric strength of the interfacial water. In particular, such a fractional mixing substantially decreases the amide coupling efficiency at the surface, as manifested by the corresponding pK_a decrease. The SERS-based platform we demonstrated can be widely applied for atomically precise control and molecular-level characterization of hydration water on various heterogeneous surfaces of biological and industrial importance.

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Development of Raman Spectroscopic analysis techniques to assess quality biomarkers in fish

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Fish have been a major source of food for the world's population for thousands of years. As fish production trending is directed away from wild capture and more towards aquacultural practises, the analytical techniques used to measure and monitor fish quality will be required to be further developed, improved, and optimised. The development of rapid, non-destructive analysis techniques is highly sought after in food production, and which is well suited to the use of Raman Spectroscopy coupled with chemometrics.

We present here our results highlighting several ways this technology can used to measure quality parameters, several different Raman techniques have been used to develop to analytic methods targeting quantitation of biomarkers in fish. Spatially Offset Raman Spectroscopy was utilised to determine if biomarkers could be measured in the tissues of whole fish using sub-surface measurements [1]; a method to quantitate carotenoid concentration through-skin in Atlantic Salmon with a portable Raman instrument using defocused measurements; and a SERS based method for detection of histamine, which is linked to immunogenic responses in seafood.

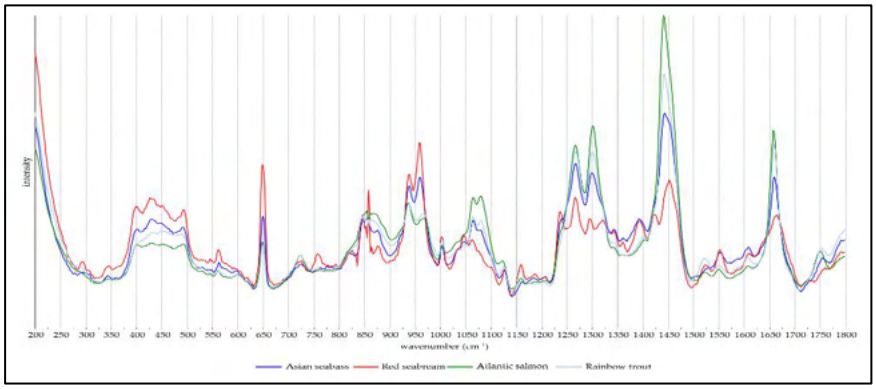

Figure 1 - Mean process SORS spectra of some commercial fish

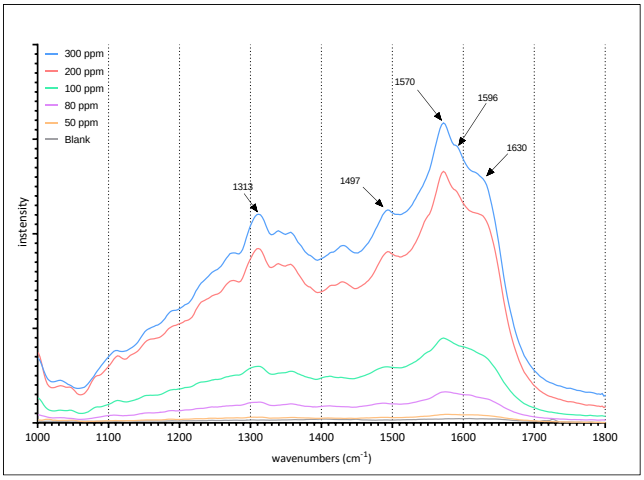

Figure 2 - SERS spectra of histamine at different concentrations

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Raman spectroscopy and semi-supervised learning for the investigation of biochemical response in patients receiving HDR-brachytherapy

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High-dose-rate-brachytherapy (HDR-BT) is an increasingly attractive alternative to external beam radiation-therapy (EBRT) for patients with intermediate risk prostate cancer. Despite this, no bio-markers or methods exists to monitor treatment response, and the changes which take place at the biochemical level in hypo-fractionated HDR-BT remain poorly understood.

Our group has shown that Raman spectroscopy (RS), combined with principal component analysis (PCA), can be used to identify biochemical changes associated with radiation exposure. However, PCA often has limitations when used to interpret Raman spectra. Difficulties can arise in deciphering the overall contribution to sample variation from individual bio-components within a spectrum.

We demonstrate an alternative approach in which a library of reference spectra containing individual cellular bio-components are used as inputs to group and basis restricted non-negative matrix factorisation (GBR-NMF). Using GBR-NMF we have successfully reproduced previously known metabolite response profiles in post irradiated MCF7 breast cancer cells[1] such as those demonstrated for glycogen by Matthews *et al*.[2] using PCA. We here show that with GBR-NMF we now gain the ability to map profiles of other biologically relevant chemicals.

We have used the RS-GBR-NMF approach to elucidate biochemical expression patterns across a preliminary group of patients, identify clusters of individuals with similar profiles and shown some correlation of these expression profiles to the following pre-treatment clinical prognostic indicators; Gleason score, CAPRA score and Ki67 expression. The ability to identify HDR-BT induced responses within individuals opens up a number of new treatment pathways that could be exploited to both increase the radio sensitivity of the tumour as well as the possibility to explore new, combination therapies.

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BCARS down to the nanometer length scale

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Broadband coherent anti-Stokes Raman scattering (BCARS) offers an increased signal intensity as compared to spontaneous Raman scattering while maintaining the spectral sensitivity, and thus sees widespread use for the chemo-sensitive imaging in biomedical applications. Recently, BCARS has been applied also to fully crystalline materials [1], impressively demonstrating how to become an indispensable tool for hyperspectral imaging of ferroelectric domain walls (DWs) [2]. While single crystalline systems are ideal model systems to explore the fundamental physics (signal generation, phase matching, coherent interaction length, etc.) of BCARS, DWs offer to extend that study to the nanometer length scale.

In this work, we explored these fundamental limits in the model system lithium niobate (LN) using two different BCARS setups, i.e. using forward and backward detection; BCARS spectra recorded well within LN domains were rendered comparable by accounting for the non-resonant background (NRB) and applying the Kramers-Kronig transformation [3] (see Fig. 1a). At DWs, the BCARS signal around 625cm^{-1} changes significantly and thus enables analysis of these Delta-function-like DWs down to the diffraction limit (see Fig. 1b). Understanding in detail the signal generation from DWs and any influence of other layers including the NRB, will not only improve the understanding of BCARS image contrast in solid state systems, but furthermore, pave the way towards BCARS applicability for 2D materials and nanoscale systems.

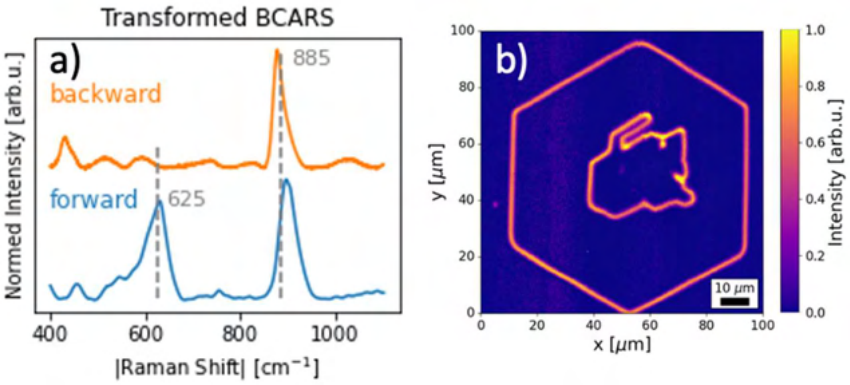

Figure 1: a) Comparison of retrieved BCARS spectra in forward- (blue) and backward-detection (orange); b) Backward detection BCARS imaging of ferroelectric domain walls (orange) in lithium niobate.

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Towards excited state Raman scattering studies of single-molecules

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Gold nanoparticles efficiently catalyse a wide range of industrially-important reactions via plasmons, charge transfer and heating. To understand the underlying mechanism at nanoscale, it is crucial to track the intermediate states at picosecond time scale with single-molecule sensitivity. Traditional single-molecule approaches detect background-free fluorescence and are thus limited by irreversible photobleaching with typical observation times of tens of seconds.[1] An alternative is detecting surface-enhanced Raman scattering (SERS) where the molecule is placed into vicinity of a nanoplasmonic structure yielding Raman signal levels equal or even superior to single-molecule fluorescence with an exceptional stability over time.[2] Moreover, since a typical Raman scattering cross-section is 10 orders of magnitude smaller than a corresponding absorption cross section, in resonant SERS, the optical transition is saturated making SERS naturally well-suited to study ultrafast excited state dynamics of molecules.

The main challenge of ultrafast SERS experiments is avoiding photodamage of the molecules and nanostructures by the pulsed laser source. Here, we employ a nanoparticle on a mirror (NPoM) plasmonic system that consists of a gold mirror and a gold nanosphere separated by a molecular spacer. NPoM is easy to assemble, the cavity resonance can be tuned by varying the nanoparticle diameter and we show that it withstands high laser flux marking it as an ideal nanocavity for ultrafast studies of single molecules. We present a fully automated Raman microscope capable of measuring thousands of single NPoM SERS spectra per hour at a few-wavenumber resolution that allows us to systematically characterise the SERS response as a function of the cavity and emitter resonance at 633 nm and 785 nm for both continuous and pulsed excitation. Under the pulsed illumination, we observe a non-linear phenomenon where we “carbonise” the molecule inside the cavity. We will present the effects of molecular resonance, laser fluence and peak power and provide insight into the mechanism of this reaction, laying basis for single-molecule excited state Raman experiments using the NPoM platform.

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Sensing dopamine with Fe(III)-sensitised AuNP monolayer 1nm-gap SERS films

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Self-assembled colloidal Au nanoparticles (AuNPs) are attractive for surface-enhanced Raman spectroscopy (SERS) substrates due to their reproducibility, ease of fabrication, and accuracy [1]. However, aggregates suspended in solution suffer Brownian fluctuations and aggregating agents can interfere with analyte detection. Here we show monolayer aggregates on transparent supports can now be accessed efficiently from opposite sides by fluids and light, allowing repeated flow sensing of neurotransmitters when combined with Fe(III) sensitization [2].

To develop compact integrated chemical flow sensors which can be reused requires solid supported implementations. Here, we demonstrate sensing of neurotransmitters utilising self-assembled plasmonic AuNP aggregates immobilised into random close-packed arrays termed monolayer aggregates (ML agg) on glass substrates [2]. Detection of neurotransmitters in urine is a challenge due to their low concentration (<μM) and biofouling/interference. Conventional techniques (LCMS, NMR) are costly, slow, and ineffective for continuous sensing.

By exploiting the complexation of Fe(III) and catecholamines [3], we fabricate highly reproducible and sensitive SERS substrates (Fig.1a). We demonstrate reusability of these films using plasma cleaning, while the NP monolayer enables efficient backside interrogation. Multiplexed sensing of neurotransmitters including epinephrine reveals unusual complexation and vibrational coupling. These devices are extremely promising for widespread microfluidic integration and implementations such as smart toilets for continuous monitoring of drug effects and compliance.

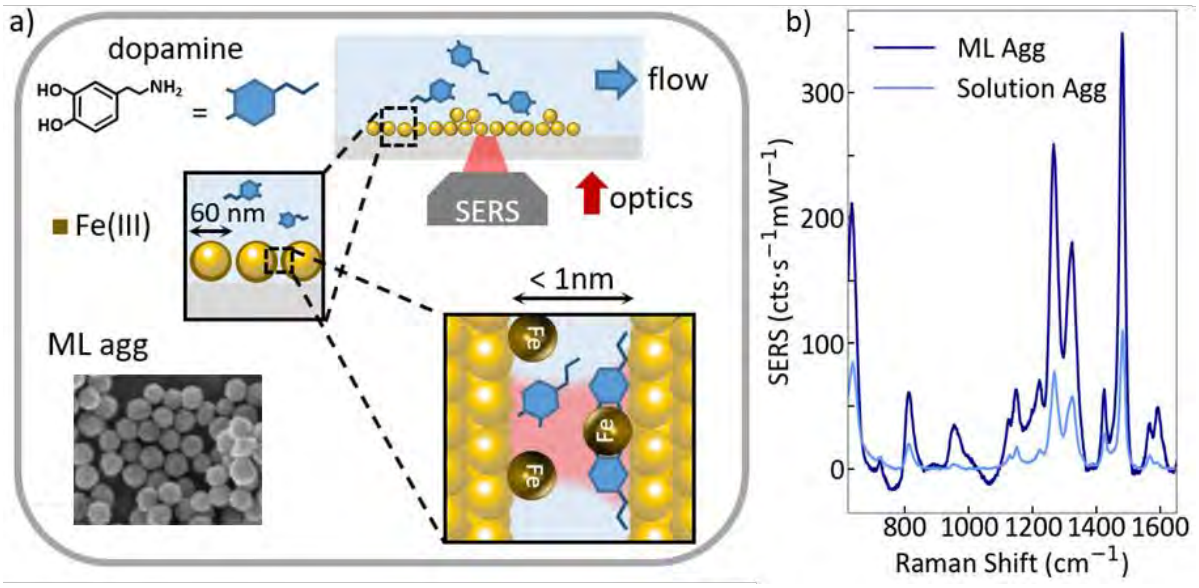

Figure 1: a) Monolayer aggregate (ML agg) film of 60 nm gold nanoparticles coated with Fe(III) on glass (grey), used for dopamine sensing by SERS. b) SERS spectra of Fe(III) + dopamine complexes measured using both ML agg and solution agg systems.

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Raman Metrology for Live Cell Imaging

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For a technique to be applied to any environment with a need for robust reproducibility, it must undergo rigorous testing and have well defined protocols and procedures.[1] Raman spectroscopy still currently suffers from instrument and user variability, as well as not having enough well defined, set procedures which can be transferred from lab to lab.[2] Raman spectroscopy therefore, still requires better established standard operating procedures and data handling methods before it will be able to be translated as a benchmark technique for chemical analysis.

As Raman spectroscopy is a technique which uses a high-powered light illumination source, there is a risk of sample damage due to laser exposure. This is particularly concerning for light and temperature sensitive samples such as live cells or biological tissues. To uphold data integrity, it is important to ensure chemical changes – which can be indicating factors to aid medical diagnosis - are due to the process under study, and not artefacts of sample damage. To help overcome some of these issues of sample damage and in order to develop improved operating procedures, this project focusses on systematically establishing how much laser irradiation a live cell sample can withstand before damage occurs during Raman spectroscopy measurements, in parallel with well-defined biological viability techniques. Initially this project utilises MG-63 mammalian osteosarcoma cells to determine the safe limits for laser exposure, however this limit could vary in different cell lines, therefore the aim of this project is to develop a type of framework to assess the safe irradiation limit which can be applied to any biological sample. This will enable the design of robust standard operating procedures for Raman analysis avoiding photodamage of live cells and delivering confidence in future Raman analysis results through reduced artefacts.

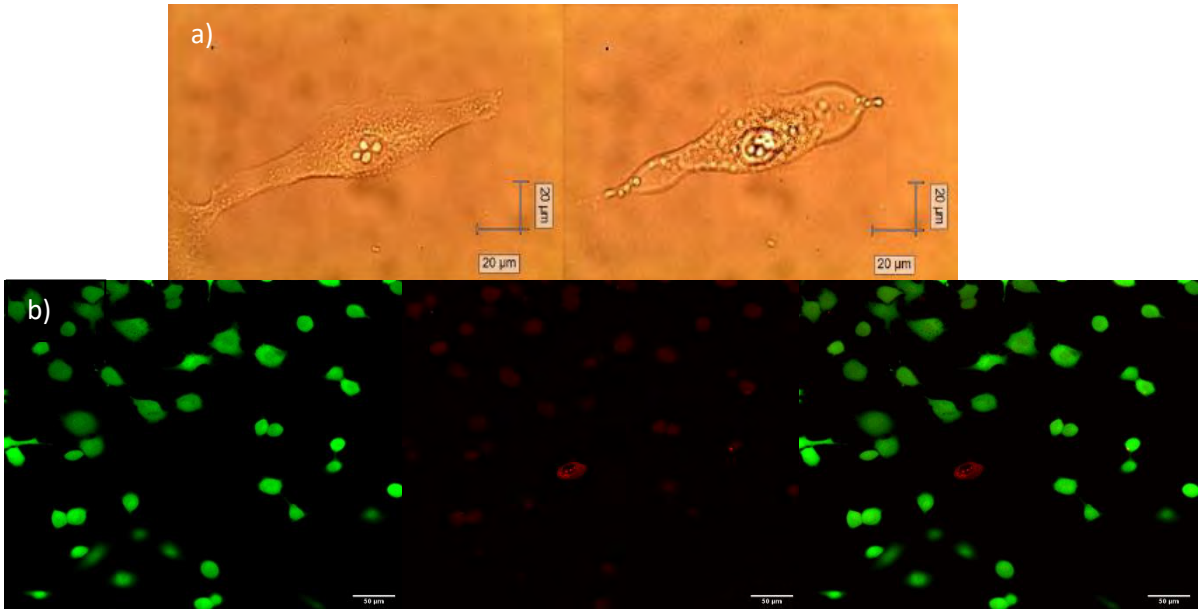

Figure 1: a) Visible light images of a single MG-63 cell before and after Raman analysis b) Biological fluorescent staining of a sample in which one MG-63 cell was analysed using Raman spectroscopy. The analysed cell is stained red indicating it has died while all surrounding cells are stained green indicating they are still alive.

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Random Illumination Wide-field Coherent Anti-Stokes Raman Scattering Microscopy

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Wide-field coherent anti-Stokes Raman scattering (CARS) imaging holds the potential for the highest image acquisition rates far beyond standard laser scanning microscopy approaches. As two major challenges, pulse energies generating nonlinear CARS photons across larger areas may damage high numerical aperture (NA) objective lenses. Furthermore, conventional wide-field microscopy misses the capacity for optical sectioning. Here, we solve both challenges using speckle illuminations. As a great advantage, laser speckle pattern feature a much higher damage threshold within any plane of the excitation or collection optics enabling conventional objective lenses and a co-propagating pump and Stokes illumination. To achieve optical sectioning, we transform first the coherent speckle wide-field CARS images into quasi-incoherent images by averaging over large numbers of speckle pump illuminations. In a second step, a stack of quasi-incoherent CARS intensity images under different but static speckle Stokes radiation is acquired. The square root of the variance of this stack of CARS images provides a quasi-confocal axial sectioning power ($1/z_c$) as show analytically, numerically and experimentally. The same laser system allows also to activate other nonlinear effects as sum frequency generation (SFG), which allows investigating the tissue's collagen. As shown in Fig.1, computing the variance over a large number of acquired images using both techniques (CARS and SFG) shows enhanced resolution and z-sectioning ability compared to the average over the acquired images.

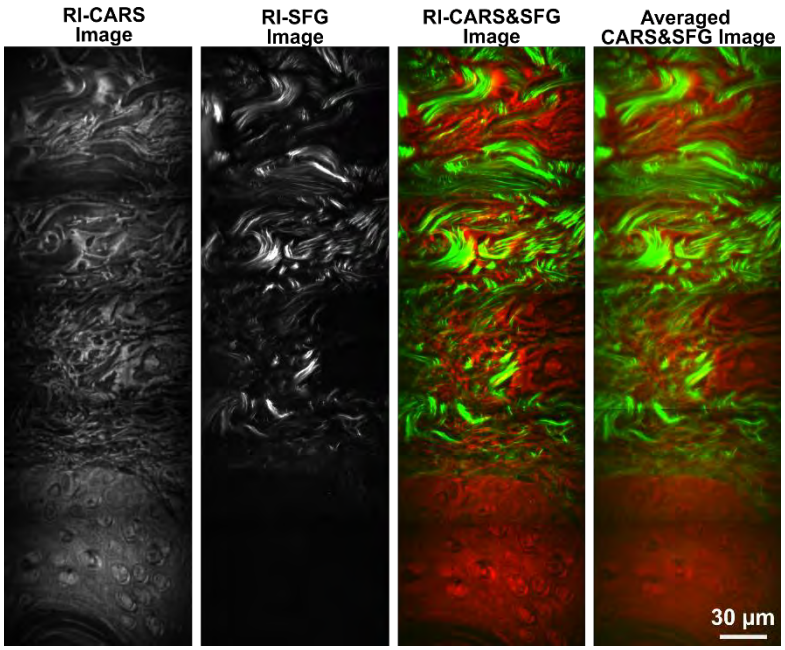

Figure 1: mosaic of a sectioned biopsy. From left to right: random illumination wide-field CARS signal; random illumination wide-field SFG signal; overlap of the random illumination wide-field CARS signal in red and of the random illumination wide-field SFG signal in green; overlap of wide-field CARS (red) and SFG (green) signal.

Spectral focusing coherent Raman scattering microscopy using the triple output dual optical parametric oscillator CRONUS-2P

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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 and can be detected in different ways [1]. Coherent anti-Stokes Raman scattering (CARS) is free from excitation background but contains non-resonant responses which make isolating chemically specific signals challenging. Stimulated Raman loss or gain (SRL or SRG) detects the intensity change of pump or Stokes beams by their interference with CRS, given by the imaginary part of the CRS response which is resonant to the molecular vibrations similar to Raman scattering. It requires shot noise limited intensity noise of the detected beams to achieve similar sensitivity as CARS, and features a typical relative modulation in the 10^{-4} to 10^{-6} range [2].
Achieving the shot noise limit at the typical 10mW detected power is a challenge for many laser systems. Suited sources are free space oscillators combined with detection of the modulation at high frequencies in the MHz range, where mechanical noise is suppressed. In this work we demonstrate the optical parametric oscillator (OPO) Light Conversion CRONUS-2P (Yb pumped) for spectral focusing SRL microscopy. The system features three synchronized outputs, enabling two independent vibrational frequencies to be addressed, as well as using pulses of wavelengths of 900nm and 1200nm to address the CH range avoiding three-photon absorption into DNA. Here we show SRL detecting the 820nm pump and find that its intensity at 2.2mA detected current is shot-noise limited at 2.5MHz modulation (see figure), as required for highest sensitivity. We perform SRS, CARS and transmission imaging in the lipid vibration region with on Calu-3 cells (see figure) for practical microscopy demonstrations.

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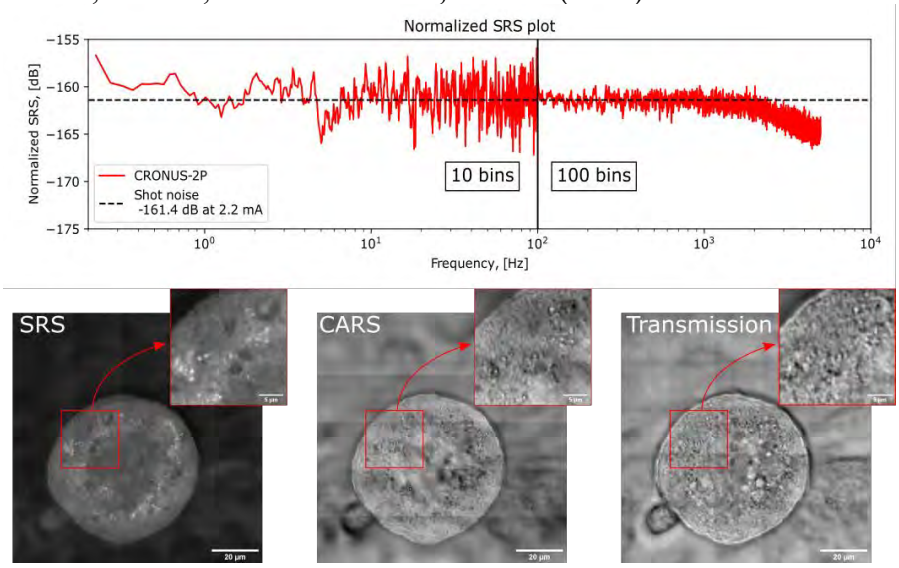

Top: SRL reference signal noise for 1Hz bandwidth sampled at 0.1ms at 820nm.
Bottom; SRL, CARS and pump microscopy of Calu-3 cells, at 2935/cm and 41/cm spectral resolution.

This work has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 812992.

In vivo monitoring disease progression in rodent models of inflammatory arthritis using fibre-optic Raman spectroscopy

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Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder characterized by synovial inflammation and pannus formation leading to destruction of local articular structure, bone erosion and functional disabilities [1]. Common research models of inflammatory arthritis in rodents such as collagen antibody-induced arthritis (CAIA) in mice [2] are associated with pain, discomfort, and distress for the animals involved. The severity and degree of arthritis can be assessed by a variety of methods such as histopathology, X-ray, CT and MRI, however these suffer from being either destructive, requiring many animals for longitudinal studies or time-consuming and expensive [3]. Raman spectroscopy can potentially provide a non-invasive, non-destructive alternative, capable of revealing disorder mediated biochemical alterations of tissue in live animals.

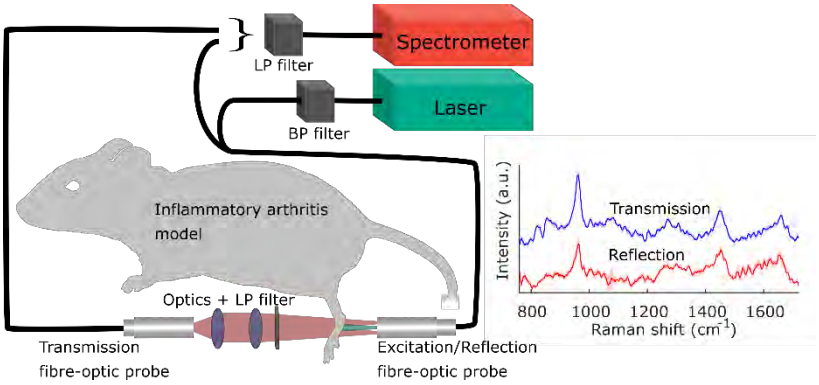

Figure 1: Conceptual illustration of Raman spectroscopy system for monitoring inflammatory arthritis disease progression in rodents.

Here we demonstrate *in vivo* assessment of biochemical changes in CAIA mice using a transflection Raman setup (Fig. 1). Mice with induced arthritis and controls were clinically and spectroscopically assessed for 14 days. Raman derived measures of tibiotarsal joint bone density correlated well with volumetric bone mineral density (vBMD) from *ex vivo* CT scans with low vBMD in mice exhibiting clinical symptoms of arthritis. The technique could potentially lead to a reduction in the number of animals needed, while improving research and development of novel therapeutic agents within the fields of bone and joint disorders.

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In vivo monitoring tissue development in bone scaffolds using Raman spectroscopy

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The development of novel biomaterials for regenerative therapy relies on the ability to assess and monitor tissue outcome *in vitro* and *in vivo* [1,2]. Non-invasive imaging modalities such as X-ray computed tomography offer high spatial resolution but limited biochemical information while histology and biochemical assays are destructive [3]. Here we demonstrate the use of fibre-optic Raman spectroscopy for non-invasive, label-free, and non-destructive quantitative monitoring of tissue development in subcutaneous bone scaffolds in mice over 16 weeks [4]. Raman spectroscopy was able to quantify the time dependency of different tissue components related to the presence, absence, and quantity of mesenchymal stem cells. Scaffolds seeded with stem cells produced 3-5 times higher amount of collagen-rich extracellular matrix after 16 weeks implantation compared to

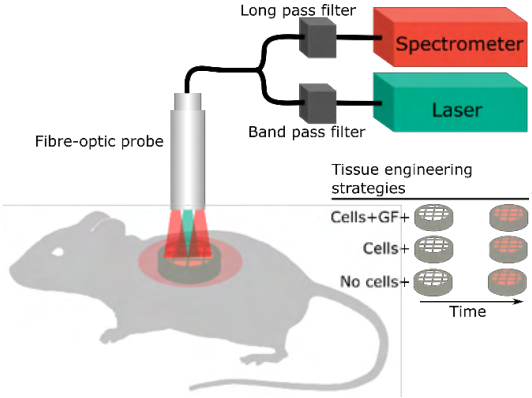

Figure 1: Conceptual illustration of spectroscopic system for biochemical monitoring of tissue development in bone scaffolds *in vivo* [4].

scaffolds without. These however, showed a 2.5 times higher amount of lipid-rich tissue compared to implants with stem cells. *Ex vivo* micro-computed tomography and histology showed stem cell mediated collagen and bone development. Histological measures of collagen correlated well with Raman derived quantifications (correlation coefficient *in vivo* 0.74, *ex vivo* 0.93). In the absence of stem cells, the scaffolds were largely occupied by adipocytes. The technique developed here could potentially be adapted for a range of small animal experiments for assessing tissue engineering strategies at the biochemical level.

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Nanoscale coherent Raman detection via the local field enhancement at a single plasmonic nanorod

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To date, we are still missing a label-free non-invasive detection method which can directly measure lipid nanodomains in living cells with high spatio-temporal resolution. Here, we show proof-of-principle experiments to chemically detect lipid molecules label-free using a epi heterodyne coherent anti-Stokes Raman scattering (eHCARS) technique [1] and exploiting the local field enhancement (LFE) occurring in the nanoscale region near a single plasmonic gold nanoparticle (AuNP).

We investigated spherical AuNPs, bare gold nanorods (AuNRs), and silica-coated AuNRs (SiAuNRs) (see sketch Fig.1c). Individual AuNPs were covalently bound to a glass surface, surrounded by silicone oil as the chemical substance to be detected. For optimum LFE effect, the longitudinal localized surface plasmon resonance of AuNRs was chosen to coincide with the CARS wavelength of the CH stretch vibration in silicone oil (~2900cm^{-1}), i.e. 660nm in our set-up. For this purpose, the extinction cross-section spectrum of each individual AuNR was measured (Fig.1a) [2] correlatively with LFE-eHCARS. The relative signal enhancement at each AuNP, named R factor, was evaluated as the difference between the CARS field measured at the AuNP position and away from it, normalized to the CARS field amplitude without AuNP (an in-plane eHCARS scan at the AuNP-glass interface is shown in Fig.1b).

Spherical AuNPs provided a small enhancement (R<1) compared to AuNRs (R~10) (Fig.1c). However, bare AuNRs and SiAuNRs with 5nm thick shell showed irreversible shape changes upon high laser power exposure, while a stable response was observed with SiAuNRs having a 10nm thick shell. These were then used in sensing measurements to detect small (30nm size) polystyrene beads, diffusing in a glycerol-water mixture. It should be noted that R=10 equates to a local field enhancement of more than 1000 in the nanoscale volume near the AuNP, as shown by COMSOL simulations of LFE-CARS which are ongoing and will be presented.

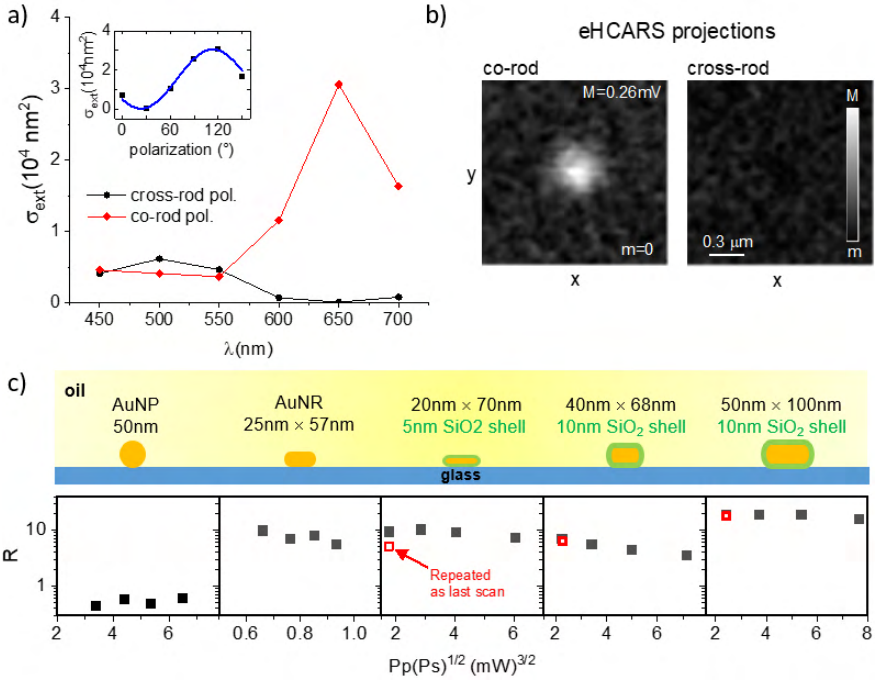

Figure 1: a) Extinction cross-section (σ_{ext}) of a silica-coated gold nanorod using wide field extinction micro-spectroscopy [2]. Inset: σ_{ext} at 700nm versus in-plane polarizer angle in the illumination. b) eHCARS amplitude spatially resolved in-plane at the CH vibration of silicone oil (2904cm^{-1}) showing the enhancement at the AuNR location. Circularly polarised excitation was used, and the detected field was projected along (co) and across (cross) the AuNR long-axis; c) Relative enhancement R factor of the AuNPs represented in the sketch. Exciting powers of pump (Pp) and Stokes (Ps) beams are shown as the product Pp(Ps)$^{1/2}$ in units of mW$^{3/2}$. A 1.45NA oil-immersion microscope objective was used for epi-CARS excitation and collection.

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Looking for significance of lipid droplets in vascular inflammation

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Lipid droplets (LDs) are cellular organelles with a unique architecture consisting of a hydrophobic core of neutral lipids, enclosed by a phospholipid monolayer decorated by a specific set of proteins [1]. For a long time, the functions of LDs were underestimated, and they were treated as a simple fat storage sites. Nowadays, it is known that LDs act as critical hubs of cellular metabolism to buffer levels of toxic lipid species and are implicated in numerous human diseases, including obesity, diabetes, fatty liver, and atherosclerosis [1]. Although general knowledge on endothelial LDs is growing, neither the precise function of LDs, nor the pathway of their biogenesis have been fully revealed [2]. This was the motivation for the microscopic and spectroscopic characterization of LDs formed in vascular inflammation and in the response to the excess of lipids.

Endothelial inflammation was triggered by pro-inflammatory factors: tumor necrosis factor (TNF), lipopolysaccharides (LPS), angiotensin II (AngII), or by hypoxia. We have demonstrated that in the TNF-, LPS-, or AngII-activated endothelium in the isolated murine aorta, endothelial LDs are formed but quickly metabolized. To overcome these limitations for the characterization of LDs in isolated aorta, we used atglistatin, a selective inhibitor of adipose triglyceride lipase (ATGL), which suppressed endothelial LDs lipolysis. Rapidly metabolized LDs in the TNF-, LPS-, or AngII-activated endothelium stood in opposition to the formation of stable endothelial LDs after hypoxia or oleic acid (OA) overload, when atglistatin usage was not necessary to observe stable LDs. Additionally, by comparing the Raman signature of endothelial LDs under hypoxic/OA conditions in the presence or absence of atglistatin, we have shown that atglistatin does not affect the biochemical composition of LDs.

Spectroscopic and microscopic characterization of LDs revealed that LDs formation is an integral part of vascular inflammation. Lipolysis of LDs was ATGL-dependent for all factors, including TNF, LPS, AngII, hypoxia, OA, thought with quantitatively different effects for atglistatin. The analysis of Raman spectra of LDs in the isolated vessels stimulated by TNF, LPS, AngII or hypoxia uncovered that they were all rich in highly unsaturated lipids and had a negligible content of phospholipids and cholesterols. Consequently, the biochemical composition of LDs in the activated endothelium was similar for all types of pro-inflammatory stimuli or hypoxia, contrary to the endothelial LDs in the aorta incubated with OA. Based on the presented results, *de novo* formation of LDs upon pro-inflammatory agents seems different than the pathway of LDs formation in response to the uptake of fatty acids and at least partially depend on intracellular sources of lipids rather than their uptake from the extracellular microenvironment.

ACKNOWLEDGMENTS

This work was supported by the National Science Centre, Poland, SONATINA1 No.: DEC-2017/24/C/ST4/00075. The participation in the conference was supported by the BioS Priority Research Area under the program "Excellence Initiative – Research University" at the Jagiellonian University in Krakow.

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Reference-free X-ray fluorescence for the molecular quantification: determination of SERS enhancement factor

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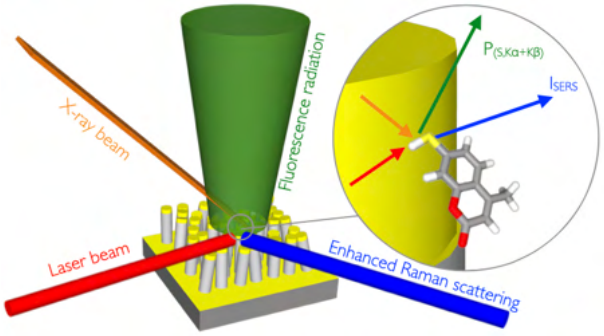
The enhancement factor (EF), indicating the magnification of the Raman signal of molecules interacting with the surface of plasmonic nanostructures, is a crucial parameter in the field of surface-enhanced Raman spectroscopy (SERS). Metrological calculation of EF requires a careful evaluation of both the signal intensities and the number of molecules in SERS and normal Raman conditions. The determination of the surface density of molecules adsorbed on the substrate is fundamental to estimate the number of active molecules contributing to the enhanced Raman signal on a plasmonic substrate and, for this reason, strongly impacts the estimation of the enhancement factor. A viable methodology for this challenging task is reference-free X-ray fluorescence (RF-XRF). We determined the EF using 7-mercapto-4-methylcoumarin (MMC) as probe molecule on gold-coated silicon nanowires, integrating SERS and normal Raman spectroscopy with synchrotron-based RF-XRF data that provide an absolute quantitative measurement of the molecular surface density [1]. In addition, the surface coverage of MMC on the substrate is modelled by molecular mechanics (MM) and molecular dynamics (MD) simulations. RF-XRF analytical quantification can be extended to other molecules or common analytes for SERS or fluorescence spectroscopy. The adoption of standardized methodologies for the characterization of nanostructured systems promotes inter-laboratory comparison and boosts the applicability and progress of SERS.



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XXVII International Conference on Raman Spectroscopy
Low-Level Organic Detection on Icy Worlds using the Compact Integrated Raman Spectrometer (CIRS)

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The Compact Integrated Raman Spectrometer (CIRS) as configured for the Europa Lander Mission Concept[1] is designed to analyze samples in their frozen, melted, and desiccated states. CIRS is equipped with an integrated sample handling system that would accept 3-5 sample cups each filled with approximately 1 cm^3 of icy regolith acquired nominally 10 cm below the surface. The bottom of each sample cup contains a transparent window through which Raman spectroscopy would be performed using 532 nm (green) continuous-wave laser excitation. We present results that explore the benefits of lyophilizing the melted sample onto the cup's window in order to concentrate its non-volatile constituents, as well as determining the organic detection limits that could be achieved using surface enhanced Raman spectroscopy (SERS).

While SERS has been shown to be capable of providing single-molecule detection[2], all SERS substrates lose their enhancement capabilities over time [3], many over the course of a few days or weeks, and some even if stored under vacuum[4]. Here we present results of coating the CIRS window with a layer of AgCl crystals which can be photo-reduced in-situ using light from the CIRS's laser or UV LEDs to form a SERS-active layer of silver nanoparticles. This method[5] of preserving SERS activity during long- duration space flight could potentially increase the sensitivity of in-situ planetary Raman instruments by orders of magnitude. Photo-reduced AgCl crystals have been shown to provide significant levels of SERS enhancement after photo-activation following storage for over 20 years. In this paper we will demonstrate the capabilities of this technique using organic laden icy simulants and explore the influence of salinity and pH on the limits of detection.

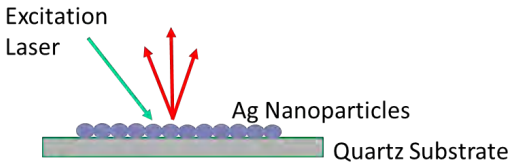

Figure 1: Surface Enhanced Raman

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XXVII International Conference on Raman Spectroscopy (SERS, a Single-molecule and Label-free Technique for Drug Discovery)

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The field of drug discovery relies on sensitive, specific, and rapid methods to identify hits. The ultra-high sensitivity and rich information obtained using Surface-enhanced Raman spectroscopy (SERS) render it an emerging technique for drug discovery.

Previously, we have developed a SERS platform with single-molecule sensitivity to detect a protein-linker adduct at a single-molecule level.[1] This platform has also shown the possibility of differentiating the proteins' spectral contribution from the linker's via visual inspection and statistical analysis. Therefore, we extended the application of this platform to detect the binding of a peptide ligand to targeted RNA repeats at a nanomolar concentration. The selected ligands are potential drug molecules that interact with disease-related RNA repeats. The binding trends found using SERS detection correlated with the binding affinity studies of different ligands. Furthermore, the binding ligand was also differentiated from the non-binding ligand and the RNA based on the analysis of the collected SER spectra. These differentiations were possible via visual inspection and statistical analysis.

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Raman Spectroscopy on Europa: A Radiation Challenge.

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Planetary exploration is a relatively new area for Raman Spectroscopy, but is gaining momentum, particularly on missions with goals surrounding the search for extra-terrestrial life and habitability. Three instruments have been built for Mars, and 2 flown [1,2,3]. With the technological advances this brings, Raman spectrometers are being considered for future missions elsewhere, including Jupiter's moon Europa. NASA's Europa lander science definition team have identified Raman spectroscopy as a suitable analytical technique for assessing the habitability on Europa and identifying surface composition including potential biosignatures [4].

Europa presents significantly different environmental hurdles to overcome than is typically associated with a mission to Mars. High levels of energetic particle radiation (such as high energy protons, electrons and ions [5]) and the extremely cold surface temperatures [6] must be carefully considered, and instrumentation appropriately designed to cope with the harsh environment. The radiation levels in particular prove the most challenging as damage to key spectrometer subsystems (for example semiconductor detectors and optics) has the potential to jeopardise the scientific performance and return of a Raman spectrometer. It is therefore paramount to ensure appropriate selection of robust components and mitigation strategies for instrument subsystems. In addition to the effects of radiation on the instrumentation, Europa's surface material is expected to be heavily radiation processed up to depths of below 10cm, altering the spectra.

We present an analogue sample set suitable for Raman spectrometer instrument optimisation within the scope of a mission to Europa. Samples have been curated to include minerals associated with biological processes, including hydrated salts (for example hydrated magnesium/sodium sulphates). The key science goals of recent mission proposals also informed sample selection which include the need to distinguish subtle changes in band position and/or shape, which must still be possible when the instrument has been subject to damage via radiation. In addition, we also present data collected from a series of radiation damage experiments where spectrometer subsystems and samples were exposed to radiation levels expected to be experienced on the harsh Europan surface. As well as better recreating mission conditions these experiments also test the instrument's ability to meet future science goals.

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Identification of immune cell phenotypes to study cell-material and tumor-immune interactions

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Immune cell differentiation and polarization are essential parameters to access their functionality, e.g. in response to an implant material or tumor. Conventional methods to characterize immune cells - such as surface marker or cytokine expression - often require invasive sample preparation and do not allow for time- or single cell-resolved measurements. Raman spectroscopy has become an established tool for the analysis of molecular composition in tissues and single cells, as it does not require fixation or labelling of a sample. It is thus especially suited for the continuous in situ monitoring of living cells in both static and dynamic cell culture systems.

Single-cell Raman microspectroscopy and imaging were established to (i) discriminate different immune cell subsets derived from peripheral blood mononuclear cells (PBMCs), (ii) monitor differentiation of monocytes to macrophages and dendritic cells, and (iii) to study the response of T-cells and macrophages to extrinsic stimuli such as cytokines or material surfaces. In this work, a set of immune cell Raman spectra was acquired comprising human primary monocytes, macrophages, dendritic cells, T-cells, and B-cells of healthy volunteers. All major PBMC subsets were discriminated and identified via multivariate data analysis based on their specific Raman bands. Furthermore, phenotypic changes within the subpopulations were analyzed. T-cell activation under static and dynamic culture conditions in a microfluidic tumor-immune in vitro model as well as macrophage polarization towards a pro- or anti-inflammatory phenotype were demonstrated and monitored via Raman microspectroscopy.

Our results demonstrate the sensitivity of Raman microspectroscopy to not only classify different immune cells, but to allow the monitoring of maturation and differentiation processes as well as cellular activation patterns. Raman imaging has the potential to become a non-invasive and low-effort alternative to conventional immune cell phenotyping techniques.

A study on the effect of functional groups of NIR Raman reporter dyes in quantitative analysis by NIR-SERRS-based LFA

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Lateral flow assays (LFA) are rapidly developing into widely employed point-of-care test (POCT) platforms beyond a pregnancy test kit due to its simple structure consisting of a nitrocellulose membrane and functional paper pads.[1] Due to the recent pandemic, research interest in rapid and accurate POCT technology has increased, and the LFA platform is being spotlighted. We recently reported a surface-enhanced resonance Raman scattering (SERRS)-based LFA platform for COVID19-related antibodies test that overcomes the shortcomings of conventional methods and dramatically improves detection sensitivity.[2] In addition, our previous work included the development of a miniaturized reader suitable for SERRS measurements based on 785 nm laser excitation.[3] This system enabled fast scanning through line focus illumination along the entire width of the test line; it was possible to obtain the most reliable trending signal by maximizing the averaged ensemble effect for the heterogeneous distribution of nanotags, which cannot guarantee the uniformity of the signal based on point mapping methods in the low-concentration range. To utilize this system suitably, it is necessary to carefully consider the reporter molecule of nanotags, which is a key factor in the SERRS sensitivity. Here, we studied the effect of functional groups of NIR Raman reporter dyes constituting SERRS nanotags on the quantitation of SERRS-LFA using this system. We investigated five similar NIR dyes containing isothiocyanate (ITC) and/or sulfonic acid (SA) groups (Fig.1 (a)). A strong SERRS response was shown by efficient adsorption on nanoparticles according to the number of ITC groups (I). On the other hand, as the number of SA groups (S) increased, the stability of nanotags was secured, however, it had a negative effect on adsorption efficiency as shown in Fig.1 (b). We constructed SERRS nanotags using each molecule and investigated the effect on quantitation by using the LFA strip for the beta-hCG known as a pregnancy marker (Fig.1 (c)). As I was increased, the quantitative sensitivity was positively affected, and as S was increased, the stability of molecular adsorption was reduced, making quantification impossible. Based on this study, we identified the factor of the optimal reporter molecule that should be considered for an optimized SERRS nanotags-based quantitative assay platform.

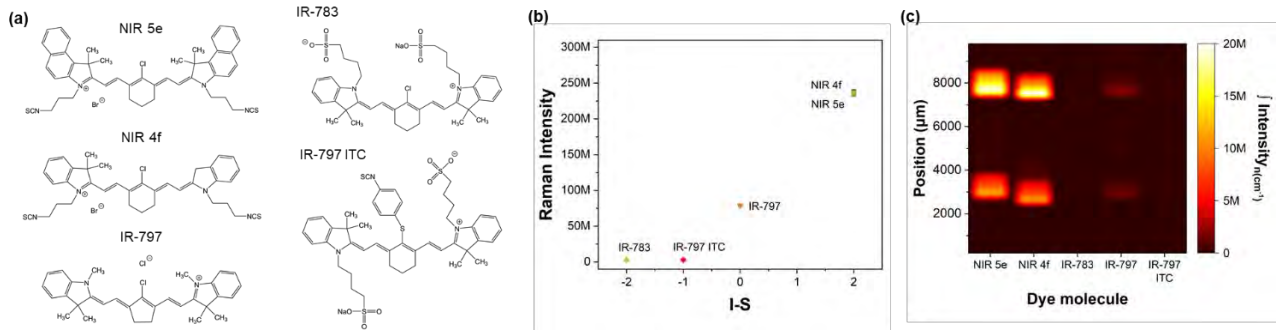

Figure 1. (a) Molecular structures of five similar NIR Raman reporter dyes, (b) a comparative plot of Raman intensity according to I-S (I = the number of ITC groups, S = the number of SA groups) value of each reporter dye, and (c) sensitivity comparison through SERRS scanning of beta-hCG LFA strips

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Raman scattering study of phase transition in methyl ammonium lead halide perovskite single crystals

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Over the years, many studies on photovoltaic absorber materials have been conducted to improve the efficiency of photovoltaic devices. However, there are many challenging issues to overcome for the solar cell materials research ensuring longer lifetime of the cell and better chemical stability for practical application, for example. In particular, for organic-inorganic hybrid perovskite materials, the role and characteristics of organic cations that remain unclear. In this study, we focus on understanding the fundamental structural properties.One of the remarkable features of organic-inorganic hybrid perovskite is that it has different phases as the temperature varies. For example, $CH_3NH_3PbBr_3$ shows a phase transition from cubic to tetragonal structure at ~235K and $CH_3NH_3PbCl_3$ shows similar phase transition at ~179K. We measured temperature-dependent Raman spectra in single crystalline $MAPbBr_3$ and $MAPbCl_3$ samples and observed abrupt changes in spectra from both samples at the phase transition temperatures. Our results show that contributions to the phase transition in each atomic/molecular vibration are different but there are common features existing for different compounds. We show that Raman scattering spectroscopy is a very effective way for studying structural phase transitions in complex materials.

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ULF TERS imaging –a novel technique for assessing the layer interaction in vertical heterostructures of 2D semiconductors.

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Tip enhanced Raman scattering (TERS) and tip enhanced photoluminescence (TEPL) are effective tools for the assessment of nanoscale heterogeneity in mono- and a few- layer 2D semiconductors and their heterostructures. Due to their non-destructive nature, TERS and TEPL imaging can be cross-correlated with other images conveniently recorded using scanning probe microscopy (SPM), such as topography, surface potential, photocurrent etc, which further improves our understanding of 2D semiconductors. Recent interest of the community in vertical heterostructures of graphene and transition metal dichalcogenides (TMDs) with precise control of the twist angle between the layers motivated us to apply and extend TERS imaging to the characterization of the WS_2-WSe_2 vertical heterostructures.

WS_2-WSe_2 heterobilayer samples were exfoliated to PDMS and then transferred to a gold-coated silicon wafer and were confirmed to feature large areas (tens of microns across) showing the presence of a lattice-like Moire pattern with 6-8 nm pitch. Additionally, as it almost inevitably occurs with the stacked layers exfoliated in air, large number of nanobubbles were present in the samples. TERS imaging of larger bubbles with 785 nm excitation revealed extreme heterogeneity of the TERS response across the bubble- at the outer edges we observed strong bands coming from WSe_2 and additionally- a predominant ultralow frequency (ULF) TERS peak at around 23 cm^{-1} that arises from the interlayer phonon mode was observed. To the best of our knowledge this is the first time demonstration of ULF TERS that track low frequency inter-layer phonons, and therefore, directly report on local structure with nanometer spatial resolution. Interestingly, this peak disappeared towards the center of the bubble, while an A'/A_{1g} peak of WS_2 simultaneously appeared at ~416 cm^{-1}. Careful correlation analysis of the recorded spectral nano-images showed that this WS_2–like peak was correlated to a local exciton. The same analysis paints a unique picture about the two layers in the bubble region, which seems to be comprised of WS_2-WSe_2 bilayers that are separated at the apex of the nanobubble. It's a rather remarkable observation taking into account that the lateral spatial extent of this decoupling took place on the 10's of nm length scale.

Again, somewhat surprisingly, TERS response of the areas in between the larger bubbles looked patchy showing great similarity to the response from larger bubbles which indicates that the sample had a developed network of smaller and larger nanobubbles that inevitably affected the electronic band structure of WS2-WSe2 vertical heterostructures, which brings us to the conclusion that any far field Raman spectra obtained on exfoliated vertical heterostructures should be taken with certain caution unless a solid proof of the lack of nanobubbles in the investigated sample is provided.

Finally, we should note that despite the demonstrated utility for characterization of the layer interaction in vertical heterostructures, ULF TERS of 2D semiconductors requires significant work for proper understanding of the resonant effects and the resulting band assignments. Unlike the case of conventional ULF Raman response in TMDs, ULF TERS response changes quite dramatically as the excitation laser wavelength changes, both the intensity and the spectral position-wise. Examples of this apparently resonant behavior will also be presented.

Assessment of formulated product performance with stimulated Raman scattering microscopy

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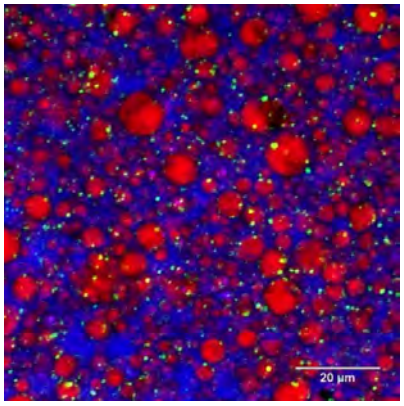
Confocal Raman spectroscopy is a well-established tool to map chemical distribution in formulated products, for example pharmaceutical tablets. However, for high resolution imaging, or investigating dynamic processes, the relatively long acquisition times to generate 3D maps can be limiting.

Stimulated Raman scattering (SRS) microscopy is a 3D imaging technique based on Raman-contrast capable of real-time examination of formulated products and their permeation into biological tissues such as skin, with lateral resolution of approximately 400 nm, 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 a wide range of materials, including the study of topical drug formulations, including the real-time disposition of actives and excipients, the "metamorphosis" of applied formulations, and nanoparticle distribution.[1-3] This technique offers unique insight into formulation properties and behaviour post-application, and can reveal mechanistic information regarding the penetration pathway.

Figure: SRS and SHG multimodal image of sunscreen formulation showing the oil phase in red, the aqueous phase in blue, and the zinc oxide in green.

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Frontiers of synchrotron-based UV Resonance Raman spectroscopy for exploring biological macromolecules

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Thanks to the resonance effect, UV Resonance Raman (UVRR) spectroscopy offers several advantages with respect to spontaneous Raman, such as a significant increment of the detection limit and the selectivity needed to incisively monitor specific chromospheres within the sample. These conditions, together with the strong reduction of the interfering fluorescence background, determines the usefulness of UVRR spectroscopy as highly sensitive and selective spectral probe for exploring the structure and dynamics of a wide range of complex systems [1]. However, the full exploitation of UVRR has so far been limited by the lack of tunable excitation sources of appropriate intensity that allow to finely approach the resonance conditions of specific targeted molecular groups. Additionally, the chance of extending the unique capabilities of UVRR to the UV domain (i.e. up to 10–15 eV) opens the possibility to cover the whole range of outer electronic excitations in matter. In this contribution, we will present the unique in the world UVRR setup working with the synchrotron radiation (SR) source available at the BL10.2-IUVS beamline (Elettra synchrotron facility, Trieste, Italy [2]). The SR-based UVRR set-up at Elettra enables to perform UVRR experiments with a fine tunable source in the range of excitation wavelengths 127-270 nm, resulting in an innovative spectroscopy facility for approaching open issues in physics, chemistry, materials and life sciences. Selected case studies will be discussed in order to show the useful of SR-based UVRR method and the areas of interactions with other research interests, with particular attention to the study of biological macromolecules.

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The Impact of Nanoparticle on Early Developing Mammalian Embryos Evaluated using Raman Spectroscopy

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Laser based spectroscopic methods can be versatile tools in investigating early stage mammalian embryo structure and biochemical processes in live oocytes and embryos. The limiting factor for using the laser methods in embryological studies is the effect of laser irradiation on the ova. Our previous work had explored the optimal parameters of the laser exposure in Raman spectroscopic measurements applicable for studying live early embryos *in vitro* without impacting their developmental capability [1]. In this work, using Raman Spectroscopy, we propose a method to evaluate the embryo quality before and after interaction with amine-functionalized nanodiamond (NDA); NDA is a surface functionalized ND that usually served as a platform for further conjugation on bio or medical molecules on the ND surfaces for applications. In this report, we study the interaction of the nanodiamond with the developing mammalian embryos using Raman spectroscopy in an effort to address the Nano-safety of nanoparticle interaction with the embryo in the mammalian reproduction systems.

Embryos at the 2-cell stage were recovered from the oviducts of the female mice after successfully mating with male mice. Embryotoxicity was tested for hybrid and homogeneous 2-cell live mice embryos. Recovered 2-cell embryos were treated with 37.5 μg/ml of NDA mixed with embryo culture media and exposed in different time frames of 30min, 1h, and 24h. Monitoring was done by estimation the morphological appearance of the embryo. Experimental results and statistical results suggest that exposition to the ND does not exhibit a significant detrimental effect on embryonic development. Spectroscopic analysis using Raman spectroscopy is presented as an additional method of estimation of the embryo by determining the embryo quality based on molecular information obtained which is non-invasive and under safe parameters for the live embryo. The Raman spectra of the embryo blastomeres were measured using 785 nm NIR and 532 nm wavelengths laser excitation. Spectra were taken on different embryo areas, mainly the Cytoplasm and lipid drops in the cytoplasm (LRA). Raman spectra variations are minimal, mostly arising from the distribution of Cytochrome c, which is proposed as a determining marker for quality, and hence the safety of the embryo development.

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Molecular platform for frequency upconversion at the single-photon level

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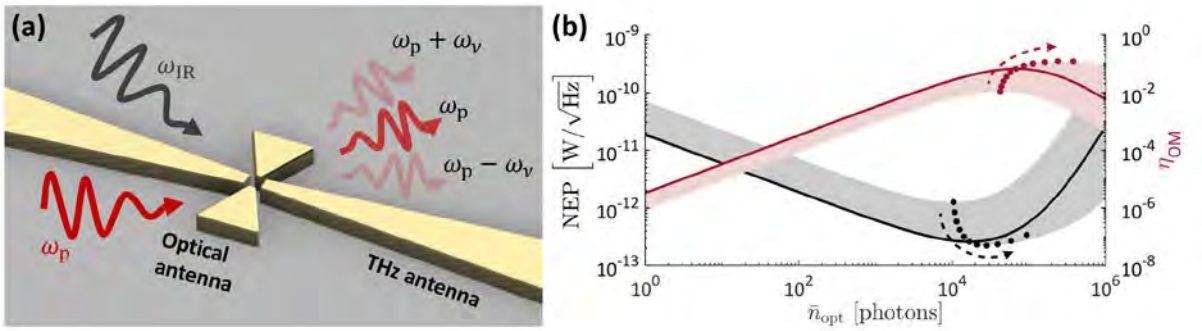
As applications in fields like security or medicine require sensitive schemes in order to detect THz photons, an interesting strategy consists in converting weak THz signals into the optical domain where detectors with single photon sensitivity are readily available. We introduce here a novel platform for ultra-sensitive conversion and detection of THz and mid-infrared signals, which is inspired by our previous work where we describe the interaction between molecular vibrations and plasmonic antenna using the model of cavity optomechanics [1]. Our study quantified the nonlinear coupling rate g_0 and revealed that it could be as high as tens of THz. We also predicted signatures of optomechanical amplification that should be observable in state-of-the-art systems [2]. Novel plasmonic platforms could thus enable the realization of protocols inspired by cavity quantum optomechanics.

The protocol that we suggest here [3] benefits from the intrinsic ability of specific molecular vibrations to interact both with optical and THz fields as routinely observed in Raman and resonant absorption spectroscopy. To insure an optimal overlap between the two beams and the molecular system, doubly resonant nano-antennas confine the fields into similar mode volumes (Fig. 1a) and increase therefore the efficiency of the conversion process.

In this conversion scheme, an incoming THz field drives resonantly a vibrational mode and modifies its excited state population, which is mapped onto the scattered anti-Stokes Raman signal produced during the interaction between the same vibrational mode and an optical pump beam (Fig. 1a). When the optical beam is red-detuned from the plasmonic resonance the interaction Hamiltonian reduces to a state swapping Hamiltonian: $\hat{H}_{\text{eff}} \propto -\hbar g_0 \left(\hat{a}^\dagger \hat{b} + \text{h.c.}\right)$, enabling an efficient optomechanical conversion process [4]. Consequently, the modified vibrational population gives rise to an additional emission of coherent optical photons on the anti-Stokes sideband that can be detected with existing single photon counting techniques.



The noise equivalent power (NEP, left axis, black line) and the internal conversion efficiency (η_{OM}, right axis, red line) of the molecular platform as a function of intracavity optical photon number are shown in Fig. 1b. Our study demonstrates that the NEP can be as low as few pW·Hz$^{-1/2}$, improving on the state of the art for devices operating at room temperature. In addition to its low noise figure the sub-wavelength dimensions of the proposed converter promises the development of multi-spectral systems designed for THz source recognition and novel technological platforms harnessing the coherent nature of the conversion process.

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XXVII International Conference on Raman Spectroscopy
Raman fingerprint as biomarker for the diagnosis of neurodegenerative diseases

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The pathogenesis underlying specific neurodegenerative diseases remains incompletely understood. The lack of quantitative and easily accessible biomarkers demands the development of new powerful, fast and sensitive techniques to efficiently probe, monitor and evaluate the disease onset and progression, as well as the different phenotypes onset. In this contest Raman spectroscopy is an established technique for the creation of a label-free, repeatable, fast and automatable signal that represent a global overview of all the biochemical specimen present in a selected biofluid [1]. The term "Raman fingerprint" indicates the output uniquely attributable to a specific subject in a particular physiological/pathological state (Figure 1). Taking advantage of Raman spectroscopy, at Laboratory of Nanomedicine and Clinical Biophotonics (LABION) we are developing Raman-based classification models that can easily discriminate and stratify the onset of different diseases including Amyotrophic Lateral Sclerosis, Alzheimer's disease and Parkinson's disease. Using this approach, we were able to detect a pathological Raman fingerprint from the analysis of different biofluids including saliva, serum and extracellular vesicles, identifying also correlations with the clinical and paraclinical tests used nowadays for the diseases identification and monitoring.

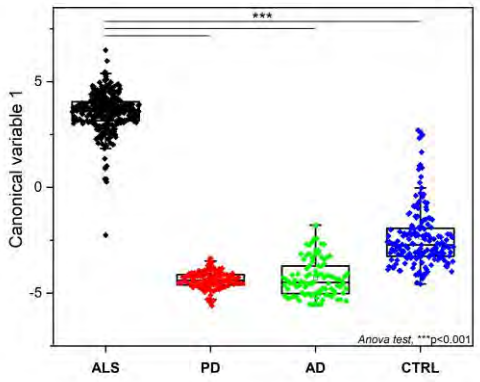

Figure 1: Multivariate Analysis on salivary Raman fingerprints collected from Amyotrophic Lateral Sclerosis patients (ALS), Alzheimer's (AD) and Parkinson's (PD) patients, and from healthy controls (CTRL).

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Single-cell Raman coupled with stable isotope labelling to study antibiotic resistance and its spread

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Antibiotic resistance that reduces the effectiveness of antibiotics against bacterial pathogens is posing a great threat to public health. This problem is being aggravated by the misuse and abuse of antibiotics. More seriously, resistance continues to spread rapidly in both clinical settings and environments via horizontal gene transfer (HGT). HGT allows antibiotic resistance genes to exchange within and cross a variety of microbial species and even concentrate in the same cell, driving the evolution of untreatable superbugs. To mitigate the occurrence and spread of antibiotic resistance, not only rapid antibiotic susceptibility testing (AST) is urgently needed to guide fast and tailored antibiotic prescription before treatment, HGT must also be understood and included in strategies to prevent the emergence of resistant organisms in clinical settings and environment.

Here, we developed a rapid activity-based phenotypic AST approach directly applicable for clinical urine samples without the need of pre-cultivation via single-cell Raman spectroscopy coupled with heavy water labeling [1]. The total assay time from receiving urine to binary susceptibility/resistance (S/R) readout was shortened to only 2.5 h, enabling a rapid diagnosis and timely guidance of antibiotic therapy for clinician (Figure 1). More than 7 antibiotics of different action mechanisms and 14 bacterial pathogens were demonstrated to work well with Raman-heavy water AST assay, including *Klebsiella variicola, Escherichia coli, Providencia rettgeri* etc. We further modified the labelling strategy to greatly improve the detection sensitivity, and applied the new strategy to study horizontal gene transfer via transformation from extracellular DNA to human pathogens. The subpopulation of transformants with phenotypic antibiotic resistance was revealed and the transfer frequency was calculated based on Raman signature to evaluate the transformation risk of different resistance genes. These new approaches are important for halting antibiotic resistance.

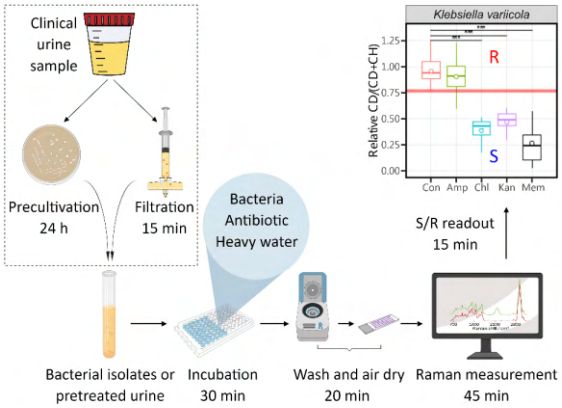

Figure 1: Workflow for antibiotic susceptibility testing via single-cell Raman-heavy water from clinical sample collection to susceptibility/resistance (S/R) readout.

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RLS, a Raman Spectrometer for Mars Exploration

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The Raman Laser Spectrometer (RLS) instrument (Figure 1) has been the first Raman spectrometer fully qualified for space [1] inside the framework of Exomars 2020 Mars mission, now postponed for launch in 2022. In parallel two other spectrometers have also been qualified for NASA Mars 2020 mission. A remote Raman included in the combined SuperCam instrument [2]. And Sherloc, a contact Raman instrument working in the deep UV excitation range [3]. In this work the final stages of the RLS flight model (FM) development and performance tests are presented before and after delivery for integration inside the ExoMars Rosalind Franklin rover [4]. In particular, a comparison between the results obtained at room conditions and relevant Martian conditions are discussed. This work is mainly focussed on spectra acquired on the RLS calibration target, which are related with the capability of the instrument to perform collaborative analysis inside the rover's Analytical Laboratory (ALD) with the two other ALD instruments MicrOmega [5] and MOMA [6]. A key point of this analysis is to correlate the spectrum with a particular position in the target. This will provide unique capabilities to the mission allowing the combined analysis (in the same spot) of powdered samples at the mineral grain scale with different and complementary techniques.

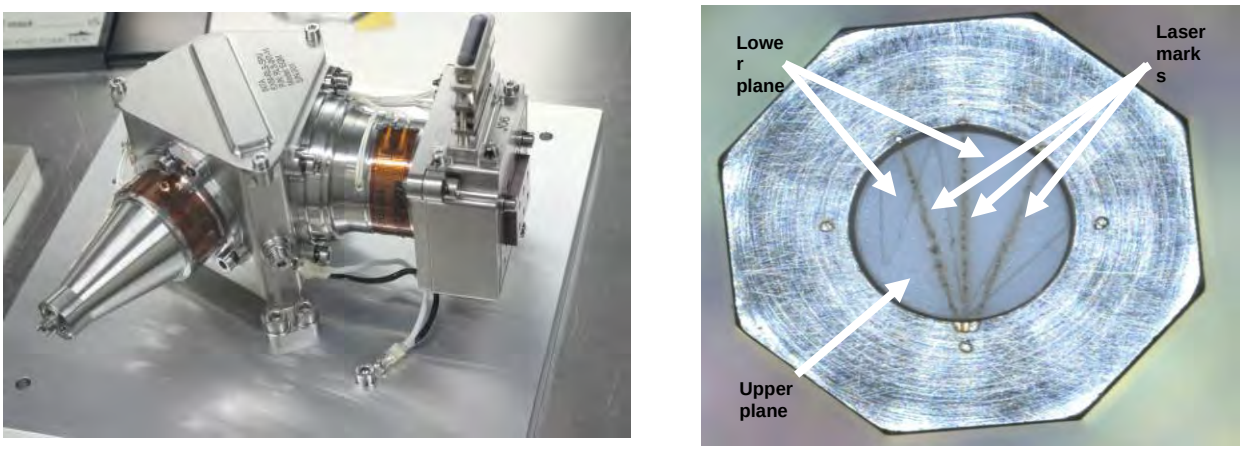

Figure 1: The RLS-FM spectrometer (left) and the special designed calibration target (right)

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Label-free characterization of rare-cell populations by high-throughput Raman flow cytometry

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Characterizing cellular heterogeneity is important for understanding functions of entire biological systems, which consist of many cells. In such a system, rare-cell populations often play biologically important roles while their detection is technically challenging in conventional bulk analysis. Flow cytometry is a suitable tool for investigating heterogeneous ensemble of cells by virtue of its high-throughput analytical capability with single-cell resolution. However, target molecules are limited to those stainable by fluorescent probes, which exclude single-cell quantification of many small molecules such as metabolites. Here, we present label-free characterization of rare-cell populations based on the concentration of metabolites in microalgal cells with our broadband Raman flow cytometer that builds on a rapid-scan Fourier-transform coherent anti-Stokes Raman scattering (FT-CARS) spectrometer [1,2], by which we can perform label-free quantification of biomolecules in many cells (> 3,000 cells/s).

With our Raman flow cytometer, we investigated time-dependent change of carbohydrates and chlorophyll in *Muriella zofingiensis* cells. Single-cell Raman spectra of *M. zofingiensis* cells 1- and 3-days after the replacement of the culture medium were measured (N=10,500 for each condition). As shown in Fig. 1a, the distribution of carbohydrates' content shows significant heterogeneity both on Day 1 and 3. While the amount of carbohydrates decreased on average, a small population of cells indicated by the boxes behaved differently from the average. Fig. 1b shows the spectra obtained by averaging different regions. The Raman spectral profiles seen in the rare-cell populations are overwhelmed in the spectra averaged over the total population, which shows the unique capability of our method for finding rare-cell populations based on the concentration of intracellular metabolites in a label-free manner.

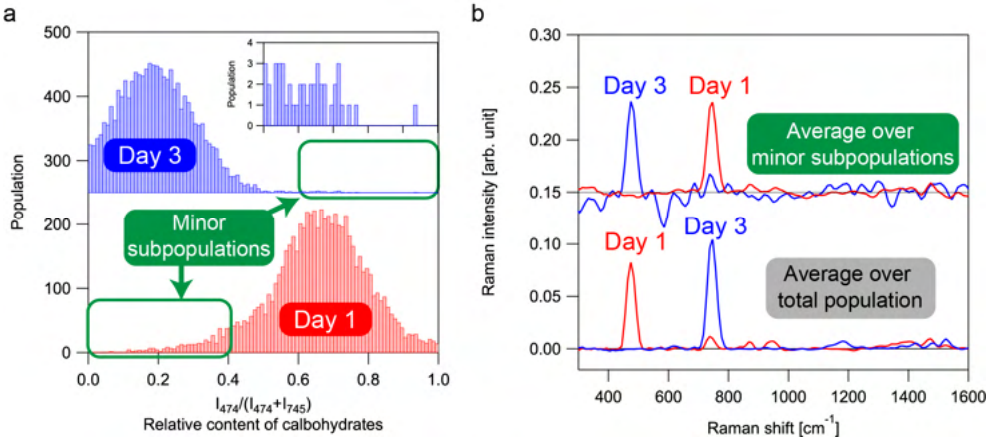

Figure 1: Characterization of rare-cell populations by high-throughput Raman flow cytometry a: Histograms of relative carbohydrates' content in single *M. zofingiensis* cells on Day 1 and 3. b: Raman spectra of *M. zofingiensis* on Day 1 and 3 obtained by averaging the population shown the indicated region in panel a (Top) and the total population (Bottom).

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Adsorption and plasmon-assisted dimer formation of aromatic thiols from surface-enhanced hyper Raman scattering (SEHRS) and SERS

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Humboldt-Universität zu Berlin

Surface-enhanced hyper Raman scattering (SEHRS), the two-photon excited analogue of SERS, has been shown to be particularly sensitive to changes in local environment and orientation of molecules that interact with a metal surface, due to the selection rules of the hyper Raman scattering (HRS) process and the particular requirements of the strong enhancements observed for SEHRS [1, 2]. We discuss the non-resonant SEHRS spectra of six aromatic thiol molecules during their interaction with gold and silver nanostructures. Spectra were obtained from thiophenol, benzyl mercaptan, and phenylethyl mercaptan and from the three isomers 2-aminothiophenol (2-ATP), 3-aminothiophenol (3-ATP), and 4-aminothiophenol (4-ATP). All SEHRS spectra were excited off-resonance at a wavelength of 1064 nm. The SEHRS spectra show a different interaction of thiophenol, benzyl mercaptan, and phenylethyl mercaptan with silver and gold nanostructures. This leads to a variety of combined SEHRS and SERS experiments for applications in the characterization of surfaces and for analytical sensing and labelling.

2-ATP, 3-ATP, and 4-ATP show a different interaction with gold nanostructures that was found to depend on pH. [3] Based on SERS experiments at many different conditions, we find that under very low excitation intensities, at high pH, an in the presence of metal cations, the plasmon-assisted oxidation of 4-ATP to DMAB takes place via formation of a metal oxide from ionic species. [4] In the two-photon excited SEHRS experiments, the plasmon-catalyzed dimerization can take place due to the high peak intensities coming from the ps laser pulses used for excitation. It is found that dimer formation by 2-ATP, leading to formation of 2,2'-dimercaptoazobenzene (DMAB), is very efficient. [3] The observation of the plasmon-catalyzed reaction by SEHRS suggests that the combination of SEHRS and SERS could be very beneficial for the further elucidation of plasmon-assisted reaction mechanisms, especially in the NIR frequency range.

Funding by ERC Starting grant No. 259432 MULTIBIOPHOT to J.K., a Chemiefonds Fellowship (FCI) to F.M, and DFG GSC 1013 SALSA to Z.Z. and Z.H. is gratefully acknowledged.

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35 word Abstract

We observe the non-resonant SEHRS spectra of six aromatic thiol molecules during their interaction with gold and silver nanostructures. Among possible applications, the plasmon-catalyzed formation of dimercaptoazobenzene in the two-photon excited SEHRS experiments is discussed.

Nanoscale Structural Characterization of Biological Systems Using Combined Nano-Raman and Nano-Infrared Spectroscopices

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The rise of nanotechnology and the desire to investigate the hierarchical structure of biological materials to the finest scale has rendered the characterization of materials and biological samples at the nanoscale a priority. However, the best possible spatial resolution obtainable with conventional Infrared (IR) and Raman microscopies is limited by light diffraction to approximately half of the wavelength of light, i.e. 1.5 μm to 10 μm in the IR, and 250 nm to ≈ 500 nm for Raman, which typically relies on a visible or near-IR laser. Not only the spatial resolution of conventional IR and Raman microscopies is insufficient to capture nanoscale details, the wavelength dependent resolution of IR spectroscopy also makes the comparison of chemical maps obtained with the two techniques somewhat difficult.

The coupling of Raman and IR spectroscopy with Scanning Probe Microscopy (SPM) has provided scientists with a common platform to overcome the limitations imposed by light diffraction, pushing the spatial resolution to the nanoscale and beyond. In my talk, I will discuss two nanoscale analogs of Raman and IR spectroscopy, namely: tip-enhanced Raman spectroscopy (TERS) and photothermal induced resonance (PTIR), which is also commonly known as AFM-IR in the IR spectral range.[1] Despite sharing the same scanning probe platform, TERS and AFM-IR have evolved independently and are based on different physical mechanisms. While the SPM tip is the key enabling factor in TERS measurements, details of the thermal expansion dynamic of the sample and the oscillation dynamics of the SPM cantilever are critical to understand the fine details of AFM-IR spectral intensities.

In my talk, I will discuss complementarity of AFM-IR and TERS for structural characterization of biological samples.[1] On the example of plant epicuticular waxes, I will show that AFM-IR and TERS provide completely different but complimentary information about structure and composition of this biological polymer.[2,3] I will also discuss complementarity of AFM-IR and TERS for structural characterization of amyloid fibrils and oligomers, protein aggregates that are directly linked to a broad spectrum of neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases.[4] Lastly, I will show how AFM-IR and TERS can be used to probe structure of viruses.

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Moiré phonons in twisted bilayer MoS_2

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The van der Waals heterostructures (vdWHs) have ultraclean and atomically sharp interfaces, providing a versatile platform for studying interface physics. Also, the material choice of the components, layer thickness and interlayer twist angle θ widely enrich the vdWHs and provide additional degrees of freedom to engineer their optical and electronic properties. The moiré patterns in vdWHs create a periodic potential for electrons, excitons and phonons to yield many interesting phenomena. Here, in the twisted bilayer MoS_2 (tBLM), one of the simplest prototype of vdWHs, we show how the periodic potentials of moiré patterns modulate the phonon behaviors to generate the moiré phonons. We report the observation of new Raman modes related to moiré phonons in as-grown/transferred tBLMs with different twist angles, which are folded from the off-center phonons in monolayer MoS_2. By varying the twist angle, the moiré phonons of tBLM can be used to map the phonon dispersion of the constituent layer (Figure.1). The lattice dynamics of the moiré phonons are modified by the patterned interlayer coupling resulting from periodic potential of moiré patterns, as confirmed by density functional theory calculations. Furthermore, with the excitation energy approaching the C exciton energy, the moiré phonon modes in all tBLMs can be strongly enhanced. This study can be extended to investigate Raman spectra in various twisted systems to deeply understand their moiré phonons, lattice dynamics, excitonic effects and interlayer coupling.

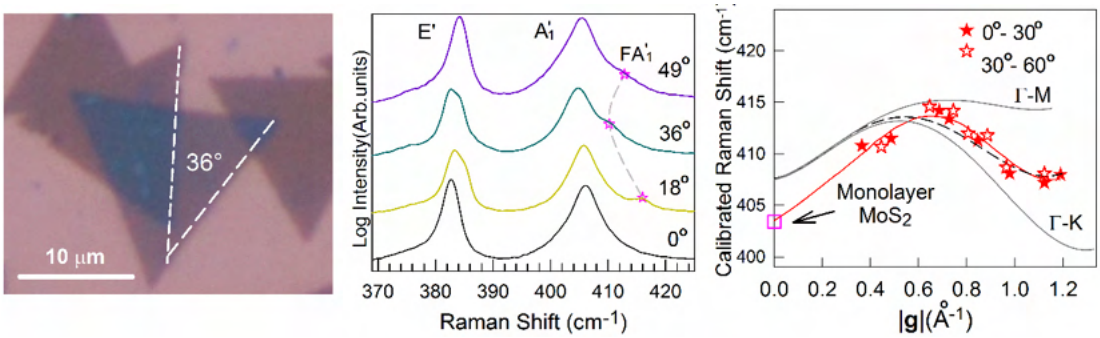

Figure 1: The optical images and Raman spectra of tBLM in the A'_1 spectral region by varying the twist angle. The wavevector-dependent frequencies of Moiré phonon related to mode are summarized, along with the theoretical phonon dispersion of A'_1 mode along the Γ-K and Γ-M directions are shown.

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Unsupervised Raman spectroscopy imaging of bio-interfaces in analogue samples in preparation for space missions

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In 2020 and 2022, Raman spectrometers will be deployed on Mars to study the composition of the first two meter of Mars' surface when the NASA's Mars 2020 and the ESA/ROSCOSMOS'ExoMars rovers are launched [1]. Raman spectrometers have the capability to detect geological substances constituting the rocky surface of Mars (inorganic molecules and inorganic molecular ions), which will provide information on the habitability of the planet, either past and present. In addition, Raman instruments have the ability to detect potential biological-derivative substances, often referred to as biomarkers (organic molecules originating from extent or extinct living organisms). In preparation for space mission, intended to use dedicated instruments developed with constraints such as minimal power budget, mass budget and data budget, documenting the detection capability of miniaturized Raman spectrometers is essential. Terrestrial analogue samples are important to address that capacity [2]. Amongst other analogue samples, those comprising bio-interfaces are of particular interest. Bio-interfaces are almost omnipresent in biological systems. In analogue samples, microorganism colonies are often thriving on or in rocks, using the mineral as both nutrient sources and protection against deathly UV irradiation, mechanical treats and desiccation [3]. Often, the functional properties of the bio-interface are significantly affected by their close micro-chemical environment. Actually, the molecular composition of the bio-interfaces is spatially affected in response to various changes of the micro-environment. To follow the variation of the chemical composition across analogue samples presenting a bio-interface, Raman molecular imaging approaches (implying that spectral data are recorded at different locations on the surface of a sample) have emerged as powerful implementation of molecular analytical techniques [4]. Raman spectroscopy was successfully applied to study the chemical composition of a wide variety of biological samples: from plants to animals, from single cells to tissues (either healthy or diseased). Raman imaging enables to obtain molecular images with a high spatial resolution. Yet, the power of Raman imaging was scarcely implemented with space designed instrumentations for future missions.

Here, we discuss the challenges associated with the integration of Raman Spectroscopy Imaging on analogue samples such as meteorite and biocrusts. From the experimental preparation of the sample to the implementation of simple chemometrics statistical tools to identify underlying molecular trends in association with the microstructure of the samples. In particular, we will compare spectral imaging data recorded with benchtop instruments and miniaturized spectrometers with operating modes similar to the future ExoMars Raman Laser Spectrometer. We will also discuss the possibility and the challenges associated with the combination of Raman imaging with Mass Spectroscopy Imaging, offering a higher molecular specificity, but a higher spatial resolution.

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Non-invasive monitoring maturation process of hepatocytes by Raman Microscopy

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Along with the development of regenerative medicine, which is aiming at fabricating artificial tissues and organs for transplantation, there is an increasing demand for non-invasive methods to monitor the growing process of different cell populations and evaluate the quality of the final tissue constructs. However, conventional methods generally require disruption of the tissue transplants, such as tissue sectioning and immunostaining. A method which can discriminate and access different cell population in a label-free and non-destructive manner is necessary for this field.

Raman microscopy has emerged as a powerful tool in label-free observation and characterization of biological samples since it can detect vibrational frequencies given by the chemical structure of the molecules. After simply shining the laser light onto the living specimens, specific biochemical information of cells and tissues can be collected and analyzed without additional treatment, enabling non-destructive quality control of cells and tissue constructs for the subsequent transplantation.

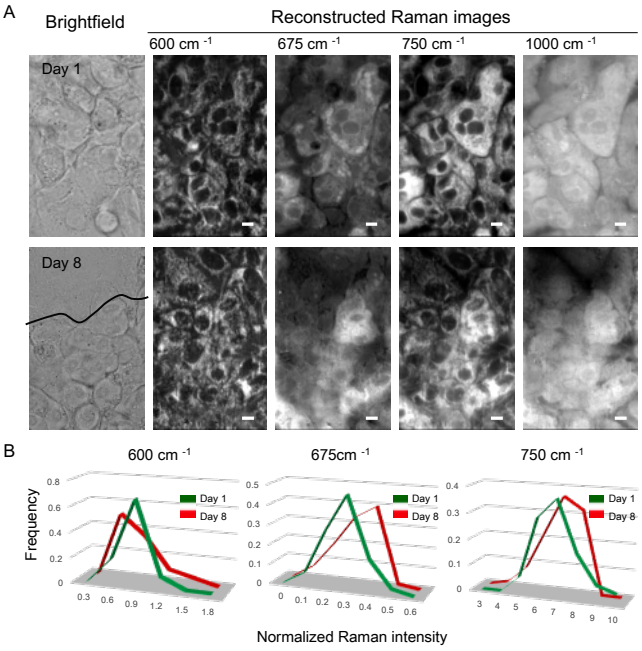

Figure 1: Time-course monitoring maturation of hepatocytes. (A) Reconstructed Raman images at 600, 675, 750 and 1000 cm^{-1}. Scale bar: 10 µm. (B) Plot of Raman intensity histogram at different culture day. Raman intensity was normalized by intensity at 1000 cm^{-1} peak (phenylalanine).

We reported several Raman peaks which can be assigned for the differentiation and maturation markers of the hepatocytes. Reconstructed Raman images depicted the distribution of different cellular components, showing the variance of Raman intensity at the single-cell level (Fig. 1A). To quantify this variance, the averaged spectrum of individual cell was extracted and Raman intensity at each peak was calculated. A plot of Raman intensity histogram was shown in Fig. 1B. After 8 days of culture, cell population with less 600 cm^{-1} and more 675 cm^{-1} intensity increased which correlates with hepatocyte maturation. Different cell populations were also identified at the end of culture (data not shown). In this research, cell maturation process was monitored and different cell population was identified by Raman Microscopy. It suggests that Raman microscopy has a great potential in evaluating the quality of cell products for regenerative medicine.

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SERS spectroelectrochemical study of the first stages of electrochemical and chemical aniline oxidation at different pH

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There are several types of aniline oligomers that can be formed in the early stages of aniline oxidation: linear oligomers with repeating units joint in para positions (*p*-AO) and various branched and polycyclic oligomers containing ortho linkages (*o*-AO) being the two most important groups. At various reaction conditions, specifically pH, different oligomer groups prevail. N-phenyl-phenazinium cation is a typical marker for the *o*-AO easily detected by resonance Raman scattering (RS) and surface enhanced resonance Raman scattering (SERRS) on gold with 633 nm excitation [1,2].

In this contribution, the analysis of the first products of the chemical and electrochemical oxidation of aniline at (starting) pH 1 and 7. The conditions of formation of phenazine-like *o*-AO are searched in order to test the theory that they have an important role in polyaniline film formation [2]. We have confirmed that phenazine-like oligomers do not form at pH 1 in neither chemical nor electrochemical [1] oxidation of aniline; however, it formed in both chemical and electrochemical [1] oxidation of aniline at pH 7. It is thus definitely not a necessary intermediate for PANI film formation even in chemical polymerization of aniline.

In addition, the redox behaviour of phenazine-like oligomers was demonstrated in a medium of pH 1 by SERS spectroelectrochemistry (Figure 1). The N-phenyl phenazinium cation is oxidized at ca 0.2 V and this process is connected with a SERRS turnoff.

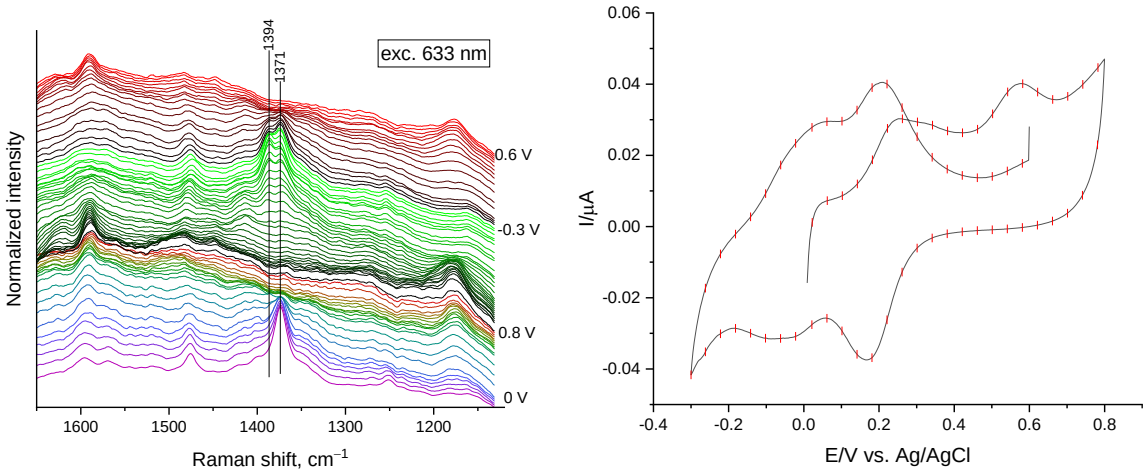

Figure 1: Raman spectra of gold SERS electrodes during the spectroelectrochemical study at pH 1 of the AO formed electrochemically in aqueous medium of pH 7. Excitation 633 nm (a) and the corresponding CV (b). Red marks separate potential ranges connected with one Raman spectrum.

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Tip-enhanced Raman Spectroscopy Protocol for Nanoscale Chemical Imaging of Commercial Functionalized Few-layer Graphene

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Non-destructive and label-free molecular imaging at nanometre length-scales in ambient environment challenges the limits of most analytical techniques. Tip-enhanced Raman spectroscopy (TERS) is one of the very few techniques capable of achieving this and has successfully emerged as a promising nanoanalytical tool over the last two decades [1]. In only a relatively short period of time, TERS has been effectively utilised for nanoscale chemical characterization in a wide range of research areas [2].

However, despite its advantages, most of the TERS research to date has been limited to model test samples and applications to real systems have been met with a limited success. This apparent failure to translate the success of TERS characterisation to real-world samples could be attributed in part to the limited lifetime, low stability and low yield of TERS probes [3].

In this talk strategies to improve the plasmonic lifetime, chemical and structural stability, and yield of Ag-coated AFM-TERS probes are presented [3,4]. Furthermore, it is shown that in addition to model systems, TERS is equally capable of revealing significant novel insights into real-world samples, such as biological cells, 2D materials and heterogenous catalysts [3,4]. Finally, the versatility of this protocol will be demonstrated through the application of TERS to the nanoscale chemical characterisation of commercially functionalized few-layer graphene. TERS imaging supported by X-ray photoelectron spectroscopy (XPS) and secondary ion mass spectrometry (SIMS) investigations reveals the presence of defects, as well as nanoscale variations in functionalization, which have a direct impact on the material properties [5,6].

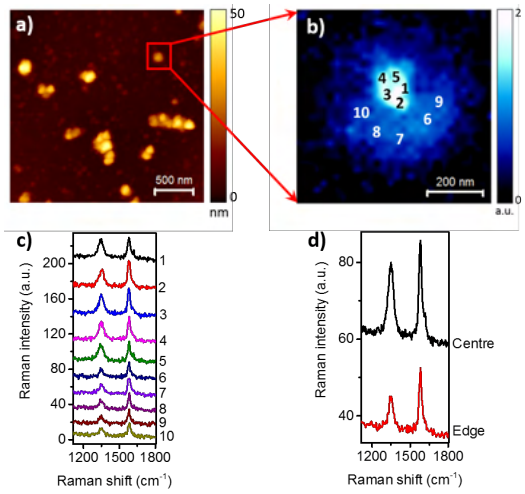

Figure 1. a) AFM topography image of few-layer graphene flakes plasma-functionalized inside an industrial reactor and deposited on a glass substrate. b) TERS image of the D band intensity of the graphene flake highlighted in (a). Integration time: 1s. Step-size: 20 nm. c) TERS spectra measured at the locations marked in (b). Integration time: 30 s. d) Average TERS spectra measured at the center and edge of the graphene flake shown in b. A higher I_D/I_G ratio is observed in the TERS spectra measured in the center of the flake compared to the edge indicating a higher density of defects at this location.

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Fluorescence-Encoded Time-Domain Coherent Raman Spectroscopy

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Fluorescence-encoded spectroscopy has become increasingly more popular by virtue of its high chemical specificity and sensitivity. Several papers based on this concept have already been published from as far back as IR fluorescence-encoded spectroscopy [1] to as recently as stimulated Raman spectroscopy fluorescence-encoded spectroscopy [2]. As of now, these fluorescence-encoding methods are narrowband and lack sensitivity in the low wavenumber region which if addressed could further enhance this method. To this end, we have developed a low-frequency, broadband spectroscopy technique to perform time-domain Raman spectroscopy in a fluorescence-encoding manner, which we named fluorescence-encoded time-domain coherent Raman spectroscopy (FLETCHERS).

The FLETCHERS setup is based on the Fourier-transform coherent anti-stokes Raman scattering (FT-CARS) spectrometer [3]. As shown in Fig. 1a, a pulse from a Ti:sapphire laser is split into a pulse pair which is used to excite and probe different vibrations in the sample as a function of the time delay between the two pulses. In FLETCHERS, these vibrations are then recorded onto the fluorescence intensity modulation which is measured in the backwards direction by time-correlated single photon counting. With FLETCHERS it is possible to obtain a broadband spectra even in the low wavenumber region as seen with rhodamine 800 in Fig. 1b. Additionally, we are also able to realize a high sensitivity at the same time, seeing a signal from samples of concentration as low as 100 nM and whose FLETCHERS intensity increases linearly with sample concentration as seen in Fig. 1c. We believe that this method would ultimately increase the efficiency and diversity of current multiplexing methods. One such example would be in flow cytometry multiplexing, where low concentration detection would increase the detectivity of minor tags and could even allow the visualization of cell-to-cell interactions.

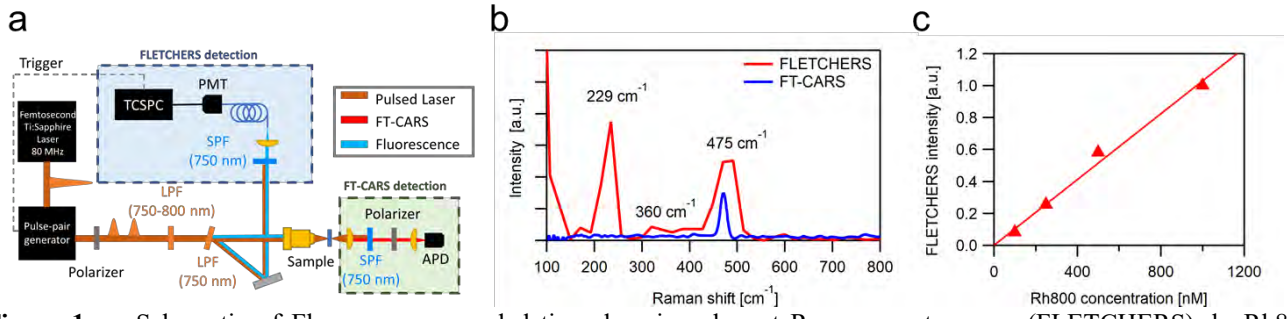

Figure 1: a: Schematic of Fluorescence-encoded time-domain coherent Raman spectroscopy (FLETCHERS). b: Rh800 spectrum in ethanol from both Fourier-transform coherent anti-stokes Raman scattering (FT-CARS) (100 µM with laser power of 300 mW) and FLETCHERS (10 µM, laser power of 70 mW). It is evident that FLETCHERS can produce a 475 cm^{-1} Raman peak as well as the lower 229 cm^{-1} and 360 cm^{-1} peaks which were not seen in FT-CARS even with a higher sample concentration and laser power. c: Concentration dependence of 475-cm^{-1} FLETCHERS peak of Rh800 concentration vs. 475 cm^{-1} peak intensity (laser power of 70 mW).

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Effects of Sulfation and the Environment on the Structure of Chondroitin Sulfate Studied *via* Raman Optical Activity

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Glycosaminoglycans are linear carbohydrate polymers with essential roles in many biological processes. Chondroitin sulfate (CS) is one of them, omnipresent in living organisms as an important structural component of cartilage. It provides much of its resistance to compression. Despite its biological importance, little is still known about the relation of the CS structure to chemical composition and interaction with the environment.

We therefore measured Raman and Raman optical activity (ROA) spectra of five CS samples of different biological origin and variously sulfated CS building blocks (GlcA, GalNAc, and basic disaccharide units) in a wide frequency range between 200 cm^{-1} and 1800 cm^{-1} and analyzed them with respect to specific structure marker bands. We show that ROA spectroscopy is sensitive to the conformational stability and rigidity of pyranose rings of saccharides, the orientation of sugar hydroxyl groups and the secondary structure of the CS's backbone. The CS secondary structure has been found to be quite stable, with a minor variation as a reaction to physicochemical parameters (concentration, pH, temperature, and the presence of cations). Larger changes were observed under chemical changes (sulfation) of the CS chain.[1] ROA spectroscopy thus exhibited useful potential to study the structure of similar biopolymers.

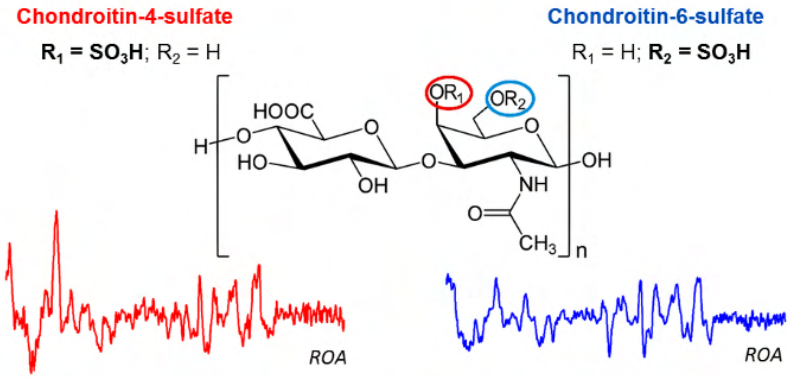

Figure 1: Raman optical activity reflects differences in the secondary structure of chondroitin sulfate caused by 4-O- and 6-O-sulfation

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Super-multiplex flow cytometry with cyanine-based Raman tags

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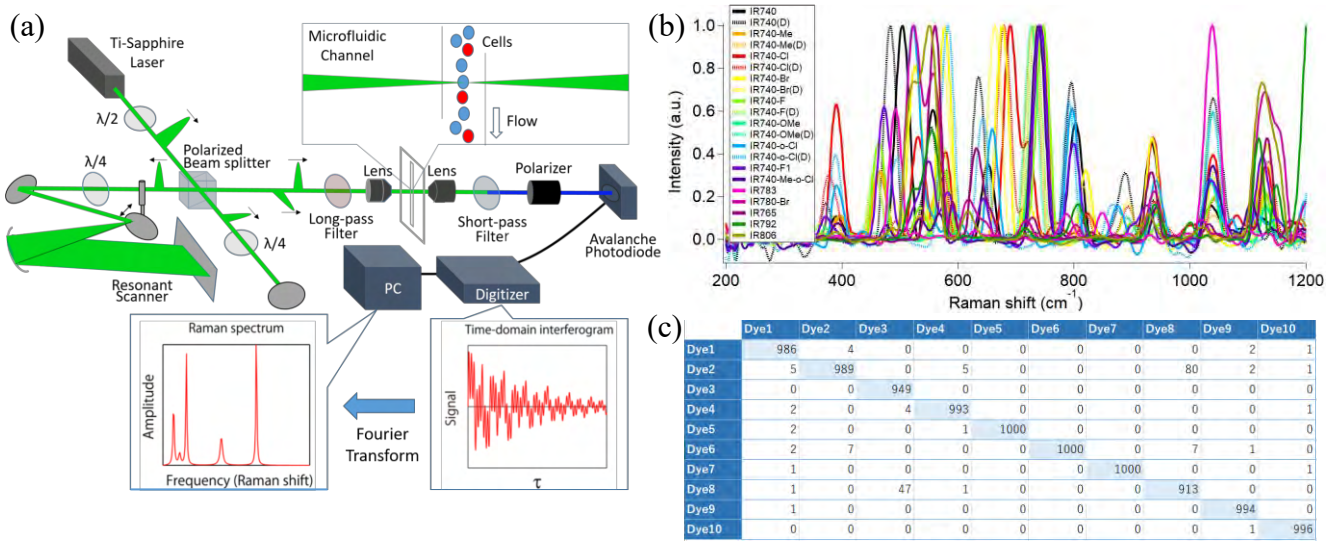
Simultaneous detection of different molecular species in living cells is increasingly important for understanding their physiological functions. While fluorescent labeling is widely used for this purpose, particularly in bioimaging and flow cytometry, its inherent problem is the limit on the number of colors: typically up to 10 due to the broad linewidth of their fluorescence signals (30-50 nm per fluorescence signal). To overcome this limit, super-multiplex imaging of living cells with more than 20 colors has recently been performed with the use of Raman probes in combination with stimulated Raman scattering (SRS) [1]. However, super-multiplex flow cytometry with Raman probes has not been performed to date, primarily because it requires much faster Raman spectral acquisition compared with imaging.

Here, we propose and demonstrate super-multiplex vibrational flow cytometry based on a high-throughput Fourier-transform coherent anti-Stokes Raman scattering (FT-CARS) flow cytometer (Fig. 1a) [2] employed with cyanine-based Raman tags that have intense, characteristic vibrational peaks in the fingerprint region (Fig. 1b). With this method, we measured single-cell Raman spectra of 10,000 cells stained with 10 different Raman tags. The stained cells are well classified with accuracy of 98.2% by analyzing the obtained broadband Raman spectra using a spectral unmixing algorithm (Fig. 1c).



(c)

	Dye1	Dye2	Dye3	Dye4	Dye5	Dye6	Dye7	Dye8	Dye9	Dye10
Dye1	986	4	0	0	0	0	0	2	1	
Dye2	5	989	0	5	0	0	0	80	2	1
Dye3	0	0	949	0	0	0	0	0	0	0
Dye4	2	0	4	993	0	0	0	0	0	1
Dye5	2	0	0	1	1000	0	0	0	0	0
Dye6	2	7	0	0	0	1000	0	7	1	0
Dye7	1	0	0	0	0	0	1000	0	0	1
Dye8	1	0	47	1	0	0	0	913	0	0
Dye9	1	0	0	0	0	0	0	0	994	0
Dye10	0	0	0	0	0	0	0	0	1	996

Figure 1: Super-multiplex flow cytometry: (a) Schematic of our FT-CARS flow cytometer, (b) Raman spectra of the 21 different dyes in methanol, (c) Confusion matrix obtained by spectrally unmixing the Raman spectra obtained from the stained cells.

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Probing lattice dynamics and electronic resonances in hexagonal Ge and Si_xGe_{1-x} alloys in nanowires using Raman spectroscopy

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Semiconducting nanowires (NWs) are well established and very interesting systems for their optical and thermoelectrical properties, when compared to the corresponding bulk material. Their great potential lies in the possibility of tuning their functional properties through the synthesis process. Much work has been done to assess and to gain control of the optical and electronic properties of NWs. In this regard the use of group IV semiconductors (notably Si and Ge) in optoelectronics is limited by the indirect nature of their band-gap, that precludes the realization of optoelectronics devices based on the widespread Si-compatible technologies. While this is true for the bulk cubic materials, it does not always apply to the hexagonal phase, which is only recently attainable and only in nanoscopic objects[1].

For this work, the crystal structure transfer technique has been employed to produce hexagonal (2H) GaAs-Si_xGe_{1-x} core-shell NWs[2]. The five compositions studied go from x = 0.14 to x = 0.59, range in which these materials are expected to exhibit a direct band-gap[3],[4].

Combining first-principles calculations with position-, polarization- and excitation wavelength-dependent μ-Raman spectroscopy studies performed on single NWs, we explore the lattice dynamics of these novel materials. In particular, we obtain frequency-composition calibration curves for the main phonon modes[5]; we experimentally retrieve the computed polarization selection rules and we observe their anisotropic relaxation due to alloy disordering; we assess the NWs crystalline quality for different Si composition; by observing resonance-induced enhancements of the Raman scattering process; we unveil the coupling between lattice vibrations and electronic transitions, inferring symmetry properties of the electronic bands involved. Good agreement is found between the observed enhancements and theoretical energies of electronic transitions [4],[6].

The results of this work constitute thus a first step towards a better understanding of direct band-gap group IV semiconductor NWs, promising materials for optoelectronic applications, and can be the basis of phonon engineering in alloy NWs.

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Visualizing cell wall dynamics during yeast sporulation process by Raman microspectroscopy and MCR-ALS technique

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Cell wall is a dynamic and an essential organelle in fission yeast. It plays a vital role, particularly in cellular growth, elongation and division. Any modification or disruption of the wall leads to lysis and cell death thereby serving an excellent target for anti-fungal drugs. Chemical structure of cell wall in fungi is complex which mainly comprised of various polysaccharides. Electron microscopy and biochemical extraction methods have been traditionally employed to study cell walls. However, the former lacks chemical specificity and requires genetically modified cells while the later involves fractionation methods that makes distinguishing spore walls from the mother cell wall impossible. Here, we have recently developed a label-free method based on confocal Raman microscopy to visualize distribution of various polysaccharide components and elucidated molecular structure of fungal cell and spore walls in detail [1]. By employing multivariate curve resolution-alternating least squares analysis (MCR-ALS), we successfully separated structurally similar polysaccharides such as α- and β-glucan. In this study, we will report on our recent findings on cell wall dynamics of living fission yeast cells whose cell cycle is synchronized. Time-resolved Raman images after MCR analysis is presented in Figure 1. Detailed results will be discussed during the symposium.

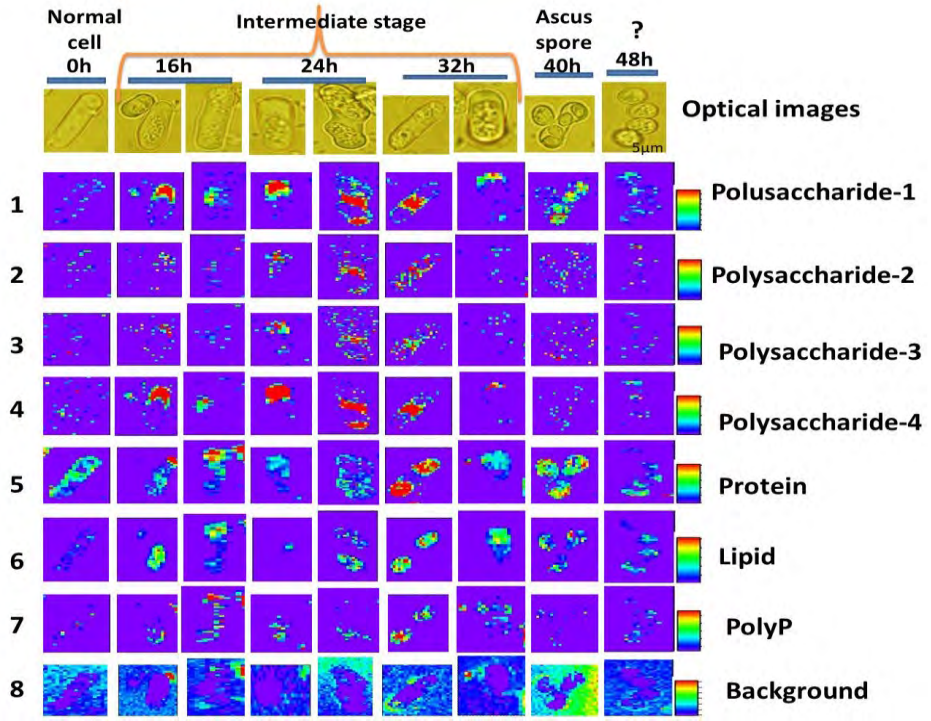

Figure 1: Time-resolved MCR component distribution images from wild type yeast cells during sporulation. (1) polysaccharide-1, (2) polysaccharide-2, (3) polysaccharide-3, (4) polysaccharide-4, (5) protein, (6) lipid, (7) polyP and (8) background. Corresponding optical images are given at the top. Scale bar measure 5 µm.

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Study of chemical enhancement mechanism in various semiconductor substrates based Surface enhanced Raman spectroscopy (SERS)

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Surface enhanced Raman spectroscopy (SERS) has been studied since 1970s to improve detection of electromagnetic fields near nanostructured metal surfaces. Both theory and techniques of SERS have developed rapidly ever since, but understanding the chemical enhancement mechanism of SERS is still incomplete. To study details of chemical enhancement mechanism, various low-dimensional semiconductor substrates such as ZnO and GaN nanowire arrays have been prepared that can safely exclude surface plasmonic effect in visible region. High quality ZnO and GaN substrates were fabricated through metal organic chemical vapor deposition process. Three types of molecules (4-MPY, 4-MBA, 4-ATP) were used as analytes to measure the SERS spectrum under non-plasmonic conditions to understand charge transfer mechanisms between a substrate and molecules.

It has been observed that there is a preferential pathway for charge transfer that is responsible for chemical enhancement. [1,2] In other words, there is a dominant process for charge transfer in non-plasmonic SERS. To further confirm our idea of the charge transfer mechanism, we used a different system, a combination of a two-dimensional dichalcogenide substrate and an analyte molecule (R6G). We observed a significant enhancement in the Raman signal from molecules adsorbed on the two-dimensional dichalcogenide surface, which is fully consistent with our previous results. [3] We also investigated heterostructures (MoS_2/WS_2, WS_2/MoS_2) to understand the path of charge transfer. Based on our observations, we will discuss important factors for determining enhancement factor in chemical enhancement of Raman signal that does not involve surface plasmonic resonance.

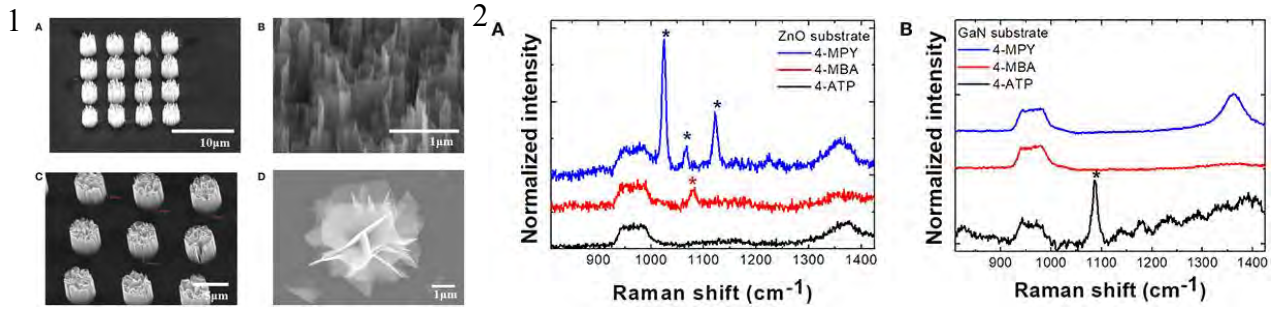

Figure 1: SEM images of (A) GaN microrod array (B) nanowall structure in a microrod (C) ZnO microrod array (D) CVD grown WS_2 nanoflower.

Figure 2: Raman spectra of three different molecules (4-MPY, 4-MBA, 4-ATP) adsorbed on (A) ZnO microrod array substrate and (B) GaN microrod array substrate. Phonon intensity in each spectrum is normalized to Si phonon (520 cm^{-1}) intensity. Raman peaks of 4-MPY and 4-MBA molecules are enhanced only on the ZnO substrate and Raman peak of 4-ATP molecules is enhanced only on the GaN substrate. *Indicates the peak positions of each molecules (4-MPY, 4-MBA, 4-ATP).

3

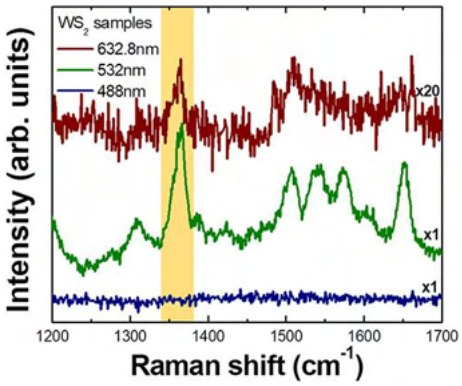

Figure 3: Wavelength dependent Raman spectra of R6G molecules adsorbed on CVD grown WS_2 nanoflower substrate measured with 3 different excitation lasers. Phonon intensity in each spectrum is normalized to Si phonon (520 cm^{-1}) intensity. The largest enhancement is observed at 532.0 nm excitation and significantly weaker but observable enhancement is observed at 632.8 nm excitation. No observable enhancement is seen under 488.0 nm illumination.

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Raman spectroscopy for biodegradation monitoring of anthropogenic organic contaminants in a diffusive fluid matrix

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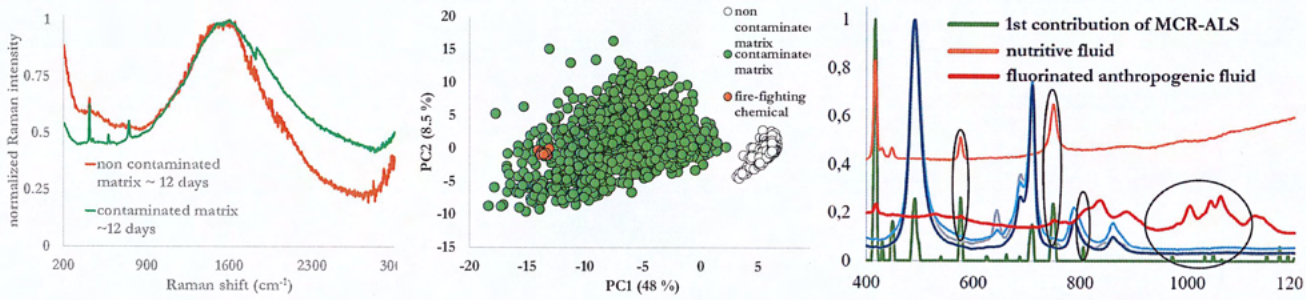
Anthropogenic activities are generating many chemicals, and their environmental fate raises many concerns. Furthermore, water pollution is extensively discussed in the literature. In the specific case of airports management, at least two chemicals are used, formates/acetates (runways, taxiways) and fluorinated ethers, respectively for winter maintenance and fire-fighting operations. Their biodegradation is estimated through the OECD 301F protocol [1], but providing information only about total organic carbon. To obtain an accurate insight about the biodegradation of these molecules, Raman spectroscopy was added to current OECD protocol to first eventually identify if some specific molecules are generated during the biodegradation, and to identify to what extent the process is complete. A Raman probe was immersed in an aqueous environmental diffusive liquid matrix containing living micro-organisms, a nutritive fluid and a chemical which biodegradation is monitored. Another one was placed in another fluid without the investigated chemical, and used as a reference. For each of the four tested chemicals, the evaluation lasted 28 days, with on Raman spectrum every 15 minutes and an integration time of 30s. To reduce the fluorescence and avoid water spectral signature, for a Kaiser RXN-2 spectrometer was selected operating with a 785 nm-laser wavelength. The large volume of spectral data obtained after each experiment was handled through principal components analysis (PCA) and multivariate curve resolution (MCR). Chemometric-assisted analysis of Raman spectra revealed a partial biodegradation, and therefore the persistence of such chemicals into the environment.



(a) (b) (c)

Figure 1: Raman spectra of a pristine and contaminated environmental matrix (a), scores plot of PCA conducted on data from the biodegradation monitoring of a fire-fighting foam (b), and 1st contribution from MCR-ALS

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Monitoring of metabolic alterations in tumor microenvironment by surface-enhance Raman scattering

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The tumor microenvironment, where numerous cell types interact to create a distinctive physiology, is characterized by deregulated metabolic features. Within this ever-changing microenvironment, cancer cells rewire their metabolic program to sustain cancer cell growth.This metabolic rewiring has a profound impact on the properties of the microenvironment, to an extent that monitoring such perturbations could harbor diagnostic and therapeutic relevance [1]. In this context, surface-enhanced Raman scattering (SERS) can be used for the label-free detection and imaging of diverse molecules of interest among extracellular components [2]. Herein, the application of nanostructured plasmonic substrates comprising Au nanoparticles, self-assembled as ordered superlattices, to the precise SERS detection of selected tumor metabolites, is presented.

The potential of this technology is first demonstrated through the analysis of kynurenine, a secreted immunomodulatory derivative of the tumor metabolism and the related molecules tryptophan and purine derivatives [3]. We thus detected the accumulation of the Methylthioadenosine (MTA) oncometabolite in tumor environment of glioblastoma cells with complete depletion of MTAP enzyme, monitoring the active transport of this metabolite to the extracellular milieu. Finally, the effective plasmonic SERS substrate is combined with a hydrogel-based three-dimensional cancer model for the real-time imaging of metabolic interactions among the different components and cell types of tumor environment. This strategy provided new insights into cancer metabolism studies.

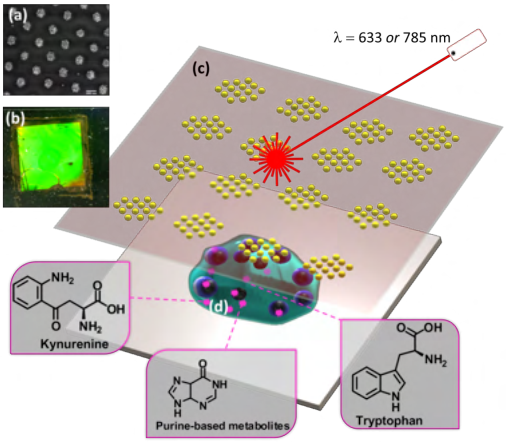

Figure 1: Schematic illustration of the SERS-based system to detect the accumulation of metabolites in the extracellular tumor milieu. a-c) Nanostructured plasmic substrate comprising a superlattice of Au nanoparticles. d) Chemical structure of the different tumor associated metabolites accumulated in the tumor environment.

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Waveguide-Coupled Plasmon Resonance for SERS

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Metal nanostructures with the configurations of narrow gaps or sharp tips show superior performances in plasmonic sensing and surface-enhanced spectroscopy. However, fabricating such elaborate structures with a large area is a great challenge in the nano processing. By considering the excitation and emission ways, we proposed a strategy for the existed metal nanostructures by optimizing the incident light field and increasing the collection of scattered light *via* a waveguide mode. The planar dielectric waveguide can harvest most incident light by a prism and generate an enhanced leaky mode evanescent field on the surface of the waveguide, the waveguide consists of a 35 nm Ag film, a 600 nm silica, and an air layer. In this way, the incident light field can be improved about one order of magnitude [1, 2, 3]. Plasmonic nanostructure arrays are ideal emitters to increase radiator density. In our study, non-sharp nanocone arrays, nano hemisphere arrays [4] and dimer arrays [5] with dozens of nanometers' gap were fabricated by depositing silver with the anodic aluminum oxide (AAO) as mask templates. These configurations can provide an enhanced electromagnetic field, whose position can be changed flexibly by adjusting polarization. We applied this configuration for label-free sensing of cell membranes by isolating the cell membrance protein and coated with metal nanoparticles. This is a useful configuration for high sensitive detection and surface analysis.

This work is supported by the National Natural Science Foundation of China (NSFC No. 21873039, 21573092, and 21573087).

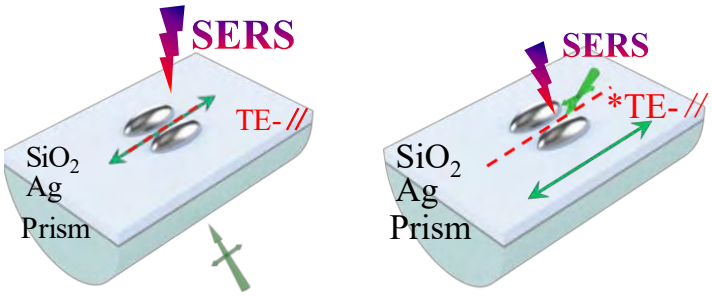

Figure 1: The excitation ways for a dimer

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Surface-enhanced Raman spectroscopic study of nanoparticle catalysis

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Rational design of nanoparticle catalysts is generally challenging because of the unknown catalytic sites and the reactive intermediates that are difficult to be detected experimentally. During the last years, surface-enhanced Raman spectroscopy (SERS) has attracted great attention in the detection of nanoparticle-based catalysis.[1,2] As an ultra-sensitive and surface-selective vibrational spectroscopic technique, SERS exhibits remarkable capability of interfacial characterization, including finding out catalytic sites and identifying intermediate species on catalyst surface.

To detect nanoparticle-catalyzed reactions, bifunctional substrates with both SERS and catalytic activity have been explored.[3,4] Typically, large Au or Ag nanoparticles are employed as plasmonic cores and small catalyst nanoparticles are assembled on the core surface as satellites.[5] These core-satellite superstructures can serve as a general platform for in situ detection of various catalytic reactions (Figure 1), when different satellite nanoparticles are used.[6] Herein, we introduce several synthesis methods for preparing bifunctional SERS substrates. Specifically, the use of these substrates in two types of nanoparticle catalysis, i.e. plasmon-free catalysis and plasmon-induced photocatalysis,[7] are also summarized. Our works show the great potential of SERS in mechanistic study of interfacial chemistry toward rational design of high-performance nanoparticle catalysts.

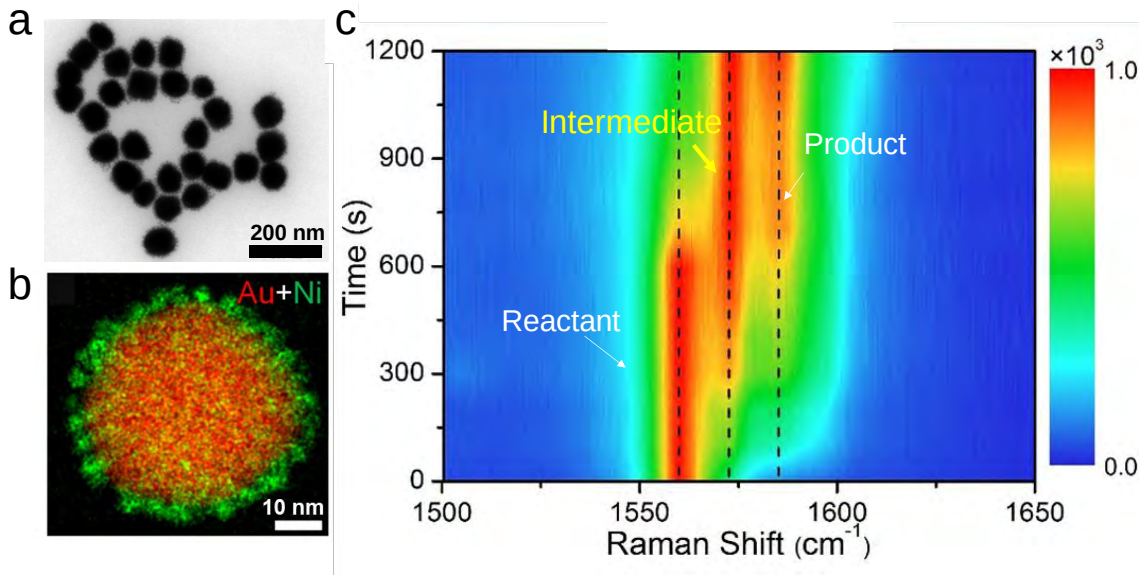

Figure 1: Bifunctional core-satellite (Au and Ni) SERS substrates and the corresponding in situ SERS spectra of Ni nanoparticle-catalyzed reaction.

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SPPs controlling and plasmonic catalysis on nanomaterials

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Surface plasmon interference and catalysis recently designed as a new exciting topic, opens a route to concentrate and direct the energy of visible light to adsorbed molecules and nanomaterials. Here, a silver microplate is employed in studying the far-field radiation of interfered surface plasmons polaritons (SPPs) on the metal-dielectric interface.[1] A strong SPPs with certain beat period can be excited and propagate on the silver-dielectric interface of the microplate. And the beat period and propagation strength of SPPs can be well controlled by switching the focusing point and the polarization angle of the incident light.

Moreover, an easy and rapid in-situ achievement of single crystal luminescent material is realized by taking advantages of plasmon induced thermal and catalysis effects.[2,3] With the assistance of localized surface plasmon resonance of Au nanoparticles, polycrystalline transforms to single crystal in tens of milliseconds, resulting in remarkable improvement of luminescence emission. It is important to point out that the single crystal transformation is also achieved even at very low temperature, which is impossible with conventional approach. Such a convenient and efficient plasmon assistant scheme provides a new technology for rapid achievement of single crystal material and extends the application of surface plasmon to a much broader field.

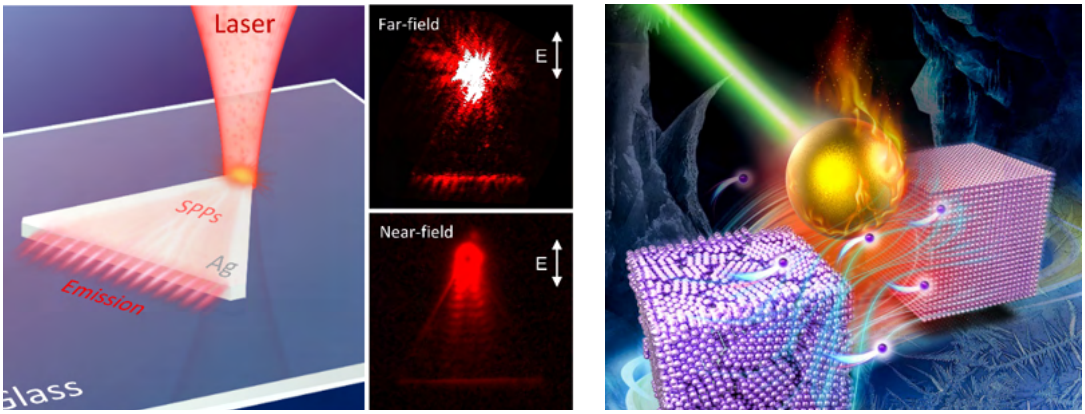

Figure 1: SPPs controlling on silver microplate (left) and plasmon catalyze crystal transformation (right).

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Theory for photoluminescent background in SERS experiments

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The photoluminescence of granular plasmon nanostructures is observed in SERS experiments [1, 2,3], when a plasmon nanostructure is illuminated by a laser with a narrow line whose frequency is close to the plasmon resonance frequency. Photoluminescence manifests itself in the scattering spectrum as a wide frequency signal, the shape of which is close to the shape of the plasmon resonance line of an individual granule, in particular, the photoluminescence spectrum has a maximum at a frequency close to the plasmon resonance frequency. Photoluminescence is also observed at frequencies higher than the frequency of the incident wave, and its spectrum in this range no longer tracks the plasmon resonance spectrum and is determined by the temperature of the metal, namely, decreases with frequency according to the Gibbs distribution [2].

In this paper, we propose a theory for the appearance of photoluminescence of plasmon nanostructures made of noble metals. We use Lindblad master equation for the plasmonic modes with frequencies inside plasmon scattering spectrum and optomechanical Hamiltonian to describe interaction between them. We show that mechanism of excitation of plasmonic modes is partly close to that which takes place in Raman lasing. Namely, inelastic Brillouin scattering leads to the stimulated coherent oscillations of the dipole moment at the frequency of the incident field excite oscillations at the Stokes and anti-Stokes frequencies. Since the Stokes shift during Brillouin scattering is, as a rule, much smaller than the plasmon resonance width, if the incident radiation frequency is close to the plasmon resonance frequency, then the Stokes frequency also falls into the plasmon resonance line. As a result, inside the plasmon resonance line at the Stokes frequency, which differs from the frequency of the incident field by the frequency of the excited phonon, stimulated resonant oscillations of the dipole moment will be excited. Further, the oscillation at a displaced frequency itself excites oscillations at a displaced frequency relative to it, and so on. As a result, a whole cascade of oscillations at frequencies inside the plasmon line is excited. Considering that phonons have a continuous spectrum, we get continua of cascades that fills the entire plasmon resonance line.

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Nanostars – decorated microfluidic devices for SERS targeting of biomolecules in liquid samples

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Liquid biopsies represent a minimally invasive tool for the precocious diagnosis of widespread diseases as well as for routinely patients monitoring by tracking selective biomarkers. Optical detection techniques based on surface enhanced Raman spectroscopy (SERS) capable of screening the molecular content of analysed samples constitute the most promising analytical method in clinical research, as alternative to traditional bioassays.[1] With the attempt to realize point-of-impact diagnostic devices, in the present study advanced and traditional manufacturing processes were combined with plasmonic nanoparticles (NPs) for the development of hybrid lab-on-chips (LOCs) integrating SERS sensors for liquid probing. As a matter of fact, LOCs enable to easily handle small volumes of samples as well as to perform multifunctional analyses on the same restricted volumes avoiding cross-contaminations.[2] This is crucial for pathologies whose diagnosis relies on the ratio of more than one biomarker. To this end, being based on a 3D printing process, the overall design of the devices was rapidly prototyped to integrate channels and detection chambers aligned with optical fibers and portable Raman probes for signal delivering and collection. SERS functionality was achieved by immobilization of gold NPs engineered in terms of shape, size and surface chemistry to play with plasmonic properties as well as to guarantee reproducibility to the NPs immobilization step and consequently to the SERS effect for signal enhancing. To assess the feasibility of the measurements for molecules optical targeting, SERS-microfluidic systems were synergically coupled with a portable fiber-based set-up and Raman spectra of rhodamine 6G at different concentrations were acquired. To further demonstrate the potentiality of developed SERS-based substrates as Point-of-Care (POC) devices, Raman analysis were successfully implemented on aqueous solutions of amyloid-β 1-42 (Aβ), considered the main biomarkers for Alzheimer's disease (AD).

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Low-Wavenumber Fourier-Transform Impulsive Stimulated Raman Spectrometer with Single Femtosecond Oscillator

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Low-frequency Raman spectra (<200 cm^{-1}) are interesting for studies of intra-molecular vibrational modes of large molecules or heavy functional groups, and for studies of inter-molecular force constants. This region is traditionally accessed by spontaneous Raman spectroscopy using triple monochromators, which however represents an expensive and cumbersome solution with relatively low signal yield. More recently, special Bragg filters have been developed to overcome these shortcomings, however they have the disadvantage of being specific to the laser-excitation wavelength.

A different approach for low-wavenumber Raman spectroscopy is based on coherent Raman techniques such as Impulsive Stimulated Raman Scattering (ISRS) allowing excitation of vibrational modes within the bandwidth of ultrashort laser pulses [1], and generation of intense signals resulting from the coherently prepared vibrational state.

In a recent paper [2] a method for measurement of ISRS signals suitable for setups using a single low-energy femtosecond laser was proposed, enabling realization of relatively simple systems for characterization of transparent samples. The setup is a Fourier-transform spectrometer based on a Michelson interferometer. The use of a position-sensitive detector enables the detection of the small oscillations of the center of mass (COM) of the probe pulse spectrum carrying information about Raman loss and gain. In this work, we present the results of a joint experimental and modeling activity aimed at the study and optimization of the performances of the above described setup. In particular, we modified the set-up in order to provide quasi-real-time data acquisition useful for vibrational imaging. A typical dependence of the time-resolved COM signal from a reference liquid sample is shown in Figure 1. The experimental signal is well predicted by a model based on an analytical solution accounting for chirped fields in the approximation of Gaussian pulses.

This work was performed in the frame of the "Grande Rilevanza" Project US19GR13, co-funded by MAECI.

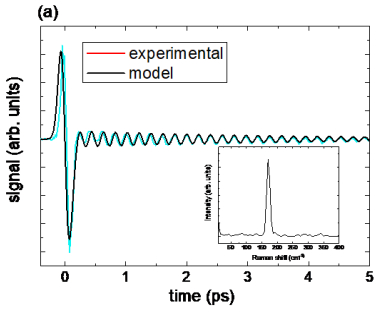

Figure 1: Experimental time-dependent ISRS signal measured on a CH_2Br_2 reference sample, and theoretical fit with the analytical model; inset: Raman signal obtained by FFT of ISRS signal.

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Drop Coating Deposition Raman Spectroscopy as a Valuable Tool for Sensitive Detection of Biologically Important Molecules

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The drop coating deposition Raman (DCDR) scattering technique is a special method based on drying of a small drop of molecular solution or suspension deposited on a special hydrophobic surface [1]. The drying process of the drop very efficiently accumulates the molecules in the forming pattern from which Raman spectrum of very good quality can be acquired. The hydrophobic surface enables to dry the droplet in a manner of either "coffee-ring effect" [1] or a small pattern without a clear peripheral ring [2]. In the former case the dispersed material is carried by the flow of a liquid in the evaporating droplet to its edge where it forms a ring [1,3]. Latter case was proposed as a universal "solvent removal" method applicable to samples in aqueous solutions deposited on highly hydrophobic surfaces [2]. Both approaches lead to a highly reproducible Raman signal by using confocal Raman microspectrometer [3, 4]. This is possible in case of the small volume of the deposited droplet (several μl) and very low initial concentrations as well. We have recently reported DCDR spectra for the detection of biologically important molecules including lipids, porphyrins, dipicolinic acid as anthrax marker, etc. [4].

In this contribution, we will compare commercial DCDR substrates with non-commercial ones. We will introduce the novel type of hydrophobic surfaces with metallic nanoparticles as well. Their preparation is based on nanostructuring of magnetron sputtered hydrophobic C:F films by a base layer of metallic nanoparticles that were fabricated employing a gas aggregation source of original construction. The influence of surface roughness of resulting coatings on surface wettability and properties of dried patterns will be investigated using biologically important molecules (lipids, methylene blue). DCDR spectra of food contaminants (melamine) and pesticides (thiram, bentazon) will be presented, too.

Acknowledgments: Support by grant 18-10897S from the Czech Science Foundation and grant 290120 of Grant Agency of Charles University.

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A fingerprint of amyloid plaques in a bitransgenic animal model of Alzheimer's disease obtained by hyperspectral Raman data

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The global prevalence of Alzheimer's disease (AD) points to endemic levels, especially considering the increase of average life expectancy worldwide. AD diagnosis based on early biomarkers and better knowledge of related pathophysiology are both crucial in the search for medical interventions that are able to modify AD progression. In this study we used unsupervised spectral unmixing statistical techniques to identify the vibrational spectral signature of amyloid β aggregation in neural tissues, as early biomarkers of AD in an animal model. We analyzed spectral images composed of a total of 55051 Raman spectra obtained from the frontal cortex and hippocampus of five bitransgenic $APP_{swe}PS1_{\Delta E9}$ mice, and colocalized amyloid β plaques by other fluorescence techniques. The Raman signatures provided a multifrequency fingerprint consistent with the results of synthesized amyloid β fibrils. The fingerprint obtained from unmixed analysis in neural tissues is shown to provide a detailed image of amyloid plaques in the brain as shown in Figure 1 [1]. Moreover we will show more recent results of the amyloid plaque formation as a function of mice age.

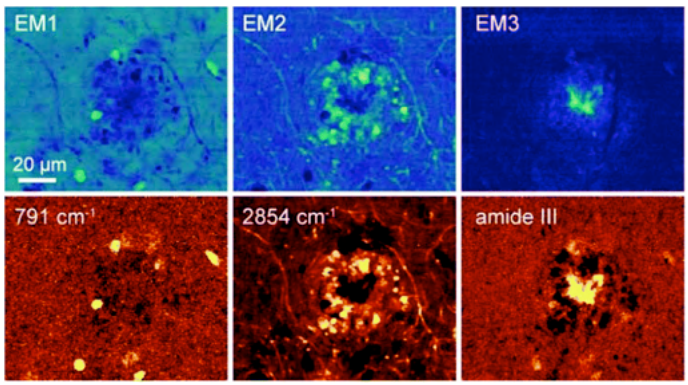

Figure 1: Hyperspectral imaging of amyloid plaques. Top – Results from the unsupervised spectral unmixing: EM1, cell nucleus; EM2, the surrounding tissue; EM3, amyloid plaque spectral image. Bottom – Analysis in selecting the Raman intensity for DNA frequency (centered at 791 cm^{-1}), lipid (2854 cm^{-1}) and amide III (1203–1263 cm^{-1}).

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Distinct Magneto-Raman Signatures of Spin-Flip Phase Transitions in 2D Magnet CrI_3

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Recent observations of long-ranged magnetic ordering in van der Waals bonded, layered magnetic materials down to the single layer limit has led to a plethora of research dedicated to the study of two-dimensional (2D) magnets, with plenty of opportunities to investigate fundamental physics and potential quantum applications. With these materials, the properties are typically strongly correlated to the number of layers, with new physics occurring in the few-layer (~few nm) regime. In this sense, common techniques used to measure magnetic behaviors such as neutron scattering and SQUID are at a disadvantage due to sample size requirements. On the other hand, Raman spectroscopy, which has diffraction-limited spatial resolution, is a powerful, non-destructive optical method to probe magnetism in 2D layered materials through inelastic scattering as a function of temperature, laser energy, polarization, and magnetic field. One material of particular interest is chromium tri-iodide (CrI_3), a ferromagnet at bulk thicknesses below the Curie temperature but with the remarkable property of layered antiferromagnetism in thin multilayers. Here, we will report on a magneto-Raman spectroscopy study on multi-layered CrI_3 [1], focusing on two new features in the spectra which appear at temperatures below the magnetic ordering temperature and were previously assigned to high frequency magnons. We observe a striking evolution of the Raman spectra with increasing magnetic field applied perpendicular to the atomic layers in which clear, sudden changes in intensities of the modes are attributed to the interlayer ordering changing from antiferromagnetic to ferromagnetic at a critical magnetic field. In addition, through DFT-calculations and symmetry arguments, we conclude that the new modes are not magnons as previously believed, but instead are zone-folded phonons.

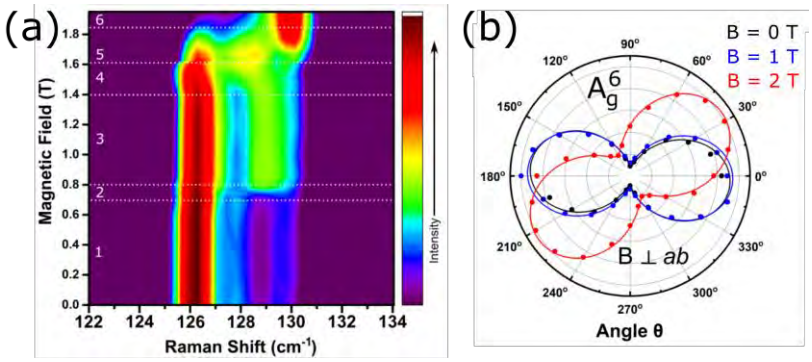

Figure 1: (a) False-color map of Raman spectra of 10-layer CrI_3 at low temperature and as a function of magnetic field. (b) Magnetic-field induced phase transition from FM to AFM stacking can be seen in symmetry changes of the Raman modes.

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Raman Microscopy of Microalgae: New Challenges and Opprotunities in the World of Photosynthetic Microorganisms

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Autotrophic microalgae use sunlight, carbon dioxide, and inorganic nutrients to biosynthesize complex organic compounds. Algae represent a large group of phylogenetically diverse organisms adapted to live at different, often extreme habitats, and capable of producing various substances that may be used as renewable raw materials. To better understand the peculiarities of their metabolism, as well as for efficient search and phenotyping of new strains suitable for industrial use, methods allowing rapid detection, simultaneously with imaging and quantification of various biomolecules directly within the intact cells are more than desirable.

Confocal Raman microscopy, which combines the molecular specificity of vibrational spectroscopy together with the spatial resolution of the confocal optical microscopy, may be a method of choice for chemical mapping of various microorganisms. Nevertheless, its routine applicability to photosynthetic microorganisms, especially microalgae, has long been hindered by a strong autofluorescence of photosynthetic pigments interfering with Raman spectra. Recently, we have developed a simple methodology [1] for fast and efficient suppression of the chlorophyll fluorescence, which opens the door wide into this exciting but little-explored field.

Besides the simultaneous detection, visualization, and quantification of already known compounds [1, 2], conventional Raman microscopy based on commercially available Raman microscopes can be of great help for identifying the real chemical nature of various intracellular structures [3, 4], frequently visualized by electron microscopy but still of unknown or questionable molecular composition. In such a way, inclusions found in some microalga were identified as crystalline guanine [3]. Similarly, enigmatic particles embedded into the plastids of extremophilic Arctic microalga *Cylindrocystis* were for the first time identified as polyphosphate granules [4]. As Raman spectra are sensitive also to isotopic labeling, Raman microscopy can be useful for metabolic studies at a single-cell level, representing thus less laborious alternative to nanoscale mass spectrometry [5]. Recent progress in the application of confocal Raman microscopy in algal research will be presented and demonstrated using our original research results. Advantages and perspectives, but also limitations and pitfalls of the method will be pointed out and discussed.

This work was supported by the Charles University Grant Agency (grant No. 796217), and the Czech Science Foundation (grants No. 17-06264S and 21-26115S).

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New approaches in preparation of metallic nanostructures for SERS by means of low-pressure plasma

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Optimization of metallic nanostructures for surface-enhanced Raman scattering (SERS) by tuning their localized-surface plasmon resonance (LSPR) properties has received much attention in recent years. In this contribution, we report some new approaches in the preparation of metallic nanostructures for SERS by means of plasma-based techniques such as:

(i) gradient nanostructured Ag surfaces, i.e. nano-islands with gradually changing optical properties (LSPR) in one direction, prepared by magnetron sputtering. It allows studying the dependence of the SERS enhancement factor on the excitation wavelength and the LSPR position [1].

(ii) nanostructured bi-metallic Ag/Au films prepared by magnetron sputtering. We found that by Ag-coating of Au nanostructures it is possible to tune in a certain range independently the position and the intensity of LSPR peak [2].

(iii) nanostructures with double plasmon resonance. When magnetron sputtering of Ag nano-islands and a gas aggregation source of Ag nanoparticles is combined, it produced nanostructures with two independent LSPRs (see Fig. 1) in the visible part of the spectra [3].

The nanostructured surfaces have been tested for SERS of biologically important molecules and the impermeability of polymer pCBAA brushes by SERS.

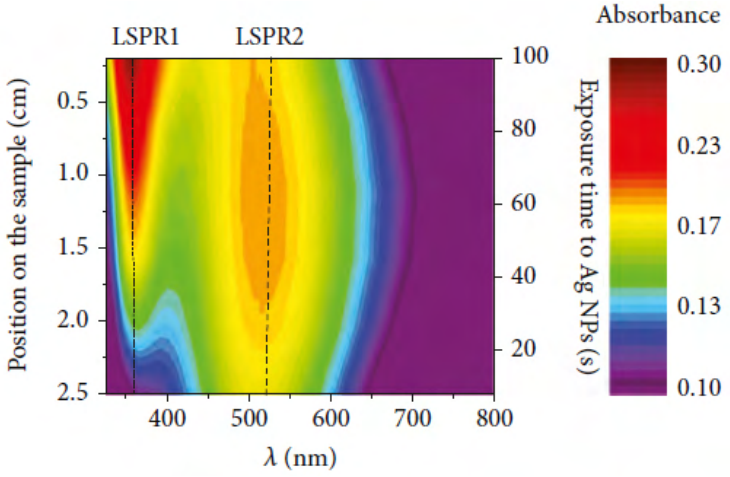

Figure 1: The nanostructure with double plasmon resonance. LSPR1 corresponds to Ag nanoparticles (Ag NPs), while LSPR2 is due to the Ag nano-island films.

Acknowledgements: Support by grant 21-19779S from the Czech Science Foundation.

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Quantitative Raman Imaging for Crystal Orientation Analysis

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In the present study, we demonstrate quantitative Raman Imaging for Crystal Orientation analysis (qRICO) by means of Polarized Raman Microscopy (PRM). While it is known that PRM is sensitive to orientation changes[1], an actual orientation map has to our knowledge never been presented before. Using a novel concept of ambiguity free orientation determination data analysis and simultaneous registration of multiple Raman scattering spectra obtained at different polarizations, our approach allows for 2D, 3D and 4D quantitative orientation mapping of multigrain materials[2]. It has been demonstrated that at least nine polarized channels are necessary to reach orientation determination accuracy around 1 degree for polycrystalline Si[2]. The optomechanical design of qRICO technology is shown on **Figure 1a**. Here, we were able to realize simultaneous acquisition of two on-axis and eight off-axis polarized Raman channels. Due to the fast polarization switching qRICO can produce up to twenty polarized channels necessary for orientation mapping with accuracy less than 1 degree for any crystal symmetry.

First results for silicon, a pharmaceutical tablet and for sapphire reveal favourable specifications: sub-micrometre resolution, fast data acquisition, and a high orientation resolution. A volumetric orientation map of polycrystalline sapphire non-destructively obtained by qRICO technology is shown on **Figure 1b**. qRICO applies to all Raman active materials independent of their crystal symmetry. Sample preparation is not required. Notably, Raman technology is relatively cheap and as such, the method may move orientation-mapping experiments into conventional optical laboratories.

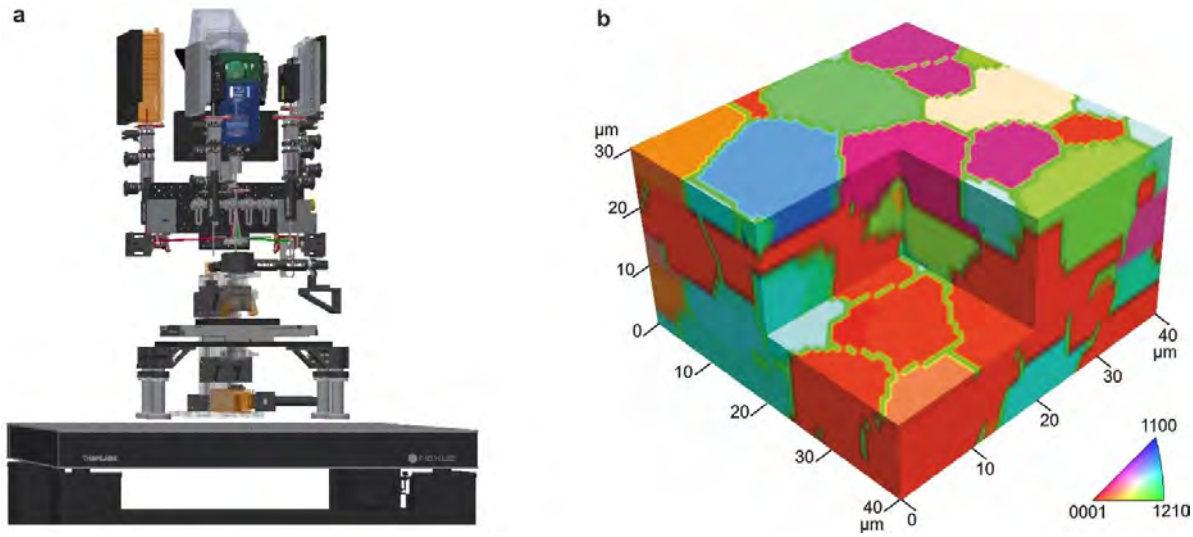

Figure 1. qRICO technology. **a.** *Optomechanical design of qRICO device;* **b.***Volumentic orientation map of polycrystalline sapphire non-destructively obtained by qRICO technology.*

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AI Powered Drug Classification by Mobile Phone based Raman Spectroscopy

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Raman spectroscopy is an powerful tool to analyze the chemical components of unknown materials because it is a non-destructive method. Portable spectrometers have been deveoped to improve its usability at various occasions compared to bulky commercial spectrometers. Recently, mobile phones have been technically evolved to operate numerous applications to provide diverse informations to consumers. Morever, cameras installed in the mobile phones offer good opportunities as image sensors to detect optical siganls from objects of interest through them. Many efforts have been made to develope mobile phone based sensors[1] to evaluate food freshness, skin cares, and etc by making use of cameras as detectors and utilazing mobile phone as operating system. To distinguish the object of interest or evaluate its condition, verifying system is essentially needed using data base either stored in the hardware or in the cloud system.

In this work, on-chip spectrometers have been fabricated by integrating distributed Bragg reflectance(DBR) filters in the mosaic pattern operating in the range of 800~900nm on the real wide camera sensor. The Raman spectrum images of ~60 drugs for diabetes, hyperlipidemia, hypertention, pain killers and nutritional supplements, were measured by using attachable Raman module with 785nm laser excitation. The classification of the drugs from the measured Raman images were conducted using artificial intelligence(AI), convolutional neural network(CNN) trained by using hundreds of Raman spectrum images from each drugs. The accuracy to determine the generic names belonging to specific types of medicine and exact brand names were ~95% and 85%, respecively. Un-trained drugs from the same types were tested with the CNN algorithm and its accuracy was almost closed to 100%.

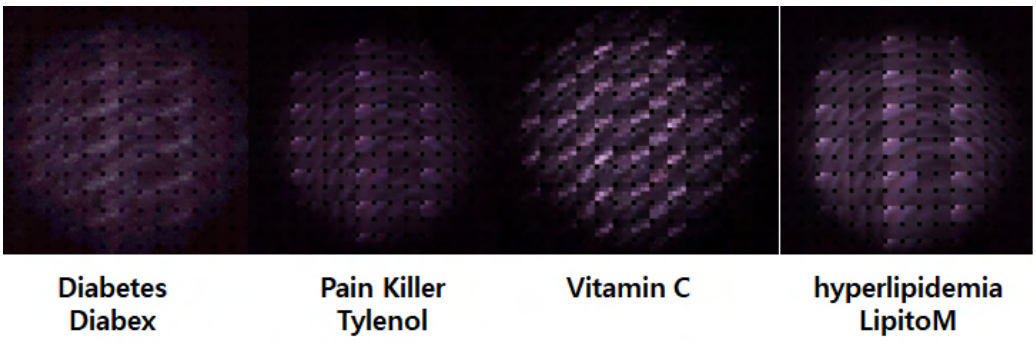

Figure 1: Mobile phone based Raman spectrometer and Raman spectrum images of Tylenol and Vitamin C

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Janus Monolayer-Induced Abnormal Interlayer Coupling in 2D Heterostructures

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Janus transition metal dicalcogenide (TMD) is a newborn of the two-dimensional (2D) materials family [1]. Its structure is similar to TMDs such as MoS_2, but one layer of chalcogen is different from the other layer, one example being MoSSe. Due to the unique crystal structure of Janus TMD, unconventional phenomena have been theoretically predicted, including out-of-plane piezoelectricity and exciton disassociation by the intrinsic out-of-plane dipole moment. In this work, we have studied the fundamental phonon properties of Janus monolayer MoSSe and interlayer coupling of MoSSe/MoS_2 heterostructures [2]. Interlayer breathing and shear modes of high-symmetry 2H and 3R heterostackings are probed by low frequency Raman spectroscopy. Unintuitively, interlayer coupling strength in the heterostructures is stronger than their pure MoS_2 counterparts possibly due to the compressive (tensile) strain in MoSSe (MoS_2) introduced during synthesis. Difference in high frequency modes between MoSSe/MoS_2 and pure MoS_2 supports the strain hypothesis. These spectroscopic features can serve as a fingerprint of stacking configurations, interlayer coupling in heterostructures, and degree of selenization in the fabrication process from TMDs to Janus TMDs.

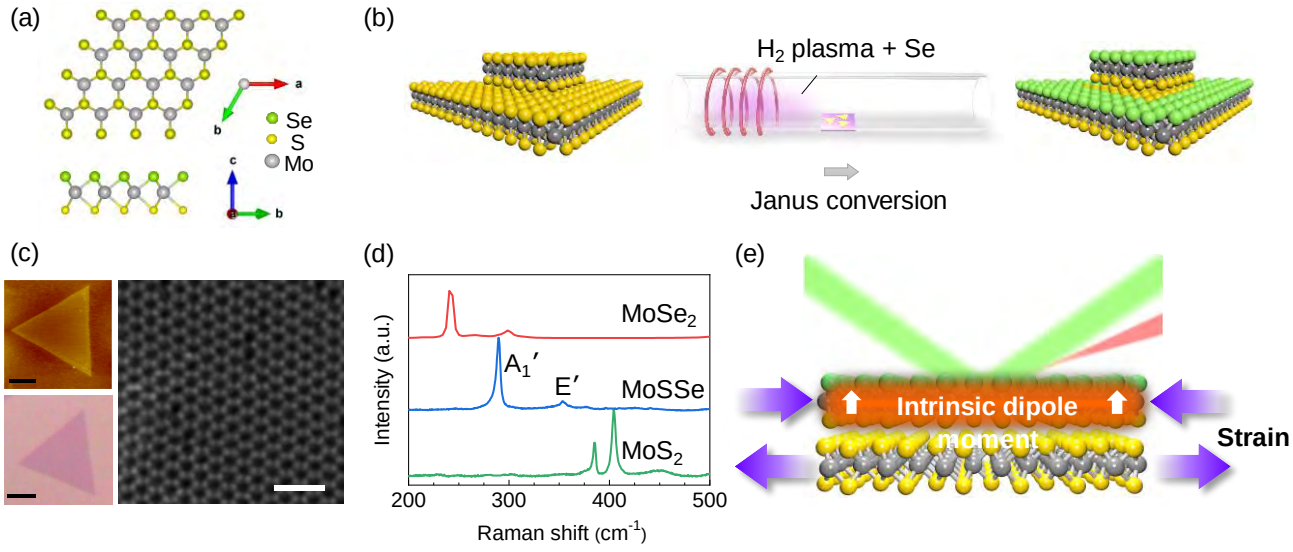

Figure 1: Janus MoSSe. (a) Crystal structure of MoSSe monolayer: bottom view (up) and side view (down). (b) Schematics of the synthesis procedure. (c) AFM (upper left), optical microscopy (OM) (down left), and MAADF-STEM images (right) of monolayer MoSSe. Scale bars: 2 μm for AFM and OM, 1 nm for MAADF-STEM. (d) Raman spectra of monolayer $MoSe_2$, MoS_2 and MoSSe. (e) Illustration of the effect of strain and dipole moment on optical response.

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Non invasive depth determination of target in *Ex vivo* animal tissues using deep Raman Spectroscopy

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In many applications, it is beneficial to identify both the chemical information on a buried object in diffusely scattering media as well as its depth within it. For example, in a clinical environment, the *in vivo* identification and localisation of a cancer lesion or SERS target located deep inside biological tissues could potentially facilitate more accurate diagnosis or improve the effectiveness of subsequent treatments (eg photothermal therapy).

This work demonstrates the use of spatially offset (SORS) and transmission Raman (TRS) spectroscopy for simultaneous non-invasive detection and depth prediction of an inclusion buried inside biological tissues. The concept exploits the differential attenuation of two Raman bands of the inclusion due to their different tissue matrix absorption to retrieve depth information[1-2]. The relative degree of the Raman band intensity changes due to matrix absorption is directly related to the pathlength of Raman photons travelling through the medium thereby encoding also the information on the depth of the object within the tissue. Four different calibration models, based on internal and external measurements, were tested and evaluated for predicting the depth of an inclusion, made up of surface-enhanced Raman scattering (SERS) labelled nanoparticles (i.e. NPs), within an up to 40 mm slab of ex-vivo porcine tissue. An external measurement carried out in transmission mode, with a noninvasively calibration model on the analysed sample, was shown to be insensitive to variations of the overall thickness of the tissue and the amount of SERS NPs yielding an average root mean square error of prediction of 6.7 % of total depth[3].

Our results pave the way for future noninvasive deep Raman spectroscopy in vivo enabling to localize cancer biomarkers for early disease diagnosis and targeted treatment.

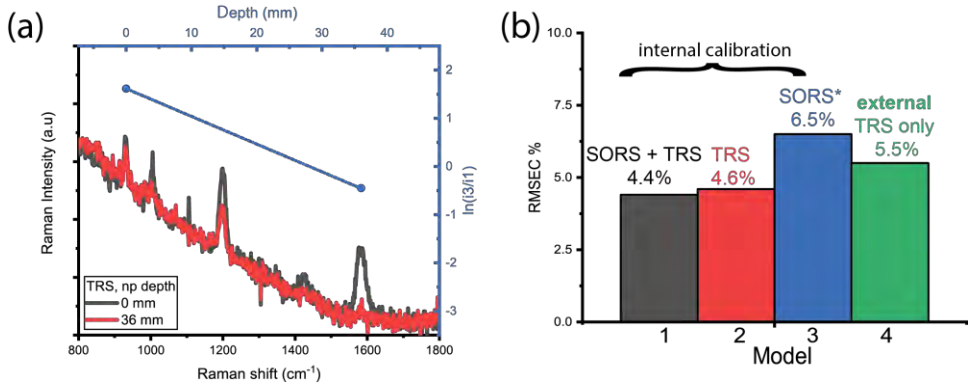

Figure 1: Raman Calibration model: (a) TRS spectra of the NPs measured at the illumination (depth = 36 mm, red line) and Raman collection (depth = 0 mm, black line) surfaces. Natural log of the I3/I1 ratio (top axes) vs depth (top axes) of the external measurement (blue dot) (b) Root Mean Square Error of Calibration (RMSEC) % for all different models. *SORS model refers to a maximum depth of 24 mm. (RMSEC % calculated with n-2 point)

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Removing Non-Resonant Background from CARS spectra via Deep Learning

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Broadband Coherent Anti-Stokes Raman Scattering (B-CARS) is a powerful label-free nonlinear spectroscopy technique allowing one to measure the full vibrational spectrum of molecules and solids [1-2]. B-CARS spectra, however, suffer from the presence of a spurious signal, called non-resonant background (NRB), that interferes with the resonant one, distorting the line shapes and degrading the chemical information. While several numerical techniques are available to remove this unwanted contribution and extract the resonant vibrational signal of interest, they all require the user's intervention and sensitively depend on the spectral shape of the NRB, which needs to be measured independently.

We present a novel approach to remove NRB from B-CARS spectra based on deep learning [3]. Thanks to the high generalization capability offered by the deep architecture of the designed neural network, trained through realistic simulated spectra, our fully automated model (SpecNet) is able to process B-CARS spectra in real time and independently of the detailed shape of the NRB spectrum. This results in fast extraction of vibrational spectra without requiring user intervention or the measurement of reference spectra. We expect that this model will significantly enhance the detection capabilities of B-CARS spectroscopy and speed up the imaging and detection capabilities of B-CARS microscopy.

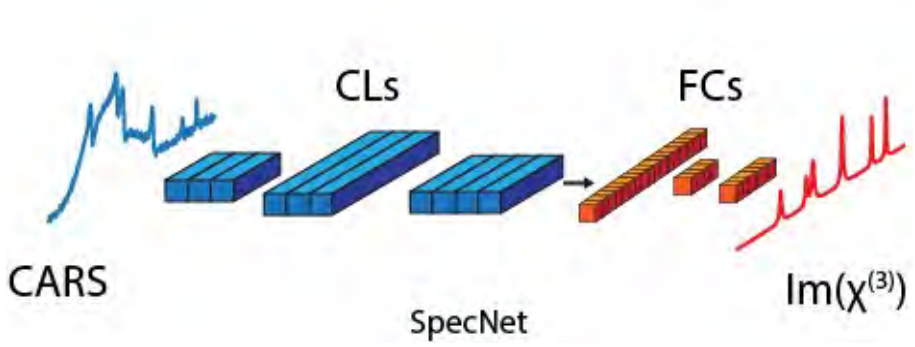

Figure 1: Schematic representation of the SpectNet model and related working principle. CL: convolutional layer; FC: fully connected layer.

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SERS-detection of osteogenic differentiation in stem cells cultured on simple gold nanoisland substrates

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Mesenchymal stem cells have been at the epicentre of regenerative medicine and therapeutic applications since their identification, due to their ability to differentiate into various cell types, such as osteoblasts. In vitro, osteogenic differentiation is induced by a cocktail of dexamethasone, ascorbic acid and β-glycerophosphate, however, it strongly depends on the donor-specific osteogenic differentiation potential. Since native MSCs demonstrate such variability, it is possible that their response to experimental manipulation may also vary, so it is of high importance to study cells with minimally invasive methods. The most commonly used procedures for following cell differentiation include e.g. immunofluorescence staining, qPCR, colorimetric assays. However, a label-free method that does not require the termination of the cell culture prior the analysis, is still lacking. Here, we show the direct and label-free approach for studying the extracellular matrix and membrane changes during differentiation in mesenchymal stem cells with surface enhanced Raman scattering (SERS). We have used SERS as a sensitive tool to study the structure of cellular compounds, providing comprehensive information on the molecules in the nm-scale proximity of gold nanoisland substrates. We fabricated the substrates by repeated gold deposition and thermal annealing, providing sufficient enhancement and a homogenous distribution of "hot spots" [1]. In order to demonstrate their applicability as in-vitro sensing platforms for long-term cell proliferation, we cultured MSCs and recorded spectra of the cellular membrane at different timepoints during differentiation (Fig. 1). While Raman scattering might not fully replace other biological procedures for studying cells, it can still serve as a great complement to the more invasive approaches.

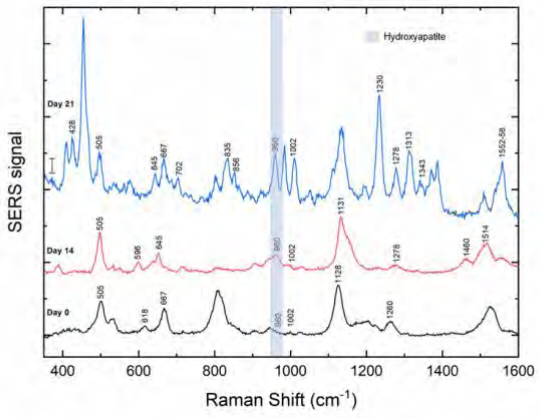

Figure 1: Representative SERS spectra of MSCs during different stages of osteogenic differentiation with highlighted peak at 960 cm-1 assigned to hydroxyapatite (laser excitation. 785 nm, intensity: 3mW, acquisition time: 3s, scale bar: 50 cps).

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Research progress of trace uranyl ions detection by SERS-based microfluidic devices

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Surface-enhanced Raman scattering (SERS) is a new trace analysis technology with high sensitivity, low sample dosage and easy identification of characteristic spectrum, especially suitable for the detection of dangerous chemicals such as uranyl ions.[1] In recent years we have been committed to the development of the highly sensitive detection of trace uranyl ions by SERS-based microfluidic devices. Therefore, here we introduce a series of our achievements in this field, which can be summarized into two aspects as follows: (1) the soft DNAzyme-based hydrogels modified on flexible SERS substrates as biofilms were applied to directly detect the aquatic products (such as fish and kelp) polluted by UO22+ ions;[2] (2) some novel SERS-based microfluidic devices were developed for real-time ultrasensitive fast detection. [3-4]

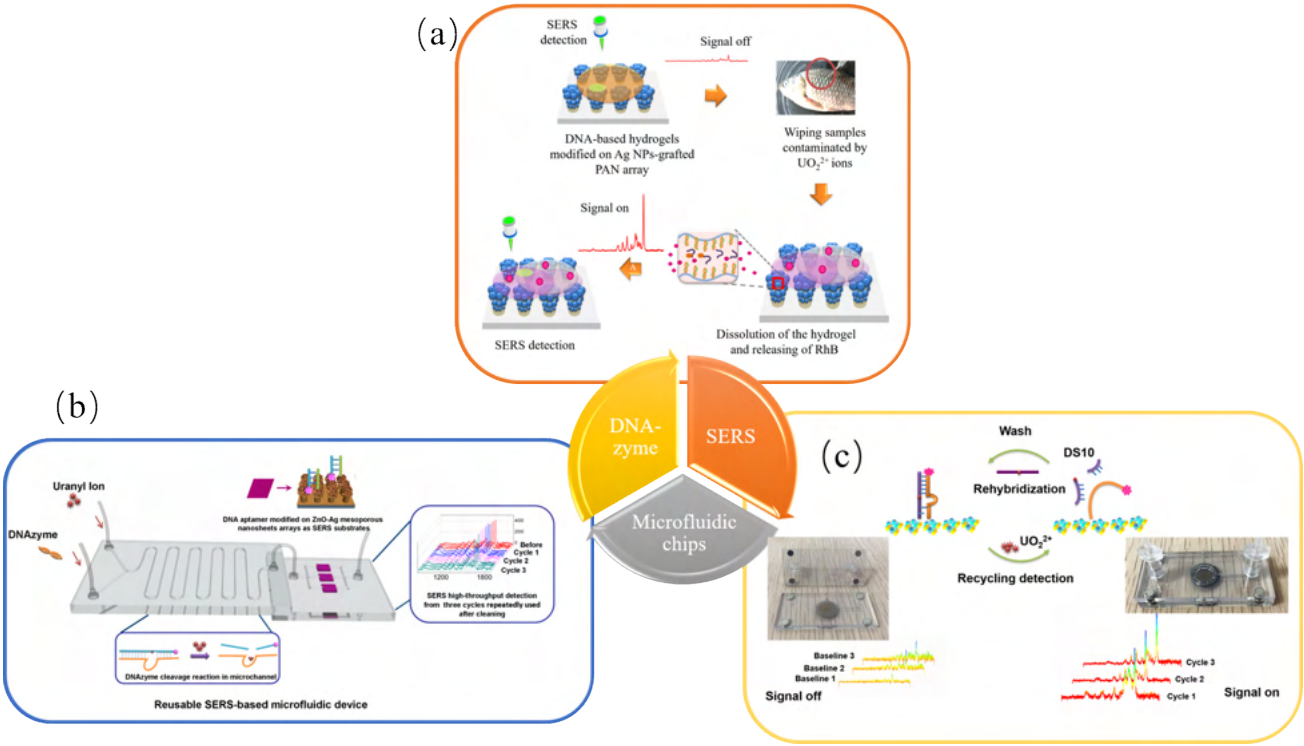

Figure 1 Trace uranyl ions detection by SERS-based microfluidic devices

As shown in Figure 1(a), highly ordered arrays were prepared under flexible SERS substrates, then coated with DNAzyme hydrogel for wiping test. Then, high-throughput screening microfluidic devices were designed for ultrasensitive detection of uranyl ions (Figure 1b). The complex device was improved and explored as recyclable devices for rapid sensing (Figure 1c).

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Nanoscale Chemical Imaging of Supported Lipid Monolayers using Tip-Enhanced Raman Spectroscopy

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DPPC monolayers are an important constituent of pulmonary surfactants, which can assemble in either liquid condensed (LC) or liquid expanded (LE) phases [1]. The phase transition from LE to LC phase and vice versa modulates the surface tension of the lungs. This stabilizes alveolar collapse during expiration and minimizes the work required to expand the alveoli during inhalation [2]. Therefore, understanding the molecular organization of DPPC monolayers into distinctly different phases is key to fully comprehending their biological functions.[3]

In this work, we have investigated DPPC monolayers supported on Au(111) using highly sensitive tip-enhanced Raman spectroscopy (TERS) imaging. Hyperspectral TERS imaging enabled reproducible visualization of molecular disorder in the DPPC monolayers, with a spatial resolution of 40 nm as shown in Figure 1.

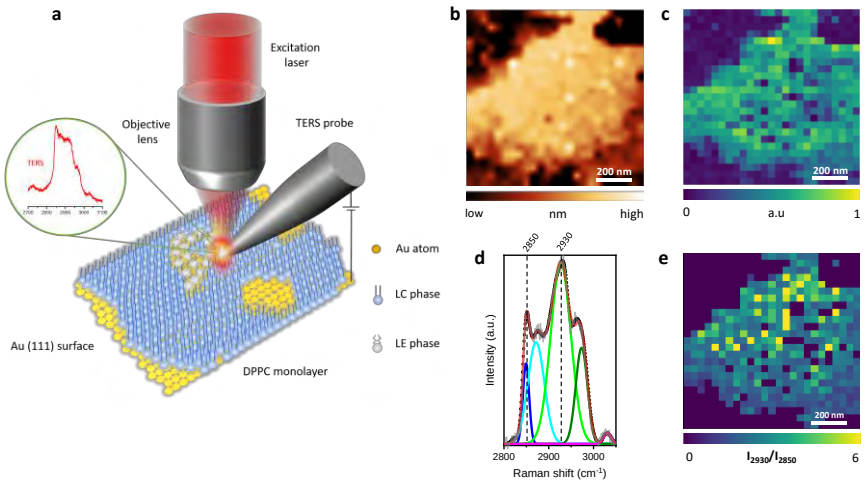

Figure 1. a) Experimental set-up used for TERS imaging of the supported DPPC monolayer. b) STM topography image of the DPPC monolayer on Au(111) single crystal. c) TERS image of C-H stretching (2800 – 3000 cm^{-1}) intensity. d) Average TERS spectrum of the TERS image in (b). e) TERS image of I_{2930}/I_{2850} ratio.

We obtained reproducible TERS spectra with a significantly better signal-to-noise ratio than reported to date, which allowed a direct correlation of high-resolution STM topography and TERS images for the first time. TERS imaging also revealed nanoscopic structural defects of size 40-120 nm in the DPPC monolayer. This is the first direct hyperspectral detection of such nanoscale features inside a supported lipid monolayer. Finally, using the I_{2930}/I_{2850} Raman band ratio, we were able to reveal the nanoscale packing order of the lipid monolayer on the same single crystal terrace [4,5]. This work demonstrates that TERS is a promising tool for the characterization and imaging of model lipid membrane systems. Furthermore, it opens the door for direct, label-free, non-destructive characterization of more complex biological membranes at a nanoscale level.

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Multi-Wavelength Raman Spectroscopy of Poly(Furfuryl Alcohol)

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Poly(furfuryl alcohol) (PFA), produced through polymerization of furfuryl alcohol, is a thermosetting polymer and basis of thermoset resin systems, and it has been investigated in several studies (IR, ^{13}C-NMR, Raman, DSC), new aspects being considered each time [1-5]. Nevertheless, PFA still remains an intriguing polymer, since curing [1-2], apart from being promoted by the presence of an acid catalyst, can be also induced by heat or suitable radiation, the resulting molecular structure of PFA thermoset compared to PFA resin not being that obvious. At present a clear and unambiguous assignation of the Raman bands observed [1,3] in the non-resonant and resonant Raman spectra of PFA thermoset is still missing, being the knowledge about the contribution of conjugated structures and cross-linking in PFA thermoset still under debate [1-4]. The purpose of the present study [1, 5] is the characterization of both PFA resin and of PFA thermoset by multi-wavelength Raman spectroscopy in the visible and in the ultraviolet spectral range, using excitation wavelengths from several laser sources and from a synchrotron light source. By taking advantage from all previous findings, the foreseen structures were modeled, simulating the corresponding Raman spectra, and exploiting them to evaluate their matching with experimental ones, for a possibly correct interpretation of the Raman spectra of PFA resin and PFA thermoset, recorded with the different excitation wavelengths. The study has been financially supported by the research project ITAT1059-InCIMa4, which is funded by the European Regional Development Fund (ERDF) and Interreg V-A Italy-Austria 2014-2020.

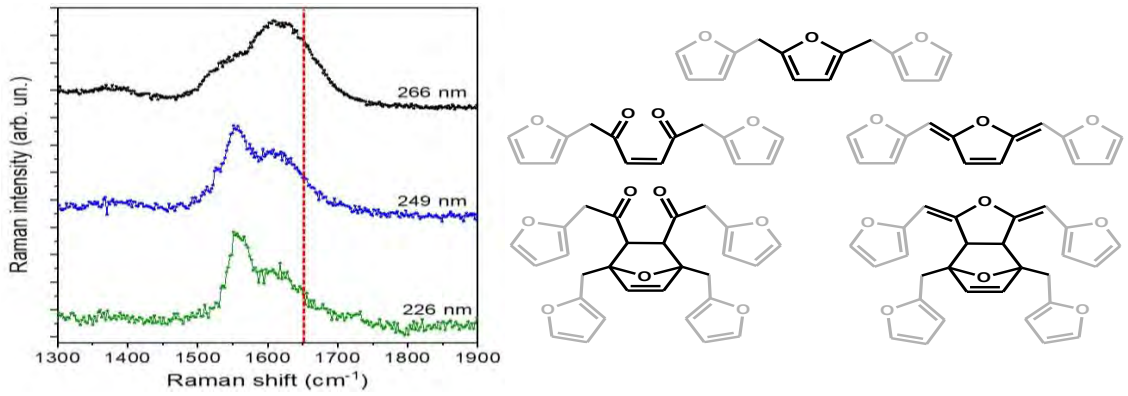

Figure 1: UV Raman spectra of PFA thermoset, and proposed molecular structure units of the PFA polymer [1]

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Raman Spectroscopy for Detecting Traces of Explosives at Security Checkpoints

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Remote detection of hazardous materials is of great interest as it allows for real-time threat detection without putting people in harm's way. It is thus highly important to develop new chemically selective and sensitive methods to detect such materials from afar. There are a number of techniques, such as for example infrared spectroscopy, laser-induced fluorescence, and Raman spectroscopy [1-3], that can be used for remote detection of explosives. Especially UV Raman spectroscopy is a very promising technique as it is highly selective and far more sensitive than conventional Raman spectroscopy using visible or near-infrared light sources. Additional advantages of using UV light as the excitation source are the reduced interference with the fluorescence background and a possible resonance enhancement when the excitation wavelength approaches electronic transitions in the sample [2, 4]**.**

In order to monitor for hazardous materials at e.g. security checkpoints, the spectroscopic unit has to be combined with an optical tracing unit to locate the position of the sample and align the optics toward the sample. In such a scenario, variations in target distance and the angle of incidence have to be considered. Other important parameters influencing the systems detection sensitivity include interfering signals from background materials and overall material coverage, as well as penetration depth and energy density of the laser.

Here, we present a concept of using a UV laser for remote Raman scattering detection of trace amounts of explosive materials at security checkpoints. The influence of the above-mentioned parameters on UV explosives Raman spectra recorded with our system will be evaluated.

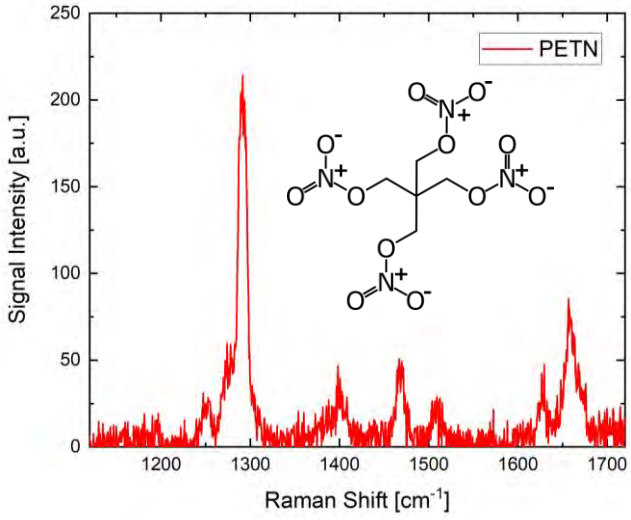

Figure 1: Exemplary standoff Raman spectrum and structural formula of the explosive PETN.

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Development of a Versatile SERS Sensor using Tyramine-medicated Crosslinking Chemistry

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Improving the design of surface-enhanced Raman scattering (SERS) substrates is essential in terms of detection sensitivity and signal reproducibility.[1,2] In past decades, several studies have focused on the generation of active hot spots with varied morphological structures. However, controlling the capture of analyte molecules, which have weak surface affinities toward metal surfaces, into the hot spot sites still remains challenging.

In this presentation, a novel strategy for generating active hot spots using tyramine-mediated crosslinking chemistry will be discussed.[3] Tyramine molecules, which are known to initiate radical reactions resulting in crosslinking networks in the presence of enzymes and hydrogen peroxide, induced a controlled aggregation of the Ag nanoparticles (NPs), and the analytes were physically captured inside these dynamic hot spots. The nanogap between two tyramine-mediated crosslinking NPs was estimated to be 1.3 nm by DFT calculation. The SERS intensity obtained using this strategy was 3.5 times more intense than that obtained using traditional SERS method using colloidal Ag NPs. The expandability of our pesticide SERS sensor was demonstrated by the detection of thiabendazole and 1,2,3,5-tetrachlorobenzene, which have different binding affinities toward metal surfaces. It is expected that our study makes a significant contribution to achieve a reliable SERS sensing which is expandable to a wide range of analyte molecule.

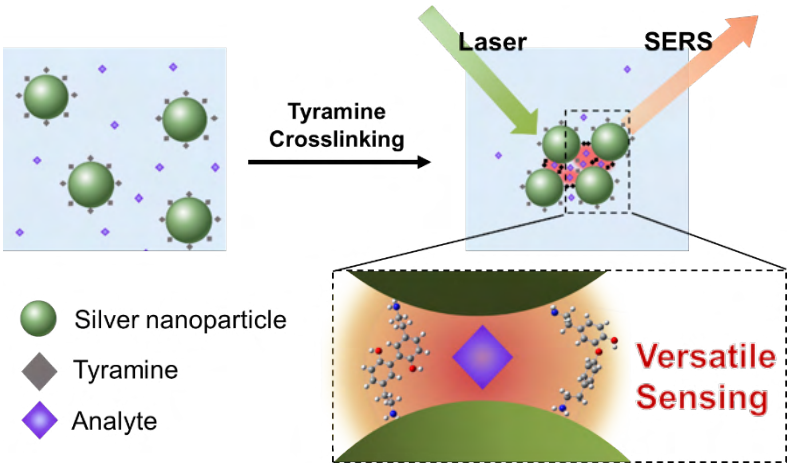

Figure 1: Synthesis and characterization of Ag nanogap-shell (Ag NGS)

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Shifted Excitation Raman Difference Spectroscopy as a Promising Tool for Precision Agriculture

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Precision agriculture becomes of global importance, e.g. to meet the food demand of a steadily increasing world population. Site-specific fertilizer application can not only ensure sustainable use of limited resources, but it is also crucial for environmental protection by avoiding excess usage of fertilizers. We address this important point by applying Raman spectroscopy as non-destructive optical method for soil characterization to pave the way for efficient nutrient management.

Shifted excitation Raman difference spectroscopy (SERDS) is applied as a powerful technique to effectively separate Raman signals from background interference, e.g. fluorescence or luminescent bands, which is a common issue for a wide range of natural samples. An in-house developed specialized Y-branch dual-wavelength diode laser emitting at 785 nm serves as excitation light source with two distinct emission lines (spectral distance: 0.6 nm) required for SERDS [1]. The measurement principle has already been successfully demonstrated for *in-situ* outdoor investigations in an apple orchard [2] and is now translated towards soil analysis.

For our study, SERDS spectra of 33 soil samples collected from an agricultural field in northeast Germany were recorded. The intrinsic soil heterogeneity at the millimeter scale has been considered using a raster scan approach comprising 100 individual measurement positions for each sample. SERDS enabled the recovery of characteristic spectral signatures of selected minerals (quartz, feldspar, anatase, calcite) and amorphous carbon from interfering background contributions and the recorded SERDS spectra allowed for the clear distinction of individual soil constituents. Quantitative soil analysis by means of partial least squares regression was demonstrated to predict soil organic matter content as important parameter affecting soil fertility ($R^2 = 0.82$, RMSECV = 0.41 %).

These results highlight the large potential of SERDS as a promising tool for soil nutrient analysis in the context of precision agriculture to improve soil nutrient management [3,4]. The findings of our investigations provide a much-needed basis for future applications of portable SERDS systems for *in-situ* field measurements on soil.

This study was funded by the Federal Ministry of Education and Research (BMBF) under contracts 031A564C and 031B0513C through the funding measure BonaRes (Soil as a Sustainable Resource for the Bioeconomy) within the consortium I4S (Intelligence for Soil) and it was also partly funded under contract 16FMD02 (Research Fab Microelectronics Germany - FMD).

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Chemically stable surface bound thiolate intermediates in surface enhanced Raman spectroscopy

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4-aminothiophenol (ATP) and 4-nitrothiophenol (NTP) are two thiols (or the respective thiolates) commonly used in surface enhanced Raman spectroscopy (SERS) towards the investigation of plasmon-induced photocatalysis.[1] Numerous projects[2] investigated the specific redox reactions involved. Particularly, one dimerized intermediate dimercaptoazobenzene (DMAB) is well assigned and generally accepted. Vibrational modes at 1560 cm^{-1} to 1600 cm^{-1} are frequently used to distinguish these three compounds. However, overlapping modes render an assignment of intermediates difficult.

In order to investigate potential intermediates, we extensively studied the fate of NTP, ATP, and DMAB under various conditions specifically on vapor deposited SERS substrates. We found that ATP and NTP result in similar spectra after identical treatment that are clearly different from DMAB (Figure 1). In addition, a new band around 1350 cm^{-1} clearly differs from the nitro group in NTP (~1330 cm^{-1}). Thus, we propose there is at least one stable intermediate and will demonstrate that with specific chemical treatment of these intermediates the relations between these structures and DMAB can be shown including the pathways of NTP to DMAB and ATP to DMAB. The results provide experimental basis to previous theoretical structure elucidations[3] and we expect a more complete assessment of the involved intermediates formed in these reactions.

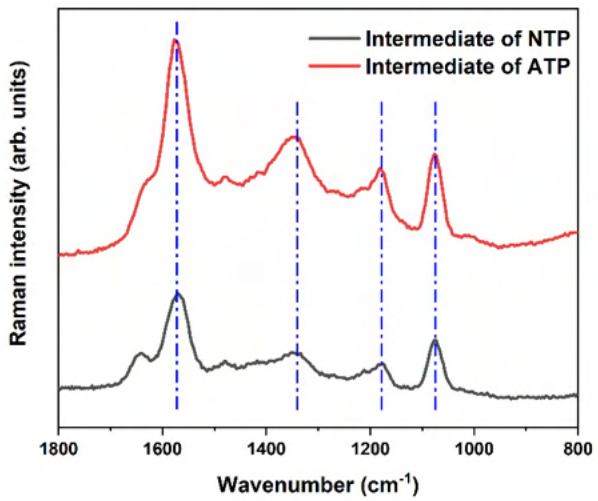

Figure 1: Similar and stable intermediates of ATP and NTP in SERS measurements. ATP and NTP were immobilized on Ag-SERS substrates by immersing substrates into their ethanol solutions for 1-2 hours, respectively. Experiments were conducted at ambient conditions using a 532 nm laser with a power of around 280 µW.

ACKNOWLEDGEMENTS
The authors acknowledge the financial support from the Leibniz Science Campus InfectoOptics SAS-2015-HKI-LWC and thanks Prof. Dr. Stefanie Gräfe group providing help on simulations!

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Raman spectroscopy in monitoring of adipogenesis and carotenoid delivery to adipocytes

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A growing interest in the role of adipose tissue (AT) under physiological conditions and upon the development of the disease has led to increasing demand for its representative *in vitro* models. Primary adipocytes, as well as mature preadipocytes differentiated from stromal vascular fraction (SVF) cells, are suitable for mechanism studies and drug testing. There are many natural compounds tested for positive effects in overcoming lifestyle diseases i.e. obesity and obesity-related cardiovascular events. Carotenoids are an example of such molecules used to counteract excess adiposity by taking advantage of their anti-inflammatory and adipocyte browning potential [1]. However, carotenoids have low solubility in water and are sensitive to environmental factors, i.e. light and oxygen, which result in their low bioavailability and bioaccessibility.

In this work, we compared the phenotype of isolated murine primary adipocytes and differentiated SVF cells derived from epididymal (eWAT) and interscapular (iBAT) adipose tissues of C57Bl/6 mice during adipogenesis and *de novo* lipid droplets formation. Then, we tested various conditions to increase the stability and efficient delivery of selected carotenoids to primary adipocytes. Due to the resonance and chiral properties, we monitored the processes of carotenoid assembly and delivery to fat cells by Raman imaging and chiroptical spectroscopies. The aim of this work was to investigate the role of the carotenoid carrier, its accumulation, and structural changes due to the uptake of adipocytes.

Our results show, among others, that considerable chemical and functional changes occur along with SVF cell differentiation and maturation, resulting in mature adipocytes of similar chemical composition, independent of cellular origin, while markedly different compared to primary adipocytes. On the other hand, studies on carotenoid uptake indicate that the efficiency of carotenoid transport to primary adipocytes depends on the way of delivery, i.e. suspension, protein binding, or micelle encapsulation, which is accompanied by the aggregation of carotenoid molecules. Our work demonstrates the applicability of Raman spectroscopy to evaluate and determine the alterations in *in vitro* models of adipocytes that may serve as a future methodology to test the effect of i.e., fat-burning drugs in obesity-reducing therapies.

Acknowledgments
The project was supported by the National Science Centre (NCN, OPUS17, 2019/33/B/ST4/0087). The participation in the conference was supported by the BioS Priority Research Area under the program "Excellence Initiative – Research University" at the Jagiellonian University in Krakow"

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Direct measurement of mode specific second order nonlinear susceptibility of collagen using vibrational sum frequency imaging

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Collagen is the most abundant protein in the human body, where it is a key component in connective tissue such as skin, cartilage, tendons and bones. Collagen is implicated in wound healing and the progression of numerous diseases, including cancer, which is typically associated with structural imperfections in collagen organization. Second-harmonic generation (SHG) microscopy is a popular tool for probing collagen structure and organization. Nonetheless, SHG is spectroscopically nonresonant and thus provides limited information about changes in collagen's conformational and chemical structure. Sum-frequency generation (SFG) microscopy is an alternative method that, like SHG, visualizes collagen through the material's second-order nonlinear susceptibility $\chi^{(2)}$. Unlike SHG, SFG adds spectroscopic information to $\chi^{(2)}$-based contrast [1,2], probing molecular vibrations that exhibit both Raman- and IR-activity.

In this contribution, we present mode-specific measurements of the $\chi^{(2)}$ of collagen using vibrationally sensitive SFG microscopy. We perform polarization sensitive measurements to reveal all non-zero tensor elements of $\chi^{(2)}$ of various modes in the C-H stretching range of the vibrational spectrum. Comparing these polarization sensitive measurements with the crystal structure of collagen, we assign the definite mode symmetries of the different spectral features observed in this range.

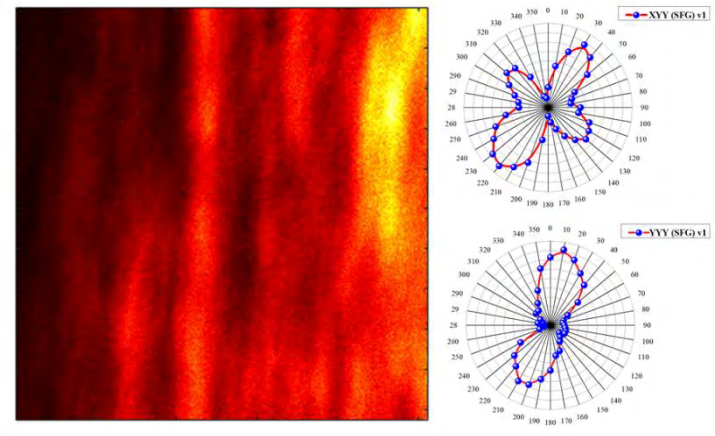

Figure 1. V-SFG image of collagen tissue and its anisotropic responses for the parallel and perpendicular detection geometry.

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Vibrational tags for Raman and infrared-based imaging

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Vibrational tags are chemical motifs with distinct vibrational signatures that, when incorporated into a target molecule, can be used to probe and identify target structures with the aid of vibrational microscopy. Recent developments have shown the utility of vibrational tags in both Raman-based microscopy and infrared-based microspectroscopy. In this context, the availability of probes that feature a strong response in both Raman and infrared imaging modalities is highly desirable. In this contribution, we develop vibrational tags which strong and narrow vibrational lines in the cell silent region of the spectrum that can be used in both vibrational imaging modalities. For this purpose, we screened numerous chemical motifs with density functional theory (DFT). Design criteria included a distinct resonance frequency, a long vibrational lifetime for narrower linewidths, and overall strength of the vibrational response. DFT simulations revealed several promising candidates that were subsequently synthesized as tags to lipid and glucose targets. In this presentation, we discuss the utility of these optimized vibrational probes for cellular imaging studies with stimulated Raman scattering and Fourier transform infrared microspectroscopy.

Vibrational and electronic properties of sp-carbon chains probed by synchrotron-based UV resonance Raman spectroscopy

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Besides the most known form of carbon allotropes, linear sp-carbon chains are appealing 1D systems made of sp-hybridized carbon bonds that generate highly conjugated molecular wires. Sp-carbon chains have been predicted to possess outstanding mechanical, thermal, and optoelectronic properties [1]. In particular, sp-carbon chains show appealing vibrational properties [2]. Indeed, they feature a peculiar collective vibration, called α mode, that is intrinsically connected to the structural and optoelectronic properties of sp-carbon chains [2,3]. Moreover, this vibrational mode is located in a region of the Raman spectra where no other carbon nanostructures feature any signal, allowing direct detection of sp-carbon bonds [1,2].

In this framework, we investigated the vibrational and electronic properties of size-selected linear sp-carbon chains via synchrotron-based UV resonance Raman scattering (UVRR). Indeed, thanks to the wavelength tunability of the synchrotron radiation in the UV range, we could precisely select the vibronic absorption peak of each size-selected H-capped polyyne (HC_nH, n=8,10,12) at which collecting the resonance Raman spectra (Fig. 1a). In this way, we detected for the first time vibrational overtones and combination bands up to the fifth order (Fig. 1b,c,d), allowing for the determination of the vibrational levels of both the ground and excited states (Fig. 1e). We also observed an interesting intensity modulation of the overtones determined by the selected vibronic transition that can be explained by a simple analytical model based on Albrecht's theory of resonance Raman scattering. Moreover, we investigated the optoelectronic properties of H-capped polyynes by combining experimental UV-Vis absorption and first-order resonance Raman spectra, finding the precise values of the displacement parameters, and discovering a peculiar size-dependent electron-phonon coupling given by the Huang-Rhys parameter. These results will help to understand the appealing properties of sp-carbon chains for future applications in the field of electronics and optics.

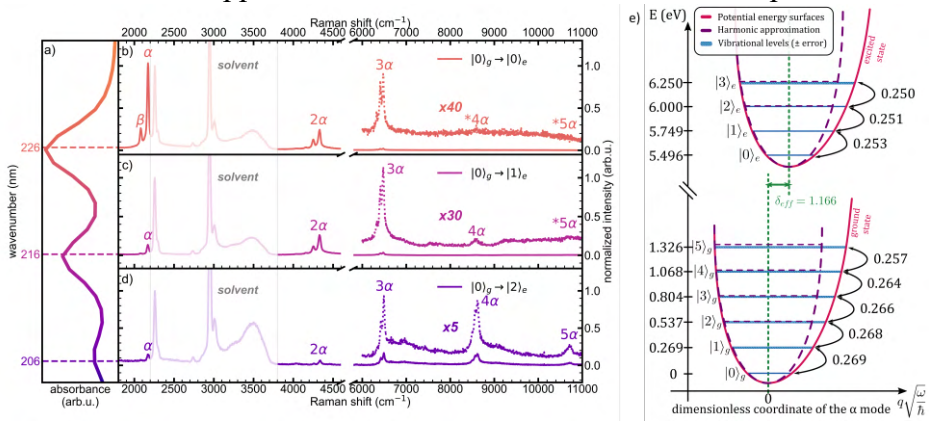

Figure 1: UV-Vis absorption spectrum (a) and UV resonance Raman spectra of HC_8H, excited using synchrotron radiation at 226 nm (b), 216 nm (c), and 206 nm (d). e) Vibrational diagram of the ground and excited potential energy surfaces extracted from experimental UVRR and UV-Vis absorption spectra of HC_8H and referred to the normal coordinate q of the α mode. The effective displacement parameter δ_{eff} is reported in green.

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Liquid-Liquid Phase Separation in Synthetic Polymers *via* Femtosecond Stimulated Raman Microscopy

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Liquid–liquid phase transitions in biological macromolecules are of central importance for the synthesis of stiff proteinaceous biomaterials such as silks [1] or underwater adhesives, [2] and for the formation process of functional microstructures in cells.[3] Phase separated liquid–liquid macromolecules are referred to as coacervates and it is believed that complex coacervates had a part in the origin of life.[4] To study the phase separation process, natural and synthetic coacervate systems (e.g. polyelectrolytes) are used.[5] Two major challenges need to be met when studying phase-separation processes: (i) The quantity of material required and (ii) the need to use multiple complex measurement techniques.

We want to tackle these difficulties using femtosecond stimulated Raman microscopy (FSRM). Raman microscopy was already successfully used in the study of coacervation.[6,7] FSRM is a non-linear imaging technique able to achieve full spectral coverage for each pixel with an acquisition time as fast as 0.1 ms and was already successfully applied to polymer characterization.[8,9] First FSRM experiments on polymeric coacervates will be presented.

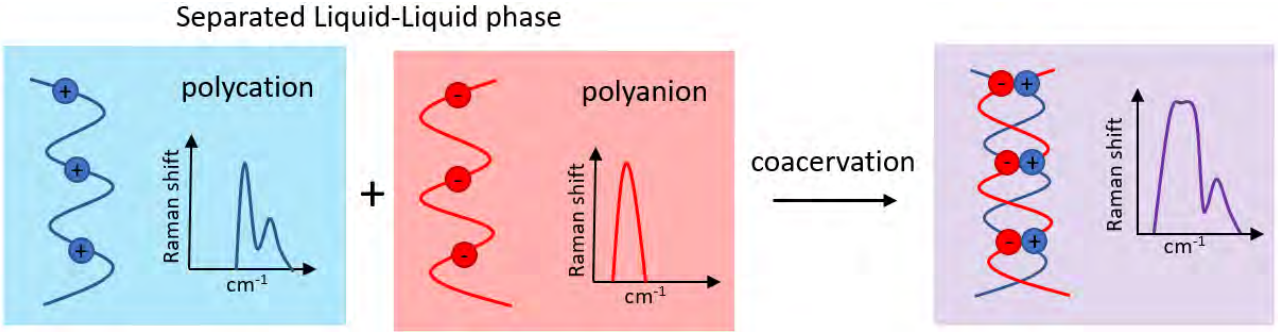

Figure 1: Schematic representation of a conventional polyelectrolyte coacervation.

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Raman Analysis of Nanoparticles in Reflection Mode Nanoaperture Optical Tweezers

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Optical tweezers have been combined with Raman in several studies of isolated objects (e.g., cells [1]); however, for the analysis of smaller individual nanoparticles in solution, nanostructured metals are highly desired to: 1) localize the trapping to the size of a single nanoparticle and 2) provide surface-enhanced Raman scattering (SERS). In the past, our group and others have demonstrated this for identifying nanoparticles using apertures in metal films [2, 3]. A common challenge with these approaches is that the trapping is usually measured with transmission through the aperture, whereas a simple reflection microscope geometry is desired for Raman analysis. To address this challenge, here we present a novel reflection geometry setup that shows enhanced signal steps upon trapping and is combined with Raman detection, where Fig. 1 shows preliminary trapping data for hBN nanoflakes. It is possible that this approach may be combined with nanopores and/or used in the analysis of DNA [4], or other biomolecules and their interactions [5]. Our group and others have already shown extensive applications in protein analysis and combining this approach with Raman spectroscopy opens further analytical capability.

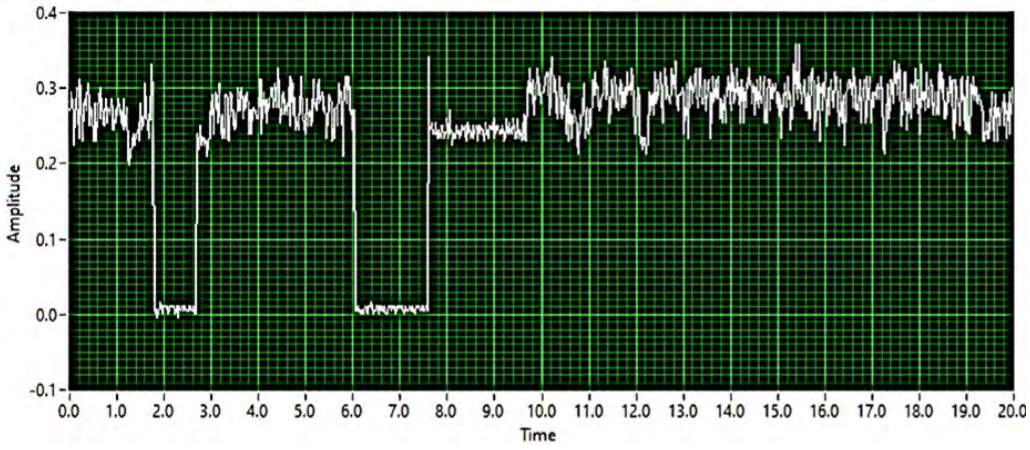

Figure 1: Trapping of hexagonal Boron Nitride nanoflakes in solution (50-200 nm diameter, few nm thickness) with double nanohole aperture in metal film in reflection geometry. Laser is blocked and unblocked twice, each time showing trapping, which is clear from the increase in the voltage amplitude detected at the avalanche photodiode.

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Non-destructive investigation of diffusion of conservation products by micro-SORS

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A crucial issue in Conservation Science is the investigation of the penetration depth and diffusion of conservation products within the substrates, essential for assessing the efficacy of treatments. Another important topic is the study of the undesired diffusion of solvents into a substrate during cleaning procedures. To date, the most common approach to obtaining this information is destructive cross sectional analysis.

Here we propose Micro-Spatially Offset Raman Spectroscopy (micro-SORS) as a non-destructive method for studying the diffusion of conservation products and solvents into substrates. Micro-SORS combines macro-scale SORS with microscopy concept to enable the collection of sub-surface Raman signals in micro-stratified turbid materials, at depths beyond the reach of conventional confocal Raman microscopy [1]. To date, micro-SORS has been mainly used to investigate layer sequences; here, instead, we used micro-SORS to study the diffusion of a product (agent) that spreads into a substrate (matrix). Laboratory mock-up samples were prepared mimicking typical situations encountered in Cultural Heritage materials where an agent diffuses in a matrix, including treated plasters and stuccos, and a painted surface cleaned with a solvent [2-3]. The micro-SORS experiments demonstrated its capability to discriminate, in a non-destructive way, different diffusion depths of products into matrix. The efficacy of the method was also demonstrated on a case study: treated plasters collected from the painted façade of Palazzo Besta, an historical Italian building, were analysed with micro-SORS providing relevant information about the distribution of the treatment inside the plaster matrix in a non-destructive way.

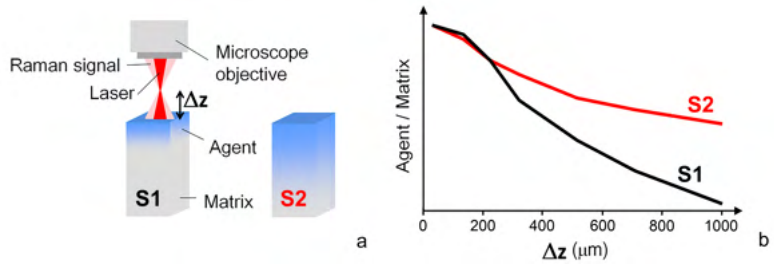

Figure 1:(a) Scheme of a micro-SORS measurement; (b) Agent/Matrix Raman intensity ratio over the degree of defocusing.

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Highly sensitive SERS sensor based on Ag nanoparticles for heavy metals detection in water

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Surface-enhanced Raman scattering (SERS) is a powerful vibrational spectroscopic technique that enables ultrasensitive molecule detection due to the enhancement of its characteristic Raman signals when it is attached or in close proximity to a plasmonic nanoparticle (NP)/nanostructure. The low Raman cross-section of many interesting analytes (i.e. heavy metals) requires the use of highly efficient, robust and reproducible SERS substrates.[1] However, despite the inherently higher SERS activity of Ag compared to Au, its higher reactivity arising from oxidation limits its applicability. [2]

Here, we report the fabrication of a SERS sensing platform based on the electrostatic layer-by-layer (LbL) assembly of Ag NPs.[3] The optical properties and SERS efficiency were analyzed as a function of size and loading of Ag NPs. This study provides accurate information on the plasmonic platform, elucidating the relationship between optical properties and SERS efficiency in hot-spot containing systems. Finally, the most efficient SERS substrate was used for the ultradetection of toxic heavy metal cations in water. The design and optimization of this type of plasmonic platforms pave the way for the detection of other relevant (bio)molecules in a broad range of fields such as environmental control, food safety or biomedicine.

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Anti-Stokes Raman scattering and sound velocity of monolayer graphene

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Raman spectroscopy associated with double resonance process in carbon materials is a unique technique to reveal the relationship between their characteristic electronic band structures and phonon dispersion. Here, we report the Raman scattering of the 2D[1], 2D' and combination phonon modes of LOLA and LOTA[2] in graphene. The excitation energy (E_{ex})-dependent frequency discrepancy between anti-Stokes and Stokes components of the 2D mode ($\Delta\omega(2D)$) is observed, which is attributed to the nonlinear dispersion of the in-plane transverse optical (iTO) phonon branch near the K point, confirmed by the nonlinear E_{ex}-dependent frequency of the 2D mode ($\omega(2D)$) in the range of 1.58–3.81 eV. The wavevector-dependent phonon group velocity of the iTO phonon branch is directly derived from $\Delta\omega(2D)$. We also report E_{ex}-dependent frequency of 2D', LOLA and LOTA modes, associated with intravalley double resonance process. The corresponding sound velocities (v_{TA}=12.9 km/s, v_{LA}=19.9 km/s) of graphene have been accessed, which are about 10% smaller than those of graphite. Based on v_{TA} and v_{LA}, the two-dimensional (2d) elastic stiffness (tension) coefficients c_{11} and c_{66}, Young's modulus and Poisson's ratio can be estimated. The results demonstrate again that double resonant Raman spectroscopy is a powerful tool to probe the fundamental properties of graphene.

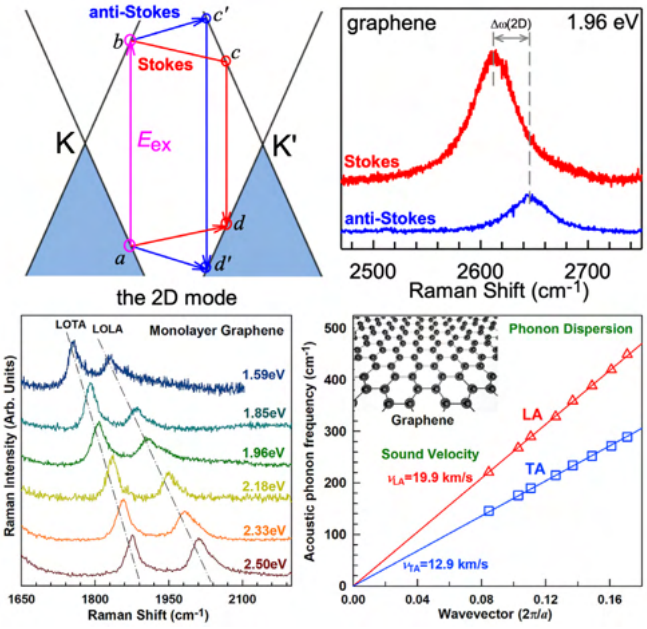

Figure 1: Stokes and anti-Stokes Raman process of 2D mode. Raman spectra of 2D, LOTA and LOLA modes. Obtained sound velocity of graphene.

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Chemically resolved pump-probe investigations of molecular dynamics

Riccardo Mincigrucci[1], Emiliano Principi[1], Claudio Masciovecchio[1]

1 Elettra Sincrotrone Trieste SCpA

FERMI is a peculiar pulsed source which allows a fine control of the energy and energy of the emitted photons. These properties have recently been used to investigate the possibility of observing impulsively stimulated vibrational dynamics of thin molecular layers. In particular, experimental and theoretical protocols have been developed to understand the excited and visible modes on the Hydroxychloroquine compound, while on an Ibuprofen racemic mixture the fine tunability and polarization control the FERMI capabilities, have been applied to observe the vibrational dynamics of chemically inequivalent carbon atoms belonging to the two deposited enantiomers. Such preliminary experiments, are necessary steps toward the realization of more complex studies where, e.g. we envision that the chemical / enantiomeric selectivity demonstrated here, could help in visualizing the vibrational dynamics occurring during a binding process and unveiling the fine, dynamical details of e.g. a drug-target interaction.

Optical tweezing combined with confocal Raman microscopy detects the metastable amorphous intermediate responsible for laser-induced nucleation

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Laser-induced crystal nucleation through optical tweezing, and in particular polymorph selection through laser polarization, promises unprecedented control over crystallization. However, in the absence of a nearby liquid–liquid critical point or miscibility gap (G,H),the origin of the required mesoscale clusters remains unclear. A number of recent studies of so-called nonclassical nucleation have suggested the presence of large amorphous clusters. Here we show that supersaturated aqueous glycine solutions form metastable intermediate particles that are off the direct path to crystal nucleation (K).Laser-induced crystal nucleation only occurs when the laser "activates" one of these particles. *In situ* low-frequency Raman spectroscopy is used to demonstrate their amorphous or partially ordered character and transformation to various crystal polymorphs. The requirement for solution aging in many previously reported laser-induced crystal nucleation experiments strongly suggests that the presence of amorphous intermediates is a general requirement.

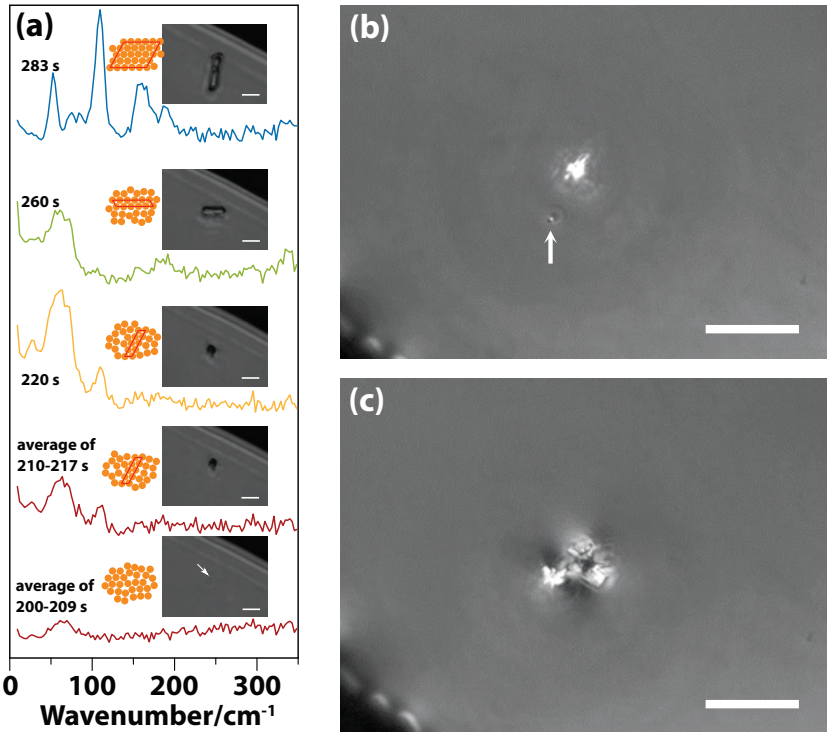

FIG. & Spectral evolution due to intermediate stages between microscopic particle and a fully-fledged crystal. Selected difference Raman spectra (with the spectrum at t = 78 s subtracted) in the low-frequency region at key times, along with the time-matched microscopy images and cartoons of proposed structure of intermediates. Scale bars, B µm; (a) One of the micrometer sized glycine particle in solution, indicated by the arrow. The bright spot is scattered light from the focus of the 7HH-nm pulsed laser set to JKH-mW in the sample; (b) The glycine particle is brought into laser focus by manually moving the stage instantly triggering crystallization; Scale bars, JH µm.

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XXVII International Conference on Raman Spectroscopy

Coherent Raman scattering-guided real-time precision molecular control

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Controlling the activities of biomolecules in living biological samples is a challenging task. The conventional method of culturing cells with compounds does not have spatial specificity, and thus cannot rule out potential complexity or misunderstanding of biological functions due to off-target effects. We developed a real-time precision opto-control (RPOC) technology that can control molecular behaviors in live cells at sub-micron precision, in real-time, and with high chemical selectivity [1]. Coherent Raman scattering (CRS) signals allow for label-free control of chemical processes using vibrational signatures from molecular targets. The concept of RPOC is illustrated in Figure 1. An excitation laser is scanned throughout the biological sample and generates a CRS signal at specific pixels of interest. The CRS signal is sent to comparator circuits for real-time comparison with pre-set conditions. Once satisfied, the comparator circuits will command an acousto-optic modulator to turn on the control laser beam only at the pixels where the optical signals are detected. The feedback loop response time is less than 20 ns, which is much faster than the pixel dwell time of the laser scanning. Using CRS signals, we can turn on the control lasers at only selected molecular targets based on the chemical signatures of the molecules. Digital logic circuits further enable smart laser activation based on logic combinations of different detection channels. Using this CRS-based RPOC system, we demonstrated precision control of photochromic molecules at different parts of the cells and with different CRS intensities. Furthermore, we synthesized photo-switchable tubulin inhibitors that can be activated by blue light [2]. Using RPOC, we can selectively inhibit the polymerization processes of microtubules at different subcellular locations and stop the active transport of lipid droplets. RPOC allows for precisely manipulating molecular processes using Raman or fluorescence signals in live cells at sub-micron precision without affecting unwanted targets.

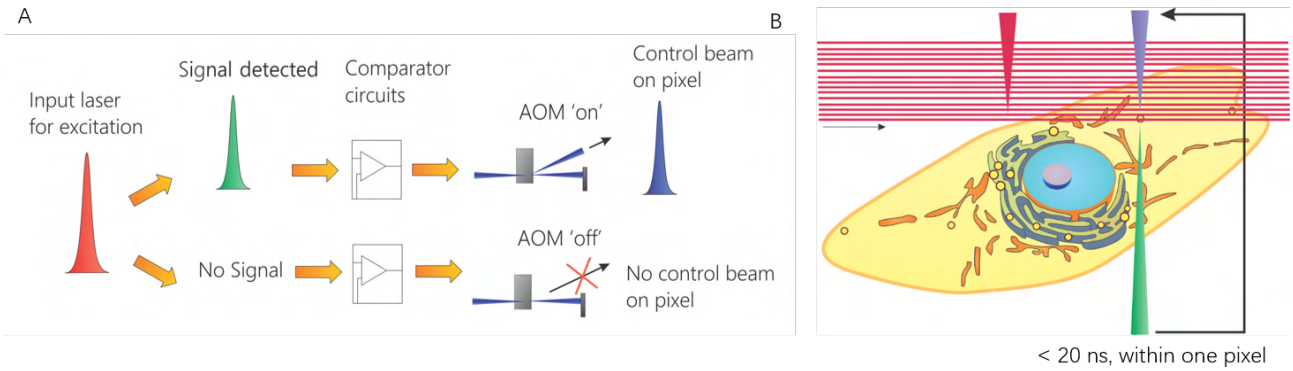

Figure 1. (A) An illustration of the RPOC concept. The excitation laser beam(s) is raster scanned across the field of view. If a chemical-selective optical signal is detected in certain pixels, the signal will trigger an acousto-optic modulator to turn on another laser beam to interact only at the same pixel. If no signal is detected, the interaction laser beam is turned off. (B) The illustration of RPOC for selective control of biomolecules in a cell during laser scanning.

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Improving SERS Reproducibility and Throughput by Affordable Custom-Made Spinning Cell Device

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Lee-Meisel colloids are widely used for Surface-Enhanced micro-Raman applications. However, once aggregated, such colloids exhibit spatial non-uniformities at the μm scale. These are the culprit for the wide scatter of SERS intensity measurements at a given analyte concentration, which seriously hinders quantitative analysis [1]. Moreover, on aggregated colloids, the SERS signal throughput is necessarily limited by photodamaging, which may occur at low incident power densities. These are serious issues for SERS applications in medicine, such as therapeutic drug monitoring (TDM), where one seeks to reliably relate the SERS signal of drugs to their concentration in controlled situations [2]. In this contribution, we developed a custom-made spinning cell device by modifying a spare computer hard drive (Fig. 1c) to obtain reproducible measurements of anti-epileptic drug (AED) Perampanel (PER). The rotating disk ensures stability and high speed, *de facto* allowing a very effective spatial averaging over a large area of the deposited colloids. This experimental setup also allows using greater laser power, as the residence time of the laser on a single spot is considerably low, and no photodegradation occurs (Fig. 1a). By this approach we could record SERS spectra of Perampanel using 1 mW laser power through a standard 50X microscope objective, thus reaching a high throughput without observing any photoinduced sample degradation. A higher degree of reproducibility in the SERS spectra was also achieved. The statistical analysis (Fig. 1b) of characteristic peak heights showed a remarkable decrease in the relative error (σ/μ) from 0.36 (static) to 0.025 (dynamic). This technique paves the way for the reliable use of Lee-Meisel colloids for SERS micro-Raman measurements in therapeutic drug monitoring of AEDs and all the applications where an affordable and reliable Raman method is required.

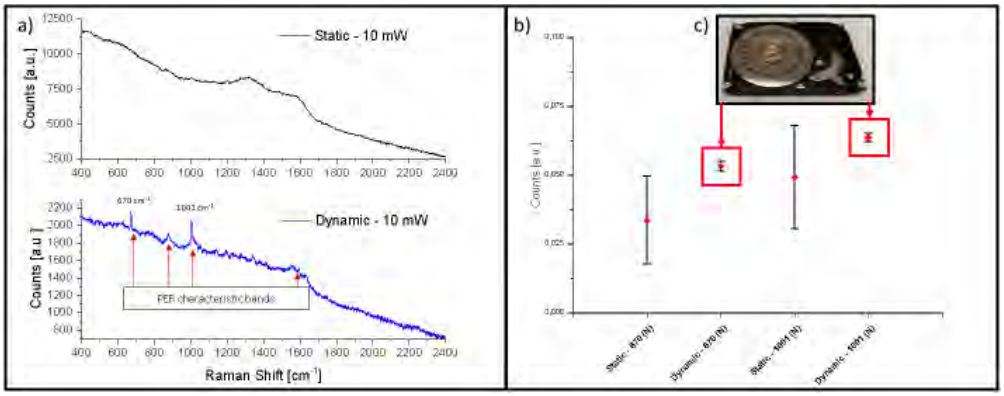

Figure 1. a) SERS spectrum of PER deposited on Lee-Meisel Au colloid, 785 nm excitation, 10 mW power. Static (black), dynamic conditions (blue). b) Repeatability assessment on 12 measurements taken at equally spaced points of the aggregated colloids.

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Fabrication of SERS active substrates through Langmuir–Blodgett and self assembly techniques for screening human cancer cell lines

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Cancer is one of lethal diseases that demand early diagnosis. However, the overall survival rate of cancer patients can be prolonged significantly in the event of early detection. In contrast to the existing diagnostic tools for cancer detection such as histopathology, fine needle aspiration cytology, spectroscopic techniques like SERS are non-destructive and offer fast molecular-level signatures directly with minimal surgical intervention or cellular biopsies [1]. In this lecture fabrication of efficient and reproducible SERS active substrates for the screening of normal and cancerous cell lines associated with prostate and breast cancers will be discussed. SERS is label free technique and with the freedom of multiple biomarker bands associated with normal and cancerous cell lines help one to identify and isolate clinically relevant vibrational signatures associated with malignancy [Fig.1.(c)]. Multivariate data analyses technique like principal component analysis (PCA) and loading spectra will be further discussed on the SERS results to identify and isolate the vibrational signatures of cancer cells from their normal counterparts. The loading spectra nearly correspond to the difference spectra of the SERS signals emanating from normal and cancerous cell lines [Fig.1.(e)]. To our knowledge, such one to one correspondence between the peak positions of loading and difference SERS spectra has been noticed for the first time and can act as a diagnostic algorithm to identify the biomarkers for cancerous cell lines in the molecular scale [2].

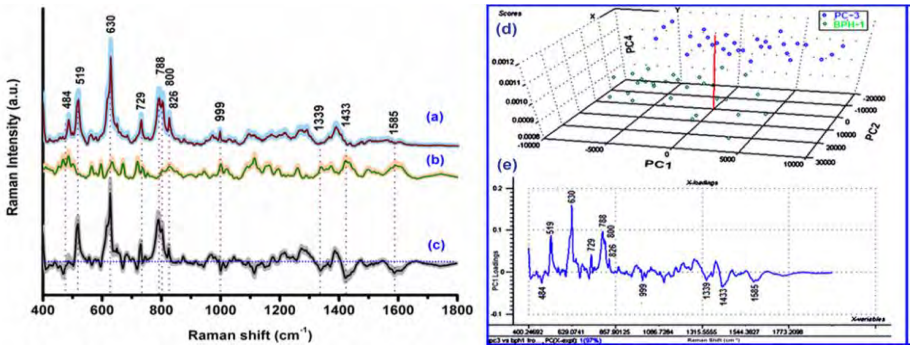

Figure 1: Mean SERS spectrum (n = 30) recorded from (a) PC-3 (cancerous), (b) BPH-1 (normal) cell lines and (c) difference SERS spectra obtained from [(a)-(b)]. [The shaded contours represent the standard deviations of the means.] (d) 3D PCA scatter plot and (e) loading spectrum for the set of PC-3 and BPH-1cell lines.

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Broadband Hadamard spectral acquisition for a high speed and high spectral resolution Stimulated Raman Microscope

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Stimulated Raman Scattering (SRS) microscopy represents a label-free technique for imaging based on chemical contrast in the specimens. Conventional SRS microscopes provide images probing one single Raman shift (wavenumber) at a time.

Here we present an implementation of an alternative multiplexed acquisition method in a SRS microscope based on a dual beam femtosecond laser that exploits spectral shaping through a high speed, narrowband (7 cm^{-1}) and multichannel Acousto Optical Tunable Filter (AOTF) [1]. In this implementation, the signal is collected by a single photodiode and the 8 independent channels of the AOTF are used to generate spectral masks, given by the Hadamard matrix [2], by turning on and off different subsets of channels, corresponding to different wavelengths available within the broad bandwidth of the "pump" femtosecond laser. After the acquisitions, the inverse Hadamard matrix is used as the deconvolution matrix to reconstruct the single contribution of each wavenumber to the overall generated signal and obtain the SRS spectrum.

The implemented multiplexed acquisition scheme is able to provide an up to a two-fold factor increase of the signal-to-noise ratio (SNR) in spectral measurements, when compared to a conventional Raster scan spectral acquisition method. This improvement allows to perform spectral acquisitions using a 4 times shorter integration time maintaining the same SNR in the measurements, potentially giving a valuable boost in hyperspectral imaging acquisition. Furthermore, this technique maximizes the flux of radiation into the photodiode, giving thus the chance to use broadband laser sources with a low power spectral density.

The multiplexed acquisition method presented here is applicable in the fingerprint and in the CH-stretch spectral regions in a completely automated set-up, allowing to perform high spectral resolution and fast broadband acquisitions. We demonstrate the obtained SNR improvement in the SRS spectral measurements of the fingerprint and the CH-stretch spectral regions of various samples, including edible oils and lipid droplets in cancer cells.

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Characterization of Fibrotic and Epigenetic Alterations in Endometriosis

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Endometriosis is a benign hormonally dependent condition affecting a great number of women in reproductive age leading to various symptoms like chronic pelvic pain, excessive bleeding during menstruation and even infertility. Up to now laparoscopic surgery is the only technique to confirm the diagnosis of endometriosis. The aim of this study is to molecularly characterize endometrium and endometrial lesions.

Human endometrium and endometriosis tissue from the sacrouterine ligament of all menstrual cycle phases were obtained from laparoscopic surgery and preserved as cryosections. To localize gland positions, tissue sections were stained with established H&E and CD-10 staining. Raman images of the endometrial gland regions were acquired in consecutive non-processed tissue sections. Raman spectra of major subcellular structures identified in endometrium and endometrial glands were compared by multivariate data analysis. Prominent differences between endometrium and endometriosis were revealed for collagen and nuclei signatures across all menstrual cycle phases. Spectral deconvolution of collagen Raman spectra allowed the identification of a Raman biomarker indicative of fibrotic changes in endometriosis. Structural changes in collagens were in alignment with picrosirius red staining. In addition, localization of epigenetic 5mC foci was demonstrated within cell nuclei based on the spectral signature of methylations. A significant increase in the number of epigenetic foci and signal intensity was detected in endometrial tissue across all menstrual cycle phases. Neural network-based classification of Raman data led to high classification accuracies, specificity and sensitivity highlighting the potential of Raman spectroscopy being utilized as an intraoperative tool for disease detection.

Overall, Raman imaging is a versatile technology in the discrimination of endometriosis while providing insight into the pathogenesis of the disease.

Miniaturized dual laser Raman spectrometer with real-time spectral and intensity calibration for in-vivo skin diagnostics

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Hand-held Raman spectrometers are very popular for identification of drugs, liquids, plastics and powders nowadays. However, hand-held Raman devices are still rather large and are mostly used in scientific and industrial laboratories. Moreover, sensitivity of hand-held Raman spectrometers is lower than sensitivity of research-grade systems with deep cooling spectroscopic sensors, that limits their applicability in challenging biological applications. Here we introduce miniaturized high-sensitivity Raman spectrometer (Figure 1a). To reduce the cost price, we used consumer-level infrared-optimized imaging detector. The device optics (lasers, lenses and the spectrometer) was customized for the highest throughput (up to 88% from the sample to the detector) and diffraction limited spot size for the best performance [1]. Moreover, miniRaman contains two lasers with wavelengths 660 nm and 785 nm to cover wider spectral range. Lasers don't have any temperature stabilization, which allows to save battery power and further reduce the cost of the device. To compensate wavelength drift of non-stabilized lasers, we implemented an in-built reference channel that collects a Raman spectrum of polystyrene. Therefore, our Raman spectrometer is automatically calibrated on Raman shift and laser intensity during each spectrum registration. The device has the following specification:

- two lasers at the wavelengths: 785 nm and 660 nm;
- laser power can vary between 15 to 150 mW at 785 nm, 2 to 30 mW at 660 nm;
- spectral range 400-2500 cm^{-1} at 785 nm, 2750-4500 cm^{-1} at 660 nm;
- spectral resolution 10-12 cm^{-1} (varying across the spectral range);
- wavenumber accuracy ±2.5 cm^{-1}.

The device is controlled by smartphone via Bluetooth (Figure 1b). In Figure 1c,d we demonstrate the applicability of our device for in-vivo skin measurements. Raman spectra of human skin at signal-to-noise ratio better than 500:1 were obtained at 1 second exposure time (averaged over 10 repetitions). Based on our knowledge, it is the first in-vivo Raman measurements of skin with hand-held device with such signal quality. We believe that presented technology could be applied for numerous in-vivo applications in the future including skin disease diagnostics, wound bacteria identification, etc.

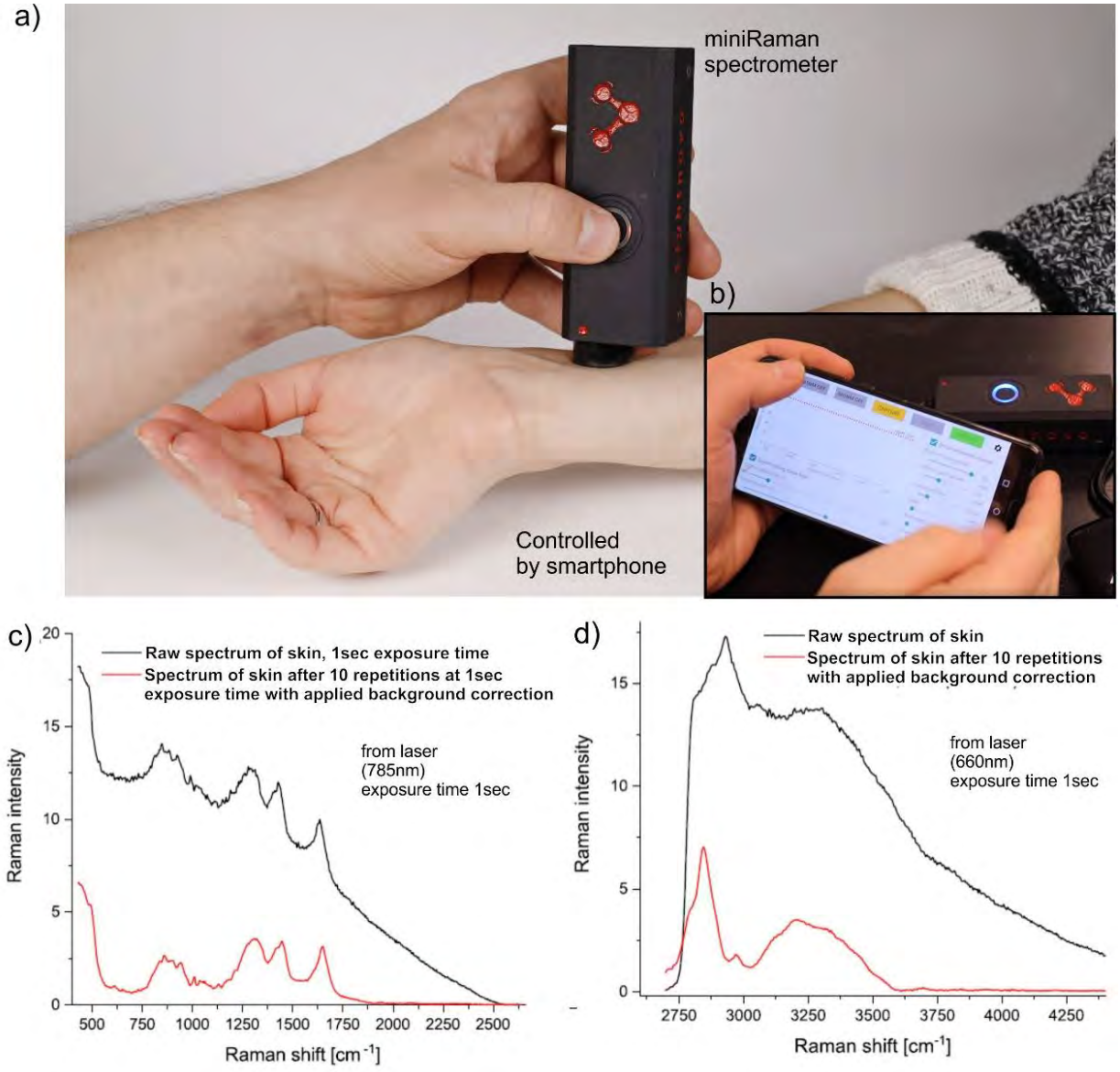

Figure 1. miniRaman spectrometer applied for *in-vivo* skin measurements:
a) miniRaman spectrometer applied to hand for skin measurements, **b)** data collection application at the smartphone **c)** Raman spectra of skin in the range 400-2500 cm^{-1} obtained from laser excitation 785 nm, **d)** Raman spectra of skin in the range 2750-4500 cm^{-1} obtained from laser excitation 660 nm.

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Monitoring phytoplankton population using NIR Raman spectroscopy, excitation/emission spectroscopy and chemometric analysis

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Phytoplankton communities constitute the basis of the food chain in marine environment and are rapidly changing in response to ecological and environmental factors. Tracking health and diversity of those communities is crucial in monitoring nutrient availability and environmental impacts in aquaculture industry. Herein, the taxonomic differences, cell viability and growth phase characterization of several species of phytoplankton were investigated using NIR confocal Raman microscopy and excitation-emission fluorescence spectroscopy. The data was analysed using a combination of the two multivariate data analysis methods: principal component analysis (PCA) and parallel factor analysis (PARAFAC). Subsequently, partial least squares discriminant analysis (PLS-DA) was used to classify the respective Raman and fluorescence data sets. [1] Our results demonstrate high cross-validation and prediction accuracy for different growth phases, taxonomic groups and cell viability. Findings of the current study illustrate potential for future developments of NIR Raman and fluorescence-based systems for use as label free, highly specific in-situ method in remote water sensing.

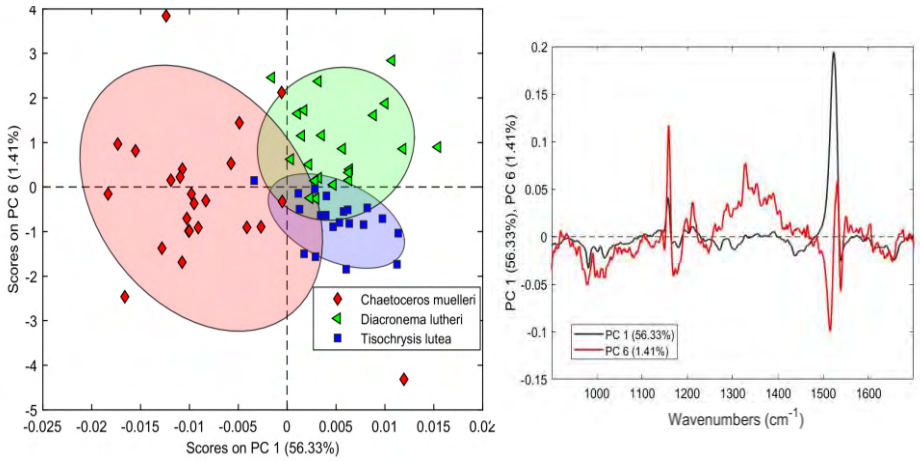

Figure 1: PC1 vs PC6 scores plot and the respective loading plots from PCA of viable and heat-treated cells of three phytoplankton species [1]

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Integrating Sphere Measurements for Paper SERS Sensors

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Paper-based SERS sensors have a number of advantages over counterparts fabricated on rigid substrates. They are flexible, easy to handle, economical and sensitive. Most importantly, they provide simple point-of-need sampling (e.g. filtration, swabbing or chromatography) that is particularly suitable for field applications when used with a handheld Raman analyzer. We have recently developed paper-based SERS sensors that are fabricated by inkjet printing of a colloidal Au sol onto a filter paper substrate [1] and we have demonstrated improved detection of narcotics through surface functionalization. [2] We will discuss the surface functionalization strategy for improved SERS detection of weakly binding chemical target.

A commercial inkjet printer was modified and used for the printing of SER sensors. Multiple printing passes are generally required to achieve the best sensor performance as the amount of AuNP deposited in a single printing passes is limited. As the number of printing passes increases, the AuNP loading on the paper substrate as well as the size of Au nanoclusters both increase. This leads to improved SERS performance. Figure 1(a) shows SERS spectra obtained from sensors after 1-8 printing passes followed by exposure to a 1 µM benzenethiol (BT) solution. SERS intensity increases markedly with the number of printing passes.

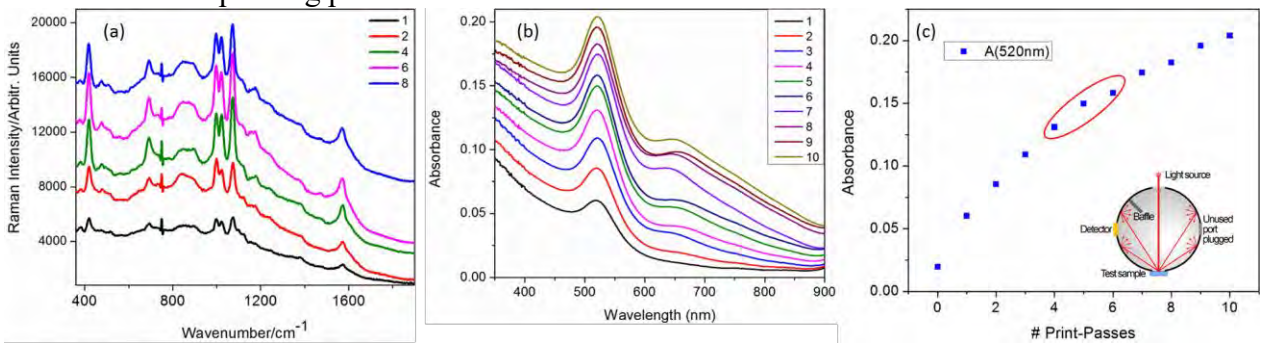

Figure 1: (a). SERS spectra of Inkjet-printed SERS sensors with various printing passes exposed to 1 µM BT. (b) Diffuse Reflectance spectra of sensors after the indicated numbers of printing passes (c). The maximum absorbance of the 520 nm peak measured for the sensors plotted against the number of printing passes. The Inset shows the integrating sphere setup used for diffuse reflectance measurement.

To better monitor the amount of AuNP deposited on the paper substrate and ensure best batch-to-batch reproducibility, absorbance measurement of the localized surface plasmon resonance (LSPR) were carried out. LSPR absorbance is a good indicator of the loading of AuNPs on the paper substrate. By using an integrating sphere, all of the diffusively scattered light from the AuNP decorated paper substrate can be collected by the detector. [3] Figure 1(b) shows the diffuse reflectance spectra of the SERS sensors after 1-10 printing passes and Fig. 1(c) plots the maximum absorbance of the 520 nm peak from the sensors. In this presentation, we will highlight the use of the integrating sphere measurement technique to monitor the AuNP loading on the paper substrate throughout the fabrication process and to achieve excellent batch-to-batch reproducibility as well as optimal sensor performance.

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Parametric Nanopore Array fabrication for visible spectrum SERS of molecule translocation

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The use of plasmonic nanopores, for surface enhanced Raman spectroscopy of molecules which pass through the pore is known,[1] as is the fabrication of plasmonic nanopore arrays through colloidal lithography[2,3]. Here we present a parametric optimization to provide nanopore arrays with a tunable visible-NIR pore-specific hot-spot like localized surface plasmon resonance for single molecule translocation SERS. These arrays are shown to enhanced capture rate for molecular translocation for use in time resolved studies. The resonance wavelength is tuned through template size and film material, and the enhancement factors are reported for non-adsorbed SERS probe. Silver and gold are used individually and in combination, with considerations to the high quality of silver plasmonics and the chemical stability of gold[4]. The relative spectral contribution of absorption and scattering is considered as this has been shown to reduce background in SERS.[5] Different pore dimensions (0-60 nm) are realized through thermal template modification and the effect this has on the optical and Raman intensities. Ultimately, nanopore arrays suitable for single molecule SERS with pore-localized enhancement for many common visible Raman excitation wavelengths are realized.

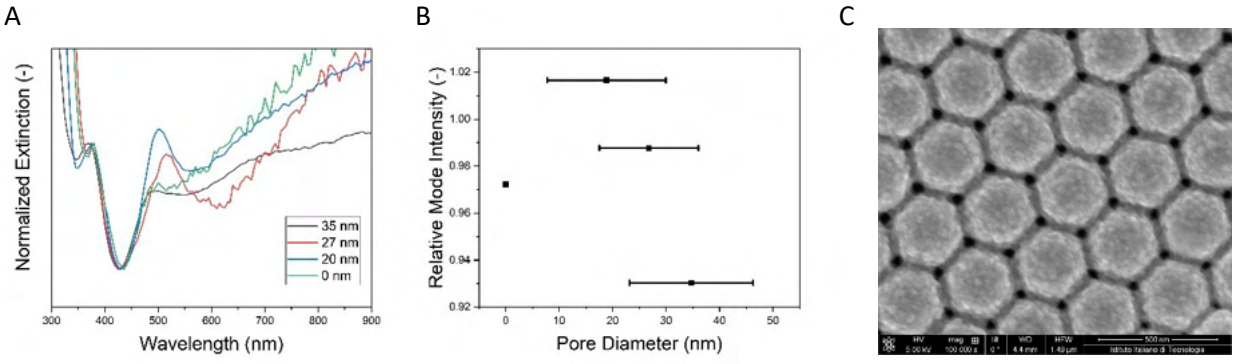

Figure 1: The normalized spectra of silver nanopore arrays formed using a 304 nm colloidal template (A) showing the variation of the intensity of the pore mode (~515 nm) with respect to the surface mode (~375 nm) with pore diameter. This shows a local maximum which allows for spectral characterization of the materials (B). Electron micrographs show the presence of 35 nm pores in the silver coated array.

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Structure-function correlation studies of a non-canonical heme oxygenase from Mycobacterium tuberculosis

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Heme oxygenase (HO) proteins catalyze the physiologically important process of heme catabolism. Until the recent discovery of a few HOs from pathogenic bacteria, it was believed that all heme degradation proceeded through the canonical pathway that generates biliverdin, free ferrous iron, and a CO molecule.[1] MhuD is a noncanonical HO from Mycobacterium tuberculosis (Mtb) that degrades heme to novel mycobilin products without releasing CO.[2] It has been identified as an auspicious antitubercular drug target due to its crucial role in the Mtb heme uptake pathway.[3] MhuD can bind either one or two hemes within the same active site, a unique feature among known HOs. It was previously reported that only the monoheme form is catalytically competent, whereas diheme MhuD is inactive. Resonance Raman (rR) spectroscopy is an ideal technique to reveal information about the active site architecture and environment of heme proteins in solution under dynamic conditions by using an excitation laser line in resonance with the heme chromophore's electronic transition to selectively enhance modes associated with the heme macrocycle, its peripheral groups, and its endogenous/exogenous axial ligands.[4] Therefore, this study employed rR spectroscopy to investigate the structure-function relationship of mono- and diheme MhuD and analyze factors that were previously suggested to dictate their reactivity, such as heme pocket hydrophobicity, heme (non)planarity, and interactions between the heme and crucial active site amino acids. Both forms of the wild-type (WT) protein were studied in the ferric, ferrous, and ferrous-CO ligated states. Heme degradation assays and ESI-MS product analysis confirmed the spectroscopic evidence for the enzymatic activity of both mono- and diheme WT-MhuD,[5] contrary to previous reports.[4] These results redefine the functional paradigm of diheme MhuD and expand its role in Mtb metabolism.

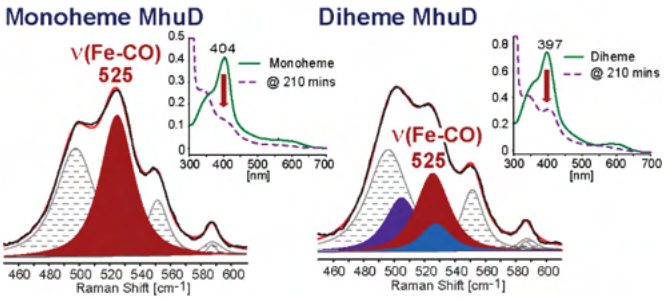

Figure 1: The deconvoluted rR spectra of ferrous-CO adduct and activity assays of mono- and diheme MhuD

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Stratification of saliva of healthy, habitués and oral cancer subjects using Raman and FTIR spectroscopic approaches.

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Minimally invasive cancer detection using bio-fluids is been actively followed due to practical limitations of in vivo methods. Saliva is one such clinically informative bio-fluid that offers advantage of easy and multiple sample collection. Despite its potential in cancer diagnostics, saliva analysis is challenging due to its heterogeneous composition. Recently, there has been an increase in saliva exploration by Raman spectroscoy [1-5]. In current study unstimulated morning saliva sample of Control (C-16), Habitues (HT-21) and Tumour (T-26) were examined by both Raman and IR Spectroscopy. Raman spectra of air dried 20 µl of sample, on a calcium fluoride (CaF2) window, were recorded by Raman microscope WITec alpha300RS (WITec GmbH, Ulm, Germany, 532 nm, 50X, 20 mW, 5s and 10 accumulations). Infrared spectra were collected using Spectrum 2 (Perkin-Elmer, USA, 12 scan, resolution 4 cm-1) on air dried thin film, on CaF2 . Pre-processed Raman and IR, spectra were analysed by principal component analysis (PCA) and PCA-based Linear Discriminant Analysis (PC-LDA). Multivariate analysis of Raman spectral data revealed 91 % correct classification of C group, 52 % classification of HT group and 77% classification of T group. On the other hand, analysis of IR showed 31% correct classification in C group, 80% in HT group and 48% in T group. Findings of this preliminary study suggest, Raman spectroscopy provides better stratification of control and tumour groups than IR.

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Raman spectroscopy to rationalize the electrochemical mechanisms of the positive electrodes in Li-ion batteries

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The redox mechanisms operating in Li-ion batteries and more particularly within the electrode materials are often complex and difficult to characterize without the use of sophisticated experimental techniques. The most common positive electrode materials are based on metal transition oxides, but they are subject to a variety of competitive and not always understood mechanisms that can affect both the transition metal (metallic redox) and/or the ligands (anionic redox). The rationalization of electrochemical processes within the electrode is crucial to understand, for example, why some materials, such as Li-rich, while possessing very interesting energy densities due to a double metallic and anionic redox, suffer from a capacity and/or potential drop during charge/discharge, thus limiting their future commercialization. [1]

For this type of issues, Raman spectroscopy can be a very powerful and efficient technique. Indeed, the information provided by Raman spectroscopy in particular, the intensities and wave numbers, governed by the polarizability and the force constant, are closely related to the variation of the nature of the M-O and O-O chemical bonds during the insertion/disinsertion of Li into and from the host structure and thus may allow to rationalize the nature of the reduced and oxidized species that are formed during the charge/discharge process of the battery. [2]

To do this, we have firstly characterized, using DFT-CPKS calculations, the Raman signature of the $(O_2)^{2-}$ species frequently announced when an anionic redox is involved and the structural and electronic factors that govern the stability of this entity which is actually only observed in the presence of M(s,p) metals. Then this study was extrapolated to Li-rich systems containing an M-3d (Li_2MnO_3) to elucidate its most likely electrochemical mechanism. After evaluating the vibrational signature of Mn under different environments and oxidation states, we show that the $(O_2)^{2-}$ entity is not viable in the presence of a (3d) metal thus discarding several electrochemical mechanisms proposed in the literature.

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On-Chip Raman Spectroscopy for the Characterisation of Oral Biofilms

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Biofilms are a structured community of microbes encased in a complex extracellular matrix (ECM) of proteins, polysaccharides, lipids, and DNA [1]. Biofilms are physiologically distinct from planktonic bacteria as the ECM not only acts as a structural scaffold but helps with surface adhesion and protects the bacteria from detergents and antibiotics, therefore biofilms are shown to have up to 1000x more antibiotic resistance compared to planktonic cells [2]. Subgingival oral biofilms can cause inflammation of the gum, gingivitis and periodontal disease if left untreated, and are known to be associated with other systemic diseases such as Alzheimer's [3] and cardiovascular disease [4].

Current methods of Biofilm analysis are time consuming and destructive, therefore here we present a method of growing multispecies biofilms on microfluidic chips where Raman Spectroscopy can be used for real time, in-situ characterisation of biofilm maturation over time.

Dual species oral biofilms were grown on chip over the course of 5 days, with Raman Spectra and confocal live/dead images taken every 24 hours. Figure 1 shows Raman spectra for planktonic species

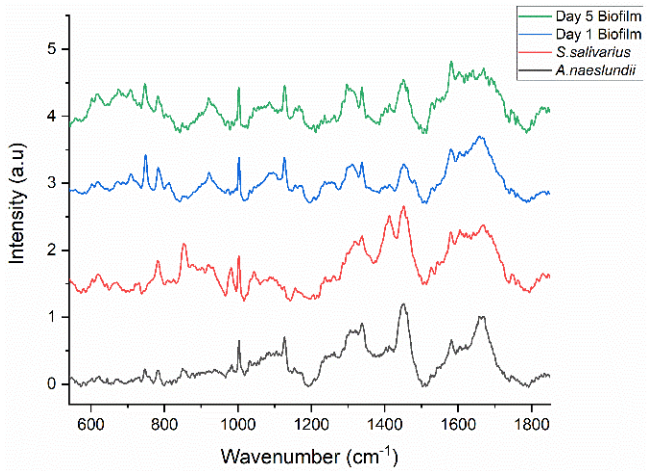

Figure 1: Raman Spectra of Planktonic species compared to multispecies biofilm at Day 1 and 5.

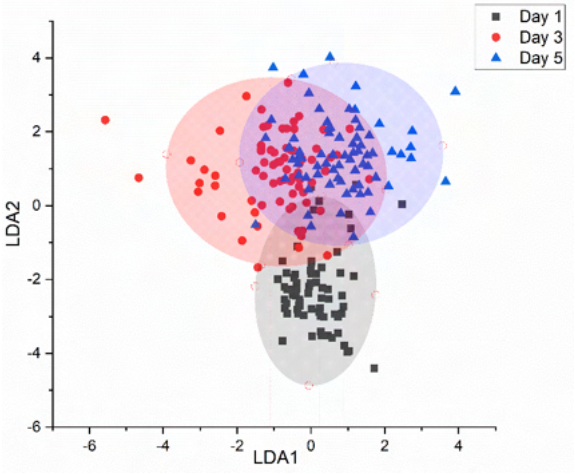

Figure 2: LDA plot of multispecies biofilm showing Days 1, 3 and 5, with 95% confidence ellipse.

compared to biofilm at Day 1 and 5. PCA can successfully differentiate between the two planktonic species. Figure 2 shows how LDA can be used to highlight the changes in the biofilm over time, likely due to growth of ECM and an increase in dead bacteria. Future works include increasing the number of species and treatment of the biofilm with microbubbles.

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Impact of the Molecular Dimensions on the Sensitivities Using Engineered Gap Hotspots at Nanoscale Resolution for Plasmonics Based Biosensing.

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Biosensors based on plasmonic sensing typically rely on light-matter interactions that concentrate Electromagnetic (EM) fields between the geometries. Electromagnetic (EM) field intensities at these hotspots tend to increase exponentially as decreasing separations between the metal structures, whereas such confining dimensions affect the ability of the three-dimensional analyte molecule that faces spatial constraints. Therefore, the trade-off between geometrical parameters, optical response & biomolecular interaction becomes more significant to integrate the plasmonic-based bio-sensing into the diagnostics applications. Self-assembly fabrication enable tailored plasmonic nanoarray geometries with sub-10 nm resolutions, flexibility in the choice of shapes and eventually, control over the geometry of Electromagnetic (EM) hot-spots concerning analyte dimensions.

In this work, we demonstrate the rational design of plasmonic nanoarray configurations that enable the Electromagnetic (EM) hot-spots to be better leveraged by the reporter of biomolecular interaction events for reproducible and quantitative biosensing. The talk also includes the discussion and evidence from the research, the impact of the molecular dimensions on their sensitivities with respect to decreasing gaps between metallic nanostructures to concentrate Electromagnetic (EM) fields. Multiple parameters are considered including the dimension, shape, and density of hot-spots, optimal surface functionalization, and the choice of substrates. Additionally, the engineered geometries also demonstrate multiple plasmonic sensing modalities and in-situ measurements, where the interaction of the analyte molecule is not only monitored physically in real-time but, also spectroscopically.

The study paves the way to identify critical parameters that play an important role at the nanoscopic level, to rationally design promising low-cost technologies compatible with portable molecular diagnostics and Point of Care Diagnostics (POCT) markets.

Figure 1. SEM Images of sub-10 nm scale geometries fabricated over 4'' wafers and schematic demonstrating concept of QCM-D ⇔ Plasmonic Biosensor In-situ real-time monitoring.

Funding by Fonds National de la Recherche (FNR) Luxembourg for project PLASENS (C15/MS/10459961) is gratefully acknowledged.

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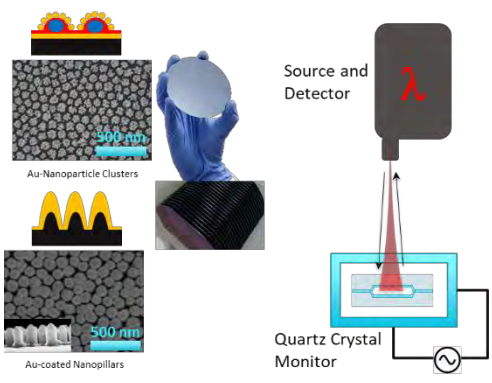

Resonance Raman Scattering Study of Edge Phonons and Defects in Molybdenum Disulfide

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Transition metal dichalcogenides (TMDs), like MoS_2, have been extensively studied over the past decade, due to their unique optical, electronic, and spin properties. One important characteristic of TMDs are the multiple valleys in their conduction bands, at the **K** (**K'**) and **Q** points, found around halfway between the **K** (**K'**) and the **Γ** point. The Raman scattering of TMDs can be used to obtain information about their electronic and vibrational structures [1], the number of layers [1], and disorder [2]. Additionally, the presence of multiple valleys is relevant for the Raman scattering effect since it allows the observation of multiple double-resonance processes. In a double-resonance Raman process, intervalley electron scattering takes place via the creation of a phonon, and momentum conservation requires the electron to return to the initial valley either by the creation of another phonon or by a defect [1,2]. In this work, we present a comprehensive characterization of the acoustic phonon double-resonance Raman processes occurring in MoS_2, in single-layer and bulk samples. We measure the Raman spectrum with multiple laser energies and different temperatures to better understand the interplay between the **KK'** and **KQ** intervalley scatterings. Moreover, we also measure the Raman spectrum of vacancy-rich samples to characterize the disorder induced peaks of MoS_2 near the resonance with its excitonic transitions. We obtained important laser dependent equations for the quantification of point defects in MoS_2 single-layers using the intensity of disorder-induced Raman bands as a key parameter. These works pave the way for new and important applications of Raman spectroscopy for the characterization and evaluation of atomically thin crystals not only of MoS_2 but also of other two-dimensional materials.

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High-pressure Raman spectra of L,L-dileucine crystals

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ABSTRACT

We have investigated the high-pressure Raman spectra of L,L-dileucine crystals up to 8 GPa. The results suggest L,L-dileucine undergoes a phase transition between 2.3 and 2.9 GPa. Broadening of X-ray diffraction peaks suggests disorder of the crystal lattice during compression.

The hydrophobic dipeptide L,L-dileucine is an aliphatic peptide performing important functions in the human body, acting in the regulation and maintenance of the amount of sugar in the blood, in the regulation of hormonal production, and in the breakdown of muscle proteins after trauma or severe stress. The crystalline structure of LL hydrate dipeptide forms supramolecular nanotubes filled by water molecules. High-pressure Raman spectra were collected at ambient temperature with a LabRam HR-Horiba instrument equipped with a He/Ne laser as the excitation source. The single crystal sample and a chip of ruby were loaded into a 120 μm hole in the stainless-steel gasket pre-indented, and the mineral oil was used as the pressure transmission medium. The spectral resolution was of ± 2 cm^{-1}. We have also performed DFT calculations on a single molecule of L,L-dileucine allowing us to assign all the vibrational modes of the crystal.

The L,L-dileucine hydrate crystallizes in an orthorhombic structure belonging to the $P2_12_12_1$ (D_2^4) space group with Z = 4, where each asymmetric unit is composed of

two water molecules and two molecules of L,L-dileucine. The four dipeptide molecules constitute a hydrophilic region through hydrogen bond interactions between NH_3^+ amide group, CO_2^- and water molecules; this generates a water-filled nanochannels by supramolecular self-assembly. Figure 1 shows the Raman and infra red spectra of L,L-dileucine as observed experimentally and calculated through DFT calculations.

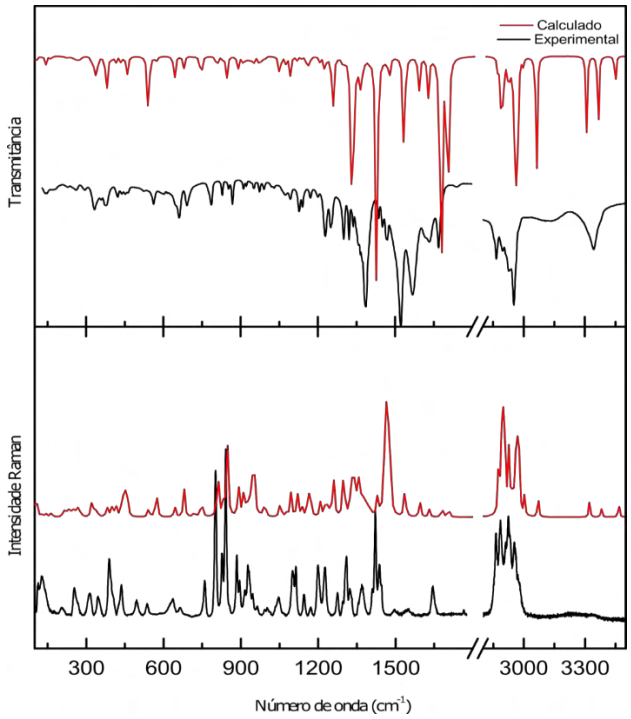

Figure 1: Raman and infra red spectra of L,L-dileucine crystals.

Measurements of synchrotron X-ray diffraction and Raman spectroscopy of L,L-dileucine under high-pressure suggested a phase transition undergone by the crystal between 2.3 and 2.9 GPa. The decreasing of intensity of bands and modifications in bands related to vibrations functional groups as CO_2^-, NH_3^+, and water molecules, directly involved in the stability of the structure were noted. These modifications indicated a change in tubular arrangement. Additionally, the X-ray diffraction results showed that the width of nanochannels decreases significantly with increasing pressure. The increase in the linewidth bands associated with stretching modes of CH_3, $C=O$, and water, suggested that a disorder was introduced in the system. Interestingly, above 16 GPa, the changes in the X-ray patterns suggested that the material is going to an amorphous phase. Finally, the phase transition seems to be reversible.

XXVII International Conference on Raman Spectroscopy (ICORS)
Human brain meningioma detection using handheld VRR analyzer

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Visible resonance Raman (VRR) spectroscopy method using 532nm wavelength excitation has been used for human organ lesions detection and diagnosis since 2011 and shows unique advantages [1-3]. Here, we report the evaluation of a VRR-LRR^{TM} analyzer based on VRR technique to identify human brain meningioma Fig. 1. In total 1,180 VRR spectra from 32 patients of two types of primary and recurrent meningioma and blood tissues were observed. VRR spectral measurements were performed during surgeries, a few minutes after resection. Data were collected using both VRR-LRR^{TM} and a micro confocal HR800 Raman systems for comparison. This study focuses on observing the characteristics of new biomarkers and their changes in meningioma. Preliminary analysis results are shown in Fig. 1 with the following observations. 1) The intensity ratio of RR peaks of protein (and collagen) to fatty acid i.e. ~2934cm^{-1} to ~2889cm^{-1} decreases with the increase of meningioma grade. 2) The ratio of RR peaks of phosphorylated protein to protein i.e. ~1584cm^{-1} to ~1637cm^{-1} increases for the high grade of meningioma. These characteristics are consistent with previous reports [1]. 3) We found that three new RR peaks at 1166cm^{-1}, 1374cm^{-1} and 1237cm^{-1} associated with molecular vibrational bonds in two types of meningioma tissues are significantly different in peak intensities in the VRR spectra. The peaks may arise from flavins, T of DNA and P-O-Phospholipids, respectively. 4) The changes in the intensities of RR peaks of carotenoids (1156cm^{-1} and 1524cm^{-1}) and amide II (1544cm^{-1}) were also found in the meningioma. These spectral features need further verification. VRR-LRR^{TM} analyzer demonstrates label-free, rapid and objective identification of primary human meningioma in quasi-clinical settings. The sensitivity for identification is ~70% compared with histopathology results of meningioma tissues.

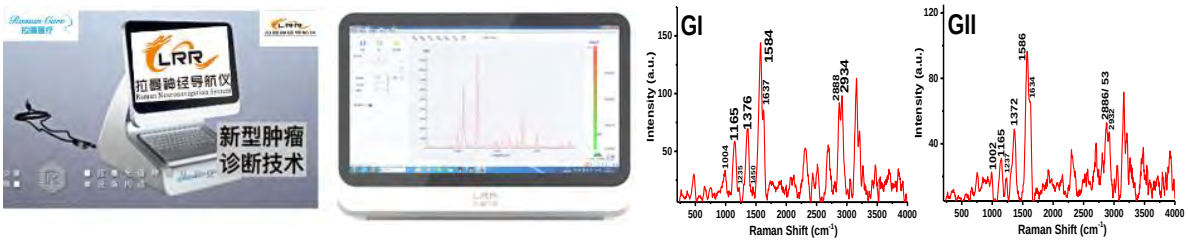

Figure 1: The left two panels: Photographs of the potable VRR-LRR^{TM} analyzer with a label free optical-fiber probe along with sample experimental data of human brain model *ex vivo*. The right two panels: VRR-LRR^{TM} spectra of meningioma of grade I (GI) and Grade II (GII).

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Label-free stimulated Raman scattering imaging reveals silicone breast implant material in tissue

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Millions of women worldwide have silicone gel breast implants. Long-term structural integrity of these implants is poor, and they can rupture or bleed silicone into the surrounding tissue. Currently, no histopathological technique exists that can specifically detect silicone in tissue, neither before or after staining. Stimulated Raman Scattering microscopy (SRS) is a fast and powerful, label-free imaging technique based on chemical contrast with sub-micron sized resolution. Therefore, silicone distribution images can be easily made with SRS to specifically identify silicone and distinguish it from other chemical components in the tissue.

Here we describe a robust method for silicone detection in ex vivo breast tissue [1]. SRS imaging was performed on H&E stained histology slides in the CH-stretching region. These samples had been obtained earlier after implant removal and had been prepared according to standard histopathological protocols. Two optimal wavenumbers were calculated, by minimizing the root mean square error (RMSE) between the silicone and protein (background) spectra. Then, the whole tissue was imaged by a quick scan with a pixel size of 1.60 µm. Finally, selected regions of interest (ROI) with high silicone content or nuclei density were imaged by a high-resolution scan with a pixel size of 0.52 µm.

Image processing operations, such as image subtraction, applying threshold and co-registration with a histological color image, were carried out using Matlab. Data provided by connected component analysis assisted the statistical interpretation of silicone distribution in the tissue.

This method was found to be very suitable for identifying silicone debris from leaking implants and quantifying silicone in the surrounding tissue. We are currently investigating the relationship between silicone particulates and the severity of capsular contracture.

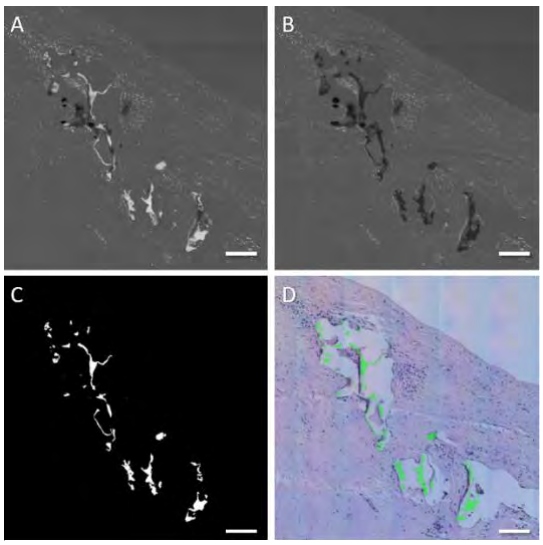

Figure 1: ROI SRS images of a breast implant capsule after explantation, taken at the silicone (A) and background (B) wavenumber. The images were thresholded and subtracted to produce a binary mask (C) which was then co-registered and overlaid (green) over an H&E stained image (D). Scale bar: 100 µm

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Raman and Raman Optical Activity of amino acids in aqueous solution: a computational investigation

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Raman scattering provides unique structural and electronic information on chemical systems [1]. Thus, it is employed in many fields, from pharmaceutical to materials science [1]. More recently, Raman Optical Activity (ROA), the chiral analogous of Raman spectroscopy, has emerged as a valid analytical technique due to its structural sensitivity. The interpretation of ROA spectra benefits from the comparison with reliable computational reference data. However, the accurate modeling of ROA spectra is a challenging task, especially for molecules interacting with the external environment (solvent or supramolecular matrices) [2].

A possible solution consists of exploiting QM/classical approaches, which retain the atomistic nature of the whole system, such as in the Quantum mechanical (QM)/Molecular mechanics (MM) approach [3]. In this contribution, we present a novel QM/MM method to accurately model Raman and ROA of systems in solution. The approach is based on the QM/Fluctuating Charge (FQ) [4,5] and QM/Fluctuating Charge and Fluctuating Dipole (FQFμ) models [6].

The performance of both approaches are tested against the reproduction of Raman and ROA spectra of solvated amino acids. In all cases, experimental data are nicely reproduced, thus highlighting the potentiality of these methods for the reliable description and prediction of Raman and ROA spectra of molecular solutes.

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N-acetyl-β-D-glucosaminidase activity assay for monitoring insulin-dependent diabetes using Ag-porous Si SERS substrate

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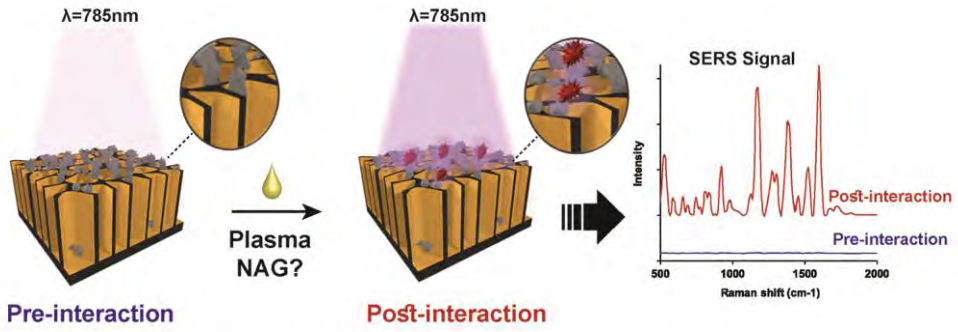
Determination of urinary or serum N-acetyl-β-D-glucosaminidase (NAG) activity as a tissue damage indicator is widely used in diagnosis of various pathologies, including diabetic nephropathy. Early and rapid biomarker detection is an important element of medical diagnosis, facilitating prompt therapeutic decisions and prognosis evaluation. Herein, we present a modified sensing approach for a rapid and reliable NAG activity determination in complex media using surface-enhanced Raman spectroscopy (SERS). Porous silicon (PSi) Fabry-Pérot interferometers were redesigned as sensitive SERS platforms utilizing the vast inherent surface area for silver (Ag) nanoparticles embedment. Interaction of the porous nanostructures with specific NAG-enzymatic products produces an indicative spectral fingerprint proportional in magnitude to its concentration. The sensitivity of Ag-PSi SERS substrates was evaluated in complex matrices presenting sufficient limits of detection compared with other advanced assays and techniques (0.07, 0.47 and 0.50 mU mL^{-1} for urine, milk and plasma, respectively). The augmented optical performance revealed recovery values of 96-109%, indicating successful and selective NAG recognition in biological fluids. Finally, the potential applicability of the suggested prototype for real-life scenarios was evaluated *in vivo,* in a model of insulin-dependent diabetes induced in sheep. Overall, the robust data confirm the application of SERS analysis for early diagnosis of pathology and for evaluation of clinical responses to pharmacological treatments.



Nanostars for label-free SERS

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The development of new nanoparticle-based theranostic tools critically relies on the possibility to understand and control the uptake of nanoparticles with corresponding functions by mammalian cells. Surface enhanced Raman scattering (SERS) has become a promising tool to investigate live cells.[1] So far, spherical nanoparticles are the most commonly used for intracellular optical detection and bioanalysis,[2] but the processes that anisotropic nanostructures such as nanorods or nanostars undergo inside cells must also be explored, due to the favourable plasmonic properties of such structures. Also the properties of nanoparticle aggregates in living cells have mainly been studied for spherical, citrate stabilized gold and silver nanoparticles so far.[3,4] The composition and dynamics of the protein corona can be probed optically by SERS.

In this work, biocompatible gold nanostars were used for label-free sensing *in vivo*. The nanostars were synthesized by reduction with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and their enhancing properties evaluated. Mammalian cells were incubated with the nanostars to evaluate their application for label-free SERS. The signals observed after the uptake indicate a close interaction mainly with protein components in the cells, indicating the formation of a protein corona. The nanostar distribution in the cells and the cellular ultrastructure were characterized in detail by soft X-ray tomography.[5] The combination of these techniques can help in the improvement of the application of nanostructures as biomedical tools.

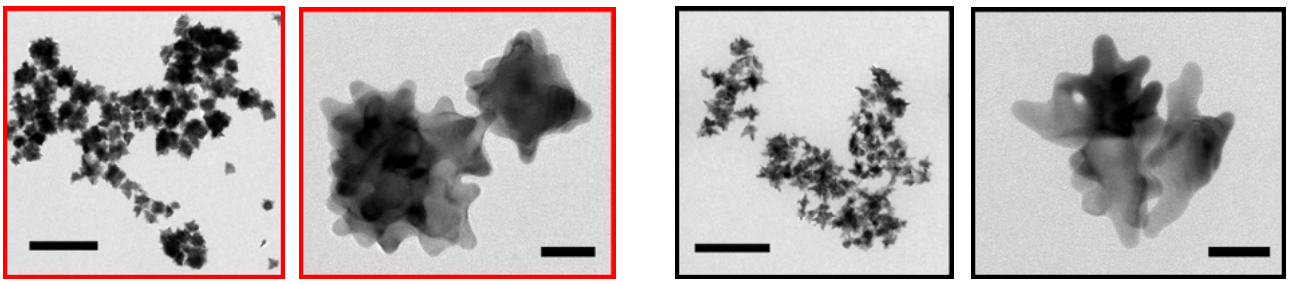

Figure 1: Gold nanostars synthesized with different concentration of HEPES. 25mM HEPES (red) and 75mM HEPES (black). Scale bars: 200nm left, 20nm right micrograph of each group.

Acknowledgements: Financial support of the project by ERC grant 259432, DFG GSC 1013 SALSA, and DFG under Germany´s Excellence Strategy – EXC 2008 – 390540038 – UniSysCat are gratefully acknowledged. We thank HZB for the allocation of synchrotron radiation beam time.

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Vibrational sum-frequency generation spectroscopy reveals glycosaminoglycan structure and its interaction with lipid membranes

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Glycosaminoglycans are found at biological barriers, where, by interacting with lipids, they can contribute to medical conditions, *i.e.,* atherosclerosis or Alzheimer's disease.[1,2] Here, we present the first vibrational sum-frequency generation (VSFG) spectra of chondroitin sulfate (CS) interacting with dipalmitoyl phosphatidylcholine (DPPC) at the air/liquid interface.[3] Using fs-pulses at 100 KHz repetition rate allowed for reduced acquisition times.[3-6] The VSFG spectra recorded in the 1050–1450 cm^{-1}, 2750–3180 cm^{-1}, and 3200–3825 cm^{-1} ranges cover the characteristic bands in the fingerprint-, C-H stretching-, and O-H stretching regions both for glycosaminoglycans and lipids. We found that the lipid head groups changed orientation, and the head-group-bound water molecules also realigned when the CS molecules interacted with the DPPC monolayer in the presence of Ca^{2+} ions. At the same time, the tail groups of the DPPC molecules remained mostly in the same conformational order. Chiral polarization combinations in VSFG spectroscopy can provide unprecedented insight into the three-dimensional conformation of glycosaminoglycans, which may lead us to a better understanding of their structure-function relationship. In our model system, spectra recorded in chiral (spp) polarization combination point towards a chiral secondary structural motive in the CS chains, which is most probably a helical coil. These observations at a physiologically relevant Ca^{2+} concentration (2.8 mM) and a CS concentration below 200 nM, exemplify the relevance of state-of-the-art VSFG technology in studying biomolecular interactions at model physiological barriers.

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Surface Modification of Plasmonic nanostructures: Enabling SERS Detection of Weakly Interacting Analytes

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In recent years, Raman spectroscopy technique has seen significant improvement towards the development of instrumentation that meet the requirement of field applications. Among these requirements are portability, robustness and sensitivity for chemical and biological analytes. Specifically, improved sensitivity in Raman spectroscopy for the fieldable detection makes use of surface enhanced Raman spectroscopy (SERS) substrates. Over the last few decades, there has been tremendous effort invested in the development of a variety of SERS substrates with a number being currently commercially available. These substrates all make use of carefully engineered coupled plasmonic nanostructures that are key to enabling SERS. It is well understood that coupled plasmonic nanostructures are able to sustain highly localized electromagnetic fields known as the plasmonic "hot spots". The majority of observed SERS spectra are measurements of the very small number of analytes that have managed to make their way to these "hot spots". SERS has demonstrated extraordinary sensitivity in laboratory settings with ideal binding molecules, typically through the thiol functional group chemisorbed onto Au or Ag surfaces. However, in most of the real-world applications, molecules of interest do not always come with a handy thiol functional group and often their interaction with the plasmonic surfaces is through a weaker physisorption or electrostatic interaction. This is one of the most visible challenges for the broad application of SERS technique. We will present a couple of strategies through the use of surface modification such as partition layers and naturally present surface passivation layers on the plasmonic nanostructure to achieve a high sensitivity of detection of weakly interacting analytes.

We will focus the SERS substrate optimization upon the detection of narcotics and scheduled substances to address the tremendous increase in the handheld Raman analysis carried out by the first responder community. This includes SERS optimization for the detection of opioids and cannabinoids. We will also address the challenges in the quantification of SERS measurements. The well recognized SERS enhancement factor that is often used to compare different types of SERS substrates provides no information on substrate sensitivity. In this presentation, we will introduce the concept of receiver operating characteristics (ROC) analysis, for the SERS sensors. ROC analysis is the gold standard for chemical sensors qualification.

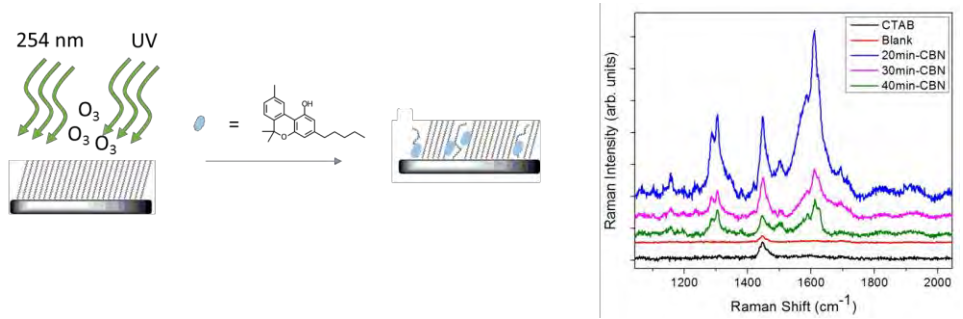

Figure 1. Irradiation of CTAB covered Au nanorad serves as a natural partition layer for the sequestration of cannabinol. SERS detection of the self-assembled structure is then achieved on Au nanorod arrays. [1]

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Surface Enhanced Resonance Raman spectro-electrochemistry of DyP type peroxidases

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DyP-type peroxidases (DyPs) couple the reduction of hydrogen peroxide with oxidation of a number of structurally different molecules that include anthraquinone-based and azo dyes, complex phenolic molecules, carotenoids, aromatic sulphides and metal ions. DyPs possess a number of unique properties in comparison with classical heme peroxidases: i) distinct amino acid sequence, catalytic residues and tertiary structure; ii) a broad substrate specificity; iii) an unusually low optimal pH for the catalytic activity; and iv) largely unexplored physiological role [1-3]. Resonance Raman spectroscopy (RR) demonstrates that multiple heme spin species, including high spin (HS), low spin (LS) and quantum spin state admixture (QS), can co-exist in DyPs of different origin. In contrast to classical peroxidases, a surprisingly high abundance of catalytically incompetent LS heme population is observed in a number of DyPs from different sources in physiological conditions, which we tentatively relate to an alternative non-peroxidase biological function [1].

Due to their broad substrate specificity and catalytic efficiency, DyPs immobilized on biocompatible metal electrodes can be explored for design of devices for the detection of hydrogen peroxide and/or for the electrocatalytic decomposition of oxidizing substrates (e. g. chemically inert dyes). We combine the insights about molecular details of DyP heme active site in solution elucidated by RR, with those on immobilized DyPs obtained by surface-enhanced resonance Raman (SERR) spectro-electrochemistry, to reveal promising enzyme candidates for biotechnological applications. in these experiments, modified plasmonic metal that serves as a surface amplifier of spectroscopic signal simultaneously acts as a working electrode, allowing us to probe the electrocatalytic, thermodynamic and structural properties of the immobilized enzyme. To that end, we show here several examples of immobilized DyPs, demonstrating how SERR spectro-electrochemistry governs rational development of DyP based biosensors and bioelectrocatalysts.

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Transforming Treatment of Patients with Drug Induced Liver Injury using SERS based Lateral Flow Testing

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The bestselling non-prescription drug, acetaminophen, is used by millions of people worldwide as a safe and effective method of pain relief. However there are a large number of acetaminophen overdoses, both accidental and intentional. Due to the increase in toxic metabolites in the liver caused by overdose, drug induced liver injury (DILI) occurs which, if left untreated, can result in a patient requiring a liver transplant or even death. Currently, when a patient presents to hospital following an overdose, the severity of it is determined by drawing blood intravenously and sending the sample to a central lab within the hospital where the level of alanine aminotransferase (ALT), a biomarker for hepatocyte injury, is measured. Although ALT is an established clinical biomarker for detecting DILI, its level in blood following acetaminophen ingestion is slow to rise, resulting in missed or delayed DILI diagnosis. Recently a new protein biomarker, keratin-18 (K18), has become a candidate for the accurate and early detection of DILI. In a recent study it was shown that K18 could accurately separate patients with and without DILI at an early time when ALT levels were still in the normal range.[1] K18 also has European Medicines Agency and US Food and Drug Administration regulatory support for both predicting DILI and prognostic assessment of outcome. To maximise the benefit of K18 a rapid, quantitative detection test, which can take place next to a patient, has to be developed.

For the rapid detection of K18, a paper-based lateral flow strip combined with surface enhanced Raman scattering (SERS) analysis has been proposed. Conventionally, SERS analysis of lateral flow strips has been carried out on large Raman microscope systems by mapping the test line. This analysis can take a number of hours, uses instrumentation with no portability aspect and has to be carried out by trained personnel. An alternative, which allows measurements to be taken next to the patient, is to use a portable SERS spectrometer. [2] By adapting point and shoot spectrometers to include accessories which can accommodate lateral flow cassettes and line illumination optics, DILI status has been successfully determined in patient serum samples rapidly and sensitively. Incorporating this platform as part of the standard care protocol would lead to quicker administration of treatment and potentially reduce the number of liver transplants and deaths associated with acetaminophen overdoses.

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Raman scattering obtained from laser excitation of $MAPbI_3$ single crystal

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Finding renewable energy sources is of paramount importance to meet the increasing global energy demand whilst minimizing the impact on the environment. The research community has focused on solar energy as it is endlessly available, and it has ranked methylammonium lead iodide ($MAPbI_3$) as one of the most promising candidates amongst perovskite solar cells. Despite its high efficiency, $MAPbI_3$ is sensitive to humidity, light, and temperature, its instability affects primarily on the crystalline structure and eventually leads to degradation [1]. Three crystalline structures are known for this material, orthorhombic, tetragonal, and cubic, which exist in different temperatures. Here we report on several processes detected from laser excitation of $MAPbI_3$ single crystal at ambient conditions. A phase transition from tetragonal to cubic phase was induced by excitation of over 15 mW laser power. The phases were characterized by LF-Raman and photoluminescence, taken simultaneously with the increase of exciting laser power. Spectral changes were assigned to the structural differences. In addition, Raman stimulation of iodine vaper signal was observed [2]. Those vapers were generated from the core of the focus wherein the highest temperature led to degradation. The stimulated Raman phenomenon was enabled due to the unique properties of the $MAPbI_3$ single crystal and revealed viability to use this material for additional applications in other research fields [3].

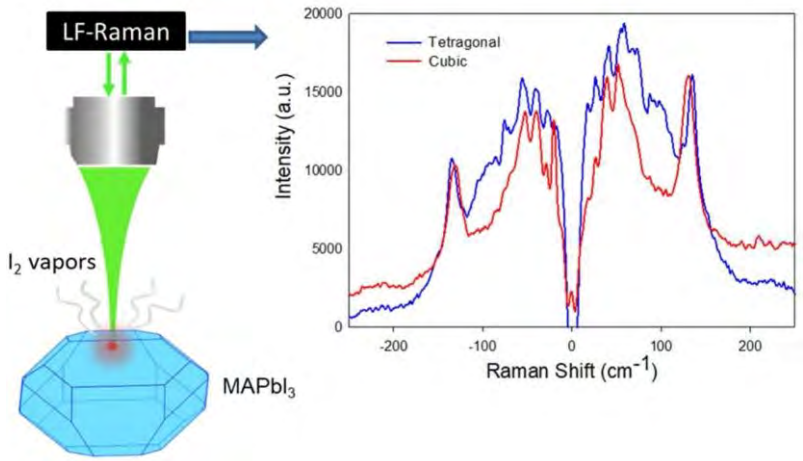

Figure 1: A scheme representing the processes detected by Raman scattering such as phase transition and decomposition.

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High-speed fingerprint broadband CARS with supercontinuum generation in bulk media and deep learning spectral denoising

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Broadband Coherent anti-Stokes Raman Scattering (B-CARS) is a powerful technique for high-speed vibrational imaging, providing chemical maps of heterogeneous biological samples in a label-free and non-invasive manner. In this work, we demonstrate an approach to B-CARS microscopy based on an amplified ytterbium laser system (Fig. 1(a)), working at 2-MHz repetition rate and delivering femtosecond pulses at 1035 nm with high energy (≈µJ level). This unlocks the possibility to produce broadband red-shifted Stokes pulses, covering the whole fingerprint region (400 – 1800 cm^{-1}), employing white-light continuum generation in a bulk crystal rather than in photonics crystal fibers. Moreover, the reduced repetition rate allows illuminating the sample with high pulse energy, generating a strong B-CARS signal without compromising sample integrity. This entails state-of-the-art acquisition speed (<1 ms/pixel) and unprecedented sensitivity (≈14.1 mmol/L) for multiplex CARS. In parallel, we modeled a post-processing pipeline to extract the maximum amount of information from the recorded B-CARS hypercubes. It consists of three steps: a spectral denoiser based on a convolutional neural network [1] to enhance the signal-to-noise ratio of the CARS spectra; the Kramers-Kronig algorithm [2] to remove the intrinsic non-resonant background of CARS spectra; some classification methods [3] to unmix the chemical components. After a validation of the imaging capability of the system on plastic beads (<1 ms/pixel), we imaged a murine vertebra (Fig. 1(b-c)), showing that our approach reveals excellent performances in distinguishing different chemical compounds in biological tissues.

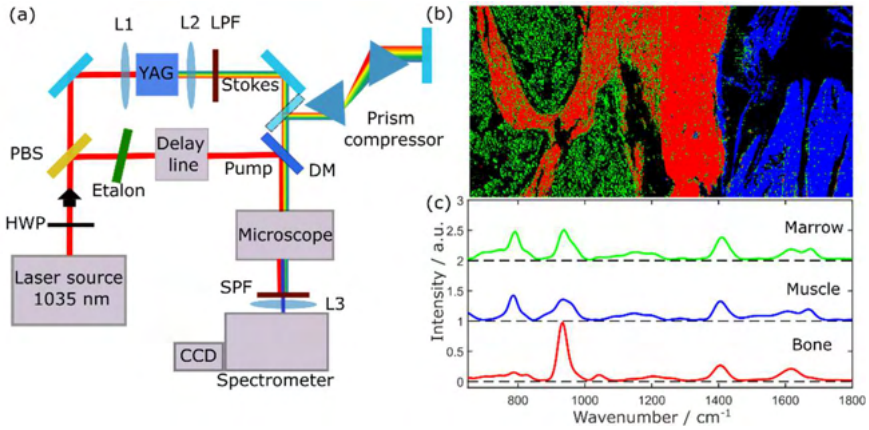

Figure 1. (a) Scheme of the B-CARS experimental setup. HWP: half-wave plate; PBS: polarizing beam splitter; L1, L2, L3: lenses; LPF: long-pass filter; DM: dichroic mirror; SPF: short pass filter. (b-c) Concentration map and spectra of 6 µm-thick slice of murine vertebra retrieved with K-means clustering analysis after the data-processing pipeline highlighting the three main clusters of bone (red), bone marrow (green) and muscle (blue). Imaging settings: 400x800 pixels, 1-µm pixel size, 1024 spectral points acquired per pixel, 10-ms pixel dwell time.

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Discrimination of Metastatic State in Prostate Cancer Cells by Identifying Metabolic Changes with Coherent Raman Imaging and Machine Learning

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Coherent Raman imaging (CRI) provides chemically specific information in a label-free manner that can be used to distinguish highly similar biological species. In particular, broadband coherent anti-Stokes Raman scattering (BCARS) microscopy is a CRI technique that affords quantitatively reproducible hyperspectral Raman images with reasonable imaging rates through simultaneous generation of a full fingerprint spectrum at each pixel [1]. Such advantages make BCARS uniquely suited for biomedical imaging. Here we exploit the rich information provided in BCARS hyperspectral images to distinguish the distinct metabolic profiles of three prostate cancer cell lines (LNCaP, DU145, PC3) and an epithelial prostate cell line (RWPE-1). By manipulating the metabolic state in cell lines with distinct metastatic potentials, growth rates, and invasion capabilities we can detect slight concentration changes in key metabolites related to the Warburg effect. Machine learning methods such as neural networks (NN) and support vector machines (SVM) have demonstrated the ability to deconvolute this spectrally complex and data rich Raman information into actionable characterizations [2,3]. By leveraging the complementary advantages of BCARS microscopy and machine learning classification, we can better understand the distinct metabolic changes sustained by prostate cancer cells and the corresponding relationship with alterations in proliferation.

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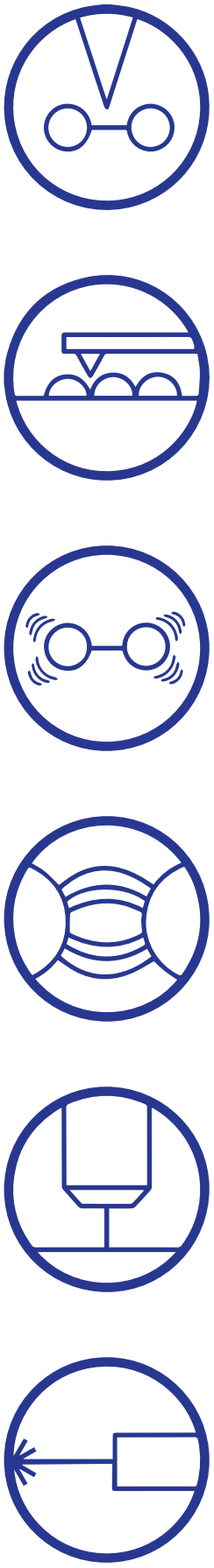
POST DEADLINE



XXVII International Conference on Raman Spectroscopy

In Vivo Biomolecular Imaging of Zebrafish Embryos using Confocal Raman Spectroscopy

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2) London School of Hygiene & Tropical Medicine, Department of Infection Biology, London, United Kingdom.
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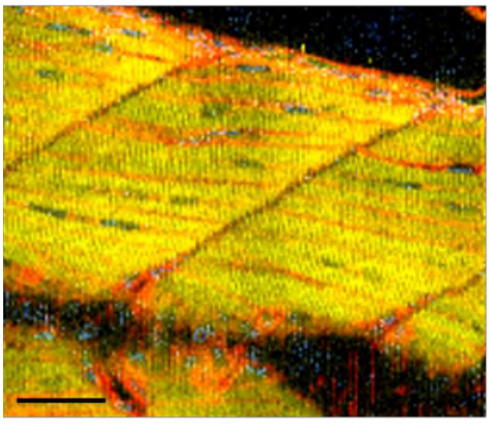
Zebrafish embryos is an important biomedical model with applications across fields such as disease modelling, developmental biology and neuroscience. Due to their optical clarity zebrafish embryos can provide direct visual access to biological processes and makes the model ideally suited for imaging approaches. However, most zebrafish imaging rely on label-based modalities, restricting the biochemical information that can be extracted. Spontaneous confocal Raman spectroscopic imaging represents a promising alternative for multivariate biomolecular analyses [1], but is still mostly unexplored in the zebrafish field. Here, we demonstrate how confocal Raman spectroscopic imaging can be applied for biomolecular imaging and analysis of zebrafish embryos. We outline a workflow of sample preparation, imaging and analysis and validate this method by collecting three-dimensional biomolecular images of whole zebrafish embryos and resolving fine anatomical features at micron scale resolution. We further apply confocal Raman spectroscopic imaging for the biomolecular profiling and discrimination of wild-type and ΔRD1 mutant mycobacteria in a zebrafish embryo model of tuberculosis. Finally, we demonstrate the use of confocal Raman spectroscopic imaging for in vivo temporal monitoring of the wound response in living zebrafish embryos. Overall, confocal Raman spectroscopic imaging constitutes a promising imaging modality for zebrafish research, enabling the first comprehensive biomolecular imaging analysis in fully intact and living zebrafish embryos [2].



High-resolution confocal Raman spectroscopic image of zebrafish embryo muscle tissue. Scale bar: 50 µm.

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Covariance-based stochastic Raman spectroscopy

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Covariance-based techniques have shown that a wealth of information can be extracted from noise that is lost when averaging multiple measurements. We have recently applied covariance-based detection to nonlinear optical spectroscopy, and show that noise in a femtosecond laser is not necessarily a liability to be mitigated, but can act as a unique and powerful asset. In this presentation, I will introduce and review our new approaches to time domain spectroscopy going beyond mean photon number observables [1-3] and show that the statistical features of light can provide richer information than standard linear and non-linear optical spectroscopies.

I will focus on femtosecond covariance based Raman spectroscopies, show their operational principles and demonstrate their applicability to the transient measurements of coherent phonon modes in case study material α-quartz[4,5]. I will then show preliminary results on archetypal strongly correlated cuprate superconductors which demonstrate the possibility of measuring electronic scattering from superconducting fluctuations on ultrafast time scale.

Our results, demonstrate how nonlinear processes in the sample can encode correlations between uncorrelated stochastic fluctuations in ultrashort pulses which can be used to unveil the Raman response of complex materials with unmatched time and frequency resolution. Finally, I will elaborate on our current directions on leveraging both the electromagnetic field fluctuations and the strong driving of materials to control the onset of quantum coherent states in complex materials.

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Picosecond energy transfer in a transition metal dichalcogenide–graphene heterostructure revealed by transient Raman spectroscopy

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Intense light–matter interactions and unique structural and electrical properties make van der Waals heterostructures composed by graphene (Gr) and monolayer transition metal dichalcogenides (TMD) promising building blocks for tunneling transistors and flexible electronics, as well as optoelectronic devices. The performance of such devices is critically ruled by interlayer interactions which are still poorly understood in many respects. Specifically, two classes of coupling mechanisms have been proposed, charge transfer (CT) and energy transfer (ET), but their relative efficiency and the underlying physics are open questions [1]. Here, building on a time-resolved Raman scattering experiment, we determine the electronic temperature profile of Gr in response to TMD photoexcitation, tracking the picosecond dynamics of the G and 2D Raman bands. Compelling evidence for a dominant role of the ET process accomplished within a characteristic time of ~ 4 ps is provided [2]. Our results suggest the existence of an intermediate process between the observed picosecond ET and the generation of a net charge underlying the slower electric signals detected in optoelectronic applications.

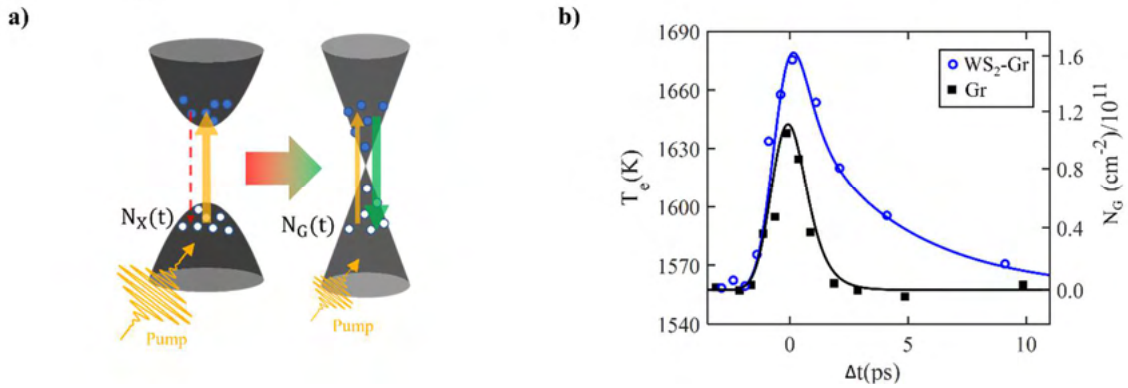

Figure 1: Modeling energy transfer in a WS2–Gr heterostructure. a) Sketch of the kinetic model used in the simulation. The pump pulse generates an exciton population in WS2 which is transferred to Gr due to an energy transfer with a characteristic time τ_T.b) Electronic temperature T_e at different time delays extracted from the dynamics of the 2D Raman band for WS2–Gr (open symbols) and bare Gr (full symbols) are compared with the simulated profiles (solid lines).

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Gold nanomesh for wearable SERS

Kotaro Hiramatsu[a], Limei Liu[a,b], Pablo Martinez Pancorbo[a], Ting-Hui Xiao[a], Saya Noguchi[a], Machiko Marumi[a], Julia Gala de Pablo[a], Siddhant Karhadkar[a], Hiroki Segawa[c], Yasutaka Kitahama[a], Tamitake Itoh[d], Junle Qu[b], Kuniharu Takei[e], Keisuke Goda[a,f,g]

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Wearable sensor technology, which is mainly represented by flexible, on-skin electronic sensors that provide rich information of the wearer's health conditions and surroundings, has attracted great interest by virtue of its high potential in biomedical applications. Surface-enhanced Raman spectroscopy (SERS) has emerged as an attractive approach to next-generation wearable sensors in recent years due to enabling highly sensitive, multiplexed chemical sensing of complex analytes in a non-invasive and label-free manner without the need for prior knowledge of the analytes. Several wearable SERS sensors have been reported recently [1], but they suffer from low scalability for widespread use beyond small-scale human health monitoring due to their complicated fabrication processes and limited multifunctional sensing capabilities. Here we report our demonstration of a highly scalable wearable SERS sensor based on an easy-to-fabricate, low-cost, ultrathin, flexible, stretchable, adhesive, and bio-integratable gold nanomesh (Fig. 1) [2]. The wearable sensor is moldable and can be fabricated in any shape and worn on virtually any surface for label-free, large-scale, in-situ sensing of diverse analytes from low to high concentrations (10 – 10^6 nM). To show the practical utility of the wearable SERS sensor, we used the sensor for the detection of sweat biomarkers, drugs of abuse, and microplastics. Our wearable SERS sensor provides a promising alternative for wearable sensor technology.

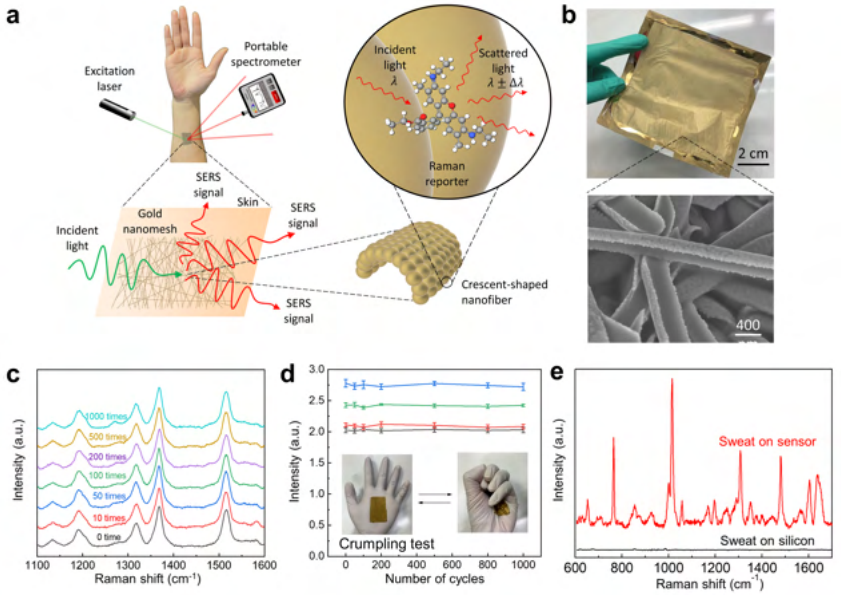

Fig. 1. Highly scalable, wearable SERS. a: Concept of the wearable SERS sensor. b: Photo and SEM image of the fabricated gold nanomesh. c: Raman spectra and d: characteristic Raman peaks of R6G molecules on the gold nanomesh adhered to a glove during the 1000-cycle crumpling test. e: Measured Raman spectra of human sweat.

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Beyond the Tunneling Limit of Quantum Plasmonics in Tip-Induced GO-Enhanced Raman Spectroscopy

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We demonstrate the application of graphene oxide (GO) as a nonmetallic substrate to enhance the nanoscale imaging capability of scanning probe microscopy based on a combination of GO-based SERS and tip-enhanced Raman scattering (TERS). We performed tip-sample distance (TSD) dependent measurements with high precision of 10 picometers beyond the tunneling contact distance. TSD measurements in a hybrid Au-GO-Au plasmonic picocavity formed by a GO junction on Au substrate showed more than 4 orders of magnitude signal enhancement compared to the Au-Au cavity without GO. Additionally, we provide the first measurement of sub-nanometer scale TSD dependence of the SERS chemical mechanism in the quantum tunneling regime. Our work paves the way to new opportunities to perform sub-nanoscale imaging of a wide range of molecular systems, investigate and control the complex interplay of enhancement mechanisms to further improve sensitivity and resolution.

Nonlocal Nonlinear Phononics

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Nonlinear phononics involves the resonant optical excitation of infrared-active lattice vibrations to an anharmonic regime in which they can selectively drive other modes. This unique form of lattice control has led to the manipulation of superconductivity, magnetism, and ferroelectricity over ultrafast timescales in complex materials. However, studies in nonlinear phononics have thus far focused primarily on the response of the material within the few-micron depth of the evanescent mid-infrared excitation field.

Here, we show that nonlinear phononics extends to propagating polaritons and the altering of ferroelectricity in regions of a crystal untraversed by the excitation field. In our experiments, mid-infrared optical pulses were used to resonantly excite an 18 THz phonon below the surface of ferroelectric $LiNbO_3$. Femtosecond stimulated Raman scattering (FSRS) revealed that the ferroelectric polarization was altered over the entire 50 μm depth of the sample, far beyond the few μm depth of the optically-driven phonon. At the highest excitation field, we observed a transient reversal of the ferroelectric polarization, as evidenced by a sign change in the Raman coefficients. These results build upon a class of *nonlocal* materials control in which a functional property is manipulated in a region separate from that of the excitation field.

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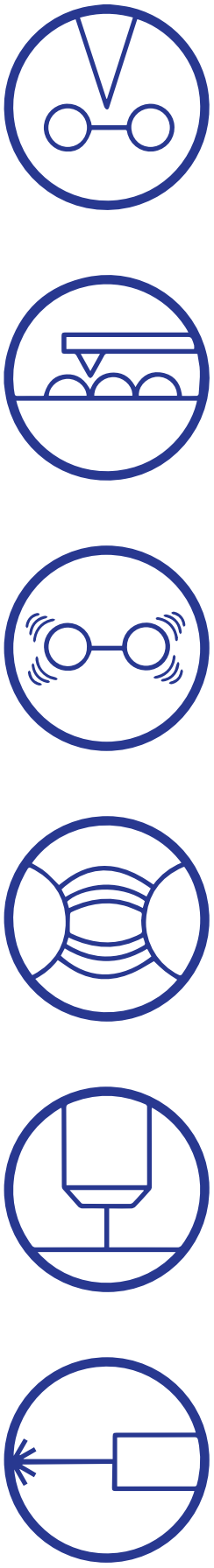
POSTER PRESENTATIONS



A SERS-based capillary sensor for Galactose Detection using 4-Mercaptophenylboronic Acid-Immobilized Silver Nanoshells

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Increased galactose level in blood have been known as indications of galactosemia, which is an inherited metabolic disorder generated from disruption of the normal galactose metabolism by enzyme deficiency. Early diagnosis of galactosemia can prevent developmental disorders and infant deaths, therefore, development of a sensitive and reliable detection of galactose is needed. In this presentation, we report a surface enhancement Raman scattering (SERS) sensor based on capillaries for fast and simple detection of galactose. The SERS sensor was fabricated by decorating the inner wall of capillary with silver nanoshells (AgNSs) and attaching 4-mercaptophenylboronic acid (4-MPBA), a label compound, on the AgNS. In our experimental design, 4-MPBA was converted to 4-mercaptophenol (4-MPhOH) by hydrogen peroxide (H_2O_2) produced from catalyzed reaction including galactose oxidase (GOx). The chemical changes of Raman label compounds were observed through the SERS signal shifts during the reaction. Through the optimization processes, selective galactose detection in the range of 0.01 to 20.0 mM was successfully developed. These results indicate that the developed capillary SERS sensor have great potential for galactose detection for early diagnosis. Details of the results will be discussed in the presentation.

Development of dual SERS substrates using silver nanoshells and two kinds of graphene quantum dots

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Recently, advanced Raman enhancement platforms using 2D materials such as graphene or graphene quantum dots (GQDs) and plasmonic nanoparticles (NPs) have been reported, named 'dual enhancement' platforms.[1] The dual enhancement platform exhibits several advantages from charge transfer-induced chemical mechanism enhancement (CM) between the molecules and graphene and attracting the analyte molecules even with low surface affinity to plasmonic NP surfaces. Compared to conventional graphene, GQDs have more accessible edges, which lead to a more effective adsorption of analyte molecules. GQD has two kinds of edges called zigzag and armchair that have different characteristics. Energy level of GQDs can tune by controlling edge of GQDs, leading to different CM effect. In this study, solid support substrates were designed based on bumpy Ag nanoshells (Ag NSs) and two type of GQDs with different edge structures as dual SERS substrate. The fabricated SERS substrates were characterized by SEM, EDS, AFM and Raman spectroscopic analysis. Rhodamine 6G and malachite green were used as probe molecules to compare dual-SERS effect between the two types of substrates that used zigzag GQDs and armchair GQDs.

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Electro-Inductive Effect—Electrodes That Act as Functional Groups to Control Electronic Properties and Chemical Reactivities of a Molecule

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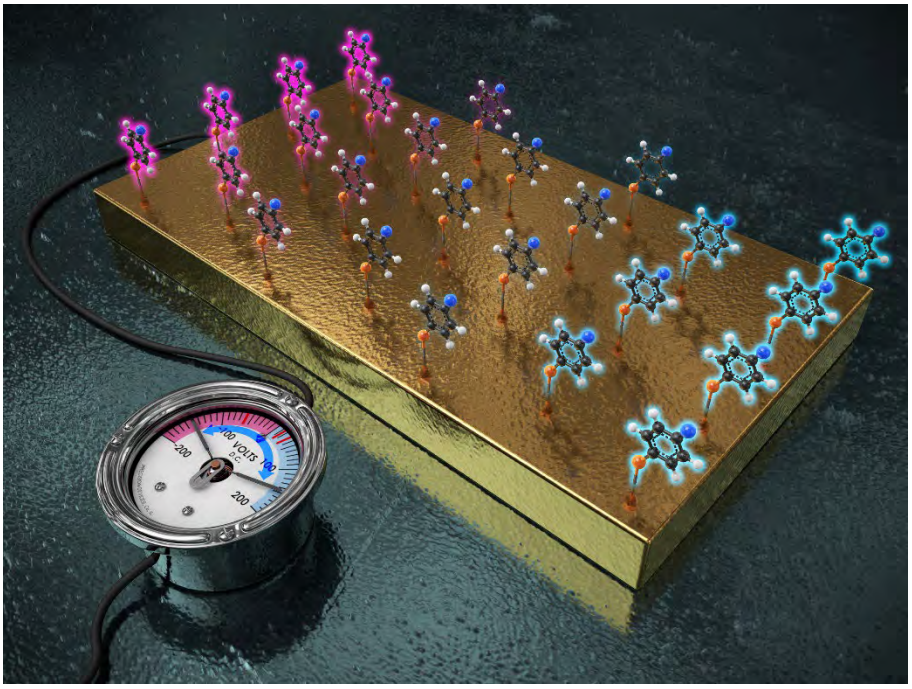
Abstract: In place of functional groups that impose different inductive effects, we immobilize molecules carrying thiol groups on a gold electrode and monitor reaction progresses using SERS technique. By applying different voltages, the properties of the immobilized molecules can be tuned. The base-catalyzed saponification of benzoic esters is fully inhibited by applying a mildly negative voltage of –0.25 volt versus open circuit potential. Furthermore, the rate of a Suzuki-Miyaura cross-coupling reaction can be changed by applying a voltage when the arylhalide substrate is immobilized on a gold electrode. Finally, a two-step carboxylic acid amidation is shown to benefit from a switch in applied voltage between addition of a carbodiimide coupling reagent and introduction of the amine.



XXVII International Conference on Raman Spectroscopy

Surface-Enhanced Femtosecond Stimulated Raman Spectroscopy: Effects of the Energy of Plasmon Resonances on Dispersive Line Shapes in Spectra

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The first successful combination of surface-enhanced Raman spectroscopy (SERS) and femtosecond stimulated Raman spectroscopy (FSRS) was done in 2011. [1] SERS provides spectroscopic detection down to a single-molecule level, and FSRS enables the acquisition of Raman spectra at ultrafast time scales. Booth techniques combined have the potential of studying molecular reactions of single molecules. However, an understanding of surface-enhanced FSRS (SE-FSRS) spectra is necessary. Unexpected, dispersive shapes of the spectral bands have been registered on the optical nanoantenna with the adsorbed *trans*-1,2-bis(4-pyridyl)-ethylene molecule. Then studying the influence of excitation wavelength has shown that the dispersive shape of bands changes with changing the excitation wavelength. [2] Those changes were not possible to explain by using only three different excitations. Here we present the result of a systematic scan of the excitation wavelength on the dispersive shape of the SE-FSRS bands and the assisting all spectra background. Also, the influence of plasmon resonance spectral position, the delay between pulses, and the pulse energy on the SE-FSRS spectra were studied. The results of this study have shown that the energy of the plasmon resonances, the line shape of the SE-FSRS signal, and the background variability associated with the SE-FSRS spectra are closely connected. The results allowed to optimize the SE-FSRS signal conditions.

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Raman Hetero Two-Dimensional Correlation Spectroscopy: A powerful technique for monitoring active centres in complex environments

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Many fields of current research in chemistry, material science and biology are trying to understand, modify or design functionalities governed by small active moieties in large, complex environments. Prominent examples are smart polymers in material science, soft-matter embedded catalysts in chemistry or functional proteins in biology. Raman spectroscopy has successfully been used in all these fields to yield knowledge about the active centres as well as the surrounding environments in isolation. While knowledge about the separate domains is already providing helpful information to the respective research fields, it is paramount to understand the interaction between the active centres and the environment to fully understand and tune these systems.

We have explored the combination of Raman spectroscopy, as a molecularly specific method with broad applicability to many kinds of environments, together with a method that targets the active centre, e.g. Resonance Raman spectroscopy,[1] utilizing the power of two-dimensional correlation spectroscopy (2DCOS) in the form of PC-2DCOS.[2] 2DCOS, an established technique for the analysis of spectral data sets under external perturbation, can visualize the cross-correlation function between two spectroscopic data sets and thereby give direct insights into the interaction between environment and active centre, which was demonstrated on the denaturation of cytochrome c and was able to reveal the interaction between secondary structure and spin-state of the active heme centre.

Since Raman spectroscopy and 2DCOS are broadly applicable, this approach can be transferred to arbitrary systems and active-centre specific techniques, enabling targeted investigation of structure-function relationships in a broad range of fields.

Acknowledgement: This work was Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project Number 364549901 – TRR 234 and BO4700/4-1.

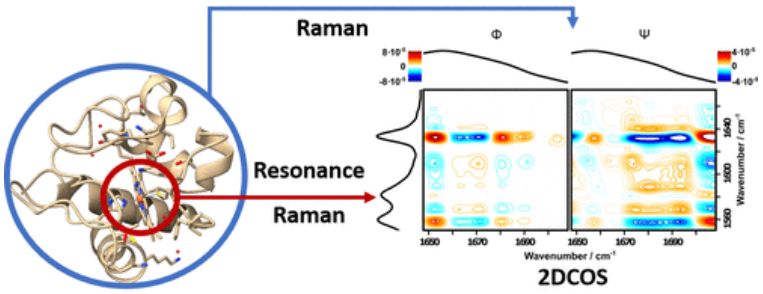

Figure 1: Schematic representation of the combined non resonant / resonant Raman approach to the analysis of an active center in a complex environment demonstrated on cytochrome c. While non resonant Raman spectroscopy is able to monitor the protein structure during an external perturbation (e.g. denaturation), resonance Raman specifically targets the photoactive heme center, which governs the functionality of the protein.

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Phonon hydrodynamic transport in 2D materials by ultrafast laser-based techniques

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In macroscopic solids, heat transport is typically based on Fourier diffusion theory. However, 2D materials like graphene and hexagonal boron nitride, may exhibit hydrodynamic heat transport which resembles wave propagation in fluids [1]. This occurs when normal scattering is dominant compared to resistive scattering and, therefore, a thermal impulse can propagate inside the material as a wave with no significant damping. This phenomenon is known as *second sound* and it was firstly observed in solid helium [2], NaF [3], Bi [4], and Al_2O_3 [5], at cryogenic conditions. Recently, the observation of the second sound above 100 K in graphite [6] brought a renewed interest in this field.

Observing second sound in 2D materials is extremely challenging. Ultrafast time-resolved techniques are well-suited for measuring the thermal response of a material after impulsive excitation and, therefore, detect this elusive hydrodynamic heat transport. In our work, we use a pump-probe setup, which consists of an ultrafast pump laser pulse (<30fs), which brings the system out of equilibrium. Then, a probe pulse monitors the sample response after pump excitation as a function of delay time between the pump and the probe, which is controlled by a mechanical delay line.

Our versatile pump-probe setup, can be used to perform either time-resolved Raman spectroscopy (TRRS) or transient reflectivity (TR) measurements, when the probe pulse monitors Raman spectra or change in reflectivity, respectively. In the TRRS scheme, we employ a pulse shaper to stretch out the probe pulse duration, and therefore, achieve higher spectral resolution (~10 cm^{-1}). A triple spectrometer is then used to collect Raman spectra at the best trade-off between time and spectral resolution as a function of time delay, which allows to track the temperature evolution of single phonon modes. Moreover, we developed a TR setup that enables us to further investigate the different energy flow mechanisms in materials. Both techniques benefit from a broad wavelength tunability, ranging from 400 nm to 900 nm, which makes this setup a powerful tool to investigate 2D and other complex materials under different excitation and detection conditions. This great flexibility is a key asset to investigate the novel properties of the hydrodynamic heat transport.

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Ultraviolet resonance Raman (UVRR) spectroscopy for label-free monitoring of peptide recognition by supramolecular ligands

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Ultraviolet resonance Raman scattering (UVRR) has been frequently used for studying peptide and protein structure and dynamics, while applications in supramolecular chemistry are quite rare. Since UVRR offers the additional advantages of chromophore selectivity and high sensitivity compared to conventional non-resonant Raman scattering, it is ideally suited for label-free probing of relatively small artificial/supramolecular ligands exhibiting electronic resonances in the UV.[1] We present results of UVRR spectroscopy in supramolecular chemistry in the context of peptide/protein recognition and focus on selected artificial ligands, which were rationally designed as selective carboxylate binders (guanidiniocarbonyl pyrrole, GCP, and guanidiniocarbonyl indole, GCI), shown in figure 1 with an emphasis on mono- and multivalent GCP/GCI-based ligands. [2][3] The supramolecular ligands bind via a combination of non-covalent interactions involving electrostatics, hydrogen bonding, and hydrophobic effects/van der Waals forces. These intermolecular interactions upon recognition induce subtle changes in the molecular properties such as the electronic structure and bond strengths, which can be monitored with UVRR spectroscopy.[4]

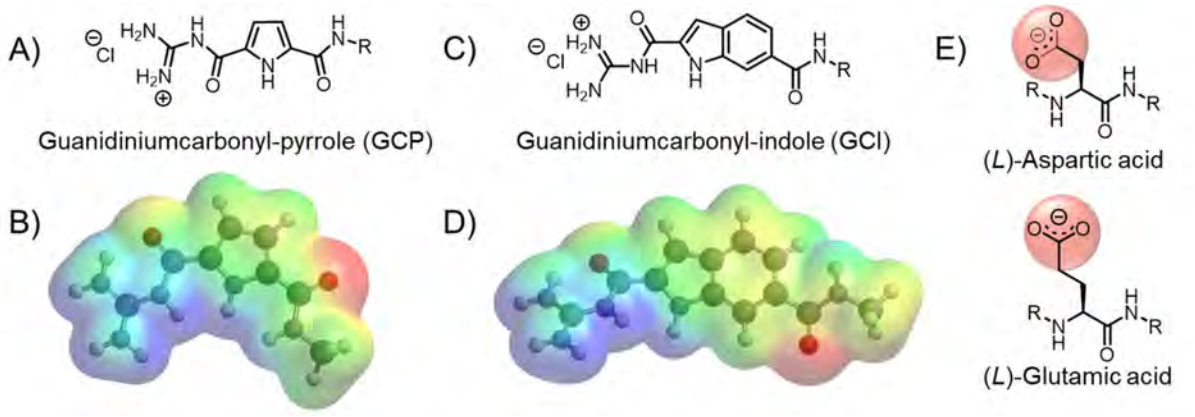

Figure 1: Molecular structure and electronic surface potential of GCP (A,B) and GCI (C,D), as well as target amino acids in a peptide backbone (E).[1]

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Detecting Hydrogen Passing Through Graphene by Surface-Enhanced Raman Spectroscopy of 4-Nitrothiophenol

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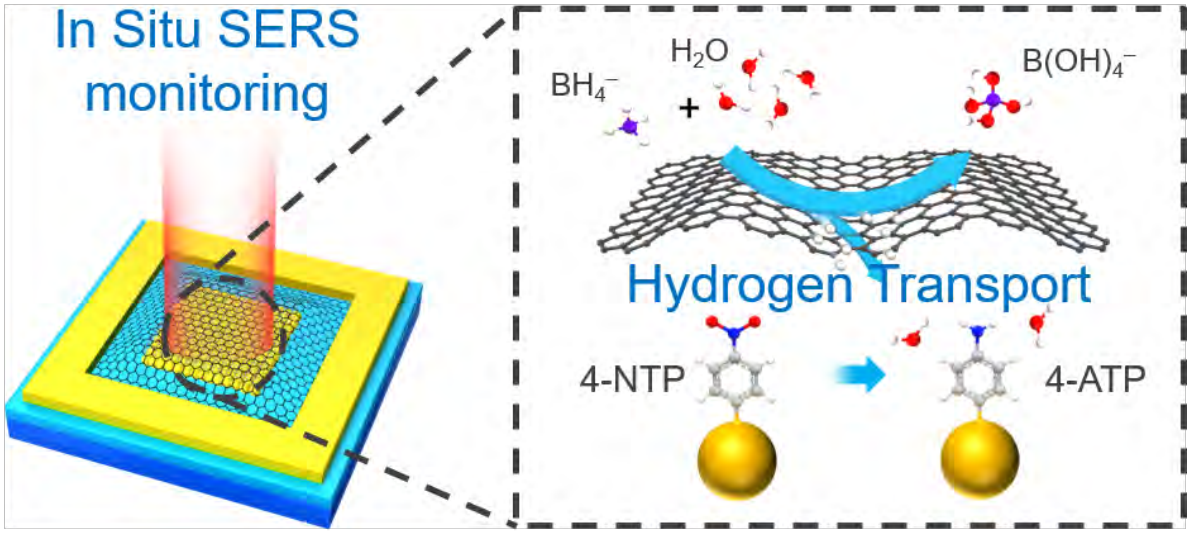
Abstract: Observing the atomic or molecular transport through a two-dimensional (2D) material is important to understand its physicochemical properties as a membrane. Here, we used in situ Surface-enhanced Raman spectroscopy (SERS), a highly sensitive and rapid analytical tool, to observe hydrogen passing through the graphene membrane. This was possible by separating Raman dye and the reducing agent into a graphene membrane and monitoring the hydrogenation of Raman dye. The reduction reaction of 4-nitrothiophenol by sodium borohydride was chosen as the demonstration reaction. We could see that the energy barrier when hydrogen passes through graphene is less than 0.47 eV in our system. Our work shows that in situ SERS can be an effective technique to measure the transport properties of 2D materials.



Raman study of undoped and Si-doped orthorhombic κ-Ga_2O_3 thin-films

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In the last decade Gallium Oxide (Ga_2O_3) has been raising interest among the scientific community because of its potential in the field of power electronics [1]. Five different polymorphs have been identified for Ga_2O_3, with the monoclinic β being the thermodynamically stable one and by far the most investigated.

Recently, considerable interest has been rising on various metastable polymorphs; among them the orthorhombic κ-Ga_2O_3 (often named ε because of its quasi-hexagonal symmetry produced by 120° domains) has significant potential due to a spontaneous polarization along the (001) direction [2]. Nonetheless, the microstructure of epitaxial (001)-oriented κ(ε)-Ga_2O_3 thin films is characterized by a peculiar columnar structure induced by the presence of the 120°-rotated domains [3]. The width of these domains is usually in the range of 5-10 nm and they are supposed to have a detrimental role in the in-plane electronic transport.

Raman spectroscopy is a powerful and non-destructive characterization technique for κ(ε)-Ga_2O_3 epitaxial layers [4]. In this work we employ Raman spectroscopy to investigate a series of undoped and Si-doped κ(ε)-Ga_2O_3 layers deposited on c-plane sapphire substrates. These layers are characterized by a variable domain size (ranging from about 10 to about 300 nm wide) and different transport properties (resistivity ranging from 10^7 to 10^{-1} Ωcm). Significant differences emerged between the analysed samples. In particular, the incorporation of Si consistently resulted in a variation of the intensity ratio between specific Ga_2O_3 Raman modes while the domain size mainly affected width and position of several modes. The role of phonon confinement is evaluated.

These results were also correlated with XRD and TEM analysis.

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Acute liver failure (ALF) has a high mortality. The commonest cause is paracetamol overdose. Paracetamol is a readily available drug for treatment of minor ailments; however, it is ingested in overdose by 40% of self-harm patients in the UK (approximately 100,000 hospital presentations per year). The antidote to paracetamol overdose is n-acetylcysteine (NAC). It is only fully effective when treatment is commenced within around 8 h of taking the overdose. Therefore, treatment must be started as quickly as possible in those patients at risk of liver injury. The current gold standard for diagnosing liver injury is serum alanine aminotransaminase (ALT) activity. Unfortunately, ALT activity only increases in the later stages of the overdose resulting in the failure to correctly diagnose liver injury in the early stages of the disease. Cytokeratin-18 (cK-18) has been identified as a potential biomarker that will provide improved diagnose of the patients suffering from drug induced liver injury (DILI) following paracetamol overdose. cK-18 is elevated significantly higher that ALT measurements in the early stages of onset DIL. In order to fully utilise the benefits of cK-18 as a DILI biomarker the development of a rapid point of care test (POC) must be carried out.

This work investigates the development of a rapid surface enhanced Raman scattering (SERS) based lateral flow immunoassay (LFIA) diagnostic test which can be used to quantify ck-18 associated with the early onset of DILI. Interrogation of the SERS-LFIA device with a purpose-built portable Raman probe will provide health-care professionals with an improved diagnostic test for efficient patient stratification. The SERS-LFIA device and portable Raman probe outlined in this research has been developed to provide a limit of detection as low as 5 ng/mL 30 minutes after application of patient serum samples.

Comparison of Gold and Silver nano thin films for chemical sensing of Tenofovir using Surface Enhanced Raman Spectroscopy.

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Abstract

Nanothin films for chemical sensing applications have gathered much interest in recent years. As such, the manufacturing of these sensors for quality control purposes has pivoted across many chemical and biochemical molecular research activities [1]. However, the right combination of sensor fabrication methods and signal acquisition techniques remains a challenge that requires optimization [2]. In this work, we synthesized nanometallic thin films using physical vapor deposition and compared them in adsorption and detection of antiretroviral medication Tenofovir. Our results show successful deposition of the nanometals on the plain glass where the Raman peak intensities of the glass decreased with every layer deposition. Secondly, the peak intensities across the three sensing platforms showed the best signal from silver nanofilms, which is attributed to the surface plasmon properties of the metal in the presence of 532 nm green laser photons [3]. Lastly, a linear regression analysis was performed using a serial dilution of the analyte and the R-squared values were compared. The results showed an improved R-value on the silver substrate and thus we concluded silver thin films performed optimally for surface-enhanced Raman spectroscopy of Tenofovir. Future work will involve, enhancement factor and sensitivity studies using these platforms.

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SERS-based microdroplet sensor for sensitive and reproducible detection of SARS-CoV-2

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The coronavirus disease 2019 (COVID-19) has been labeled an ongoing pandemic by the World Health Organization (WHO). Real-time quantitative polymerase chain reaction (RT-qPCR) has been considered a gold standard for the quantitative evaluation of a target gene. However, it still suffers from the problem of a long detection time. Commercially available lateral flow assay (LFA) kits can be taken within 30 min, but it has problems in terms of low sensitivity and poor accuracy. To address these issues, we developed a surface-enhanced Raman scattering (SERS)-based immunosensing platform for the rapid and sensitive detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In this work, SERS nanotags, anti-SARS-CoV-2 nucleoprotein antibody-conjugated gold nanoparticles, and magnetic beads have been used for the detection of SARS-CoV-2. The Raman signals of SERS nanotags in 140 droplets were measured under flowing conditions inside a microdroplet channel. Total analysis time from droplet generation to SERS detection takes less than 10 minutes because all experimental conditions were automatically controlled inside the exquisitely designed microfluidic channel.[1] Moreover, limit of detection was improved from 341 to 0.22 PFU/mL, compared to that of the assay performed in a LFA strip. This novel SERS-based immunosensing platform using a microdroplet channel is expected to be a powerful analytical tool to detect SARS-CoV-2.

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Effect of conductivity and SERS activity by temperature-mediated crystallinity changes of PEDOT:PSS organic semiconductor

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In recent years, semiconductor materials have become a research focus in optoelectronic materials due to their unique properties of energy band structures. In particular, the π-π coupling formed by the organic semiconductor provides more possibilities for improving SERS activity based on the charge transfer. Based on this property, we used a compound semiconductor, poly(3,4-ethylenedioxythiophen):poly(styrene sulfonate) (PEDOT:PSS), as a SERS substrate and adjusted the molecular crystallinity by changing the temperature. Raman, UV-vis, XRD, and simulation methods were used to study the specific changes of molecular crystallinity at different temperatures. Especially, the thermal annealing induced this change of crystallinity was studied by the Raman band and UV-vis absorption peak. The effect of this change on conductivity and SERS activity were analyzed using methylene blue as the probe molecule. This study is expected to provide more solutions to the problem of organic semiconductors-based optoelectronic devices based on SERS method. The details of the mechanism will be discussed in this presentation.

SARS-CoV-2 Screening Using Raman Spectroscopy Enhanced with Flexible Nanoparticle Substrates

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Background: The SARS-CoV-2 virus and its variants have impacted everyone's health and life, and a high-throughput, rapid screening methodology and device for SARS-CoV-2 and related viruses to identify infected personnel and reduce spreading are needed. We propose to detect SARS-CoV-2 virus using surface enhanced Raman spectroscopy (SERS) based upon the enhanced interaction of light with the chemical bonds within a material adsorbed onto a nanostructured metal surface. Studies were conducted to determine if flexible substrates with embedded nanoparticles could be used to generate an enhanced Raman spectrum of the virus. This flexible substrate can be manufactured as stickers placed on the inside or outside surfaces of any face mask. Scanning of the stickers using a Raman spectroscopy system with a combination of software-analytics can automatically detect small numbers of SARS-CoV-2 (or detect and differentiate any other virus, using spectra template-matching) absorbed on the nanoparticle surface. In addition, mutation of the virus only affects the software-analytics which can be easily and rapidly updated.

Materials and Methods: We employed a portable Raman spectroscopy system (6"x6"x4") that utilizes a handheld, fiber optic probe (3"x1.5"x0.5") to record Raman fingerprint spectra (400-1800 cm^{-1}) using near-infrared (NIR) excitation at 785 nm. The flexible substrates include 1) quartz fiber with gold nanoparticles deposited with vacuum evaporation; and 2) cellulose with inkjet-printed gold nanoparticles. We acquired Raman spectra from 1) substrate only; 2) substrates with SARS-CoV-2 spike protein solution of different concentration (1.25 and 12.5 μg/mL); 3) substrate with the base solution used to disperse the spike protein; and 4) substrate with influenza vaccine. Each spectrum was acquired over a 1 second period with an excitation laser power of 50 mW.

Results: For the quartz fiber substrate, we observed strong SERS spectral bands with SARS-CoV-2 spike protein that were unique compared to either substrate alone or base solution on the substrate. Raman spectra peaks located at 942, 1167 and 1259 cm^{-1} are only present in the spectra of SARS-CoV-2 spike protein and locations of those peaks agree with that measured using spike protein powder [1, 2]. For the cellulose substrate, the intrinsic Raman bands observed with the substrate alone which resulted from the cellulose background are much higher than the quartz fiber substrate. Only when the protein concentration was increased to 250 μg/mL, can the peaks of above bands be observed. Raman bands from the influenza vaccine observed on the quartz fiber substrate do not overlap with those of SARS-CoV-2 spike protein.

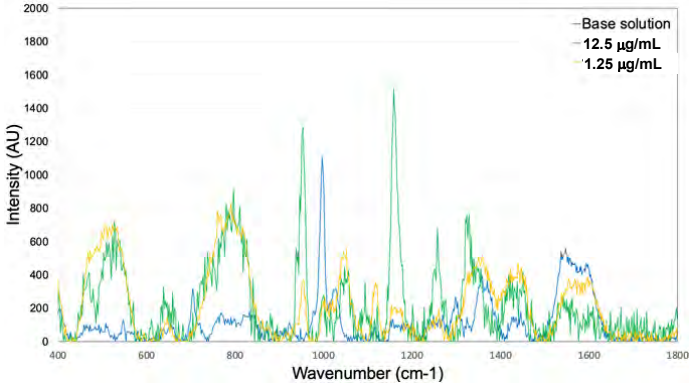

Fig.1 Raman spectra of SARS-CoV-2 spike protein solution of 1.25 (yellow), 12.5 (green) μg/mL or base solution (blue) used to disperse the spike protein. Quartz fiber substrates were used.

Conclusions: Our preliminary data demonstrate SERS from SARS-CoV-2 on a flexible nanoparticle substrate have unique bands that can be used to identify SARS-CoV-2 virus. The substrate can be fabricated as stickers placed on any facemask. The unique spectral peaks recorded from SERS SARS-CoV-2 suggest the approach can have high specificity.

Acknowledgements: AFOSR, FA9550-20-1-0052; NIH, HHSN272201400008C (BEI Resources)

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Who's who? Discrimination of Breast Cell Lines by Raman Microspectroscopy

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Despite all efforts, cancer is still a growing health problem worldwide, metastatic breast carcinoma being the second most lethal cancer among women [1]. Discriminating between healthy and malignant cells is of primary importance, as being able to identify the different subtypes of breast cancer in a rapid and accurate way in order to apply a more effective treatment [2,3].

The aim of this work was to use Raman microspectroscopy in order to identify possible biomarkers of cancer, that may allow to distinguish among different human breast cancer cells, as well as between these and healthy cells [4]. Four human breast cell lines - triple-negative and non-triple negative (MDA-MB-231, MDA-MB-468, HCC-1143, MCF-7), and one healthy cell line (MCF-12A) were measured by Raman microspectroscopy, with a 532 nm excitation wavelength. For both the fingerprint and high wavenumber spectral regions, data was statistically analyzed by Principal Component Analysis (PCA) allowing to obtain a good discrimination between the different sets of samples.

The vibrational modes found to play a more significant role in the discrimination between cancer and healthy cells were ascribed to lipids and proteins, the lipid/protein content being identified as a reliable biomarker of malignancy. Breast cancer cell lines displayed a higher lipid contribution relative to their non-tumourigenic counterparts. When comparing triple-negative and non-triple negative cell lines a good discrimination was obtained, mainly based on the with lipid CH stretching bands (at 1350-1450 cm^{-1}).

A particular Raman signature was obtained or each cell line under study, evidencing the occurrence of specific spectral biomarkers allowing the detection of triple-negative breast cancer, which may contribute for an early identification of this low prognosis type of cancer leading to the development of more effective chemotherapeutic approaches.

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SERS-PCR ASSAYS OF SARS-COV-2 USING AU NANOPARTICLES-ANCHORED AU NANODIMPLE SUBSTRATES

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The reverse transcription-polymerase chain reaction (RT-PCR) method has been adopted worldwide to diagnose severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although this method has good sensitivity and specificity, there is a need to develop a more rapid diagnostic technology, given the virus's rapid spread. However, the RT-PCR method takes a long time to diagnose SARS-CoV-2 because it requires thermocycling steps. Therefore, we developed a surface-enhanced Raman scattering (SERS)-PCR detection method[1] using an AuNP-anchored Au nanodimple substrate (AuNDS)[2] to shorten the diagnosis time by reducing the number of thermocycling steps needed to amplify the DNA. For the envelope protein (E) and RNA-dependent RNA polymerase (RdRp) genes of SARS-CoV-2, when the initial DNA concentration was 1.00×10^5 copies/μL, 25 RT-PCR thermocycles are required to reach a detectable threshold value, while 15 cycles are required for magnetic bead-based SERS-PCR. However, only 8 cycles are needed for the AuNDS-based SERS-PCR, and the corresponding detectable target DNA concentrations were 3.36×10^{12}, 3.28×10^9, and 2.56×10^7 copies/μL, respectively. Therefore, AuNDS-based SERS-PCR is seen as being a new molecular diagnostic platform that can shorten the time required for the thermocycling steps relative to the conventional RT-PCR.

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A Portable SERS-based Lateral Flow Assay Strip for Rapid and Sensitive Detection of SARS-CoV-2 Antigen

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COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been affecting the world for over two years. Reverse transcription-polymerase chain reaction (RT-PCR) has been considered a gold standard method for diagnosing COVID-19. In RT-PCR, however, the total diagnostic time takes a long time, approximately 3-4 hours. Therefore, as the number of infected people increases rapidly, a lateral flow assay (LFA) strip has been used to diagnose COVID-19 instead of RT-PCR. However, the commercial LFA strip has a high false-negative rate due to the limitation of detection sensitivity. [1] To resolve this issue, we developed a surface-enhanced Raman scattering (SERS)-based lateral flow assay (LFA) strip [2] with a portable Raman reader for rapid and sensitive detection of SARS-CoV-2 nucleocapsid protein. The SERS-LFA strip was performed using SARS-CoV-2 lysate with different concentrations of the virus (0 – 1000 PFU/mL) and had a limit of detection (LoD) of 3.53 PFU/mL. As a result of testing clinical samples using the SERS-LFA strip, it was confirmed that the false-negative rate was improved compared to the results of the commercial LFA strip. Our SERS-LFA strip shows a strong potential to resolve the problems in terms of low sensitivity inherent in the conventional LFA strip.

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Biochemical Origin of Raman-Based Diagnostics of Huanglongbing in Grapefruit Trees

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Abstract:

Biotic and abiotic stresses cause substantial changes in plant biochemistry. These changes are typically revealed by high-performance liquid chromatography (HPLC) and mass spectroscopy-coupled HPLC (HPLC-MS). This information can be used to determine underlying molecular mechanisms of biotic and abiotic stresses in plants. A growing body of evidence suggests that changes in plant biochemistry can be probed by Raman spectroscopy, an emerging analytical technique that is based on inelastic light scattering. Non-invasive and non-destructive detection and identification of these changes allow for the use of Raman spectroscopy for confirmatory diagnostics of plant biotic and abiotic stresses. In this study, we couple HPLC and HPLC-MS findings on biochemical changes caused by *Candidatus Liberibacter* spp. (*Ca. L. asiaticus*) in citrus trees to the spectroscopic signatures of plant leaves derived by Raman spectroscopy. Our results show that *Ca. L. asiaticus* cause an increase in hydroxycinnamates, the precursors of lignins, and flavones, as well as a decrease in the concentration of lutein that are detected by Raman spectroscopy. These findings suggest that *Ca. L. asiaticus* induce a strong plant defense response that aims to exterminate bacteria present in the plant phloem. This work also suggests that Raman spectroscopy can be used to resolve stress-induced changes in plant biochemistry on the molecular level.

Ensemble-level single particle characterisation of SERS nanoparticles

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Placing molecules close to metallic surfaces and recording their enhanced Raman scattering has revolutionised spectroscopy and analytical techniques. The brightness of the surface-enhanced Raman Scattering (SERS) can outcompete fluorescence allowing single-molecule SERS studies that shed light into the fundamental interactions at nanoscale interfaces. In analytics, sensitivities down to zeptomole can be achieved [1] paving way to a new generation of chemical sensors. The main challenge of exploiting SERS on a large scale is its irreproducibility stemming from the lack of nanoscale control in the substrate preparation and the placement of molecules on the substrate. Complicated methods using DNA origami can partially overcome the problem, however, for industrial-scale applications, simplicity and cost are crucial.

We have analysed the most commonly used SERS substrates loaded with a model thiol dye to systematically characterise their performance. Using a fully automated Raman microscope, we measure thousands of spectra, one particle at a time, achieving ensemble-level insight at a single-particle precision. We present the brightness, stability and morphology distributions for gold and silver nanospheres, nanorods and nanostars spanning resonances across the visible and near infrared spectrum. Correlating the intensities and morphology under 633 and 780 nm illuminations gives us a detailed insight beyond the standard ensemble experiments "in a cuvette" and will pave a way for a better design of SERS substrates for large-scale technological applications.

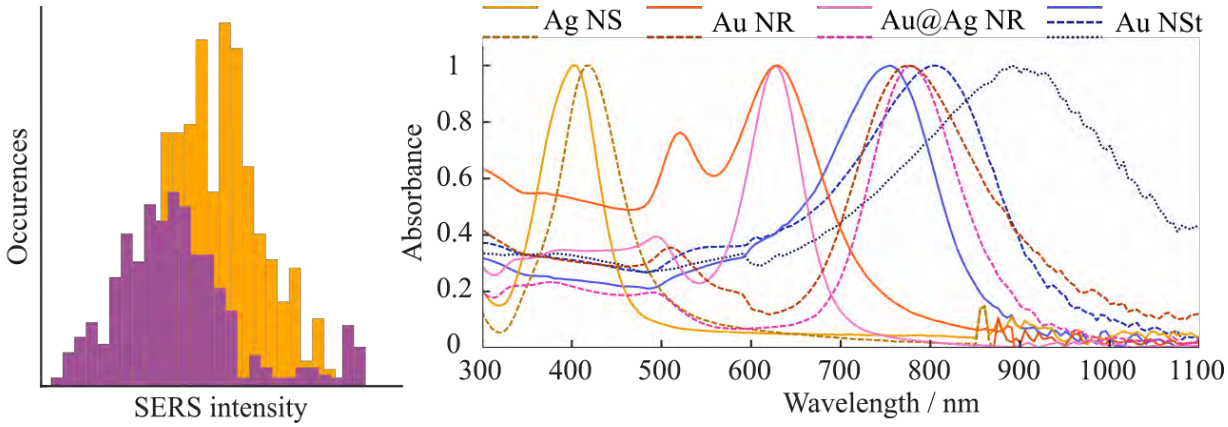

Figure 1 Left: Example SERS intensity distributions of particles from two samples that would appear the same in an ensemble experiment, but are distinguishable in a statistically significant single-particle measurement. Right: Normalised absorbance spectra of studied particles: nanospheres (NS), nanorods (NR) and nanostars (NSt).

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SERS-based Kinetic Monitoring of the Platinum-catalyzed Hydrogen Reduction of the Three Nitrothiophenol Constitutional Isomers (2/3/4-NTP)

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4-nitrothiophenol (4-NTP) has been widely used in surface-enhanced Raman-scattering (SERS)-based reduction studies for testing the catalytic performance of a variety of noble metal nanoparticles using hydride or hydrogen as reducing agent. The catalytic conversion of 4-NTP to 4-aminothiophenol (4-ATP) strongly depends on the catalyst's morphology, size, and material composition.[1] This reduction reaction proceeds via a Langmuir-Hinshelwood mechanism (s. **Fig.1a**).[2] After the formation of the Pt-H species (**Fig.1a** first step) two alternative reaction pathways concerning the transferred hydrogen species can be envisioned. The upper pathway (hypothesis 1) describes the direct transfer of a hydrogen radical or hydride to the molecule, while the lower pathway (hypothesis 2) describes an electron transfer from the platinum to the 4-NTP followed by a proton transfer. If the first hypothesis is valid, we expect that the distance of the nitro group to the metal surface would be decisive for the reaction rate. Thus, 2-NTP should react the fastest, followed by 3-NTP, and 4-NTP as the slowest. However, if the second hypothesis is valid, we would expect similar rate constants for 2-NTP and 4-NTP, while for 3-NTP the reaction would proceed more slowly. In order to falsify one of these two contrary hypotheses, we varied the position of the nitro group concerning the metal-bonded sulfur, i.e. the distance to the metal surface. We studied the hydrogen reduction kinetics of the three constitutional isomers of NTP (2-NTP, 3-NTP, and 4-NTP) with catalytically and SERS-active platinum-coated gold nanorods. Based on our obtained SERS kinetic data ($k_{2\text{-NTP}} > k_{3\text{-NTP}} > k_{4\text{-NTP}}$; s. **Fig1b**), we conclude that the reduction does not proceed according to the second pathway, but via a hydrogen radical/hydride transfer.

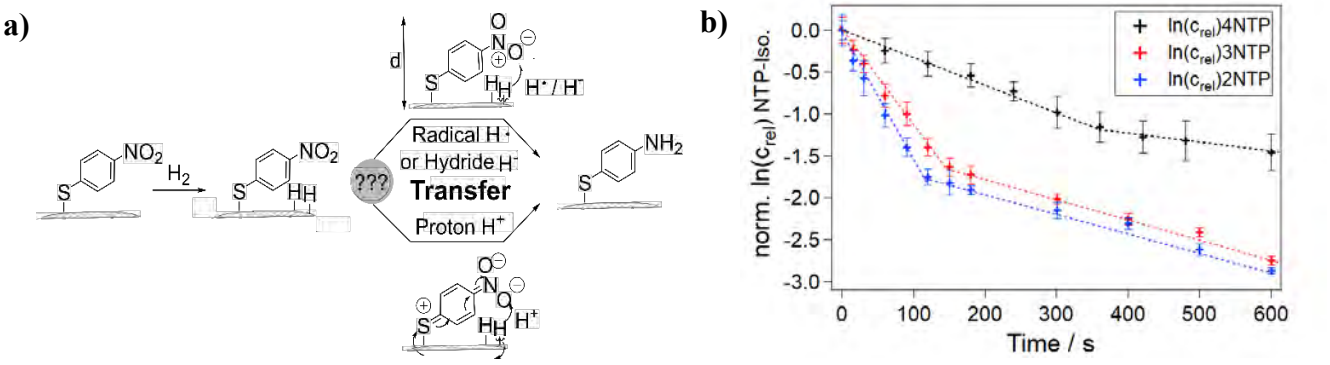

Figure 1. a) Reaction scheme showing the two possible pathways of the reduction of 4-NTP to 4-ATP on Pt@AuNR. **b)** SERS kinetic monitoring of the catalytic reduction of the NTP-isomers (4-NTP = black; 3-NTP = red; 2-NTP= blue).

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Dynamic DNA Origami/Gold Nanoparticle Hybrid Device for Distance-controlled Dimer Assembly

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Hybrid devices comprising DNA Origami and plasmonic nanostructures in various morphologies strongly benefit from the capability of DNA nanotechnology to provide precise positioning of molecular objects. [1] Hybrid materials, especially designed for nanoparticle dimerization, are used for sensing[2], spectroscopy [3] and biomedical applications[4]. The gap size in dimers plays a crucial role for the local electric field enhancement in the narrow gap (hot spot) upon resonant excitation[5]. Here, the design and assembly of a nanophotonic DNA hybrid device for gold nanosphere (AuNP) dimerization has been developed. A DNA Origami structure comprising a dynamic platform and boxes for AuNP incorporation was created (Fig. 1a). Incorporation of bare gold nanoparticles inside lidless 3D Origami boxes for the hybrid device was performed using a thiol-based technique. To obtain dynamic properties, toehold-mediated strand displacement is employed for highly precise adjustment (sub-nanometer range) of gap distances between the two halves of the dynamic DNA platform. Based on transformation from a flexible state to five different fixed states, defined gap distances between 0 and 15 nm are achieved, as evidenced by single-particle TEM and agarose gel electrophoresis (Fig. 1b). This approach enables AuNP dimerization with a free hot spot, allowing site-specific functionalization for highly sensitive detection of molecules. Consequently, this programmable hybrid device facilitates its use for spectroscopic applications such as surface-enhanced Raman scattering (SERS) and surface-enhanced fluorescence (SEF) by adjusting gap distances in the range of ca. 0-5 nm and 5-15 nm, respectively.

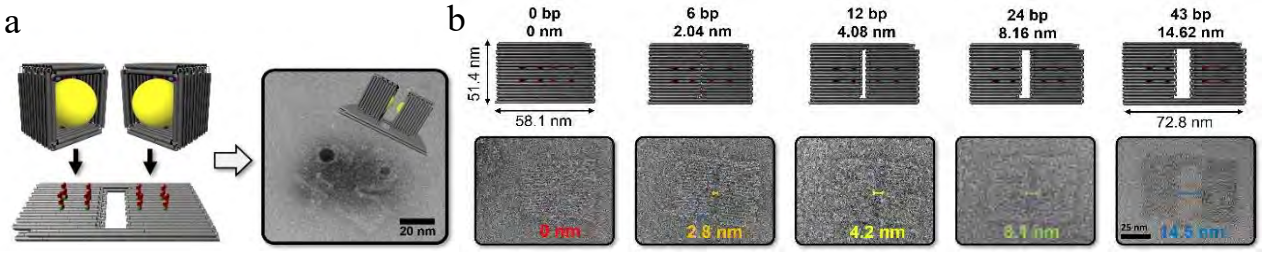

Figure 1: (a) Hybridization of DNA boxes with incorporated AuNP onto DNA platform resulting in desired hybrid device. (b) Dynamic DNA platform with varying calculated gap distances (top) and TEM images showing the measured distances (bottom).

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Raman spectroscopy: A tool for analyzing phase transitions in hypophosphite coordination polymers under high pressure

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The metal-organic frameworks (MOFs) with the perovskite-like $AM^{II}X_3$ architecture have received notable attention in recent years because of their unique features that make them appropriate for a variety of applications. They form a large group of metal (M^{II}) coordination polymers linked by small ligands (X) into a three-dimensional network with small molecular cations (A), mainly ammonium, embedded in the crystal voids. Compounds containing formate, dicyanamide, and halide ligands are the most common. Hypophosphite-based ($H_2PO_2^-$) perovskites stand out among these materials because they allow for uncommon structural properties such as unusual shifts of cations in the voids and tilts of the metal-ligand framework [1]. Temperature-dependent phase transitions are substantially less prevalent in hypophosphites than in formates, according to structural investigations, due to the reduced stiffness of the metal-formate framework. This property makes them ideal model materials for studying the stability and structure-related properties of materials under high-pressure conditions [2, 3].

Raman spectroscopy is well-known as the best technique for investigating lattice dynamics under the influence of external stimuli like temperature and pressure. It can be used in combination with X-ray diffraction studies to elucidate the mechanisms of phase transitions and to understand the essential factors that cause instability of perovskite-type structures.

Our recent reports on temperature and pressure Raman and X-ray diffraction studies for compounds containing formamidinium (FA^+) and methylhydrazinium (MHy^+) cations will be presented. We will show results that allow us to comprehensively explain the role of individual ions in the mechanism of the transition and how they might lead to technologically important features like second-harmonic generation (SHG) or ferro-, pyro-, and piezoelectricity, which could be used in the future [2, 3].

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Automated and Reproducible Synthesis of Gold Nanoparticles for SERS

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Nanoparticles are widely used due to their unique chemical and physical properties. A prominent example are gold nanoparticles that support a localized surface plasmon resonance (LSPR), which is exploited in surface-enhanced Raman scattering (SERS). The reproducibility of their synthesis is the most important aspect. [1] Surprisingly, the vast majority of all available syntheses are still performed manually nowadays. The idea of reproducible nanoparticle synthesis using an automated pipetting robot comes into play here.[2]

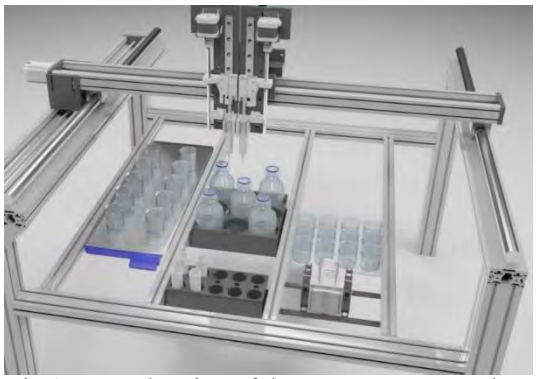

Fig 1: CAD drawing of the BONAPARTE robot

Here, we present our BONAPARTE (BOttom-up NAnoPARTiclE) synthesis robot (Fig. 1): it is designed to take over most of the synthesis steps that are usually performed manually. The advantage of this approach is that these tasks can be scheduled and executed with high reproducibility, with the benefits of reduced personal workload and elimination of human error sources, especially for complex protocols. Recently, we focused on the synthesis of highly SERS-active gold nanorods and gold nanostars with the BONAPARTE robot. Figure 2 shows UV/Vis absorption spectra of gold nanorods (ca. 25 nm x 10 nm) produced manually (left and middle) compared to those produced by the BONAPARTE robot (right). Our preliminary results demonstrate that the reproducibility of the automated synthesis is better than the manual synthesis performed by a master student and comparable to that of an experienced postdoc. Ideas for potential improvements will be discussed.

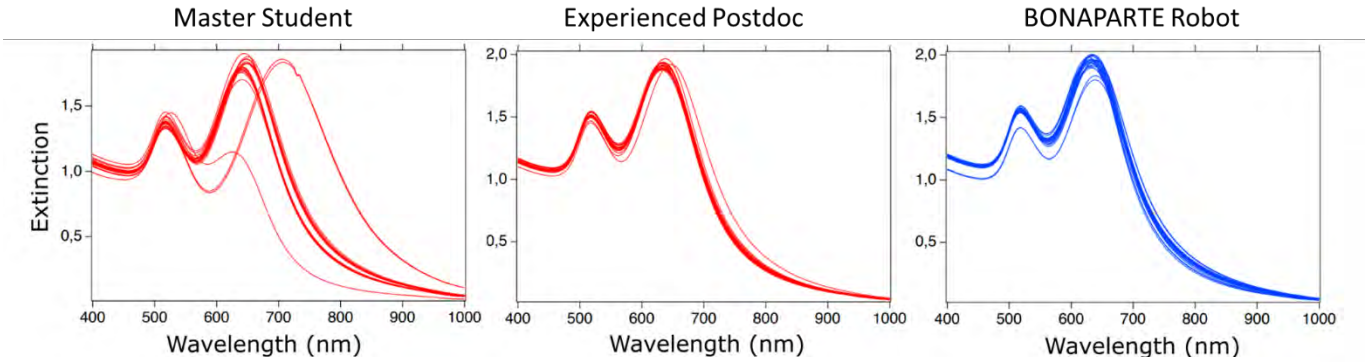

Figure 1: UV/Vis absorption spectra of gold nanorods (ca. 25 nm x 10 nm) produced manually (left and middle) and by the BONAPARTE robot (right). Each set contains 20 repeated syntheses.

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Mechanisms of the phase transition in imidazolium lead bromide perovskites studied using Raman spectroscopy

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The enormous interest of the scientific environment in hybrid organic-inorganic perovskites, and especially ammonium lead halides, can be explained by their great optical and electrical properties. Although 3D perovskites are well-known, attention is turned to lower dimensional perovskites, which still exhibit unique optoelectronic parameters but surpass their bulk counterparts [1]. The dimensionality of received materials strongly depends on the used ionic radii. Relatively larger organic cations like imidazolium (ImH^+) cannot be incorporated into the 3D structure, so lower dimensional compounds are created [2]. Such a framework can be distorted and tilted significantly, resulting in parameter tunability. Vibrational spectroscopy appears to be the most crucial in understanding the variety of factors yielding towards desirable materials.

Although X-ray diffraction and differential scanning calorimetry are necessary for general characterization of phase transitions, they do not provide crucial information about lattice dynamics. The structure-property relationship, such as the behavior of selected functional groups, local distortion, and ion ordering, can be studied using temperature-dependent Raman spectroscopy. Because of the high sensitivity of this method, even hydrogen bonds can be investigated [3]. Deep understanding of phase transition mechanisms seems to be beneficial in understanding the influence of octahedral distortion on optoelectrical parameters.

The goal of this work is to provide crystal structures and propose an assignment of the observed IR and Raman bands for the newly discovered $[ImH]_2PbBr_4$ and $[ImH]_3PbBr_5$, as well as previously reported $[ImH]PbBr_3$ [4]. To elucidate the molecular mechanisms of observed phase transitions in these compounds, we used factor group approach and temperature-dependent analysis.

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Excited State Dynamics of Arylazopyrazole Photoswitches

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Arylazopyrazoles (APP) are a class of molecular photoswitches with exceptional two-way photoisomerization yields (up to >98%) along with good thermal stability [1]. They share the azo-moiety with the famous azobenzene; the difference of AAP compared to azobenzene is that one of the two phenyl rings is replaced by dimethylpyrazole (Fig.1 top). Switching from the E- to the Z-isomer and back is achieved by excitation with UV and green light, respectively.

Using time-resolved nonlinear Raman spectroscopy, we want to characterize the structural dynamics of AAP. The ground state CARS spectrum of AAP, obtained using three-color CARS (Fig.1 bottom-left), shows a characteristic reduction in Raman intensity of dominant vibrational modes for the Z-isomer. A preliminary transient absorption study (Fig.1 bottom-right) of AAP using a UV pump to find the excited state absorption (ESA) maximum shows increased ESA from 640 nm to 660 nm. These preliminary measurements also display a picosecond relaxation which is comparable to the dynamics of azobenzene [3].

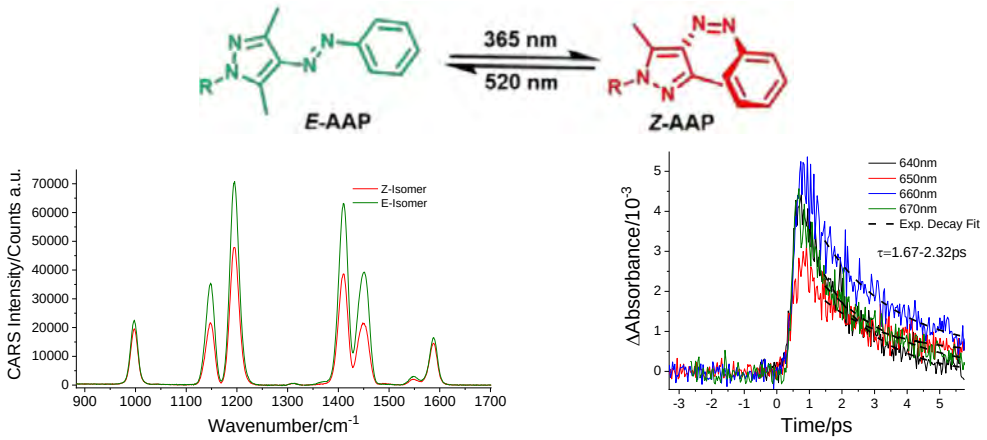

Figure 1: Top: Basic structure of arylazopyrazole E and Z-isomers [2]. Bottom left: Ground state CARS spectra for Z and E isomers of AAP1 in water. Bottom right: Transient absorption decay curves of AAP1 E-Isomer with varying probe wavelength after excitation by 365nm pump beam.

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Drop Coating Deposition Raman Spectroscopy of Liposomes on Substrates with Different Roughness

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Drop coating deposition Raman (DCDR) method - as a special method of Raman spectroscopy - is based on evaporation of solvent from a small droplet of studied solution or suspension. Drying of a droplet with a pinned contact line on a hydrophobic substrate typically leads to the accumulation of dissolved material in the ring-shaped pattern [1]. By using a confocal Raman microspectrometer, the acquisition of Raman spectra of good quality from the pre-concentrated analyte ring is possible. Because of this, the DCDR technique enables to measure samples at very low initial concentrations and small volumes with high sensitivity in comparison with conventional Raman measurements. It was shown that it is a very useful tool to detection of different biologically important molecules, e.g. lipids, as major components of biological membranes [2]. The shape of dried pattern, which is formed after the complete evaporation of the volative phase of the solution is influenced not only by the properties of the solution but it depends also on the substrate characteristics such as wettability and roughness that governs the droplet drying dynamics.

We focused our attention on liposome suspension dried on nanostructured hydrophobic substrates with different roughness. These substrates are prepared by a novel method that is based on the deposition of arrays of nanoparticles (Cu or Ag) fusing a gas aggregation source and subsequent overcoating of such prepared substrates by sputter-deposited thin C:F film. The nanoroughness and with it connected wettability of produced coatings is controlled by the number of nanoparticles in the base layer including an option of a gradient surface coverage. It was shown that in the case of liposome suspension the nanoroughness leads to stronger preconcentration as well as to the reduction of the ring diameter, i.e. two parameters crucial for the DCDR measurements.

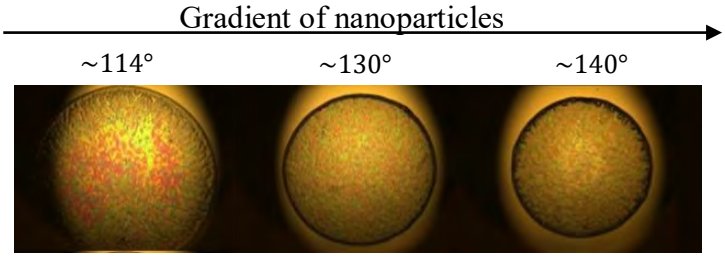

Figure 1: Ring-shaped dried patterns of liposome suspension at the initial concentration of 0.5 mg/ml on the substrate with gradient hydrophobicity characterized by different contact angles

Acknowledgements: Support by grant 18-10897S from the Czech Science Foundation and grant 290120 of Grant Agency of Charles University.

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Novel insights into the oxidation behaviour of Nitride Bonded Silicon Carbide (NBSC) by in-situ Raman spectroscopy

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Nitride bonded silicon carbide refractory materials are an important material group especially for the lining in waste incineration plants, because they are highly resistant against alkalines, Cl and SO_x in vaporous state. Furthermore, their high thermal conductivity makes these materials ideally as a protective barrier between the combustion chamber and the metallic pipes of the recuperator system. There, they are exposed to oxidation by O_2 and H_2O. The oxidation of NBSCs by these gases causes a small passivation layer of SiO_2 on the surface of the shapes [1]. In addition, a small layer of silicon oxynitride (Si_2N_2O) is formed between silicon nitride (Si_3N_4) and the SiO_2 layer (Fig. 1) [1, 2]. If a passivation layer of SiO_2 forms throughout the whole sample surface, the barrier can protect the shape from further oxidation because SiO_2 has a low oxygen permeability [1]. However, a badly adjusted porosity during production can lead to oxidation within the shape. This can ultimately cause failure due to a volume increase. Another factor known to influence the oxidation behaviour are the different morphologies of alpha-Si_3N_4 and beta-Si_3N_4 [3].

Purpose of this study is to investigate the influence of different factors like porosity and the phase ratio of alpha- and beta-Si_3N_4 on the oxidation of NBSCs. In-situ Raman spectroscopy at high temperatures can give new insights that cannot be obtained by ex-situ experiments. The combination of Raman spectroscopy and in-situ high temperature measurements was used by [4] and [5] and gave new insights on the CaO-Al_2O_3-SiO_2 system.

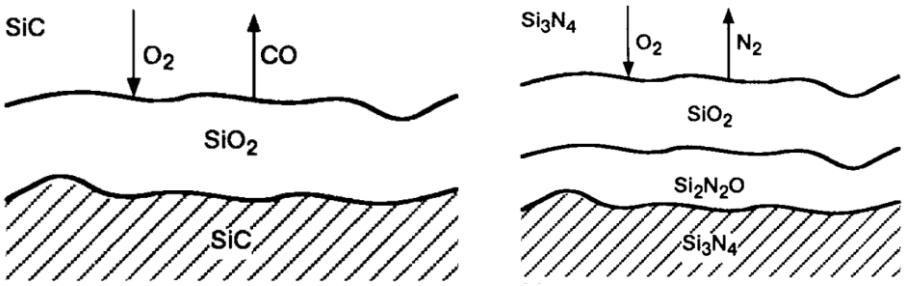

Figure 1: Schematic depiction of the formation of protective SiO_2 and Si_2N_2O layers on SiC and Si_3N_4 [1]

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New Insight of Charge Transfer Enhancement: Carrier Density Effect

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Semiconductor can change the density of a carrier in the range of 10^{15}-10^{21} cm^{-3} by means of chemical doping, electronic doping or optical excitation, which opens a new way for adjusting the band gap distribution and charge transfer (CT). The robust control of carrier density distribution is very important for the use of widely studied optical properties, and it can be used as an optical probe medium to complete the observation of CT [1-2]. In this work, we discussed the influence of controllable carrier density on the band gap distribution and CT in the metal semiconductor doping system based on different proportion. Ag and semiconductors with different doping content were composited into a new material by cosputtering technology. The interface CT process of the composite system is very sensitive to the energy level position of the contact interface. By adjusting the energy level, the control of the CT process in the system can be realized. We used Hall effect to characterize the carrier density, and proposed to calculate the band gap position of different doped composite materials by using the ultraviolet electronic energy spectrum (UPS) technology. The basic discussion of the relationship between carrier density, band gap distribution and CT was confirmed by surface-enhanced Raman scattering (SERS) spectrum.

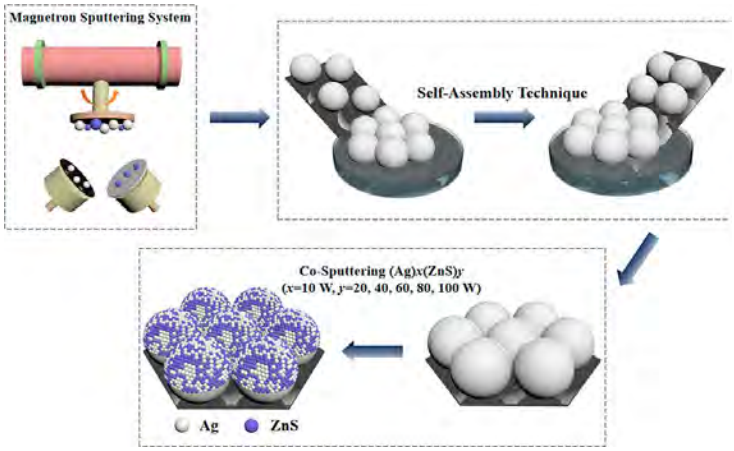

Figure 1: The schematic of the cosputtering of the Ag/ZnS.

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Raman and Surface Enhanced Raman Scattering (SERS) for the Detection of Trace Components in Drug Mixtures

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Portable instruments have potential to be used as part of an overdose prevention measure in drug-checking as a response to the increasing number of fentanyl-related deaths. Our project uses infrared absorption spectroscopy, gas chromatography-mass spectrometry (GC-MS), Raman scattering, surface enhanced Raman scattering (SERS), and immunoassay-based testing to run a drug-checking service in downtown Victoria, British Columbia. By collecting orthogonal instrument data of street drug samples, we build a large spectral dataset fitting for robust pattern recognition algorithms [1].

SERS has recently been in the spotlight due to its ability for trace detection and prospect in detecting potentially harmful ingredients (e.g. carfentanil, benzodiazepines) present at a concentration too low to be detected by other popular portable technologies (e.g. FT-IR) [2, 3]. SERS is attractive for point-of-care applications due to its simple instrumentation and sample preparation, when compared to the current gold-standard of GC-MS. However, without chromatographic separation the SERS signature of trace analytes can easily be masked by overwhelming signals from cutting agents and other components in a drug mixture. With these challenges in mind, the aim of this study is to explore unique approaches to SERS sampling and spectral analysis. This work uses a suite of supervised and unsupervised chemometric methods to push the limits of trace drug detection, differentiation, and classification in real-world mixtures using SERS.

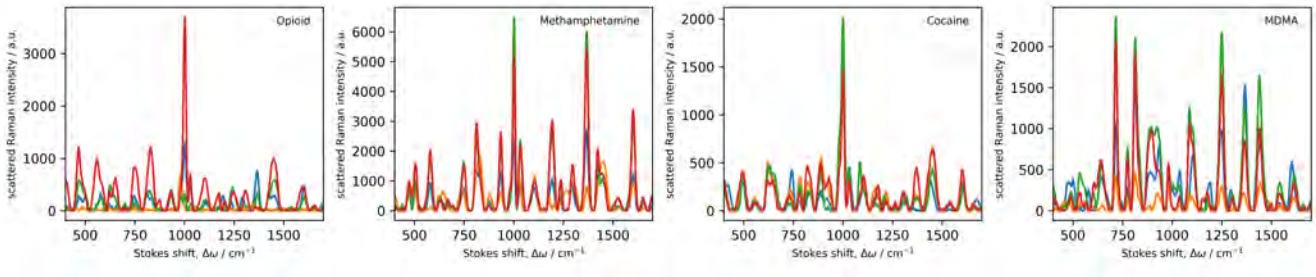

Figure 1: SERS spectra of real drug samples within various drug classes.

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In situ study of the mineral reactions during hydration in calcium aluminate cements (CAC)

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Calcium aluminate cements (CAC) are used for refractory applications because of their chemical resistance as well as their early strength development [1]. It is mainly applied as a binder for refractory monolithics, as linings in furnaces, for melting glass or metal. From precursor material to the final product, three processes are of great importance: hydration (setting and curing), dehydration, and high-temperature behaviour.

A highly-detailed understanding of the aforementioned underlying processes is necessary to improve the physical and chemical properties of the final product. Therefore, *in situ* Raman imaging, a powerful tool to investigate the chemical and microtextural development of refractory materials, was applied. A special closed cell, developed at the University of Bonn, was used in which the mineral reactions during hydration can be measured by Raman imaging through a sapphire window. During first experiments the different stages of hydration could be observed, including the formation of two calcium aluminate hydrates. In further experiments the dehydration and high-temperature behaviour will be investigated by *in situ* Raman imaging at temperatures up to 1200 °C. This method was described by [2] and [3] for ceramics in the CaO-Al_2O_3-SiO_2 system.

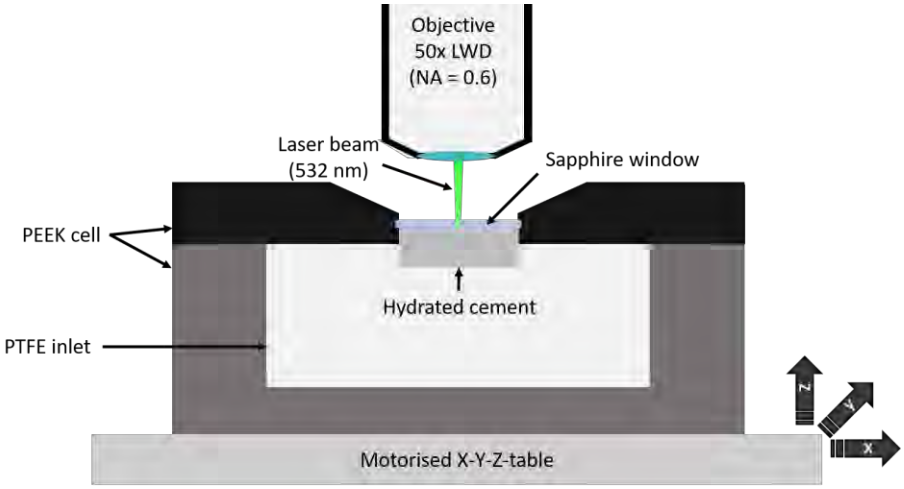

Figure 1: Schematic drawing of the cell used for the hydration experiments

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In situ or colloidal prepared metal nanoparticles on cyanobacteria for improved reproducible surface-enhanced Raman spectroscopy

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Surface-enhanced Raman spectroscopy with in situ or colloidal produced metallic nanoparticles is a powerful tool for fast and reliable selection of cyanobacterial strains, which produce desired product molecules, such as PHB or glycogen. [1, 2]

To apply SERS properly the distribution of the analysed molecule on a metallic surface is decisive. It is possible to reduce pure salts of precious metals, such as gold and silver, in order to obtain colloidal metallic nanoparticles. [3] Some microorganisms like cyanobacteria or ***E. coli*** possess a negatively charged surface, which makes it possible to coat them with nanoparticles in situ and to investigate their status by Raman spectroscopy regarding the storage of specific molecules of interest. It is essential to distribute the metallic Raman signal enhancer regularly on the subject analysed.

The aim is to improve the reproducibility and the quality of the gathered enhanced Raman scattering spectra, and to shorten the analysis time. Both, the Au-colloids (> 40 nm) and the in situ designed Ag-colloids (>100 nm) synthesized directly on the cell surface of the MOs, were evaluated. Using the Bruker Senterra instrument with 538 and 785 nm laser, the in situ Ag- colloids (Figure 1a) enabled an increased reproducibility of the data (98%) compared to classical 40 or 80-100 nm Ag-colloids (58%), and the measurement time was reduced by a factor of 12. The Au-colloids (Figure 1b) mixed with cyanobacteria delivered reproducible measurements up to 82%. Here the reproducibility is defined as the percentage of measurements that delivered reasonable spectra in the wavenumber range of interest with signal to noise ratio of above 3.

The acquired Raman data were pre-processed (detrend in prospectr, RStudio; 1280-920 1/cm) and assessed by principal component analysis (PCA). It was possible to distinguish between cyanobacterial strains producing different levels of desired molecule on a cellular level (Figure 2), revealing heterogeneities within sample groups as well.

The tested SERS enhancers were applicable for specified microorganisms and according to the analyte of interest. For PHB producing cyanobacteria the in situ Ag-colloids performed the best. Gold-colloids, applied in solution, may be more useful in investigations of other cells.

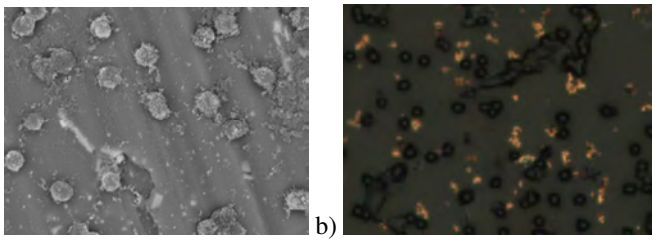

Figure 1: a) in situ Ag-***Synechocystis***, SEM, 600x
b) Au-colloid mixed with ***Synechocystis***, 500x

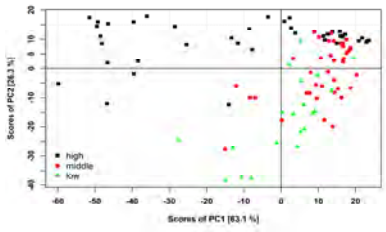

Figure 2: Amount of accumulated product in cells, PCA

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Novel strategy for hot spots generation using tyramine-mediated crosslinking chemistry

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Surface-enhanced Raman scattering (SERS) has been actively studied as a highly sensitive and selective analytical technique with high photostability and multiplexing capacity. SERS offers molecular information based on vibrations, benefiting from the plasmonic enhancement of metal surfaces. The enhancement of SERS resulting from the localized surface plasmon resonance strongly depends on the distance between the molecule and the metal surface. For signal reproducibility, it is important to consider a strategy for controlling hot spots and trapping the analytes in the hot spots.

In this study, we study a novel strategy for generating hot spots using tyramine-mediated crosslinking chemistry. Tyramine molecules, which are known to initiate radical reactions resulting in crosslinking networks in the presence of enzymes and hydrogen peroxide, induced a controlled aggregation of the Ag nanoparticles (NPs), and the analytes were physically captured inside these dynamic hot spots. The SERS signal obtained using this strategy was 3.5 times higher than that obtained using the traditional SERS method using colloidal Ag NPs. Using this method, different kinds of pesticides that have different binding affinities toward metal surfaces were successfully detected. Furthermore, this proposed sensor was evaluated as a promising tool to achieve a solution-based sensitive detection of universal pesticides. [1]

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Animal Feedstuff Inspection using Shifted Excitation Raman Difference Spectroscopy

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In animal feeding, high-quality pellets with homogenous distribution of ingredients should be used to achieve best performance and health in farm animals. Nutritive values and physical characteristics of pellets are strongly dependent on the properties of raw materials involved in the production process. Raw materials and pellets should thus be monitored by comprehensive quality control measures. Incoming and outgoing goods control using rapid analytical methods could be very beneficial, e.g., in the case of raw materials and feed additives at processing plants but also for final pellet assessment before further distribution. Optical measurement techniques are very promising in this instance due to their ability for fast, simple, and non-destructive analysis. Our study addresses this important point by using Raman spectroscopy for the inspection of intact feed pellets and their constituents thus opening up new avenues for feed characterization.

Shifted excitation Raman difference spectroscopy (SERDS) is applied as a well-demonstrated and powerful technique to effectively separate Raman signals from background interference, e.g. fluorescence, which is a common issue for a wide range of natural samples. An in-house developed Y-branch dual-wavelength diode laser emitting at 785 nm serves as excitation light source with two distinct emission lines (spectral distance: 0.6 nm) for SERDS [1].

Results demonstrate that SERDS permits for efficient extraction of Raman signals from background interferences enabling a qualitative inspection of chicken feed pellets. This opens up the possibility to perform spatially resolved analyses of the heterogeneously distributed constituents of feed pellets. Based on their characteristic spectral signature, individual ingredients present at concentrations down to 10 g/kg, e.g. soybean meal, soybean oil, calcium carbonate as well as starch and ferulic acid from wheat and maize, were successfully detected within intact pellets using SERDS. These results highlight the large potential of SERDS as a promising tool for animal feedstuff analysis and quality assessment [2]. The findings of our investigations provide a much-needed basis for future applications of SERDS for *in-situ* inspection of raw materials and pellets at selected points along the process chain.

This study was funded by the Leibniz Science Campus Phosphorus Research Rostock through the project “Shifted excitation Raman difference spectroscopy testing for analysis of inorganic phosphorus, inositol phosphates and myo-inositol in environmental and animal samples (SERAIP)” and it was also partly funded by the Federal Ministry of Education and Research (BMBF) under contract 16FMD02 (Research Fab Microelectronics Germany - FMD).

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Monocrystalline Gold Platelets as a Platform for Reproducible High-Performance SERS Substrates

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Metallic nanostructures play an important role in many different fields, e.g. biosensing or surface-enhanced Raman spectroscopy (SERS). Fabrication of these structures is either achieved through a bottom-up chemical synthesis of crystalline particles or via top-down nano-lithography methods performed on polycrystalline films. While chemically grown particles have a very limited range of geometries, which are rarely optimized for a desired task, the nano-lithography approach suffers from the disadvantages of polycrystalline films, e.g. graininess of the material.

We propose a combination of both approaches by utilizing chemically synthesized monocrystalline gold-platelets (see Fig. 1a) [1] with top-down structuring methods. The uniformity of the platelets not only allows homogeneous structuring, but also improves the optical enhancement of the resulting structures [2]. Combination with structuring through focused ion-beam milling results in structures with well-defined features on the nanoscale (see Fig. 1b). The deterministic control of the exact structure's geometry allows the optimization of near-field enhancement at well defined locations as well as to different excitation- and detection schemes in the far-field.

The final structures may find applications in many different fields where metallic nanostructures are imperative, as for instance biosensing, e.g. surface plasmon resonance imaging, SERS or tip enhanced Raman spectroscopy.

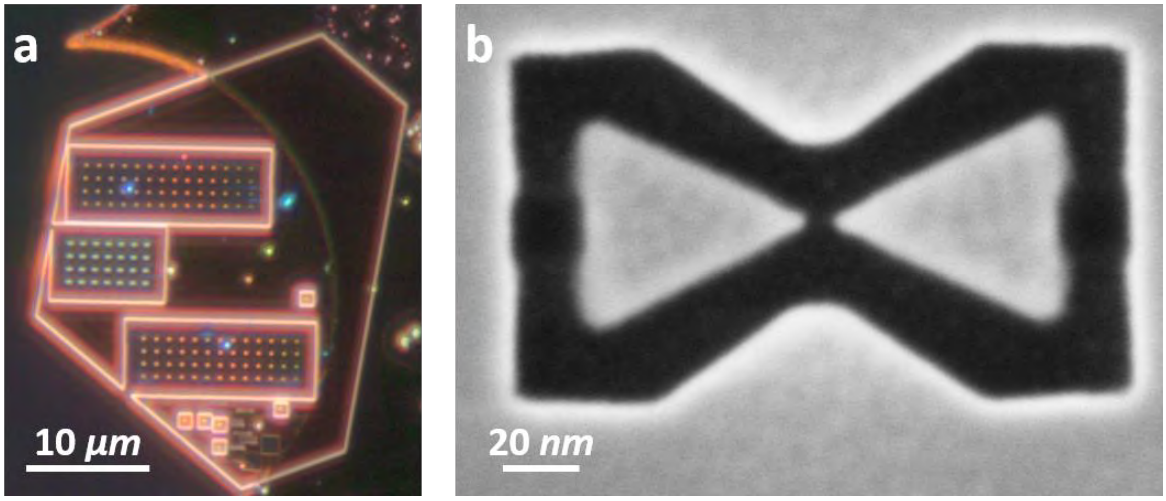

Figure 1: a) Dark-field optical microscope image of a monocrystalline gold platelet with arrays of multiple optical antennas with shifting resonances **b**) Scanning electron microscope image of a high precision nanostructure fabricated through ion-beam milling.

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SERS-active Fe_3O_4@TiO_2-Au nanocomposites as A Reusable Photocatalyst

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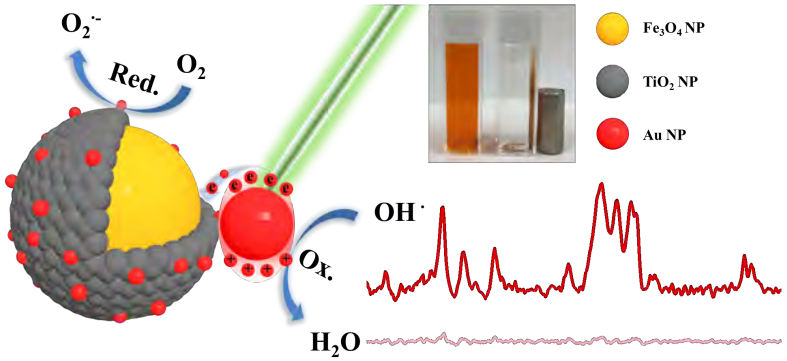
The photocatalytic reaction shows various mechanisms depending on the target material. Good photocatalysts have excellent durability because they are chemically and biologically inert and react with only light. Au nanoparticles are an excellent photocatalyst, but their easy aggregation between nanoparticles decrease catalytic efficiency rapidly.

In this study, we fabricated Fe_3O_4@TiO_2-Au nanocomposites (FTN-Au) to increase surface-enhanced Raman scattering (SERS) activity with good photocatalytic efficiency, reuse, and stability. To confirm the fabrication of FTN-Au, the size of Fe_3O_4 nanoparticles, the thickness of the TiO_2 shell, and the amount of Au nanoparticles were measured using TEM. Their stability and catalytic ability were determined by methyl orange (MO), one of the organic dyes, absorbance spectrum changes during reduction.

The difference in photocatalytic efficiency, stability, and SERS enhancement, which depends on the amount of Au nanoparticles adsorbed on the surface of Fe_3O_4@TiO_2 nanoparticles was related on the aggregation degree of Au nanoparticles. In XPS spectra, binding energies of Au and TiO_2 were decreased, which means that the electron mobility of the semiconductor was improved. Therefore, it can be used as a photocatalyst at a longer wavelength than the conventional TiO_2 photocatalyst. Both charge transfer between the Au nanoparticles and the TiO_2 shell and strong absorption of surface plasmon resonance in the visible region by the Au nanoparticles can be expected to improve solar energy application efficiency by increasing the visible light absorption of semiconductor photocatalyst materials. Details on its characterization and analysis will be discussed in this presentation.



Reference

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Limitations in the detection of Cancerous Human Colorectal Tissues by micro-Raman Spectroscopy

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Colorectal cancer is the third most common and one of the deadliest cancers worldwide and demands non-invasive early diagnosis and eradication in the curable stage. One of the most promising techniques to achieve this goal is Raman spectroscopy [1,2]. Most reports in this area have been concentrated on the clinical use of Raman in order to discriminate cancerous from precancerous polyps and healthy tissues where Raman probes adapted in portable systems are used either ex-vivo or employed in endoscopes in order to perform in vivo analysis. All these studies aim on the quick identification of cancer by acquiring a large number of spectra from many patients defining a correlation of the subtle Raman spectroscopic differences with the cancerous state via machine learning algorithms. However, the lateral areas examined are very large in the order of hundreds of μm^2, thus the spectroscopic identification of different parts and constituents of the tissue is averaged. Furthermore, the low spectroscopic resolution of the portable instruments and the parasitic signals and limitations of the Raman probes hide the spectroscopic information at the molecular level and the correlation of the Raman bands with the biological characteristics of the malignant tissues.

In this work, 10x5 mm human colorectal specimens were collected from 15 patients who underwent open surgery. The samples were preserved in a based fixative (Z7) [3] and were examined after 1-5 days. Micro-Raman spectra were recorded from random 2 μm^2 spots by stabilizing the tissue at 2 °C in order to minimize dehydration effects and consequent focus drift during measurement. Excitation was performed at 785 nm in order to avoid high level of autofluorescence. An extended vibrational spectral range of 500-3200 cm^{-1} was examined with a high resolution of 1.8 cm^{-1}. Distinct spectral fingerprints of the tissues body, lipids and blood vessels are recorded from different spots, which require careful treatment in the analysis and evaluation of multiple data. The average spectra were used for least square fitting analysis while principal component analysis PCA identified several Raman bands of amino acids, proteins and lipids with high PCA weights which allow the efficient discrimination of cancer tissues.

Acknowledgment: This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T1EDK-01223).

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Characterization of PHBHx-based SPEs for Li polymer battery

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As the use of batteries increases due to the development of electric vehicles and smartphones, concerns about environmental pollution caused by waste batteries are increasing. To solve this problem, it is necessary to develop an environmentally friendly and renewable battery. Recently, studies on solid polymer electrolytes (SPEs) using biodegradable polymer have been actively conducted in Li polymer battery fields.[1]

In this study, poly(hydroxybutyrate-*co*-hydroxyhexanoate) (PHBHx), which is one of well-kwon biodegradable polymer, was applied as SPEs to Li polymer battery for the first time. Ion conductivity and mechanical properties of PHBHx-based SPEs were investigated to verify the performance of SPEs. To characterize SPEs, IR and Raman spectra were also measured. Based on analysis of Raman spectra, the degree of binding of electrolyte ions, which greatly affects the movement speed of lithium cations, were investigated. In addition, the analysis of IR spectra provide information of chemical moiety of PHBHx-based SPEs. Details of these results will be discussed in this presentation.

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Application of Raman spectroscopy in studies on mechanisms of phase transitions in lead halide hybrid perovskitoids templated by hydrazinium derivatives

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[c]*Institute of Physics, Wrocław University of Science and Technology, Wrocław, Poland*

Hybrid organic-inorganic perovskites (HOIPs) are well-known for their unusual physicochemical properties, which make them potentially useful in a wide range of optoelectronic applications [1]. Depending on the mutual dependence of the ionic radii of the structural components, HOIPs are predestined to adopt diverse structures with varying dimensionalities. Lead halides, for example, can form a three-dimensional (3D) perovskite lattice when templated by relatively small organic cations such as methylammonium, formamidinium or methylhydrazinium [2]. Using protonated bulky amines, on the other hand, favors the formation of two- (2D) or one-dimensional (1D) structures [3]. In recent years, the attention of researchers has been drawn to 1D hybrids, known as perovskitoids, because of their significantly different properties from archetypical 3D perovskites, such as greater intrinsic stability, which allows them to be used to expand the application potential of perovskite solar cells [3-5].

Perovskitoids, like perovskites, can undergo structural phase transitions driven by the ordering of ammonium ions or the tilting of metal-halide octahedra. The family of multimetylated and alkyl derivatives of hydrazinium cation was chosen to investigate the influence of ammonium ions changing steric hindrance on the mechanisms of structural phase transitions in lead halide perovskitoids.

Temperature-dependent Raman spectroscopy, in combination with infrared spectroscopy, differential scanning calorimetry, and X-ray diffraction methods, allowed us to investigate in detail differences between phases and the behavior of selected functional groups in the compound during phase transition, including changes in ammonium ion ordering, bond lengths, angles between them, and influence on hydrogen bonds.

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Potential of Raman spectroscopy in on line analysis of photocatalytic glyphosate degradation using Rh-P25

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Abstract

To fulfill the global demand for food the use of pesticides and herbicides is necessary. Unfortunately, these chemicals end up to wastewater and the conventional treatment technologies are not effective enough to remove them. These pollutants called the attention of scientist because of their potential harmful effects for life [1]. Glyphosate, one of the emerging pollutants, is a herbicide that is commonly used in soy crops. It has an organophosphate group that is toxic. [2] One promising option for the treatment of emerging pollutants is photocatalysts. Photocatalysts can convert light into chemical energy creating active species that can be further used in the degradation of emerging contaminants [1]. Generally, the photocatalysts are active in the UV range of light, but remarkable efforts are made in material development to induce visible light activity in the catalysts. [3] However, deeper understanding on degradation mechanisms as well as material characteristics are required. Time-gated Raman spectroscopy offers a novel option for this purpose.

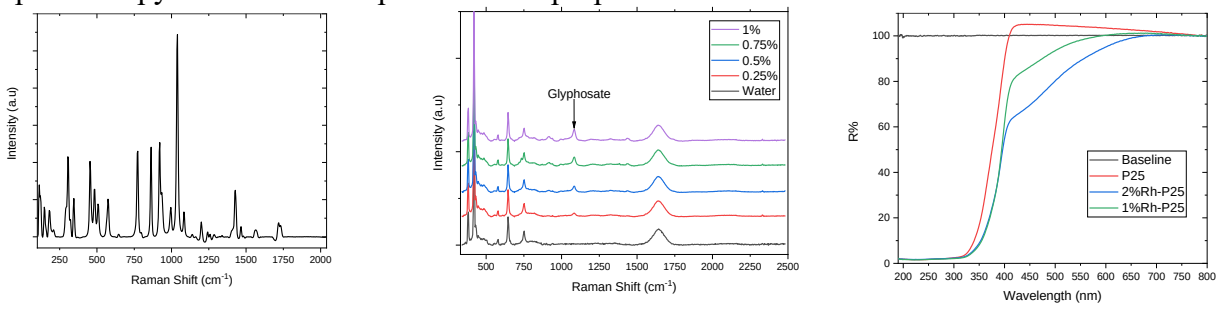

Figure 1: (left) solid glyphosate spectrum, (center) Raman spectra of dissolved glyphosate, (right) UV-vis reflectance of the photocatalyst.

Detection of glyphosate in water cannot be realized directly with conventional methods, such as UV-vis spectroscopy. However, it has rather strong Raman signal (Figure 1, left). The dissolved glyphosate can be detected from water solution at a level of 0.25 w-% using TG-Raman. Furthermore, TG-Raman is very sensitive for TiO_2, which opens possibilities for on-line characterization of photocatalytic water purification. In this work we plan to present the potential of TG-Raman as an on-line measurement for the glyphosate photocatalytic degradation along with the potential of Rh-doped TiO_2 catalysts

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Development of a new Raman measurement system for in situ measurements during high temperature microwave synthesis of inorganic materials

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Non-conventional microwave heating has become an increasingly popular technique for the preparation of inorganic materials. Compared to conventional (furnace) heating methods, extreme heating rates and selective heating of reactants can be achieved in microwave ovens unlocking novel syntheses and microstructural control. However, significant difficulties are still encountered in the interpretation of these methods which is largely due to the lack of widely available in situ monitoring techniques that can accurately track reaction progression and temperature. Raman spectroscopy is uniquely suited to this task allowing unambiguous phase identification and simultaneous temperature measurement without physical contact with the material. Unfortunately, currently available Raman probes cannot be used inside high temperature microwave reactors due to the use of metallic components and typically very short working distances. Therefore, we have developed a new microwave-compatible Raman probe and measurement system designed to operate in laboratory microwave reactors (in this work, a 2.45 GHz multimode reactor supplying 100-1800W power levels). The new Raman probe is completely composed of microwave transparent materials. It is long enough to enter the microwave reactor through an access port, keeping all of the optics (except the objective) outside of the chamber. Additionally, it accepts most 1in./25.4mm unmounted objective lenses allowing free choice of working distance based on the intended application. As a proof-of-concept for our in-house built system, in situ Raman spectroscopic observation of the phase change in TiO_2 (anatase to rutile) at high temperatures under microwave heating was analyzed using the new probe which allowed detailed tracking of the phase transition with excellent time and temperature resolution. These results demonstrate the great potential of this new probe/measurement system and its expected impact on experimental synthetic chemistry by shedding light on intermediates and formation mechanisms that might be unique to microwave heating.

Optimising and understanding the spectroscopic signatures associated with planetary surface processes

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The most recent Mars rovers developed by ESA and NASA carry infrared and Raman spectrometers, in order to exploit molecular composition data obtained from the surface materials. Due to a broad range of recent advances in instrument miniaturisation and robustness, Raman spectrometers are frequently included in payload proposals for planetary exploration missions. An example of this includes the ESA ExoMars mission, which incorporates the Raman Laser Spectrometer (RLS) [1], an analytical instrument which main aims include identifying organic compounds as well as searching for signs of extinct or extant life in materials found on the surface or near sub-surface of Oxia Planum. Another example is the NASA Mars2020 mission, which also includes two instruments that can operate in Raman spectroscopy modes (i.e. SuperCam and SHERLOC [2,3]).

Following the instrument development of the RLS instrument and NASA's Mars2020 Raman spectrometers, Raman spectrometers are being seriously considered for NASA's potential Europa Lander [4] and lunar missions. These spectrometers are ideal instruments for identifying molecular signatures associated with planetary surface and sub-surface processes. However, the radiation environment experienced during transit to or on the surface of these locations (i.e. high energy particle irradiances) can affect the performance of both detectors and electronic components, significantly reducing the overall instrument performance, science capability and reliability. Consequently, it is important to fully model all of the physical processes involved in such extreme environments, in order to comprehend and account for the impact that they will have on the overall scientific performance of the spectrometer. Following the development of these models, analysis of data obtained during preparations for the ExoMars2022 (and Mars2020) is critical in verifying such models.

Here, we present an overview of the models developed for the various instrument concepts and compare the prediction to data obtained in the laboratory (i.e. from the prototype systems developed for the ExoMars 2022 mission, and the prototype systems developed for the Europa lander). The results are described in the context of the primary science goals of instruments developed for lunar, Mars and Europa exploration (i.e. including Mars Sample Return and icy moons missions). The work presented includes: assessing and prioritising the key science goals and requirements for future missions (e.g. identification of biosignatures, habitability, hydration conditions, and geological context), instrument performance modelling, environment modelling (including orbital analysis and radiation effects), and data interpretation and algorithms/systems (specifically focussing on real time, autonomous systems for analysing spectral data acquired by the instrument, and optimised operation during surface operations).

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Transfer of chirality from chiral capped silver nanoparticles to achiral adsorbate evidenced by surface-enhanced resonance Raman optical activity

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Raman spectroscopy (RS) is blind to chirality. Although Raman optical activity (ROA) spectroscopy is extensively used to study chiral molecules, its intensity is extremely low compared to the Raman signal. On the other hand, surface-enhanced Raman scattering (SERS) is a well-established technique to obtain enhanced Raman signal. Combining the SERS technique with ROA appears to be a promising approach to investigate molecular chirality at low concentration, via observation of surface enhanced ROA (SEROA) spectra. This technique, however, was so far restricted by the lack of reproducible experimental procedures.

We report that at resonance condition, chirality is transferred from chiral capped silver nanoparticles (AgNPs) surface to an achiral analyte (~10^{-4} M) adsorbed on the metal surface. At near resonance condition, mono-signate ROA spectral feature is recognized as a hallmark of resonance ROA (RROA). Using colloidal silver nanoparticles (AgNPs) as SERS substrate, we observe mono-signate SERROA spectra of the achiral analyte (2-mercaptopyridine i.e., 2-MPY) as mirror-image for the two enantiomers of the chiral capping agent (D- and L- tartaric acid) used to cap the AgNPs. The detected SERROA signal is quite strong, having circular intensity difference (CID) up to 10^{-3} and the spectra are reproduced on several occasions. The intrinsic chirality present in the AgNPs capped with chiral capping agent is observed in their mirror image electronic circular dichroism (ECD) spectra.

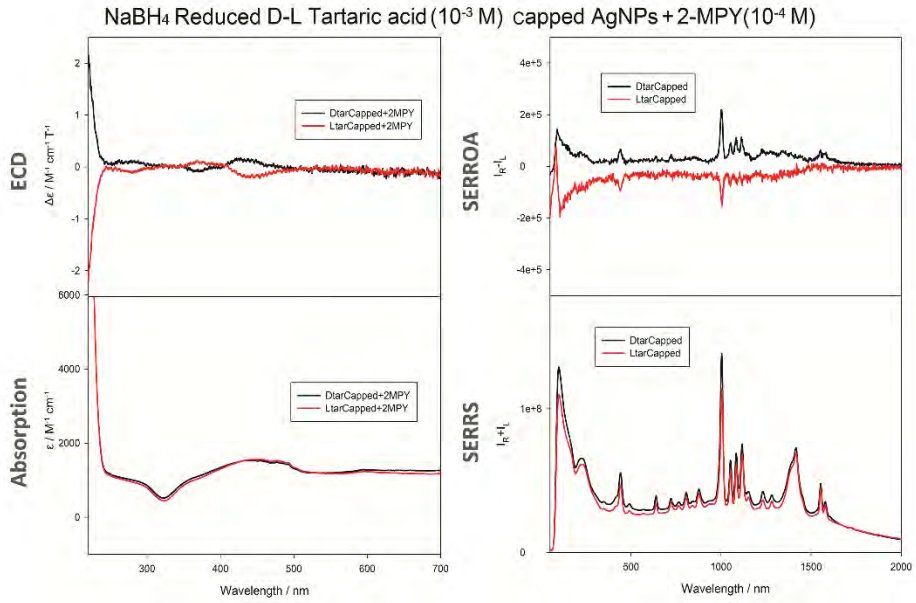

Figure 1: Transfer of chirality from the chiral capped (by L- and D- tartaric acid) AgNPs to achiral analyte (2-MPY) evidenced by SERROA and ECD spectra.

Such observation of strong mono-signate SERROA spectra from achiral molecules may be useful in detection of chirality of nanoscale entities, which cannot be observed by other spectroscopic techniques having low sensitivity. Chiral amplification via asymmetry induction, resonance, and surface effects promises to be a potential method for biomolecular detection and analysis in the future.

Line-Scanning VSFG Hyperspectral Microscopy for Imaging Self-Assembled and Biomimetic Materials

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Nonlinear spectroscopy has been implemented in various research fields to study interface/surface, system dynamics, molecular structures *etc*. The two-fold progress: 1. A clear and fundamental understanding of nonlinear spectroscopy; 2. Advancements of instrumentation design, has enabled its novel applications and led to many scientific breakthroughs.

Here we first present a new and tutorial approach based on Neumann's principle and eigensystem to efficiently derive non-vanishing tensor elements for 2^{nd}-5^{th} and higher order nonlinear optical susceptibilities, which would benefit the design of polarization-resolved measurement as well as spectra interpretation to gain insights on structure-property correlations. [Ref 1]

Secondly, we demonstrate our advancement on coupling polarization resolved collinear vibrational sum frequency generation spectroscopy (VSFG), an interface and symmetry selective second order nonlinear optical technique, with a line scanning microscopy platform to create a line scanning VSFG hyperspectral microscope which characterizes different chemical environment with spatial fidelity. We applied the VSFG microscopy to study self-assembled materials such as cell-derived and lyophilized collagen as well as biomimetic materials comprised of β-cyclodextrin/sodium dodecyl sulfate complex (2β-CD@SDS) and L-phenylalanyl-L-phenylalanine (FF). Molecular self-assemblies (MSA) are common in living organisms and their hierarchical organization is crucial to the ultimate function of many biological systems. Using 2β-CD@SDS as an example, spatial and polarization resolved images were collected of individual 2β-CD@SDS sheets and machine learning function approximation techniques were applied to extract the orientation of 2β-CD@SDS subunits within the self-assembled sheets. Interestingly, it was found that 2β-CD@SDS subunits are slightly tilted rather than parallel to the principal axis of the sheet. This work signifies the importance of understanding the hierarchical orientations of molecular self-assembled materials and is beneficial to future research on how such orientation could potentially affects the morphology and functions of MSAs. [Ref 2-3]

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Synthesis monitoring of Silver/Gelatin crosslinker nanocomposites on silver nano thin films, for molecular sensor applications using Raman spectroscopy.

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Abstract

Chemical sensor fabrication for small molecule detection applications has become a major subject of interest in nanomaterial research. Silver nanoparticles are often coupled with stabilizing polymers to form analyte crosslinkers for analyte adsorption [1]. However, reproducibility and stability of the sensors remains a challenge [2]. Opportunely, nanomaterial research has ventured into nondestructive photonics-based methods which offer rapid, nondestructive molecular analysis [3]. In this work, we synthesized silver-coated nano thin films as a substrate for adsorption of gelatin functionalized silver nanocomposites (Ag@Gelatin). Each fabrication step was analyzed using Raman spectroscopy where changes in vibrational modes were observed and correlated to the chemical synthesis reactions. The results show consistent and reproducible Raman shifts which can be used to provide chemical reaction monitoring methods during nanomaterial sensor fabrication. Future work will involve the adsorption of analytes on the Ag@Gelatin crosslinker substrates for surface-enhanced Raman spectroscopy research.

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Spectral Characterization of High-Speed SERS Fluctuations

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Surface enhanced Raman spectroscopy (SERS) is a well-known and highly-studied effect that effectively represents both the challenges and opportunities inherent to nanophotonics and nanotechnology [1]. This is due to the extremely localized nature of SERS, where intense plasmonic "hotspots" increase Raman scattering by orders of magnitude, generating signals from single molecules [2]. These signals often show significant fluctuations, both in intensity and spectral features due to the dynamic nature of light-matter interaction at the atomic scale. Recent experiments have shown these SERS intensity fluctuations (SIFs) to occur over an extremely wide range of timescales, from seconds to micro-seconds [3]. While many mechanisms have been proposed for these fluctuations, such as molecular diffusion or transient plasmonic hotspot generation, the underlying source of these fluctuations are likely to be a complex interplay of several different effects. Furthermore, while high-speed intensity fluctuations provide important information on the overall timing statistics, high-speed spectral information has so far been lacking. Figure 1 demonstrates initial progress on a high-speed acquisition system capable of taking more than 100,000 spectra per second. Characterization of SERS fluctuations at these speeds can provide further clues as to the source of these events.

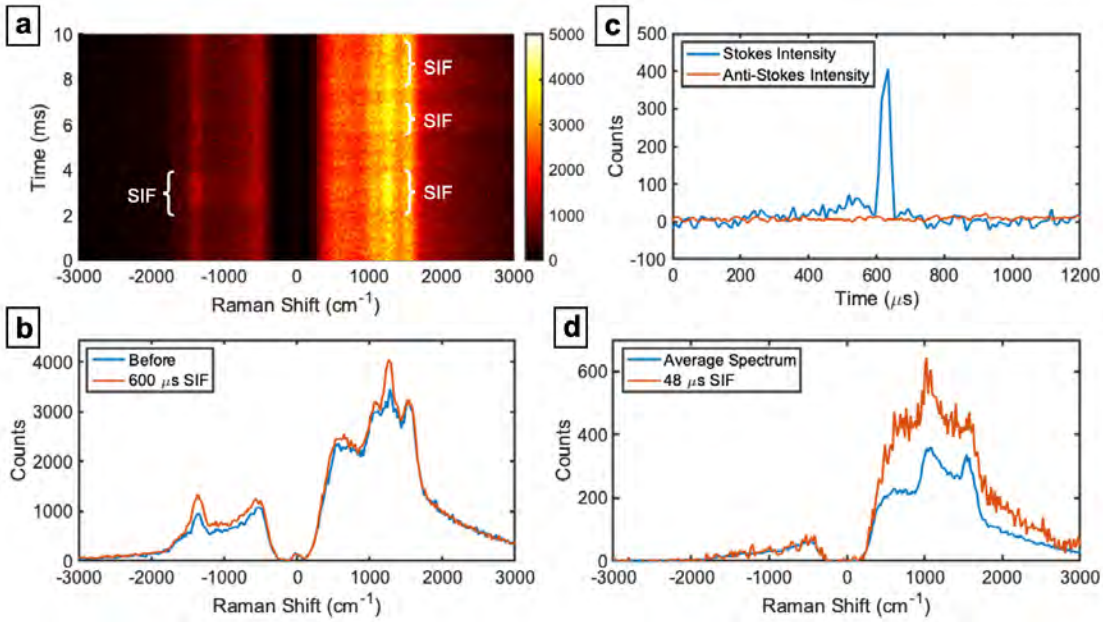

Figure 1. SERS from a benzenethiol-coated Ag film-over-nanosphere sample. (a) Waterfall plot of SERS spectra showing (b) fluctuations of both the Stokes and Anti-Stokes peaks near ±1360 cm^{-1}. These fluctuations are on the millisecond timescale. (c) Faster scanning captures a 48 µs event, with (d) the entire Stokes region being involved.

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High-speed time-domain coherent Raman spectral imaging with compressed sensing

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Raman spectra in the fingerprint region contain rich structural information of target molecules. Time-domain coherent Raman (TDCR) microscopy [1] is a promising method for rapid acquisition of broadband Raman spectra covering the entire fingerprint region. However, its acquisition rate is lower than the state-of-the-art stimulated Raman scattering methods for probing the CH stretching region and thus require further enhancement for realizing video-rate Raman spectral imaging.

In this report, we combined TDCR spectral imaging and compressed sensing to improve the spectral image acquisition rate. Specifically, we sampled time-domain interferograms coarsely with the 3D Lissajous scanning method [2], whose scanning trajectory is obtained by scanning all three axes simultaneously at slightly different frequencies (Figure 1). After the scanning, corresponding Raman spectral images $\boldsymbol{u}$ were reconstructed from coarsely sampled time-domain interferograms $\boldsymbol{f}$ by solving the following total variation regularization problem:

$$\min_{\boldsymbol{u}} \left\{ \frac{1}{2} \|\boldsymbol{W}\boldsymbol{u} - \boldsymbol{f}\|_2^2 + \lambda \, \mathrm{TV}(\boldsymbol{u}) \right\},$$

where $\boldsymbol{W}, \lambda, \mathrm{TV}(\boldsymbol{u})$ are the measurement matrix, the hyperparameter, and the band-wise total variation of $\boldsymbol{u}$, respectively.

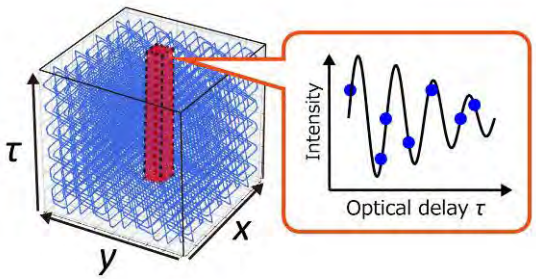

Figure 1: Illustration of a Lissajous scanning trajectory in 3D space spanned by positions (*x*, *y*) and optical delay *τ*.

Figure 2 shows numerical simulation results of a spectral image reconstruction assuming the number of measurement points was reduced by a factor of 10 (i.e., measurement was 10 times faster) compared with raster scanning. Usual Fourier transform of under-sampled data at each pixel failed to recover the spectra due to aliasing, while five input peaks were recovered in the spectral image reconstructed via compressed sensing (Figure 2(c)).

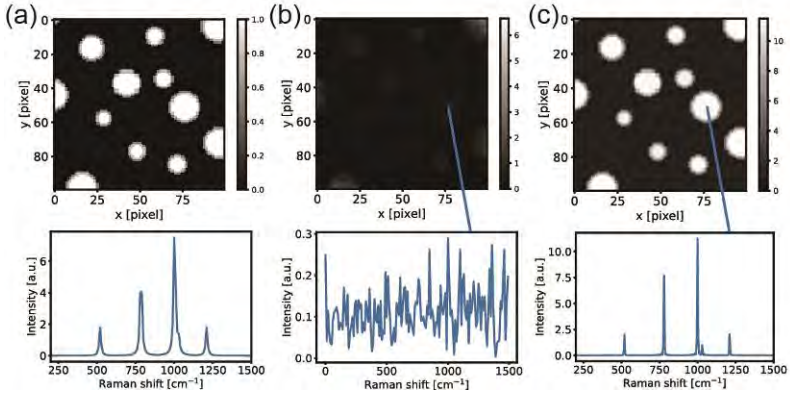

Figure 2: Numerical simulation of recovering under-sampled data using compressed sensing. (a) Assumed spectrum of a chemical and its concentration map. (b) Typical spectrum and the intensity map at 1000 cm^{-1} of sparsely sampled data obtained by Fourier transform. (c) Typical spectrum and the intensity map at 1000 cm^{-1} of sparsely sampled data reconstructed by compressed sensing.

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Tunable aluminum nanocrescents as a platform for circular dichroism spectroscopy and surface enhanced Raman spectroscopy

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Several noble metals, such as gold, silver, and copper have been the focus of studies of the behavior and applications of plasmonic nanostructure.[1] However, aluminum (Al) has several advantages over those more commonly studied metals and is a promising material for nanoscience studies and applications. Al nanostructures exhibit plasmons across a broad spectral range, are resistant to corrosion due to the native oxide layer[2] and are economical as an abundant material. Even so, the complication of the ubiquitous Al_2O_3 film and challenges of fabricating Al nanostructure are still the reasons for a limited number of studies for Al nanostructures. With copper mask nanosphere template lithography[2], the Shumaker-Parry group has successfully fabricated size controllable Al nanocrescent (NC) arrays (Fig.1A) on a variety of substrates, overcoming the challenge from the native oxide layer.

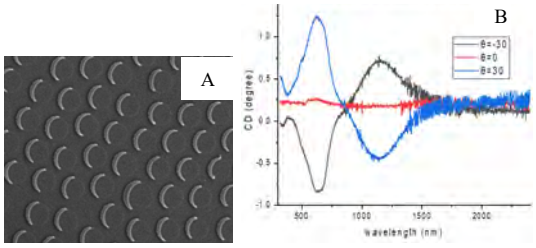

Figure 1. A) Scanning electron micrograph of Al NCs array. B) CD spectrum of Al NCs.

The Al NCs exhibit multimodal, polarization-dependent plasmons**:** long-axis dipole along tip-to-tip of the structure, short-axis dipole orthogonal with long-axis, and quadrupole mode. These plasmonic modes can be tuned in different spectral regions by control of size. Furthermore, Fig.1B presents large chiroptical responses of Al NCs when tilted to non-normal angles with respect to the incident angle of circularly polarized light, referred to as extrinsic chirality. There is no circular dichroism (CD) response at $\theta = 0°$, normal incidence. At $\theta = \mp 30°$ out-of-plane incident, the CD response is equal in ellipticity but opposite in handedness.

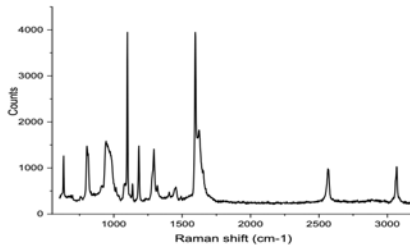

Figure 2. SERS spectrum of PMBA on Al NCs substrate

The Al NCs array is being explored for surface enhanced Raman spectroscopy (SERS). Although coupling molecules with oxide layer is a challenge, 4-mercaptobenzoic acid (MBA) coupling with Al NCs to study the SERS behavior of the molecules has been demonstrated. The SERS spectrum in Fig.2 shows the expected peaks for MBA, including intense bands for aromatic ring vibrations, aromatic stretching band, C-S stretch, etc.[3] The Al NCs arrays allow for highly sensitive plasmonic modes and exhibit CD response. The strong enhancement of system for MBA has proven that the Al NCs will be an effective performance, affordable cost plasmonic substrate for SERS. The research will expand the understanding of the plasmonic behavior of Al nanostructures, including the CD response and application in SERS.

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Low Frequency Raman microscopy for API polymorphisms analysis

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Since the physical state can affect the pharmaceutical behavior of drug substances, it is important to know what controls crystallization, solid state reactions, phase stability, and solubility. There are numerous methods that have been used to measure the solid state composition of pharmaceuticals; these include X-ray diffraction, optical microscopy, thermal analysis, dissolution testing, particle size analysis, NMR, and infrared (IR) spectroscopy. Raman spectroscopy is a now a validated technique in this industry as a very powerful characterization technique.

Indeed, Raman spectroscopy can provide qualitative and quantitative information of the polymorphy, with 1 µm spatial resolution when necessary. The new generation in Raman technology provides many advantages over the other techniques. Thus, it is a non-destructive analysis, samples can even be examined in transparent glass or plastic containers. Microscopic samples as small as 1µm can be easily characterized, and finally little or no sample preparation is required. Moreover, polymorphic and pseudo-polymorphic phases in microscopic samples can be mapped. This last point is important as the pelletizing can create pressure-induced polymorphic transformation.

By definition, the differences between two polymorphic phases is in the crystal modes, which can be characterize on the low Raman frequencies region. That increases the difficulty for the discrimination of the phases. Thanks to the standard Super Low Frequency standard module available on LabRAM Soleil^{TM}, it becomes easy to reach 30 cm-1 frequency, and so to characterize polymorphisms without additional options. This provides so low frequency spectra with no intensity compromise.

In this presentation, we present an example of polymorphisms characterization by Raman microscopy using the Super Low Frequency module.

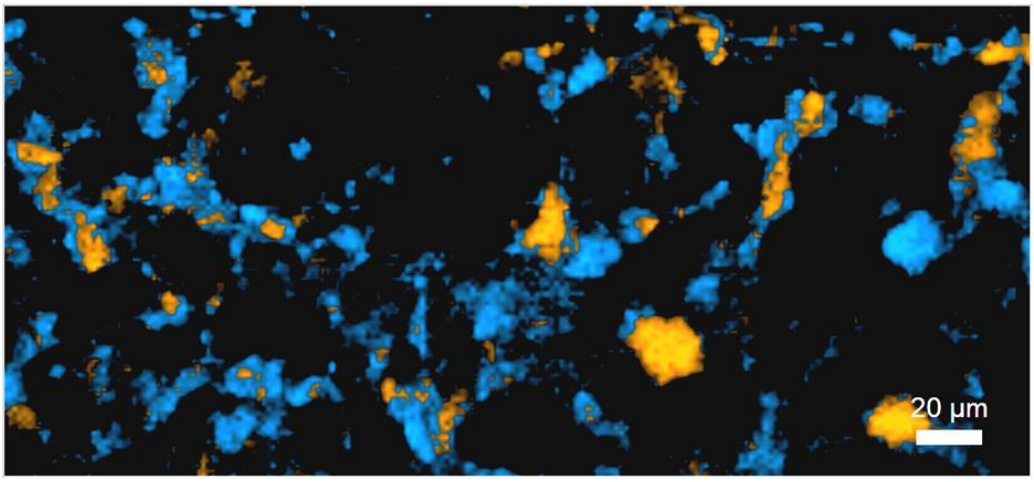

Figure 1: Carbamazepine distribution in tablet (blue: Form I, orange: Form III, black: excipients)

Defects in polymer multilayer films: a new way to investigate based on Raman microscopy

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Polymer multilayer films are presents everywhere in our world. Thus we can found them in food packaging, on car coatings, in phone protection films, among many other applications. But their characteristics are questioning as soon as a defect is present. Unfortunately, it's not easy to locate this defect, and consequently chemically characterize and identify it.

Confocal Raman microscopy is a perfect candidate for such issue, combining the high spatial resolution of optical microscopy with the chemical identification through the spectral characterization. Moreover, we demonstrate in this paper how the QScan patented-technology is a great improvement for defect investigation applying this tool on the analysis of a defect in a multilayer polymer film.

This combination makes our Raman confocal microscope the ideal solution for non-destructive highly resolved characterization of the defect realizing a very fast survey mapping of the sample.

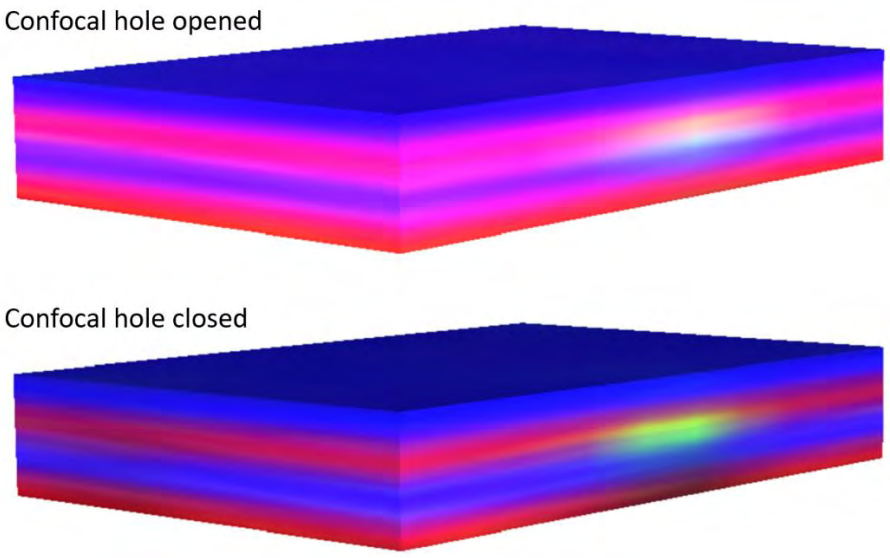

Figure 1: 3D Raman map of an feature in a multilayer polymer sample. The colors represents the different chemical fingerprint. Blue: Plastic tape. Red: Glue. Green: Feature. Map dimension: 500x500x100µm with 50x50x1µm steps. (Top) Map with confocal hole opened. (Bottom) Map with confocal hole closed.

Use of complementary techniques for depth profiling of mobile screen protection covers

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Despite the improvement in glass manufacturing, it is still annoying when a brand new mobile phone falls on the ground and its screen shatters. To avoid (or at least minimize) this scenario, a new industry has risen and is supplying mobile screen protection covers. Besides protecting mobile screens from breaking, such protection films avoid scratching of the display when carrying the phone in the pocket, and they are also dirt-repellent. The cheapest ones are usually plastic films that can be stuck on the telephone screen. But have you ever wondered what they are made of?

As simple as such polymer foils look, the polymer technique behind them is quite demanding. Controlling the production process to ensure reliable protection capability for large batches is required to guarantee consistent quality.

At HORIBA Scientific, thanks to our wide characterization technique portfolio, we can provide some of the necessary instruments for the correct analysis and process control of polymer films.

In this presentation we will focus on analytical depth profiling methods, providing both composition and layer structure. We will show how micro Raman Spectroscopy and pulsed Radio Frequency Glow Discharge Optical Emission Spectroscopy, coupled with the Ultra Fast Sputtering, can provide a comprehensive understanding of the molecular footprints and the elemental composition of generic commercially available mobile screen protection covers.

Cavity Enhanced Transmission Raman for Content Uniformity Analysis of Low Dosage Pharmaceutical Tablets

Authors:
B&W Tek

Abstract

We have developed a transmission Raman system intended for content uniformity analysis of solid pharmaceutical dosage forms, based on a portable high throughput spectrometer and a cavity enhanced transmission Raman probe. With a replaceable aperture ranging from 2 to 8 mm diameter, and sample thickness up to 10 mm, most solid pharmaceutical dosage forms can be analyzed. Both theoretical analysis and experimental results have shown that reflective cavities placed at both sides of the sample not only enhance the overall signal, but also improve sampling uniformity across the penetration depth. The performance of the system is tested on two model tablet formulations, one containing 0.5% w/w acetaminophen of good uniformity, and the other a few percent of poorly blended caffeine. Partial least square models are used to quantify the concentrations of active pharmaceutical ingredients. Results show that for the well blended acetaminophen tablets, a small number of calibration samples are required, gravimetric concentrations of the blends can be used as reference values for model building, and sufficient specificity, linearity, accuracy, precision, and detection limits are achievable for content uniformity analysis.

Probing Zeolite H-ZSM-5 Deactivation using Correlative Hyperspectral Confocal Raman, Fluorescence and Tip-enhanced Fluorescence Spectroscopies

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Zeolite H-ZSM-5 is a widely used solid catalyst in the chemical industry to convert methanol into gasoline as well as base chemicals, such as ethylene and propylene, in the so-called methanol-to-hydrocarbons (MTH) process [1]. However, the deactivation of zeolite H-ZSM-5 catalysts via the formation of coke species during MTH is not yet well understood, primarily due to the lack of analytical techniques with sufficient specificity, sensitivity or spatial resolution. Herein, we demonstrate that hyperspectral imaging using confocal Raman spectroscopy is an effective tool to investigate the structure of large, pristine zeolite ZSM-5 crystals without any sample preparation and in a non-invasive manner. Raman chemical imaging of the intensity ratio of 380 cm^{-1} and 832 cm^{-1} Raman peaks [2] revealed a higher amount of extra framework aluminum (EFAL) in the edge region compared to the central region of zeolite ZSM-5 crystals as shown in Figures 1a-1c. This correlates very well with the previous observation of general Al enrichment at the edge of ZSM-5 crystals [3].

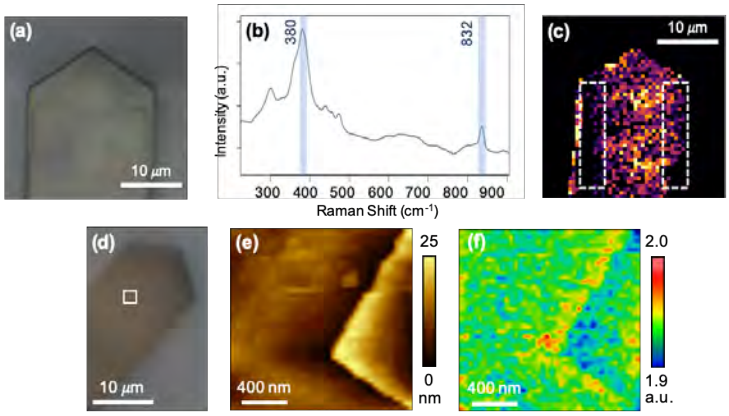

Figure 1. (a) Optical image of a pristine zeolite ZSM-5 crystal on a Si substrate. (b) Average Raman spectrum of a pristine zeolite ZSM-5 crystal. Raman bands at 380 cm^{-1} (P1) and 832 cm^{-1} (P2) are highlighted in blue. (c) Confocal Raman image of the P1/P2 ratio measured from the zeolite crystal shown in (a). Step size: 600 nm. Spectrum acquisition time: 1 s. A low P1/P2 ratio is observed in the edge regions indicating a higher EFAL compared to the center. (d) Optical image of a zeolite 10-ZSM-5 crystal. (e) AFM topography image of the marked area in (d). (f) TEFL image of the area shown in e. Spectrum acquisition time: 0.25 s. Step size: 25 nm.

Furthermore, to probe formation of coke species during the MTH process and to understand the correlation of microscale structural imaging using confocal Raman spectroscopy, we performed confocal fluorescence imaging of zeolite ZSM-5 crystals subjected to 10 (10-ZSM-5) and 90 (90-ZSM-5) min of reaction. These measurements revealed a preferential formation of smaller coke species in the central region of the crystal, whereas larger coke species preferentially formed at the edge and the apex regions. Additionally, the 90-ZSM-5 crystals were found to produce more graphite-like species compared to the 10-ZSM-5 crystals.

Finally, nanoscale tip-enhanced fluorescence (TEFL) imaging showed a higher amount of coke formation at certain topographic features (such as the crystal steps) on the 10-ZSM-5 and 90-ZSM-5 crystals as depicted in the representative example, shown in Figures 1d-1f. Our study demonstrates that correlative hyperspectral Raman and fluorescence imaging could be a powerful tool to investigate deactivation of catalytic materials of industrial importance, such as zeolite crystals.

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At-Line Monitoring of Downstream Process by Time-gated Raman technology

Mari Tenhunen and Amutha Daniel, Timegate Instruments Oy

Process Analytical Technology (PAT) approach for pharmaceutical manufacturers was issued by the US Food & Drug Administration (FDA) in the Guidance for Industry PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance. This framework highlighted a paradigm shift from traditional quality assurance methods to quality by production process. In this regard, the understanding of this process must also be applied to downstream bioprocesses to produce high-quality biological products. Currently the critical quality attributes (CQAs) in downstream process are being monitored using enzyme-linked immunosorbent assays (ELISA) or sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), which are laborious and time-consuming. Recent advances in spectroscopic techniques for process analytical technology are burgeoning since these technologies offer simultaneous quantification of various analytes. Among various spectroscopic techniques, Raman spectroscopy is a sensitive technique and requires no sample preparation and have relatively low water signal, aiding it to be employed in online or at-line monitoring during downstream process. Yet the wide use of Raman spectroscopy is limited by the long-lived fluorescence of the protein of interest which masks the weak Raman signal. Time-gated Raman spectroscopy has resolved this impediment by detecting the Raman scattered photons before the emission of fluorescence. At-line time-gated Raman spectroscopy was used in this work for monitoring monoclonal antibody titer and the antibody aggregate amount in samples obtained from different stages of recovery and purification process.

Single-photon-sensitive infra-red luminescence spectroscopy

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Affiliation: Lawrence Livermore National Laboratory

Abstract.

The development of superconducting nanowire single-photon detectors allows detection of mid-IR photons with wavelengths up to 15 um with high efficiency, high time resolution – down to ~20 ps – and a low dark count rate. A multi-pixel detector array can be placed in the focal plane of a cooled monochromator to count photons in multiple spectral channels in parallel. Applying IR photon sensors for IR luminescence spectroscopy should allow spectral and temporal information similar to Raman spectroscopy. When used with live cells, the method will avoid the common problem of Raman spectroscopy of phototoxicity caused by exposure to intense visible light. The issue of overloading the IR photon detector with the room temperature thermal radiation background has a solution: placing a small warm sample holder, cooled monochromator, and detector array inside a cryogenic environment and acquiring IR radiation only in a cell-size region. Light-induced IR luminescence, IR emission due to cell processes, and thermal radiation background can be detected and time-stamped at the single-photon level. Analysis of these time-and spectral- data arrays will allow looking for signatures of cell metabolic processes, interactions, and time correlations between processes, chemical and non-chemical (IR, phonons) signaling passways. Searches for quantum features like squeezing and entanglement in cell-emitted IR radiation could be possible. IR photoluminescence technique will be compatible with other spectroscopic methods, non-linear optics, and visible bio-photonics methods. When applied in the cryopreservation industry, the method can provide non-invasive chemical imaging of precious samples like human eggs or a few cells embryos with single-molecular sensitivity as the thermal background vanishes for cooled cells
The development of these techniques will address breakthrough biological/biochemical/biophysical questions in experiments leading to new disruptive technologies.
This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. LLNL-ABS-833946-DRAFT

The impact of graphene derivatives additives on polymer membranes analysed by Raman microspectroscopy

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Nowadays, taking advance of the rapid growth in the field of nanotechnology, the tissue engineering medicine offers novel nanocomposites that are capable to surpass traditional transplant procedures. The scaffold composed of such biomaterial would behave as a native extracellular matrix supporting growth and coordinating the regulation of host cells [1].

The objects of presented study are the nanocomposites composed of poly(ε-caprolactone) polymeric matrix (PCL) and graphene derivatives: graphene oxide (GO) and partially reduced graphene oxide (rGO). The poly(ε-caprolactone) is a highly suitable synthetic polymer for application in the field of bone tissue engineering due to its distinctive mechanical qualities, long-term biodegradability and great solubility [2,3]. In order to improve its hydrophilicity and therefore the biocompatibility, and overall its mechanical properties, it was blended with nanoadditives of GO (PCL/GO) and rGO (PCL/rGO).

The main aim of the study was to characterize the structure of graphene derivatives and to decode the phenomenon that occurs in the polymer in contact with the GO that is rich in hydrophilic functional groups, and with rGO that is less rife with these groups. Raman microspectroscopy was the method of choice on account of its exceptional ability to test short-range ordering. For the studied graphene derivatives the degree of disorder, dispersion of the graphitic G- and D-band, crystallite size [4], and the distance between point defects was assessed. It reveals that oxidized graphene after reduction presents more defects then oxidized graphene itself, also for the GO the D-band is most disordered comparing to G- band. The analysis of composites membranes clearly shows the higher level of polymer crystallinity in PCL/GO, however PCL/G membranes are characterised by significantly higher homogeneity of graphene distribution within the polymeric membranes. Also, it can be concluded on the base of Raman spectra that the reductive process of oxidized graphene leads to exfoliation of graphene layers.

Acknowledgments

AK has been partly supported by the EU Project POWR.03.02.00-00-I004/16.

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Machine Learning Analysis of Spectral Data using Bacterial Metabolic Networks for Signal Amplification

Bacterial metabolism responds to chemical changes in the local environment, and these responses can amplify the signal of analytes in solution. Nutrient deprivation, for example, results in a large change in intracellular bacterial metabolites and can be useful for process monitoring in biotechnology industries. Similarly, bacterial stress responses to toxic heavy metals can be used to monitor water quality. Surface enhanced Raman scattering (SERS) sensors with controlled surface chemistry and gold nanogap spacing produce spectra that are highly sensitive to changes in bacterial metabolism associated with these metabolic responses, and these responses are robustly differentiable by using machine learning (ML) algorithms to analyze the spectral data. Using this approach, we show that carbon deprivation of *E. coli* is discernable due to changing carbon source from glucose to the less prefered sugar xylose, and to sucrose, which is minimally usable by *E. coli*. We also show that detection of arsenic (Ⅲ) ions (As^{3+}) and chromium (Ⅵ) ions (Cr^{6+}) is possible at ultralow concentrations, comparable to state-of-the-art analytical methods. The limit of detection (LOD) for As^{3+} is 0.065 ng/L and a limit of quantification (LOQ) of 0.65 ng/L is achieved when utilizing a convolutional neural network (CNN) regression algorithm.

Characterising Graphene and 2D Materials by Confocal Raman and Photoluminescence Microscopy

Analysis of graphene and transition metal dichalcogenides (TMDCs) is crucial for understanding the characteristics and quality of samples and thus the effectiveness of different growth methods. Confocal Raman and photoluminescence (PL) microscopy is a non-destructive technique used to determine number of layers, defects, strain, and functionalisation. This work details the analysis of 2D materials, such as graphene and MoS_2, via mapping the same sample areas using Raman, PL, and PL lifetime microscopy.

Subtle changes in the Raman spectra aid in detecting the number of layers present and locate defects in graphene samples. Three main bands of interest are studied by Raman spectroscopy; the G-band, which is used for layer and strain analysis, the 2D-band, used for layer analysis, and the D-band, studied to detect defects. A confocal Raman microscope can be used to precisely map and plot where defects occur on the sample, Figure 1(a), of graphene.

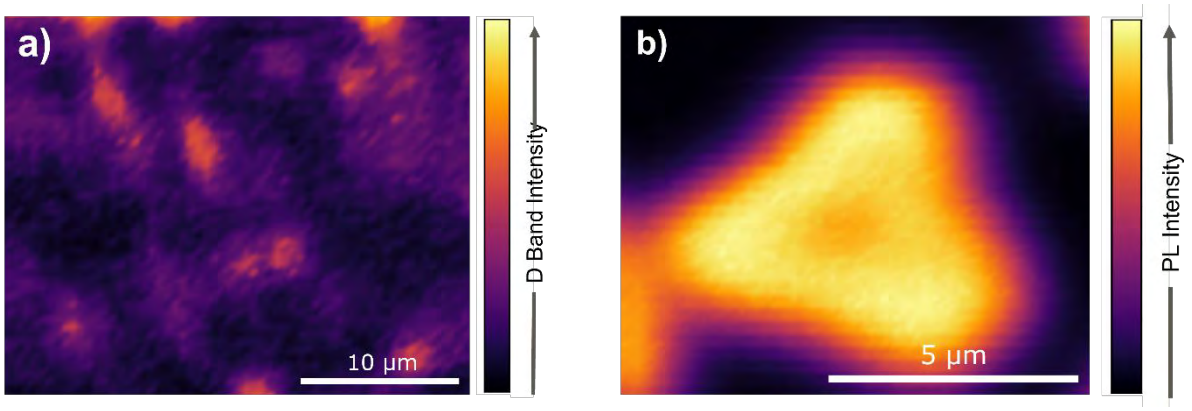

Figure 1: (a) Raman map highlighting defects in a sample of graphene, (b) PL intensity map of MoS_2

Using Raman and photoluminescence techniques on the same sample can also provide extremely useful information in the study of 2D materials. In TMDCs, for example, the position of two Raman bands is indicative of the number of layers present, as the number of layers increase these bands move farther apart due to interlayer vibrations. Mapping TMDC samples allows plotting of these two peak positions to see distribution of layer thickness. PL mapping is also used in the search for monolayer TMDCs, the PL peak, for example at ~ 680 nm for MoS_2 (Figure 1(b)), will significantly decrease as layers increase due to the direct bandgap that is only present in mono-layer TMCDs. Additionally, as the layer thickness increased the PL peak position will shift to the red. TMDCs PL lifetimes also show important sample information, as the number of layers increase the lifetime will become shorter. Analysing single flake by lifetime mapping allows for grain boundaries and structural defects to be investigated.

De-noising and differentiation of low-SNR Raman-spectra of EV's

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UV-written silica waveguides are investigated as a potential material to construct a lab-on-chip for high-throughput Raman-spectroscopy of bio-nanoparticles, primarily extracellular vesicles (EVs). A significant challenge of this approach is the Raman-signal induced in the waveguides by the high-power trapping field, thus inducing a significant background in the obtained signal. Neural networks are used to mitigate the impact of the high Raman-background by de-noising the signal and extracting latent information from it. The aim is to differentiate between EVs of different origins in presence of the Raman-background of the waveguides. The method may have general relevance for analysis of Raman-spectra with low signal-to-noise ratio.

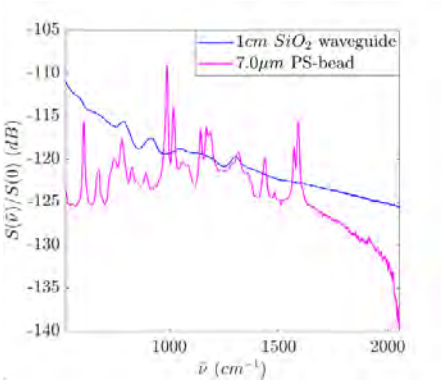

Figure 1 Absolute intensity Raman-spectrum of SiO_2 waveguide

The measured, absolute intensity of the Raman-background of the waveguide is shown in **Figure 1** with the spectra of a polystyrene bead as an analogue of a trapped particle. The signal-to-noise ratio (**Figure 2)** is estimated from the waveguide background relayed via Mie-scattering, Rayleigh-scattering, and by crosstalk between the waveguides and the collecting aperture. De-noising and differentiation are performed by a specialized convolutional variational autoencoder (ConVAE) neural network that is evolved using a genetic algorithm. The hyperparameters of the evolution scheme allow for the evolution of networks with varying depts, widths and channels at every level of both the encoder and decoder.

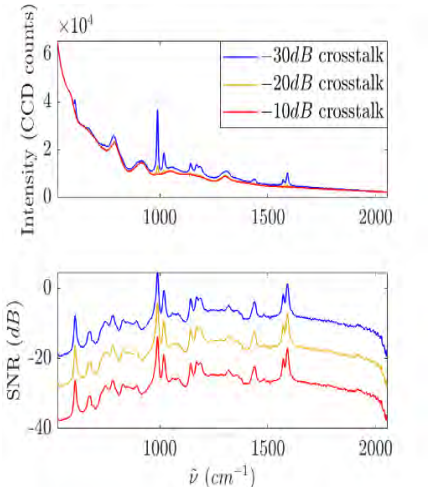

Figure 2 Expected SNR of 200nm nanoparticle in waveguide trap

The versatility and adaptability of the network is enhanced compared to a standard VAE by employing a ResNet-based skip connection scheme to allow the deep layers of the network to access more of the input. This allows for the spectra and the corresponding wavenumber shift to be passed in parallel to the network. Having both spectra and wavenumber shifts as input enables the network to explicitly consider the spectral features in context with the corresponding wavenumber shift, thus allowing for flexibility in wavenumber range and resolution such that truncation of data is not required.

The network is shown to be able to reconstruct the original data from the latent representations with an accuracy (RMS) of 90.1% for low-noise spectra (SNR>14dB) and 74.5% for spectra with induced background and gaussian noise (SNR<-15dB). Furthermore, it is predicted that the architecture will perform label-free differentiation of EVs of different origin and activation state of the parent cell. This differentiation is possible both for the low- and high-noise spectra, with consistent patterns of differentiation and with similar lipid- and protein-association of the spectral components used for differentiation.

Fabrication of a prototype Raman-waveguide chip is scheduled for Q3 2022, and a trapping Raman-microscope is under construction with expected completion mid-Q3 2022. The ConVAE is under continuous development through additional data supplied by the project collaborators at MCBP (U.Twente, Enschede, Netherlands) and UPMC (Sorbonne.U, Paris, France).

Raman Spectroscopy in Extreme Environments

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One of the key analytical instruments commonly proposed in the baseline payload designs of most planetary exploration missions is a Raman spectrometer [1,2,3,4,5]. This powerful and largely non-destructive molecular spectroscopy technique is often included alongside other analytical instruments in order to complete the comprehensive characterisation of planetary samples [6,7]; for example: XRF, XRD, UV Fluorescence and LIBS (which are sensitive to elemental/mineralogical components) can provide complementary scientific data to the molecular information inferred by the Raman spectrometer [8,9,10].

Following a significant investment across a number of decades by numerous space agencies [2,11], the Technology Readiness Level (TRL) of such baseline Raman spectrometer systems is relatively high, with a range of low mass and low power technically capable instruments readily available [12,13]. This TRL-raising instrument development work has specifically focused on improving the autonomous operation of such spectrometers, including developing sample scanning algorithms, optimising data acquisition modes and improving auto focus and thermal stabilization systems. Other key areas of development include optimization of:

i. Thermal and mechanical robustness in order to ensure the spectrometer systems can operate to requirement across a wide range of challenging environments (e.g. very low temperatures for Europa lander missions, very high temperatures for Venus missions, and very high levels of radiation for landers/orbiters operating around Jupiter and its moons), and
ii. Radiometric models in order to adequately inform sampling strategies and instrument design trade-off studies [14,15].

In this talk, we present initial results from two separate instrument design studies that were performed in preparation for anticipated future mission opportunities, one for a Europa lander and one for a lunar lander. Specifically, the presentation includes a summary of the laboratory measurements performed with two prototype camera systems specifically developed for the unique challenges associated with these missions: one of the camera systems is based on a recently developed CMOS sensor and the other is based on a CCD sensor. This talk also includes a report on the development of radiometric modelling software that specifically accounts for the high levels of radiation expected on Europa. Overall, the camera system performance is described in terms of the primary scientific goals of both missions and we conclude with a report on measurements performed on appropriately selected analogue samples demonstrating the overall spectral capability of each instrument design.

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Raman Analyses of Planetary Analogue Materials in Preparation for Future Exploration Missions

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Raman spectra obtained from planetary analogue materials recovered from terrestrial sites is presented. Results are relevant to instruments on the Perseverance and ExoMars rovers and recently proposed instruments for potential future lunar and Europa landers.

Raman microspectroscopy highlights new features on hair greying.

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Hair greying (*i.e.* canities) occurs during aging and even though the involvement of oxidative stress is well detailed at the biological level, there is a lack of study on its consequences at the hair shaft level. The aim of this study was thus to investigate by Raman microspectroscopy the molecular signature of pigmented, grey and unpigmented hair shaft in order to better understand hair greying.

For this purpose, hair samples were taken from 29 volunteers. For each of them, 2 pigmented, 2 to 5 grey and 2 unpigmented hair were isolated. Raman microspectroscopy acquisitions were conducted on 5 points by hair, by recording a signal covering from the surface to 10µm of depth. A Hierarchical Cluster Analysis (HCA) was conducted on Raman spectra in order to automatically classify them in groups. The HCA revealed the classification of hair into 5 groups, corresponding to 1 group of pigmented hair, 3 groups of grey hair (light, intermediate and dark) and 1 group of unpigmented hair. The discriminating spectral regions were identified between spectra of each group previously described and relevant molecular features were then quantified. Statistical analyses highlighted several molecular descriptors (*i.e* melanin content as well as protein oxidation and peroxidized hydrogen) as significantly modified within hair greying. Very interestingly, other spectral vibrational features, linked to hair structural quality, were identified as significantly modulated in the course of hair greying. Indeed, *trans/gauche* lipid ratio was decreased with hair greying suggesting an alteration of barrier function. Moreover, α-helix/β-sheet ratio was also impaired with hair greying, which may lead to an impairment of biomechanical properties in grey hair.

Hence, this Raman microspectroscopy investigation allows identifying 3 groups within grey hair for a more precise analysis of hair greying. Analysis of spectral markers allows identifying for the first time the link between hair greying and structural changes in hair shaft at the molecular level. This study paves the way to the use of Raman microspectroscopy for the substantiation of new active ingredients to reverse hair greying.

Deep Ultra-Violet Raman Spectroscopy for Eyesafe Standoff Chemical Threat Detection

Shayne Harrel, Adam Wise, Jenny Goulden

Andor Technology, Belfast, UK

We report on portable chemical threat detection instrumentation which employs deep ultra-violet Raman spectroscopy. We discuss general system aspects such as basic optical design, fluorescence rejection, and characterize its performance with narcotic excipients and narcotics.

Multimodal label-free nonlinear optical microscopy on murine cortical bone to study skeletal diseases

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INTRODUCTION: We home-built an innovative label-free multimodal nonlinear optical microscope with off-the-shelf components, able to perform coherent anti-Stokes Raman scattering (CARS), stimulated Raman scattering (SRS), two-photon excitation fluorescence (TPEF) and second harmonic generation (SHG) microscopy [1]. This set-up is highly versatile, can be easily reconfigured, and allows the observation of vital, complex, and thick biological samples without invasive laser sources and staining. In this work, we performed nonlinear imaging of different biological samples, from single cells to highly heterogeneous tissues to validate our acquisition system.

METHODS: The microscope is fed by a multi-branch Erbium-doped amplified fiber laser. In the CARS/SRS modalities, pump (at 780 nm) and tunable Stokes pulses (in the 940-1200 nm range) with ps duration are employed, thus covering the CH-stretch region (2700-3100 cm^{-1}). TPEF/SHG signals are excited by employing the sole pump pulse. The sample is mounted on an x-y translational stage, enabling us to image large sample areas.

RESULTS: Figure 1 shows results on a murine vertebra section. The distribution of collagen is detected in the SHG modality (green areas), while the CARS signal at 2940 cm^{-1} Raman shift is colored in red. We employed this microscope also to investigate unknown components of vital breast cancer cells [2], thus enhancing the potentiality of nonlinear microscopy with respect to conventional imaging techniques. This technique represents a turning point toward clinical applications [3].

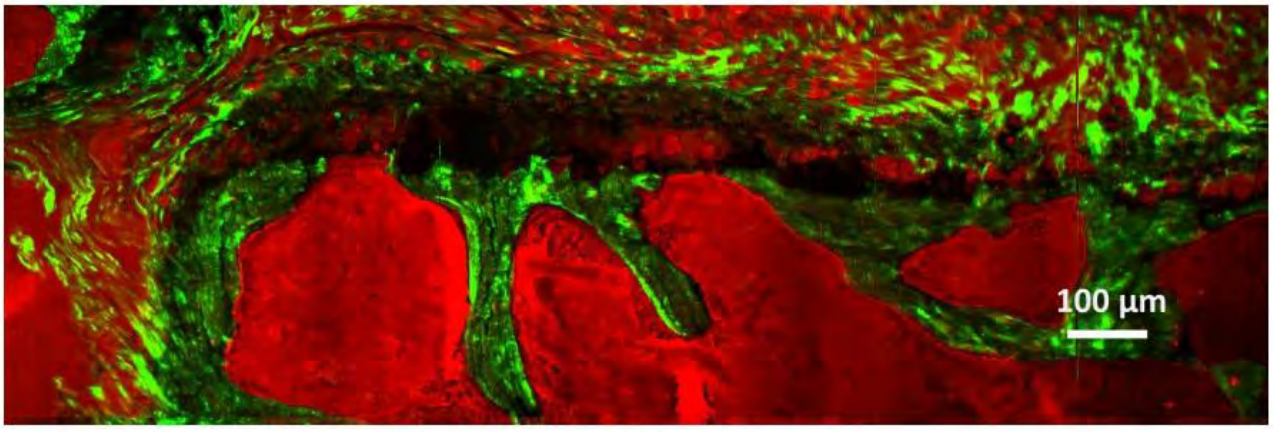

Figure 1. Multimodal image of murine vertebra section. In red CARS signal at 2940 cm^{-1} Raman shift, in green SHG signal from collagen. Furthermore, each channel was acquired with a total dimension of 1500 µm x 500 µm, 1500 x 500 pixels, and a pixel dwell time of 5 ms.

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Confocal Raman Particle Analysis on the Micron Scale Applied to Microplastics, Bacteria and 2D Materials

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Particle analysis is gaining more and more attention. Apart from microplastics research [1-2], where the necessity of establishing particle materials and their size distribution in water andfood is of growing importance, there are also now biological applications in which single bacteria are localized using white light microscopy and characterized using confocal Raman spectroscopy. Additionally, the growing field of 2D materials not only aims to refine production of large homogenous sheets, but also of smaller particles of regular shape and size. Analysing these properties along with determining whether the flake consists of a single, double or multi-layer structure can deliver highly useful information for the optimization of growth processes and parameters. To obtain statistical relevance in all of the aforementioned cases, hundreds or thousands of particles must be measured. To be able to achieve this on the scale of single micron-sized objects, the measurement's spectral acquisition speed, positioning accuracy, stability, and the quality of the white light image acquisition and particle recognition are all crucial.

In this presentation, results from the fields of microplastics research, bacteriology, and 2D materials will be presented to illustrate the benefit of automatically analysing hundreds of particles, bacteria, or 2D flakes for each specific application. The effects of advanced data-acquisition algorithms, which vary the integration time from particle to particle based on their scattering efficiencies in order to the minimize the total acquisition time will also be shown.

Once the spectra have been recorded, a highly efficient database search can accelerate the analysis. For this purpose, the hit quality index (HQI) enables the researcher to objectively evaluate the accuracy of the database search. This HQI, however, may be heavily influenced by noise and substrate signals, especially as they may change with the particle being measured. These effects and possible methods to overcome the challenges that they present will also be covered.

Figure 1 shows as an example the colour-coded particle map of a sample consisting of different polymers and their corresponding Raman spectra.

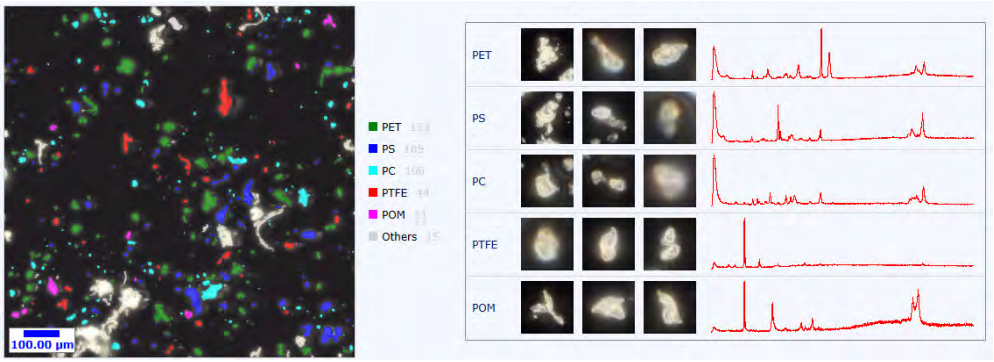

Figure 1: Particle distribution of polymers and their respective spectra. Total number of particles measured ~ 400; Total acquisition time: ca 45 Min.

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Development of a new unique concept for accurate sample measurement across different microscope based molecular spectroscopy system

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Raman and infrared spectroscopy are well known as complementary techniques to study molecular structure and function. In addition, UV-visible spectroscopy can also be important as it offers an orthogonal assessment. Although orthogonal assessment has been proven to be useful to improve the quality of analysis, it has been difficult to accurately synchronize the measurement position at the microscopic level across different systems, such as Raman, IR and/or UV-visible microscopy. To overcome this barrier, we have developed 'IQ Frame'. (Figure 1), a sample holder that can be easily transferred among Raman, FTIR and UV-visible micro-spectrometers. IQ Frame uses information about the measurement coordinates stored in a spectrum data file to roughly select the measurement position. Then, using imaging analysis of the image stored in the data file and the live visible image, fine adjustment can be made to the stage until the sample position is perfectly matched to the previous measurement.

Figure 1: Concept of IQ Frame: Shared holder with Micro-Raman, Micro-FTIR and Micro-UV-visible systems

Figure 2, shows the spectra for colored fabrics measured using IQ Frame with a UV-visible, Raman and IR micro-spectrometers. The measured location was identical for each technique. Firstly, micro-UV-visible spectra were used to analyse the fabric colors. In addition, the UV-visible spectra were useful to select the excitation laser wavelength for Raman measurement. From the Raman data, we could identify information about the dyes using the phenomenon of resonance Raman. Lastly, using FTIR with micro-ATR measurement, the chemical composition of the fabrics could be determined. The result is a complementary and detailed chemical analysis.

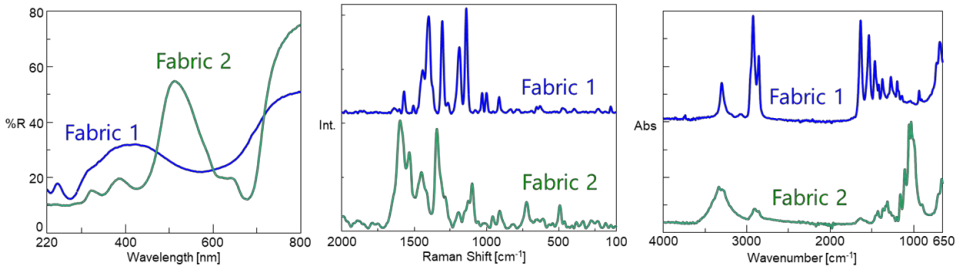

Figure 2: Micro-UV/Vis (left), Micro-Raman (center) and Micro-FTIR (right) spectra of the fabrics at the same point using IQ Frame

In this presentation, some applications included imaging measurement using IQ Frame and the features of these systems are introduced.

Optimizing SERS Structures beyond the monochromatic E^4-Model

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Arrays of metallic nanostructures play an important role in many different optical applications, e.g. biosensing or surface-enhanced Raman spectroscopy (SERS). To optimize the performance of SERS substrates, often simulations are performed with the goal to maximise the electrical near-field intensity $\boldsymbol{E}$ around the nanostructures at the excitation wavelength, as the SERS enhancement is expected to increase proportional to $\boldsymbol{E}^4$ [1,2]. This is, however, only an approximation, where excitation and emission are considered to be at the same wavelength.

We conduct a more detailed analysis, with optimization for both the excitation and the emission wavelengths taking advantage of the grating properties. While the emission wavelengths are enhanced via a low qualityfactor plasmonic mode, the excitation wavelength is in resonance with a high quality factor grating mode, tailored to a perpendicular collimated excitation. Simulations as well as experiments show a strong increase of the SERS performance compared to the case of utilizing only a near-field or a grating resonance.

This approach has the capability of boosting the SERS performance, not only by taking into account all the involved wavelengths, but also tailoring the structures to the illumination and detection angle. The approach also goes beyond SERS, as it enables to optimize arrays of metallic nanostructures for applications where multiple wavelengths are involved (e.g. Coherent Anti-Stokes Raman scattering, Four-wave mixing), or special excitation and emission schemes are used.

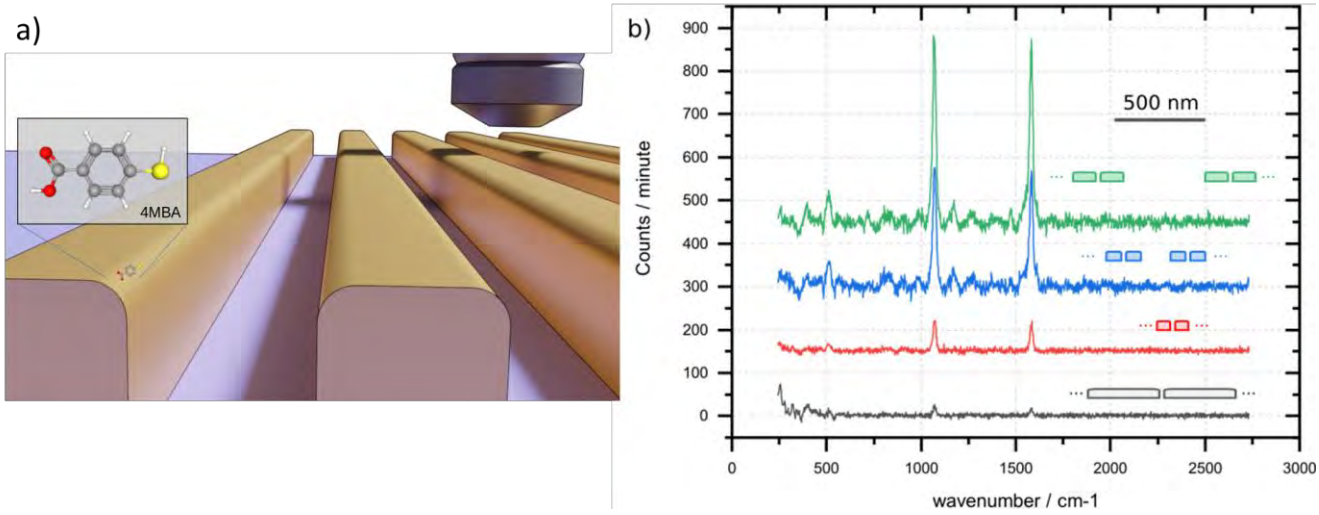

Figure 1: a) Schematic of grating arrays with analyte 4-MBA (4-Mercaptobenzoic acid). b) Measured SERS-performance in dependence of different grating geometries.

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Simultaneous Raman and Infrared testing for better microplastic identification

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1. Introduction

While mircorplastic contamination in our oceans, lakes and rivers continue to grow, the problem trickles down to our sources of water used for human consumption. Municipalities are looking to implement regulations to identify and chatacterize the types and amounts of MP's in drinking water so they can determine safe levels for human consumption. A robust method for MP characterization is often hampered by the tools most redily avaiallble to to characterize and chemically specify MP's. FTIR microscopy systems are often utilized but suffer from spatial resolution and scatter artifacts for MP's smaller than 30µm. Raman microscopy is another method and can measure smaller MP's in the 5µm and below but suffers from fluorescence interference, lower chemical specificity and samples can often be burnt by the high laser power required to get qualtiy spectra.

While new IR technologies based on IR Quantum Cascade Lasers (QCLs) are entering the market, the fundamental issues of direct IR measurements are still applicable and suffer from the same spatial resoution and scatter artifacts as traditional FTIR microscopy.

A new approach to IR microspectroscopy, termed "Optical Photothermal Infrared (O-PTIR)" spectroscopy has demonstrated a unique ability to generate submicron IR spectra in reflection mode without common scatter artifacts and the resulting IR spectra are comparable to FTIR transmission or ATR databases and spectra. This technology uses a pump (IR laser) -probe (vis/NIR laser) that provides the ability to measure mm's to submicron MP's with infrared chemical specificity in a non-contact reflection geometry. Since the vis/NIR laser used is a high quality Raman grade laser it can provide for the simultaneous aquasition of Raman spectroscopy from the same spot with the same submicron resolution.

In this presentation we will introduce, with examples, the various types of artifacts observed, from dispersive Mie-Scattering, diffuse/specular mixtures to band saturation issues. The issues with Raman spectroscopy, though it possesses excellent (and equivalent to O-PTIR) spatial resolution at the submicro level, are quite different, with it is often being plagued by auto-fluorescence interference and poor sensitivity, necessitating longer and sometime prohibitively measurement times.

2. Materials and methods

O-PTIR and simultaneous Raman spectra were collected on a mIRage IR and Raman microscope (Photothermal Spectroscopy Corp, Santa Barbara, CA). The IR pump laser was a CH/FP QCL with a spectral range of 3000-2700, 1800-950cm-1 operted at 100kHz pulse rate. O-PTIR spectra were collected at 6 cm-1 spectral resolution with 5-10 scans co-added (~5-10 secs). Simultaneous Raman spectra were collected using a 600lines/mm grating with a spectra range of 4000-200cm-1. Typical measurement parameters were 2sec integration with 2-5 aveages. IR and Raman data, as well as visible images were collected using a 40x, 0.78NA Cassegrain objective. Standard polymer beads were deposted into CaF_2 windows with real-world MPs being measured directly off gold coated polycarbonate filters through which the samples were filtered.

2.1. Results and Discussion

The key to overcoming these otherwise fundamental IR limitations, is to employ an indirect IR measurement technique - (O-PTIR) spectroscopy, which provides all the valuable chemical specificity of traditional IR spectroscopy, but via its unique pump-probe design, with a visible (532nm) beam for sample measurement, both spatial resolution is enhanced by up to 30x and particle size/shape dependent artifacts are eliminated.

Through the use of a single frequency probe beam (532nm), the wavelength dependent dispersive scattering (Mie-scattering) issues associated with variable IR probe wavelengths (such as in FTIR and direct QCL measurements) are circumvented. Additionally, the other issues often encountered with such methods of band saturation and competing specular and diffuse reflectance are also eliminated. The end result is a series of measurements that are now no longer confounded with particle morphology (shape/size) but are

only dependent on their chemistry, which is what is needed for robust and repeatable MP analysis, independent of particle shape and size.

Both spatial resolution to 500nm and lack spectral independence to particle shape and size is shown in figure 1. Here, we see that excellent IR spectral quality obtained in seconds, for both the 500nm single polystyrene bead and the cluster of particles with 4 micron size. It is noteworthy that the IR spectra shown are raw, without any spectral processing. Furthermore, excellent Raman signal quality is observed for this sample being collected at the same time, from the same spot at the same spatial resolution.

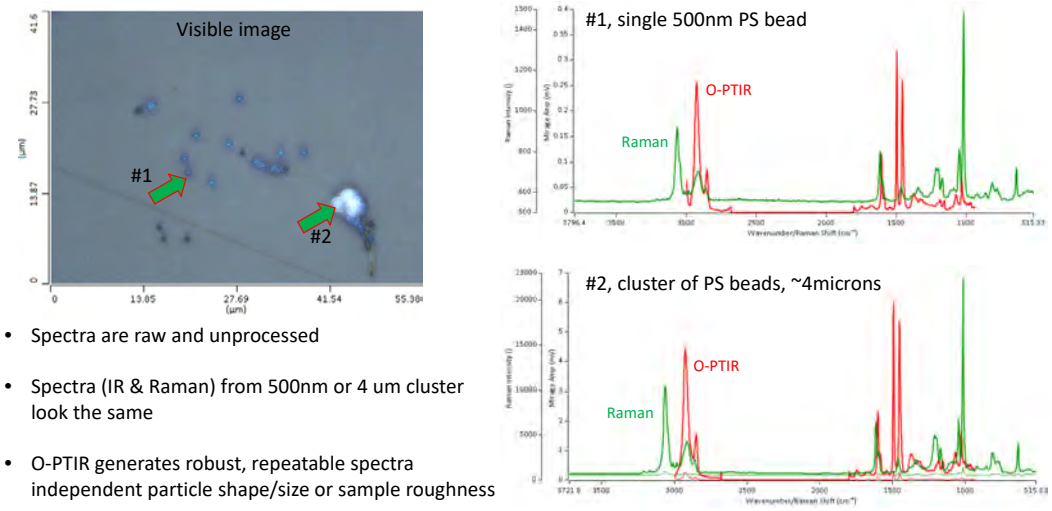

Figure 1. Submicron infrared and Simultaneous Raman microspectroscopic analysis of individual 500nm polystyrene (PS) beads and clusters of PS beads up to 4 micron in size

As seen in figure 1, Raman spectra can simultaneously be collected when appropriate but not all samples are compatible with Raman measurements Raman autofluorescence can obscure the Raman signal giving a broad spectral response without any spectral information to lead to identification. But if the MP being tested is organic the O-PTIR data is still used to identify the MP of interest. Problems also arise with small dark color samples which are often times melted or brunt with Raman but suing our low power enhance measurements we can use 1/10 of the visible lasers power and still get good quality O-PTIR data used for identification.

3. Conclusions

The capabilities of submicron IR measurements in reflection mode simultaneously with Raman measurements, provides an as-yet unexplored plethora of opportunities in the analysis of MPs, specially the very small end of the size range (<10um, even <1um) which is known to be both more biologically relevant because of its ability to cross cell membranes, but also with increased surface-area to volume ratios with the smaller particles, these are known to then possess enhanced capabilities to act toxin vectors in the environment. We have demonstrated the importance of using IR analytical techniques like O-PTIR that do not confound the collected spectra with sample morphology (sample shape/size) and thus generate far more robust and repeatable spectra, thus improving MP identification accuracy.

The outlook for not only improved MP identification accuracy, but also improved throughput looks positive with recent developments allowing the coupling of fluorescence microscopy to O-PTIR microscopy to deliver “Fluorescence-guided O-PTIR”. This opens up the exciting possibility of using well established fluorescent staining (with Nile Red) to selectivity target only the MPs in amongst other perhaps inorganics (sand) and biological matter, thus speeding up the analysis by pinpointing which particles are MPs for O-PTIR analysis.

4. References

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Simultaneous Raman and Optical Photothermal Infrared Spectroscopy of Bioplastics at Submicron Spatial Resolution

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[c] *Danimer Scientific, USA*

Simultaneous Optical Photothermal Infrared and Raman (O-PTIR+R) spectroscopy is an emerging spectral microscopy technique requiring no contact with the sample. It provides highly spatially resolved Raman and IR hyperspectral images down to about the 500-nm level, well below the IR diffraction limit. This range of spatial resolution is well suited for the analysis of multicomponent and multiphase samples, including composites and laminates. Such systems often exhibit varying degrees of molecular level mixing and spatial segregation of constituents at their interfaces, which in turn strongly affect the end use performance of the material. Although O-PTIR+R spectra are obtained in the reflection mode, the spectral profiles closely follow those obtained under the transmission mode without distortion. Recent advances have also made it possible to simultaneously measure Raman spectra at the same co-registered position with similar spatial resolution. In this study, hyperspectral IR and Raman image data were simultaneously obtained using the mIRage IR+Raman microscope for a biodegradable laminate sample, comprising macroscopically immiscible polylactic acid (PLA) and a polyhydroxyalkanoate (PHA). The result was subjected to various forms of two-dimensional correlation (2D-COS) analysis to extract pertinent information about compositional distribution and possible molecular level interactions among the constituents around the interface.

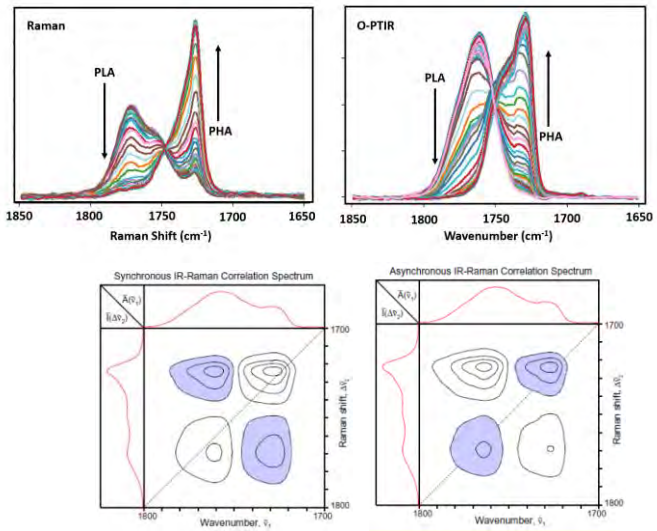

Figure 1: Simultaneously collected Raman and IR spectra collected with 100-nm spacing across the interfacial boundary of a PLA and PHA polymer laminate (top). Corresponding synchronous and asynchronous IR-Raman heterospectral 2D correlation maps generated from these spectra (bottom).

Surface-Enhanced Raman Spectroscopy-Based Detection of SARS-CoV-2 Through In Situ One-pot Electrochemical Synthesis of 3D Au-Lysate Nanocomposite Structures on Plasmonic Electrodes

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The ongoing COVID-19 pandemic, caused by the SARS-CoV-2 virus and is gradually shifting to an endemic phase which implies the outbreak is far from over and will be difficult to eradicate. Global cooperation has led to unified precautions that aim to suppress epidemiological spread (e.g., through travel restrictions) and reach herd immunity (through vaccinations) however, the primary strategy to restrain the spread of the virus in mass populations relies on screening protocols that enable rapid on-site diagnosis of infections. Herein, we employed surface enhanced Raman spectroscopy (SERS) for the rapid detection of SARS-CoV-2 lysate on Au-modified Au nanodimple (AuND) electrode. Through *in situ* one-pot Au electrodeposition on the AuND electrode, Au-lysate nanocomposites were synthesized generating 3D internal hotspots for large SERS signal enhancements within 30 s of the deposition. The capture of lysate into newly generated plasmonic nanogaps within the nanocomposite structures enhanced metal-spike protein contact in 3D spaces and served as hotspots for sensitive detection. The limit of detection of SARS-CoV-2 lysate was 5 x 10^{-2} PFU/mL. Interestingly, ultrasensitive detection of the lysates of influenza A/H1N1 and respiratory syncytial virus (RSV) were possible but the method showed ultimate selectivity for SARS-CoV-2 in lysate solution mixtures. We investigated the practical application of the approach for rapid on-site diagnosis by detecting SARS-CoV-2 lysate spiked in normal human saliva at ultralow concentrations. The results presented demonstrate the reliability and sensitivity of the assay for rapid diagnosis of COVID-19.

Keywords—Label-free detection, nanocomposites, SARS-CoV-2, surface-enhanced Raman spectroscopy

Enhanced Tri-modal Optical-Photothermal Infrared (O-PTIR) Spectroscopy – Advances in Spatial Resolution, Sensitivity & Tri-modality (IR, Raman & Fluorescence)

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IR and Raman have long been known to be complementary, but their true complementarity has been hindered by the inability to collect data simultaneously, from the same spot and the same spatial resolution. The advent of O-PTIR, a pump (IR) -probe (vis) optical micro-spectroscopy technique has now, for the first time, provide the ability for sub-micron, simultaneous IR and Raman micro-spectroscopy. Optical Photothermal Infrared (O-PTIR) spectroscopy has established itself as a cutting edge vibrational microspectroscopy tool, offering significant advantages over the traditional FTIR/QCL & Raman spectroscopic tools, providing submicron simultaneous IR+Raman microscopy, in non-contact mode with high sensitivity. The ability to collect, for the first-time submicron IR spectroscopic data in an optical microscope has enabled new research outcomes across a range of application fields, such as life sciences (cells, tissues, bacteria), polymers, cultural heritage and microplastics. A new modality, "counter-propagating" has been engineered to provide for enhanced IR (and Raman) spatial resolution and sensitivity, through decoupling the need for a reflective objective. The IR pump beam can now be directed to the sample via the underside, thus allowing the collection objective for the visible probe (and Raman excitation beam) to be a high-NA refractive objective. This improves spatial resolution to ~300nm for both IR and Raman, whilst improving sensitivity, image quality and facilitating immersion objective studies. To further integrate vibrational spectroscopic tools into life science workflows, we coupled widefield epifluorescence to facilitate a novel concept – fluorescence guided (or fluorescence co-located) O-PTIR microspectroscopy. Rather than, or in addition to the visible image, the fluorescence image can now be used to guide the user to the region of interest, thus combining the well-established specificity of fluorescence imaging with the broad macromolecular profiling capabilities of IR spectroscopy

Several life sciences examples from bacteria, cells and tissues will be provided to demonstrate these new capabilities and how they can enable new experiments and research findings.

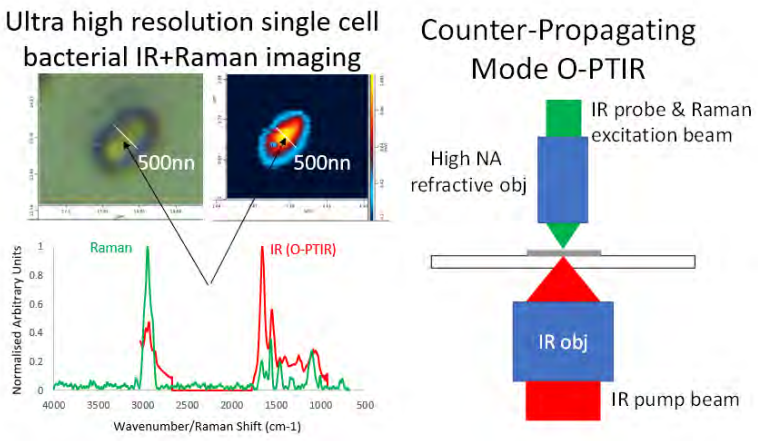

Fig 1. Left: Single E.Coli cell imaged in counter-propagating mode with 50nm pixel/step size and submicron simultaneous IR+Raman spectra (few second measurement) from centre of bacterial cell. Right: Schematic of counter-propagating layout.

Raman Spectroscopy Study of Commercial Activated Carbons Aging Processes

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Abstract

Raman spectroscopy is a non-destructive non-invasive method to characterize the chemical structure of various materials. In this research, Raman spectroscopy was used to characterize the aging of commercial impregnated activated carbon samples. Activated carbon is the most common absorber material for the removal of hazard materials. The activated carbon is usually being impregnated with metal oxides in order to improve its adsorbing capabilities to non-hydrophobic substances. During storage, impregnated activated carbons might be exposed to humidity causing water adsorption by the activated carbon, meaning the activated carbon is being aged. The effect of aging processes on activated carbon include the oxidation of the carbon surfaces as well as modification of the impregnating metals availability thus changing the chemisorption capacity of the activated carbon, and its adsorption capabilities.

Commercial activated carbon samples were accelerated aged in a thermally and humidity-controlled environment. The fresh samples, as well as the accelerated aged samples were then characterized by Raman spectroscopy. It was found that the aging process, which causes oxidation of the activated carbon can be noticed by Raman oxides vibration band at 418 cm^{-1}, which increase its intensity with aging. Furthermore, the carbonaceous Raman vibrations D and G bands at ~1350 and ~1580 cm^{-1} intensity ratio was found to increase with aging compare to the fresh samples. Thus, Raman spectroscopy is a powerful tool to study packed AC samples aging in a non-destructive non-invasive mode.