



BOOK OF ABSTRACTS



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ORAL PRESENTATIONS

Mid-Infrared Photonics: From Emerging Tool to Enabling Technology

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Vibrational spectroscopies - and especially infrared spectroscopy - play an increasingly important role in modern biodiagnostics. This has led to the evolution of mid-infrared photonics from an emerging tool in the clinical/medical domain to an enabling technology. With applications ranging from non-invasive exhaled breath analysis to in-vivo assessment of cartilage damage, midinfrared (MIR; 3-20 μm) photonics ranges among the most flexible molecular sensing platforms nowadays available. With the emergence of quantum and interband cascade laser technology, the on-chip hybridization and/or integration of entire MIR sensing devices is on the horizon ultimately leading to IR-lab-on-chip systems. The inherent molecular selectivity of MIR signatures enables studying small molecules (e.g., volatile organic compounds; VOCs) in the gas phase, as well a biomacromolecules (e.g., proteins) in the liquid phase at unprecedented detail in a label-free and non-destructive fashion. Last but not least, the combination with advanced multivariate data evaluation and deep learning algorithms facilitates analyses in real-world complex mixtures of biomedical and clinical relevance. The discussion of latest MIR photonic technologies in this presentation we will be augmented by highlight applications underlining the utility of next-generation MIR photonics.

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Colloidal Synthesis and Applications of Plasmonic Nanoparticles

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The ability of noble metal nanoparticles to couple with electromagnetic radiation in the near IR and visible regions stimulates research worldwide because of possible applications in several technological fields such as sensing, solar cells, spectroscopy, signal enhancement for imaging and several biological and medical issues. It is also known that the geometrical features of the nanoparticles, their coupling and the nature of the metal mostly define the optical properties in terms of scattering, absorption and extinction cross-sections. In this talk I will review some of the most challenging methods to obtain variously shaped metal nanoparticles in the colloidal form, either using conventional chemical methods and contaminant free procedures. Strategies for the deposition of plasmonic layers onto solid substrates will be also presented in order to obtain suitable sensing devices. Applications of the obtained nanostructures for plasmon sensing and surface-enhanced phenomena are used as excellent examples showing the advantages of these tools.

Novel approaches to broadband coherent Raman microscopy

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Coherent Raman scattering (CRS) microscopy is a powerful nonlinear optical technique for chemical identification of (bio)-molecules based on their intrinsic vibrational spectrum and for high-speed label-free imaging of cells and tissues. Single-frequency CRS microscopy allows the detection of molecules with a specific Raman response but is not sufficient to distinguish different components within complex heterogeneous systems in which chemical species display spectrally overlapped resonances. For this reason, there is ongoing research aimed at extending CRS to broadband detection, combining the speed of coherent Raman spectroscopy with the information content of spontaneous Raman [1]. In this talk we present two innovative approaches to broadband CRS, both in the stimulated Raman scattering (SRS) and coherent anti-Stokes Raman scattering (CARS) modalities. We first present a broadband CARS (B-CARS) system based on a high power ultrafast solid-state laser and white light continuum generation in a bulk material, acquiring high-quality spectra in a 1-ms time, limited by the spectrometer refresh rate, covering both the fingerprint and the CH stretching region [2]. A convolutional neural network is used to denoise the B-CARS spectra and remove the unwanted non-resonant background [3]. Our instrument delivers high quality label-free images of therapy-induced senescent cells and liver tumor tissues. Next, we present a broadband SRS system equipped with a home-built multichannel lock-in amplifier, simultaneously measuring the stimulated Raman signal over 32 frequencies within 44 μ s, allowing for detailed, high spatial resolution mapping of spectrally congested samples [4]. We demonstrate the performance of our SRS microscope by discriminating the relative concentrations of different fatty acids in cultured hepatocytes at the single lipid droplet level and by identifying fibrosarcoma tumor lesions embedded within healthy tissue. Finally, we describe our effort to develop a commercial SRS microscope [5] based on a compact,

turnkey fiber laser system for application to histopathology and label-free tumour identification, staging and grading.

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Advances in FTIR Spectroscopic Imaging

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Recent advances in spectroscopy, such as the development of FTIR spectroscopic imaging using infrared array detectors, have opened up a window of opportunity within materials science, with applications to polymers, biomaterials, pharmaceuticals, and also facilitate research in areas ranging from biomedical to forensic and conservation sciences. FTIR spectroscopic imaging enhances research at the interdisciplinary interfaces, stretching the boundaries between traditional science and engineering. This talk will outline the research we are developing in this area with focus on macro ATR (Attenuated Total Reflection)-FTIR spectroscopic imaging applied to heterogeneous materials. The advantages of macro ATR-FTIR imaging include its versatility and the relative ease of sample preparation. Recent new developments in macro ATR-FTIR imaging with the use of inverted prism crystals show good potential with applications to protein crystallization, imaging of live cancer cells and microfluidics. The opportunity to obtain macro ATR-FTIR images of the same sample from different depths using a diamond ATR imaging accessory has been demonstrated. This approach involved the introduction of a movable aperture to restrict the angles of incidence to certain values. A recently developed application includes the introduction of a new high-throughput crystallization screening method for permitting in situ chemical imaging of protein crystallization and aggregation through the use of macro ATR-FTIR imaging. Exciting recent applications of this imaging approach has been demonstrated by the ATR- FTIR images of flows in the channels of microfluidic devices. This opens many new possibilities of using macro ATR-FTIR imaging as a novel detection method in the studies of dynamic systems.

Uncovering hidden dynamic structure in biological systems using Brillouin Light Scattering Microspectroscopy

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Since first being predicted 100 years ago Brillouin Scattering has found diverse applications in the material and physical sciences. More recently it has become possible to also perform Brillouin Light Scattering (BLS) measurements on living cells and tissue under near physiological conditions. While 4D mapping a viscoelastic modulus inside living cells has the potential to open a plethora of biophysical and medical applications, the distinct time scales and boundary conditions probed in BLS mean their relevance in regard to describing mechanics in soft matter, is not so trivial. I will discuss some unexpected insight that BLS can give us where it serves as more than a proxy to the quasi-static tensile or shear properties commonly probed in elastography and rheology, spanning COVID-19 prognostics, directed cell growth, and gene transcription in the cell nucleus.

Vibrational spectroscopy for the study of cultural heritage materials: recent developments in hyperspectral macro imaging

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Tangible cultural heritage poses interesting challenges to the field of vibrational spectroscopy because of the need to develop methods to discriminate compounds that are often mixed, layered, and altered over time, but at the same time contained in valuable and unique samples. Much work has been done in recent decades to develop non-invasive methods that can operate directly on the artwork, based on infrared spectroscopy and Raman spectroscopy, in point mode, thus contributing fundamentally to the development of research in conservation, art history, and archaeology. The next stage of development has been to push toward hyperspectral applications in the macro mode with the goal of combining the potential of chemical information obtainable from point vibrational techniques with the spatial information provided by imaging techniques. The state-of-the-art applications of macro-scale hyperspectral imaging/scanning methods in the near-infrared range will be presented by discussing cases of particular importance for the value added to the solution of challenging issues in the art-historical, codicological, and archaeological fields. The case studies will be chosen as part of the international activities of E-RIHS' MOLAB access platform of the European research infrastructure for heritage science, also to present the potentials and challenges of multimodal applications. Recent developments to shift the macro-hyperspectral application from near to mid-infrared will also be discussed in order to take advantage of the strong specificity and sensitivity of this spectral range in the recognition of compounds of great importance for understanding the conservation status of artworks.

A systematic study of protein-fiber interaction in asbestos body model using FTIR microscopy and IR nanoscopy

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Occupational exposure to asbestos is associated with severe lung diseases such as asbestosis and mesothelioma [1]. The mortality peak of exposed subjects is predicted in the next 10 years. One key process in pathology development is the interaction between asbestos fiber and proteins, which determines the formation of asbestos bodies (AB) described as asbestos fiber coated with mucopolysaccharides and proteins (e.g. ferritin) [2]. Understanding this mechanism is essential to shed light on the onset of asbestos-related disease and to set up therapeutic approaches for its treatment. In this framework, we present a recent project exploiting IR microscopy and nanoscopy to study the protein misfolding caused by asbestos fiber interaction, performed at SISSI-Bio beamline at Elettra Sincrotrone Trieste [3]. The present contribution is based on the fine-tuning of *in vitro* AB models (AB-M) to figure out the fiber-protein interaction mechanism and to highlight the protein structural changes following their binding to asbestos. AB-Ms are made with iron storage proteins holo- and apo-ferritin (HoloF, ApoF), and chrysotile fibers (Chry) [4]. AB-Ms are prepared mimicking cytosol and phagosome-like steps in the lung environment, after fiber ingestion, at different incubation times. FTIR microscopy analyses are able to reveal time-depending aggregation of both proteins, but more pronounced for ApoF (iron free) than HoloF. In addition, infrared s-SNOM spectroscopy and nanoimaging with PsHet detection are fundamental to establish the strong interaction of Holo/ApoF-Chry and the role of iron as an active reaction partner. HoloF binds to asbestos fibers intercalating itself into the silicate structure. Instead, ApoF cannot penetrate the structure and binds indeed onto fibers by creating a protein skin around them. These findings suggest that ferritin-chrysotile interaction triggers the protein misfolding process and iron may have a key role in the AB-M formation. In conclusion, these data add new experimental evidence in AB assembling and provide solid bases for better dissecting their pathogenicity.

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Charge Instability in Multimodular Push-Pull Systems: Excited-State Symmetry Breaking Under the Gaze of Ultrafast Time-Resolved Infrared Spectroscopy

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The interest for symmetric multi-branched electron donor–acceptor dyes has considerably increased over the last decade. These compounds are usually characterized by a large two-photon absorption cross-section and have also been introduced as promising chromophores for thermally activated delayed fluorescence providing low singlet–triplet gap. Additionally, these multibranch dyes can be viewed as simple models of organic conjugated polymers and are particularly useful for understanding the electronic excited-state properties of these materials [1]. Whereas, as expected for compounds that do not possess a permanent electric dipole moment, their absorption spectra show almost no solvent-dependence, their fluorescence spectra display a strong solvatochromism suggesting a dipolar S_1 state. This behavior was rationalized in terms of a break-up of the symmetry in the excited state driven by structural and solvent fluctuations [2, 3]. In this regard, the aim of our research has been the investigation of three symmetric and multidonor-acceptor compounds bearing one or two C-C triple bonds per branch [4] (Figure 1.)

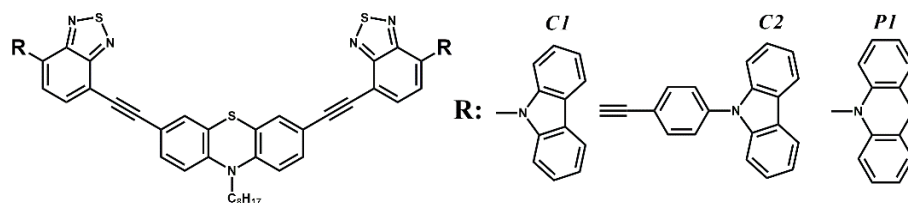


Figure 1. Molecular structures of the multimodular push-pull systems investigated.

Specifically, in this work, these compounds were investigated in a wide set of organic solvents, to evaluate the impact of the medium, the nature of the donor group and the presence of multiple C-C triple bonds on their excited state dynamics. The first part of this study was carried out by means of steady-state and advanced spectroscopic techniques, such as nanosecond and femtosecond UV-Vis transient absorption and femtosecond broadband fluorescence up-conversion as well as non-linear two-photon excited fluorescence. Despite useful information on the excited-state lifetime, the data collected by electronic spectroscopies do not present any clear feature that could be assigned to the transition from a symmetric and quadrupolar excited state to an asymmetric dipolar state. For this reason, we decided to employ, for the first time in this kind of push-pull systems, the Time-Resolved Infrared Spectroscopy to resolve the spatial distribution of the excitation within the molecules and then to follow the occurrence of the Excited-State Symmetry Breaking.

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Doping of P3HT electrospun fibers: monitoring through infrared (IRAV) and Raman (RaAV) polaronic features

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Regioregular poly(3-hexylthiophene -2,5-diyl) P3HT fibres blended with high-molecular-weight polyethylene oxide (PEO) to support fibre formation are deposited by electrospinning. Optionally, the scaffold PEO polymer can be washed, without evident damage to the fibres morphology. Vibrational spectroscopy is an effective method to study the structural properties of pristine and doped P3HT in 1D structures, like electrospun micro- and nano-fibers. By means of FT-IR spectroscopy in polarized light, we were able to investigate the orientation of the polymer in the pristine fibres showing that samples exhibit a high molecular orientation, with the polymer chains mainly running parallel to the fiber axis. The most straightforward method of doping P3HT electrospun fibers is the exposure to Iodine vapours. Polymer fibers, are properly doped, as proved by the appearance of the doping-induced IR bands (IRAVs) and by means of FT-Raman spectroscopy by following the clearly recognizable shift of the ECC band of the doped samples (RaAV) [1].

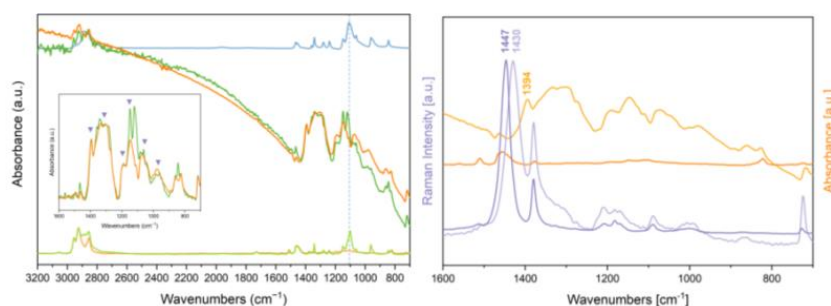


Figure 1: Left: infrared spectra of iodine-doped nanofibres of P3HT (dark orange line) and of P3HT:PEO (dark green line). The inset in the IRAV bands region shows the main peaks (triangles). The IR spectrum of nanofibres of pure PEO (blue line), of the pristine P3HT (light orange line), and of pristine P3HT:PEO fibres (light green line) are shown. Right: violet lines are FT-Raman spectra of P3HT nanofibres. Orange lines are IR spectra of P3HT nanofibres. The darker line refers to the pristine sample, the lighter line refers to the iodine-doped sample [1].

Nevertheless, the doping is not stable over time, as evidenced by the evolution of the IRAV bands, which rather quickly fade over time, leaving the sample un-doped after some hours. [1] Therefore, we decided to explore another dopant species, namely 2,3,5,6-Tetrafluoro-7,7,8,8-tetracyanoquinodimethane (F4TCNQ), which is commonly employed for doping P3HT. We herein compare, via vibrational spectroscopy methods, the effectiveness and stability of the doping of P3HT fibers with both iodine vapours and F4TCNQ. It is demonstrated that depositing films from a solution that already contains the dopant results in doping level which is stable over time. Therefore, we prepared electrospun fibers from a solution which already contains the dopant, both for iodine and F4TCNQ, thus showing the feasibility and the limits of this process. By preparing doped fibers in a one-step method, we could also perform a quantitative analysis of the doping level reached by the different samples, which allows obtaining an estimate of the absolute IR intensity of the IRAV bands.

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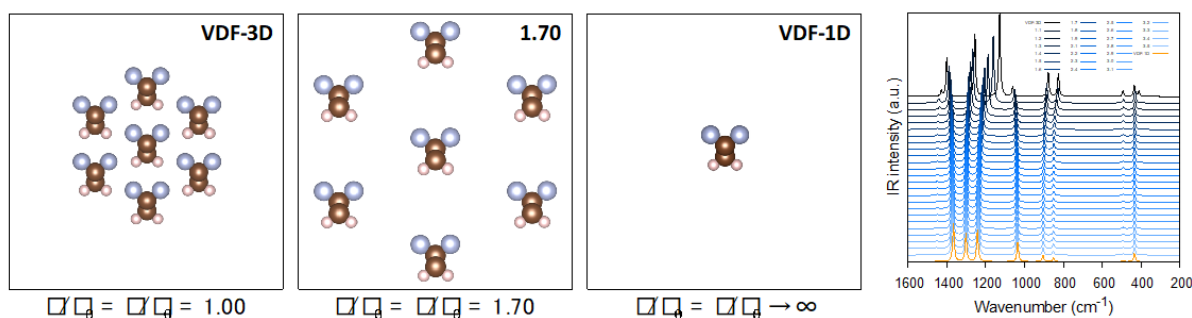
Solid state effects on the vibrational spectra of the β phase of polyvinylidene fluoride

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Polyvinylidene fluoride (PVDF) is the most famous ferroelectric polymer. As such, it finds applications ranging from flexible electronics to biocompatible sensors. In one of its polymorphs, all the polymer chains arrange themselves in an *all-trans* fashion, with the backbone of each chain belonging to one plane. Such polymorph defines the β phase of PVDF, which has a non-zero electric dipole moment, resulting from the sum of the moment of each chain. Thus, in the β phase of PVDF, two types of interactions are found: short-range dipole-dipole interactions, and long-range electrostatic interactions. Starting from a previous spectroscopic investigation [1], we study how these two types of interactions affect the vibrational spectra of the β phase of PVDF. To do so, we build computational models that gradually and selectively exclude, partially or totally, these interactions from the starting three-dimensional crystalline system, and then compute the corresponding infrared spectra. We observe that different infrared bands are differently affected by these exclusions.



Computed equilibrium geometries at the B3LYP/6-31G(d,p) level of the β phase of PVDF and their infrared spectra. The three panels on the left show portions of periodic models as viewed from the *ab* plane of an orthorhombic crystal system, where *c* is the cell parameter along the chain axis: **VDF-3D** is the system at thermodynamic equilibrium, **1.70** is the system when *a* and *b* are constrained to be 1.70 times larger than the equilibrium values *a*₀ and *b*₀, and **VDF-1D** is an isolated chain. The panel on the right shows the calculated infrared spectra (at the same level of theory) of these and other analogous models.

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Raman spectroscopy for combustion gas diagnostic

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In this work is proposed a laser-based system to perform gas analysis through Raman spectroscopy, the system is designed to be applied to in-line combustion diagnostic. The Raman approach also provides information about H₂ and N₂ concentrations not commonly available from analysis with absorption spectroscopy techniques. The experimental setup is based on a multi-pass cell (designed to increase the generated Raman signal) and allows high-frequency (7 Hz) acquisitions.

The use of hydrogen as a green fuel has seen notable growth in interest in recent years, new tools and diagnostic techniques need to be developed to fully cover its use in industrial applications. Furthermore, in the case of combustion that involves the use of atmospheric air as a comburent, nitrogen is the main component of the mixture and is a useful inert (ex. creation of oxides) to be traced and used as a reference. The low density of gas samples poses a problem, as the amount of Raman scattering is proportional to the number of molecules present in the light-matter interaction region. To boost the amount of signal collected, we have coupled the cell with a system of spherical mirrors in order to perform 16 reflections of the laser beam back on the focus point (i.e. 17 passages). In Figure 1 is reported the multi-pass setup with its main components: Nd:YAG 532 nm CW laser (1.16W) (1), spherical mirrors (2a, 2b), windowed gas cell (3) and spectrometer (4).

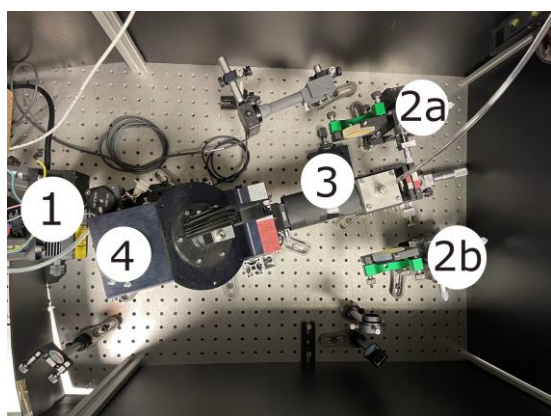


Figure 1: Multi-pass laser beam setup.

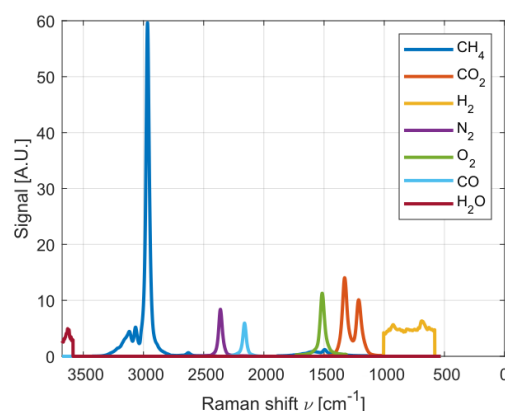


Figure 2: Spectra of different gasses at 100% of concentration used for the instrument calibration.

The instrument is able to detect Raman scattering starting from the H₂ rotational peaks with a Raman shift (ν) of 587 cm⁻¹ up to the H₂O vibrational peak at $\nu = 3657$ cm⁻¹. In Figure 2 are reported the spectra of different gasses at 100% of concentration used for the instrument calibration.

This research demonstrated that a Raman spectroscopy approach meets the main requirements of combustion diagnostic since it is capable to follow the time scale of the process. The system is able to perform analysis with an integration time of 0.15 seconds per acquisition, which is a significant achievement in Raman spectroscopy on gaseous samples.

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Hydration water and ionic aggregation in aqueous solutions of imidazolium-based ionic liquids by UV Resonance Raman

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Water molecules, present as additive or as contaminant of Ionic Liquids (ILs) can compete for the hydrogen bond sites leading to important modifications of the local order of these liquids and to the modulation of their physical chemical properties. In this work, aqueous solutions of a set of imidazolium-based ILs [MIM][X] (X = NO₃, TfO, HSO₄, and Cl), [BMIM][HSO₄] and [BMIM][MeSO₄] are investigated by deep UV Resonance Raman spectroscopy in highly diluted regime where ionic aggregates and bulk-like water should coexist. A differential method was used to analyze the OH stretching profile to extract the so-called solute-correlated (SC) spectrum which is particularly informative of the hydration features of the ILs. Moreover, specific bands of the cation, sensitive to the hydrogen bonding, were comparatively investigated. The progressive evolution from contact ion pairs (CIP) / larger ionic aggregates to solvent-shared ion pairs (SSIP) and/or solvent-separated ion pairs (SIP) can be efficiently monitored as a function of the hydration level. Various combinations interactions of water-water/water-IL/IL-IL interactions lead to alteration in ionic liquids C-H groups of the imidazole ring (3000-3200 cm⁻¹) and O-H stretching of water. The analysis of SC Raman spectra points out that, upon ILs concentration increase, the formation of cation-anion aggregates occurs, especially in the case of [MIM][Cl]. Chloride ions tend to perturb a larger number of water molecules than the other anions, consistently with a progressive substitution of water by the cation within its solvation shell by increasing PIL concentration. The unique response of [MIM][Cl] to hydration found by SC-UVR data is related to the synergy of different factors such as the reduced size (higher charge density), spherical symmetry, and the high H-bond basicity.

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Huge Raman intensity due to intra-molecular charge oscillation in BEDT-TTF₂⁺ dimerized salts

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The salts of bis(ethylenedithio)-tetrathiafulvalene (BEDT-TTF) molecule (Fig. 1, right) and its variants have played and play a central role among molecular quantum materials [1]. The salts have stoichiometry (BEDT-TTF)₂⁺X⁻, and a variety of molecular packings, almost all characterized by the presence of dimerized BEDT-TTF pairs. Besides superconductivity, many other interesting phenomena are occurring in these salts, such as charge fluctuations, inter-molecular charge-ordering with consequent ferroelectricity, and so on. Vibrational spectroscopy has been instrumental in the characterization of BEDT-TTF salts, as for instance it has provided information about the strength of electron-phonon coupling that might be involved in the superconductivity mechanism [2]. In particular, the totally symmetric C=C stretching phonons a_g ν_2 and ν_3 are strongly coupled to the Charge Transfer electronic system, and within a dimer give rise to huge intensity infrared (IR) bands polarized perpendicularly to the BEDT-TTF planes. On the other hand, the IR active antisymmetric b_{1u} ν_{27} C=C stretching appears with strong intensity with polarization along the BEDT-TTF long molecular axis.

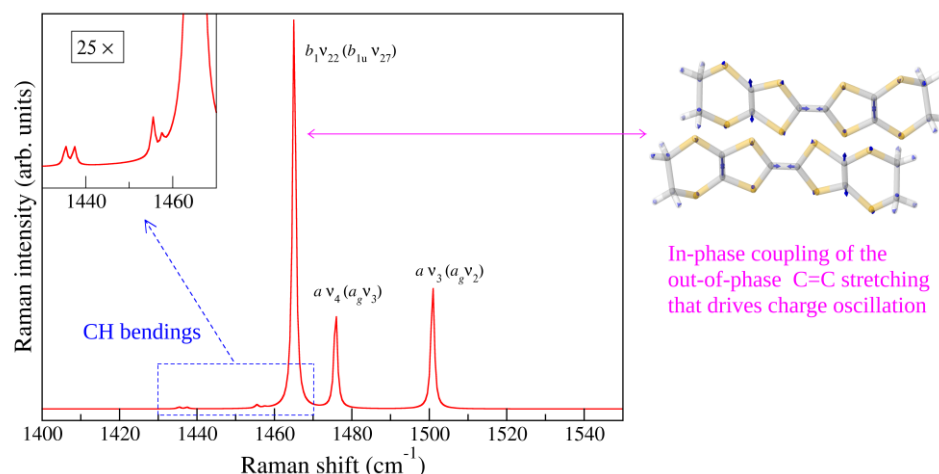


Figure 1. Calculated (BEDT-TTF)₂⁺ Raman spectrum in the C=C stretching region.

The IR spectra of (BEDT-TTF)₂⁺ dimers are well understood [2]. On the other hand, Raman spectra in the C=C stretching region appear more complex than expected, and a clear interpretation is still lacking. Here I present the results of DFT calculations of the dimer's Raman intensities (Figure 1). Rather surprisingly, the most intense band does not correspond to the monomer's a_g phonons, but to the in-phase coupling of the b_{1u} ν_{27} mode, driving charge oscillation along BEDT-TTF long axis. Proper transformation from the molecular to the crystal axes allows one to satisfactorily reproduce and interpret the experimental polarized Raman spectra of the so-called k-phase BEDT-TTF salts.

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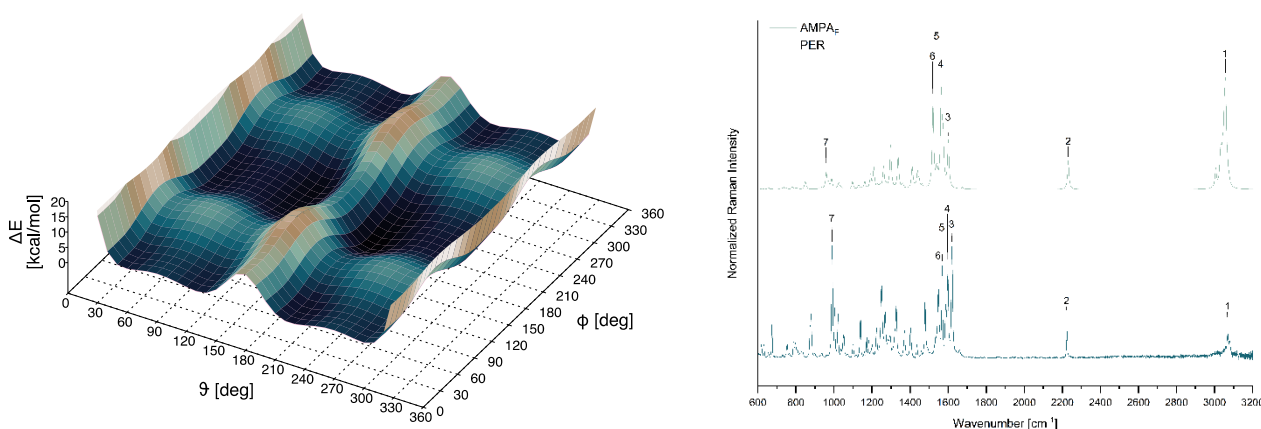
Investigating Perampanel drug by DFT and Raman spectroscopy

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Perampanel (PER) is a novel anti-epileptic drug used to treat patients affected by epilepsy to control and prevent the onset of seizures. The PER dosage range suitable for effectively treating patients with no collateral effects is very narrow [1]. *Therapeutic Drug Monitoring* (TDM) is thus used to control the concentration of PER in the patient biological fluids, e.g., blood plasma [2]. Since TDM can be burdensome on patients, research efforts are ongoing on alternative and non-invasive techniques that may improve their quality of life. Specifically, Raman spectroscopy and SERS have been considered for the detection of PER [3][4], but a detailed computational investigation of the molecular and vibrational structure of PER is still lacking. Hence, in such a context, this work is the computational effort to thoroughly investigate the molecular and vibrational structure of PER. We relied on the DFT method B3LYP/6-31G(d,p) to investigate the *Potential Energy Surface* (PES) of PER. A systematical exploration of the PES was carried out, which provided the equilibrium structures of PER conformers upon full geometry optimization. This study brought to light a conformer matching the structure of PER within its target receptor as reported in the Protein Data Bank [5]. From the equilibrium conformers, we could determine the theoretical Raman spectrum of PER. Furthermore, since SERS is performed in acidic conditions, by protonating such structures also, the theoretical SERS spectrum could be theoretically investigated. Comparing the theoretical Raman and SERS spectra with the experimental data [4] gave a promisingly good match, which validates this model as a benchmark for future vibrational spectroscopy studies on PER.



Representation of the PES of PER (left) and comparison of the experimental vs. DFT-simulated Raman spectra (right).

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Raman gas spectroscopy for biological survey

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Despite the advances in the dairy industry, the contamination of milk (and therefore its derivatives) by Clostridium remains both a public health and an economic problem. Current investigation techniques are microbiological but are costly both in terms of money and time (usually a few days). The aim of the project is to develop a Raman spectrometer capable of measuring (mainly) CO₂ and H₂ in the headspace of vials and being able to discriminate between tubes containing contaminated milk samples from negative ones. The instrument is automated in such a way as to rotate vials to minimize the scattered light created by the imperfections of the glass, since it is not realistic to require the use of high optical quality and therefore high cost test tubes. The instrument in its definitive form should allow the rapid, inexpensive analysis of a large amount of samples contained in low-cost, disposable vials without requiring initial procedures on samples other than sample injection and incubation¹. Since Raman spectroscopy provides information on multiple gases (H₂, O₂, N₂, CO₂) as shown in Figure 1, the instrument in the final stage could be applied in other fields, such as for example in the health sector.

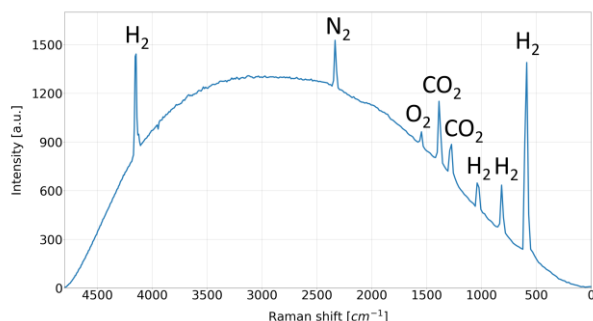


Figure 1: Raman spectrum

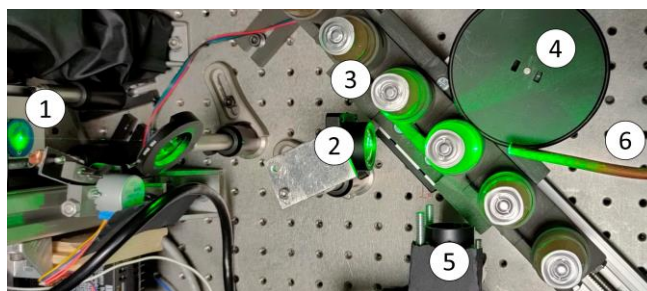


Figure 2: Experimental setup

Figure 2 shows the instrument, it is composed of a 532 nm Nd:YAG 1 W CW laser, a focusing lens (2), a batch of six vials (3), a rotating pulley (4), a Czerny-Turner spectrometer coupled with a CMOS camera (5) and a beam dumper (6). As already mentioned, the main problem is the presence of impurities in the glass which lead to saturation of the detector, not providing any useful information. To control vials rotation, an algorithm was written that takes into consideration two different parameters to determine the goodness of a measurement position: the integral relative to the N₂ spectral band at 2331 cm⁻¹ and the weighted average of the signal intensity not relative to the peaks of the gases involved. Once two optimal threshold were found, the performances in determining the concentrations of H₂ and CO₂ were evaluated from the images considered good. A calibrated 5% concentration of both gases is measured with a sigma of 0.4% (absolute value), which is sufficient for an instrument that rejects vials at threshold.

The activity is performed in the framework of the CNR project PROGETTI@CNR "Scan4Food-Early detection of contaminants in food".

Solid SERS substrates for water pollution monitoring

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The drastic increase of environmental pollution requires new sampling strategies that allow to perform in-situ monitoring and rapid identification. In this regard, Surface Enhanced Raman Spectroscopy is a good candidate among the possible analytical techniques. The consolidation of SERS as a routine analytical method requires to find the best compromise between different issues: higher enhancement factors (EFs), low-cost substrates manufacturing, stable and reproducible performances and environment-friendly chemical routes. Thus, enormous efforts are in course to select best nanoplatfoms capable of enclosing all the listed properties in order to reach the lowest limit of pollutant detection. In this work we have investigated the SERS response of gold nanostars (GNS), GNS covered by silver and properly functionalized by polymeric covering layer in view of the detection of pollutant molecules in the framework of water pollution. In a first instance, we have studied the optimization of the Ag shell thickness to maximize the SERS response of our colloidal GNS [1]. The experimental results, corroborated by full field electrodynamics modelling, have pointed out that the optimal configuration is the one in which small parts of the original GNS branches protrude from the silver coating. Subsequently, these GNS@Ag have been grafted on glass slides to prepare SERS chips that exhibit EFs of the order of 10^7 , in non-resonant conditions. The homogeneity on a millimetre scale of the SERS response has been studied as well as the stability over time. These SERS chips have been used to detect molecules representative of different classes of pollutants. Among them, norfloxacin, an antibiotic, has been detected down to 3 ppb, and a pesticide, thiram, down to 100 ppb. Trying to lower the limit of detection and at the same time to simplify the synthesis approach, we have explored a slightly different procedure: the GNS have been firstly anchored on the glass slides and subsequently they have been covered by silver. Through this new configuration, we have been able to lower our thiram limit of detection down to 20 ppb.

In a second instance, we have decided to further functionalize our nano-metallic solid substrates with a layer of polydopamine, an emerging biopolymer, which has been recently found to be useful in the realization of SERS sensing of plasticizers [2]. The effect of polymer adhesion on solid SERS substrates has been carefully evaluated by monitoring spectral changes in Raman and SERS features of targeting molecules as a function of polydopamine layer thickness, as a preliminary step towards the preparation of sensing chips able to selectively reveal plasticizers.

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In situ Raman-based probes explore the formation of polyynes by pulsed laser ablation in liquid

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Polyynes are linear chains of sp-hybridized carbon atoms that have attracted the interest of material scientists due to their remarkable predicted properties [1]. Raman spectroscopy is a powerful tool used to investigate these properties, as polyynes possess a Raman-active collective vibration whose position and shape are strongly influenced by chain structural and electronic properties [2]. Pulsed laser ablation in liquid (PLAL) is the most efficient and versatile physical synthesis technique of polyynes, but the dynamics of polyynes' formation during PLAL remain unclear [3]. Here, we investigated polyynes' synthesis through *in situ* Raman-based spectroscopy. First, we studied the process of formation and degradation of polyynes by PLAL using an innovative surface-enhanced Raman probe able to monitor the entire synthesis, even at very low polyynone concentrations (Fig. 1a) [4]. We observed a peculiar evolution of the SERS signal of polyynes and carbon byproducts, which we attributed to the degradation of polyynes due to crosslink reactions occurring during the synthesis. We developed a computational method to compare the formation of long and short chains during the ablation and noticed that shorter chains grew more as the ablation progressed [4]. Thus, we employed synchrotron-based *in situ* UV Resonance Raman (UVR) spectroscopy to track the formation of size- and termination-selected polyynes during PLAL (Fig. 1b). UVR spectroscopy was exploited as a signal enhancer based on the results of our recent work [5]. The production rate of polyynes was higher in solvents with a high C/H ratio, low polarity, and low oxygen solubility and was dependent on the chains' structure. We analyzed the impact of laser fluence, discovering an almost zero threshold for polyynes' formation by PLAL. These studies shed light on the dynamics of polyynone formation by PLAL and have implications for improving the scalability of PLAL and the functionalization of polyynes.

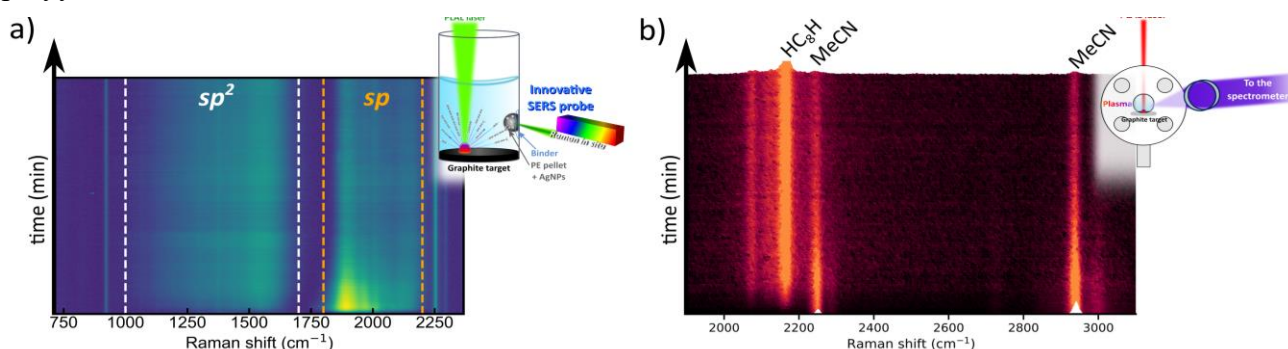


Figure 1 a) Evolution of the SERS sp and sp² (byproducts) signals during ablation in acetonitrile. Inset: *in situ* SERS setup. b) Evolution of the UVR signal of HC₈H during ablation in acetonitrile (MeCN). Inset: *in situ* UVR setup.

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Molecular beacon decorated SERS nanosensor for quantitative detection of miRNA

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In recent years microRNAs (miRNAs) have shown great promise as a novel class of biomarkers for the detection of various diseases, including cancer and inflammatory chronic diseases. Due to its high sensitivity, SERS has emerged as a powerful technique for biomarker detection, including oligonucleotide sequences of DNA, messenger RNA (mRNA) and miRNA. Research in the field is currently focused on the diagnostic capabilities of biological nanosensors, that provide the required sensitivity, increased specificity and offer significant advantages in cost and response times, making them suitable for high-throughput applications^[1]. Herein we present the engineering of a SERS biological nanosensor for miRNA that exploits a signal-off mechanism by means of a labelled molecular beacon (MB) as the oligonucleotide biorecognition element immobilized on a platform made from a self-assembled layer of silver nanowires (AgNWs). The miRNA selected as target of this work is miRNA-183, and a specific MB for its selective detection is designed to be coupled with the engineered SERS nanosensor. We exploit the sensitivity of a spot-on AgNWs substrate to incorporate the MB molecules and increase the selectivity against the selected miRNA target^[2].

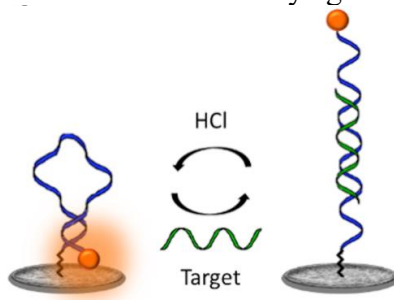


Fig. 1 Mechanism of operation and reversibility of the MB-decorated SERS nanosensor for miRNA target.

The efficacy of the MB-decorated SERS nanosensor is tested in the quantitative detection of miRNA-183 down to femtomolar concentration, aligning with the best limit of detection (LOD) values obtained until now. The engineering of multi-well holder and the in-depth optimization of the SERS nanomaterials as well as MB bio-interface, are carried out to enhance the sensitivity and achieve optimal selectivity for miRNA-183 target. Additionally, the SERS nanosensor is obtained by means of cost-effective materials and simple fabrication procedures without losing reproducibility in the SERS response. Finally, an unprecedented advantage of the SERS MB nanosensor presented in this work lies in its capability of regeneration after the miRNA target capture through an ease reversible process, that adds great value to the biosensor engineered by making it extremely reusable and recyclable.

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Strain characterization in SiGe epitaxial samples by Tip-Enhanced Raman Spectroscopy

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The progressive downsizing of semiconductors is driving information processing technology into a broader spectrum of new applications and capabilities. Strained silicon has become one of the best solutions for integrated circuits thanks to the advantages in terms of miniaturization. Indeed, a biaxial tensile stress applied to the silicon in the channel region of a MOSFET increases the mobility of carriers. This stress can be imposed by doping the silicon underneath with germanium, causing a mismatch between the lattice constant thus improving the electrons' mobility [1]. Over the years, there has been an increasing need, especially in the industrial sector, to develop faster and non-destructive characterization techniques to monitor strain during the manufacturing phases of semiconductor devices. Currently, Tip-Enhanced Raman Spectroscopy (TERS) is one of most powerful methods for strain characterization, as it is a non-contact and non-destructive technique with a lateral resolution of a few nanometers and the capability of analyzing and collecting signals from the most superficial layer of a sample. The enhanced field is strongly restricted to the surface plasmons region, just a few nanometers deep [2], thanks to the simultaneous use of a nanometric tip of an Atomic Force Microscope (AFM) and a laser from a Raman spectrometer (Figure 1a) [3].

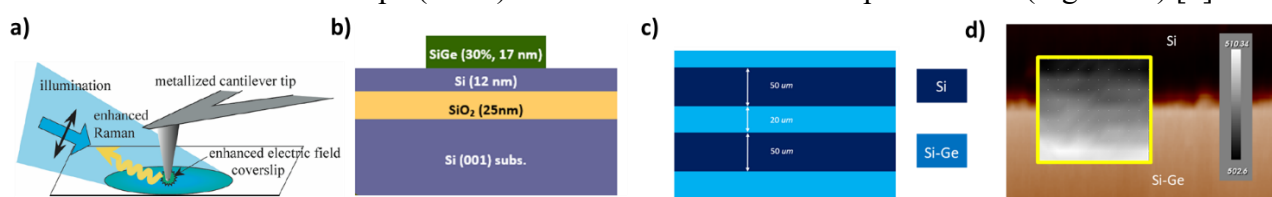


Figure 1. (a) Scheme of TERS configuration in Reflection Mode; (b) section and (c) top view of the analyzed sample; (d) TERS map of the peak position of strained-Si along the sample.

The analyzed sample was provided by CEA-Leti (Laboratoire d'électronique des technologies de l'information, Grenoble) and consists of a (001) silicon substrate where an epitaxial layer of Si_{0.7}Ge_{0.3} (Figure 1c) with thickness of 17 nm (Figure 1b) is grown following several patterns. The AFM probe employed is characterized by an innovative coating which enables its implementation in the clean room for in-line characterization. TERS is used to map the variation in the position of the silicon peak in the local Raman spectrum ($\approx 520.5 \text{ cm}^{-1}$) along the sample pattern (Figure 1d) in order to identify the strain profile with a resolution of a few nanometers. The results confirm that TERS represents a powerful tool in monitoring the quality of production lines in the semiconductor industry and currently provides the best resolution among the Raman techniques for the strain characterization.

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THz Raman spectroscopy to investigate structural order and polymorphism in charge transfer cocrystals

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Vibrational spectroscopic techniques, as IR and Raman, are powerful tools to investigate crystal packing, polymorphism and phase transitions of molecular materials. Raman spectroscopy in the THz region probes the intermolecular vibrations (lattice phonons), that reflect the intermolecular interactions and unit cell symmetry, thus giving the fingerprint of a crystal phase. The use of polarized light on oriented single crystals also gives phonon symmetry species, associated with directional information on the molecular displacements involved. Thus, the interactions within and between chains or layers inside a crystal can be evaluated separately, eventually revealing the occurrence of low-dimensional disorder along specific directions [1,2]. Following this approach, we investigate structural order and phase transitions in some charge transfer cocrystals we recently prepared [3]. The THz Raman measurements were integrated with the polarized IR and Raman spectra in the mid-IR range to assess the molecular orientation, the degree of charge transfer and the stack symmetry.

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Sub-Micron Simultaneous IR+Raman Microscopy with Co-Located Fluorescence Imaging: Advances in Spatial Resolution, Sensitivity & Tri-modality (IR, Raman & Fluorescence)

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The recent advent of Optical Photothermal IR (O-PTIR) spectroscopy, has enabled for the first time, true submicron spatial resolution (20x better than FTIR) infrared microscopy in far-field reflection mode, generating “FTIR transmission-like” spectral quality, without spectral artefacts and distortions such as Mie Scattering associated with traditional FTIR or other emerging QCL based IR microscopy systems. Furthermore, it is now possible to combine O-PTIR with Raman and widefield fluorescence for trimodal correlative microscopy. A new modality, “counter-propagating” has been engineered to provide for enhanced IR (and Raman) spatial resolution and sensitivity, through decoupling the need for a reflective objective. The IR pump beam can now be optionally directed to the sample via the underside, thus allowing the delivery/collection objective for the visible probe (and Raman excitation beam) to be a high-NA refractive (glass) objective. This improves spatial resolution to ~300nm for both IR and Raman, whilst improving sensitivity, image quality and facilitating immersion and water dipping objective studies. To further integrate vibrational spectroscopic tools into life science workflows, we coupled widefield epifluorescence to facilitate a novel concept – fluorescence guided (or fluorescence co-located) O-PTIR microspectroscopy. Rather than, or in addition to the visible image, the fluorescence image can now be used to guide the user to the region of interest, thus combining the well-established specificity of fluorescence imaging with the broad macromolecular profiling capabilities of IR spectroscopy. Several examples from life sciences (cells, live cell imaging, tissues, bacteria) as well as polymers, microplastics and cultural heritage, will be provided to demonstrate these new capabilities and how they can enable new experiments and research findings.

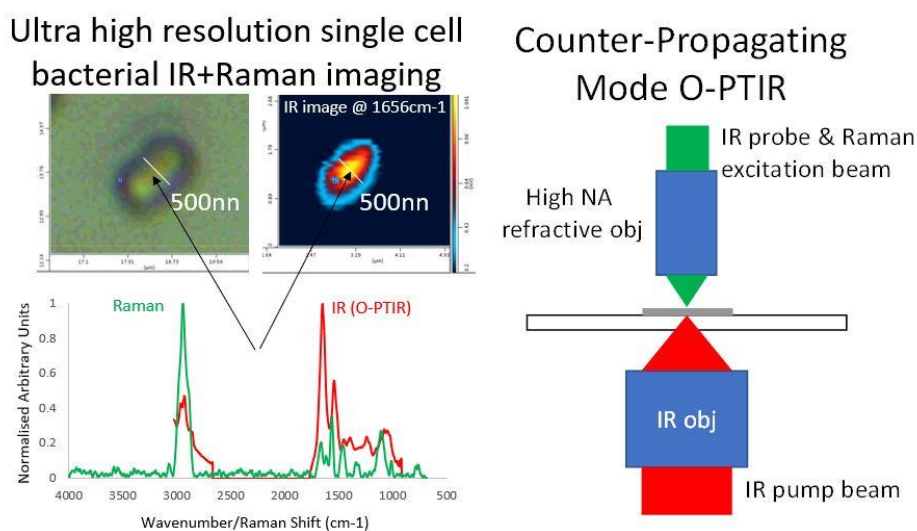


Fig 1. Left: Single E.Coli cell imaged in counter-propagating mode with 50nm pixel/step size and submicron simultaneous IR+Raman spectra from centre of bacterial cell. Right: Schematic of counter-propagating layout.

Raman characterization of Ga₂O₃ thin-films: a key tool for the study of different phases and crystalline properties

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In the last decade ultrawide bandgap (UWBG) semiconductors have been raising interest within the scientific community because of their potential in many different fields. Among UWBG semiconductors, gallium oxide (Ga₂O₃) has become very popular because it can play a key role in different applications, especially high-power electronics, UV detectors and gas sensors. Indeed, it offers some advantages over SiC and GaN: larger energy gap, tunable electric properties and cost-effective production in wafer or thin film form. Five different polymorphs have been identified for Ga₂O₃, with the monoclinic β phase being the thermodynamically stable one and by far the most investigated [1]. Despite Ga₂O₃ is not a novel material, and it has a long R&D history in the last 70 years, today there is an increasing interest towards some of its metastable phases, such the hexagonal α and the orthorhombic κ [2,3]. In this work, we present an overview of different Ga₂O₃ thin-film samples, deposited with different techniques and all characterized by micro-Raman spectroscopy, that demonstrated to be a powerful tool, complementary to X-ray diffraction, for phase determination and analysis. Thin-films of Ga₂O₃ were deposited for the first time by PED (Pulsed Electron Deposition) technique on different substrates such as glass, amorphous quartz and *c*-plane sapphire. The best crystalline layers were obtained on *c*-oriented Al₂O₃ substrates, while films deposited on glass were mostly amorphous. The improvement of crystalline quality was also studied as a function of the different growth conditions, such as substrate temperature and electron beam accelerating voltage. A series of undoped and Si-doped $\kappa(\epsilon)$ -Ga₂O₃ layers were also deposited on *c*-plane sapphire substrates by MOVPE (Metal Organic Vapor Phase Deposition). These layers are characterized by a variable size in crystalline domains and different transport properties. Significant differences emerged between the analyzed samples. In particular, the incorporation of Si consistently resulted in a variation of the intensity ratio between specific Ga₂O₃ Raman modes, while the domain size mainly affected width and position of several modes [4]. The role of phonon confinement is still under evaluation. Finally, Raman spectroscopy was employed to identify the α phase among the others in thin-film samples with mixed Ga₂O₃ phases, in order to better understand the energy gap and transport properties of such complex samples.

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Mode softening driven bilayer to monolayer phase transition in Ph-BTBT-10 organic semiconductor

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The asymmetric organic semiconductor Ph-BTBT-10 crystallizes at room temperature in a unit cell with a bilayer structure, which on heating undergoes a transition to a monolayer smectic E phase [1, 2]. The transition strongly affects the electronic properties of Ph-BTBT-10, but its mechanism is still unclear. We followed the temperature evolution of Ph-BTBT-10 lattice phonons by polarized micro-Raman spectroscopy on single crystals, probing the crystal planes both parallel and perpendicular to the molecular layers. Although the phase transition is first order, a phonon with soft behavior was observed, with interlayer polarization (Figure 1). This suggests that the transformation from crystal to liquid crystal is firstly driven by the soft mode through interpenetration of adjacent layers, followed by a discontinuous in-plane rearrangement of the molecules.

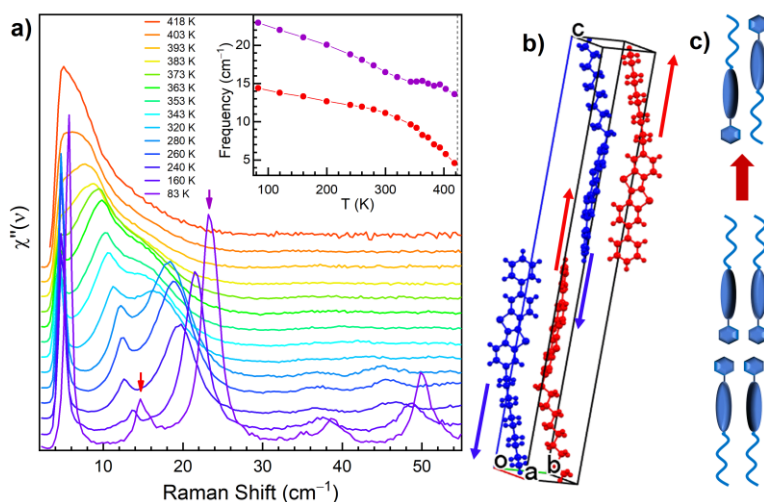


Figure 1: a) Ph-BTBT-10 low frequency Raman spectra with bc polarization recorded on heating from 83 to 418 K. Inset: frequency shifts vs temperature for the two lattice modes indicated by the purple and red arrows. b) Molecular motions in the lattice vibration of B_g symmetry undergoing the softening in the transition. c) Schematic representation of the molecular re-organization of the bilayer into the monolayer following the mode softening.

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Vibrational spectroscopy techniques for the study of cardiorenal syndrome in rat models

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Increasing evidence from clinical studies underscores an interaction between the heart and kidney, in which impairment of one organ contributes to progressive and combined failure of both organs: cardiovascular damage drives a worsening of kidney function, and, in turn, kidney failure worsens the cardiac injury. Experimentally, chronic kidney disease produced by uninephrectomy (UNX) in rodents resulted in early cardiac fibrosis with mild diastolic impairment and preserved systolic function [1]. Much has been written on this subject, but pathophysiological mechanisms continue to be unraveled, implications for management continue to be debated and there is still a lack of valid non-invasive tools capable of prematurely diagnosing a pathologic condition [2]. In this preliminary study of cardiorenal pathology, FTIR imaging was used to investigate the physiological and pathological conditions of the cardiac tissue and evaluate the presence of any structural and chemical changes, following the cardiorenal damage. To this aim, spectroscopic results were compared with histology methods, which are the gold standard in tissue characterization, carried out in collaboration with the Institute for Experimental Medical Research of Oslo University (Norway).

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IR characterization of thin silica film

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Amorphous silica coatings are important mirror components of gravitational wave detectors currently used in the Virgo experiment hosted by the European Observatory of Cascina (PI-Italy). Exceptionally high sensitivity is required for the detection of gravitational waves and thermal noise from mirror coatings is one of the main limiting factors in the spectral region where the detector is most sensitive [1]. The main challenge for many research teams around the world is to identify new materials and define the deposition and post-deposition procedures that ensure thermal noise reduction. Possible tools to obtain structural information and detect the presence of impurities on thin films are specular reflection (SR) and attenuated total reflection (ATR) IR spectroscopic techniques [2]. In our work, these two approaches are employed to analyze amorphous silica coatings produced by ion-beam sputtering on a silicon and SiO₂ substrates, and treated after deposition at different annealing temperatures, from 500°C to 1000°C. We highlight how such techniques can be applied to the structural analysis of thin films of nanometer thickness. In particular, we demonstrate how the spectroscopic techniques are powerful tools to detect impurities and inhomogeneities. In addition, they can provide estimates of optical properties, such as the refractive index, and of the effective thickness which are sensitive to annealing.

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Amyloid and Non-Amyloid Aggregation of β -Lactoglobulin in Self-Crowded Regime

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Globular proteins in aqueous environment fold into their native and biologically active conformation with a compact shape to minimize the interactions among water molecules and protein hydrophobic chains [1]. Varying the system properties such as solvent composition, solution pH, ionic strength and temperature, globular proteins can unfold losing their native structure. The unfolding process promotes the exposition to the solvent of the hydrophobic chains and the possibility to activate the aggregation among monomers [2]. Proteins aggregates can be of different complexity, going from small transient oligomers up to mature amyloid fibrils [3]. Proteins aggregates can further interact forming extended polymeric network leading to the formation of hydrogels, soft materials capable to self-sustaining and retain into their meshes a great amount of water. Protein aggregates and hydrogels are of increasing interest in biomedical field due to their involvement in amyloid pathologies [4] and in material science thanks to their emergent properties [5, 6] and biocompatibility. Exploiting the natural propensity of proteins to self-assemble is a smart strategy to realize biomaterials using a bottom-up approach, bypassing the synthetic effort. Simply acting on the aggregation conditions, the chemical-physical properties of the material can be tuned. In this work, molecular insights on the β -Lactoglobulin (β -Lg) thermal unfolding and aggregation are obtained by means of structural-sensitive techniques. The commonly used Fourier Transform Infrared (FTIR) spectroscopy gives information on the secondary structure variation of β -Lg during thermal unfolding and on the formation of amyloid aggregates thanks to the marker amyloid signal at 1620 cm^{-1} . The UV Resonance Raman (UVR) spectroscopy with an excitation wavelength at 226 nm is a tryptophan-sensitive probe and gives information on the environmental changing around this amino acid residue. Depending on the solution pH, two different aggregation pathways have been identified; one is amyloidogenic while the other is of different nature.

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Raman spectroscopy for the biochemical characterization of extracellular vesicles as nanotherapeutics and clinical biomarkers

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Extracellular vesicles (EVs) are natural nanoparticles released by cells in both physiological and pathological conditions that can be isolated from accessible biofluids, like blood and saliva [1]. EVs are carriers of bioactive molecules with extraordinary potential for both therapeutic and diagnostic applications. Still, technical challenges are now limiting their translation to clinics. Raman spectroscopy (RS) can represent a valuable characterization method for EVs, as it provides a biochemical fingerprint of the sample, in a fast, cost-effective and sensitive manner, without focusing on a specific component, but rather looking at the true complexity of EV composition. We report herein the RS bulk analysis performed on EV preparations from both conditioned medium of in vitro cultured cells and human liquid biopsies (serum and saliva). EV preparations were analysed after air drying on calcium fluoride slide by means of a Raman microspectroscope (LabRAM Aramis, Horiba Jobin Yvon) equipped with 532 laser line, in the spectral ranges 500-1800 cm⁻¹ and 2600-3200 cm⁻¹ [2]. Baseline correction, normalization and multivariate statistical analysis were performed using LabSpec 6 (Horiba) and Origin2021 (OriginLab). For EVs isolated for therapeutic purposes, our results demonstrated that the Raman approach is effective in the evaluation of the reproducibility of different isolation methods, the purity of the preparations and the quality of the cell sources by calculating the spectroscopic protein-to-lipid ratio and performing the multivariate statistical analysis of the collected spectra [3,4]. Our data suggest that RS can represent a valuable tool in the quality control of cell-free products to be used, for example, in the pipeline of stem cell-derived preparations for regenerative medicine. For EVs isolated from human body fluids, our approach was proved to be effective in identifying Parkinson's disease (PD) associated biomarkers. In particular, correlation was found between the Raman spectra of blood-derived EVs and the clinical scales used to neurologically evaluate people with PD [5]. Using a comparable protocol, salivary EVs were also evaluated from healthy subjects, showing greater purity in terms of co-isolates (both lipoproteins and protein corona) compared to blood EVs, with Raman bulk characterization being able to provide a comprehensive biochemical overview, in a high throughput and repeatable manner, despite their low concentration [6]. Taken together, our data demonstrate that the RS approach can represent a turning point in the application of EVs in clinics, being able to help in the quality control of EV therapeutics and in the identification of new complex biomarkers associated to EVs isolated from human biofluids.

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New Findings from UV Resonance Raman RNA investigations: sperm-cells RNA analysis and nucleotides/drugs interactions in SARS-CoV-2 RNA G-quadruplexes

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UV Resonant Raman (UVR) has long been used to the investigation of nucleic acid systems. This is due to the possibility of selectively enhance the Raman signal coming from nitrogenous bases with respect to the ones of ribose/deoxyribose and phosphate [1]. We have recently proved that UVR is able to infer about hyper- and hypo-methylation from Jurkat leukemic T-cell lines [2], and therefore potentially able to detect methylation in human sperm RNA. With this aim we have developed a purification protocol applied to human sperm RNA samples, aimed to minimize the presence of protein residual on the samples, to warrant that absence of phenolic compounds due to the RNA extraction procedures, as well as to identify the correct excitation wavelength able to provide the best signal-to-noise ratio in this class of samples [3]. Furthermore, UVR at 266 nm of excitation wavelength has been used, successfully, to characterize the interaction between SARS-CoV-2 RNA G-quadruplex and selected FDA-approved drugs, namely Netarsudyl, Pranuklast and Quercetin [4], identifying the sites of interactions.

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Correlative Raman and Brillouin analysis for the mechanical and chemical characterization at nano and micro-scale

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Using light-matter interaction, spectroscopic techniques provide a nondestructive and contactless method for the accurate analysis of material properties. Correlative investigations can disclose the rich scenario linking structural, chemical and dynamic properties of complex materials [1]. Here we present some case studies analyzed by the recently developed simultaneous Brillouin and Raman micro-spectrometer [2-3], capable of characterizing mechanical and chemical properties at the nano and micro-scale [4-5]. The performance of the set-up will be presented in terms of spectral and spatial resolution, contrast and acquisition speed in a large number of applications ranging from material science to life science, including also the analysis of turbid samples whose spectrum is affected by a non-negligible multiple scattering contribution [6].

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The influence of Piezo1 on Cell Mechanics investigated by correlative Brillouin and Raman Microscopy

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Spectroscopic techniques represent important tools in the analysis of biological materials, thanks to their contact-free and label-free character. Here we present the use of the combined Raman and Brillouin apparatus developed in our Lab [1] in the study of single cells. Raman Spectroscopy is a well-established technique, which provides information on the chemical composition investigating

the molecular vibrations modes of the sample. On the other hand, Brillouin spectroscopy determines the mechanical properties by measuring the spontaneous acoustic waves present in the material. Traditionally, Brillouin spectroscopy was applied in condensed matter physics to investigate homogeneous samples. However, in recent years, technological advances allowed to couple confocal microscopes to Brillouin spectrometers, achieving higher spatial resolutions. Enabling the mechanical characterization of single living cells and tissues, Brillouin spectroscopy is becoming an emerging technique in mechano-biology. We applied the combined use of Raman and Brillouin microscopy to investigate an in-vitro system consisting of HEK 293T cells, HEK overexpressing the mechanosensitive ion channel Piezo1, and HEK knockout for Piezo1. [3]. The mechanical properties of subcellular compartments were analysed for the different cell types. The comparison of the spectroscopic results with fluorescence imaging and biological assays, allowed to relate the modifications in cellular mechanics to the variations induced by Piezo1 to the sub-cellular structures.

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Studying anisotropic and porous materials with Brillouin light scattering

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Cellulosic fibers are the main component of paper and textile products; however, their mechanical properties are not well understood. The reason is their complex structure and hygroscopic properties. Brillouin light scattering (BLS) spectroscopy was recently found to be a promising non-contact optical technique that can measure mechanical properties of cellulosic fibers in short acquisition times [1]. This offers new perspectives in the micromechanical characterization of cellulosic materials. The anisotropic properties of cellulosic fibers can be described by using different scattering geometries with BLS and obtaining the full elastic stiffness tensor [2]. Furthermore, the influence of relative humidity on the mechanical properties of the fiber can be studied in the milliseconds range which gives access to wetting and drying dynamics which are crucial to understand the interaction of cellulose with water better. Another material class with anisotropic properties are metal organic frameworks (MOFs) [3]. MOFs consist of inorganic nodes connected by organic linkers and thereby forming highly porous structures, with a plethora of possible applications, e. g., gas storage or

catalysis. Many of these applications rely crucially on the MOFs heat transport properties. Despite its importance there is up to this date an insufficient understanding of the concrete structure-to-property relationships governing thermal transport in MOFs [4]. Using BLS to determine acoustic phonon velocities and the full elastic tensor of such materials is of great importance to shed further light onto the issue of thermal transport in MOFs. In this talk, we are going to show first results which have been obtained for cellulosic materials to study anisotropy and water interaction and give an outlook on the approach to study MOFs with BLS.

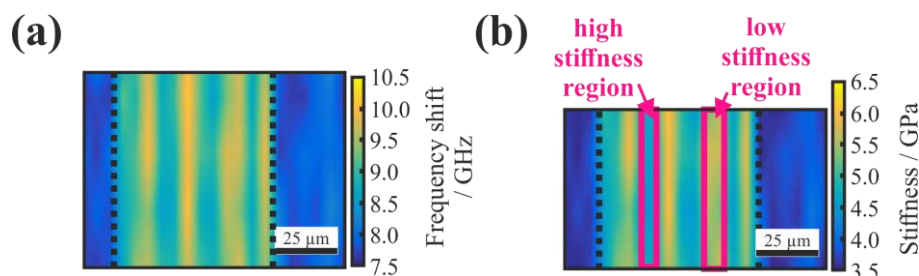


Figure 1. (a) Frequency shift map of a cellulosic (viscose) fiber ($100 \times 100 \mu\text{m}^2$, $21 \times 5 \text{ px}^2$). (b) Regions with a high frequency shift correspond to regions of higher stiffness in the fiber.

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Identification of dyes and pigments in artwork by Raman/SERS and agar gel microextraction: new applications and advances.

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The use of hydrogels as tool for surface cleaning of artworks is a well established procedure [1]. A few year ago we proposed the use of agarose hydrogels (agar gel) as a tool for micro-sampling of dyes and we devised solvent [2,3] or chemically supported [4] strategies to enhance its effectiveness. After extraction, the dyes were characterized by Raman or SERS methods. Here we report on new applications of this approach on different classes of materials. For instance, we have applied this method to the investigation of anthraquinonic lakes in paintings by exploiting their (although limited) solubility in ethanol/ethanol rich agar gel. The use of both Raman and SERS detection schemes was possible, even if with different sensitivity and experimental easiness [5,6]. Furthermore, some new results on the study of flavonoids (e.g. luteolin) will be presented, focusing on their extraction in agar gels and on the theoretical modeling of their SERS spectra [7].

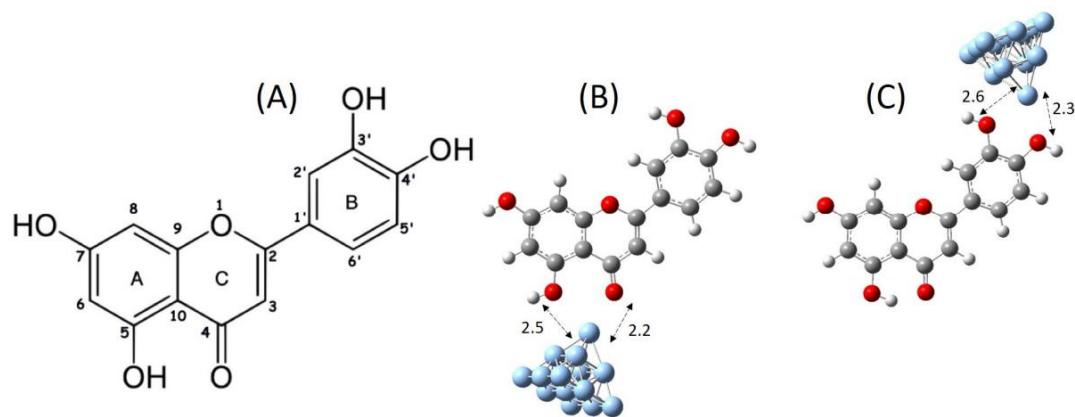


Figure 1: Luteolin (A) and two theoretical models (B,C) used for modeling its SERS spectrum

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Assessing surface and sub-surface modifications of chemical and mechanical properties of ABS items in cultural heritage by a non-invasive analytical approach

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Plastic has been definitively established as an essential element of everyday life, that due to its versatility, it has also conquered the global market for toys, vehicle parts, food packaging, design, and art. Understanding the physical and chemical characteristics of these complex systems is critical for identifying their degradation tendency, monitoring their conservation condition, and establishing suitable preservation strategies. In this work, a new mechano-chemical approach is presented to reveal the combined compositional and structural properties of plastic materials at both surface and sub-surface levels informing about the conservation state and monitoring of the degradation. Acrylonitrile-Butadiene-Styrene (ABS) is chosen as a case study widely used in art and design objects. It is a copolymer made of acrylonitrile–styrene continuous phase (SAN) and partially grafted polybutadiene (PB, as a dispersed phase) acting as an impact modifier and giving excellent mechanical properties to the material. Photochemically aged ABS was investigated using traditional spectroscopic methods (external reflection FT-IR spectroscopy in the mid and near-IR ranges) in combination with Brillouin and Raman micro-spectroscopy (BRaMS), a novel approach only recently applied in the field of heritage science [1]. BRaMS enabled combined measurements of Brillouin light scattering (BLS) and Raman spectroscopy in a micro-spectroscopic setup providing for the simultaneous monitoring of chemical and mechanical changes that occur with photo-ageing, mainly at the sample surface [2]. Modifications of the mechanical properties were also assessed by Unilateral NMR spectroscopy, which allowed to perform non-invasive bulk measurements of relaxation times and profilometry directly related to the molecular mobility of the material [3]. Finally, the knowledge achieved from model samples was employed to monitor the conservation condition of design plastic items made of ABS and non-invasively investigated through the MOLAB platform of the European Research Infrastructure for Heritage Science-ERIHS.

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Reflection Infrared Spectroscopy: a promising tool to monitor the degradation state of paintings containing emerald green

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Reflection FT-IR spectroscopy, from the near-IR (NIR) range up to 400 cm⁻¹, is one of the most informative and reliable molecular technique in the field of Heritage Science, allowing the identification of painting materials (both organic and inorganic constituents) [1] and the

characterization of degradation state [2] by fully respecting the artwork integrity. The present contribution shows the results of investigation campaigns focused on the in-situ characterization of emerald green-containing paintings' conservation state with a portable FT-IR spectrophotometer working in reflection mode. Emerald green (EM, $3\text{Cu}(\text{AsO}_2)_2 \cdot \text{Cu}(\text{CH}_3\text{COO})_2$) is a synthetic Cu- and As-bearing pigment [3] widely used by post-impressionist painters, such as Vincent van Gogh, Robert Delaunay, and James Ensor. In oil painting, it undergoes a degradation process that leads to the formation of Cu^{2+} -carboxylates and As^{5+} -compounds as alteration products [4]. The FT-IR analysis, along with investigation in the X-ray range, of EM-based mock-up samples artificially aged allows fingerprint identification of the degradation products in the mid-infrared range and, therefore, the assembly of a dedicated database for real-time interpretation of the IR spectra and informing in a non-invasive way about the conservation state of this pigment in paintings. Non-invasive in situ access underlined by FT-IR the presence of EM alteration and enabled the selection of representative of original paint samples to be taken (Fig. 1). The non-invasive study has been thus supported by micro-scale spatially resolved. ATR-FT-IR microspectroscopic imaging analysis of the embedded paint cross sections confirms the observations and assumptions made in situ regarding the artworks' conservation state and the advancement of emerald green degradation. These results will be shown in this contribution as well.



Figure 1: Paintings analyzed during the non-invasive campaigns. From left to right: “The Intrigue”, James Ensor, 1890, oil on canvas, Royal Museum of Fine Arts, Antwerp, Belgium; “Paysage au Disque” and “Autoportrait”, Robert Delaunay, 1906, oil on canvas, Centre Pompidou, Paris, France.

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Tourmalines analysis with portable Raman for in-situ application

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Tourmalines are a borosilicate mineral supergroup with high mechanical and chemical stability.[1] These properties and their complex structural formula $(XY_3Z_6(T_6O_{18})(BO_3)_3V_3W)$ make the study of this minerals useful to gather geological information, related to their genesis and provenance. Tourmalines crystallizes in granitoid and metamorphic rocks and are widespread in all type of detrital sediments. Tourmalines are ultra-stable both mechanically and chemically and their chemistry represent a powerful proxy studying ancient sediments, to reconstruct their parent lithology. Among all the 33 tourmalines species [2,3] we focused on the most common three species belonging to the alkali group, where X-site is mainly occupied by Na^+ or (rarely) K^+ , i.e. dravite, schorl and elbaite. The main difference between these three species is the Y-sites occupation: Mg^{2+} is the dominant element in dravite, Fe^{2+} is more abundant in schorl, while elbaite has both Li^+ and Al^{3+} . Raman spectroscopy represents an optimal technique for distinguish different tourmalines species as it allows to perform quick and easy analysis without any sample preparation and degradation. The main advantage of portable Raman is that it can be applied for in-situ measurement directly in mining sites or sediments, thus avoiding sample collection for laboratory analysis. Many works on the characterization of Raman vibrational modes in tourmalines have been done in both low frequency region [4] and OH stretching region [5], correlating variations in peaks positions and intensities with change in sample composition. The aim of the present study is the discrimination between dravite, schorl and elbaite using a portable Raman spectrometer. The spectra have been obtained using a RaPort handheld spectrometer by EnSpectr with a 532nm wavelength excitation line. Our attention was focused on the variations in different region of the spectrum like 200 - 315 cm^{-1} , where we have vibrational modes of YO_6 octahedra, but also 360 - 375 cm^{-1} (ZO_6 octahedra modes), 600 - 750 cm^{-1} (SiO_4 tetrahedra modes) and OH stretching region in 3400 - 3770 cm^{-1} range. Each of these vibrational modes are influenced by Y-sites occupation and thus could yield information about tourmalines composition. For this reason we correlated variations in spectra parameters with change in composition measured by SEM-EDS and LIBS.

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Raman Spectroscopy on salivary samples: a new approach for diseases diagnosis

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Saliva is a complex biofluid that contains a wide range of biological molecules shared with blood [1]. The presence or the alteration in the concentration of specific proteins, carbohydrates, metabolites, nucleic acids and hormones are correlated with neurological, respiratory, viral infections and cardiovascular diseases]. Salivary samples are useful for diagnosis but also for patients monitoring and management in several diseases. In this context, the application of the Raman Spectroscopy (RS) is an innovative approach. RS is a label free and a highly sensitive method, considered a promising tool in molecular diagnostics and biomarkers discovery. We propose the analysis of saliva with RS in order to obtain the biochemical fingerprint of saliva and to provide clinicians with a valid diagnostic tool. Salivary samples were collected using Salivette® swabs from patients with neurodegenerative disorders (Amyotrophic Lateral Sclerosis and Parkinson's disease), chronic respiratory diseases (Chronic obstructive pulmonary disease) cardiovascular disease and viral infections (COVID-19) [1-4]. Samples were then centrifuged and a saliva drop was deposited on an aluminium substrate. Taking advantage of a 785 nm laser line, Raman spectra were acquired at the edge of the drop in the spectral range 400-1600 cm⁻¹. The Raman shift was calibrated using silicon as reference, spectra were fit with a polynomial baseline and normalized. Multivariate statistical analysis combined with machine learning algorithms were performed in order to create a classification model and assess the principal differences between the groups [1,4]. Our results demonstrated the differences in saliva biochemical composition of the experimental groups, with modifications in specific spectral regions. We could discriminate and stratify all considered patients from healthy and pathological controls, finding also correlations between specific spectral features and the clinical and paraclinical tests used nowadays for the disease identification and monitoring. Accuracy, sensitivity, specificity, and precision of the optimized classification models were more than 89% [1,2]. This innovative Raman approach has the potential to make the diagnosis more efficient, supporting clinicians in their clinical practice. Increasing the Raman and clinical data, we expect to significantly improve the performance of our discrimination models and validate the methodology in different clinical settings.

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Structure and properties of grafted silk fibroin fibers for biomedical applications

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In biomaterials, growing interest is devoted to naturally-derived materials, which possess excellent biological and mechanical properties [1]. Fibroin produced by silkworms *Bombyx mori* are protein-based biomaterials with outstanding characteristics, which can be easily tuned to meet specific functional requirements by several strategies, such as grafting [2]. This study discussed the structure and properties of silk fibroin fibers grafted with methacrylonitrile (MAN) with different weight gains (in the 10-60% range): the nitrile group of MAN is of particular interest since it is an acceptor of H-bonds and can bind metals. This last property was evaluated to explore silver ions' adsorption (for possible use as anti-microbial materials) and bioactivity (in terms of calcium phosphate nucleation) for bone tissue engineering applications. IR and Raman spectroscopies were used to study the structure of the grafted silk fibers: combining the two techniques gave complementary information since the former is sensitive to the surface and the latter to bulk. Spectroscopical data gained insights into the efficiency of the grafting mechanism, which involved polar amino acids (i.e., serine and tryptophan) and induced an increase of fibroin-ordered domains. The IR technique also allowed the study of the orientational order of fibers after grafting, relevant to their mechanical and biological properties (such as the morphology and proliferation of cells [3]). Bioactivity test in SBF (Simulated Body Fluid) solutions showed the nucleation of a thin calcium phosphate layer which increased with the amount of grafting agent; a similar trend was observed in the silver ions uptake assay. These preliminary results were encouraging about the positive effect of grafting on the bioactivity and metal adsorption properties of MAN-grafted silk fibroin fibers.

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Ferrous iron insertion in coproporphyrin ferrochelatase from the *Listeria monocytogenes* bacterium

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Chelatases are enzymes that catalyze the insertion of metal ions into porphyrins. Coproporphyrin ferrochelatase (CpfC) catalyzes the penultimate step in the coproporphyrin-independent (CPD) biosynthesis pathway. This process, discovered in 2015, is unique to Gram-positive bacteria, as eukaryotes use the well-characterized protoporphyrin-dependent biosynthetic pathway to accumulate heme b [1]. During infection, heme biosynthesis is a highly efficient strategy for bacterial pathogens to survive since the heme is otherwise sequestered in high-affinity proteins of the host [2]. In detail, CpfCs incorporate ferrous iron into coproporphyrin III (cpIII) to give coproheme, which is eventually decarboxylated to yield heme b by the coproheme decarboxylase enzyme at the end of the CPD biosynthesis pathway [3,4]. The porphyrin metalation reaction of ferrochelatases has been studied for decades, but mainly with non-physiological model substrate analogues [5,6]. The general reaction mechanism includes several steps, involving the binding of the porphyrin and the metal ion to the enzyme, deformation of the porphyrin, deprotonation of the porphyrin nitrogens, and metalligand exchange with the release of the product [1,7]. Here, we disclose the ferrous iron insertion mechanism, in solution and in crystallo, into the physiological substrate cpIII by the coproporphyrin ferrochelatase from the firmicute, pathogen and antibiotic-resistant, *Listeria monocytogenes* bacterium. By following the metal titration in vitro by means of resonance Raman spectroscopy combined with time-resolved X-ray crystallography, we prove that a saddling distortion, resulting from the readjustment of the hydrogen bond interactions of the propionates of the porphyrin with the protein matrix [8] during the metalation, represent a catalytic intermediate. As the enzymatic reaction proceeds further, the distortion slowly decreases, to reach the almost planar final coproheme product [9].

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POSTER PRESENTATIONS

Two-dimensional Broadband Mid-Infrared Spectroscopy for Molecular Structure-Function Analysis

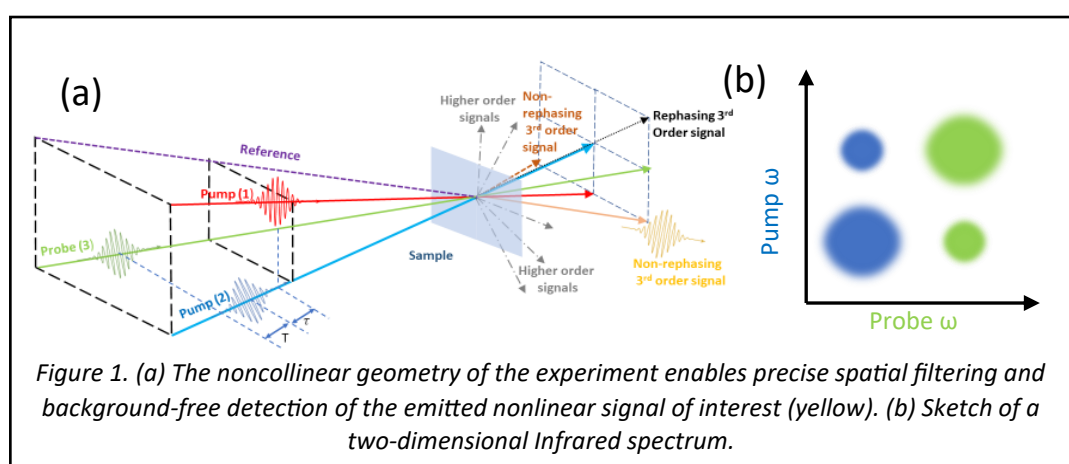
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DNA molecules ‘speak and understand’ the natural language of bio-molecular processes as they occur in cells and organisms. Experimental techniques, such as nuclear magnetic resonance (NMR), X-ray diffraction, and cryo-electron microscopy [1,2], despite their high sensitivity, are unable to resolve convoluted structures of DNA, its conformation, and its coupling with proteins. Here we develop an approach based on time resolved optical spectroscopy to investigate the relationship between molecular structure and its function by implementing an experimental setup that enables the generation and coherent superimposition of ultra-broadband pulses in the mid-infrared (MIR) spectral region (2.5 to 15 μm). This setup allows for direct interrogation of vibrational resonances via electro-optic sampling (EOS) of the MIR pulses and will be coupled with a homemade confocal microscope to add spatial resolution. Using ultrafast two-dimensional MIR spectroscopy, we aim to investigate the optical response of DNA-nano-transducers (DNA-NT) and of proteins such as the transcription factor GCN4, and the neurodegenerative biomarkers α -synuclein (a-syn) as well as of the coupled DNA-NT-protein system. In this way, we will try to link the optical response to the molecular system structure, and eventually understand the related molecular functions depending on the complex structural interactions between the DNA-NTs and the target protein. Experiments are performed on a substrate and in solution to test the stability of the biological systems and their optical signal. The results of this research will contribute to the understanding of the relationship between molecular structure and function and may have implications for various applications, including medical diagnostics and personalized medicine.



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AgNFs as single-particle, multi-wavelength SERS active platforms

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Surface Enhanced Raman Spectroscopy (SERS) has been successfully employed in several fields of interest such as biosensing, in-situ catalysis studies, and archeology. Usually, the greatest contribution to the Raman enhancement is explained by an electromagnetic mechanism [1]. To amplify the SERS effect, the research studies report the production of nanostructures that allow the creation of hot-spots on the surface [2]. Furthermore, the possibility of amplifying Raman signals is linked to the excitation wavelength of the laser used [3]. In this work, we present a SERS active substrate with a high enhancement in a wide range of excitation wavelengths. We propose Silver Nanoflowers (AgNFs), metal microparticles with a nanorough surface that creates a pattern of petals. Thanks to their dimension is possible, by using a Micro-Raman spectrometer, to quickly find a single microparticle and study the signal enhancement of an analyte adsorbed onto its surface. The SERS properties at a single-particle level were studied using a standard molecule 4-mercaptobenzoic acid (4-MBA). Because of the AgNFs extinction spectra cover the entire visible range, we were able to study the enhancement for the single AgNF with 532, 633, and 785 nm laser wavelengths (Fig.1), finding a maximum enhancement factor in the order of 10^8 - 10^9 , due to several number of hot-spots on the tips and between the petals. This suggests that the AgNF could be an excellent SERS substrate on the entire visible and near infrared spectral region and demonstrates that such nanomaterial can be easily used to study analytes at low concentrations with any exciting wavelength, opening the possibility to study several biological and medical interest analytes without the interference of not desired optical phenomena such as luminescence.

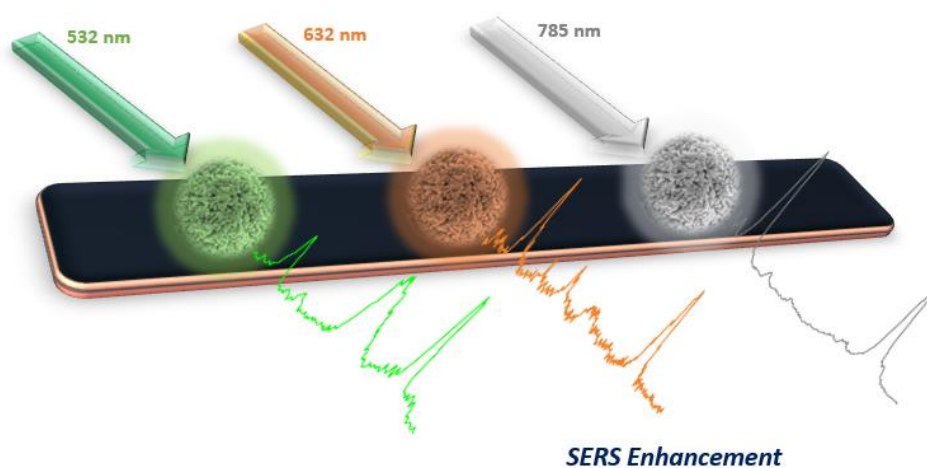


Figure 1. Representative scheme of the SERS study conducted on single AgNFs with different excitation wavelengths.

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An in situ SERS methodology to study the effect of polyynes length and terminations on their thermal stability

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Polyynes represent a novel class of 1D materials constituted by finite chains of sp-hybridized carbon atoms connected through alternated single-triple bonds. Their outstanding mechanical and transport properties, also tunable by the modification of their length and terminations, make them highly appealing in view of future technological applications [1]. Despite their exciting potentialities, polyynes are highly reactive, and easily undergo degradation via crosslinking and oxidation reactions. Embedding polyynes inside solid polymeric matrices appears as a highly efficient strategy to stabilize them over long periods at ambient conditions [2, 3]. However, still very little is known about polyynes stability upon thermal treatments, an aspect of great importance within an application-oriented vision. In this respect, we developed a methodology to investigate polyynes thermal behaviour and its dependence on their length and terminations. HCnH and HCnCl polyynes were obtained via pulsed laser ablation in liquid, then selected by length and terminations via high-performance liquid chromatography, and finally embedded in a polyvinyl alcohol matrix. We established a strategy to optimize the films composition independently from the concentration of the embedded chain, finding a relationship between the polyynes stabilization within the nanocomposites and their relative amount with respect to Ag nanoparticles, needed to perform films characterization through Surface Enhanced Raman Spectroscopy (SERS). We designed a proper experimental setup consisting of a Peltier thermoelectric modulus coupled with an in situ SERS mapping procedure to monitor the thermal behaviour of the embedded polyynes up to 110°C. The SERS laser heating effect was also investigated, proving to be non-negligible in polyynes degradation. Thus, by decoupling the laser and thermal effects, we provided a clearer understanding of the effect of the thermal treatment alone on polyynes degradation. SERS was employed as it provides a high sensitivity towards carbon hybridization and local order, an improved capability to detect poorly concentrated polyynes, and a metal nanoparticles-induced stabilizing effect on polyynes themselves [4]. Longer chains and higher thermal treatments temperatures resulted in faster polyynes degradation processes in both HCnH and HCnCl polyynes-based films. On the other side, considering the different chain terminations, we observed a much larger thermal stability of HCnCl polyynes compared to HCnH ones, hypothesized to be related to the chains-nanoparticles interaction and to the endgroups steric hindrance. Through our methodology it is possible to perform long SERS measurements on polyynes-polymer nanocomposites in safe conditions, and to eliminate the issue resulting from films inhomogeneity, allowing for a statistical description of the samples thermal behaviour. Having proved to be valid to study the effect of polyynes length and terminations on their thermal behaviour, our methodology

results in principle very promising also for a wider class of materials, such as carbon nanostructures and biological samples.

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In-situ Raman spectroscopy of on-surface synthesized graphdiyne nanowires on noble metal surfaces

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Graphdiynes (GDYs) are novel allotropes of carbon that combine sp and sp^2 hybridized carbon to form 1D and 2D nanostructures, with potential applications in the fields of energy storage and conversion, catalysis, and molecular sensing [1]. Depending on the atomic structure, GDYs can be either metallic or semiconductive, going beyond the capabilities of graphene and its derivatives. In particular, GDY nanowires (NWs) consist of a sequence of benzene rings linked by two C–C triple bonds in the *para*-positions, thus generating linear carbon chains that easily assemble as 2D networks. To obtain such a system on Au(111) and Ag(111) substrates, we combined organic molecular-beam epitaxy (OMBE) and on-surface synthesis (OSS), through which we previously fabricated hexagonal 2D GDY networks [2,3]. A brominated molecular precursor is evaporated onto the metallic surface on which, aided by temperature, bromide atoms are replaced by metal adatoms and a linear metalorganic framework is produced. A subsequent increase in the annealing temperature promotes the removal of the bridging adatoms, i.e. enabling the C–C homocoupling, while also partially disrupting the long-range order towards an amorphous 2D sp - sp^2 carbon network. We followed such structural changes with scanning tunneling microscopy (STM), as shown in *Fig. 1(a,b)*.

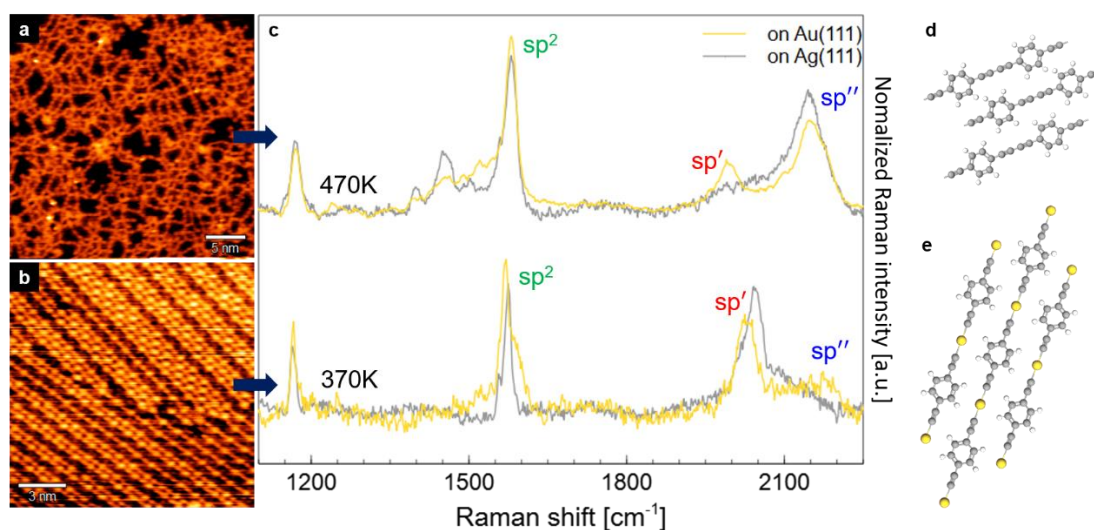


Figure 1 STM imaging of GDY NWs on Au(111) after an annealing process at 370 K (b) – MoF, in which gold adatoms are still present – and at 470 K (a) – CoF, in which the removal of the adatom is concomitant with the disordering of the network. (c) Raman signal (at 532 nm) collected after both annealing treatments for GDY NWs evaporated on Au(111) and Ag(111). (d, e) Schematic model of MOF and COF configurations, respectively.

Raman spectroscopy is extremely sensitive to the structural arrangement of 2D systems and can be used as a tool to monitor their evolution; as shown in *Fig.1(c)*, we observe a modification in the spectral range associated with *sp* carbon upon increasing the annealing temperature, which can be directly linked to the transition from a metalorganic to a covalent framework (MOF-to-COF transition).

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Raman chemical fingerprint of breast cancer cell lines, expressing different levels of HER2.

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Raman spectroscopy (RS) is a reliable system to study biochemical variations in cell biology. The chemical fingerprint of samples is obtained through the unique vibrational configuration of composing molecules, recorded as a shift of inelastically scattered light [1]. Advancement in optics and photonics over the last decades has dramatically increased the number of biological and biomedical applications of RS, which is by itself a fast, non-invasive, non-destructive, multiplexing analytical method, and requires minimal sample manipulation [2]. In this frame, many Raman biology studies have been developed to evaluate the chemical differences among different cancer cell lines [3,4]. Specifically, breast cancer is one of the most attractive research fields in which the characterization of cell lines and correlation with a prognosis is still challenging and relies on immunocytochemistry or immunofluorescence, which are expensive and time-consuming methodologies [5]. Specifically, we have investigated the Raman fingerprint of multiple breast cancer cell lines expressing different levels of HER2 protein, a molecular marker highly correlated with the diagnosis and a dismal outcome [6]. Hence, we propose a cell lines classification based on their Raman signal.

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Calibration of reflectance imaging spectroscopy using MA-XRPD for 16th century illuminated manuscript

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This study presents a method for predicting the relative abundance of the artistic pigments used on illuminated manuscripts by using reflectance imaging spectroscopy (RIS) from visible to short-wave infrared range (400-2500 nm), calibrated using X-ray diffraction data. A folio of an illuminated manuscript, likely from the 16th c., was analysed using multiple hyperspectral cameras (RIS) and a macroscopic X-ray powder diffraction scanner (MA-XRPD). From the MA-XRPD analysis, we were able to identify a number of common pigments found in historical manuscripts such as: azurite ($\text{Cu}_3(\text{CO}_3)_2(\text{OH})_2$), malachite ($\text{Cu}_2\text{CO}_3(\text{OH})_2$), lead white (hydrocerrusite, $\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$), vermilion (cinnabar, HgS), calcite (CaCO_3), gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) and lead tin yellow (Pb_2SnO_4). Also the less commonly found pigment, posnjakite ($\text{Cu}_4\text{SO}_4(\text{OH})_6$), was found to be present, which allows to date the manuscript to the 16th c. The MA-XRPD data was used to calculate a relative mass percentage of the identified pigments in each pixel. Although the MA-XRPD measurements result in detailed information, the acquisition time is long when compared to RIS measurements.

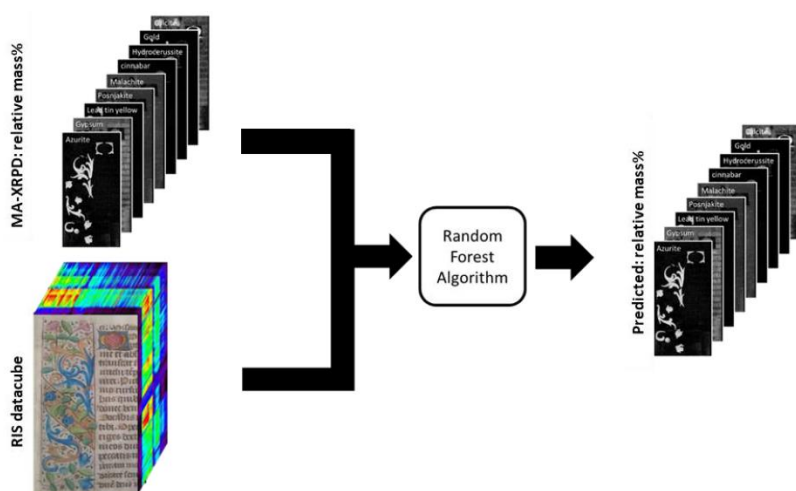


Figure 1: Schematic overview of calibration method

The disadvantage of the RIS measurements is that they are difficult to interpret. Thus the MA-XRPD-based relative concentrations were used as baseline data for training machine learning algorithms to interpret the RIS dataset of the same manuscript folio. Using the trained models, it is possible to derive concentration maps of some of the pigments based on the RIS images alone, such as azurite and malachite. Suggesting the possibility to predict the concentration maps in other non MA-XRPD scanned areas of the same (or other) manuscript(s).

Structural modification of a polymer matrix composite with inorganic dopants.

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Polymer matrix composites are used for various applications in engineering ranging from bio-medical sector to marine sector. They have shown high fracture resistance, high durability, flame resistance and other interesting characteristics due to which they have found a place in the manufacturing of safety structures. The combination of PMCs with different organic/inorganic/metallic compounds have resulted in enhancing of various desirable characteristics by affecting their structure. In our work, we study the structural modification of a polymer matrix developed by thermal polymerization of 2,4-diamino-6-phenyl-1,3,5-triazine at 400°C due to the doping of H₃BO₃ and TiO₂. The polymer part is phenyl-modified carbon nitride with a structure analogous to graphene. For the first nanocomposite- the organic polymer and TiCl₄ are used as precursors in ethanol, followed by prolonged high-pressure treatment at a certain temperature. Different calcination temperatures and ratios between the inorganic and organic part are employed to study the trend of variation in structure. The second composite was prepared via solid-state method using the polymer precursor and industrial grade boric acid in a furnace and again the ratios between the polymer and dopant were varied. The structural and optical properties of these composites were rigorously studied by XRD, SEM, EDX, BET; RAMAN, UV-Vis, PL and Time resolved PL spectroscopies. In our investigation, these composites have proven to be efficient in degrading organic dyes in water (RhodamineB, Methylene Blue and Methyl orange) in the presence of light, hence acting as photocatalysts. We have studied the effects of different dopant concentrations in the polymer matrix on its structural properties using the spectroscopic techniques and correlated them with their photocatalytic properties. These materials have promising applications in the water treatment industry.

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Ketorolac loaded PLGA coating of AZ31 alloy: development and characterization

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AZ31 is a biodegradable magnesium alloy potentially suitable as support for favoring bones repair. Despite positive aspects, it is characterized by rapid degradation in physiological medium [1]. PLGA could slow down the degradation process [2] and control the release kinetic of active pharmaceutical ingredients. In this work, ketorolac tromethamine (KT) loaded PLGA was used for AZ31 coating. AZ31 disks were activated in a sodium hydroxide (NaOH) solution and successively heated to stabilize the activated layer. Then, samples were coated with PLGA containing 5% w/w KT using the solvent casting technique. Raman spectroscopy, SEM and DSC were used to characterize the coated samples. *In vitro* release profile and blank PLGA mass loss were determined. *In vitro* cytotoxicity studies were performed to evaluate KT [3,4] and AZ31 coated samples effects on BSCL138 fibroblasts and primary human osteoblasts. Raman spectra highlighted the formation of new hydroxyl groups on sample surface, after activation, as indicated by the bands at 3664 cm⁻¹ and 3690 cm⁻¹ (Figure 1) [5]. The same technique allowed to approximate PLGA coating thickness of 300 μm thick. SEM photomicrographs allowed to distinguish between AZ31 and polymeric coating. Besides, from the analysis of non-activated and activated alloy, a better adhesion of PLGA was identified for activated AZ31. PLGA Tg was ~ 14 °C lower than raw PLGA due to KT plasticizing effect. Since only one face was exposed to solvent, AZ31 coated samples showed a slower release (80% after 15 days) than KT loaded PLGA film and no burst release was observed. Blank PLGA mass loss showed a sigmoidal trend reaching 95% after 45 days. KT toxicity on osteoblasts and AZ31 toxicity on both cell types were significantly reduced in PLGA coated samples. PLGA coating was successful in improving cytocompatibility of the alloy and in slowing down AZ31 degradation preserving the alloy mechanical properties. Moreover, KT release kinetics was compatible with its use in bone fracture pain relief.

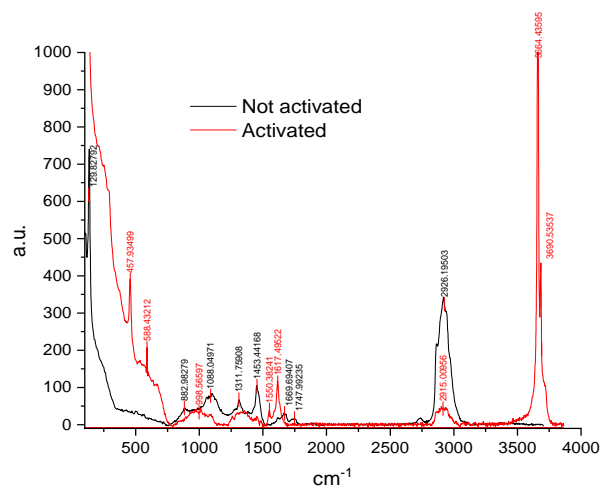


Figure 1. Raman spectra of activated (red) and non-activated (black) AZ31 samples.

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3D Printed Material for Electrocardiogram Monitoring Systems: PEGDA-PANI Composites Using In Situ Aniline Photopolymerization

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Nowadays, stereolithography 3D printing and additive manufacturing (AM) technologies have expanded beyond the development of testing prototypes and are widely used in various production processes as essential fabrication tools. [1] However, the wide use of this technology is still limited by the availability of printable materials. In this context, the use of conjugated polymers (CPs) as conductive component in a 3D printer ink can open new possibilities for the fabrication of flexible conductive objects with complex geometries. [2] However, the use of 3D printing for CPs is still in its early stages and faces many challenges, mainly related to their solubility and printability. To overcome these challenges, a promising approach is to produce 3D printed electrically conductive materials using the photopolymerization of conjugated monomers during the stereolithography process. In this study, an ink containing aniline is formulated, which can photopolymerize within the insulating printable polyethylene glycol diacrylate (PEGDA) polymeric matrix directly during the 3D-printing process. [3] The resulting PEGDA-polyaniline (PANI) composites (Figure 1) exhibit suitable morphological and structural features, as well as electrical and electrochemical performances, making them potentially useful for various soft electronics applications. As a proof of concept, 3D printed PEGDA-PANI samples are employed as soft electrodes in an electrocardiogram (ECG) device, and their efficiency is monitored under real-time conditions. The collected data exhibit reproducible ECG patterns, demonstrating the potential of 3D printed PEGDA-PANI electrodes for biosignal monitoring applications.

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The SuPerStAr project: an integrated spectroscopic non-invasive approach for the study of street art murals

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In recent years, street art and graffiti have become very popular, accounting for the growing interest of art historians, sociologists, and the art market. Their perception within the socio-cultural context has radically changed, raising the need to protect them. Since street art murals are directly exposed to environmental agents and vandalism, developing effective methods for their in-situ analysis, monitoring, and treatment is fundamental. In this context the project *SUPERSTAR Sustainable Preservation Strategies for Street Art* [1] (coordinator Prof. Modugno, University of Pisa) sees the light for the first time. The project aims at developing integrated analytical protocols and optimizing innovative cleaning procedures and protective coatings for the preventive conservation and long-term conservation of street art murals. However, knowing the nature and behavior of the constituent materials is the first step to finetuning the best conservation approach for these artworks and preserving them from degradation [2]. For this reason, we focused on developing a combination of single-point techniques (Raman and external reflection FTIR spectroscopies, UV-Vis-NIR reflection spectroscopy, and X-Ray fluorescence spectroscopy), and hyperspectral mapping/imaging techniques in the X-ray (MAXRF), Vis-NIR and SWIR ranges and finalized to a multi-technique non-invasive approach for in-situ investigations of street art murals [3]. The methodology has been implemented with the support of laboratory spectroscopic equipment to study sample mock-ups. This analytical approach led to understand the composition of the constituting materials and allowed us to monitor the state of conservation of some iconic mural paintings of the cities of Milano and Torino (Italy) selected as case studies in collaboration with the *Politecnico di Milano* and the University of Torino, partners of the project. The artworks represent some of the most recent struggles humanity has been dealing with: from the largest painted wall in Italy (1300 m²), “*Necesse*” by Smoe (2021), dedicated to those who worked intensively to safeguard the community during the COVID, to the mural by the street artists Corn79 and Mrfijodor representing the fragile balance within an endangered ecosystem. The data collected on the murals allowed us to characterize the colorful “palette” of spray paints as regards the inorganic/organic pigments and fillers, and to give indications about the binder and the protective possibly applied. The analytical results of non-invasive single-point analyses combined with the macro-scale compositional distribution obtained by very close-range hyperspectral imaging methods on limited portions of the murals have been integrated and extended to the overall surface by remote multispectral and thermal imaging analyses by Unmanned Aircraft Systems (UAS) combined with the relative virtual reconstruction by a laser scanner.

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All-optical investigation of biocompatible plasmonic colloids: surface chemistry, colloidal stability and pH responsivity

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In the past few decades, a large variety of biocompatible colloidal nanomaterials have emerged as powerful nanomedical diagnostic sensors and drug carriers [1]. These nanosystems can show selective ability to target specific bioreceptors, cells or tissues, and to locally measure biologically relevant parameters, as extra- or intracellular pH, with nanometric resolution [2]. Within this context, silver nanoparticles (AgNPs) represent a promising candidate material, combining the enhanced optical response of the NP surface plasmon resonance, the Ag antimicrobial properties, and the possibility of functionalization of the NP surface with biocompatible ligands [3]. Further to this, in recent years, light transmission spectroscopy (LTS) has been proposed for the quantitative study of colloidal nanomaterials. Indeed, the optical extinction spectrum contains information on the system scattering properties and, if interpreted through Mie's scattering theory, it can serve as a solid diagnostic tool to assess the particle size, shape, concentration and aggregation level. [4] In this work, we will report on the synthesis of AgNPs functionalized with two types of hydrophilic thiol species, characterized by amino and sulfonate ending groups [5]. The presence of functional groups confers high stability to the NPs under physiological conditions and a controllable surface chemistry, with interesting pH responsiveness. Surface Enhanced Raman Scattering (SERS) spectroscopy is applied for the nanoscale investigation of the molecular layer surrounding the NPs at varying environmental pH conditions, suggesting the potential application of the system as biocompatible SERS pH nanosensor. Additionally, real-time light transmission spectroscopy (rt-LTS) is applied to characterize the NP colloidal system and to follow its aggregation dynamics under variations of the environmental pH.

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Sonochemical Synthesis and Performance of New Mesoporous Inorganic Matrices to Tackle Poor Drug Solubility Issues

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Poor bioavailability of many drugs often impaired their therapeutic effectiveness, usually because of limited solubility and/or permeability. This problem has become a major challenge in drug formulation. Mesoporous silica materials (MSMs) have been proposed to be used as matrices for enhancing the apparent solubility and dissolution rate of different drug molecules due to their high surface area, regular pore structure, specific pore volume and high thermal stability. In this work, MSMs have been prepared according to the method reported earlier [1] with a sonochemical synthesis an additional step [2]. The advantages of the sonochemical method applied to the support matrix are remarkable as it allows to obtain a significant reduction of the reaction time (from 2 days to 1 hour). To follow the rules of green chemistry during the sonochemical process, the synthesis of new matrices has been obtained by combining non-ionic surfactant (Pluronic P123) with cationic surfactants (CTABr, CTBABr). Prepared matrices have been characterized by particle size analysis, SEM and TEM analysis. Chosen MSMs have been later loaded with a model IV class drug (furosemide) to analyze their capacity to entrap and release an insoluble model drug. Achieved particles have been analyzed for drug content and later a dissolution test has been performed.

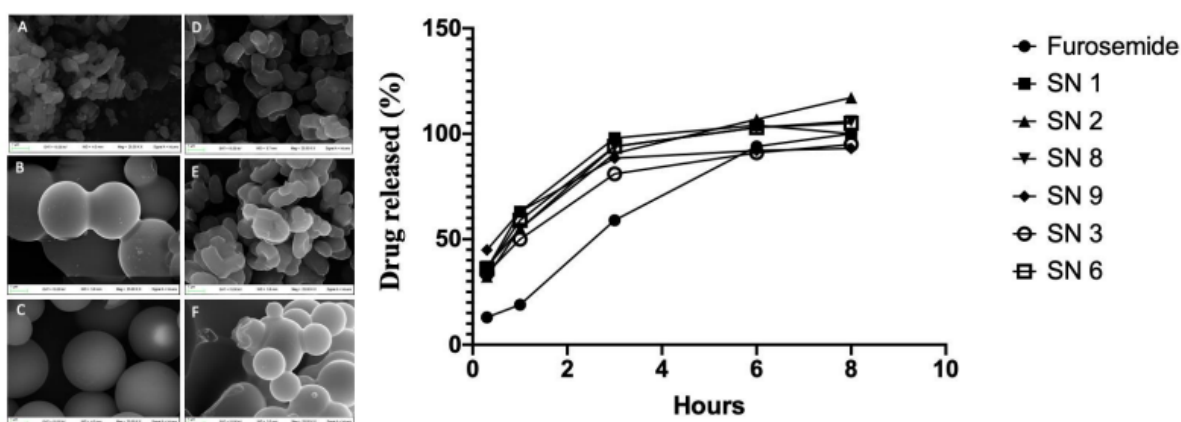


Figure 1: SEM (A-F) micrographs and drug release profiles of matrices obtained by combining non-ionic surfactants P123 with CTABr A) SN1, B)SN2 C)SN3 and CTBABr D) SN6, E)SN8 and F)SN9.

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Flexible Nanocomposites for the Detection of Additives

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In food industry, many different chemicals were used for increasing the quality and stability of the products. Although, use of these chemicals is necessary for the recent production systems, their use should be regulated with limits due to their possible undesired effects on human health with exposure such as nervous, immune, and reproduction [1-3]. For the detection, mainly used methods are chromatography and spectroscopy based such as liquid chromatography and mass spectrometry which has high selectivity and sensitivity but require complex instruments, complex sample preparation procedures, long time, and skilled operators [4]. Thus, effective detection systems should be developed which can provide on-site real time detection with high sensitivity and selectivity in short time. In this study, flexible nanocomposites were used for the detection of additives in beverages using vibrational spectroscopy. Consequently, proposed system provided detection of additives from real samples at ppm levels.

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