



November  
09-10  
**2023**

# RamanFest

Paris | FRANCE

**International Conference on  
Advanced Applied Raman Spectroscopy**

**PHANTOMS**  
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**HORIBA**

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## FOREWORD

On behalf of the Organising Committees, we take great pleasure in welcoming you to Paris (France) for this new edition of the RamanFest International Conference (RamanFest2023).

The 10th International Conference on Advanced Applied Raman Spectroscopy (RamanFest2023) will feature presentations from world-leading Raman experts and researchers using the technique across varied applications within life science, materials science, and energy and environmental analysis. It will bring together the world's Raman community to share, learn and discuss how Raman spectroscopy is being applied to today's problems and pioneering tomorrow's capabilities.

We are indebted to HORIBA Scientific for their help and financial support. We also would like to thank all the speakers, partners, exhibitors and participants that joined us in person this year.

Hope to see you again in the next edition of RamanFest.

RamanFest2023 Organising Committee.



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In 2019 HORIBA has celebrated the 200th anniversary of its optical spectroscopy roots. The Maison Soleil was founded in 1819 by Jean-Baptiste Soleil, becoming in 1923 the company Jobin Yvon through association with Amédée Jobin and Gustave Yvon. The technical foundation given by the Maison Soleil and the subsequent Jobin Yvon company remains at the heart of HORIBA's spectroscopy products, which are renowned for their performance and quality.





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# Optomechanical approach to Surface-Enhanced Raman Spectroscopy

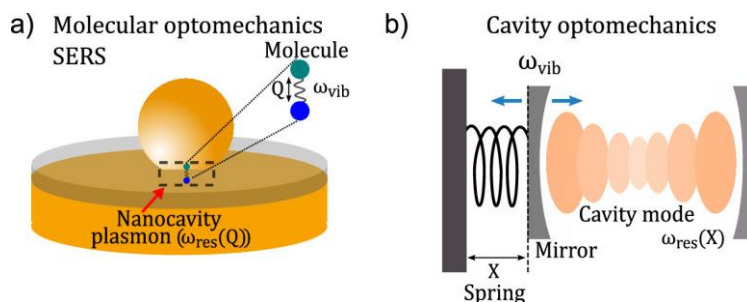
Molecular vibrations constitute one of the smallest mechanical oscillators available. The energy and strength of molecular oscillations depend on the attached specific functional groups as well as on the chemical and physical environments. Raman scattering can access the information contained in molecular vibrations, however, the low efficiency of the Raman process typically allows only for characterizing large numbers of molecules. To circumvent this limitation, plasmonic resonances supported by metallic nanostructures and nanocavities can be used because they localize and enhance light at optical frequencies, enabling surface-enhanced Raman scattering (SERS), where the Raman signal is increased by many orders of magnitude. This enhancement enables few- or even single-molecule characterization. The coupling between a single molecular vibration and a plasmonic mode constitutes an example of an optomechanical interaction [1], analogous to that existing between cavity photons and mechanical vibrations (see Fig. 1). Optomechanical systems have been intensely studied because of their fundamental interest as well as their application in quantum technology and sensing. In this context, SERS brings cavity optomechanics down to the molecular scale and gives access to larger vibrational frequencies associated with molecular motion.

Here, we describe this novel approach to SERS [2], and note that the molecular optomechanics framework naturally accounts for a rich variety of nonlinear effects in the SERS signal with increasing laser intensity. In this context, we review collective phenomena involving many molecules [3], and the modification of the effective losses and energy of the molecular vibrations due to the plasmon–vibration interaction. The quantum description of SERS also allows us to address the statistics of the Raman photons emitted, enabling the interpretation of two-color correlations of the emerging photons [4], with potential use in the generation of nonclassical states of light.

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- [3] Y. Zhang, J. Aizpurua, R. Esteban, *ACS Photonics* 7, (2020) 1676-1688.
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## Figures



**Figure 1:** a) Schematics of a plasmonic nanocavity with resonance frequency  $\omega_{res}$ , and a molecule with vibrational frequency  $\omega_{vib}$  located in it. It is possible to explore molecular optomechanics effects in this SERS configuration. b) Schematics of a canonical optomechanical system composed of a Fabry-Perot cavity with resonance frequency  $\omega_{res}$ , and a vibrating mirror with frequency  $\omega_{vib}$ .

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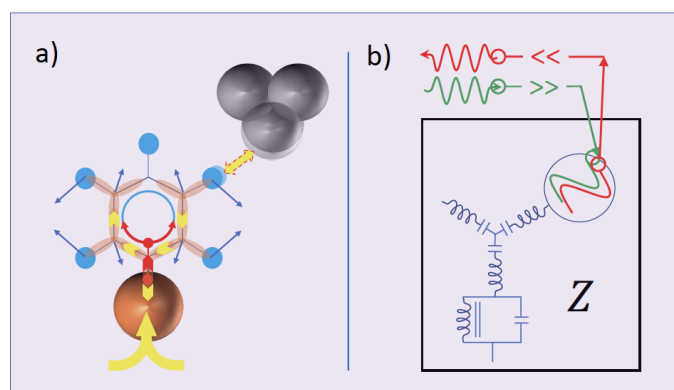
## Direct Wiring of Photons Into Molecules: TERS in the ANF

The atomic limit in optical microscopy was attained through Tip-enhanced Raman scattering (TERS) in the atomistic near-field (ANF). [1,2] By replacing the compound optical microscope with an atomically terminated Ag needle that functions at once as plasmonic antenna, waveguide and focusing lens, an image resolution of 1.6 Å, equaling the radius of the Ag atom, is attained.[1,2] While the images make it clear that light is atomically confined, it is possible to further establish experimentally and theoretically that the photon is atomically confined at the tip apex. Atomically confined photons acquire mass, charge and momentum of an electron, which is measured in tunneling TERS microscopy. In this limit, photon and electron become indistinguishable, optical and electron microscopy merge, and TERS functions as a network analyzer of molecular electronic circuits that can probe atom-resolved point contacts. This is illustrated in Fig. 1a for a pyridine molecule chemisorbed on copper, probed through its meta-CH bond terminal. The equivalent circuit in Fig. 1b is illustrative of a network analyzer. The far field photon impinging on the tip antenna (green wave) is focused into the apex mode and injected into the  $\sigma$ -network of the molecular circuit (yellow arrows), which is interconnected to the  $\pi$ -network (red arrows) through the  $\pi$ -backbond at the copper atom. As in a supply line terminated at an impedance,  $Z$ , the reactive optical current is radiated back into the far field. Vibrational line intensities now measure conductivity of the molecule along paths traced by the normal modes and governed by quantum interference among the open channels. The fundamental processes of electron transport and bond selective intramolecular conduction is for the first time accessible through TERS in the ANF.

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### Figures



**Figure 1:** a) A pyridine molecule chemisorbed on copper, probed with an atomically terminated Ag tip at its meta-CH bond terminal. The photon is injected into the molecule as optical current that connects the Ag tip to the Cu substrate through the intramolecular circuit traced by the normal modes. b) TERS as a network analyzer operating at optical frequency, with the tip acting as transimpedance wire connecting the radiation in the far field to the intramolecular current in the atomistic near-field (ANF).



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## High-sensitivity Raman imaging of cryofixed biological samples

Raman imaging is used in various applications to provide spatial distribution while analyzing molecular components in a sample. In recent years, the improvement in spatial resolution has enabled the acquisition of spectral distributions in complex samples such as cells and advanced studies for new applications of Raman spectroscopy in biological research. However, there have been challenges, such as the weak Raman scattering that makes it difficult to detect low-concentration molecules and to visualize moving samples. In this research, we developed a Raman microscope that can extend the exposure time while maintaining a cellular environment by freezing the cells [1]. On the microscope stage, cell samples were rapidly frozen [2] and measured while keeping the sample at a low temperature. This approach significantly improved the signal-to-noise ratio from prolonged observation time, confirming practical enhancements in wavenumber and spatial resolution. Additionally, due to the high sample stability at low temperatures, the bleaching effect of resonant Raman scattering is reduced, and Raman peaks, which are not observed at room temperature, were observed. Furthermore, this technique can be easily combined with other observation methods, such as fluorescence microscopy, and high-sensitivity and motion-artefact-free observation can be achieved in multi-modal imaging, allowing us to visualize intracellular molecules and chemical environments in biological samples more accurately.

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## Novel Instrumentation for 2D Characterization: Combined Magneto-Optical Magneto-Transport

Raman spectroscopy, imaging, and mapping are powerful non-contact, non-destructive optical probes of quasiparticles and fundamental physics in graphene and other related two-dimensional (2D) materials, including layered, quantum materials. An amazing amount of information can be quantified from the Raman spectra, including layer thickness, disorder, edge and grain boundaries, doping, strain, thermal conductivity, magnetic ordering, and unique excitations such as magnons and charge density waves. Most interestingly for quantum materials is that Raman efficiently probes the evolution of the electronic structure and the electron-phonon, spin-phonon, and magnon-phonon interactions as a function of laser energy and polarization, temperature, and applied magnetic field. Our unique magneto-Raman spectroscopic capabilities will be detailed, enabling spatially resolved optical measurements while simultaneously measuring electrical transport in a back-gated graphene Hall bar device. Raman and electrical data from an hBN-graphene-hBN device operating in the quantum Hall regime will demonstrate our novel capabilities. In addition, unconventional quantization plateaus from a PNP junction created via spatial photodoping by the Raman laser will be presented.

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## On-the-fly Raman microscopy guaranteeing the accuracy of discrimination

We present our recent study combined a multi-armed Bandits algorithm in reinforcement learning with spontaneous Raman microscope for the acceleration of the measurements by designing and generating optimal illumination pattern “on the fly” during the measurements while keeping the accuracy of the discrimination of the sample. Here accurate diagnosis means that a user can determine an allowance error rate  $\delta$  a priori to ensure that the diagnosis can be accurately accomplished with probability greater than  $(1 - \delta) \times 100\%$ . We present our algorithm and our simulation studies using Raman images in the diagnosis of follicular thyroid carcinoma, and show that this protocol can accelerate in speedy and accurate diagnoses faster than the point scanning Raman microscopy that requires the full detailed scanning over all pixels. The on-the-fly Raman image microscopy is the first Raman microscope design to accelerate measurements by combining multi-armed bandit algorithm utilized in the Monte Carlo tree search in alpha-GO. Given a descriptor based on Raman signals to quantify the degree of the predefined quantity to be evaluated, e.g., the degree of cancers, anomaly or defects of materials, the on-the-fly Raman image microscopy evaluates the upper and lower confidence bounds in addition to the sample average of that quantity based on finite point illuminations, and then the bandit algorithm feedbacks the desired illumination pattern to accelerate the detection of the anomaly, during the measurement to the microscope. The realization of the programmable illumination microscope using a spatial light modulator will be presented.

**Acknowledgements:** This research project is supported by Japan Science and Technology Agency (JST) / Core Research for Evolutional Science and Technology (CREST), Grant Number JPMJCR1662, Japan (PI: T. Komatsuzaki, coPI: K.F., Y.H.), JSPS (no.25287105 and 25650044), Grant-in-Aid for Scientific Research on Innovative Areas (Singularity biology) (No.18H05408).

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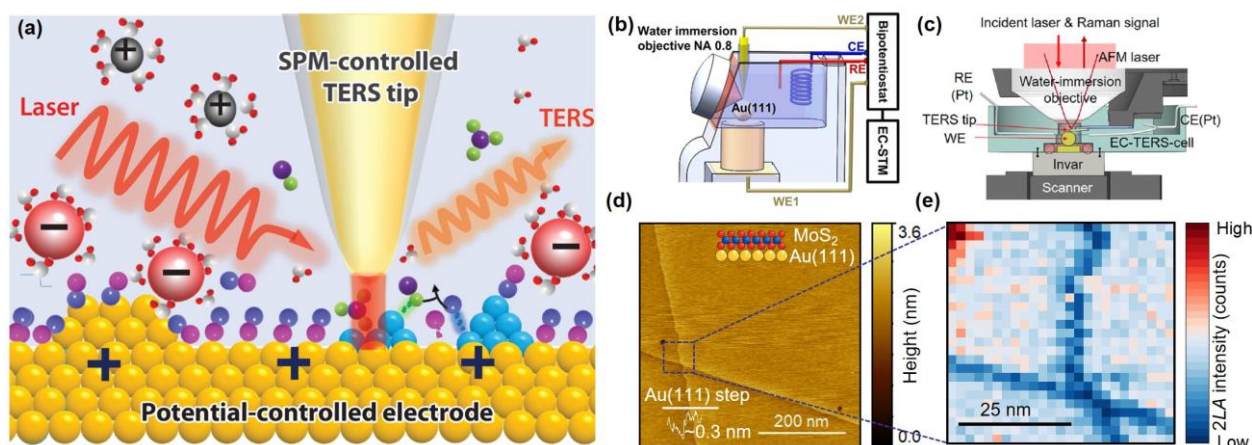
## The road to the successful electrochemical tip-enhanced Raman spectroscopy

Electrochemical tip-enhanced Raman spectroscopy (EC-TERS), which combines electrochemical scanning probe microscopy (EC-SPM) and plasmon-enhanced Raman spectroscopy (PERS), is a powerful technique for the in-situ characterization of the electrochemical interface at the nanoscale and molecular level<sup>[1]</sup>. It is challenging to realize this technique since both a high sensitivity and a high stability are required. In the past 10 years, we have been developing EC-TERS so that it becomes possible to study the electrochemical interface<sup>[2][3][4]</sup>. We introduced water-immersion objectives to EC-TERS to avoid the optical path distortion between air and water, which allowed us to achieve a higher sensitivity. Both home-designed rigid EC-TERS cells and SPM heads were adopted to achieve a high stability. TERS tips with high TERS activity were fabricated by electrochemical method or nanofabrication (focused ion beam, FIB) method. These tips were then carefully insulated or protected for EC-TERS to give a long-time TERS enhancement. Benefited from the above improvements, we achieved EC-TERS imaging with a spatial resolution around 4 nm. With this powerful tool, we successfully probed the spatial distribution of plasmonically excited hot carriers<sup>[5]</sup> and visualized the structural evolution of individual active sites of MoS<sub>2</sub> during hydrogen evolution reaction (HER). These discoveries offer new insights into our understanding of the electrochemical interface and may help the design of more active catalysts.

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### Figures



**Figure 1:** (a) Schematic illustrations of EC-TERS. Side-illumination EC-STM-TERS (b) and top-illumination EC-AFM-TERS (c) setups with water-immersion objectives. (d) EC-STM image of MoS<sub>2</sub> on Au(111). (e) EC-TERS image of the blue region in Figure 1 (d).

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# Raman microscopy meets microfluidics: an integrated approach for rapid diagnosis and cell sorting

Individual phenotypic differences exist ubiquitously in biology. The importance of identifying and isolating key individuals from populations has become increasingly recognized in many fields. While fluorescence-based technologies dominate current methods, the desire not to interfere with the “natural” cell state and/or the lack of known biomarkers makes label-free sorting strategies extremely attractive. In particular, for naturally occurring microorganisms, most of them are not cultivable in the lab, and our knowledge of their specific biomarkers is limited. Single cell Raman spectra provide an intrinsic chemical ‘fingerprint’ of individual cells, which can characterize cell types and metabolic activities. By integrating microfluidics with Raman spectroscopy, we have developed several platforms to study cell populations at the single-cell level. Our approaches enable quantitative and real-time analysis of individual cells without the need for external labelling processes. With this capability, we have recently realized rapid diagnosis of pathogens from sample to results within minutes. We have also developed a series of flow-based Raman-activated cell sorting (RACS) platforms using single cell Raman spectra as a readout for cell identification. Our platforms enable automated, high-throughput isolation of individual cells with desirable traits in a community for downstream culture or genetic analysis. They can be applied to a wide range of samples (from 1  $\mu\text{m}$  bacteria to mammalian cells) and provide a versatile tool for function-based flow cytometry and sorting applications in the fields of microbiology, synthetic biology, life science, and diagnostics..

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## Photonic Data Science: Data pipelines for modeling of Raman effect related data

Raman spectroscopic techniques are increasingly used in various disciplines such as chemical analytics, life science and medicine. This increase in Raman based applications is being driven by improvements in measurement techniques and instrumentation, but also by the development of data science methods. When data science is applied to Raman effect related data the aim is to extract high-level information and knowledge from subtle data differences. The high-level information depends on the task and the sample, e.g., disease types, tissue types and other properties of the samples such as concentrations of constituents. Raman spectroscopy and its related methods have several advantages, for example they can be used as non-destructive fingerprinting techniques. To unlock their full potential the whole spectroscopic data lifecycle needs to be studied. This includes aspects like data generation, data modelling and data archiving. In particular, experimental design, sample size planning, data pre-treatment, data pre-processing, chemometric and machine learning based data modelling, model transfer methods and transfer learning are important. All procedures are sequentially combined in a data pipeline that standardizes the vibrational data and extracts reliable high-level information.

Here, we present our studies researching a standardized data analysis pipeline for biomedical Raman spectra [1] and describe studies dealing with the comparability of Raman spectra between conditions and setups [2,3]. Additionally, we describe our efforts to generate a vibrational spectroscopic data base (VibSpecDB) within the NFDI4Chem project [4].

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# The interplay of spontaneous and stimulated Raman spectroscopy, high-resolution microscopies and the use of machine learning methods in the assessment of the impact of micro- & nanoplastics on human / animal health

## Abstract

The driving force behind this research stems from the growing occurrence of harmful health effects like various sorts of cancer and kidney diseases in both humans and animal models and the potential role of micro- and nanoplastics particles. These effects are now affecting younger individuals more frequently. Additionally, the increasing environmental concerns related to microplastic particles and endocrine-disrupting chemicals (EDCs) add to the urgency of investigating their potential role in the development and advancement of the aforementioned diseases.

To delve into this subject, we align spontaneous and stimulated Raman spectroscopy with high resolution microscopies (with light, electrons, ions) and their analytics with the help of the nanoGPS<sup>R</sup> technology<sup>1,2</sup>.

Together with the use of machine learning algorithms for a quantitative assessment of plastic particles and their cell uptake, existing knowledge gaps will be bridged.

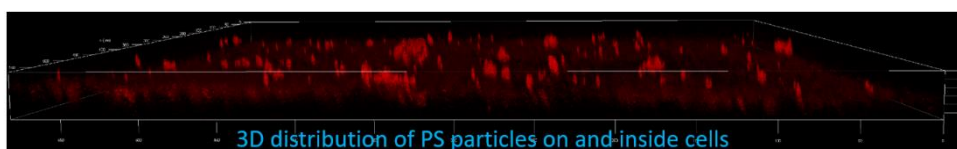
The specific use of mouse models provides a nuanced approach to understanding the intricate dynamics between disease progression and plastics particle presence and the details of the particle size, shape, chemical composition, etc.

The use of the aforementioned innovative analytical technologies in their interplay is crucial to ultimately promote a translation of novel findings into clinically relevant procedures / therapies.

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## Figures



**Figure 1:** Particle localization (polystyrene particles) in and on biological material (individual cultured cell). The 3D Z-Scan was carried out with a Stellaris 8 (Leica) in an area of 581  $\mu\text{m}$  (X) x 581  $\mu\text{m}$  (Y) x 28  $\mu\text{m}$  (Z) with a resolution of X, Y: 1.13  $\mu\text{m}$ , Z: 0.91  $\mu\text{m}$  within a scan time of ~2 min.

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## Surface enhanced Raman spectroscopic (SERS) detection of antibiotics and metabolites in complex biological matrices

Raman spectroscopy is known as powerful analytical tool in biomedical application schemes. Its limitation due to the intrinsic weak Raman effect is overcome by applying powerful plasmonic nanostructures creating the surface enhanced Raman spectroscopy (SERS) technique. SERS became attractive to identify and estimate the trace concentration of biomolecules such as drugs and its metabolites even in complex matrices. [1, 2] To perform SERS investigations, we applied as sensing principle the direct approach, which allows all molecules to contribute to the overall SERS spectrum. The specificity is increased for those molecules with high affinity towards the metal surface.

The bacterium *S. multivorans* is known to form PCE reductive dehalogenase (PceA) within the membrane, which is the key enzyme in respiration of a major groundwater contaminant, perchloroethylene (PCE). PceA harbors B12 which was detected by means of SERS after coating the SERS-active surface with the bacterial membrane. [3]

Further on, the SERS technique was applied to estimate the antibiotic ciprofloxacin in pharmaceutical formulations. For formulations with high background signal, a dilution by 1:5000 was applied and the recorded SERS spectra were only dominated by the contribution of the target ciprofloxacin, which is associated with the strong affinity of this drug towards the metal sensing surface. [4]

Finally, we illustrated the SERS-based detection of pyrazinoic acid (POA), a metabolite of the tuberculosis-relevant prodrug pyrazinamide (PZA). To be specific for POA, gold nanoparticles equipped with a Prussian blue modification were applied, complexing the POA molecules via Fe (II) and allowing its sensitive detection. This scheme has a high potential in assessment of PZA resistance in *M. tuberculosis* bacteria, as only sensitive bacteria convert PZA into POA. [5]

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### Acknowledgements

Funding “EXASENS” (13N13856) and InfectoGnostics (13GW0096F) by BMBF, Germany as well as the project 465289819 (CI 344/3-1) by DFG, Germany is gratefully acknowledged.



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# Discovering Biology with Broadband Spectroscopic Coherent Raman Imaging

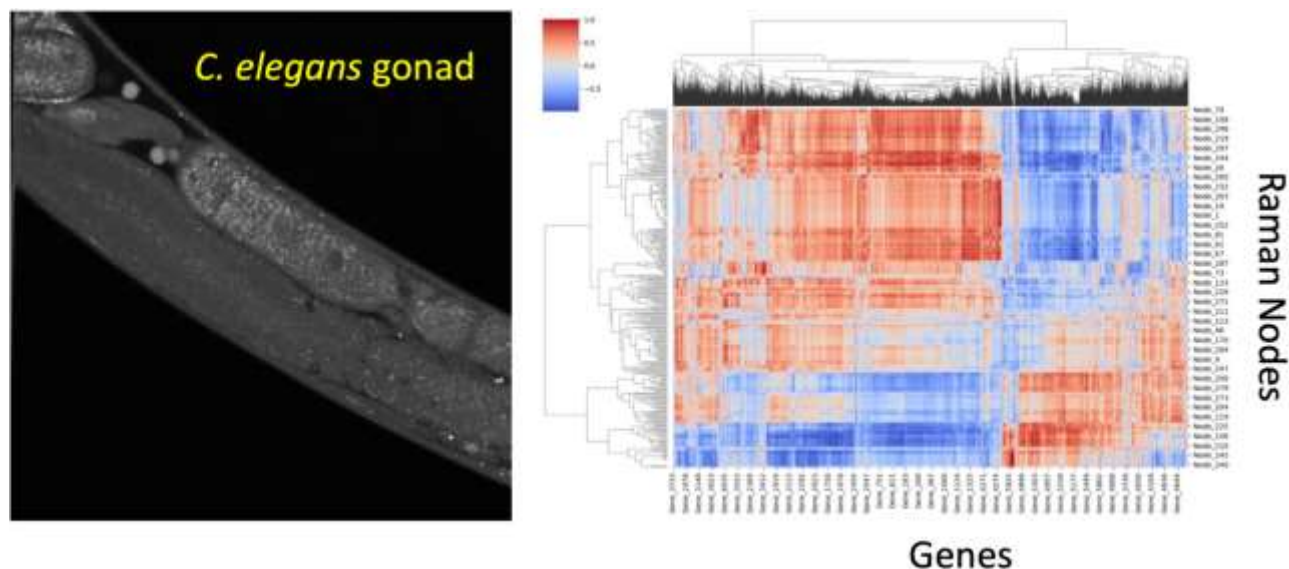
## Abstract

Broadband coherent anti-Stokes Raman scattering (BCARS) microscopy facilitates the rapid acquisition of high-quality, amplitude-normalized Raman spectra, even in delicate samples such as live organisms [1,2]. I will briefly introduce BCARS microscopy, focusing on approaches we have adopted to optimize signal generation with a limited photon budget. I will also show how we have used BCARS to help solve long-standing questions about lipid metabolism in *C. elegans* and about *de novo* organelle biogenesis in human cells. Finally, I'll discuss the potential of BCARS spectra as a rapid, high-spatial-resolution surrogate for transcriptomics.

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## Figures



**Figure 1:** Left: BCARS image of a live *C. elegans* gonad region. Each pixel contains a full Raman spectrum. Right: Co-localization-based correlations between Raman spectral features and transcriptomic activity of specified genes.

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# A learning Raman microscope for high-speed imaging: the compressive Raman concept

Raman imaging is recognized as a powerful label-free approach to provide contrasts based on chemical selectivity. Nevertheless, Raman-based microspectroscopy still have several drawbacks related to its 3D hyperspectral data format and throughput that will soon be bottlenecks when pushing this technology to clinical biomedical and industrial applications scenarios.

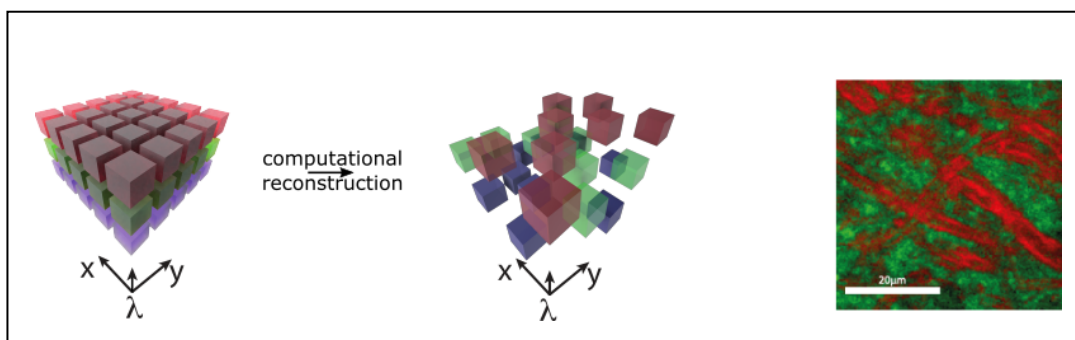
Compressive sensing has shown a paradigm shift approach where one can obtain accurate information from fewer samples than assumed by Nyquist-Shannon sampling theorem. A key concept in compressive sensing is to recognize that data sparsity can be exploited to computationally reconstruct data that has been undersampled. Such approach opens interesting perspectives not only to compress data sets during its acquisition, but also to concomitantly speed up the imaging process as less data is needed to obtain useful information.

In this contribution, I will introduce the concept of compressive Raman imaging: by exploiting sparsity [1] and redundancy [2] in Raman data sets, one can considerably simplify and speed up the spectral image acquisition, nowadays reaching high-speed imaging [3]. I will discuss the different ways of performing compressive Raman, in particular focusing on challenges for bio-imaging, and how we recently tackled them. With these outcomes, compressive Raman imaging soon may be routinely used by non-specialists of vibrational spectroscopy: that is, in a “blind” manner, due to the simpler workflow provided by the compressive Raman imaging framework.

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## Figures



**Figure 1:** (left-center panel) Conceptual description of the compressive Raman framework, where the hyperspectrum is sparsely sampled and the information is reconstructed aided by advanced algorithms. (right panel) one example of biological application of this technology: lipid-rich (red) and protein-rich (green) chemical images of brain tissue.

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# (Nano)spectroscopic fingerprints of strong interactions between 2D layers and their substrate

Common spectroscopic investigation of two-dimensional materials and their van der Waals (vdW) heterostructures mostly relies either on diffraction-limited micro-Raman or photoluminescence (PL). However, these methods do not adequately capture local variations caused by, for example, nanometre-sized heterogeneities ensuing from contamination trapped between the layers or from intricate lattice deformation and charge-doping patterns caused by strong out-of-plane interactions.

Tip-enhanced spectroscopy techniques enable to gather information on the local lattice strain as well as on the interaction between the individual layers forming the heterostructure. What can appear as peak splitting in micro-Raman or PL spectra of vdW heterobilayers or transition metal dichalcogenides (TMDC) on metal substrates, may, actually, often stem from summing up signals from various regions within the laser spot, including new or discretely shifted peaks. In other cases, however, peak splitting can indicate lifting the degeneracy of the phonon, due to, for example, uniaxial deformation. Spectroscopic signatures, both on micro- and nanoscale, of variously interacting vdW layers will be discussed, including TMDCs on metals [1-3] and TMDC heterobilayers [4-6].

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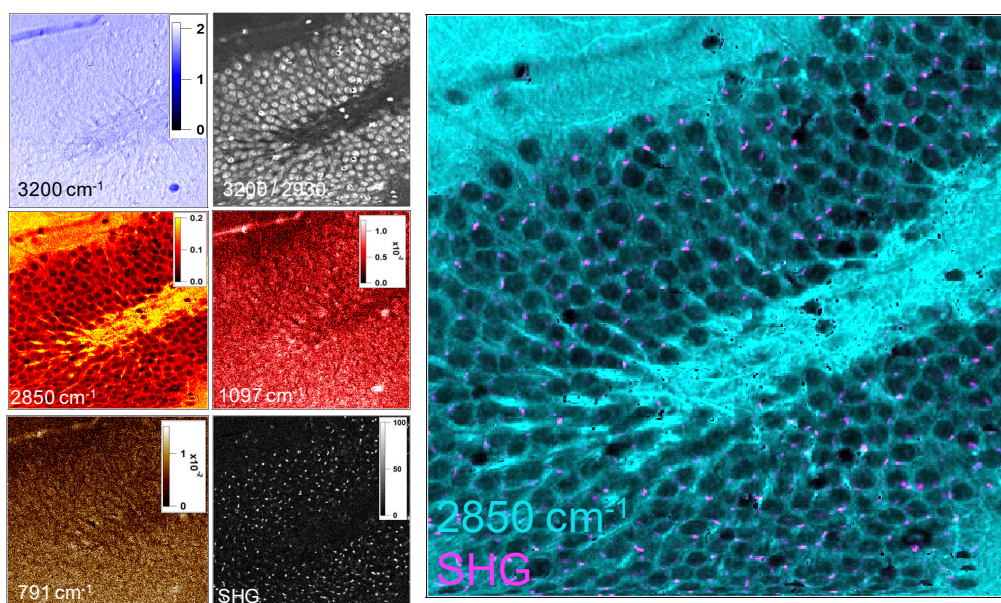
# CARS molecular fingerprinting using a supercontinuum light source !

Spectroscopic imaging, leveraging advanced nonlinear Raman scattering techniques such as coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS), offers a robust approach for label-free microscopic molecular visualization. Notably, multiplex CARS delivers an extensive spectral coverage of around  $3000\text{ cm}^{-1}$ , encompassing the entirety of fundamental vibrational modes [1,2]. This methodology typically utilizes a solitary master laser source paired with supercontinuum (SC) radiation. Within our research, we harnessed a multimodal nonlinear optical microscope to visualize mouse brain tissue, integrating both multiplex CARS and second harmonic generation (SHG) channels. Our imagery provides compelling insight into the hippocampus. Beyond visualizing signals associated with the myelin sheath at  $2850\text{ cm}^{-1}$ , the ratio image of OH stretching ( $3400\text{ cm}^{-1}$ ) to  $\text{CH}_3$  stretching mode ( $2930\text{ cm}^{-1}$ ) distinctly delineates cell nuclei. Moreover, we pinpointed pronounced SHG spots in the dentate gyrus. These SHG spots were identified not only in the dentate gyrus but also in CA1, regions dense with mature neurons. Consequently, we have ascertained them to be Rootletin, an integral molecule of the primary cilium's rootlet.

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### Figure



**Figure 1:** On the left, we have the Multiplex CARS images captured at 3200, 2850, 1097, and 791  $\text{cm}^{-1}$ , as well as the ratio image contrasting 3200  $\text{cm}^{-1}$  to 2930  $\text{cm}^{-1}$ . On the right, there's a composite image blending the CARS visualization at 2850  $\text{cm}^{-1}$  (depicted in cyan) with the SHG image (presented in magenta).

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## Raman spectroscopy to probe the amyloid proteins involved in Alzheimer's disease

Alzheimer's disease (AD) is the most common neurodegenerative disorder and cause of dementia. The disease is pathophysiologically characterized by aggregated amyloid protein, as A $\beta$  (A $\beta$ ). The disease is identified pathologically by amyloid plaques composed of aggregated amyloid peptide, neurofibrillary tangles composed of aggregated, hyperphosphorylated tau protein and neuron loss.

Our group aim at understanding the process of aggregation of A $\beta$ <sub>1-42</sub> and Tau proteins involved in Alzheimer disease, and their interaction with membrane. Tip-Enhanced Raman Spectroscopy is very relevant to probe at the nanoscale the morphology and associated structure with the different species detected during the assembly of the A $\beta$ <sub>1-42</sub> peptide and Tau protein. TERS allows an analysis of the surface of oligomers or fibers at the scale of the single object. [1-3]

We are also interested to determine what damage is created within the tissue in the vicinity of the amyloid plaques of the A $\beta$ <sub>1-42</sub> peptide. To probe this damage, we chose to study brain sections of mice and humans with severe Alzheimer's disease by vibrational microscopy methods. These methods require no labeling and are non-destructive. Fourier transform infrared and Raman imaging on Alzheimer's diseased mice and human brain tissue were performed. Our finding suggests the accumulation of hemes in the senile plaques of both murine and human samples. We compared the Raman signature of the plaques to the ones of various hemeoproteins and to the hemin-A $\beta$ -42 complex. The detected Raman signature of the plaques does not allow identifying the type of heme accumulating in the plaques [4]. With the same approach, by FTIR and Raman imaging, we evidenced a reorganization of phospholipids in brain tissue from AD diseased tissues of mice with severe AD.

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## Multiplex CARS microspectroscopy: advances in instrumentation, data analysis and applications

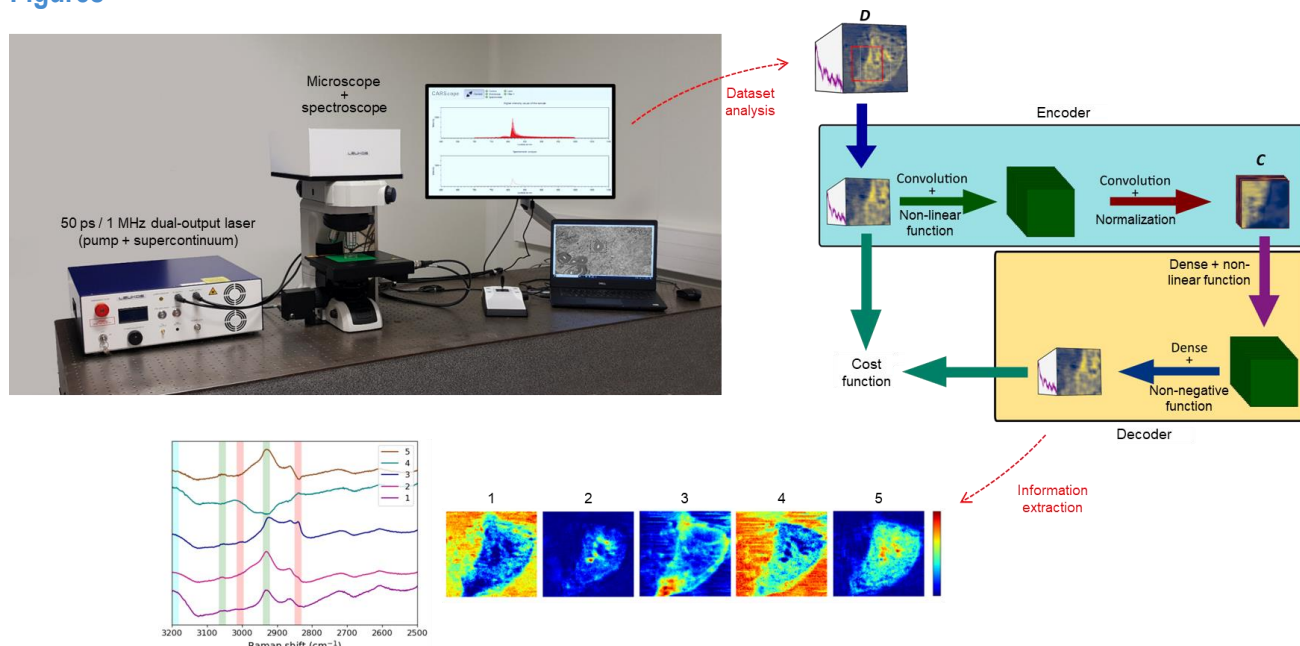
Multiplex coherent anti-Stokes Raman scattering (MCARS) microspectroscopy based on sub-nanosecond supercontinuum excitation [1,2] has become a reliable technology for label-free bioimaging. Owing to recent developments in fiber-based laser systems, the associated instrumentation could be significantly simplified and compactified. In addition, new numerical methods have been introduced to perform the unsupervised analysis of MCARS hyperspectral data, based on multivariate [3] or deep-learning [4] approaches. These advances pave the way for the development of a complete chain of acquisition, processing and analysis of MCARS data (Fig. 1), which should foster applications of coherent Raman technology on a wider scale.

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### Figures



**Figure 1:** Development of a complete chain of acquisition, processing and analysis of MCARS hyperspectral data for biomedical applications.

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## Ramanome, FlowRACS and RACS-Seq/Culture: functional dissection and mining of microbiomes at single-cell resolution

A single cell is the atomic unit of function and the basic step of evolution for life forms on Earth. Thus, a platform to rapidly and cost-effectively profile the “metabolic phenome” and its matching “genome” at single-cell resolution can answer “who is doing what” at the ultimate resolution. To accomplish this mission, we established the Ramanomics Platform (RAMP), which consists of a series of single-cell analysis instruments we invented, including Flow-mode Raman-activated Cell Sorter (FlowRACS), Raman-activated Cell Sorter coupled to Sequencing or Culture (RACS-Seq/Culture), Clinical Antimicrobial Susceptibility Test Ramanometry (CAST-R), etc. By producing high-quality single-cell multiomics data (metabolic phenome, genome, culturome, etc) for both microbiome and human/plant cells, RAMP have ushered in many new industrial and medical applications that demand rapid, low-cost yet in-depth functional understanding of a highly heterogeneous system, such as mechanistic dissection of microbiota function, efficient probiotics-mining from microbiota, culture-free functional mining of enzymes and cell factories from mutant libraries, rapid microbial ID and drug-resistance test for precision medicine, etc.

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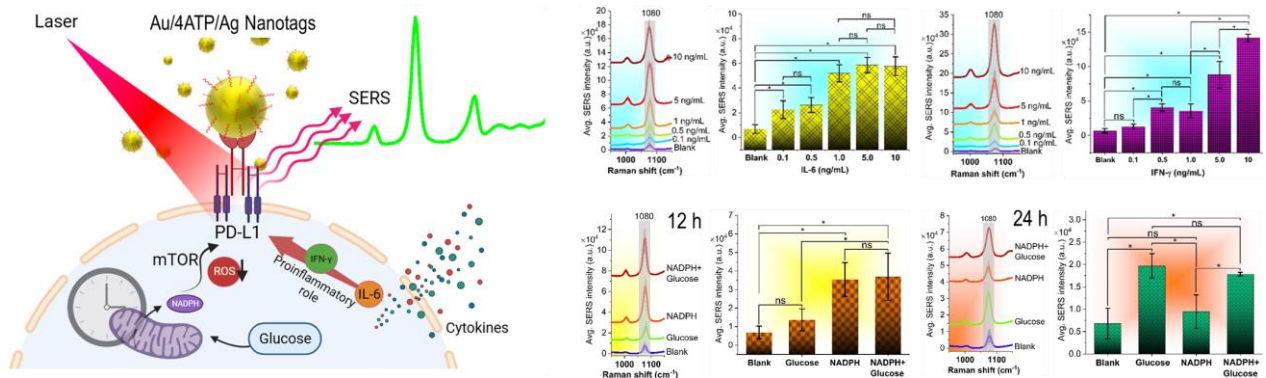
## Unmasking Cellular Secrets: SERS-Enhanced Immunological Biomarkers Studies

In recent years, the expression and progression of programmed cell death ligand 1 (PD-L1) as an immunomarker in the context of cell metabolic environment has gained significant attention in cancer research. However, intercellular bioprocesses which control the dynamics of PD-L1 immunomarker has been largely unexplored. This study aims to explore the cell metabolic states and conditions which govern dynamic variations of PD-L1 expression and progression within the cell metabolic environment using Surface-Enhanced Raman Scattering (SERS). The SERS technique offers a sensitive, rapid, and powerful analytical tool that allows for targeted and non-destructive detection of immunomarker with high-sensitivity and specificity. By combining SERS with cell metabolic state-profiling, we investigated the modulation in PD-L1 expression under different metabolic states including glucose deprivation, metabolic co-enzyme activity, and altered time/concentration-based cytokines availability. The most intriguing features in our findings across different cells include the cell-specific responses, cell differentiation by revealing distinct patterns and dynamics of PD-L1 in HeLa, H1299, and A549 cell lines. Additionally, the time-dependent variations in PD-L1 expression, coupled with the dose-dependent relationship between glucose concentration and PD-L1 levels, underscore the complex interplay between immune checkpoint regulation and cellular metabolism. Finally, the measurement of rapamycin levels as an indicator of mTOR activity adds mechanistic insights, emphasizing the potential for tailored immunotherapeutic strategies based on cell type and treatment timing in cancer immunotherapy.

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### Figures



**Figure 1:** (Left) Schematic representation of SERS nanotags for studying intercellular processes regulating PD-L1 dynamics. (Right) Cytokines, and glucose-NADPH regulated PD-L1 expression detected through SERS.



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# A Large Database Of Raman Spectra Created With Optimized Computational Workflow

## Abstract

Raman spectroscopy is a widely used material analysis technique based on the vibrational properties of materials. Raman spectra provide information about the vibrational modes, atomic structure, and chemical composition of materials but spectrographic analysis relies on comparison to known spectra. Hence, experimental databases of spectra have been collected but limited to well-known materials or the materials may contain significant amount of impurities of unknown identity, for instance.

The spectra can also be simulated using atomistic first-principles methods to complement experimental databases. However, current methods for the simulations of Raman spectra are computationally demanding. Thus, the existing databases of computational Raman spectra contain only a fairly small number of entries.

We present an optimized workflow to calculate the Raman spectra which can reduce the computational cost [1] and takes full advantage of the phonon properties found in existing material databases [2]. The workflow was benchmarked and validated by comparison to experiments and previous computational methods for select technologically relevant material systems.

Using the workflow, we performed high-throughput calculations for a large set of solid materials (5099) belonging to many different material classes and collected the results in a database that can be browsed online on the CRD website [3].

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## Figures

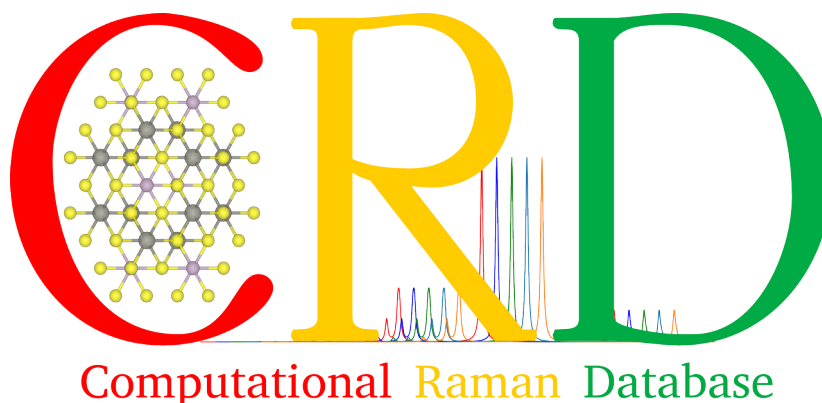


Figure 1: Computational Raman database logo

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# In situ Raman spectroscopy of crystallization: One crystal nucleation at a time

Crystallization is an important topic in science and has many applications in fundamental and industrial research. Understanding the microscopic picture of crystal nucleation is crucial for rational control of crystallization processes and crystal polymorphs. A major challenge for experimentalists to study crystal nucleation is its stochastic and heterogeneous nature at the nanoscale: it is impossible to predict where and when a nucleation occurs. Particularly, probing the details of the nucleation dynamics by optical spectroscopy has been difficult. To this regard, there is a well-established powerful concept to deal with a stochastic, complex and heterogeneous system: Single molecule spectroscopy [1]. The key to bring optical spectroscopy to the field with its full potential is to probe single nucleation event at a time, if we can predict precisely where a nucleation occurs.

Recently, we developed a method called Single Crystal Nucleation Spectroscopy (SCNS) that spectroscopically probes crystallization process in aqueous solution one crystal nucleation at a time [2]. SCNS is based on an extension of optical trapping Raman microspectroscopy combined with optical trapping induced crystallization (OTIC). OTIC allows us to spatially control a single crystal nucleation event (i.e. at a focused laser spot), so that a probe beam can be placed at the same position to track the nucleation process. We achieved measuring Raman spectral evolution of a single glycine crystal formation in aqueous solution with 46 ms time resolution at room temperature.

Even if glycine is the simplest amino acid, the glycine crystallization in solution is subject to a lot of debate on its nucleation pathway: the existence of aggregates and the alteration of polymorphs by addition of additives. Raman spectral evolution during a single glycine crystal nucleation in water measured by SCNS and analyzed by a non-supervised spectral decomposition technique uncovered the Raman spectrum of pre-nucleation aggregates as well as its critical role as an intermediate species in the dynamics. The kinetical data showed that the spectrum of pre-nucleation aggregates were replaced by that of  $\beta$ -glycine which then quickly converted to  $\alpha$ -glycine. The comparison between the Raman spectrum of pre-nucleation aggregates and our simulated spectrum from glycine solutions further suggested that there is a broad structural distribution of glycine linear networks that are held together by hydrogen-bonding interactions, and they play an important role as pre-nucleation aggregates toward glycine crystallization in water.

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## Al foil and silicon vs Gold Film: when more affordable SERS substrates may compete with gold film substrates

SERS (Surface Enhanced Raman Spectroscopy/Scattering) is a sensitive vibrational spectroscopic technique increasingly tested in bioanalytical and biomedical applications. SERS has significant advantages over fluorescence, such as relatively narrow peaks that may be used for multiplex detection, resistance to photobleaching and no need to modify the analyte molecule with fluorophore (label free nature). In spite several 10000s of publications about SERS in the last 40 years, there is still relatively limited number of clinical/analytical application of this method in everyday life. Among challenges to its applications are relatively high cost and low shelf life/stability of substrates, sometimes insufficient reproducibility of performance, including one due to substrate contamination. The sandwich SERS immunoassays have used gold film as a default substrate by at least several research groups. Our research group tested silicon and then Aluminum foil as the substrate for this sensitive bioanalytical method. Silicon and aluminum foil are not only far more economical substrates than gold, but also substrates that has demonstrated at least comparable LOD and better selectivity / lower non-specific signal in detection of human IgG on Al foil and on Si wafers in comparison to the same performance parameters in simultaneous assays on gold film. The improvement in sensitivity is likely to come from a decrease in nonspecific protein binding to the surface of aluminum and silicon relative to this kind of binding to the gold film surface. We also studied the impact of nanoparticle dimerization on SERS enhancement on various substrates including gold, silver, Al and silicon, using a novel method of AFM/SEM and Raman map combination, published in *Nanoscale Advances*.

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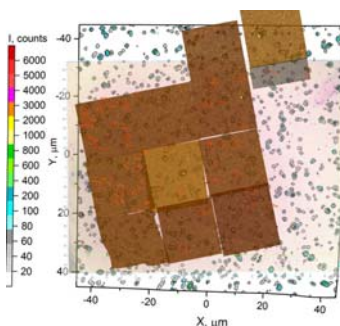


Figure 1: Combination of AFM and Raman + confocal Microscope maps for gold nanoparticles modified with C12 mercaptocarboxylic acid and Raman marker (2-Methoxythiophenol)

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# Extreme heat and strain gradients in 2D material measured by Raman spectroscopy and vibrations

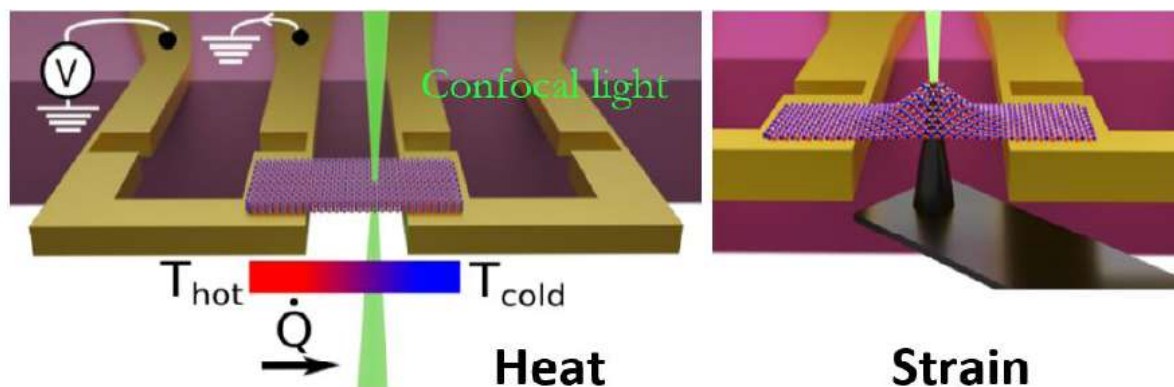
**2D materials heterostructures are atomically thin materials, sandwiched together, to form new devices and concepts.** It is the case in electronic, with subthermionic transistors, neuromorphic or memristive devices, and even transistor integration reaching the Si benchmark. but it also covers many potential topics: water sieving, exciton condensation, chiral quantum source emitters, twistrionic, atypical thermal transport, and more recently straintronics. 2D thermal phonons, 2D vibrations or 2D straintronic engineering are important and fascinating emerging topics in the field of 2D materials and 2D heterostructures. **Straintronics in 2D materials** is the modulation and control of the 2D properties through macro- and nanoscale strain engineering. Straintronics is used for optical response tuning or the creation of quantum emission sources. This is especially interesting since 2D materials can withstand more than 16% strain (theory) and the excitonic energy is shifted by 125meV/% of strain. **The engineering of heat transport** remains a key point in miniaturized devices. Integrated circuits and thermal management strategies require materials with high thermal anisotropy, for example, for heat spreaders which can eliminate hot spots along the direction of the fast axis and provide thermal insulation along the slow axis. 2D materials have a record thermal anisotropy of 900, with room for improvement.

**Theoretical deformation and heating limits applied to 2D materials are generally well beyond the scope of standard experimental tools . However, closing the gap between such theoretical predictions and experimental limit is crucial to benchmark 2D materials and integrate them optimally in novel devices. This are the activities that we propose to discuss (1,2,3).**

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### Figures



**Figure 1:** (left) Sketch of the strain/heating platform where two independent Silicon heaters can apply a calibrated heat gradient (>600K) along a suspended 2D nanomaterial. (right) A metallic tip is used to indent locally a 2D membrane by 10%. Optical methods, such as Raman spectroscopy, photoluminescence or optomechanic, are used to perform thermal and strain measurements.

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## Novel biocompatible nanoprobes for multimodal optical imaging via SERRS and fluorescence effects

**Context and Objective:** Anisotropic gold systems as nanoflowers (AuNF) are the object of intensive studies, due the unique plasmonic properties they provide that can be used in various fields, including biomedical applications. The ability of AuNFs to interact with near-IR light makes them attractive as optical imaging agents, since biological tissues are more transparent to the light in the near-IR region. In the present study, we aimed to develop AuNF-based aqueous colloids able to provide aggregation-free surface-enhanced resonance Raman scattering (SERRS) effect in NIR, due to both the tip effect on their petals and "hot spots" in the junctions between the petals (Fig1), but also fluorescent in the NIR region.

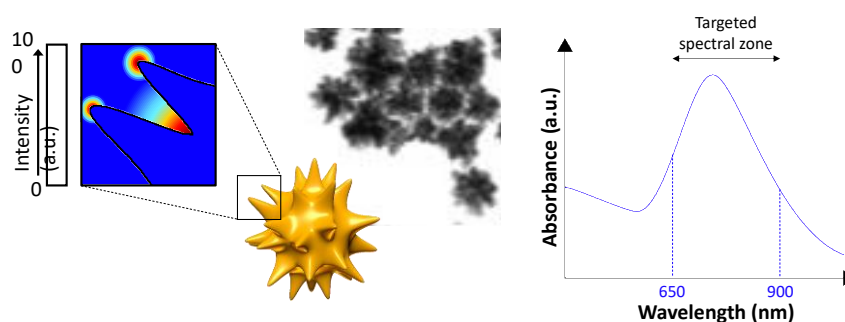
**Methods:** We have recently developed a new protocol of synthesis of the AuNF (1) which surpasses practically all the limits of the protocols published before: it is fast, single-step and uses only a small number of known reagents. In addition, our protocol allows to control the characteristics of AuNF such as the size and the position of their LSPR band, between 600 and 900 nm (Fig 1). To improve their stability in various media, in the present study, we coated our AuNF with various biocompatible materials. The physico-chemical characterization of the hybrid nanosystems was carried out using TEM (transmission electron microscope), DLS (dynamic light scattering) and UV-visible spectroscopy.

**Results and Conclusions:** We studied the effects of the coatings on the intensity and shape of SERS spectra of a fluorescent chromophore Blue Nile (NB) emitting in the near infrared. The colloidal substrates based on our AuNFs coated with biocompatible shells have shown their potential to provide the SERS effect without aggregation and to allow ultra-sensitive analysis of small chromophores. Their detection in cells has been demonstrated, using several optical imaging modalities, namely SERRS and fluorescence in NIR. In addition, our results show that these new substrates are able to deliver a molecular cargo to cancer cells. Thus, they appear promising as versatile agents, for multimodal optical imaging and also for drug delivery.

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### Figures



**Figure 1:** Left: schematic presentation of the tip effect and of the hot spot formation on a plasmonic nanoflower. Middle top: TEM image of AuNFs (diam. ca 100 nm). Right : spectral zone of the tunable plasmonic resonance of AuNF.

## Nicolás Coca-López<sup>1</sup>

Athanasia Maria Moustaka<sup>2</sup>, Aino Nielsen<sup>2</sup>, Enrique Lozano Diz<sup>3</sup>, Søren Birk Rasmussen<sup>2</sup>, Raquel Portela<sup>1</sup>, Miguel A. Bañares<sup>1</sup>, Pablo Beato<sup>2</sup>

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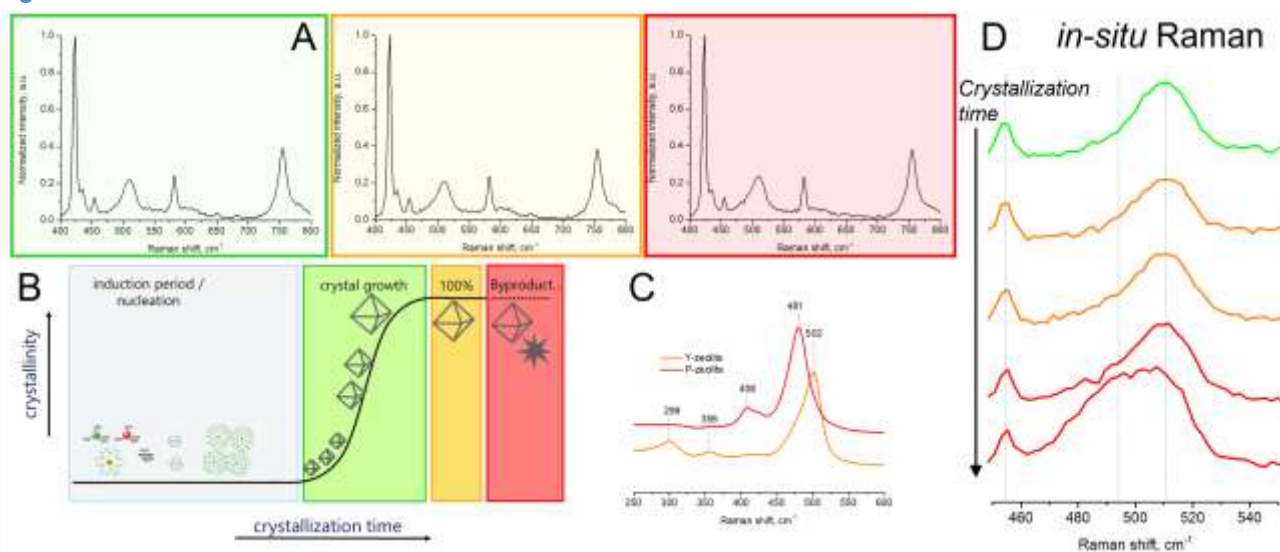
# Raman spectroscopy for in-situ characterization of interzeolite conversion during synthesis

Interzeolite conversion (IZC) is a common phenomenon occurring during zeolite synthesis that can lead to undesirable byproducts [1]. Commercial production of metastable zeolites relies on ex-situ X-ray diffraction (XRD) for synthesis monitoring. XRD allows to quantify the fraction of different zeolite structures [2]. However, this requires sample extraction, preparation, and analysis by qualified operators, which is inconvenient, time consuming and prevents from stopping the reaction at the optimal moment. Here we propose Raman spectroscopy as alternative, in-line, process control technology to speed up sample analysis and simultaneously probe solid and liquid phases. Chemometric analysis of the Raman spectra allow to establish a direct quantitative relation between in-situ Raman and ex-situ XRD data, and therefore obtain similar information. Furthermore, we show this use case to demonstrate harmonization between different Raman spectroscopy systems.

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## Figures



**Figure 1:** Raman spectroscopy spectra during different crystallization stages. The color code (green = early stage; orange = desired product; red = product of IZC) is maintained for the different figure panels. *In-situ* Raman spectra during different crystallization stages (A); schematic overview of crystallization process (B); *ex-situ* Raman spectra of Y zeolite (desired product) and P-zeolite (IZC product) (C); *in-situ* Raman spectra zoom into zeolite relevant spectral area (D).

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## Coic Laureen

Raffaele Vitale, Cyril Ruckebusch

Address: U. Lille, CNRS, LASIRE, Laboratoire Avancé de Spectroscopie pour les Interactions, la Réactivité et l'Environnement, Cité Scientifique, F-59000 Lille, France

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# Accelerating confocal Raman microscopy by capturing Essential Information in the Fourier Domain (EIFD)

Point-by-point Raman confocal hyperspectral imaging is a widespread technique through which both spectral and spatial information can readily be extracted, allowing one to have a global understanding of the samples under study. However, when the sample surface to analyze is large (i.e., for pharmaceutical specimens), its characterization may require from few hours to days, hampering specific analytical applications which require fast identification (i.e., falsified medicines) or can induce photodamage. To circumvent these issues, we propose an approach based on the assessment of Essential spectral Information in the Fourier Domain (EIFD) which permits to enhance confocal Raman imaging speed by 50-fold compared to classical point scanning [1]. The set of essential spectral profiles selected encompass the most linearly independent (dissimilar) spectra measured and the sole use of these spectra enables the reproduction of the collected data in a convex linear way [2-3]. The selection of EI has proven to be a very useful and reliable data reduction tool for linear spectral unmixing, it brings advantages in terms of speed and compression and allows challenging analytical issues such as the detection of very minor species to be tackled [4]. In EIFD, EI is evaluated from a convex hull analysis of the data point cloud in the 2D phasor plots displaying the Fourier coefficients estimated from the registered spectra for a few selected harmonics. One of the major assets of EIFD is that essentiality can be assessed independently for each spectral pixel and potentially as the data acquisition is ongoing. This paves the way to acquisition processes that would be based on the EI content of a spectral pixel and that would speed up the collection at those locations where information is not essential, considerably accelerating the whole imaging procedure. To illustrate our approach, we show outcomes obtained for several Raman hyperspectral imaging datasets resulting from the analysis of pharmaceutical samples of varying complexity (from handmade to commercial tablets) and outline a new data acquisition scheme relying on targeted sampling based on EIFD that could represent a real breakthrough in the domain of Raman confocal hyperspectral imaging. As an example, such a scheme permits to decrease the time required for the collection of a 101x101x293 Raman hyperspectral image from 14 hours to 28 min by identifying the essential information encoded in this image in the presence of noise and by increasing the spectral accumulation (and, thus, the spectral resolution) only when essential pixels are found. We show that in this way we are able to obtain very similar chemical information compared to when a standard analysis on the entire sample is conducted. Another advantage of EIFD is that it is broadly applicable since it is based on spectral frequencies and could be implemented to perform the acquisition of EI on-the-fly, opening the doors to the characterization of photosensitive biological systems.

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**Haolei Dai**

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# Enhanced Double Resonance Raman Scattering in Multilayer Graphene with Broadband Coherent Anti-Stokes Raman Spectroscopy

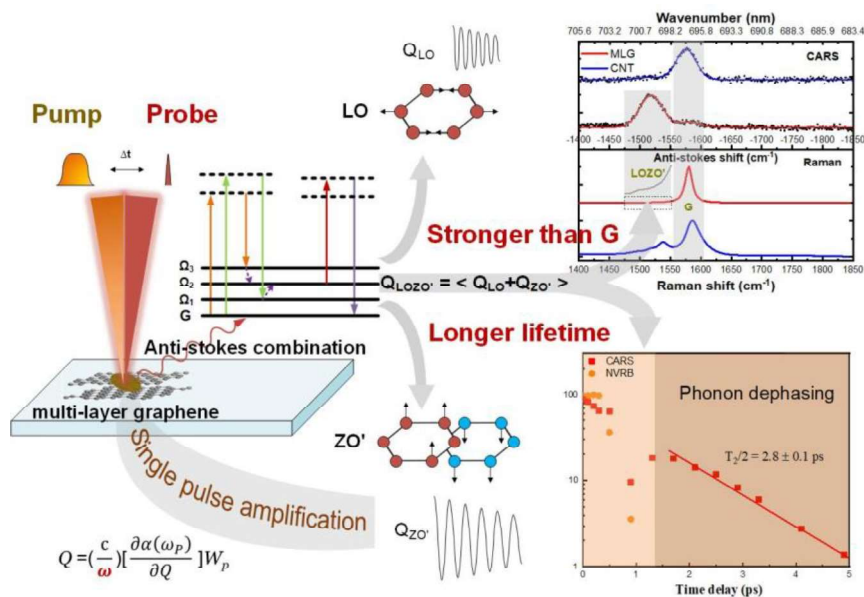
**Abstract**

Graphene's unique gapless band structure and remarkably large third-order optical susceptibility have drawn significant attention to its nonlinear optical response, particularly in the context of coherent anti-Stokes Raman scattering (CARS). Under the combined influence of phononic and electronic resonances, the CARS response of graphene has been observed to exhibit a distinctive feature of time-resolved dip-to-peak evolution. Here, we report a greatly enhanced double resonance Raman mode beyond G mode of multi-layer graphene with broadband CARS measurements. The significant difference in intensity ratio between CARS and SR for this mode may be attributed to the preferential activation of low-frequency phonons in the impulsive stimulated Raman scattering process (ISRS) and a lower dephasing rate. Our results build on foundation towards a deeper exploration of coherent Raman response of two-dimensional materials.

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**Figures**



**Figure 1:** Table of Contents



**Mohamed Elgawish**

Sang-Hee Shim

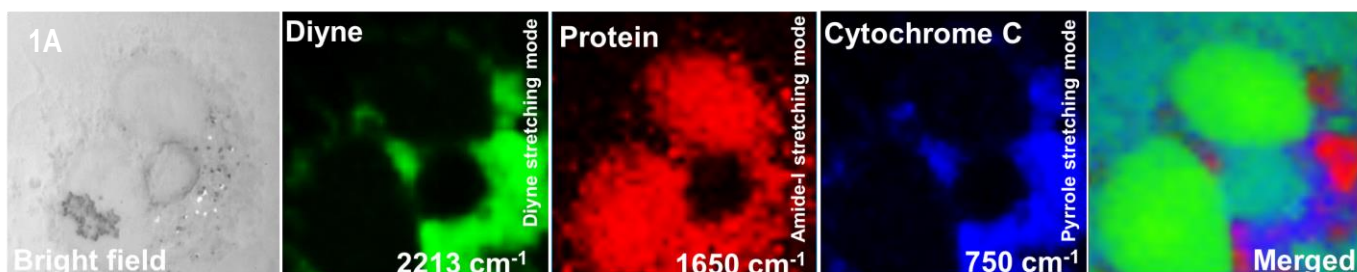
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[Mohamed\\_elgawish@korea.ac.kr](mailto:Mohamed_elgawish@korea.ac.kr)   [sangheeshim@korea.ac.kr](mailto:sangheeshim@korea.ac.kr)

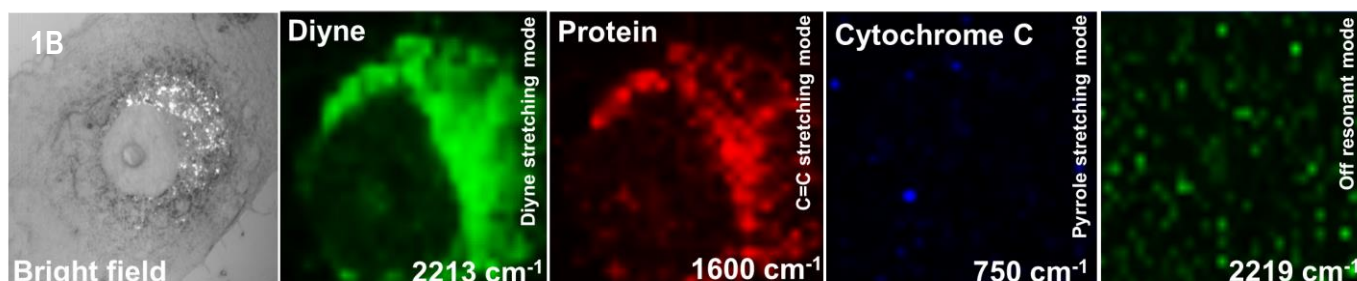
## A genetically encoded Raman probes for organelle-specific labeling: unleashing the power of Raman microscopy for bioimaging

Raman microscopy, with its spectral sensitivity to local electric fields, holds promise for probing intracellular environments. Yet, the weak Raman scattering signal, about  $10^{10}$  times weaker than fluorescence emission, has limited its use in molecular profiling of intracellular organelles. To overcome this, we combined proximity labeling with spontaneous Raman microscopy, using APEX2 for site-specific labeling of the Raman probe. This approach precisely locates new diyne probes within organelles, offers a strong signal in the cell silent window ( $1800\text{-}2800\text{ cm}^{-1}$ ), and maintains photostability. It enables rapid imaging, outperforming conventional confocal Raman microscopy in acquisition time. Organelle-specific Raman images are generated in under 5 minutes, relying on the  $2213\text{ cm}^{-1}$  peak intensity of polyiine probe. Colocalization with the pyrrole breathing mode of cytochrome C validates Raman mapping specificity (**Figure 1A**). The diyne stretching mode signal is an order of magnitude stronger than cytochrome C pyrrole breathing mode and EdU alkyne mode, resulting in shorter acquisition times and lower laser power for live cell imaging (**Figure 1B**).

### Figures



Acquisition time: 1s, Accumulation 2, laser power:  $\sim 10\text{ mW}/\mu\text{m}^2$ , exposure time: 42 m.



Acquisition time: 0.25s, Accumulation 2, laser power:  $\sim 3.25\text{ mW}/\mu\text{m}^2$ , exposure time: 11:42 m

**Figure 1:** Bright field images and Raman images show colocalization of both diene stretching mode and the pyrrole breathing mode of cytochrome C at mitochondria and confirm the strength of diene signal at very short measuring time

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# How to get Raman spectra harmonization from twinned devices

Raman spectroscopy is increasingly widespread in different industrial applications as it allows characterizing advanced materials in different industrial stages: from design and manufacturing, to performance, end of life and recycling, improving the quality of products and processes. Unlike the calibration protocols, the intercompatibility among different Raman devices in terms of comparability, reproducibility and reliability of the data, it is an aspect not sufficiently explored in this characterization technique [1,2]. For this reason, in this work, a harmonization protocol has been developed for the first time, that allows defining a correction factor (CF) to twin two different Raman spectrometers. This CF is a constant that includes all the differences that exist between both spectrometers such as spot size, density power, optical path, detector. From it, harmonized spectra measured from different Raman spectrometers have been obtained.

For the development of this protocol, a homogeneous reference sample with a high compositional control has been manufactured with a composite material of epoxy and 0.5% by wt. of anatase TiO<sub>2</sub> particles. The protocol consists of different steps for each Raman twinning, that allow obtain the CF calculating the slope of the regression line of the intensity of the main TiO<sub>2</sub> band at different laser powers in each Raman device.

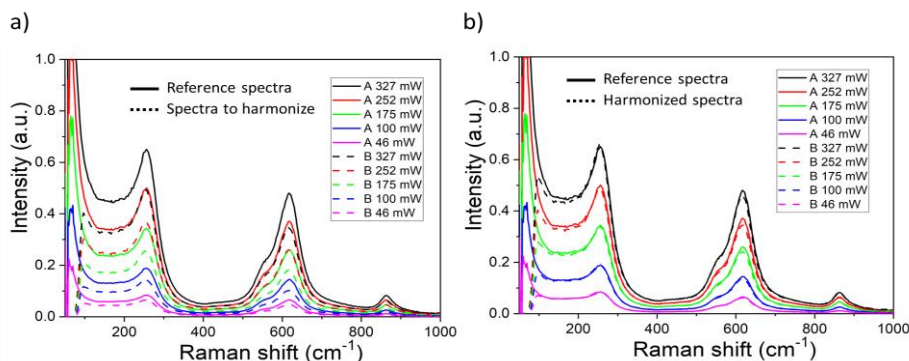
The study was performed in two different devices with different optical paths and wavelengths. Figure 1 shows an example of K<sub>0.5</sub>Na<sub>0.5</sub>O<sub>3</sub> (KNN) spectra harmonization. In Figure 1a it can be seen the reference spectra (A) and the spectra to be harmonized (B) at different laser powers measured with a 785 nm laser showing different intensities. After multiplying the spectra B by CF, both spectra are harmonized in terms of intensities (Figure 1b). The similarity between reference and harmonized spectra is greater than 95% for all laser powers, proving that high quality of harmonization can be obtained with this new protocol.

*Acknowledgment:* EU H2020 Project CHARISMA under Grant Agreement no. 952921.

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## Figures



**Figure 1:** KNN spectra of spectrometer A and spectrometer B before (a) and after (b) Raman harmonization.

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# **Synthesis of silver nanoparticles used as SERS Raman substrate , analysis of various organic samples and first tests on micro plastics**

The following work aims to develop the synthesis of silver nanoparticles used as a substrate for Micro Raman SERS analysis of various organic samples (antimicrobial) and first tests on micro plastics. There are many advantages of the Raman technique, however the limit is linked to the inelastic scattering process, its cross section is approximately 10 orders of magnitude smaller than the absorption cross section.

To address this problem, SERS spectroscopy (Surface Enhanced Raman Spectroscopy) was subsequently introduced which allows the sensitivity to be increased by several orders of magnitude and consequently obtain a more intense signal.

The amplification of the Raman signal is possible thanks to the interaction of the incident electromagnetic radiation with a metallic substrate (usually gold or silver) near the sample. In particular, we propose the development of a simple and easily reproducible synthesis of spherical silver nanoparticles which act as a metal substrate and allow the intensity of the Raman signal to be significantly increased and the concentrations of molecules of the order of nanomolar to be detected. and lower the fluorescence sometimes induced by the sample.

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## Raman and thermoelectrical study of thermally and flash lamp annealed Cu<sub>2</sub>ZnSnS<sub>4</sub> nanocrystals

The increasing demand for electrical energy has led to searching for new, alternative energy sources as well as to improving existing ones. Solar energy is one of the most promising alternative energy sources. There are huge progress and developments in this field during the last decade, but one of the main issues that should be overcome to increase lifetime and efficiency of solar panels is overheating. Around 70% of the absorbed solar energy are converted to waste heat requiring additional efforts for proper cooling systems. One of the approaches to solar panel cooling and conversion of the waste heat into additional electrical energy is hybrid photovoltaic-thermoelectric devices. Accordingly, materials with both good thermoelectric and photovoltaic properties have gained popularity in recent years. One of such materials is Cu<sub>2</sub>ZnSnS<sub>4</sub> (CZTS) and related compounds. It is quite promising due to the fact that all of the constituent elements are earth abundant and environmentally friendly. The CZTS-like compounds have been intensively studied for photovoltaic applications and the ability to produce CZTS nanocrystals (NCs) by „green” synthesis in colloidal solutions makes it even more attractive for use in so-called third-generation photovoltaics [1]. In addition, recent studies show good thermoelectric properties for CZTS-like materials [2]. This fact makes CZTS NCs very promising for hybrid photovoltaic-thermoelectric devices and can enable an improved overall energy conversion efficiency. In order to improve the properties of CZTS NCs in terms of crystallinity after their synthesis and thin film deposition, one of the most common methods is thermal annealing. However, thermal annealing can cause changes in the NC films, which are deteriorating the photovoltaic performance, namely the appearance of Cu<sub>x</sub>S secondary phases. In addition, thermal annealing usually requires extended time durations of tens of minutes and inert atmosphere to avoid oxidation. Another technique proved to be very promising for CZTS NCs treatment is flash lamp annealing (FLA). Due to duration of about 18 ms and tuneable energy densities, it is possible to improve the crystal quality and avoid formation of oxides and secondary phases even in ambient atmosphere [3].

Here, using Raman spectroscopy we investigated and compared the effects of thermal annealing up to 350 °C and FLA treatment up to 12 J/cm<sup>2</sup> on spin-coated thin films of CZTS NCs obtained by “green” colloidal synthesis. In the temperature range up to 200 °C Raman spectroscopy provides clear evidence of improved crystalline quality, while atomic force microscopy additionally reveals improved surface morphology. However, the electrical conductivity of CZTS NCs films remains very low and not sufficient for proper Seebeck coefficient measurements. At annealing temperatures of 250 °C and 300 °C, Raman spectroscopy shows the appearance of a Cu<sub>x</sub>S secondary phase and a pronounced shift of main CZTS NCs characteristic feature. A clear correlation between the Raman spectra and the thermoelectrical properties for thermally annealed samples is observed. Raman spectra for FLA treated samples, however, reveal only slight improvement of crystallinity in the whole range of studied energy densities and no significant changes in thermoelectrical properties [4]. Using higher FLA energy densities (> 15 J/cm<sup>2</sup>) leads to the appearance of other secondary phases than Cu<sub>x</sub>S or even full decomposition of the CZTS NCs. In such a way, Raman spectroscopy illuminates the differences between the two distinct annealing techniques applied to CZTS NCs.

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[4] Ye. Havryliuk, V. Dzhagan, A. Karnaukhov, O. Selyshchev, J. Hann, D.R.T. Zahn, *Nanomaterials*, 11 (2023), 1775

## Sebastian Heeg

Roman M. Wyss, Günter Kewes, Martin Frimmer, Karl-Philipp Schlichting, Markus Parzefall, Eric Bonvin, Martin F. Sarott, Morgan Trassin, Lala Habibova, Giorgia Marcelli, Jan Vermant, Lukas Novotny, Mads C. Weber, Sebastian Heeg  
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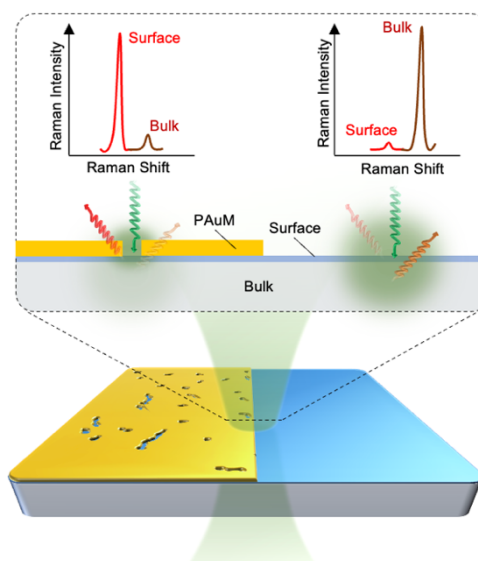
# Surface-Sensitive and Bulk-Suppressed Raman Scattering by Transferable Nanoporous Plasmonic membranes

Raman spectroscopy is a powerful technique to characterize materials. It probes non-destructively chemical composition, crystallinity, defects, strain and coupling phenomena. However, the Raman response of surfaces or thin films is often weak and obscured by dominant bulk signals. Here we overcome this limitation by placing a transferable porous Au membrane (PAuM) on top of the surface of interest. Slot-like nanopores in the membrane act as plasmonic slot antennas and enhance the Raman response of the surface or thin film underneath. Simultaneously, the PAuM suppresses the penetration of the excitation laser into the bulk, efficiently blocking the bulk Raman signal. Using graphene as a model surface, we show that these two simultaneous effects increase the surface-to-bulk Raman signal ratio by three orders of magnitude. We find that 90% of the Raman enhancement occurs within the top 2.5 nm of the material, demonstrating truly surface-sensitive Raman scattering. To validate our approach, we analyze the surface of a  $\text{LaNiO}_3$  thin film. We observe a Raman mode splitting for the  $\text{LaNiO}_3$  surface-layer, which is evidence that the surface structure differs from bulk. This shows that PAuM give direct access to Raman signals of surfaces and their structural properties.

## References

Roman M. Wyss, Günter Kewes, Martin Frimmer, Karl-Philipp Schlichting, Markus Parzefall, Eric Bonvin, Martin F. Sarott, Morgan Trassin, Lala Habibova, Giorgia Marcelli, Jan Vermant, Lukas Novotny, Mads C. Weber, Sebastian Heeg, <https://arxiv.org/pdf/2301.04054v1.pdf> under review (2023)

## Figures



**Figure 1:** Nanoporous Au membranes enhance the surface Raman signal and suppress the bulk Raman signal

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## Searching for micro-Raman LoD: The case of Tiotropium Br in Spiriva® and Braltus® formulations.

One of the advantages of micro-Raman spectroscopy is the point-by-point mapping resulting in higher sensitivity for the identification of low-content ingredients. According to International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), the common approaches used for LoD determination are based on visual evaluation, signal-to-noise or on the standard deviation of the response and the slope [1]. In micro-Raman mapping spectroscopy though, numerous spectra are collected in order to possibly identify one spectrum of a component at low concentration and thus, ICH methods are not expected to be adequate.

Currently there is no established method for the determination of the LoD using micro-Raman mapping spectroscopy in solids (mRMLD). Also, there is no established methodology for the optimization of the scanning parameters taking into account factors such as the nature of the components, the particle size and the dispersion of the API in excipients (manufacturing process) that can influence the mRMLD. Several and different attempts for the LoD calculation of micro-Raman mapping were previously performed. In some studies, the LoD was extracted from the construction of calibration line from known calibrants using multivariate methods but an LoD range was calculated and not as an absolute value [2]. Others are more often based on the visual evaluation of the detected impurity at extremely low but known concentrations. The lowest recorded LoD between two polymorphs was found at 0.01%w/w [3] although the correlation between API particle size and lens or step was not checked. Such shortcomings were addressed in this work in an effort to establish a methodology/protocol for determining the mRMLD.

As case study the Spiriva® inhalation spray (Boehringer Ingelheim) was selected which contain only 0.4 w/w% of tiotropium bromide. This API exists in two forms, as anhydrous and as monohydrate. The X-Ray diffraction technique, which is usually used for polymorph discrimination, could not be applied successfully due to the extremely low content of the API in the formulation. Tiotropium Bromide is present in the Spiriva® formulation in its monohydrate form. In order to establish the mRMLD the experimental parameters were optimized e.g. step size, scanning mode, objective lens, laser power and accumulations, by taking also into account the API particle size, area scanned, manufacturing process and Raman spectra and the sample was scanned using a long-time map. After the mapping, the value obtained from the Concentration of each of the API forms (monohydrate and anhydrous) in the test sample divided by the Number of spots (spectra) of the form detected, corresponded to the respective mRMLD. This was verified by appropriate sample dilutions and found to be inversely proportional to the number of scans. Micro-Raman mapping spectroscopy was capable to detect and discriminate between the two tiotropium bromide forms. The method was applied also on Braltus® formulation which contains anhydrous API and the manufacturing method includes spray dry instead of Spiriva's® direct mixing.

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Figures

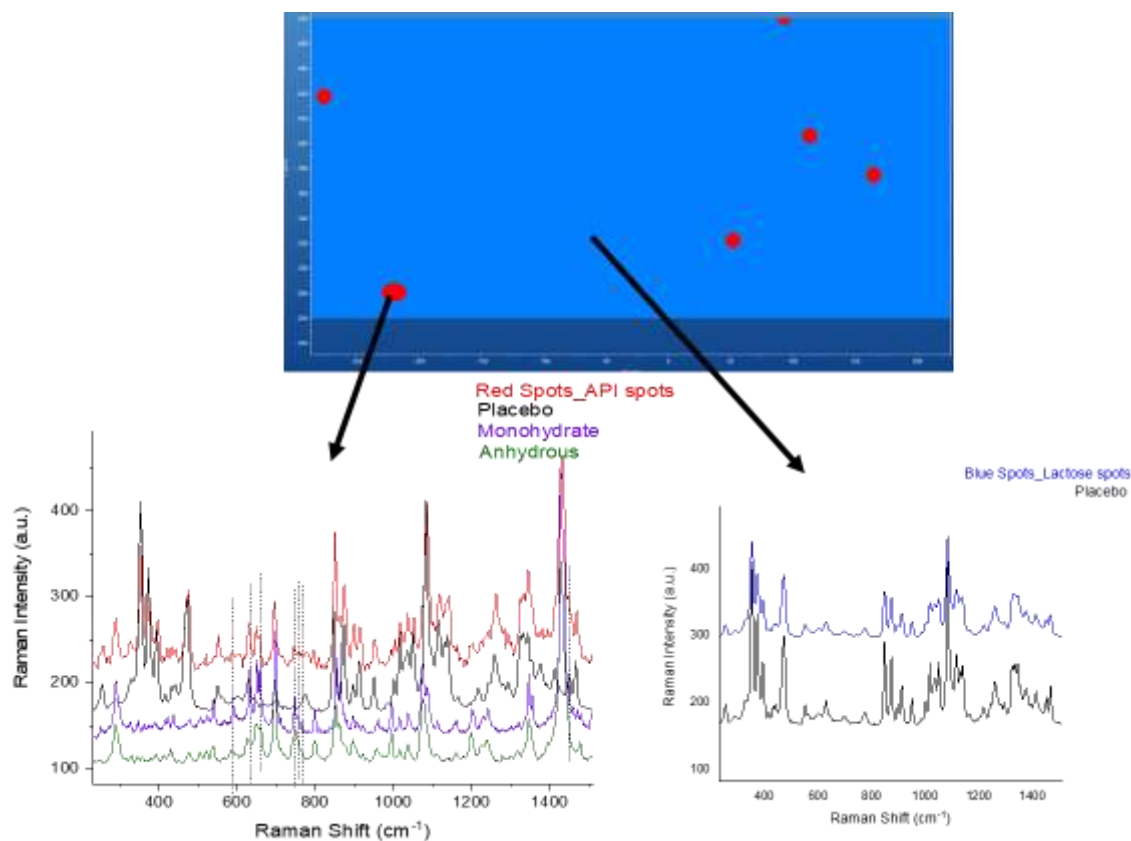


Figure 1: Typical Raman map of Spiriva® inhalation spray and the Raman spectra of the two present components.

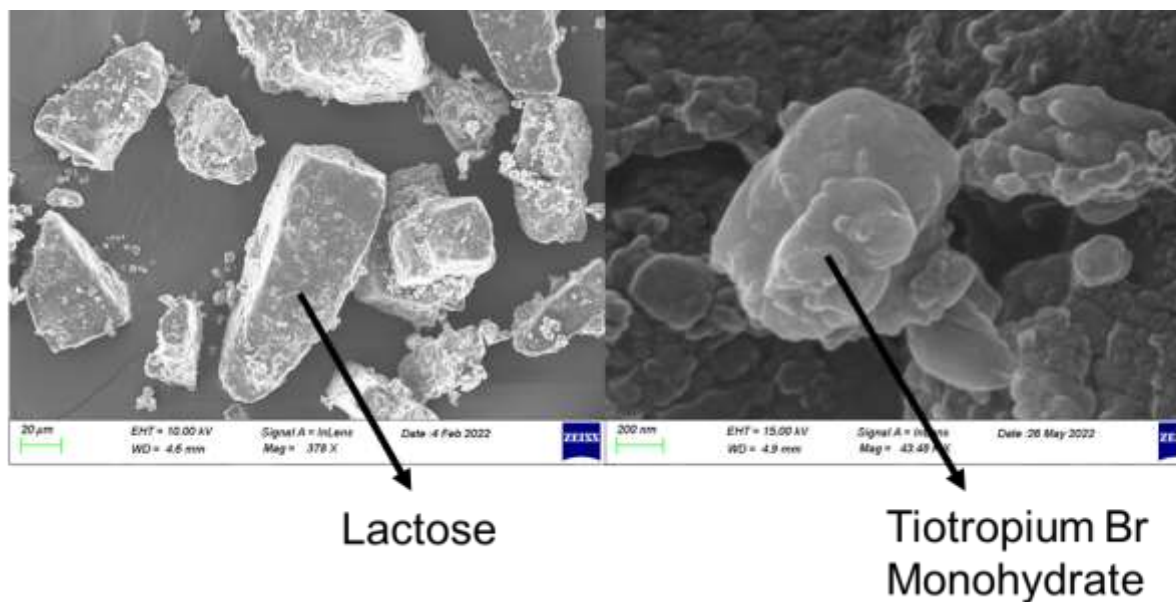


Figure 2: SEM images from EDX analysis of Spiriva® inhalation spray at 378 x magnification (only carriers are visible) and at 44k x magnification (focusing on a API particle on the lactose carrier).

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# Multi-Modal Imaging and Analysis: Combining Enhanced Darkfield Hyperspectral Microscopy with Confocal Raman Microscopy

Enhanced Darkfield Hyperspectral Microscopy is the preferred imaging and spectral analysis tool for nano-scale biological and materials science applications. This powerful technique offers rapid visualization, characterization and mapping of nano-scale entities, as small as 10-20 nm, in a wide range of translucent samples. Combining this technique with the powerful quantitative analysis offered by confocal Raman provides researchers with a tool of unequalled imaging and spectral analysis capabilities on a single microscope platform.



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# A pixel-by-pixel correcting autobalanced detector for SRS microscopy

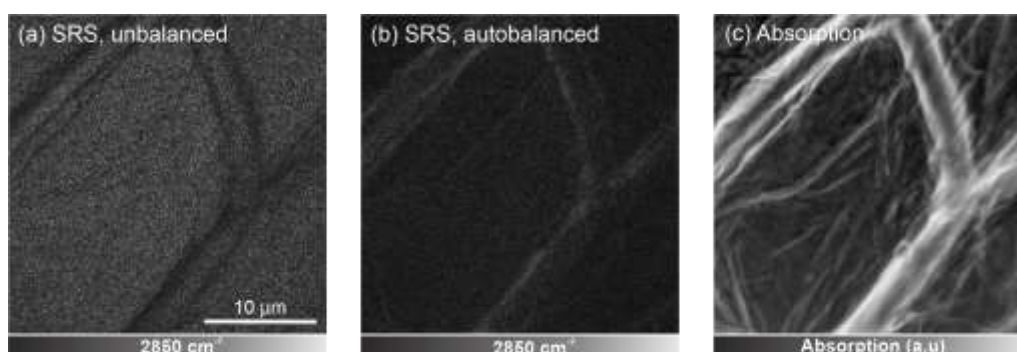
Stimulated Raman scattering (SRS) microscopy is a powerful tool in biological and medical research due to the chemically-selective and label-free nature of the Raman process. However, excess noise of the used light source limits the achievable signal-to-noise ratio (SNR) below the SNR given by the shot-noise limit [1,2].

In this submission we present an easy to use, compact, and modular autobalanced detector capable of up to 60 dB correlated noise suppression, 110 ° possible phase compensation at 20 MHz, and 300 ns PID settling time allowing for true pixel-by-pixel sample absorption compensation for pixel scanning rates of up to 3 MHz. Our autobalanced detector is able to provide an SRS image which is absorption compensated as well as shot-noise limited for photocurrents above 2 mA. As a novel addition, a noise-suppressed absorption image of the sample is simultaneously provided, allowing for combined SRS and absorption imaging. Our autobalanced detector was applied to a home-built SRS microscope setup utilizing a fiber-based dual-color picosecond light source with a 40 MHz Stokes and 20 MHz pump repetition rate, detecting a 20 MHz modulation on the Stokes beam. A special high dynamic range photodetector was developed, suppressing the 40 MHz repetition rate signal of the Stokes beam by up to 80 dB before transimpedance conversion, allowing for sensitive and saturation-free detection even for photocurrents above 10 mA. The image improvement by using our autobalanced detector is demonstrated in Fig. 1(a)-(b) for red onion skin. Only after enabling autobalancing the distribution of cellulose in the cell membrane became visible. Fig. 1(c) shows that this was due to high absorption in the membrane, suppressing the SRS signal to the level of the non-resonant background, which was then compensated for by the autobalancer circuit in Fig. 1(b). The SNR was increased by a factor of 1.5, however, the increase in SNR was limited only by shot-noise due to the available laser power of 10 mW per detector as well as the already low excess noise of the used light source.

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## Figures



**Figure 1:** (a)-(b) SRS image of cellulose in red onion skin, (c) absorption image of red onion skin. All images consist of 512 x 512 pixels and were acquired with a pixel dwell time of 10 μs and a mean photocurrent of 5 mA. Image (c) was simultaneously acquired with image (b).

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## Recent software developments for full spectral analysis of Raman, IR, cathodoluminescence spectra etc.

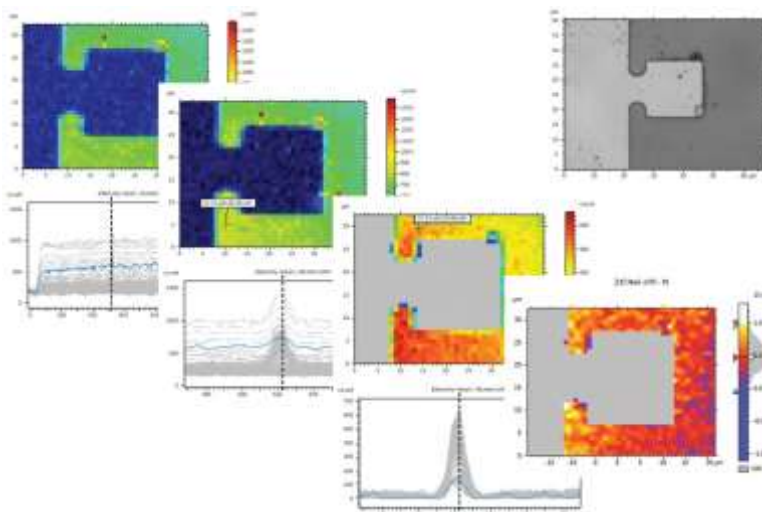
With the new release of MountainsSpectral® software, users working with spectra can now access tools for analyzing their data from A to Z and benefit from the robust and easy-to-use analysis software platform used by thousands of scientists and researchers worldwide.

Data from multiple instrument techniques (AFM, SEM, spectroscopy, profilometry etc.) can be brought together, correlated and analyzed in one single software program. Mountains® software unique document layout can be fully customized and converted to PDF format for sharing. The analysis workflow allows complete traceability in the analysis process; crucially, users can revert back to and modify any step. Automation features are also available for batch processing large quantities of data.

The most recent developments largely improve visualization, pre-processing and analysis of spectra, series of spectra (one-dimensional hyperspectral data) and hyperspectral images (2D hyperspectral data). Capabilities include:

- Baseline correction & noise filtering
- A wide range of display options (style, axis, curves to display, envelope etc.)
- Manual or automatic peak detection
- Parameter maps

### Figures



**Figure 1:** Strain analysis in crystalline silicon using Raman spectroscopy and MountainsSpectral® software.

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## Tiril Aurora Lintvedt

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# Inline Raman Spectroscopy for Characterization of an Industrial Poultry Raw Material Stream

Raman spectroscopy is a promising spectroscopic technique for in-line food analysis, with the advantage that both gross components, including fat, proteins and bone, can be targeted at the same time as more detailed information on protein composition, e.g. the concentration of collagen. However, food matrices are generally heterogeneous, and the lack of appropriate tools for addressing sample heterogeneity has traditionally been one of the main challenges preventing the widespread use of this technique in food analysis. One of the milestones in representative Raman analysis has been the development of wide area illumination (WAI) Raman probes. Here, a loosely focused laser beam is used to illuminate a larger area within the sample. The technique has found a range of applications within pharmaceutical, biomedical and chemical analysis, but surprisingly, WAI Raman has not been frequently used in food analysis. In recent years we have shown how the WAI Raman can be used to provide reliable quantitative information from samples that traditionally have been regarded as challenging in a Raman setting, from whole salmon and meat to chicken carcasses, salmon by-products and fruits like apples and strawberries. Recently, we tested the technique for measurements of a heterogeneous poultry raw material stream in a true in-line setting [5]. This work is the focus of the presentation.

In recent years, the poultry processing industry have adopted enzymatic protein hydrolysis (EPH) as a strategy to recover constituents from by-products (e.g. carcasses and mechanical deboning residues). In this process, proteins from the by-products are digested and solubilized by proteases. Today, recovered constituents from EPH usually end up as lower value feed ingredients (used for e.g. pet food), but the focus has been shifting towards protein ingredients for higher-paying markets like human consumption. This puts increasing emphasis on protein quality (e.g. producing protein hydrolysates with specific functional or nutritional properties), and not only on protein recovery. This motivates in-line measurements of the raw materials input to the hydrolysis, which could provide the means for real time process control, potentially leading to better raw material utilization (less waste of food resources) and help prevent batches of hydrolysates being discarded from the human market. The main aim of the present study was to test in-line Raman spectroscopy for characterisation of an industrial poultry raw material stream. In the work, calibrations based on Raman measurements of fat, protein, ash (proxy for bone) and hydroxyproline (proxy for collagen) in ground poultry rest raw material were established. Subsequently, the obtained calibrations were tested for continuous monitoring of a ground poultry by-product stream at a commercial hydrolysis facility over the course of two days. To the best of our knowledge, this is the first time a WAI Raman probe has been tested in-line under relevant measurement conditions in the food industry.

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## DIRECT IMAGING OF BAND STRUCTURE FOR CVD GROWN RHOMBOHEDRAL-STACKED BILAYER WSe<sub>2</sub> USING NANOSPOT ANGLE-RESOLVED PHOTOEMISSION

Twisted layers of atomically thin two-dimensional materials support a broad range of novel quantum materials with engineered optical and transport properties<sup>1,2</sup>. Transition metal dichalcogenides (TMDs) in the rhombohedral (3R i.e. 0° twist) crystal phase have been the focus of significant research interest in optical applications due to their particular broken inversion symmetry. Here, we report experimental and theoretical study of WSe<sub>2</sub> homo-bilayers obtained in stable 3R configuration by chemical vapor synthesis<sup>3,4,5,6</sup>. We investigate the electronic and structural properties of these 3R WSe<sub>2</sub> bilayers with 3R stacking using micro-Raman spectroscopy, angle-resolved photoemission nano-spectroscopy measurements (nano-ARPES) and Density Functional Theory (DFT) calculations. Our results demonstrate that WSe<sub>2</sub> bilayers with 3R crystal phase (AB stacking) show a significant spin-orbit splitting estimated to  $550 \pm 20$  meV. We derived experimentally effective hole masses of 0.48 me and 0.73 me at K point for upper and lower bands, respectively. Our work opens up new perspectives for the development of optoelectronic and spintronic devices based on 3R TMD homo-bilayers.

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### Figures



Figure 1: Optical image of bilayer CVD-grown WSe<sub>2</sub> flakes

## Simone Melesi

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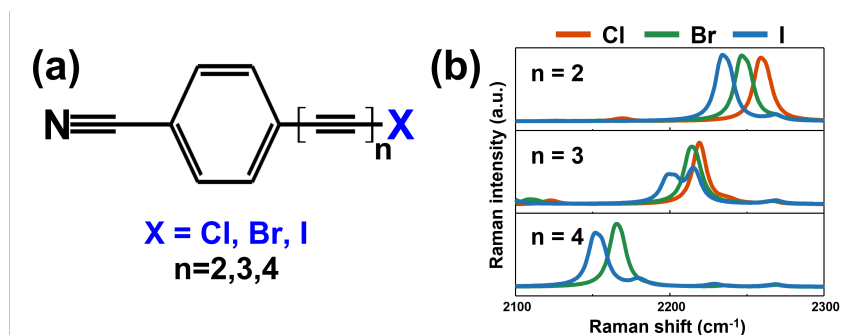
## The role of Raman spectroscopy in the investigation of the $\pi$ -conjugation properties of halogenated Carbon Atomic Wires

Carbon atomic wires in the form of polyynes are linear sp-hybridized carbon chains made of an alternation of single and triple bonds [1]. These systems possess a strong  $\pi$ -electron conjugation and outstanding optical, mechanical, and electrical properties that can be modified by changing the length of the chains and their termination groups [1]. In this framework, it is crucial to better understand the effect that the variations in the structures of these systems can have on the modification of their properties to develop in the next years polyynes-based tunable devices. To accomplish this, Raman spectroscopy is one of the most used characterization techniques since polyynes present a characteristic peak (i.e., Effective Conjugation Coordinate, ECC) from whose frequency it is possible to extrapolate important information on the electronic properties of these systems [2]. In this work, we studied a class of polyynes (Fig. 1a) with different halogen terminations (Cl, Br, and I) and with different lengths (2,3, and 4 C≡C bonds) [3]. Combining DFT calculations and experimental FT-Raman spectroscopy, we compared the extent of the tuning given by an increase in the length of the sp-chain and by a variation in the halogen end group. Both increasing the length or the size of the halogen, we observed an increase in the  $\pi$ -conjugation of the chains but with different magnitudes: a strong modulation (coarse tuning) is observed in the first case while a smoother change (fine tuning) is present in the second case (Fig. 1b). Moreover, by comparing the spectra of powders and solutions, we investigated the aggregation state of halogenated polyynes identifying some important features in the normal modes ascribed to a passage from an “isolated molecule” configuration to a “crystal” configuration in which we recognize strong halogen bonding between adjacent molecules. In this work, Raman played a crucial role in determining how halogens can affect the tunability of polyynes, thus paving the way for the use of halopolyynes in future developments of materials with tunable properties.

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### Figures



**Fig 1:** (a) Chemical structure of the halogenated polyynes characterized in this work. (b) Effect of the chain length and of the halogen termination on the modulation of the ECC peak frequency and of the electronic properties of halopolyynes.

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# Full wafer-scale characterization method for 2D materials

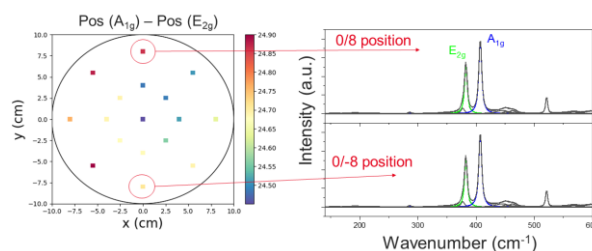
Uniform large-scale growth poses a significant challenge for 2D integration in microelectronic devices. Recently, teams have demonstrated their ability to process 200 mm or 300 mm wafers of MoS<sub>2</sub> or WS<sub>2</sub> [1, 2]. Nevertheless, in the most cases, only local characterizations are conducted, and the provided data do not accurately reflect the overall quality of the entire wafer. Comprehensive characterization of the full-size wafer is, therefore, essential to monitor the quality and uniformity of both the growth and integration processes, especially for large-scale applications.

This study focuses on wafer-scale characterization of MoS<sub>2</sub> grown by ALD on 200 mm SiO<sub>2</sub> wafer in clean room [3]. Wafer-scale Raman spectroscopy, photoluminescence, Wavelength Dispersive X-Ray Fluorescence (WDXRF), and Atomic Force Microscopy are systematically performed. Data processing, including spectrum decomposition [4], is automated (Fig. 1), followed by the subsequent plotting of mappings for each of the previously mentioned large-scale methods (Fig. 2). The thickness (*i.e.*, number of monolayers) is calculated using WDXRF. Raman and photoluminescence signatures are monitored across the entire wafer, enabling statistical analysis and providing insights of the MoS<sub>2</sub> uniformity. A non-destructive high-speed method has been developed for mapping 2D materials on 200 mm MoS<sub>2</sub> wafers, facilitating wafer-scale analysis within cleanroom environments. This characterization protocol is also applied to 300 mm wafers.

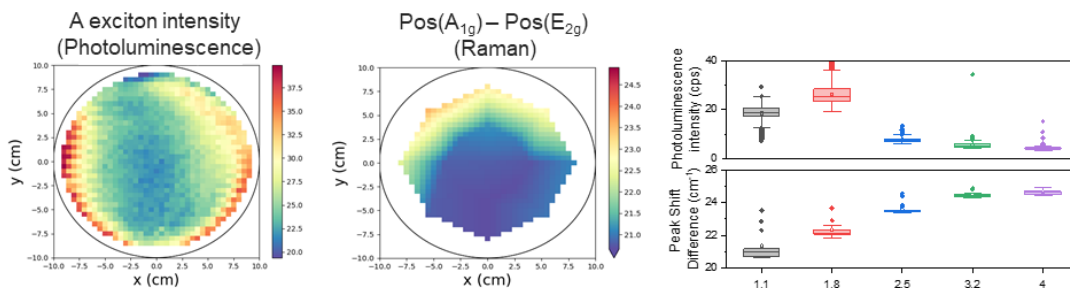
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### Figures



**Figure 1:** Example of Raman mapping (without interpolation) on 200 mm wafer.



**Figure 2:** Characterization of 1-4 monolayers of MoS<sub>2</sub> across 200 mm wafers. A linear interpolation was employed for Raman analysis. Peak Shift Difference = Pos (A<sub>1g</sub>)-Pos (E<sub>2g</sub>)

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Patricia Val-Gómez, Adolfo del Campo, José Francisco Fernández, Fernando Rubio-Marcos

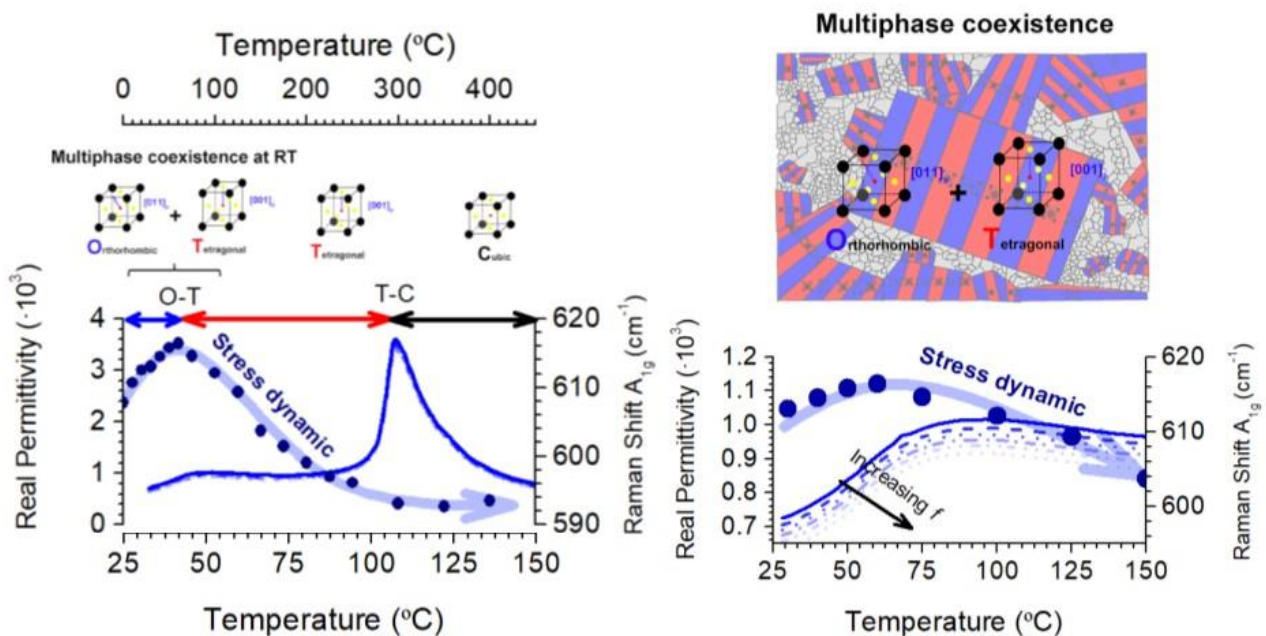
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# Stress dynamics during O-T phase transitions in lead-free KNN-based piezoelectric ceramics studied by Confocal Raman Spectroscopy

In this study, we have investigated by Raman Spectroscopy the stress dynamics arising from the Orthorhombic-Tetragonal Polymorphic Phase Boundary (PPB) in lead-free potassium sodium niobate (KNN)-based ceramics and its temperature dependence, as a means to elucidate the characteristics of PPBs in lead-free piezoelectric oxides. The dynamics are dictated by the phase transitions that occur upon cooling from the cubic phase and the coexistence of different crystal structures. Our findings reveal that the growth of Orthorhombic phases is constrained by the high-temperature tetragonal phase distributions. In particular, it is evidenced that two main mechanisms regulate the decrease of stress processes in which structural and microstructural effects are correlated; the first one is associated to a purely microstructural effect in which bimodal grain distribution hinders the formation of non-180° domains. By contrast, the second is mainly governed by ferroelectric domain distribution and the occurrence of pseudo-cubic regions around room temperature associated with local structural heterogeneity (that is, polar nano regions, PNRs). Specifically, these mechanisms generate regions with different stress state and explain the widening of the phase transition due the phase coexistence (Figure 1).

### Figures



**Figure 1:** Relations stress/widening of phase transitions due to phase coexistence

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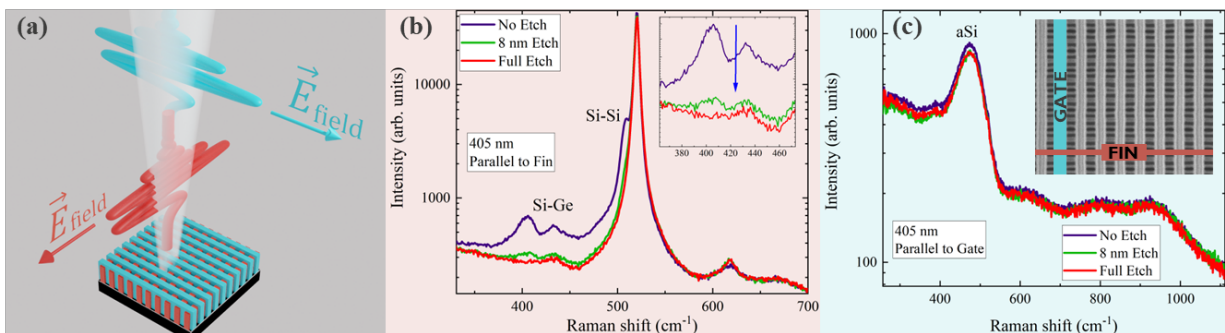
## Tunable Raman Selectivity for Semiconductor Metrology

We present significant developments in Raman spectroscopy of state-of-the-art semiconductor devices, where the sensitivity of the characterization can be fully tuned to the structures of interest. In a previous report we showed how Raman can be employed as a dimensional metrology in semiconductor manufacturing, but here we extend that concept to full selectivity to the different materials that make up a modern semiconductor device. We demonstrate the implementation on so-called forksheet transistor modules [1], which are characterized by two gratings of first the n- and pMOS transistor structures and then subsequently the tri-gate stack with both gratings orthogonal to each other. When we align the incident polarization to the transistor structures, the spectra are entirely free of signals from the gate stack, and we regain the previously enabled capabilities of e.g. extreme sensitivity to remaining SiGe cavity material during an etch process. Indeed the sample before etch shows signatures of Si-Ge and Si-Si vibrations coming from the SiGe, and these signals progressively disappear as more and more of the SiGe is etched from between the Si transistor channels. In contrast, when the polarization is aligned to the gate lines, the SiGe-like features completely disappear, and an entirely different Raman spectrum emerges that now shows the amorphous Si (aSi) signature from the gate material. As the etch process is not selective to the gate materials, the intensities of the aSi remain constant throughout the different etch steps. The result is that we can probe the different constituents of modern transistor architectures completely independently and in a fully non-destructive manner, which represents an important advancement for in-line spectroscopic semiconductor metrology.

### References

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### Figures



**Figure 1:** (a) Artist impression of the incident laser light coupling into the bimodal grating that is formed by the transistor channels and gate lines. When the polarization is aligned along the gate (blue), the light couples into that grating exclusively. Similarly, when the E-field is oriented along the transistor channels (red), the light probes that grating only. (b) Raman spectra with polarization parallel to the fin structures. A strong sensitivity on remaining SiGe content is observed, and no aSi material from the gate is detected. (c) Raman spectra recorded with polarization parallel to the gate. Only the aSi is observed in the spectra, and since this material is not subject to the etch process, the intensity is unaffected. The inset shows a top-down SEM image of the structures under investigation with gate and fin directions indicated.



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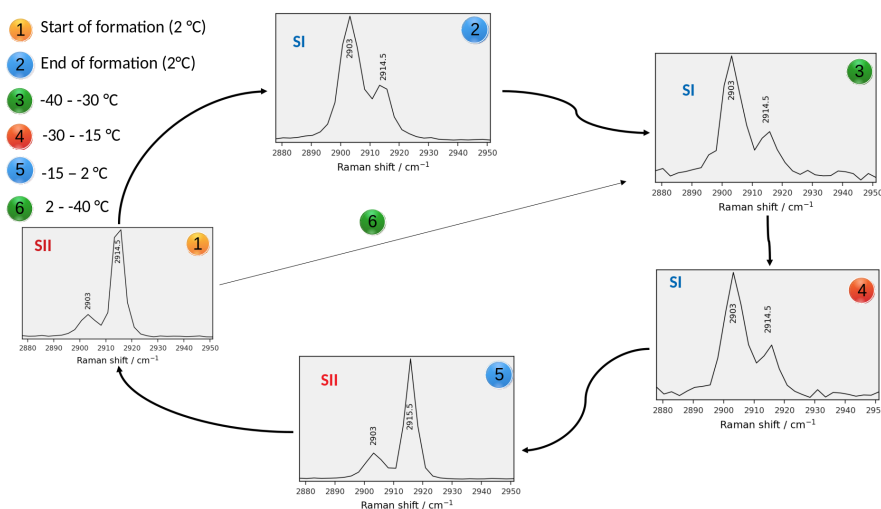
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## Climate-Resilient Technologies: Adapting Methane Hydrate Transformations to Moderate Thermodynamic Conditions

Crystalline clathrate hydrates represent solid materials characterized by a distinctive framework structure, wherein hydrogen-bonded water molecules form well-defined cavities capable of accommodating gas molecules. Notably, the formation of methane hydrates with structure II (sII) is not observed within moderate temperature and pressure ranges ( $P \leq 100$  MPa and  $T \leq +20$  °C). Instead, the dominant and thermodynamically stable phase is structure I (sI) hydrate. Consequently, both natural gas hydrate and synthetic methane hydrate, cultivated to simulate natural conditions, predominantly exhibit the sI structure.

In this study, we present the outcomes of Raman spectroscopic investigations aimed at manipulating the transformation of methane hydrates under a constant low pressure of 2.7 MPa and within a temperature range of +2 to -40 °C. Considering that the ratios of large to small cavities in structure I and structure II hydrates are 3:1 and 1:2, respectively, the relative peak areas observed in the experimental spectra serve as reliable indicators of the hydrate structure. Raman spectroscopic investigations coupled with multivariate curve resolution - alternating least squares (MCR-ALS) method investigated hydrate formation at 10 MPa and 275.2 K. The results showed that sII was formed earlier as a kinetic product, followed by a mixed sI-sII phase, which is finally transformed into the thermodynamically stable sI. Such a result indicates that forming 5<sup>126</sup>2 cavity is the rate-limiting step for hydrate formation and agrees well with previous investigations [1]. Upon reducing the temperature gradually to -40 °C, sI remains the dominant phase.

Interestingly, a phase transformation to a dominant stable sII structure was found when the temperature increased gradually from -15 °C to +2 °C, which is reported for the first time at those very moderate thermodynamic conditions. The sII two remained intact for 3 days, indicating relatively high stability of the structure. sII could be reverted to sI again by direct cooling to -40 °C. Such results have important implications related to such important issues as the energy storage, climate change, geotechnical engineering, and material science development in general.



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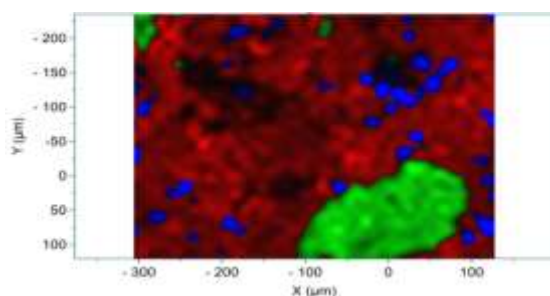
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## Pharmaceutical and Biomedical Applications of Raman Spectroscopy

Raman Spectroscopy has received great attention in solid-state pharmaceutical applications ranging from the verification of raw materials to quality control of products. On-line or even in-line process monitoring in drugs production lines is also considered advantageous. Detection of active pharmaceutical ingredients (APIs) in biological fluids in extremely small concentration levels is also a challenging task. Sophisticated and time-consuming techniques are currently used in these cases. Raman spectroscopy can be further extended to the study of bone and joints in human and animal models. Metabolic and degenerative diseases, like osteoporosis and osteoarthritis, can be identified. The procedures and factors responsible can be explored. In this work, some of such cases will be presented. Conventional, portable and bench-top micro-Raman spectrometers analyzing APIs and formulations as they are and inside packaging, in biological fluids (blood serum, urine, and saliva). Raman metrics established for the study of bony and cartilaginous tissues in several cases will be also shown.

### Figures



**Figure 1:** Left: Raman map of a section of a two-API pain-killer. Right: Raman map Human bones and joints studied with Raman spectroscopy

### Acknowledgment

University of Patras Research Committee is greatly acknowledged.

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Nicolas Coca-Lopez<sup>1</sup>, Nina Jeliaskova<sup>2</sup>, Dirk Lellinger<sup>3</sup>, Enrique Lozano Diz<sup>4</sup>, Miguel A. Bañares<sup>1</sup>

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## Raman characterisation: FAIRness and relevance

The industrial and academic use of Raman spectroscopy is becoming wider as the number of specialized techniques, devices and Raman active products increases, costs are reduced, and relevance is enhanced by measuring in realistic conditions (pressure, temperature, environment, sample). This has fostered the development of standards and norms for terminology, calibration, performance validation, data analysis, and specific applications, as well as research on harmonisation, automation, and Findable, Accessible, Interoperable, and Reusable (FAIR) repositories.

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### Figures



**CHARISMA**

**Harmonized  
and realistic**

**Figure 1:** Industrial and academic impact of advances in Raman spectroscopy

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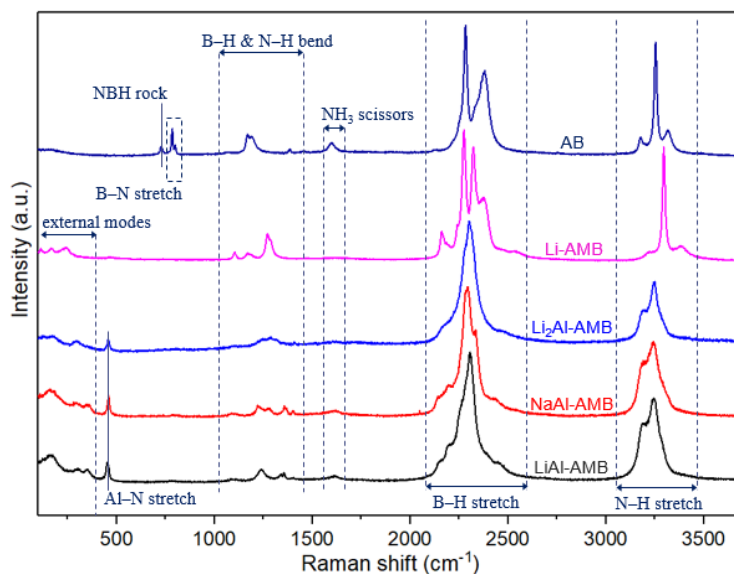
## Operando Raman spectroscopy for hydrogen energy storage

Hydrogen is now considered a major energy carrier capable of reducing greenhouse gas emission of both industrial and mobility sectors owing to the diversity of production and use of low-carbon and renewable hydrogen. The storage and transport of hydrogen energy is however still a serious technological challenge. Multiple cost- and energy-efficient alternates are actively researched beyond the current compressed gas solution, including liquid H<sub>2</sub> storage, chemical storage or solid-state hydrogen storage materials. We will show through diverse applications that Raman spectroscopy is a key technique for the development of these alternate solutions. For *materials-based* hydrogen storage, borohydrides and ammine metal borohydrides in particular (Fig. 1) are interesting compounds for the release of large H<sub>2</sub> quantity with high purity under mild temperature conditions. In-situ Raman spectroscopy is used to analyze the thermolysis dehydrogenation process and gain insight into the hydrogen storage mechanism [1]. For *chemical* hydrogen storage, ammonia shows decisive advantages in terms of energy density and existing industrial infrastructures. New multifunctional catalytic materials are being evaluated to improve the energy efficiency of NH<sub>3</sub> production and make it compatible with green H<sub>2</sub> sources [2]. Operando Raman spectroscopy at high temperature and pressure is developed in our group to elucidate the chemical reaction mechanisms at play in novel Li<sub>x</sub>NH<sub>y</sub> catalytic systems for both NH<sub>3</sub> synthesis and decomposition. For hydrogen storage *as a liquid*, cryogenic temperatures are required and the ortho spin isomer of H<sub>2</sub> needs to be converted to para-H<sub>2</sub> to improve long term storage capabilities [3]. Operando rotational Raman spectroscopy performed in cryogenic heat exchanger is developed to monitor the kinetics and catalytic activity of ortho/para-hydrogen conversion catalysts.

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### Figures



**Figure 1:** Raman spectra of various synthesized ammine metal borohydrides compounds (AMB) compared to pure ammonia borane (AB)

## Graham A. Rance

Jack W. Jordan, Alexander I. Chernov, E. Stephen Davies, Anabel E. Lanterna, Jesum Alves Fernandes, Alexander Grüneis, Quentin Ramasse, Graham N. Newton, Andrei N. Khlobystov

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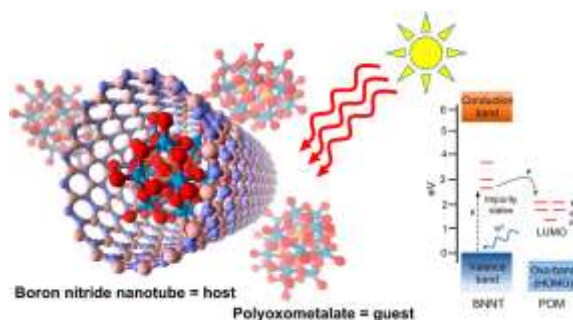
# Host-Guest Chemistry in Boron Nitride Nanotubes: Interactions with Polyoxometalates and Mechanism of Encapsulation

Boron nitride nanotubes (BNNTs) are an emerging class of molecular container offering new functionalities and possibilities for studying molecules at the nanoscale. Herein, BNNTs are demonstrated as highly effective nanocontainers for polyoxometalate (POM) molecules. The encapsulation of POMs within BNNTs occurs spontaneously at room temperature from an aqueous solution, leading to the self-assembly of a POM@BNNT host-guest system. Analysis of the interactions between the host-nanotube and guest-molecule indicate that Lewis acid-base interactions between W=O groups of the POM (base) and B-atoms of the BNNT lattice (acid) likely play a major role in driving POM encapsulation, with photoactivated electron transfer from BNNTs to POMs in solution also contributing to the process. The transparent nature of the BNNT nanocontainer allows extensive investigation of the guest-molecules by photoluminescence, Raman, UV-vis absorption, and EPR spectroscopies. These studies revealed considerable energy and electron transfer processes between BNNTs and POMs, likely mediated via defect energy states of the BNNTs and resulting in the quenching of BNNT photoluminescence at room temperature, the emergence of new photoluminescence emissions at cryogenic temperatures (<100 K), a photochromic response, and paramagnetic signals from guest-POMs. These phenomena offer a fresh perspective on host-guest interactions at the nanoscale and open pathways for harvesting the functional properties of these hybrid systems.

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## Figures



**Figure 1:** Photoactivated electron transfer from BNNTs to POMs plays a major role in driving POM encapsulation.

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## SEM and Raman analysis in a single chamber for the analysis of the environmental contamination by nano- and microplastics

Microplastics (MPs) are a form of marine debris smaller than 5 mm in size. They are usually described based on criteria such as colour, shape, polymer type and size [1]. Originating from human activities, they became ubiquitous in worldwide seawaters, and especially in the coastal zones where they contaminate living organisms. As filter-feeding organisms, mussels accumulate a wide range of contaminants from their environment, including MPs with characteristics consistent with those of their surrounding environmental media [2-5]. Since mussels are consumed entirely, without gut removal, they became one of important vectors to transfer MPs into human food chain [6]. In a context of food security, it is thus important to monitor the contamination of mussels and describe the shape, nature and size of MPI. Unfortunately, the size distribution is usually presented between 5  $\mu\text{m}$  and 5 mm whereas smaller plastics are also expected. This is related to the limit of detection of microscopic methods. In these conditions, a fraction of MPs is never described. We used the recent developments of methods from nanotechnology to solve this technical bias. We used Raman spectroscopy coupled to scanning electron microscopy in a single chamber. Model microplastics and nanoplastics of polyethylene and polystyrene were used to model small marine debris. By adapting the protocol and choosing the right parameters, we succeeded in recording Raman spectra of nano- and microplastics without destroying them. These measurements were done in the project Moustic funded by Anses (French Agency for Food, Environmental and Occupational Health & Safety).

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E-mail: [satyen.saha@gmail.com](mailto:satyen.saha@gmail.com), [ssaha@bhu.ac.in](mailto:ssaha@bhu.ac.in)

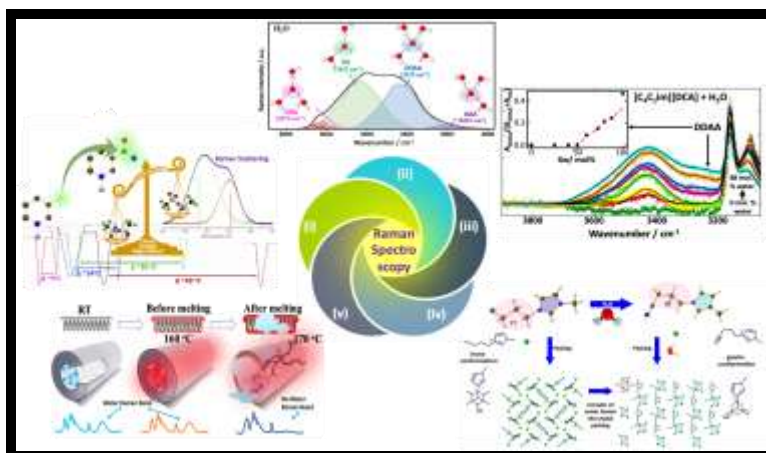
### Raman Spectroscopy: Unveiling Its Versatility as a Scientific Tool

Raman spectroscopy provides detailed molecular and chemical information in a non-destructive manner, making it a powerful and versatile analytical tool in scientific research and various industrial applications. Raman can prove as a versatile tool for fundamental studies as well. In this oral presentation, I shall be highlighting some of our recent results using Raman spectroscopy: (i) for the first time to show the equilibrium among *transoid-cisoid* of  $\text{NTf}_2$  anions considerably depending upon the alkyl chain length attached with cations (in nicotinamide ionic liquids(ILs)). (ii) To determine the type of water (DA, DDA, DDAA, or DAA), both qualitatively and quantitatively. (iii) It is used to understand the profound effect of water on the physical and chemical properties of ILs. (iv) Highlights the importance of water, considering that a considerable amount of water can creep in ILs during synthesis or from exposure to air. Lastly, (v), we have used temperature-dependent confocal Raman microscopic measurements to investigate the stability of water in a crystal environment (e.g.,  $[\text{Cu}(\text{cyclam})(\text{N}_3)_2] \cdot 4\text{H}_2\text{O}$ ), which depicts the stability of liquid water up to  $166^\circ\text{C}$ , well above the normal boiling point of water molecules. We anticipate that more investigations based on the confocal Raman microscopic technique to study small water clusters stable at high temperatures may prove useful to precisely locate water clusters deeply buried in soil samples of locations with extreme weather conditions. Such stable water at high temperatures may prove advantageous for searching/locating dormant life forms.

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- [3] A. Verma, S. Verma, Monika, M. Mondal, N. E. Prasad, J. Srivastava, S. Singh, J. P. Verma, **Satyen Saha\***. *Journal of Molecular Structure*, **2022**, 1250, 131679.

#### Figures



**Figure 1:** Raman spectroscopy shows its versatility in studies of various chemical systems.

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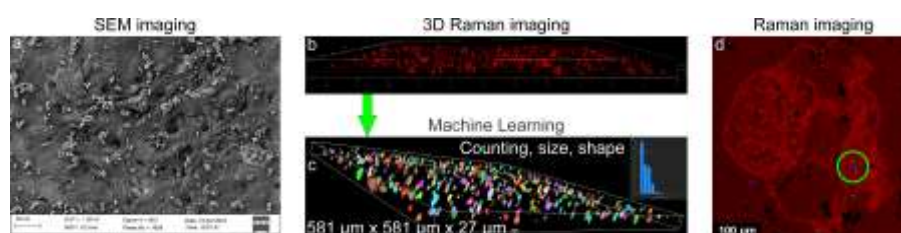
## Chemical Identification, Localization, and Counting of Ultrafine Particles in Complex Biological Matrices

There is an increasing public concern about the negative impact of ultrafine particles (UFPs) on human health. UFPs are aerosols with a diameter of 100 nm or less that are able to penetrate biological barriers, contribute the highest number of particles in the atmosphere, and have the largest surface area promoting the adsorption of additional hazardous substances [1]. Here, we demonstrate advanced analytical workflows based on correlative spectroscopy (spontaneous and stimulated Raman, X-ray fluorescence (XRF)) and microscopy (optical, electron, fluorescence) techniques using relocalization technologies from Horiba (nanoGPS and Particle Finder) [2]. These complementary methods were first applied to standard plastic particles, in this case polystyrene with the smallest diameter of 50 nm for the initial development of workflows and then to real, environmental particles (smog, break dust, and tyre wear), all being part of UFPs pollution. Investigations of chemical and elemental composition by Raman and XRF revealed a mixture of carbon- and metallic-based nanoparticles, while high-resolution microscopy showed both single and clustered UFPs. We found amorphous carbon in all environmental samples, mainly S, Na, Si, Ca, Fe (~84.6, 6.5, 3.4, 1.6, 1.1 wt%) on the smog filters, and mostly Fe, Si, Al, Ti, S, Zn, Ca, Sn (~85.3, 1.9, 1.8, 1.5, 1.4, 1.3, 1.3, 1.1 wt%) in the break dust, largely as oxide compounds. This detailed precharacterization of the UFPs allowed us to efficiently detect them inside intricate biological matrices at cell, organ tissue, and animal levels. We performed extensive exposure experiments on in-vivo human podocytes and Calu-3 cells as representative kidney and lung cells prone to accumulation of particles during lifetime, model mice similar to human ulcerative colitis, and model zebrafishes resembling human membranous glomerulonephritis affecting kidneys. Distinct functional and gene expression biomarkers were observed for UFP-treated cells compared to non-treated controls. Mice with an inflamed gastrointestinal tract showed an impaired mucosal barrier function facilitating particles to enter in the bloodstream, thereby spreading and accumulating in all organs. Zebrafishes also showed the presence of UFPs in all organs implying uptake through the digestive system rather via skin. Thus, Raman integrated in correlative workflows supported by machine learning algorithms can be used to correctly estimate the exposure level and health effects of UFPs.

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### Figures



**Figure 1:** Example of spectroscopy and microscopy workflow applied to lung cells exposed to UFPs (a, b) followed by machine learning particle analysis (c). Accumulation of UFPs in zebrafish organ tissue (d).



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[nadezhda.shchedrina@universite-paris-saclay.fr](mailto:nadezhda.shchedrina@universite-paris-saclay.fr)

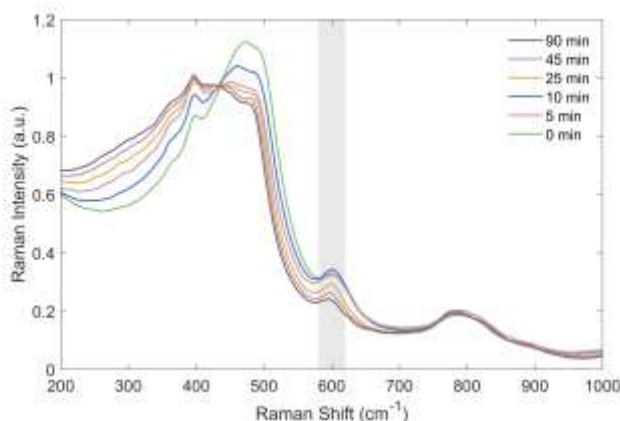
# In Situ Raman Spectroscopy during Annealing for Calculation of the Activation Energy Distribution in Densified Silica Glass

Densified silica glass, having enhanced mechanical and unique optical properties, serves critical roles in extreme environments as a material for various applications, such as optical sensors. In this study, a set of samples were prepared and analyzed using Raman spectroscopy. The range includes the same high-pressure, high-temperature (HP-HT) treated sample subjected to three different irradiation doses – 0, 10<sup>7</sup> Gy, and 11 GGy. Additionally, metamict-like samples obtained under two distinct conditions – 11 GGy with and without HPHT treatment – were compared. In this study, alterations in Raman spectra, particularly in the D<sub>2</sub> band linked to 3-membered rings in silica's matrix, serve as indicators of structural transformations in the glass during annealing. Utilizing a 'master curve' approach derived from these annealing curves, we assess the distribution of activation energy, offering a dependable method for forecasting the longevity of density properties. Samples were subjected to isothermal annealing, during which their Raman spectra were continuously monitored in real-time. The annealing was carried out at 3 temperatures between 825°C and 900°C, each for a duration of 18 hours. We used a Horiba Jobin Yvon LabRam HR 800 spectrometer. Our findings indicate that despite similar activation energy distribution widths (~0.6 eV) for both samples, the central energy was notably higher for the HP-HT treated 11 GGy sample (2.87 eV) than for the solely irradiated SiO<sub>2</sub> 11 GGy sample (1.89 eV). This suggests greater thermal stability and thus internal structure for densified silica glass subjected to HP-HT conditions. For the densified sample and the low-dose irradiated sample, we observe a bimodal activation energy distribution. This unveils the role of the high-density amorphous (HDA) transition phase in influencing the D<sub>2</sub> Raman band's non-monotonous behavior in low-irradiated densified samples. Electron irradiation appears to minimize these HDA states, leading to a transition to a lower-density amorphous (LDA) structure.

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## Figures



**Figure 1:** Evolution of Raman spectra during isothermal annealing at 850 °C of HP-HT SiO<sub>2</sub> sample irradiated at 10<sup>7</sup> Gy at different annealing times.

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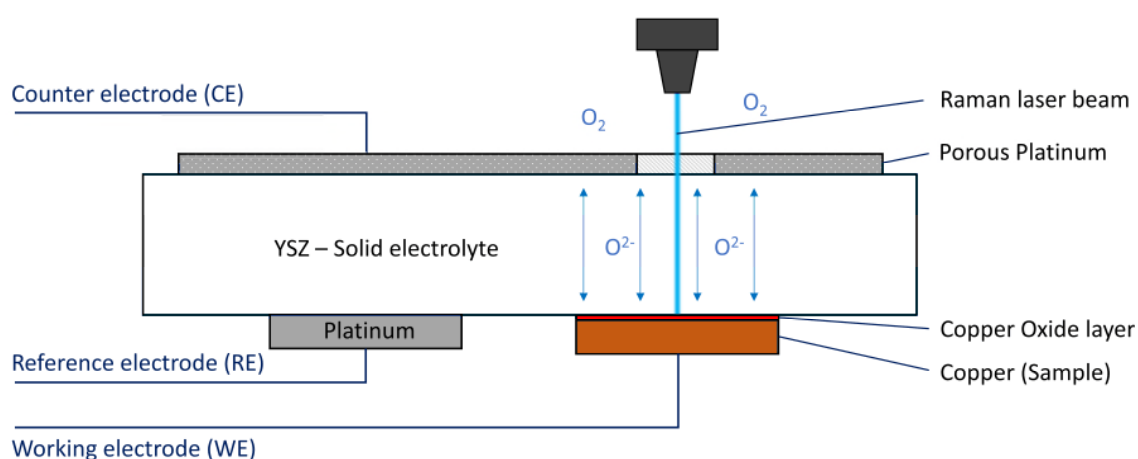
# In-situ Raman Spectroscopy of Defined Oxide Layers in an Electrochemical Solid-State Setup

Copper oxidation processes are critical because their occurrence causes a variety of issues in the subsequent processing and operation of electronic devices and other technical applications. Extensive investigations on thermal oxidation at different temperatures and oxygen partial pressures resulting in various compositions of the oxides were carried out.

In this work, a Raman heating stage is utilized to analyze thin oxide layers during their controllable formation. Controllable oxidation states are achieved in a solid-state electrochemical cell by polarizing a copper sample in contact with a single crystal of yttria-stabilized zirconia (YSZ), which is conductive for oxygen ions at high temperatures. According to the Nernst equation, the oxygen partial pressure at the interface to the metal can be set by applying specific potentials. The optical transparency of the YSZ single crystal allows the Raman laser to pass through the crystal to analyze the copper oxide formed on the copper-electrolyte interface.

Kinetic and thermodynamic information is provided by cyclic voltammograms about the oxidation processes. Thus, the potential corresponding to the oxygen partial pressure controls the various oxidation reactions, whereas the current measures the layer growth rate. In-situ Raman measurements enable more detailed chemical oxide analyses. The combination is used to develop an oxide layer growth model, focusing on the defect chemistry of copper oxide.

**Figure**



**Figure 1:** Schematic of the high-temperature setup for in-situ Raman measurement during the electrochemical controlled oxidation.

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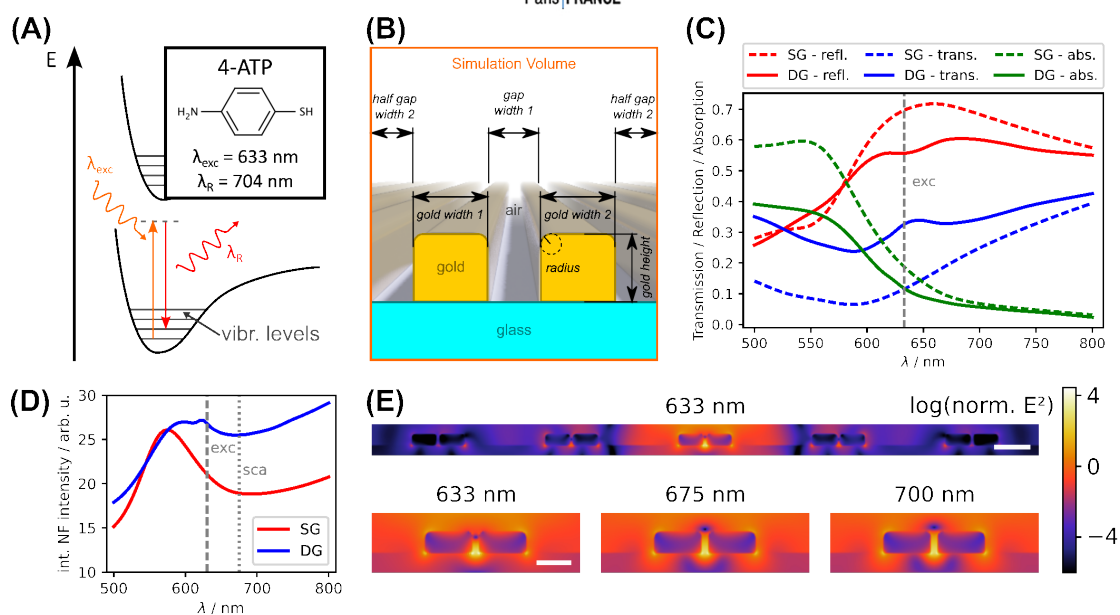
## Bioinspired Evolutionary Algorithm-Optimized Monocrystalline Gold Double Wire Gratings as a Novel SERS Sensing Platform

Achieving reliable and quantifiable performance in large-area surface-enhanced Raman spectroscopy (SERS) substrates pose a formidable challenge, demanding substantial signal enhancement while ensuring response uniformity and reproducibility. Conventional SERS substrates are typically made of inhomogeneous materials with random resonator geometries and distributions. As a consequence, they exhibit multiple or broadened plasmonic resonances, undesired absorptive losses, and uneven field enhancement. These limitations diminish signal strength and hamper reproducibility, making it difficult to conduct comparative studies with high sensitivity. This study introduces an innovative approach that utilizes monocrystalline gold flakes to fabricate plasmonic double-wire resonators with nanometer-level precision using focused ion-beam lithography. Inspired by biological evolution strategy, the double-wire grating substrate (DWGS) geometry was evolutionary optimized to enhance both excitation and emission processes involved in generating SERS peak signature of each analyte. The use of monocrystalline material minimizes absorption losses and enhances shape fidelity during nanofabrication. DWGS demonstrates notable reproducibility (RSD=6.6%), repeatability (RSD=5.6%), and large-area homogeneity over areas  $>10^4 \mu\text{m}^2$ . Moreover, it provides a SERS enhancement factor of several  $10^6$  and detection capability for sub-monolayer coverage. The DWGS demonstrates reusability, as well as long-term stability on the shelf. Experimental validation with various analytes, spanning from chemisorbed, physisorbed, plant extract and even gaseous species to proteins and DNA strains, confirms the sensitive and reproducible nature of DWGSs, thereby establishing them as a promising SERS substrate for future sensing applications.

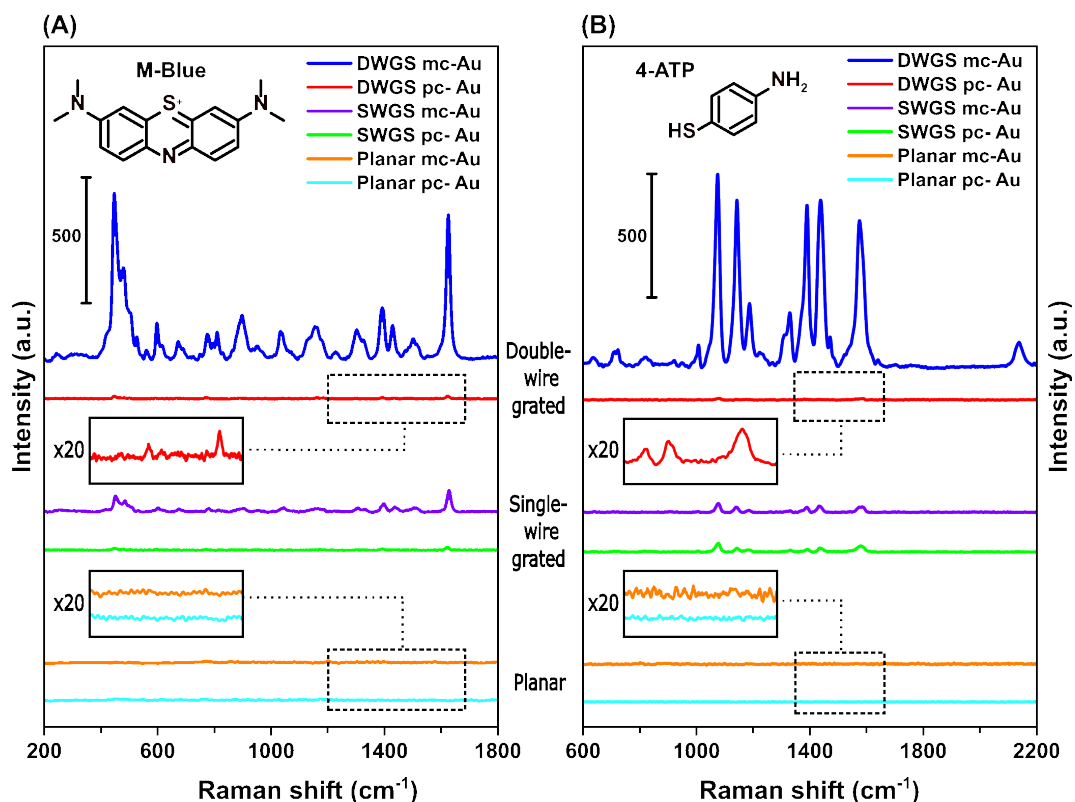
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### Figures



**Figure 1:** Numerical optimization. (A) Simplified energy level scheme of the Stokes-Raman-scattering process. The inset shows the 4-Aminothiophenol (4-ATP) molecule structure. (B) The elementary cell cross-section illustrates the potential possible free parameters for the optimization of substrates. (C) Spectra of transmission (blue), reflection (red) and absorption (green) for optimized substrates SG (dashed lines) and DG (solid lines). (D) Integrated near-field intensity enhancement for the substrates (SG, DG). (E) The optimized substrates near-fields are showcased in a vertical cross-section, illuminated from above, at three different wavelengths. Scalebar (E): top – 200 nm; bottom – 100 nm.



**Figure 2:** Panel (A) displays SERS measurements of M-Blue (1  $\mu\text{M}$ ) on dip-casted planar Au (non-grated), single wire grating substrate (SWGS), and double-wire grating substrate (DWGS). Both monocrystalline (mc-Au) and polycrystalline (pc-Au) gold materials were utilized for each substrate type. A zoomed-in view of the interval between 1200  $\text{cm}^{-1}$  -1700  $\text{cm}^{-1}$  is presented to highlight the relevant peaks. (B) Depicts SERS signals of 4-ATP SAM on the same substrates as in panel (A). Here, a zoomed-in view of the intervals between 1350  $\text{cm}^{-1}$  -1650  $\text{cm}^{-1}$  is provided.

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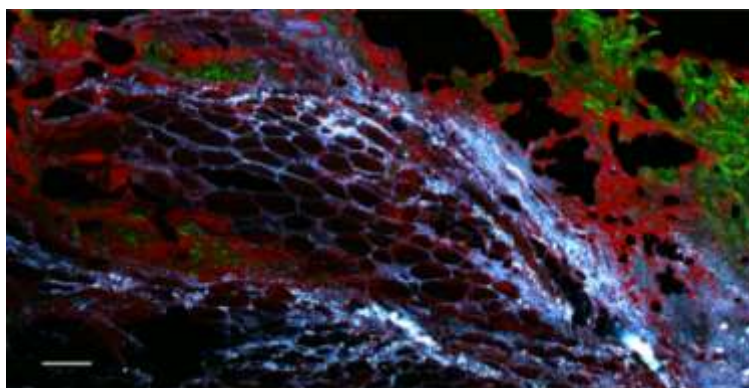
## Topical drug delivery: challenges and opportunities

Stimulated Raman scattering (SRS) microscopy is increasingly utilised within the pharmaceutical industry for its capability of performing fast, non-invasive, label-free imaging. This technique provides a signal intensity that is linear with concentration, allowing elucidation of quantitative information. SRS microscopy can also be correlated with other optical imaging modalities which can offer simultaneous visualisation of the connective tissues. Consequently, it is an ideal tool for investigating drug uptake and pharmacokinetics within skin tissues. There are various quantitative approaches that can be used in analysing samples with SRS [1]. Nevertheless, when it comes to analysing skin samples, several challenges must be overcome. These challenges include i) addressing signal attenuation with increasing depth; ii) detection and removal of parasitic signals (such as two-photon absorption processes); iii) detecting and quantifying the target signal amidst spectrally complex backgrounds; iv) correcting for optical and physical changes to the sample caused by the application of the formulations (optical clearing, dehydration etc). Addressing these challenges will enable SRS to reach its full potential as a new tool for supporting innovation in pharmaceutical product design and regulation.

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### Figures



**Figure 1:** SRS and Second harmonic generation (SHG) microscopy composite image of a rat skin section containing metallic particles. SRS contrast for CH<sub>2</sub> stretching at 2850 cm<sup>-1</sup> is shown in red, and off-resonance signals at 2770 cm<sup>-1</sup> are overlaid in cyan hot, revealing the distribution of the metal particles based on their strong signals due to absorption and photothermal lensing. Collagen (green) is visualised using SHG. The scale bar represents 100  $\mu\text{m}$ .

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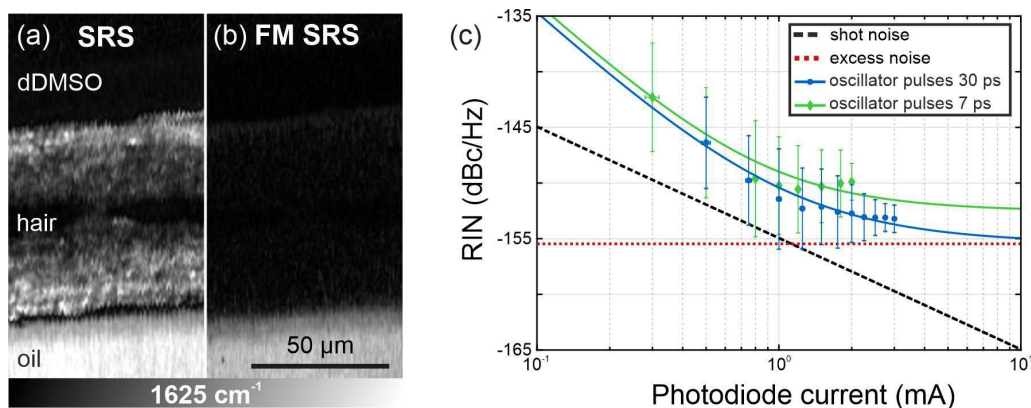
## Single low-noise fiber-based light source for FM SRS

Stimulated Raman scattering (SRS) microscopy is a powerful tool for in vivo diagnostic imaging due to the chemically-selective and label-free nature of the Raman process. The SRS process can suffer from parasitic effects such as cross-phase modulation, two-photon absorption and thermal lensing with according distortion of the SRS signal and a corresponding reduction of the image contrast [1]. Several schemes were implemented to suppress these parasitic signals, e. g. frequency modulation (FM). We substantially simplified FM SRS microscopy by using only one single fiber-based light source that is fast and widely tunable in wavelength [2], allowing for FM SRS imaging with real-time background subtraction and improved image contrast. The principle of FM SRS uses pump pulses alternating in wavelength in combination with Stokes pulses fixed in wavelength. In our setup the resonant signal as well as parasitic contributions were probed alternately by pulse-to-pulse wavelength-switching and lock-in detection allowed for direct subtraction, resulting in live imaging with background corrected signals at four frames per second. The SRS signals were acquired with a home-built detector that showed – compared to a commercially available one – an 8-times improved contrast performance. As fiber lasers typically show high noise levels, that are not compatible with sensitive microscopy applications, also the relative intensity noise (RIN) was characterized. Different parameters like the pulse duration can effect the RIN [3], hence this aspect was investigated in more detail. By increasing the pulse duration from 7 ps to 30 ps due to spectral filtering, the RIN of the amplified oscillator pulses decreased by approx. 3 dB to -153.7 dBc/Hz [4], limited by excess noise around -155.5 dBc/Hz as shown in Fig. 1(c).

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### Figures



**Figure 1:** (a) SRS and (b) FM SRS image with parasitic background suppression by a factor of 8. (c) Relative intensity noise (RIN) for different pulse durations at variable photodiode currents.

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## Van Hove singularity modulation of phonon transport in Twisted Bilayer Graphene

**Abstract**

Twisted bilayer graphene (tBLG) exhibits remarkable optical and electrical properties, rendering it a promising material for future micro/nano devices. Nonetheless, efficient heat transport poses a critical challenge, and comprehending the influence of twist angle on phonon properties is vital. In this study, we present the first analysis of the effects of twist angle on phonon scattering near the Van Hove singularity. Our investigation reveals an extended lifetime of G mode phonon due to electron-hole pair excitation near the critical angle accompanied by a substantial enhancement response. This phenomenon occurs when electron-phonon scattering is suppressed, leading to the dominance of phonon-phonon scattering in the transport process. Our study unveils the predominant control of the phonon-phonon scattering process by three-phonon scattering, with the proportion directly influenced by the twist angle. This effect arises from the alteration in the phonon band structure, aligning with theoretical predictions acquired via molecular dynamics simulations. These findings contribute significant scientific insights into the phonon scattering behavior of tBLG, thereby informing the development of advanced micro/nano devices with enhanced performance. Furthermore, our study may open avenues for investigating the potential practical applications of tBLG and other twisted materials.

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**Figures**

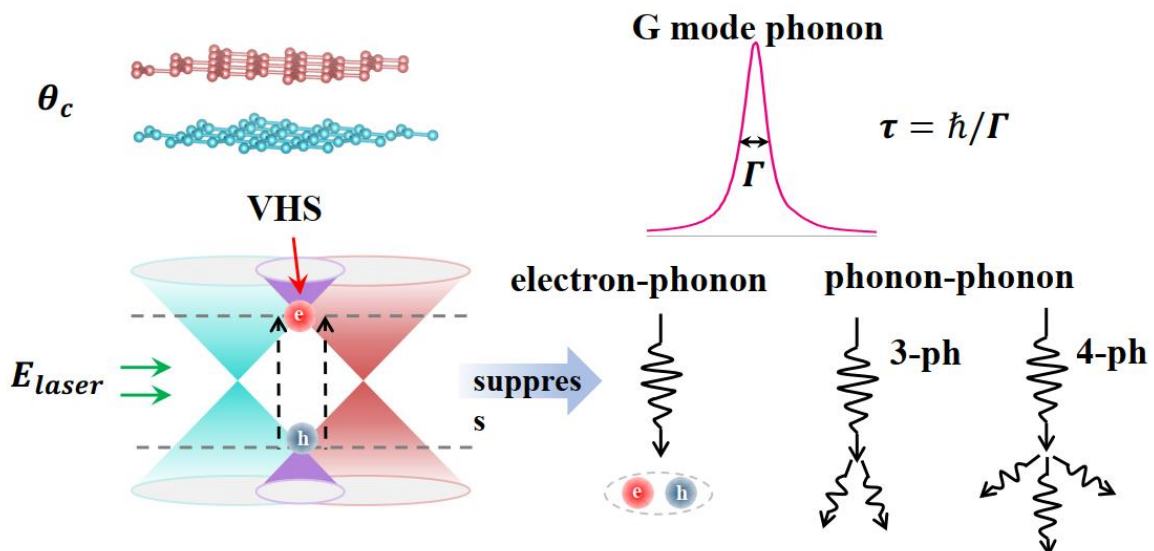


Figure 1: Table of contents

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## Surface Enhancement of Raman Scattering (SERS) of Microstructures Fabricated by Femtosecond Laser Optical Lithography

Surface enhancement of Raman scattering (SERS) is a rapid non-destructive detecting spectroscopy technique with high sensitivity, accuracy, fingerprint spectrum, and immunity from water molecules, which can achieve single molecule detection. Some kinds of methods have been developed to improve the enhancement of Raman signal. Among them, the strategy of developing good substrate with well-designed microstructure is promising. In this talk, the development of SERS substrate by using Femtosecond Laser Optical Lithography and the application will be addressed.



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# Collaborative brainstorming : Correlate Raman, Fluorescence and AFM Microscopy

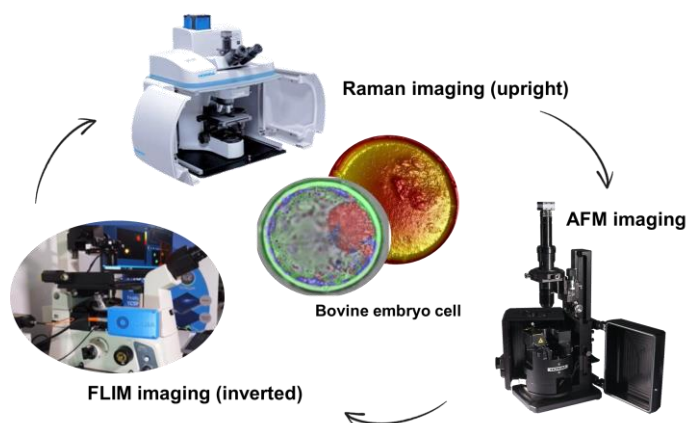
Being able to image living samples (cells, viruses, bacteria, diatoms...) and understand kinetics of their reactions to various stimuli is a must nowadays, and often requires super-expensive hardware combinations of multiple modalities.

Revisiting regions of interest between techniques capable to image living organisms such like FLIM, CLSM, SHG, Raman or Scanning Probe Microscopes is a challenge due to the different brands used, different operators, different magnifications and the various optical setups (inverted, diasopic illumination for fluorescence, upright and episcopic for Raman and AFM).

HORIBA has developed a solution based on a multiscale machine-readable pattern (nanoGPS tags, transparent for low magnification or Si based conductive for high magnification compatible with SEM) which can be attached to the sample holder (glass slide, Petri dish, flask, AFM transparent sample holder) and enable to relocate within a few microns the same points of interest.



**We would like to hear from RamanFest conference attendees where this new technique could help!**



**Please join our brainstorming sessions during the Poster Sessions**

What are the applications where you would like to test such solution ?

Do you see other modalities that would be worth combining ?

What are your pains using fiducials, shuttles, objective stamp markers ?

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## Low Frequency Raman microscopy for API polymorphisms analysis

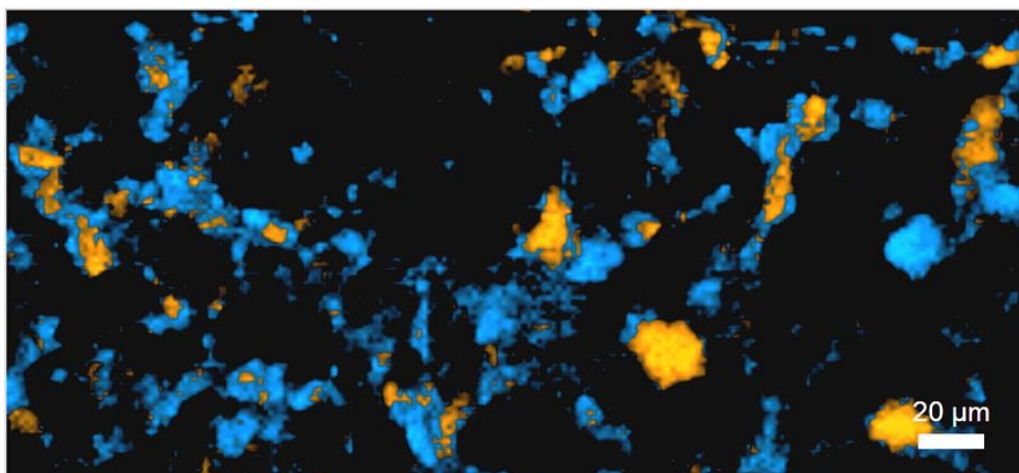
Since the physical state can affect the pharmaceutical behavior of drug substances, it is important to know what controls crystallization, solid state reactions, phase stability, and solubility. There are numerous methods that have been used to measure the solid state composition of pharmaceuticals; these include X-ray diffraction, optical microscopy, thermal analysis, dissolution testing, particle size analysis, NMR, and infrared (IR) spectroscopy. Raman spectroscopy is 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  $\mu\text{m}$  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 1  $\mu\text{m}$  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 characterized 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™, it becomes easy to reach 30  $\text{cm}^{-1}$  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.

### Figures



**Figure 1:** Carbamazepine distribution in tablet (blue: Form I, orange: Form III, black: excipients)

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## Use of complementary techniques for depth profiling of mobile screen protection covers

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.

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# Continuous raman activated cell sorting with machine learning

Raman activated cell sorting (RACS) is an emerging technology for isolating cells based on phenotypic function. Without the need for externally labelling cells, it has unique advantages in investigating natural microbial communities. However, challenges, such as low throughput and robustness, have yet to be addressed for its broad deployment. Here, we developed a continuous flow RACS system integrated with machine learning algorithms, using the full Raman spectra for on-the-fly cell classification at a high throughput of ~ 300 events/min. With its automation and robust performance, this platform provides a versatile tool for flow cytometry and cell sorting in various applications in microbiology, biotechnology, and life science.

## References

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## Figures

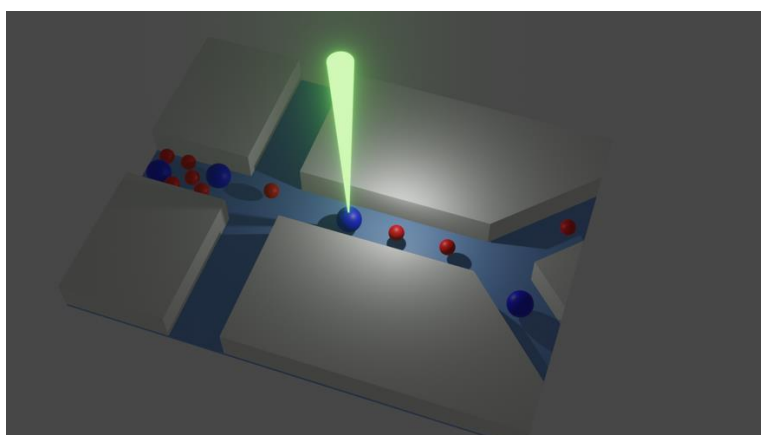


Figure 1: The simplified design of the microfluidic chip

## Nicolás Coca-López

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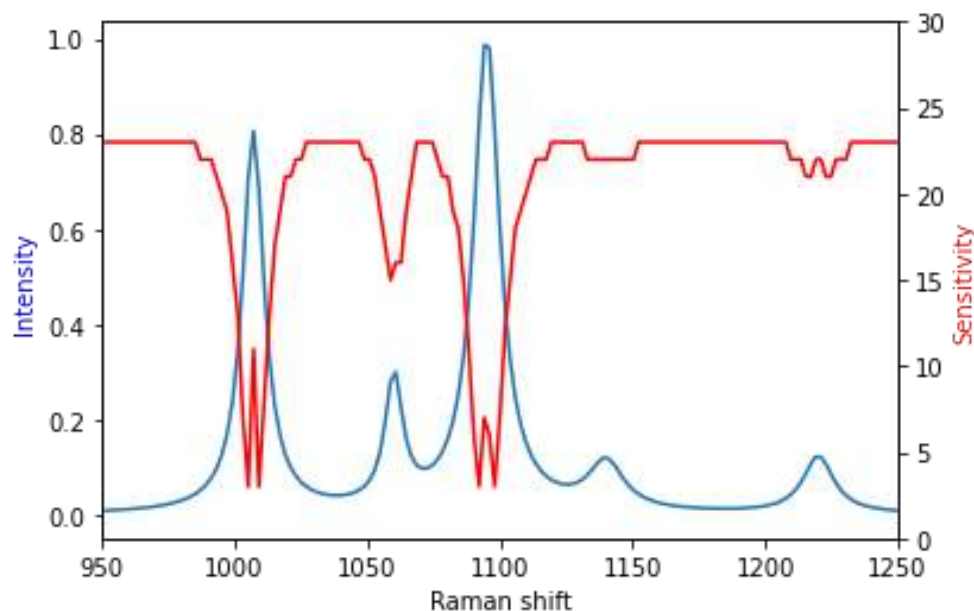
# Spike detection algorithms for Raman spectroscopy: A comparative study

For most applications based on Raman spectroscopy, spike detection and removal are required steps in data pre-processing [1]. Spike detection algorithms can be categorized depending on whether they rely on several spectra for comparison among them or they can be used with a single spectrum. The first ones require a minimal difference between adjacent spectra, whereas the second type offers greater versatility. In this study, several single-spectrum algorithms for spike detection are compared. The sensitivity of each method is evaluated across various types of spectra, different spike positions within a spectrum and different levels of noise. Furthermore, their suitability in terms of ease of implementation and computational time is also discussed.

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- [3] Maury et al., Appl. Spectrosc. 69(8), (2015), 984-992

### Figures



**Figure 1:** The blue line shows a spectrum presenting several Raman bands. The red line corresponds to the sensitivity of a given spike detection algorithm, where the sensitivity was defined as the minimum spike intensity to be detected, in percentage of the most intense Raman band intensity.

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## Analysis of microplastics in hand sanitizers using Raman spectroscopy

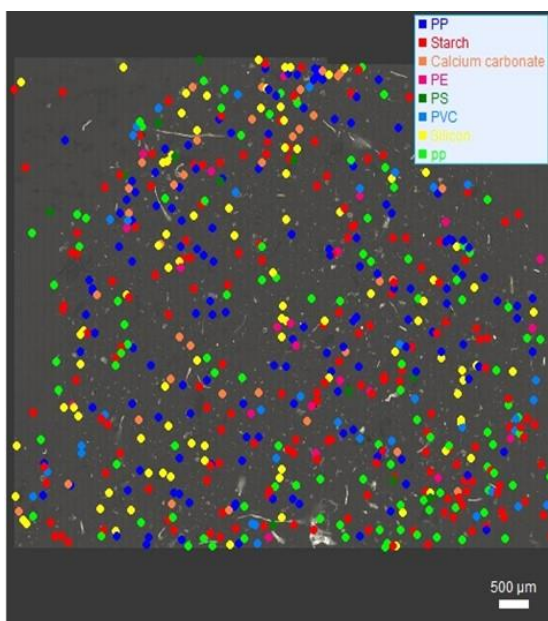
In the aftermath of the global pandemic, the ubiquitous use of hand sanitizers has become integral to daily life, yet hidden within these seemingly harmless gels lie potential hazards—microplastics. Known for slow degradation and toxin absorption, microplastics pose serious environmental and potential health risks; therefore, it is imperative to thoroughly examine the composition of these formulations.

Raman microscopy emerges as a non-destructive technique, providing morphological, quantitative, and chemical analysis of hand sanitizer composition. In this study<sup>1</sup>, we present a concise yet comprehensive solution that employs high-performance Raman microscopy, a purpose-built filtration kit, and the sophisticated ParticleFinder software to analyze hand sanitizer samples, providing a complete understanding of the microparticles content.

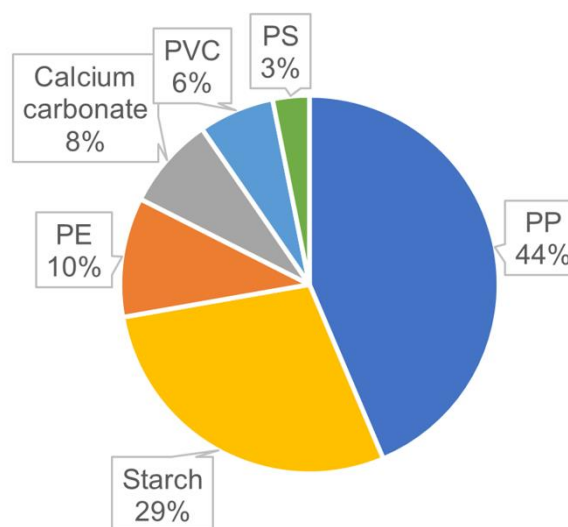
### References

- [1] <https://www.horiba.com/pol/scientific/applications/biopharma/analysis-of-microplastics-in-hand-sanitizers-using-particlefindertm/>

### Figures



**Figure 1:** microparticles spatial distribution (represented by colors) on an optical image of a silicon filter post filtration of a hand sanitizer sample.



**Figure 2:** apportionment of microparticles chemical identification given by Raman spectroscopy.

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## Electrochemical TERS setups for *in situ* measurements at the nanoscale

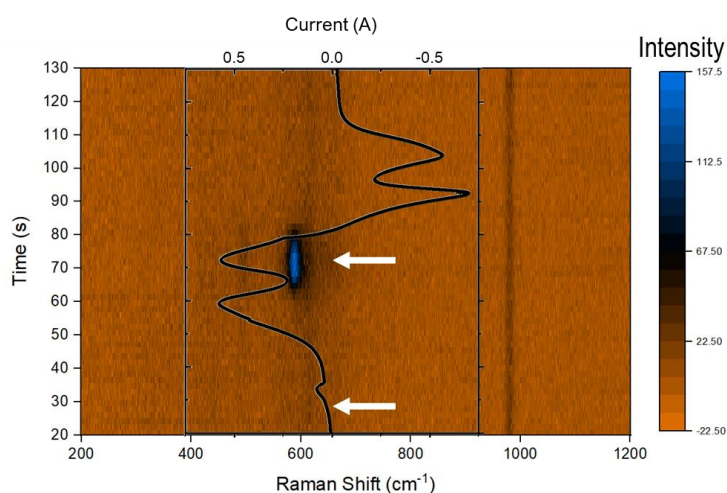
Monitoring ongoing (electro)chemical reactions on nanostructures at high resolution is a matter of prime importance in those research fields (such as nanocatalysis or nanoelectronics) where knowledge is missing on the chemical transformations and morphological changes that concomitantly occur at the molecular scale. Electrochemical Tip-Enhanced Raman Spectroscopy (EC-TERS) can answer these needs: thanks to the latest developments, both in AFM [1,2] and STM [3,4] setups, it was possible to efficiently detect spatial- and/or potential-dependent spectral modifications occurring on molecular layers, as well as short-life intermediates.

An important requirement to perform easy and reproducible EC-TERS analysis is the optimization of the instrumental setup, which must maximize the TERS signal in liquid, synchronize spectro-electrochemical measurements and be compatible with several samples and working environments. The solutions proposed by HORIBA meet these requirements and will be presented in this contribution, along with a few successful results (Figure 1) obtained within the EU project Harvestore in collaboration with the IREC laboratory.

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- [1] Kang G, Yang M, Mattei MS, Schatz GC, Van Duyne RP, Nano Letters, 19 (2019), 2106–2113
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- [3] Pfisterer JHK, Baghernejad M, Giuzio G, Domke KF, Nature Communications, 10 (2019), 5702.
- [4] Fiocco A, PhD thesis' manuscript (2022)

### Figures



**Figure 1:** TERS and current signal evolution vs time, acquired on lithium manganese oxide in an EC-AFM-TERS setup.

## Pooja Gune, Jana Geyer

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## Understanding characteristics of vaccine components using spectral decomposition

Vaccines for diphtheria, tetanus, and pertussis (DTaP vaccines) are a fundamental part of the standard vaccination schedules for both children and adults. DTaP vaccines are complex liquid formulations that result in strong spectral signals from enhancing substances (adjuvants) like aluminum salts and excipients such as phenoxyethanol. These signals need to be differentiated from the often weaker signal of the medically relevant compounds [1–4], i.e. proteins contained to elicit an immune response. In this study, we conducted comprehensive Raman spectroscopic measurements on two complete DTaP vaccines and their respective components to identify their spectral contributions. In particular, we have focused on the tetanus component of the vaccine and analyzed spectra of non-adsorbed tetanus toxoids obtained from the National Institute for Biological Standards and Control (NIBSC), UK, as a standard reference. However, this non adsorbed tetanus toxoid is formulated in a matrix of glycine, which exhibits a strong Raman signal. Consequently, we employed a series of experimental and analytical signal extraction techniques to unveil pure tetanus toxoid spectra. Eventually, we can show differential spectra of the pure adjuvant and tetanus toxoids adsorbed to the adjuvant. Our study underscores the remarkable potential of Raman spectroscopy in the identification of medically relevant DTaP vaccine components like adjuvants and tetanus toxoid, opening avenues for advanced vaccine quality control and batch testing.

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### Figures

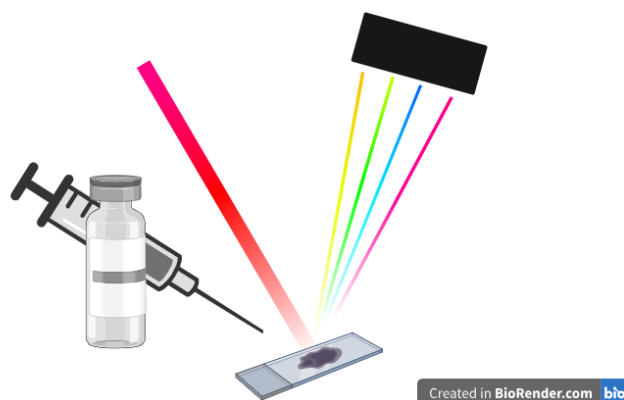


Figure 1: Analyzing vaccines with Raman spectroscopy



## Nathan I. Hammer

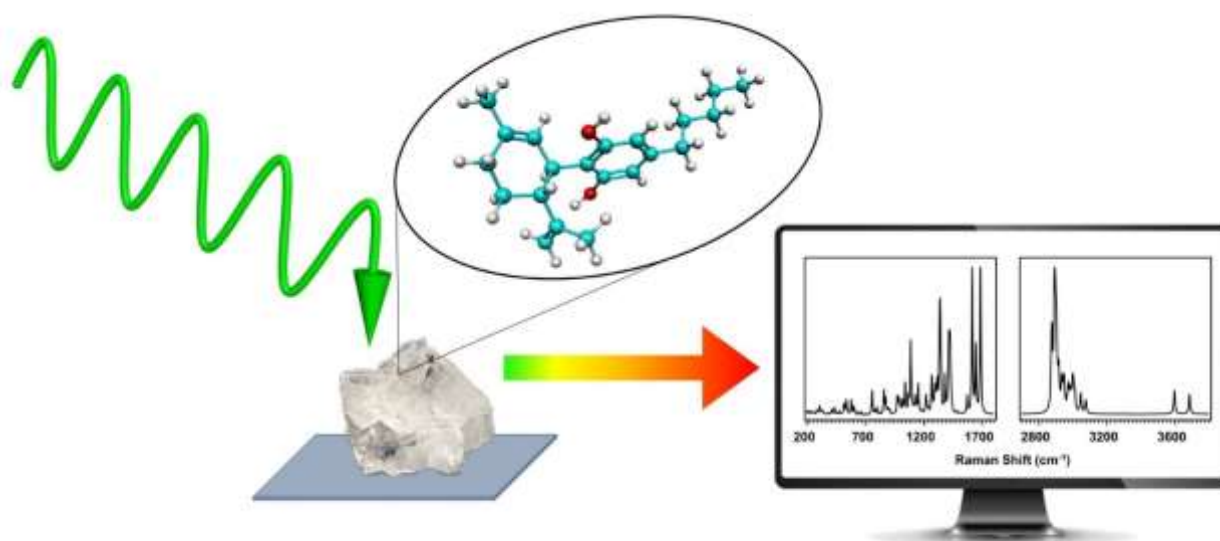
Trevor J. Wolfe, Nicholas A. Kruse, Mohamed M. Radwan, Amira S. Wanas, Kalee, N. Sigworth, Mahmoud A. ElSohly

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## A study of major cannabinoids via Raman spectroscopy and density functional theory

Cannabinoids, a class of molecules specific to the cannabis plant, are some of the most relevant molecules under study today due to their widespread use and varying legal status. Here, we present Raman spectra of a series of eleven cannabinoids and compare them to simulated spectra from density functional theory computations. The studied cannabinoids include three cannabinoid acids ( $\Delta^9$ -THC acid, CBD acid, and CBG acid) and eight neutral ones ( $\Delta^9$ -THC, CBD, CBG, CBDVA, CBDV,  $\Delta^8$ -THC, CBN and CBC). All cannabinoids have been isolated from cannabis plant grown at the University of Mississippi. The data presented in this work represents the most resolved experimental and highest-level simulated spectra available to date for each cannabinoid. All cannabinoids displayed higher peak separation in the experimental spectra than CBGA, which is most likely attributable to physical composition of the samples. The overall agreement between the experimental and simulated spectra is good, however for certain vibrational modes, especially those in the  $-OH$  stretching region, deviations are observed due to hydrogen bonding, suggesting that the OH stretching region is a good probe for decarboxylation reactions in these and related species.



**Figure 1:** Raman spectroscopy and computational chemistry are used to study eleven major cannabinoids.

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## Raman spectroscopy as a tool for simultaneous determination of diesel fuel additives

Various alternative sources are being explored as potential substitutes for conventional diesel fuel in the name of environment and circular economy. However, substitutes that are added to diesel fuel must comply with fuel quality regulations. Therefore, fuel additivation in form of diesel additives is required to maintain high quality fuel properties and its performance [1].

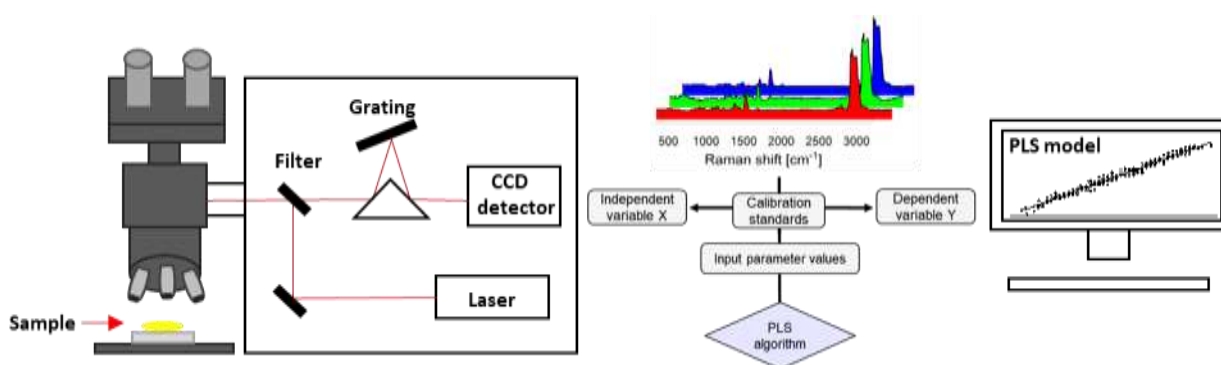
Raman spectroscopy in a combination with multivariate statistical analysis, partial least square algorithm (PLS) [2] was used as a promising tool to determine simultaneously the concentration of three types of diesel additives that improve the cetane number of diesel, the cold-flow properties and the conductivity-lubricating properties of diesel. Used cooking oil, waste tire oil and waste plastic oil was used as a replacement of 20% by volume of conventional diesel fuel. The root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV) and correlation coefficient was applied as a basic chemometric diagnostic tools to optimize the PLS models predictions. The calibration samples were equally distributed along the line of the PLS models with significant correlation  $>0.99$ .

Thus, Raman spectroscopy could be very promising technique in refinery/industrial applications, especially for online analysis, which could serve as quality control in production, prevent overdoses with diesel additives and save not only money but also time.

### References

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- [2] Marinović, S., Krištović, M., Špehar, B. et al. Prediction of diesel fuel properties by vibrational spectroscopy using multivariate analysis. *J Anal Chem* 67, (2012), 939–949.

### Figures



**Figure 1:** Raman spectroscopy scheme for PLS modelling

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# Raman microspectroscopic study of corroded bronze fragments from an Assyrian period tomb at Yasin Tepe, Iraqi Kurdistan and clayey volcanic products from the 7 March 2012 phreatic eruption on Ioto Island, Japan

Micro-Raman spectroscopy was used to identify mineral species in tiny bronze fragments from archaeological objects buried in an Assyrian period (10th-7th century BCE) tomb discovered at Yasin Tepe and mineral species in volcanic ash from the 7 March 2012 phreatic eruption at Old Crater on Ioto Island (Iwo-jima), Izu-Bonin arc, Japan. Five reference products of clay minerals, including kaolinite (JCSS1101b), dickite (JCSS1301), pyrophyllite (JCSS2101), montmorillonite (JCSS3101), and saponite (JCSS3501) were also analyzed to optimize analytical conditions and to obtain reference spectra.

Alteration minerals (malachite, azurite, and langite) and metal parts were identified with Raman analyses of the corroded bronze fragments. The absence of copper silicate minerals and soil minerals in the bronze fragments indicates that the samples have not been significantly weathered. These results are important for a better understanding of the condition and the past environment of the bronze samples. In addition, the information is helpful for evaluating the elemental and copper isotopic compositions of the original bronze artifacts [1].

Micro-Raman results revealed the presence of dickite, montmorillonite, gypsum, pyrite, marcasite, quartz, and anatase in the volcanic ash sample from the phreatic eruption. These alteration minerals were likely derived from the acidic alteration zone (150–190 °C) below the crater on Ioto Island. Marcasite, quartz, and anatase in aliquots of the sample were not detected by a previous powder X-ray diffraction study. Thus, these results demonstrate that Raman microspectroscopy is a more sensitive technique for identifying and characterizing alteration minerals in the volcanic products [2].

## References

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# High-performance miniaturized Raman systems for challenging applications

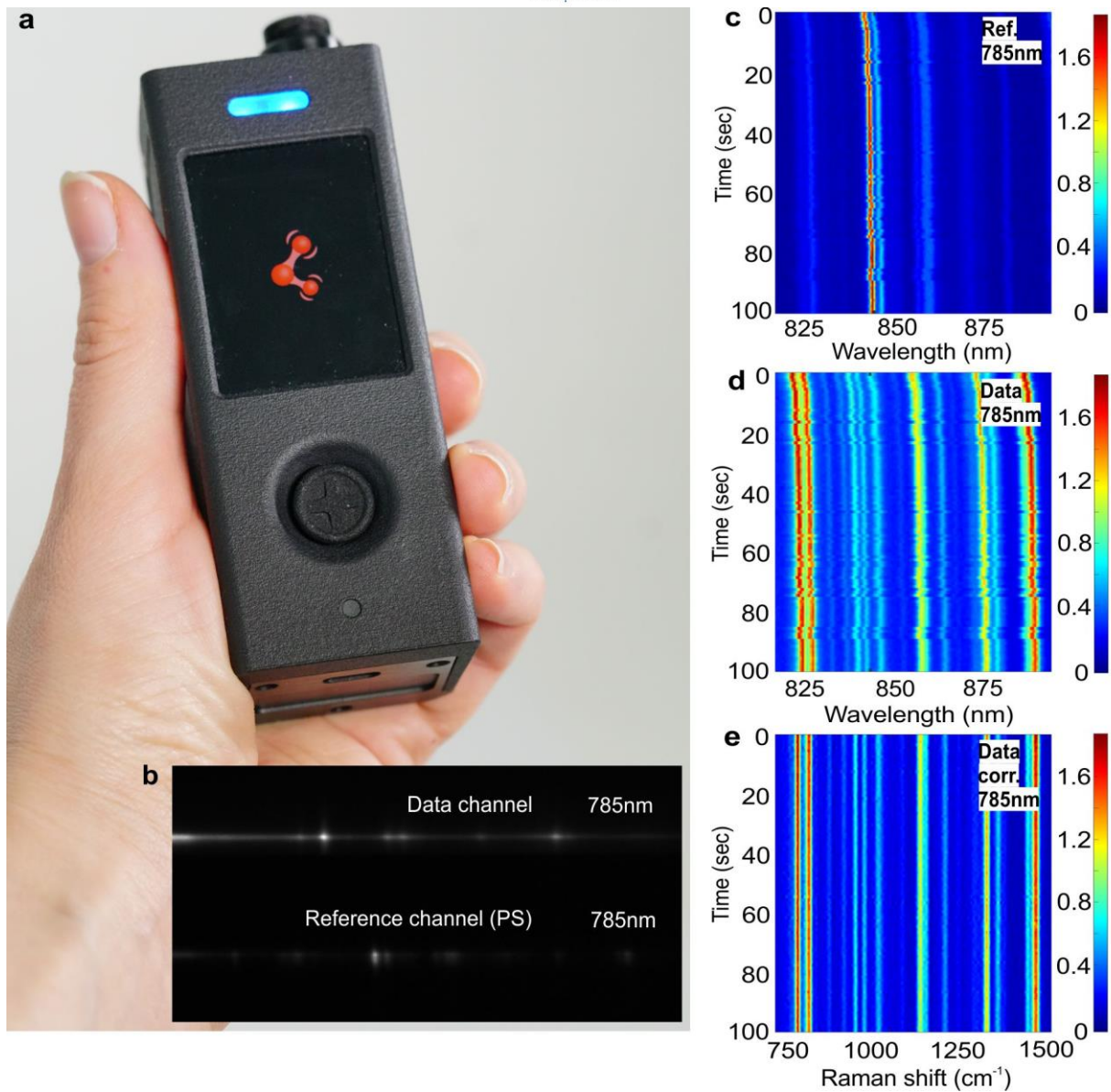
We present a novel miniaturization strategy that allows us to create versatile compact Raman spectrometers and microscopes based on cheap non-stabilized laser diodes, densely-packed optics, and non-cooled small pixel size sensors. We demonstrate that the achieved performance is comparable with expensive and bulky research-grade Raman systems. Our miniaturization concept is based on real-time calibration of Raman shift and Raman intensity using a built-in reference channel that is independent of the main optical path<sup>1</sup>. We have demonstrated the miniaturization of the whole device dimensions down to several centimeters and achieved excellent sensitivity, low power consumption, perfect wavenumber and intensity calibration combined with high spectral resolution of around 7 cm<sup>-1</sup> within the spectral range of 400-4000 cm<sup>-1</sup> [1]. We demonstrate possible solutions to the most critical Raman miniaturization challenges: need for laser temperature and power stabilization, reduction of sensor dark noise, compensation on pixel-to-pixel quantum efficiency variation, laser optical isolation and achieving high spectral resolution. Moreover, the proposed miniaturization strategy provides shifted-excitation Raman difference spectroscopy and spatially offset Raman spectroscopy functions as a derivative of the working principle [2-3].

The high performance and vast versatility offered by our strategy facilitate simple integration into various applications. As examples, we show the quantification of methanol in alcoholic beverages through a glass bottle, in-vivo Raman measurements of human skin, quantification of p-coumaric acid and serine during fermentation by *E. coli* bacteria, high resolution Raman mapping, quantitative SERS mapping of the anti-cancer drug methotrexate and in-vitro bacteria identification by Raman mapping. We foresee that the proposed miniaturization strategy will allow realization of super-compact Raman spectrometers for integration in e.g. smartphones and medical devices.

## References

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## Figures



**Figure 1:** Figure 1. (a) Photo of miniaturized Raman system with in-built reference channel, (b) CMOS sensor image that demonstrates simultaneous acquisition of main and reference Raman signals from laser excitation at 785 nm, (c) laser stability experiment versus time that represents raw Raman spectra variation of polystyrene in the reference channel under laser excitation wavelength 785 nm and (d) variation of raw Raman spectra of polypropylene in the main channel under laser excitation wavelength 785 nm, (e) Raman spectra of polypropylene in the main channel versus time after multiple calibration and pre-processing steps being applied.

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## 2D and 3D crystallographic orientation mapping using qRICO

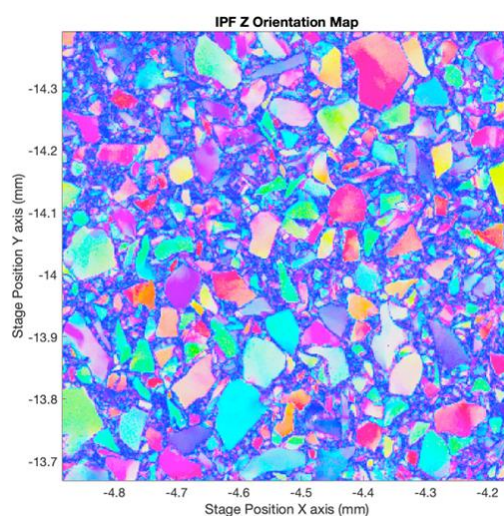
Non-destructive orientation mapping is a crucial tool in the characterization of polycrystalline materials and helps improve material properties based on the microstructure. Confocal Raman microscopy proves to be a highly effective non-invasive method for chemical mapping of organic and inorganic material. Polarized Raman microscopy (PRM) can be used as a tool for crystallographic orientation mapping, since Raman peak intensity is defined by crystal symmetry and its local crystal orientation [1]. This technology does not require a large amount of sample preparation such as scanning 3D X-ray diffraction or electronic back scattered diffraction (which can also alter the microstructural properties in some cases).

Quantitative Raman Imaging for Crystal Orientation (qRICO) analysis by means of PRM [2] is a non-destructive, cost-effective, and fast characterization technique for 2D and 3D orientation mapping of polycrystalline materials. The basic idea involves the simultaneous measurement of Raman scattering at different combination of incident and scattered polarization. The theoretical spatial resolution of the orientation map obtained by qRICO is constrained by the diffraction limit. In the present study, we demonstrate different applications of the qRICO technology (2D and 3D mapping of polycrystalline germanium and sapphire samples) and discuss possible methods to increase the spatial resolution mapping using chemometric techniques.

### References

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### Figures



**Figure 1:** Color coded IPF map of polycrystalline germanium.

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## Unlocking the potential of Raman with Artificial Intelligence: Collaborative brainstorming

Are you curious about how Artificial Intelligence can boost your Raman applications? Join us on an exciting journey of collaborative brainstorming to discover how AI can address your specific needs, alleviate pain points, and exceed your expectations in scientific workflows. Together, we will explore the boundless possibilities of AI in Raman, harnessing its power to accelerate discoveries and breakthroughs. Your insights and ideas will shape the future of scientific innovation.



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## Raman spectroscopy as a part of the PAT approach for in-line bioprocess monitoring

**Context and Objective:** Successful industrial bioprocess, in particular that concerning production of therapeutic antibodies (TAB) by animal cells in culture, requires the possibility of controlling a multitude of parameters, those of the process (CPP as critical process parameters) and those of the product (CQA as critical quality attributes). CPPs correspond to the concentrations of nutrients, catabolites and the density of the living cells. The CQAs correspond to the characteristics of the TAB produced (post-transcriptional modifications, aggregation state). Monitoring CPP and CQA in real time allows corrective actions to be carried out without leaving the quality margins. Even more interesting is to measure them without sampling (in situ), thus reducing the risks and costs linked to analyzes and helping to better understand the mechanisms involved in the successful process (Quality by Design approach).

Such a strategy becomes possible by process analytical technology (PAT) approaches, namely using molecular optical spectroscopy (MOS) where Raman spectroscopy may have a particular role. As part of a CLIMBIN collaborative project aiming to develop a PAT-MOS solution for bioprocessing, the present work evaluated the Raman spectroscopy performance in quantitative analysis of bioprocess ingredients.

**Methods:** CHO cells (ExpiCHO) suspensions were cultivated in a commercially purchased nutritive medium (ExpiCHO™), at controlled conditions (pH, temperature, gas equilibrium), for up to one week, either in flasks or in a Bioreactor (Primo, Pierre Guérin). The culture medium was analyzed either as in-line kinetics, or off-line, as the model solutions prepared to correspond to different moments of the cell culture. Both in-line and off-line Raman measurements were performed via immersion probes coupled by optical fibers with a Viserion Raman spectrometer (INDATECH, France) equipped with a 785 nm laser source and CCD detector. The Raman data have been then pre-processed and analyzed using MATLAB® (Mathworks, USA). The same samples were also analyzed by conventional off-line methods, to establish viable cells density (NucleoCounter® SCC-100™, Chemometec) and molecular composition of the medium (biochemical analyser Gallery, Thermo; HPLC, Agilent).

**Results and Conclusions:** Operating conditions were optimized for recording Raman spectra from both the off-line model solutions and the in-line bioreactor monitoring. The multivariate statistics modes were established to predict from the Raman spectra the concentrations of several molecules (nutrients, metabolites or TAB) and the cell density. The performance of the Raman-based vs conventional methods-based analysis has been compared.

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## Confocal Raman microspectroscopy study of vitelline membranes from hen eggs

**Context and Objective:** The vitelline membrane (VM) surrounds the yolk of the hen's egg and separates it from the albumen. It consists of two major layers, the inner layer, which is laid down in the ovary, and the outer layer, which is secreted in the oviducts. VM is known to stretch and become weakened with decrease of the egg quality (1). In order to get a deeper insight into VM structure and role it plays in the hen egg quality and preservation against bacteria, we investigated the effect on the molecular composition of VM induced by a series of factors such as the hen age, the egg storage duration and conditions (temperature and atmosphere). **Methods:** Lohmann Tradition laying hens were set up at the PEAT unit (INRAE Nouzilly) and reared on the ground for egg production. The eggs were collected at different ages of hens: 32, 73, and 81 weeks. The eggs were stored for 0, 14 and 21 days under controlled conditions, variable in terms of temperature (4 or 20°C), relative humidity (50 or 75-80%) and CO<sub>2</sub> (0 or 10%). The samples of VMs were extracted from the eggs, washed in cold water, disposed on CaF<sub>2</sub> substrates and dried at ambient conditions. Raman spectra were collected as hyperspectral maps (min 25 spectra each, up to 4 maps per sample, up to 2 maps on each side of the membrane) via an x50 objective of a confocal Raman microspectrometer (LabRam, Horiba Scientific, France) equipped with the 690 nm laser source. The data acquisition was made using a LabSpec software (Horiba Scientific, France). The data have been then pre-processed (EMSC, area normalization) and analyzed by ASCA (ANOVA Simultaneous Component Analysis) using MATLAB® (Mathworks, USA).

**Results and Conclusions:** For each sample of VM, selected according to chicken age and storage conditions (duration, temperature, and atmosphere composition), average Raman spectra were generated and compared. The obtained results (Fig 1) confirmed that confocal Raman microspectrometry is potent enough to reveal the specific features of protein composition/conformation of the VMs according to the hen's age.

### References

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### Figures

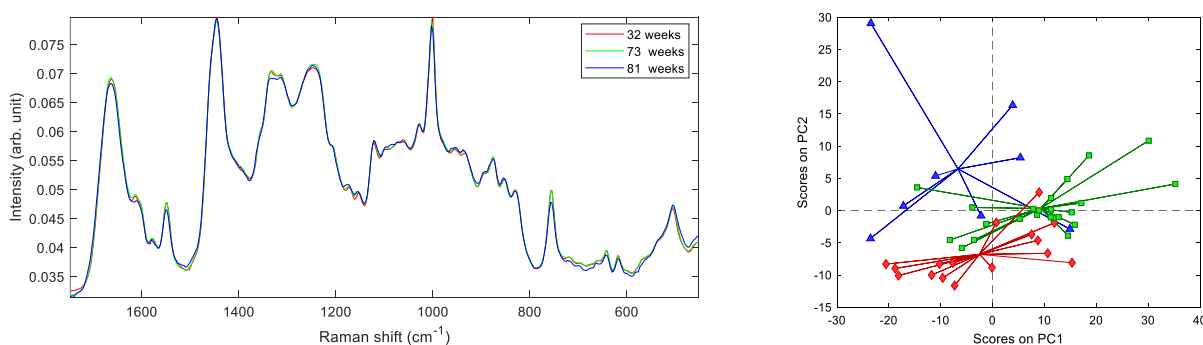


Figure 1: Distinguishing molecular changes of vitelline membranes from Raman spectra. A: Average Raman spectra of 3 groups of samples according to hen's age (Day 0). B: ASCA scores evolution for the same groups of samples.

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