Tuesday June 13, 2017

Plenary Session 2
Of Phonons and Chameleons: Specific Low-frequency Vibration Strongly Modulates the Band Gap in Perovskites

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Methylammonium lead iodide perovskites are increasingly being used for high-efficiency, low-cost photovoltaics. These perovskites are soft materials where thermally populated phonons lead to a large structural variability and peculiar opto-electronic properties at ambient temperature. For example, the material is characterized by a unique temperature-dependence of the optical band gap: the band gap increases with increasing lattice temperature, whereas thermal expansion generally leads to the opposite behavior in conventional semiconductors (silicon, gallium arsenide, etc.). Despite the presence of thermally accessible phonons at room temperature, which are responsible for many of the unique opto-electronic properties of this perovskite (and thereby photo-conversion efficiency), a fundamental understanding of how changes in temperature affect the optical properties of this material, via phonon modes has remained elusive.

To specifically address the relation between phonons and the optical band gap, we have developed a novel THz pump-optical probe experiment, which allows directly accessing the coupling between phonons and the optical gap.

We present the transient and periodic blue-shift of the optical band gap driven by a particular phonon centered at ~ 1 THz. Following ultrafast THz excitation, we monitor the band edge absorption at room temperature. We find surprisingly strong coupling between one specific phonon (vibrating at 1 THz, 33cm⁻¹) and the optical band gap, as summarized in the figure below. The coherent excitation of the phonon leads to an oscillating band gap: the phonon changes the color of the perovskite. Quantitative modeling shows that population of this phonon fully captures the macroscopic temperature dependence of the material: the temperature-dependent optical properties can be fully accounted for by the thermal excitation of this 1 THz phonon. Also, the population distribution of this particular phonon can explain the significantly broadened band gap of this perovskite at ambient temperatures. The phonon specificity of the coherent gap modulation consistently appears at various temperatures not only in the tetragonal phase but also in the orthorhombic phase.

Our results thus provide a clear picture how one phonon governs one of the key macroscopic properties of this perovskite, and likewise, demonstrate a broadly applicable approach to study isolated phonon behaviors in various semiconductors without perturbation of photo-carriers.
Figure 1. The main panel shows the macroscopic temperature dependence of the band gap (black squares). Upper left: Selective THz excitation of the 1 THz phonon simultaneously increases the effective phonon temperature ($\Delta T_{ph}$) by $\sim 1$ K and displaces the average lattice position ($\Delta Q_{avg}$). Due to the strong coupling, the lattice displacement leads to the shift of the optical band gap ($\Delta E_{gap}$) by $\sim 0.3$ meV, which is the same effect on the band gap as heating the entire sample by $\sim 1$ K (i.e. effective band gap temperature: $\Delta T_{gap}$). This quantitative agreement ($\Delta T_{ph,exp} \sim 1$ K) indicates that the 1 THz phonon is the sole origin of the peculiar temperature-dependence of the band gap.

(2) Quarti, C.; Mosconi, E.; De Angelis, F. Chem. Mater. 2014, 26 (22), 6557.
Graphene and Metal Based Mid-IR Plasmonic Biosensors

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Mid-IR spectral range is uniquely positioned for biosensing application, as it encompasses the molecular vibrations that uniquely identify the biochemical building blocks of life, such as proteins, lipids, and DNA. In fact IR absorption spectroscopy is a powerful technique that provides exquisite biochemical information in a nondestructive and label-free fashion by accessing these vibrational fingerprints. Nevertheless, vibrational absorption signals are prohibitively weak because of the large mismatch between mid-IR wavelengths (2 to 6 μm) and biomolecular dimensions (<10 nm). In addition strong absorption bands of liquid water overlapping with the major bands of proteins and lipids severely limit spectroscopy in biomolecules’ native aqueous environment. To overcome these limitations, strong optical near fields in the vicinity of resonant plasmonic nanostructures can be exploited to achieve high sensitivity.

Recently, by exploiting graphene plasmons we demonstrated a dynamically tunable plasmonic Mid-IR biosensor that can extract complete optical properties of proteins over a broad spectrum [1]. Graphene has the potential to reshape the landscape of photonics and optoelectronics owing to its exceptional optical and electrical properties. In particular, its infrared (IR) response is characterized by long-lived collective electron oscillations (plasmons) that can be dynamically tuned by electrostatic gating, in contrast to conventional plasmonic materials such as noble metals. Furthermore, the electromagnetic fields of graphene IR plasmons display unprecedented spatial confinement, making them extremely attractive for enhanced light-matter interactions for ultra-high sensitivity. Specifically, biosensing is an area in which graphene tunability and extreme light localization offer great opportunities. Our device (Fig. 1A) consists of a graphene layer synthesized by chemical vapor deposition and transferred to a 280-nm-thick native silica oxide of a silicon substrate. Graphene nanoribbon arrays (width W = 20 to 60 nm and period P ≈ 2W) are then patterned using electron beam lithography and oxygen plasma etching. A scanning electron microscope image for typical sample is shown in Fig. 1. We apply an electrostatic field across the SiO2 layer through a bias voltage (Vg that is varied between 0 and 120 V) to dynamically control the Fermi level of graphene. Extinction spectra of the device are acquired using Fourier transform infrared spectroscopy for the incident electric field polarized perpendicular to the nanoribbons. The plasmon resonance of nanostructured graphene is dynamically tuned over different frequencies in which the plasmonic mode is either on- or off-resonant with the amid-I-II absorption bands of protein. On-resonant conditions are used to determine the absorption signature (i.e. imaginary part of the refractive index) while the off-resonant ones give information about the real-part of the
protein refractive index. While the tunability offers unique advantages, one of the limitations in graphene plasmonics is the difficulty of exciting plasmonic resonances with high intensity. Additionally, graphene electrostatic tuning is bounded by dielectric breakdown and limits the reconfiguration capabilities of graphene optical devices. In our most recent work, we show that stacks composed of multiple graphene layers produce infrared plasmonic resonances that are much stronger than in single-layer graphene and that can be dynamically tuned over significantly broader spectral ranges [2]. We provide a complete theoretical and experimental framework explaining the origin of the superior plasmonic response and tunability of multi-layer graphene. Our technique could be rapidly implemented in a new family of high-performance graphene plasmonic devices, including infrared modulators, absorbers and photodetectors, and will have particular impact in recently demonstrated graphene mid-IR biosensors. If time allows we will also cover some of related works in Mid-IR metal plasmonics for biosensing on proteins and biomimetic lipid membranes [3-5]

![Figure 1.](image)

**Figure 1.** (A) Illustration of a graphene plasmonic Mid-IR biosensor. (B) SEM image of nanofabricated graphene plasmonic nanoribbon arrays. (C) Resonance tuning over the protein absorption bands (D) Extracted complex dielectric constant of a protein biolayer using tunable Mid-IR biosensor and its performance comparison.

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Parallel Session 13

Vibrational Imaging 1
Impact of Scattering and Fringes on Spatially Resolved Chemistry from Spectromicroscopy and Spectromicrotomography

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The holy grail of chemical imaging is to provide spatially and temporally resolved information about heterogeneous samples on relevant scales. Synchrotron-based Fourier Transform infrared imaging¹ combines rapid, non-destructive chemical detection with morphology at the micrometer scale, to provide value added results to standard analytical methods. Hyperspectral cubes of spatially and spectrally resolved data (x,y,z,Abs(λ))) are obtained employing spectromicrotomography², which is a label free approach. This method inherently evaluates a broad array of wide organic materials, with minimal sample preparation and modification.

Parallel windows, or parallel surfaces of samples or windows can lead to fringes in both spectral and spatial domains in hyperspectral data (see Figure 1). An algorithm has been refined to remove fringes from such hyperspectral data and will be described for temporally resolved measurements of living algal cells.

Scattering from objects of the order of the wavelength is a well-known phenomenon that can impact the spectral measurements, and can therefore effect the analysis of the spatially resolved data. This talk will demonstrate some approaches to analysis to address these issues, highlighting examples from spectromicroscopy of tissues³ (see figure 2) and spectromicrotomography of plastics.

This work was supported by the UWM Physics department and NSF grants CHE-1508240 and CHE-1112433.

![Figure 1. Infrared images from an algal cell maintained in a flow cell with parallel windows. Before: Spatial and spectral fringes are clearly present. (After) Spatial and spectral fringes are reduced with fringe correction algorithm.](image-url)
Figure 2. (Chemical images of the WT and Akita/+ retina tissue highlighting distinctive layers of retina. A) Photomicrograph of retina tissue and the red box indicates the area that was imaged. B, C) Chemical images of the WT and Akita/+ retina, covering the area of 256×600 µm$^2$ and integrated over the nucleic acid band at 1712 cm$^{-1}$ (the linear baseline is set at 1700-1725 cm$^{-1}$) to highlight the absorption strength of DNA in retinal layers. The inner, outer and nucleus segments of the photoreceptor system, outer plexiform layer, inner nucleus layer and inner plexiform layer, are shown. Scale bar is 30 µm and the color scale is from blue color (the lowest absorption strength) to red color (the highest absorption strength).

Recent Developments and Applications of FTIR Spectroscopic Imaging

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FTIR spectroscopic imaging is a powerful tool for characterisation of polymeric, pharmaceutical, biomedical samples and complex materials. This talk will outline the recent research that we are developing in this area and its emerging applications. Significant advancements range from studies of cancer biopsies and live cells [1] to studies of micro-formulations in microfluidics. [2] These studies have used FTIR imaging in transmission and Attenuated Total Reflection (ATR) modes.

Previously we have suggested [3] a solution to overcome the problem of optical aberrations in transmission FTIR microscopy by introducing a novel, yet elegant, approach to remove scattering artefacts where the tissue biopsy is inverted such that the CaF$_2$ window sits between the biopsy and objective. This was achieved by placing a CaF$_2$ lens on top of the window to form a pseudo hemisphere. The advantages of this added lens approach are the increase in magnification and the removal of chromatic aberration, the latter of which would otherwise occur when measuring samples through a window.

However, the imaged area using this approach was relatively small (170×170 μm$^2$). In order to map large tissue sections with the benefits of this added lens approach, the window and lens can be placed on an X-Y stage whereby a holder keeps the lens aligned with the objective while the window with the tissue sample slides underneath, successively acquiring a series of tiles, which are later stitched into a large data set (Figure 1). This approach, [3] demonstrates the combination of imaging and mapping methodology to produce large data-sets of high quality spectra from different areas of the sample that are free from scattering and obtaining images with a high spatial resolution. This would be useful for spectroscopic analysis of biopsy tissues for disease localisation, classification and staging. [3]

Recently, we demonstrated a novel approach that is based on combination of disrelation mapping analysis of imaging datasets obtained by macro ATR-FTIR spectroscopic imaging of polymer blends. [4] The disrelation mapping analysis localises regions where the absorbance varies in a dissimilar manner because of the contribution from species of different chemical origins. Chemical visualisation with enhanced spatial resolution in micro ATR-FTIR imaging mode broadens the range of samples amenable to study with FTIR imaging which would otherwise be ruled out by the inadequate spatial resolution in measurements by transmission. Micro ATR-FTIR imaging of live cells provided opportunity for sub-cellular imaging of live cells.[1] However, obtaining information about hydration of proteins and about molecular states of water within live cells was still
In collaboration with H. Shinzawa (AICT, Japan) we have recently studied live cells by applying approach, which is based on combination of disrelation mapping analysis of imaging datasets obtained by micro ATR FTIR spectroscopic imaging. This approach allowed us to differentiate spatial distribution of different molecular states of water within the live cell. This is related to the protein hydration in the cells and important for understanding the mechanisms of hydration of these molecules inside live cells.

**Figure 1.** Schematic of combining FTIR imaging mapping with the added lens, where the lens is held in place and the window with biopsy underneath is translated on the X-Y stage, for obtaining FTIR images of large areas.

Multisensor Hyperspectral Imaging:  
A Novel Approach to Chemical Structure Determination

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Novel developments in image-based analytical instrumentation allow the acquisition of high-resolution chemical images, using different techniques. However, in many cases analytical problems cannot be clarified by applying only one single technique. Multisensor Hyperspectral Imaging was developed to solve these limitations by allowing a combined statistical analysis of different hyperspectral datasets. After fusion of the single hyperspectral datasets to one combined multisensor datacube [1], a subsequent statistical approach [2] allows a linkage of the different methods to one virtual image with the advantage of chemical structure determination across the limitations of single analytical methods on an image-based approach (Figure 1).

The concept of Multisensor Hyperspectral Imaging will be demonstrated based on hyperspectral datacubes acquired by a WITec alpha 300 RSA+ Raman Imaging System, a FEI Quanta 200 Electron Microscope and an EDAX EDX Imaging System as well as an IONTOF TOF.SIMS 5 Secondary Ion Mass Spectrometric Imaging System. Hence, vibrational, elemental and mass-spectrometric chemical information are provided within one overall combined datacube.

**Figure 1.** Basic concept of Multisensor Hyperspectral Imaging with subsequent Multivariate Statistics.
A simple example is given in figure 2, where copper sulfide particles were analyzed by Raman micro-spectroscopy, SEM-EDX and the ToF-SIMS mass-spectrometric imaging. The subsequent multivariate statistical approach uncovers the linkage of the three Raman bands (RMS) with the elements Cu and S (EDX) and the related Cu isotopes from the ToF-SIMS (SIMS) by a cluster analysis. The application of multivariate statistics to the fused datacubes of different samples, ranging from environmental to industrial and life science applications demonstrates the ability for image-based chemical structure determination and will uncover the overvalue of this approach compared to side-by-side interpretation of separated imaging datasets.

Figure 2. Example of Multisensor Hyperspectral Imaging of Copper Sulfide particles with combined clustering of the different methods and extraction of the pure cluster spectra.

Tracing Lipids inside Macrophages - Following the Endocytic Uptake of Lipoproteins with Raman Spectroscopic Imaging

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The interaction of lipids and macrophages plays a crucial role in the development of atherosclerotic plaque depositions in arteries. As the main carrier of lipids inside the blood stream, the endocytic pathway and intracellular fate of lipoproteins is of high interest. In case of an endothelial dysfunction, macrophages and low density lipoproteins (LDL) can enter the subendothelial layer of arteries, where LDL can get modified enzymatically or by oxidation (oxLDL). Macrophages cannot regulate the uptake of oxLDL and transform into foam cells. The excessive uptake of lipids is cytotoxic and eventually leads to cell death and in turn to formation of a lipid-rich plaque inside the arterial wall \cite{1}.

To study the storage process of lipids caused by oxLDL uptake, Raman spectroscopic imaging was implemented. Due to its unique chemical specificity, cellular components like lipids and proteins can be distinguished \cite{2,3}. We present recent results on the endocytic uptake of triglyceride-enriched oxLDL by macrophages \cite{4}. As a reliable marker for the uptake of lipoproteins into macrophages, \(\beta\)-carotene was found. The administration of lipoproteins leads to small endocytic vesicles with a diameter of around 2 \(\mu\)m. Figure 1 depicts the four cellular components of a macrophage incubated for 24 h with oxLDL after applying a spectral unmixing algorithm.

![Figure 1](image_url)

\textbf{Figure 1.} False-color Raman image after N-FINDR analysis of a macrophage cell after incubation with 50 \(\mu\)g/mL oxLDL for 24 h. Four cellular components were found.
A protocol was developed to incorporate deuterated tripalmitate into oxLDL. Due to the deuteration of the triglycerides, a unique vibration band occurs in the normally silent wavenumber region between 2100 and 2300 cm\(^{-1}\) of biological samples. Figure 2 displays spectra taken from endocytic vesicles and lipid droplets of the same cells at three time points. A clear decrease of deuterated bond vibrations is visible in the marked region of spectra corresponding to endocytic vesicles, while the vibrations increase in lipid droplets. A translocation of deuterated lipids between endocytic vesicles and lipid droplets is described for the first time.

**Figure 2.** Raman spectra of (A) endocytic vesicles and (B) lipid droplets. Over time, endocytic vesicles get depleted of deuterated lipids while the content in lipid droplets is increasing. Spectra on the same line correspond to the same cell.

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**References**
Noninvasive High-speed Near-infrared Imaging of the Biomolecular Distribution and Molecular Mechanism of Embryonic Development in Medaka Fish (Oryzias latipes) egg

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NIR imaging has been a matter of keen interest because it provides the spatial distribution of chemical components nondestructively even for thick materials.\textsuperscript{1,2} It has been used for a variety of applications such as quality evaluations of foods and pharmaceutical tablets, diffusion process of solvents in tablets and polymers, and component and crystallinity distributions of polymers.\textsuperscript{1,2} In the present study, we explored the molecular mechanism underlying growth of fertilized Japanese medaka fish (Oryzias latipes) eggs using NIR imaging.

The eggs produced were approximately 1.5 mm in diameter. They were transparent and suitable for the spectroscopic analysis. A standard egg hatches approximately two weeks after fertilization under normal feeding conditions. After the fertilization, the egg consists of three major parts: oil droplets, egg yolk, and embryo. The oil droplets are rich in lipids (unsaturated fatty acids), while the egg yolk contains both lipids and proteins.\textsuperscript{3} The NIR imaging data were obtained by a microscopic NIR system with hyper spectral camera (Compovision) developed by Sumitomo Electric Industries. The measurements were performed in transmission mode with an objective lens at 5× magnification. The sample area in the present study was about 1.5 × 1.5 mm\textsuperscript{2}. NIR data in the 1000–2350 nm region of a 1.5 × 1.5 mm\textsuperscript{2} area (approximately 50,000 pixels) could be measured within a few seconds.

Figure 1(a) shows NIR spectra in 1000-2200 nm region of the three major parts of fertilized medaka egg measured on the first day. The two broad bands around 1430 and 1910 nm are owing to a combination of the antisymmetric O-H stretching mode and symmetric O-H stretching mode, and antisymmetric O-H stretching mode and O-H bending mode of water, respectively. The spectrum from oil droplets has several characteristic peaks attributed to lipids. The bands at 1217, 1722 and 1773 nm are due to C-H stretching of hydrocarbons and aliphatic as shown in Figure 1(b).\textsuperscript{4}
In Figure 2(a), the intensity at 1767 nm as attributed to the first overtone in the C-H stretching modes of CH$_2$ groups present in aliphatic compounds such as fatty acids and hydrocarbons clearly indicates the shape of the egg membrane, the contour of oil droplets, and the outline of the embryo. The images of C-H stretching band at 1716 nm of CH$_3$ groups included in aliphatic compounds are shown in Figure 2(b). The peak maximal of C-H stretching was reported to be higher with unsaturated fatty acids shifts to lower wavelengths from approximately 1725 nm to 1709–1717 nm. Thus, the band at 1716 nm in the oil droplet spectra is consistent with the fact that polyunsaturated fatty acids are present in medaka fish eggs. Figure 2(c) drawn by the band intensity at 1584 nm which is due to the first overtone of N-H stretching mode of amide groups. They show the clear shape of membranes of egg and oil droplets. Furthermore, eye shapes become clearer with the development. It is assumed that these membrane structures are related with polyamides. Figure 2(d) expresses the water distribution with different strength of hydrogen bonds extracted by principal component analysis. Pixels with a higher proportion of weakly-hydrogen bonded water are highlighted. The detailed structures in the embryo and egg membranes and the inner and the outer contours of oil droplets are apparent. These results suggest that different water structures at interfaces of different substances with various structures are in contact within the egg.

![Figure 2](image_url)

**Figure 2.** NIR images developed by using the band intensities of second derivative spectra at (a) 1767 nm, (b) 1716 nm, (c) 1584 nm, and (d) by using PCA scores.

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Parallel Session 14

Electrified Interfaces 1
IR Spectroscopy Studies of Thin Organic Films at the Solid – Liquid Interface.

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After a brief review of basic principles of IR spectroscopy and laws of reflection at the metal-solution interface and elimination of the solvent background, three methods of Infrared Reflection Absorption Spectroscopy (IRRAS) will be described. The first is the subtractively normalized interfacial Fourier transform infrared spectroscopy (SNIFTIRS) or in short the potential difference IR spectroscopy. This technique finds application to study reversible adsorption of soluble molecules at electrode surfaces. Methods of optimization of the SNIFTIRS experiment will be discussed. Its application will be illustrated by the case of adsorption of a soluble surfactant such as sodium dodecyl sulphate (SDS) at a gold electrode surface.\(^1\)

The second technique is the surface enhanced infrared reflection absorption spectroscopy (SEIRAS). It is performed using attenuated total internal reflection (ATR) element covered by nanoparticles of gold. The intensity of the IR signal is then enhanced by a factor of 100. In addition, since the film of metal nanoparticles is conductive it is used to study phenomena at electrified interfaces. I will describe application of this technique to study structure of water in a film of SDS at a gold electrode surface.\(^2\) I will emphasize complementarity of SEIRAS and SNIFTIRS in application to study thin films at interfaces.

The third technique to measure IR spectra at interfaces is the photon polarization modulation infrared reflection absorption spectroscopy (PM IRRAS). This technique is used to study films of insoluble molecules at various interfaces. I will discuss application of this technique to study model biological membranes supported at a gold electrode surface.\(^3\)

Literature


**Figure 1.** IR absorbance spectra for the sulfate stretching regions of adsorbed SDS films on the Au(111) electrode.

**Figure 2.** Central image, potential difference spectrum of water band between bilayer (E=0.6V) and hemimicellar (E=0.4V) states of the film of SDS at the Au(111) electrode surface. Cartoons on the left and right sides of the spectrum illustrate two structures of the film.
Toward Microsecond Resolved Infrared Spectroelectrochemistry using Synchrotron Radiation.

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Characterization of electrified surfaces with infrared (IR) spectroscopy provides valuable information concerning interfacial composition under equilibrium or steady-state conditions. Electrochemical reactions often provide concentration gradients that evolve in both space and time and mapping these processes can provide new information on coupled processes. Furthermore, surfaces of electrode materials are often heterogeneous and generate uneven rates of reaction. It is extremely difficult to study the spatiotemporal characteristics of electrode reactions with conventional IR spectroelectrochemical methods due to limitations in photon throughput. Infrared radiation from non-traditional sources such as synchrotrons and quantum cascade lasers offer unique brilliance advantages and provide new opportunities to study electrochemical interfaces with unprecedented spatial and temporal resolution. The synchrotron IR advantage can be realized in several electrochemical applications including 1) spectroelectrochemical studies of ultramicroelectrodes 2) mapping diffusion layers and 3) combinatorial approaches to electrocatalyst development. A methodological breakthrough (see Figure 1a) is described that represents significant progress to actualizing fast spectroelectrochemical studies on ultramicroelectrode surfaces. The new approach is shown to allow the measurement of high quality synchrotron IR spectra of adsorbed molecules confined to a 125 μm² beam spot with nearly 20 fold improvement in S/N as compared to a conventional IR source (Figure 1b). A simple electrochemical experiment involving the potential dependent adsorption of 4-methoxypyridine (MOP) provides attenuated total reflectance surface-enhanced infrared absorption spectra (ATR-SEIRAS) to be obtained as a function of irradiated electrode area (Figure 1c). Using the lowest available aperture setting on the IR microscope at the mid-IR beamline at the Canadian Light Source, an estimated 2.5 femtomoles of electrochemically adsorbed molecules are sampled with a S/N of 20 using synchrotron IR radiation (Figure 1d). This sets the limit of sensitivity of the method to be in the region of tens of picomoles. Further improvements in coupling synchrotron IR with SEIRAS-active spectroelectrochemical surfaces are described which should allow, for the first time, a means to perform surface sensitive IR spectroscopy of dynamic processes with sub-microsecond time constants. Implications for dynamic studies of biological systems such as redox-active proteins will be discussed.
Figure 1. ATR-SEIRAS using synchrotron infrared radiation (a) Schematic of SIR-ATR-SEIRAS and electrochemical cell geometry (b) Standard deviation of the 100% transmission (noise) as a function of the nominal radius of the beam spot illuminating the gold surface using the globar and the synchrotron., (c) ATR-IR spectrum of neat MOP on an unmodified Si hemisphere (top curve) and the potential difference absorbance SIR-ATR-SEIRAS spectra (colored lines) of adsorbed MOP as a function of nominal spot size radius (d) S/N as a function of nominal spot size radius for the asymmetric C-O-C stretch at \( \sim 1300 \text{ cm}^{-1} \) using synchrotron infrared radiation.

Raman Studies for Electrocatalysis and Energy Storage

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In this talk we address the use of Raman spectroscopy to provide mechanistic insight into both electrocatalysis and energy storage. In the realm of electrocatalysis we discuss our recent work developing high activity catalysts for CO$_2$ reduction, focusing on Ag, Cu, and related alloys. By interrogating the CO intermediate, we develop insight into product speciation. In the realm of energy storage, we use Raman to evaluate changes at the solid-electrolyte metal interface, focusing on electrolyte instability. This instability translates to other storage systems, such as those involved in Mg batteries.

Electrochemical conversion of CO$_2$ to value-added chemicals has the potential to reduce CO$_2$ emissions, store otherwise-wasted intermittent renewable energy, and create economic value. Presently Cu is the only known metal catalyst able to reduce CO$_2$ to hydrocarbons at fairly high Faradaic efficiencies (FEs). However, the FE for ethylene (C$_2$H$_4$) is usually lower than 35%. Finding approaches to increase the FE for C$_2$H$_4$ remains a challenge. We found that the addition of 3,5-diamino-1,2,4-triazole (DAT) into the Cu electrodes results in a more than 2-fold improvement in the FE for C$_2$H$_4$. The FE for C$_2$H$_4$ is as high as 56%, and the partial current density for C$_2$H$_4$ is as high as 190 mA cm$^{-2}$ at a cathode potential of only 0.84 V$_{RHE}$. In situ Surface-Enhanced Raman Spectroscopy of a Cu electrode in the presence of DAT showed that the addition of DAT induces bending in chemisorbed CO (a necessary precursor for ethylene), blocks adsorption of ethanol and formate species, and provides observable quantities of surface adsorbed ethylene. This work complements SERS obtained from Ag surfaces, showing that DAT inhibits CO product formation, leading to higher CO production rates.

In the realm of energy storage, we use Raman spectroscopy to interrogate solid electrolytes for Li ion batteries. Quasi-binary thiophosphate-based solid electrolytes (SEs) are attracting substantial interest for lithium batteries due to their outstanding room temperature ionic conductivities. This talk describes reactions occurring at the solid electrolyte (SE)/Au interface during Li deposition and stripping for two exemplary SE materials: $\beta$-Li$_3$PS$_4$ ($\beta$-LPS) and Li$_{10}$GeP$_2$S$_{12}$ (LGPS). We used in situ Raman spectroscopy, along with X-ray Photoelectron spectroscopy (XPS) and Scanning Electron
Microscopy (SEM) to evaluate potential dependent changes in the chemistry of these materials at active electrode interfaces. For β-LPS, a partially reversible conversion of $\text{PS}_4^{3-}$ to $\text{P}_2\text{S}_6^{4-}$ (Figure 1) was found along with the formation of $\text{Li}_2\text{S}$ during Li deposition and stripping. In contrast, LGPS exhibited only irreversible changes at potentials below 0.7 V vs. Li$^+/\text{Li}$. The different behaviors likely relate to differences in the structures of the two SE materials, and the availability of easily bridged anion components in close proximity.

Finally, we discuss another battery system, related to Mg ion batteries. The magnesium aluminum chloride complex (MACC) electrolyte is a promising system that is composed of stable chloride salts and exhibits nearly 100% Coulombic efficiencies with high anodic stability. Interestingly, efficient Mg electrodeposition and stripping behavior is not observed in the electrolyte as-prepared, instead, the MACC must be conditioned before Mg deposition and stripping is supported. In this talk, we used Raman spectroscopy and SERS to determine the complexation of the Mg complexes in the active electrolyte. Relative changes in speciation between the as-prepared and conditioned electrolyte provide vital insights into the speciation necessary to achieve efficient Mg electrodeposition and stripping behavior.
Spectro-Electrochemical Determination of Electric Fields at Model Membrane Systems.

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Electric fields play a crucial role for a wide range of biological processes. For instance, particularly strong fields arise at the interface of lipid membranes and influence the structure and activity of membrane proteins like potential dependent ion channels\cite{1}. Therefore, it is of highest importance to quantify these electric fields and provide insight into their impact on mechanisms of biochemical reactions.

However, the in-situ quantification of these electrostatic fields is still a challenge. A promising approach is based on the so-called vibrational Stark effect (VSE) in which the strength of the electric field can be determined by the shift of the vibrational frequency of a Stark reporter group \cite{2}. These reporter groups can be e.g. introduced into biomimetic interfaces that consist of an electrode coated with a self-assembled monolayer (SAM), so that defined electric fields can be applied via an electrochemical potential. Additionally, it is possible to construct a lipid bilayer membrane on the SAM and immobilize proteins.

In this project, Stark reporter groups will be incorporated in different ways into biomimetic interfaces to determine the intrinsic electric fields of these constructs. A useful biomimetic interface to study membrane proteins are SAM-coated electrodes \cite{3} or structured membrane models like tethered bilayer lipid membranes (tBLM) \cite{4}, which display similar interfacial potential distributions (Figure 1A and B). Therefore, the electrode/SAM system was first studied as the simplest model of an biomimetic interface and to determine the interfacial electric fields within a combined spectro-electrochemical and theoretical approach, using surface enhanced IR absorption (SEIRA), attenuated total reflection (ATR), electrochemical impedance spectroscopy (EIS) and density functional theory (DFT). Determining a Stark tuning-rate of 0.546 – 0.702 cm\textsuperscript{-1}/(MV/cm) and potentials of zero charge of 0.1 V and 0.15 V, interfacial electric fields between ca. -3.5 to 1 MV/cm were obtained for Au electrodes covered with SAMs of alkynitriles (C5CN or C6CN) (Figure 2). The studies were further extended to bilayer lipid membranes assembled on a gold electrode.
Figure 1. Potential curve across a SAM covered electrode (A) in comparison with the potential curve across a bilayer lipid membrane (B).

Figure 2. Interfacial electric fields of the Au/SAM interface in comparison to the applied electrode potential for a Stark-tuning rate of 0.546 cm⁻¹/MV/cm (squares) and of 0.702 cm⁻¹/MV/cm (circles) for a C5CN SAM (blue) and a C6CN SAM (red) on a gold electrode.

Researchers are always on the hunt for new and/or improved materials with novel physics and ground-breaking technological opportunities. Ever since the isolation of graphene in 2004 [1], two-dimensional (2D) layered materials, including graphene and transition metal dichalcogenides (TMDs) have become a fast growing research field with many exciting potential applications. Weak coupling between the layers of these materials allows for access to single and few-layers, which often display novel properties as the number of layers are reduced [2],[3]. At present, these materials are high contenders for use in future electronics, optoelectronics, and flexible technology due to their extremely thin nature [4], exceptional transport properties [5], and ability to be subjected to large amounts of strain [6],[7]. In addition, the combination of different 2D layered materials, such as graphene, boron nitride, and TMDs allow for the possibility of unique hetero-layer stacked structures with tunable properties depending on the material and stacking arrangements used.

**Figure 1.** Experimental setup and schematic of our unique Raman setup, which couples a triple grating spectrometer to tunable wavelength sources and a magnetic field up to 9 T.
Optical techniques, including Raman and photoluminescence (PL) spectroscopy, are vital characterization techniques for 2D materials due to their non-destructive nature, diffraction-limited spatial resolution, and extreme sensitivity to fundamental physics in a material (e.g. number of atomic layers, electron-phonon coupling, defects, doping, strain, edges/grain boundaries, low-energy excitations).

The experimental capabilities of our laboratory at the National Institute of Standards and Technology provide a very unique opportunity to investigate the properties of 2D materials (as well as other nanomaterials) under a wide variety of physical configurations. Our novel magneto-Raman microscopy system couples optical spectroscopy (Raman and PL) with simultaneous magnetic fields (up to 9 T), temperature (between 4K and 400 K), spatial mapping capabilities with diffraction-limited spatial resolution, and resonance excitations via tunable laser sources through the visible to near infrared range, as shown in Figure 1. The newest addition of electrical contacts and feedthroughs now permits in-operando Raman measurements of active devices (Figure 2). The impact this measurements system will be demonstrated using 2D materials as examples.

Figure 2. Image of electrical feedthrougths and chip where sample is placed for characterization in magnetic and electric fields.

Tuesday June 13 2017

Parallel Session 15

Aqueous Surfaces 1
Molecular surface structure of aqueous nanoscopic interfaces: adiposomes, liposomes and water droplets

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Nonlinear imaging and scattering / spectroscopy methods are promising tools that can gain label-free molecular level information about aqueous systems and nanoscopic interfaces in three dimensions. I will introduce these methods, in particular vibrational sum frequency scattering and then consider two intriguing topics related to the nanoscale properties of water: The production and characterization of lipid coated nanodrops for lipid studies, the molecular structure of liposome interfaces and the surface structure of water droplets.

Water is important for life. It is intimately linked to our well-being. Without water, cell membranes cannot function. Charges and charged groups cannot be dissolved, self-assembly cannot occur, and proteins cannot fold. Apart from the intimate link with life, water also shapes the earth and our climate. Our landscape is formed by slow eroding/dissolving processes of rocks in river and sea water; aerosols and rain drops provide a means of transport of water.

In many processes it is the interfacial region (of the membrane, the droplet, or the particle) that determines much of the physical, chemical, biological, and geological properties. Interfacial water is often considered in one of two ways: As a background, describable by a single parameter, or simply omitted. Alternatively, it is studied in great detail in an environment or condition that is precisely defined but so oversimplified that it has not much to do with the real world. Aqueous interfaces are mostly studied in vacuo, or as a planar water/air interface.

However, interfacial water occurs on different length scales, from sub-nanometer to micron sized (corrugations, organelles, membranes, liposomes), and is often buried inside another solid or liquid environment that is not at all comparable to vacuum or air. This absence of molecular knowledge of realistic interfaces is due to a lack of tools that can access buried nano- or microscopic interfaces in liquids and solids.

To do this, we have created nonlinear light scattering and imaging tools (vibrational sum frequency scattering, second harmonic scattering and second harmonic imaging) that can be used to access molecular structural information of particle interfaces. These optical methods are then combined with the development and use of small nanoscale droplets, shells and particles in solution that are closely related to the systems under study, such as oil droplets coated with lipids (adiposomes 1), liposomes (cell membranes; liposomes) 2,
and water droplets (aerosols)\(^3\). This approach provides a unique route towards accessing curved interfaces but also has the advantage that up to ~4 orders of magnitude higher surface to volume ratios can be produced in sample volumes of ~50 \(\mu\)L. In addition since the interfaces are created in solution no oxidative degradation occurs, which often hampers lipid studies.

From our spectroscopic investigations we find that it is indeed possible to obtain detailed molecular information about the surface of nanoscopic objects in solution. We also find that the molecular structure on the nanoscale is distinctly different from the planar extended interface or that the molecular structure is different from expectations: Liposome interfaces are highly curved and thus for a 100 nm liposome some 15% of additional lipid is expected in the outer leaflet. We find from SFS our experiments that are exceptionally sensitive to transmembrane asymmetry that the number of lipids in the outer and inner membrane is the same. The difference in area between the leaflets is determined by the number of hydrating water molecules. For multi-component liposomes hydrogen bonds can induce lipid asymmetry (as illustrated in Figure 1).

Water droplet interfaces of 100 nm sized droplets exhibit a much higher ordering in the hydrogen bond network than planar interfaces composed of the same chemicals. The difference in effective temperature is 50 K, and upon supercooling the water droplet interface does not change its structure.

![Figure 1. Illustration of a liposome composed of multiple lipids, their molecular structure and the vibrational surface spectrum generated by the lipid head groups.](image)

**References**


(3) Smolentsev, N.; Smit, W. J.; Bakker, H. J.; Roke, S. *submitted* 2017.
Over 150 years ago, Faraday proposed the existence of a liquid-like layer at ice surfaces below the bulk melting temperature. This layer is important for surface chemistry and glacier sliding close to sub-freezing conditions. Since Faraday’s discovery, the properties of this water-like layer have been the research topic of many scientists, which has entailed considerable controversy. The experimentally reported onset temperature for quasi liquid layer (QLL) formation varies between 200 K and 271 K. Moreover, most experimental work shows that with increasing temperature, the QLL thickness gradually and continuously increases from the onset temperature up to the bulk melting point, with reported thicknesses varying from 2 nm to over 45 nm at 271 K. In contrast, early simulations showed that the QLL is formed in a more quantized, bilayer-by-bilayer manner.

To elucidate the precise temperature variation of the QLL, and its nature, we investigate the surface melting of ice Ih by combining non-contact, surface-specific vibrational sum frequency generation (SFG) spectroscopy and spectra calculated from molecular dynamics simulations. In our SFG experiment an 800 nm and a 3 μm laser pulse are combined at the interface and the sum-frequency light is detected (Fig 1A).

Figure 1. (A) Schematics of the hexagonal unit cell of Ih ice together with the layout of an SFG experiment. The red hexagon is the basal face of ice. (B) Experimental SFG spectra at different temperature. The red line marks the peak frequency. (C) First moment of the spectral intensities shown at different temperatures for the basal face together with a sigmoidal fit.
As this is a second-order nonlinear process it is forbidden in centrosymmetric materials such as the proton disordered ice studied here. At the interface this symmetry is broken, thus allowing us to specifically probe the vibrational response of the interfacial region. The signal is strongly enhanced when the infrared laser pulse is resonant with a molecular vibration. Here we use the O-H stretch vibration of the water molecules. The amplitude of the signal depends on the number of vibrational chromophores and the amount of order present at the interface.

Using SFG, we probe the outermost water layers of distinct single crystalline ice faces at different temperatures. Distinct ice samples are grown from a melt using the seed extraction method. Afterwards the samples are oriented and cut to obtain a specific face. For the basal face, a stepwise, sudden shift in the SFG spectrum to higher frequency occurs around 257 K (Fig. 1B and C), which means that the hydrogen-bonded structure of the outermost water layers weaken at this temperature. The spectral calculations from the molecular dynamics simulations reproduce the experimental findings. Moreover, both the experimental and the calculated spectra show only a very weak change in the dangling OH bond. From the combined experimental and simulated surface-specific vibrational spectroscopy, we conclude that the thickness of the quasi liquid layer changes in a non-continuous, stepwise fashion around 257 K [1]. Below this temperature, the first bilayer is already molten; the second bilayer melts at this transition temperature.

Measuring orientation of water molecules at interfaces

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Structure and dynamics of hydrogen bonds at aqueous interfaces determine the solvation properties of interfacial water, which in turn affect chemical processes such as including heterogeneous catalysis, electrochemistry, atmospheric, and environmental chemistry. Orientation of water molecules at interface is an important ingredient, since it is the orientational anisotropy that makes interface fundamentally different from bulk water. In particular, we aim to address the interplay between H-bonding and alignment of the water dipoles in the electric field at charged interfaces. Water bend vibrational mode ($\nu_2$) presents a more straightforward probe of the molecular orientation because, unlike the OH-stretch modes, it is less affected by inter- and intra-molecular coupling.

We have previously reported the spectrum of the water bend mode at the air/water interface measured using Sum-Frequency Generation (SFG).[1] Here, we present experimental evidence to aid the assignment of the $\nu_2$ spectral features to H-bonded classes of interfacial water, which is in general agreement with the recent theoretical studies by Nagata et al. and Ni and Skinner.[2,3] The dispersive line shape (Figure 1) shows an apparent frequency shift between SSP vs. PPP polarization combinations (SFG-visible-IR). This is naturally explained as an interference effect between the negative (1630 cm$^{-1}$) and positive (1662 cm$^{-1}$) peaks corresponding, respectively, to “free-OH” and “H-bonded” species, which have different orientations and thus different amplitudes in SSP vs. PPP spectra. Surfactant monolayer of sodium dodecyl sulfate (SDS) was used to suppress the free OH species at the surface, and the corresponding SFG spectral changes indicate that these water molecules with one of the hydrogens pointing up into the air phase, contribute to the negative peak at 1630 cm$^{-1}$.[4]

In order to get quantitative information about the molecular orientation at the surface, we calculated the non-linear susceptibility for the polarization-selected SFG spectra of the water bend as a function of the molecular orientation (Figure 2). At the air/water interface, SFG spectra reveal an average orientation of $80^\circ \pm 5^\circ$ for the water bending dipole from the surface normal for a free-OH species on the surface, whereas an average of $107^\circ \pm 5^\circ$ for the H-bonded species. The water bend data are complementary to the OH-stretch spectroscopy, since their transition dipoles are not aligned in the same direction in the molecular frame.

We performed SFG spectroscopy at the air/water, positively charged surfactant/water interface and negatively charged surfactant/water interfaces using SSP, PPP and SPS polarization combinations. Line shapes at negatively charged interface and positively charged interface follow the same trend, but the signs of the two peaks are flipped for positively charged surface in PPP and SPS polarizations. Comparing the calculated amplitudes with our experiment yields the average tilt angle as a function of surface charge density. The water bend results at the (uncharged) air/water interface are compatible with literature results from based on the free-OH stretch spectroscopy. The
charged surfactant interfaces provide a picture of how the water dipole reorients in response to the electric field of the surfactant headgroups, as a function of the surface charge density.

**Figure 1.** Vibrational sum frequency generation spectra of the water bend at the air/water interface for SSP (blue) and PPP (red) polarization combinations.

**Figure 2.** Orientational dependence of the water bend SFG amplitude as a function of angle between the C$_2v$ molecular axis and surface normal, for SSP, PPP, and SPS polarization combinations.

Transient phase of ice observed by sum frequency generation at the water/mineral interface during freezing

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We observed a transient non-centrosymmetric phase of ice at water/mineral interfaces during freezing, which enhanced the intensity of the IR-visible sum frequency generation intensity by up to 20 fold. The lifetime of the transient phase was several minutes. Since the most stable form of ice, hexagonal and cubic ice, are centrosymmetric, our study suggests the transient existence of stacking-disordered ice during the freezing process at water/mineral interfaces. Stacking-disordered ice, which has only been observed in bulk ice at temperatures lower than -20 °C, is a random mixture of layers of hexagonal ice and cubic ice. However, we observed the transient phase at the ice/mineral interface at temperatures as high as -1 °C. This observation suggests that the mineral surface may play a role in promoting and stabilizing the formation of the stacking-disordered ice at the interface.

**Figure 1.** Sum frequency generation is obtained by (A) spatial and temporal overlap of two beams (B) at the interface of a silica prism and liquid water. The system is cooled via a copper cooling block and the temperature is monitored by a thermocouple inserted near the surface of the prism.
Figure 2. (A) The SFG intensity and temperature as a function of time during a cooling experiment when the IR beam was set at 3100 cm$^{-1}$. (B) SFG intensity from 2800 to 3800 cm$^{-1}$ as a function of temperature showing the transient SFG increase and (C) SFG intensity as a function of time during a cooling experiment when the IR laser is parked at various wavenumbers. (D) SFG signal as a function of time during a cooling experiment with different polarizations. The transient SFG signal at SPP polarization combination suggests the growth of ice is anisotropic as well as non-centrosymmetric.

Poly(ω-methoxyalkyl acrylate)s (PMCxA, Figure 1a) are a series of biomaterials having a different carbon number in the side chain between ester and methoxy groups. In the case of $x = 2$ (i.e., PMC2A), this polymer has been used as a coating material for medical devices due to its excellent antithrombogenic property. It has been known that PMC2A has three types of hydrating water, i.e., non-freezing water having a strong interaction with the polymer chain, freezing bound water having an intermediate interaction with the chain and freezing water having bulk-water-like structure. [1] Our recent studies have revealed that the freezing bound water interacts with the methoxy moiety in the side chain terminal and the water structure plays important roles for the blood compatibility. [2-3] In the present study, water structure in PMCxA with different $x$ was investigated using attenuated total reflection infrared (ATR-IR) spectroscopy.

Figure 1. (a) chemical structure of poly(ω-methoxyalkyl acrylate) (PMCxA) and (b) schematic illustration of the in situ ATR-IR flow trough cell

Figure 1b shows schematic illustration of an in situ ATR-IR flow trough cell designed for the measurement of wet polymer films. A polymer film was prepared on a flat surface of a ZnSe prism by solvent casting. The obtained film was enough thicker than penetration depth of near-field light generated at the prism/polymer interface. Time-dependent ATR-IR spectra were recorded when water vapor or liquid water was injected into the cell.

Figure 2 shows time-dependent ATR-IR spectra during a sorption process of liquid water into a PMC2A film. Increases of the bands assigned to water around 3600-3000 cm$^{-1}$ and at 1640 cm$^{-1}$ are observed, representing sorption process of water into the polymer matrix. It is interesting to note that the spectral shape of the O-H stretching band
round 3600-3000 cm⁻¹ gradually change with time. This demonstrates structure change in water during the sorption process induced by the polymer-water interaction. Both positive and negative signals are identified in the fingerprint region below 1800 cm⁻¹. Decreases of the polymer bands mainly represent swelling of the polymer film by the water sorption. Increases and peak position shift of the polymer bands represent structure change in the polymer chain induced by the hydration. Both structure change in hydrating water and hydrated polymer chain will be discussed in detail.

**Figure 2.** Time-dependent ATR-IR spectra of the process of water sorption into a PMC2A film

Tuesday June 13 2017

Parallel Session 16

Protein Structure and Pharmaceutical Applications
Low-Frequency Raman Spectroscopy Is Suitable For Quantitative Analysis of Multiple Solid State Forms of Pharmaceuticals

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Low-frequency Raman spectroscopy is a technique that has recently been applied for solid state studies.[1] For pharmaceuticals, it is very important to be able to control and monitor crystallinity, because the solid state structure can determine the bioavailability, stability, and manufacturing aspects of a drug. The advantage of low-frequency Raman spectroscopy is that it gives additional information on the lattice vibrations of a crystal which cannot be collected by the conventional, mid-frequency Raman spectroscopy. The resulting distinct spectral features between different solid state forms have been used for characterizing polymorphs of several pharmaceuticals.[2]

The purpose of this study is to further evaluate the practical feasibility of low-frequency Raman spectroscopy for quantitative analysis of complex ternary mixtures, and to compare the results with those obtained using the more conventional mid-frequency analysis.

Three solid state forms of piroxicam were used. Form I was used as received, while form II was prepared by recrystallization from ethanol and the monohydrate by recrystallization from water. The solutions were cooled to room temperature and vacuum filtered to harvest the crystals. Mixtures of the solid state forms were prepared without grinding. A calibration set was composed of thirteen piroxicam mixtures, with form I, form II and monohydrate. The samples were measured with two different low-frequency systems, one capable of simultaneous low- and mid-frequency analysis, and a conventional FT-Raman spectrometer. The solid state forms were confirmed using DSC, XRPD and FTIR.

Low-frequency Raman spectroscopy was able to differentiate the three piroxicam solid state forms. When combined with multivariate analysis, both low-frequency and mid-frequency Raman spectra could separate all mixtures from each other. The amount of each solid state form could be predicted with all models based on the different Raman techniques and spectral regions. When comparing the low and mid-frequency spectral data collected using the same Raman instrument, models built with the low-frequency data performed slightly better. This implies that there is an advantage in using low-frequency over mid-frequency Raman data. The signal strengths were higher at the low-frequency range, which offers potential advantage of better signal-to-noise ratio.
Figure 1. Left panel: Solid-state low frequency Raman spectra for differing forms of piroxicam. Right panel: Superposition of the tertiary mixtures of the three forms of piroxicam (orange dots) and the principal components analysis from the low frequency data.

Beyond FT-IR and Raman: Predictability and Sensitivity of ROA for Structure of Biologics

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Vibrational Spectroscopy, such as FT-IR and Raman, have long been used in Pharmaceutical industry for variety of tasks from identification of raw material, formulation, processing and quality control. But in biopharma, and specifically in structure elucidation, where the drugs are based on either peptides, proteins, carbohydrates or nucleic acids these techniques have been taking much longer to gain ground. FT-IR is best adapted but Raman has just now started to gain popularity. Its advantage is ability to detect the conformation of disulfide bonds and gain information from side-chains, in addition to secondary structure. More recently, several studies have shown an enhanced sensitivity of ROA (Raman Optical Activity) with differences observed when none are seen with any other spectroscopic technique.

Raman optical activity (ROA) is a highly sensitive stereospecific spectroscopy that combines the structure-rich detail of conventional Raman spectroscopy with chiral sensitivity of electronic circular dichroism (ECD or CD) [1]. Just as CD enhances the biological structure content of its parent UV absorption spectra, so ROA tremendously enhances the structure-sensitive content of conventional Raman spectra. Both ECD and ROA are measures of the difference in their parent spectroscopies, (UV absorption and Raman scattering) with respect to left and right circularly polarized light. ROA was discovered in 1973 by A.D. Buckingham and L. D. Barron [2] and was commercialized for general use by BioTools, Inc. in 2003 after several improvements and innovations that facilitated its routine manufacture and use. There is now an extensive literature of over 500 scientific publications involving ROA that demonstrate among other aspects that ROA is a unique and highly sensitive probe of protein secondary and higher-order structure. Over past forty plus years since its discovery ROA is now recognized world-wide, at first in academic research laboratories and increasingly throughout the pharmaceutical industry (see below) as technique that is significantly more sensitive to protein structure than either CD or Raman [3].

An ROA spectrometer is about the same size as a bench-top Raman spectrometer but is actually two spectrometers in one since ROA and conventional Raman are measured simultaneously. In operation the instrument measures in two separate channels the Raman scattering for right and left circularly polarized light. Subtraction of the two channels yields ROA and addition yields conventional Raman. So there is always a Raman reference spectrum for every ROA spectrum. ROA spectra are smaller than their parent Raman intensities by several orders of magnitude in the same way the CD spectra are similarly smaller than their parent UV absorption spectra. As a result, even though Raman spectra can be measured in a few minutes ROA spectra of high quality require a few hours to collect, although basic features of the ROA spectra can be seen in minutes. Because ROA spectra require longer collection times, the design of the spectrometer is the world’s fastest Raman spectrometer being able to measure high quality Raman spectra of protein solutions in less than a minute.
The first report on the sensitivity of ROA to structure of biological drugs was published by Dr. Li and co-workers at Amgen [4]. Figure 1 shows that Raman spectra of IgG2 at pH=7 and pH=3 are essentially identical to each other, whereas their biological activity is not. In contrast, ROA spectra of IgG2 at pH=7 and pH=3 show remarkable differences, and are consistent with melting of native β-barrel and formation of aggregates at pH=3. In more recent studies, other companies including Merck [5] and BMS [6] confirmed the unusual sensitivity of ROA to enable incisive characterization of even minor changes in biologics structure and/or early detection of structural degradation and/or aggregation.

In this lecture, we will describe the fundamentals of ROA and compare the sensitivity of the four forms of vibrational spectroscopy as applied to structural studies of proteins, with emphasis on predictability (and thus sensitivity) nature of ROA.

![Native IgG2 Structure](image)

**Figure 1.** Unusual sensitivity of ROA to proteins structure illustrated by Human IgG2 example. Raman spectra of IgG2 at pH=7 (top blue) and pH=3 (top magenta) are essentially identical. In contrast, the corresponding ROA spectra show remarkable differences that are attributed to loss of native β-barrel and formation of aggregates at pH=3. (Courtesy of Dr. Tiansheng Li – Amgen)

Study of structure, conformation and interactions in pharmaceutical cocrystals using vibrational spectroscopy

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Cocrystallization alters the molecular interactions and composition of pharmaceutical materials, and it is considered to be a better alternative to optimise the drug properties. The use of crystal engineering in active pharmaceutical ingredients (API) acts as an enabling technology to bring improved pharmaceutical products to the marketplace as well as for improved drug delivery.

Crystal engineering principles based on the supramolecular synthon approach have proved to be a powerful tool in the design of cocrystals. Supramolecular synthon can be formed between similar (homo) or different (hetero) functional groups such as amides, carboxylic acids and alcohols. The strong hydrogen bonds include (N–H⋯O), (O–H⋯O), (N–H⋯N) and (O–H⋯N) whilst weak hydrogen bonds involve C–H⋯O and C–H⋯N. Pharmaceutical cocrystal is an attractive field for pharmaceutical scientists because it can provide number of crystalline state for a particular API, in which the physical and chemical properties of pure API such as solubility, stability, bioavailability can be modified without affecting the chemical composition of API.

Characterization of cocrystals involves both structural (infrared spectroscopy, single crystal X-ray crystallography and powder X-ray diffraction) and physical properties (melting point apparatus, differential scanning calorimetry, thermogravimetric analysis). Vibrational spectroscopy is employed in the characterization of cocrystal. This method includes infrared absorption spectroscopy and Raman spectroscopy. IR spectroscopy gives qualitative and quantitative information about the molecule. Formation of cocrystal is confirmed by the lowering in the wavenumber of the functional group which is involved in hydrogen bonding leading to the increment in the bond length of that group. Terahertz spectroscopy is a versatile spectroscopy.

Theoretically, Density functional theory (DFT) proved as an efficient tool for the prediction of models and hydrogen bonding interactions whose crystal structure is not known and also for the cocrystal whose crystal structure is known. For the selection of the binding sites (reactive sites) available in both API and co-former, molecular electrostatic potential energy surface analysis (MEPS) has been performed.

Nitrofurantoin (NTF) is an antibacterial drug that is used to treat the bacterial infections of the urinary tract. It has poor solubility in water. Therefore its cocrystal with L-proline has been studied to improve its solubility and bioavailability. L-proline being a zwitterion, is an amino acid containing an amino and a carboxylic group is been used as co-former. Molecular structure of NTF-LP cocrystal using several models on the basis of hydrogen bonding present in other NTF cocrystals is shown in Fig. 1. In all these crystal structures strong O–H⋯O, O–H⋯N, N–H⋯O and C–H⋯O hydrogen bonds play an important role. In nitrofurantoin-L-proline cocrystal [1], after analysing FT-IR and FT-
Raman spectra of both NTF and LP the reactive sites (NH, C=O, NO$_2$) groups of NTF (API) and (NH$_2^+$, COO$^-$) group present in L-proline (co-former) have been predicted for the formation of cocrystal. Based on these predicted sites, several models for the formation of cocrystal have been made to get the most stable structure.

![Figure 1. Optimized structure of several models of NTF-LP cocrystal.](image)

In paracetamol-oxalic (PRA–OXA) acid cocrystal [2], the structural and spectral characteristics of a PRA–OXA cocrystal has been carried out using two models (monomer and dimer), with the aim to understand the supramolecular structure and intramolecular interactions within the cocrystal (Fig. 2). Structural and spectral calculations indicate that OH and NH groups form stronger hydrogen bond in PRA with the NH and OH groups of neighboring molecule respectively comparison to cocrystal. In case of cocrystal both the groups of paracetamol are hydrogen bonded to the neighboring oxalic acid molecule, resulting in an increment in the bond length and lowering in wavenumber. Hydroxyl and carbonyl stretching modes of oxalic acid are red shifted in cocrystal in comparison to pure oxalic acid as these groups make stronger hydrogen bonding with C=O, O–H and N–H groups of paracetamol resulting in the formation of cocrystal.

![Figure 1. Optimized structure for dimer+2OXA cocrystal.](image)

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**Space- and time-resolved imaging of pharmacokinetics and pharmacodynamics at the single cell level by Confocal Raman Microspectroscopy**

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**Introduction**

Cellular pharmacokinetics (PK) / pharmacodynamics (PD) is an emerging field in pharmacology research recently attracting much attention owing to its potential in evaluating drug efficacy. By studying the dynamic profiles of drug uptake, distribution, metabolism and efflux into the extracellular environment, we can derive detailed information about PK/PD and construct models that can help in drug discovery and development. Such an approach integrates cellular physiology/pharmacology, which needs an effective way to study single cells without involving fractionation methods. Although some studies have utilized fluorescence-based techniques to study PK/PD, the obtained information is limited to identifying accumulation of drugs and completely lacks information about intracellular metabolic changes. We successfully employed confocal Raman microspectroscopy to visualize dynamic profiles of drug at the single cell level without employing any fluorescent labels and/or fractionation methods. We further believe that application of multivariate method such as multivariate curve resolution will throw light on the drug induced metabolic changes simultaneously.

**Methods**

A diploid yeast strain of Saccharomyces cerevisiae was used as a cellular model for all measurements. Antifungal agent (Terbinafine) was used as a drug candidate. A homemade Raman microspectrometer equipped with a He-Ne laser (632.8 nm) was used with a laser power of 3 mW at the sample point [1]. Raman images were obtained to study PK/PD using exposure time of 1 s/point with a step size of 0.5 µm in X and Y directions.

**Results and Discussion**

Terbinafine is an antifungal drug that inhibits ergosterol biosynthesis by inhibiting squalene epoxidase, an enzyme that is an integral part of fungal cell membrane biosynthetic pathways. The chemical structure of terbinafine inherently contains C=C (triple bond) whose stretching vibration appears at 2200 cm⁻¹ as can be seen from Figure 1. In fact, yeast cells took up Terbinafine almost immediately upon addition of the drug. Particularly the drug seems to have localized to lipid droplets.
Time-lapse Raman imaging studies revealed intracellular accumulation of squalene (PK). Further studies provided information on how the cell reacts to the administered drug (PD). The details will be discussed during the presentation.

**Conclusion**

Space- and time-resolved information on pharmacokinetics / pharmacodynamics of antifungal agent terbinafine has been obtained at the single cell level in budding yeast model using confocal Raman microspectroscopy. Label-free imaging of cellular uptake and distribution of terbinafine has been achieved. Spatio-temporal studies revealed immediate localization of the drug to lipid droplets. Terbinafine induced squalene accumulation and ergosterol depletion has been visualized in living cells without the aid of any fractionation method.

**References**

Effects of different classes of antibiotics revealed by using SR-FTIR spectromicroscopy

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Surprisingly little is known about the path to death upon antibiotic treatment in individual bacteria. This gap in our knowledge is getting increasingly alarming as conventional antibiotics become ineffective. Besides cell wall assembly, most clinically used classes of antibiotics target RNA synthesis, protein synthesis, or DNA replication. The increase in available crystal structure data has enabled researchers to precisely define drug-target interaction sites in known antibiotics and understand the causes of antibiotic resistance. However, how these drug-target interactions ultimately cause cell growth arrest and cell death is not well understood. In particular, it is clearly evident that more research is needed to understand how different classes of antibiotics ultimately lead to cell death. It is well known that growth arrest does not automatically imply cell death. Studies of antibiotics effects are generally dependent on cellular markers and/or labeling. [1,2] Therefore, different markers have been developed to distinguish between viable and dead cells. The most common live/death markers are based on dyes that can only penetrate cells with compromised membranes (e.g. propidium-iodide). However, such indicator dyes often affect cell physiology and are not suitable for long-term studies of live bacteria.

To gain better insight into changes related to antibiotic affects, we recently developed a new method for real-time single cell tracking of energy metabolism (i.e., ATP content) in living bacteria and demonstrated the application of this method to the evaluation of antibiotics with different modes of action (Figure 1). [3] Fluorescence Resonance Energy Transfer (FRET)-based methods provide data on a single metabolite and cannot capture more global biochemical changes. Synchrotron radiation-based Fourier transform infrared microspectroscopy (SR-FTIRM) coupled with IR transparent liquid cells can provide noninvasive, label-free, spectroscopic microanalysis with necessary spatial and temporal resolution. In this contribution we present data on Mycobacteria smegmatis, a model organism for M. tuberculosis, exposed to several classes of antibiotics that inhibit different essential molecular targets, including isoniazid (fatty acid synthase II), streptomycin (ribosome 30S subunit), rifampin (RNA polymerase), and ciprofloxacin (DNA gyrase). As a control, cells were left untreated. By comparing IR signatures of mycobacterial responses to different antibiotics, we were able to confirm the target and effects of the different antibiotics, as well as to observe what kind of other cellular changes occur during the antibiotic treatment. The experiment was carried out in three different conditions: 1) dry fixed cells to observe the major effect of the antibiotics on the cells for 0h, 6h, 15h, and 24h 2) hydrated fixed cells were used to
better evaluate the changes in the nucleic acids’ structure, that are more evident in hydrated condition, and to better assess the time range of the drugs, and 3) Live cell measurements from 0 to 8 hours on one of the faster acting antibiotics (Rifampin). Some of the data are presented in Figure 2.

The presented data and method will be the foundation for further experiments to explore the effect of other antibiotics, whose mechanism of action are not completely known.

**Figure 1.** Real-time measurements of single-cell ATP using genetically encoded biosensors. (A) Time traces of FRET/YFP ratios of selected cells during 24 h of drug exposure to bedaquiline (BDQ), isoniazid (INH), streptomycin (Strep), rifampin (Rif), and ciprofloxacin (Cipro). (B) Time traces of the percentage of cells undergoing ATP switching (high to low) after 24h exposure to BDQ (n = 277), INH (n = 250), Strep (n = 111), Rif (n =125), or Cipro (n =124) followed by 24h of recovery.

**Figure 2.** (A) average spectra of five of the measured conditions at 0h and 6h (B) PCA plot of the five analyzed conditions, rifampin (Rif) and isoniazid (INH) have already affected almost the whole population, whereas ciprofloxacin (Cipro) spectra are still similar to the controls, (C) plot with the loadings 1 and 2: Controls, INH and RIF are differentiated along PC1, mainly RNA (1240 cm\(^{-1}\)) and lipids signature (1730 cm\(^{-1}\), 2925 cm\(^{-1}\), 2852 cm\(^{-1}\)), whereas PC2 are mainly DNA (1080 cm\(^{-1}\)), and proteins (1654, 1620 cm\(^{-1}\)).

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Parallel Session 17

Vibrational Imaging 2
Infrared spectroscopic microscopy\textsuperscript{1} has advanced considerably in the past few years with the availability of new instrumentation, a diverse array of applications and measurement capabilities. These have allowed for a dramatic increase in the spatial quality of biomedical images acquired in the laboratory.\textsuperscript{2} At the same time, there is much greater detail in spectral content and its understanding from fundamental developments from theory to cell culture studies. These new capabilities do not just improve the performance of conventional Fourier transform infrared (FT-IR) imaging but also provide a wide array of alternatives in terms of discrete frequency IR (DFIR) imaging,\textsuperscript{3} spectral\textsuperscript{4} and spatial\textsuperscript{5} pattern analysis, optical setup and signal to noise ratio of data. This diversity in instrumentation and capabilities complicates comparisons between various approaches and finding optimal strategies for imaging and analysis becomes challenging. Concomitant with new capabilities are challenges arising from massive data sizes, subcellular diversity in spectral signals, effectively utilizing selection of parameters for optical design and confidence in results. Here we discuss various theoretical approaches and comparison metrics to understand the trade-offs involved and the ultimate capabilities of instrumentation. Starting from the design of microscopes to matching image quality for specific uses, we describe the setup and evaluation of high-definition IR imaging for pathology. Next, we provide a description and challenges of DFIR imaging with a quantum cascade laser and report on fast imaging for multiple chemical analyses. Finally, we describe combined spatial-spectral imaging with quantitative estimates of the accuracy of results. Together, these examples cover design, selection of parameters, evaluation of systems and quantification of confidence in results for pathology.
Figure 1. Spatial and spectral comparisons obtained on similar regions of tissue across different modalities. (a) High definition (top) compared to standard definition (bottom) 6 class classification and H&E stained images (middle) (b) Spectral comparisons of QCL and standard definition imaging.

Using SERS for Super-Resolution Chemical Imaging of Cell Surfaces

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Applications of spectroscopy for chemical imaging are limited in spatial resolution by diffraction. As one solution, surface plasmon resonances in metallic nanostructures can manipulate optical energy within sub-wavelength dimensions [1]. These locally enhanced fields are often called “hotspots” and can give chemical and spectral information of proximate molecules via Surface Enhanced Raman Spectroscopy (SERS) [2]. The large field enhancement from these hotspots can also give a “blinking” SERS effect that can be processed using Stochastic Optical Reconstruction Microscopy (STORM) algorithms for super-resolved imaging [3]. However, using SERS in this way is limited in two respects: (1) due to the inhomogeneous and random nature of hotspot generation [2], these SERS images can be filled with gaps; (2) if only the intensity of the SERS blink is used for STORM imaging, spectral content is lost. To address the first problem, these gaps can be “filled in” by randomly changing the illumination profile of the laser on the sample using an optical diffuser or spatial light modulator (SLM) [4,5]. Images taken with this technique of biological samples, such as collagen protein strands, show excellent agreement with scanning electron microscope (SEM) images. To address the second problem of lost spectral content, we place either a tunable band-pass filter (obtaining an image that corresponds to a single wavelength band) or an optical transmission grating (providing both super-resolved spatial information as well as spectral content) into the imaging path, shown in Figure 1. Different chemical signatures (e.g. from gram-positive vs. gram-negative bacteria) are observed and correlated to super-resolution images.

\textbf{Figure 1.} Experimental setup. (a) A thin silver film generates hotspots when illuminated from below. (b) SEM of a sample film. (c) Cells on the surface generate (d) SERS signals from various locations. (e) Setup of the laser, microscope, filter, grating, and camera.

Various cell samples were seeded onto a thin 10 nm silver surface on a glass cover slip. The samples were illuminated from below with a 100x oil (NA = 1.25) immersion objective. Typical biological samples included gram-positive and gram-negative rod and spherical-shaped bacteria, e.g. \textit{M. Luteus}, \textit{E. Coli}, or \textit{B. Subtilis}. Emitted light was
collected through the same objective and passed through a long-pass Raman filter (Semrock) and sent to an electron multiplied (EM) CCD (Andor) for STORM imaging. While it is possible to image a single band of SERS light by passing the emitted light through a tunable bandpass filter as we have shown previously [5], the process is tedious and slow for imaging multiple wavelength bands or achieving high spectral resolution. However, by placing a transmission diffraction grating in the imaging path, some light is diffracted into a separate spectral channel. Imaging through the grating provides spatial content (zero-order undiffracted light) for STORM imaging purposes and spectral content (first-order diffracted light) for SERS identification at the same time in two different regions on the CCD. Since the SERS hotspots are blinking in time, the spectral channels are prevented from overlapping (e.g. from two side-by-side SERS hotspots). Some initial super-resolution chemical imaging results are shown in Figure 2.

![Figure 2. Super-resolution chemical imaging results. (a) A single frame from the EMCCD showing image content and spectral content. (b) Sample SERS spectra from two bacteria showing the recorded chemical information. (c) STORM image overlaid with an SEM image of an E. Coli bacteria sample. (d) STORM image overlaid with SEM of a B. Subtilis bacteria sample. (e) Zoomed in region showing 50 nm features of the cell surface.](image)

We conclude that combining SERS, STORM, and imaging through a diffraction grating is applicable for super-resolution imaging of cell surfaces and precise chemical data collection. The technique shows potential for super-resolution “snapshot” chemical imaging of biologically relevant structures with both high spatial and chemical resolution.

Functionalized Gold Nanoparticles as Hypersensitive Biosensors for Monitoring Cellular Uptake and for Cancer Diagnostics

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Noble metallic nanostructures exhibit a phenomenon known as surface-enhanced Raman scattering (SERS) in which the scattering cross sections are dramatically enhanced for molecules adsorbed thereon. In recent years, SERS has been established as one of the emerging analytical techniques, with sensitivity up to the single molecule. The present contribution reports on the preparation, characterization and functionalization of colloidal gold nanoparticles (AuNPs) to be used as a biosensing platform for cellular uptake studies and for diagnostic purposes in early cancer detection. The main tool to characterize the AuNPs morphology is Transmission Electron Microscopy (TEM). In Fig. 1 is shown a micrograph of a typical colloidal solution, highlighting the absence of aggregates; different shapes are produced (spheres, nanorods, triangles). Image analysis (inset of Fig. 1) detects a bimodal distribution: the main population (74 %) displays a spherical shape (roundness above 0.8) and a mean diameter of 31 nm. The remaining fraction (26 %) is made of nanorods (roundness less than 0.6) and has a mean diameter of 43 nm. The size distribution and shape homogeneity can be further improved by post-processing (ultracentrifugation). In general, shape multiplicity does not reduce the SERS response. Once prepared, the gold nanoparticles have been functionalized with 4-mercaptobenzoic acid (4-MBA) to form a Self-Assembled Monolayer (SAM); the resulting colloidal solution is stable. The bare nanoparticle has no Raman activity, while strong SERS signals are produced by the functionalized nanoparticles due to the chemisorbed 4-MBA. The enhancement factor (EF) in solution can be evaluated in a number of ways. The present AuNPs provide EF values ranging between $10^6$ and $10^8$, resulting in highly efficient substrates for biosensing applications. The enhancement factor depends on particle size, with the maximum occurring for diameters of around 50 nm. However, even for smaller sizes (30 and 20 nm) SERS signaling remains efficient, which adds to the versatility of the approach. Hyperspectral Raman imaging represents an emerging technique to investigate the cellular environment at a molecular level [1, 2]. It provides several advantages among which a high contrast, originating from the specificity of the Raman spectrum, molecular level information, appropriate spatial resolution, versatile sampling. Fluorescence emission may be occasionally problematic. We have investigated normal and tumor lines of human prostatic cells by SERS spectroscopy. We employed the gold nanoparticles functionalized with 4-MBA as molecular reporters. These were added as colloidal solution to a water dispersion of the cells, incubated overnight and then deposited on a quartz substrate for Raman inspection. In the case of healthy cells, we found that the
AuNPs aggregate around the cell membrane, possibly through a molecular interaction between the carboxyl groups of 4-MBA and the polar groups on the membrane surface (see Fig. 2A). Thus, an undamaged membrane prevents the nanoparticles from entering the cell body. For the tumor cells, strong SERS signaling is observed in the whole cell area (see Fig. 2B). In the latter case, SERS imaging demonstrates a conspicuous uptake of nanoparticles, which cross the more porous cellular membrane. This effect is evidenced by the spectra collected within the cell body, which are featureless for the normal cell and are very intense (more than in the periphery) for the tumor cell. These results may have important implications for diagnostic purposes. They also demonstrate the sensitivity and the specificity of SERS imaging for studies of nanoparticle uptake in cells and tissues, which are becoming very relevant to test cytotoxicity, selective uptake of drug-carriers, and more.

Figure 1. TEM micrograph and image analysis of the AuNPs.

Figure 2. Raman images of healthy (A) and tumor human prostatic cells (B). In C is reported the SERS spectrum collected in the red areas of both Raman images.

Rapid LDIR Imaging of Whole Mouse Brain Sections.

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Some years ago [1], we reported the discovery of crystalline creatine deposits, indicative of perturbed energetics, in brain tissue sections from two lines of mice expressing a double mutant form of human APP695 with two familial Alzheimer Disease (AD) mutations, K670N/M671L and V717F. The deposits were identified through FTIR mapping of the hippocampus, a key structure involved in memory. AD is characterized by learning and memory deficits. In subsequent studies, we showed that the amount of creatine appeared to increase with age of the transgenic mouse [2], though the fine details of the distribution were dependent on the freeze/thaw conditions; moreover, care had to be taken with the analysis of synchrotron source FTIR data, owing to the partial polarization of the source illumination and the orientation dependence of the crystalline creatine spectrum (Figure 1A). We have continued these studies, imaging snap-frozen hippocampal sections from male and female triple transgenic (3xTg) mice that possess amyloid beta (Aβ) plaques, neurofibrillary tangles (NFTs), and cognitive deficits, consistent with AD, as well as post mortem samples from human AD brain [3].

In this paper, we report the rapid detection of creatine across entire tissue sections, several mm in length and width, with Laser Direct InfraRed (LDIR) imaging. The LDIR method exploits the intensity of a Quantum Cascade Laser and rapid scan technology to achieve contrast images created through single frequency imaging, on and off an absorbance maximum for a given analyte. The tissue morphology, grey and white matter, and cell body location can be imaged through contrast based on the lipid carbonyl (Figure 1B). The LDIR source is inherently polarized; an additional filter can be used to ensure full polarization, when required, as is the case for creatine (Figure 1C). The results are compared to our regular detection procedures for which we employ thermal source FTIR imaging with Focal Plane Array (FPA) detection.

Our motivation for these investigations is that, despite its use in clinical trials in other neurodegenerative disorders, creatine has not been tested as a potential therapy in AD. We and others have shown cognitive enhancements with creatine supplementation (CS), including enhanced hippocampal-dependent spatial learning and memory in normal mice.

In a parallel study to evaluate the effects of CS on neuropathology and hippocampal function in the AD-like brain (unpublished), 7-month-old male and female triple transgenic (3xTg) mice were assigned either a CS (3%, w/w) or control diet for 8 weeks,
followed by training in the hippocampal-dependent Morris water maze (MWM). Results thus far indicate sex-specific effects of CS in 3xTg mice, with enhanced cognition in females in contrast to impairments in males. Our results suggest that CS may offer cognitive benefits and protection from neuropathology in women with early-stage AD.

Analysis of the distribution and, potentially, the differences in the quantity of creatine in the test animal brains is essential. Since creatine is a small, water-soluble molecule, histochemical methods cannot be used and current methods are indirect. Though detectable in vivo by MRI, the spatial resolution is insufficient. Immunostaining shows the related enzyme, creatine kinase, to be most prominent in the cerebellum and hippocampus [4]. Detection of creatine directly by FTIR FPA imaging of tissue sections is feasible [1-3], but time-consuming, given the large areas of tissue to be surveyed, and the need for multiple sections from each case. LDIR offers a rapid and accurate means of obtaining sufficient data for a meaningful analysis of the in situ presence and distribution of this important bioanalyte. Notably, the creatine distribution in the LDIR image (Figure 1C) corresponds to the reported distribution of creatine kinase [4].

![Figure 1. LDIR imaging of mouse brain. (A) FTIR spectra of pure creatine crystals under 0° and 90° polarized light. LDIR images based on intensity of (B) lipid carbonyl peak at 1735 cm⁻¹, and (C) creatine peak at 1306 cm⁻¹. Lighter blue = higher intensity.](image)

Raman Spectroscopy and Multimodal Non-Linear Imaging of Tissue-Engineered Cartilage Using Human Skeletal Stem Cells

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The use of skeletal stem cells (SSCs) for cell-based therapies is currently one of the most promising areas for skeletal disease treatment and skeletal tissue repair. The unique properties of SSCs, with their ability to self-renew and potential to differentiate along the stromal lineages, make SSCs an ideal tool for bone and cartilage reparative medicine. However, current available techniques for SSC characterization are invasive, require cell fixation or lysis, and/or are destructive. These limitations have led to the application of alternative strategies that aim to identify molecules at the subcellular level by using their inherent properties, in the absence of any dye or label [1]. Label-free methods such as Raman spectroscopy and coherent anti-Stokes Raman scattering (CARS) microscopy are minimally invasive, non-destructive, and are emerging as powerful alternatives to conventional techniques in biomedicine [1].

Here, human SSCs were differentiated under \textit{in vitro} pellet culture conditions into chondrogenic tissue (cartilage) over 21 days and examined using label-free spectroscopic methods [2].

**Figure 1.** Raman spectrum of human SSCs cultured in chondrogenic conditions for 21 days and the corresponding CARS images of (a) proteoglycans (1410 cm\textsuperscript{-1}), (b) lipids (2845 cm\textsuperscript{-1}) and (c) proteins (2935 cm\textsuperscript{-1}).
Raman spectra provided molecular information on the SSCs and the ability to distinguish biochemical changes in skeletal stem cell differentiation (Figure 1). Using CARS at different vibrational frequencies we imaged typical biological components such as lipids and proteins. Remarkably, for the first time we were able to image other structures including those composed of glycosaminoglycans providing invaluable information regarding the cartilage matrix phenotype (Figure 1).

CARS imaging can be further combined with second harmonic generation (SHG) and two-photon (auto)fluorescence imaging. The combination of CARS microscopy with complementary imaging techniques is highly advantageous and gives a more holistic insight on the development of SSCs.

Elucidation of the architecture of the differentiated tissue is a key component for cartilage tissue engineering. The application of 3D in place of 2D imaging further enabled a more comprehensive understanding of the collagen fibrous network during the chondrogenic development of SSCs. Additionally, 3D imaging harnessing and combining CARS, SHG and autofluorescence revealed for the first time the lipid distribution within the developing cartilage pellet (Figure 2). Quantification of the changes observed using multimodal label-free techniques will allow unprecedented insight into cartilage growth using SSCs and characterisation of their development stages for optimal use in therapy.

In summary, the non-invasive nature of Raman spectroscopy and CARS imaging presents an advantageous alternative to characterise and monitor human SSC cartilage development. Crucially, these studies demonstrate the immense value of non-linear multimodal imaging and label-free approaches and their application to human skeletal repair and regeneration research.

**Figure 2.** 3D multimodal imaging of SSC chondrogenic culture (red: CARS, green: SHG, blue: autofluorescence). Orthogonal views (xy, xz and yz) show the intersection planes at the position of the yellow cross-hair.

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Parallel Session 18

Electrified Surfaces 2
Understanding electric fields at the junctions of two dissimilar materials are important in a wide range of phenomena in physical sciences. Two prime examples are photovoltaic and electrochemical junctions, in which charge separation and transfer is performed by either built-in or externally applied electric fields. While such fields are important, their presence is often inferred through bulk transport measurements, which leave many questions, including their value in equilibrium conditions, unanswered. Direct measurements of such fields through Stark shift of vibrational chromophores near the surface has been pursued before. However, two fundamental aspects of the problem, namely solvation field and fields under non-equilibrium steady current have not been addressed. The work here addresses this problem.

We report two fundamental measurements of local fields for the conductor-dielectric interface under no external bias and the conductor-electrolyte junction with controllable potential. Figure 1 shows our experimental and theoretical results of vibrational Stark shift spectroscopy at conductor-dielectric interface [1]. Since no external potential is applied, just as in the classic Onsager theory of dipole solvation, the Stark shifts arise due to the local solvation field. However, to our knowledge, no theory of dipole solvation

**Figure 1.** (a) The sharp vibrational signature of the Stark shift reporter benzonitrile is measured. The fit is to a model that accounts for the interference of the non-resonant background with the resonant response of the CN stretch. The cartoon in the inset shows the location of the Stark shift reporters at the interface. (b) The nitrile frequency shift relative to air and fit to our interfacial solvation field theory with only one free parameter. (c) The estimated solvation electric field experienced by the Stark shift reporter near the surface is plotted as a function of the dielectric constant of the adjacent solvent.
near the interface existed before. We created and tested such a theory which reasonably matches with our experimental findings (figure 1.b). Furthermore, it allows us to estimate the value of the solvation field at the interface (figure 1.c).

We also report measurements of vibrational Stark shift at the junctions of biased electrodes and electrolytes. The field, in that case, is determined by the experimentally controllable parameters of ionic concentration and applied potential (figure 2.a). Our measurement of the sensitivity of the local field to ionic concentration shows a good match with a model of the interface composed of a dielectric and a diffuse ionic layer (figure 2.b). Finally, we correlate the measured local electric field with the electrochemical current and conclude that under non-equilibrium (steady current) conditions, the measured field is pinned to a constant value and does not respond to applied potential (figure 2.c), reminiscent of the field inside a leaky capacitor.

The work reported here will help establish a fundamental connection between spectroscopic frequency shifts and local electric fields at interfaces and other confined environments in the condensed phases.

**Figure 2.** (a) A model of the interfacial potential profile, accounting for the dielectric behavior of the adsorbed monolayer and ionic screening by the electrolyte. (b) Experimental data for the sensitivity of vibrational frequency to applied potential match the predicted theoretical form. (c) We have identified that the local field as measured by the vibrational frequency of the Stark reporter increases only when negligible current is drawn. Under current flow conditions, the local field does not change with potential.

Raman Observation of Monolayer Graphene on Various Substrates under Electrochemical Potential Control

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Graphene is an ideal material to control surface properties of catalysis, optics, and electronics. Most of promising properties appear based on a single atom layer structure with well-defined electronic states. Its electronic structure interacting substrates can be affected by many factors such as strain, doping and defect. Therefore, the graphene/metal and graphene/dielectric interfaces should be critically controlled [1]. We have revealed that in-situ Raman spectroscopy under electrochemical potential control is suitable tool to determine the vibrational and electronic properties of interfaces [2]. In this presentation, the method is applied to characterize the change of strain, doping and dielectric screening of graphene. It is possible to use Raman spectroscopy to study the influences from those complicated factors. The challenge comes from the interplay of those influences. Thus, to discuss the influences from the factors is of quite importance.

High-quality monolayer graphene was first characterized on Au(111) surface (Figure 1) [3]. The electrochemical properties of a monolayer graphene grown on a Au(111) electrode were studied using cyclic voltammetry and electrochemical scanning tunneling microscopy. These measurements in an aqueous solution revealed that graphene grown on the reconstructed \((22 \times \sqrt{3})\) Au(111) structure effectively inhibited potential-induced structural transitions between reconstructed \((22 \times \sqrt{3})\) and unreconstructed \((1 \times 1)\) which are intrinsic behavior of the bare Au(111) surface. The underlying reconstructed structure was significantly stabilized by covering with monolayer graphene over a wide potential range much wider than that for bare Au(111). Such high stability of surface structure is the proof of high quality of a single layer graphene. These results are considered to be key for understanding the fundamentals of graphene/metal–electrolyte interfaces.

Figure 1. Raman spectrum of monolayer graphene on Au(111).
Monolayer graphene was also synthesized with chemical vapor deposition on Cu foil. To monitor the effect of substrates, graphene was transferred to SiO$_2$ or ITO glass substrate. The influence from strain was studied by measuring the Raman spectra at different locations on these samples. It is found that the strain has proportional influence to the G band frequency ($\omega_G$) and 2D band frequency ($\omega_{2D}$), because the points of ($\omega_G$, $\omega_{2D}$) lie on a straight line in the $\omega_G$ - $\omega_{2D}$ space. The doping level of graphene was controlled by electrochemical potential of graphene. Raman spectra were measured in-situ during electrochemical control at a fixed location. It is found that the $\omega_G$ reaches a minimum at about 0.1 V vs. Ag/AgCl, where the graphene is undoped. With the increase of doping level, regardless of electron or hole doping, the $\omega_G$ increases monotonously. On the other hand, the change of $\omega_{2D}$ with doping is much smaller. As a result, the ($\omega_G$, $\omega_{2D}$) shifts to the right direction with any doping. Finally, on different substrates, there are huge difference on ($\omega_G$, $\omega_{2D}$). A significant blueshift of $\omega_{2D}$ is found on metal substrates such as Cu and Au comparing to SiO$_2$, without much shift of $\omega_G$. This phenomenon could be attributed to the dielectric screening from the substrate which reduces the Fermi velocity of graphene. Theoretical explanation is proposed based on previously reported first principle calculation results, in which the electron-electron self-energy is screened by a substrate with a high dielectric constant [4]. Even on the same sample, the nonuniformity and contamination can induce different degree of dielectric screening.

Experimentally Probing the Hydrogen Evolution Reaction on Pt and Au with Femtosecond Time Resolution

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Hydrogen evolution from noble metal electrodes during water splitting (i.e. the so-called Hydrogen Evolution Reaction (HER)) plays a crucial role in a variety of fuel cells and photoelectrochemical devices. More than a century of study of this reaction has found that its rate strongly depends on type of metal, surface structure and aqueous phase composition. Despite this work a clear view of the mechanism of this reaction, and how this mechanism may depend on surface or solution composition, is lacking. Here we address this challenge for two noble metals electrodes: Au and Pt.

Because neither protons nor water chemisorb on Au, theoretical studies suggest that the HER proceeds via an interfacial solvated electron stabilized by water molecules with one OH pointed towards the Au (Figure 1, left panel) [1]. In contrast, protons adsorb strongly on Pt and thus hydrogen evolution has long been argued to occur through either a Volmer/Heyrovsky or Volmer/Tafel mechanism (Figure 1, right panel) [2]. While important, mechanistic insight at this level is insufficient to understand the dramatic dependence of HER rate on metal surface structure and solution composition. For both Au and Pt more direct characterization of HER mechanism, and the interaction of intermediates with their local environment, is required.

Experimentally interrogating the HER mechanism on either metal requires the ability to probe species present only at the interface between two bulk phases whose lifetime is short: on Au we would like to understand how the interfacial solvated electron and on Pt how adsorbed hydrogen evolve during electron transfer. Gaining such insight requires both a probe capable of yielding interfacial structural information on sub-picosecond timescales and a way of initiating the HER at a well-defined point in time. We overcome these challenges here by initiating electron transfer at the Au or Pt electrode using an intense fs laser pulse and characterize interfacial structure with fs time resolution after the pump, using interface specific, vibrationally resonant sum frequency spectroscopy. Because all optical experiments are performed inside an electrochemical cell, relating the fs time-resolved optical response and time-averaged electrochemical observables is straightforward. Because neither water nor protons adsorb strongly on Au, a UV pump pulse is required to form an interfacial solvated electron. In contrast, the strong adsorption of protons on Pt makes it possible to induce chemistry with substantially lower energy pumping.

Using this experimental approach we show that at circumneutral pH, at the gold/water interface, the delocalized, conduction band solvated electron has a potential dependent
lifetime of 150-250 fs. These conduction band electrons relax to form localized solvated electrons the great majority of which have a potential dependent lifetime of 1-18 ps while a small fraction live for much longer, > 40 ps, and are presumably responsible for chemistry. In contrast, at the Pt/water interface at similar pH, electron induced depopulation and Pt-H structural evolution occurs on 1-2 picosecond timescales (where the extent of structural evolution and depopulation depend strongly on potential) (see Figure 2). These results are, to our knowledge, the first direct observation of the HER at its natural, femtosecond, time-scale on both metals. Our approach is straightforwardly generalizable: e.g. extending the probe to other interfacial solution phase species or utilizing other, more complicated, electrodes should be relatively straightforward.

Figure 1. (left) The HER intermediate on Au as inferred from electronic structure calculation [1] (right) Proposed mechanism(s) for the hydrogen evolution reaction on Pt [2]

Figure 2. (left) 800 nm pump / Pt-H probe data on Pt(110) in 0.5 M H2SO4 as a function of potential. Each trace is the integrated intensity over the Pt-H resonance. (right) Experimental scheme showing optically induced electron transfer over the Pt/water interface.

Graphene nanoribbons inherit physical and electronic properties from their purely 2-D parent, graphene, but have a higher proportion of edge atoms that opens the door to chemical functionalisation without perturbing the sp² graphene lattice. However the absolute number of edge atoms is low and is below the detection of conventional IR and Raman spectroscopies. We use a mechanical fracturing technique [1], starting with high purity highly oriented pyrolytic graphite (HOPG), to produce very pure graphene nanoribbons and then selectively modify the edges of the graphene nanoribbons.

Plasmon enhancement can be used to increase the sensitivity of both IR absorption and Raman scattering. For molecules, the plasmonic nanostructure is necessarily larger than the molecule of interest and it is a relatively straightforward task to adhere the molecules to the nanostructure surface. Nanoribbons are however typically larger than the plasmonic nanostructure and the problem of locating the plasmonic nanostructure at the edge of the nanoribbon must be solved to provide selective enhancement of the edge
structure and functional group.

In this work we analyse the edge structure of graphene nanoribbons obtained from HOPG by mechanical fracturing. Analysis by Raman spectroscopy, TEM and HR-TEM reveals the fractured graphene edges to be of almost pure zigzag or armchair structure. The graphene edges are functionalised with a variety of functional groups.

We show how silver nanoparticles preferentially locate at the edges of graphene nanoribbons. The nanoparticles enhance both infrared and Raman intensities and we show how surface-enhanced infrared absorption spectroscopy (SEIRAS) and surface-enhanced Raman spectroscopy (SERS) provide spectroscopic evidence for edge functionalization that cannot be obtained by standard approaches.

We also show how polarised Raman spectroscopy can determine the graphene edge structure (zigzag or armchair), and present some preliminary results from DFT calculations that show zigzag and armchair fragments should have distinct spectral features in the IR spectrum. We compare these calculations with SEIRAS spectra of two samples taken from the same HOPG starting material that show distinct spectral features that we attribute to differences in edge structure.

References:
Synthesis, Raman Spectroscopic Identification and Evaluation of Single-Particle Catalyst in Electrodynamic Trap

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Materials work in multi-component forms. A wide range of compositions must be tested to obtain the optimum composition for a specific application. We propose optimization using a series of small levitated single particles synthesized from droplets with ingredients inside. This method enables the rapid examination of the properties of particles with a wide variety of compositions. The particle composition can be controlled by merging a predetermined number of droplets containing the component materials as a solute. We aim to optimize catalytic activity of multicomponent particles which catalyze gas-phase reactions. We developed a reactivity-evaluation system of a single particle levitated in an electrodynamic trap [1]. In the present study, we measure a CO-oxidation reaction catalyzed by a single-particle catalyst of gold-supported titanium dioxide (Au/TiO$_2$). The reactivity of the catalyst is observed from temperature rise by the heat of reaction measured by the black-body radiation.

Figure 1A shows the tandem-trap apparatus, which consists of two sequential electrodynamic traps. The first and second traps are the droplet merger and measurement sections, in which liquid droplets are mixed and the properties of the trapped particle are determined, respectively. Each trap consists of an upper endcap electrode, a ring electrode, and a lower endcap electrode. Every endcap electrode has an aperture of 2 mm in the center for transferring the trapped droplet and/or gas purging of the trap apparatus. The lower endcap of the merger section and the upper endcap of the measurement section are in contact. In the present experiments, one droplet nozzle was used. The droplet was charged using an electric field between the nozzle tip and the ring electrode, trapped by an RF electric field applied to the ring electrode, and transferred to the measurement section by a pulsed electric field applied to the upper endcap electrode.

**Figure 1.** Apparatus for synthesis, characterization, and reactivity measurement of levitated single-particle catalyst: (A) cross sectional view and (B) measurement section.
The trapped particle was calcined by irradiation of a CO$_2$ laser. The trapped particle was identified by a home-built Raman spectrometer by irradiation of a Nd:YAG laser. Scanning electron microscope (SEM) measurement of the trapped particle was also performed. The catalytic reactivity of the trapped particle was measured by temperature rise of the particle by an IR thermography camera with a set of germanium lens.

Figure 2A and B show the experimental procedures to synthesize the single-particle catalyst in the electrodynamic trap. An aqueous droplet containing ingredient materials of the particle (TiCl$_4$, HAuCl$_4$, and sodium citrate) is trapped in the merger section, transferred to the measurement section, and irradiated with the CO$_2$ laser to be calcined into the trapped particle of Au/TiO$_2$. The calcination is confirmed by the Raman spectrum after the CO$_2$-laser irradiation (Figure 2C). The SEM analysis shows that gold particles with submicron diameter are supported on the TiO$_2$ particle (Figure 2D). The Au/TiO$_2$ particle is again irradiated with the CO$_2$ laser and its temperature is measured from the intensity of the black-body radiation by use of the IR camera. The radiation intensities are obtained under an atmosphere of CO + O$_2$ or N$_2$, where the heat of CO-oxidation reaction raises temperature of the particle or not, respectively. Photophoresis by the CO$_2$ laser irradiation perturbs the radiation image, so that the comparison of the images under CO + O$_2$ and N$_2$ are performed for the images giving similar shapes. Figure 2E shows the radiation intensity under the CO + O$_2$ and the N$_2$ atmospheres, where the radiation is more intense under CO + O$_2$ than under N$_2$. These results indicate that the CO oxidation reaction raises temperature of the single-particle catalyst. This study showed that the reactivity of the single-particle catalyst can be evaluated by the temperature rise of the particle measured by the intensity of the black body radiation.

Figure 2. Experimental procedures and results of synthesis and evaluation of single-particle catalyst levitated in the electrodynamic trap: (A) cross sectional view of the apparatus, (B) catalyst synthesis, (C) Raman spectra of the levitated particle before and after irradiation of CO$_2$ laser, (D) SEM image of the single-particle catalyst, and (E) reactivity measured by black-body radiation.

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Parallel Session 19

Aqueous Surfaces 2
Separating the pH-Dependent Response of Water in the Stern Layer and the Diffuse Layer with Vibrational Sum Frequency Generation

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Vibrational sum frequency generation spectroscopy (SFG) was utilized to distinguish different populations of water molecules within the electric double layer (EDL) at the silica/water interface. In contrast to previous work where the influence of ionic strength was explored,[1] here we maintain a constant high salt concentration to ensure that the Stern layer (meaning the compact part of the double layer) is present throughout our experiments. By varying the pH, we then modulate the amount of deprotonated sites on silica and consequently the surface potential (Φ0) and the Stern layer potential (ΦS). As will be shown the pH-dependent trend of two different water vibrational modes varies substantially, but only at higher salt concentration (> 10 mM). This combination of high salt concentration and pH variation reinforces that certain water populations that correspond to different peaks in the SFG spectra are associated with different layers of the EDL[1] and that these regions can experience different orienting forces as a consequence of the high electrolyte concentrations.

Figure 1. Depiction of ssp-SFG generated at the interface between silica and water (s-polarized SFG, s-polarized visible, and p-polarized IR). (Inset): A close up of the different layers of the electric double layer. The layer of counterions and water adjacent to the negative surface charge make up the Stern layer.
It was found that \textit{pss} and \textit{sps} polarization combinations primarily probe the water molecules in the Stern layer, which have a resonance at 3400 cm\(^{-1}\), while \textit{ssp} and \textit{ppp} polarization combinations mainly probe water molecules further from the surface in the diffuse part of the electrical double layer (with a resonance near 3200 cm\(^{-1}\)). According to the \textit{pss}-SFG data, upon increasing the pH from the point-of-zero charge of silica (pH 2) to higher values (pH 12), we observe an increase in alignment of waters adjacent to the surface within the Stern layer in the presence of high salt concentration (Figure 2B). We attribute this increase to a systematic increase in the magnitude of \(\Phi_0\) (or a systematic decrease to more negative potentials) as a result of silica becoming more negatively charged. In contrast, waters further from the surface probed by \textit{ssp}-SFG become less aligned upon increasing the pH from 2 to 7 than more aligned as the pH is further increased (Figure 2A and 2C). We attribute this non-monotonic behavior to a net flip in water orientation upon increasing the pH over this same range, as a result of overcharging of the EDL at low pH (resulting in \(\Phi_s > 0\)), which then changes sign as the silica surface becomes more negative with increasing pH (resulting in \(\Phi_s < 0\)). Such overcharging has been observed in zeta potential measurements of silica colloids in the presence of high concentrations of NaCl,[2] but never discussed in the context of the water structure of the EDL. As such, these results significantly impact our understanding of the water structure within the electric double layer when the Stern layer is present.

\textbf{Figure 2.} Broadband SFG of A) representative pH titrations of 100 mM NaCl at the silica/water interface using \textit{ssp} polarized SFG. B) Representative pH titrations of 100 mM NaCl at the silica/water interface using \textit{pss} polarized SFG. C) Integrated SFG versus pH. The spectra were integrated from 2950-3550 cm\(^{-1}\).

Surface tension has been the most popular measure of a molecule's surface activity. However, in many cases the complex behaviors of the surface tension are difficult to interpret. For example, the aqueous solution of sodium docecyl sulfate (SDS) and poly(diallyldimethylammonium chloride) (PDADMAC) shows a dramatic change in surface tension when the concentration of SDS is increased. (Figure 1) We found that the dramatic surface tension change is a result of a surface charge reversal. The decrease of surface entropy resulting from a better ordering of surface water molecules has a significant contribution to the change of surface tension.

Figure 1. Surface tension of aqueous PDADMAC solution (50 ppm) with various SDS concentrations. The solid line is a guide to the eye. The colored data points indicate the corresponding colored SFG spectra in Figure 2. The insets are the molecular structures of SDS and PDADMAC.
Figure 2. (I) Im($\chi^{(2)}$) spectra of (a) pure water (blue), and PDADMAC solutions (50 ppm) with (b) 0 M (cyan), (c) $7 \times 10^{-5}$ M (green), (d) $2.5 \times 10^{-4}$ M (orange), (e) $8 \times 10^{-4}$ M (red), (f) $1.6 \times 10^{-3}$ M (magenta), and (g) $10^{-2}$ M (purple) of SDS. (II) Spectra of PDADMAC. (III) Spectra of SDS. Spectra of the same color have the same SDS concentration.

Heterodyne-detected sum frequency generation spectroscopy of aqueous interfaces

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Sum frequency generation (SFG) spectroscopy is one of the most unique methods to study molecular structure and dynamics at interfaces. It is applicable to any interfaces as far as they can be accessed by visible and IR light. It provides a vibrational spectrum that allows for investigating molecular structure and identifying chemical species at interfaces. Furthermore, heterodyne-detected (HD-) SFG spectroscopy enables us to determine absolute orientation of interfacial molecules. HD-SFG has been applied to various interfaces including gas/liquid, gas/solid, and liquid/solid interfaces. Among them, the water surface has been studied most intensively and extensively because of its fundamental importance in science. Here we review the HD-SFG spectrum of the water surface with emphasis on "correctness" of spectroscopic measurements.

Figure 1 shows the Im $\chi^{(2)}$ (imaginary part of second-order nonlinear optical susceptibility) spectra of the water surface with quartz used as a reference in the SSP polarization combination. The spectra in Figure 1a, b, and c are reported by Tahara's group in RIKEN,1 my group in Saitama Univ,2 and Shen's group in UC Berkeley,3 respectively. All of them exhibit a sharp positive band at 3700 cm$^{-1}$, a broad negative band around 3450 cm$^{-1}$, and a small positive band around 3100 cm$^{-1}$. The sharp positive band and broad negative band are assigned to up-oriented free (dangling) OH at the topmost surface and down-oriented hydrogen-bonded OH at the subsurface, respectively. The small positive band around 3100 cm$^{-1}$ was assigned to a strongly hydrogen-bonded pair of water1 or ice-like structure at the surface.3 The spectra from RIKEN and UC Berkeley covered a wavenumber range above 3000 cm$^{-1}$, which was not sufficient to see the overall structure of the small positive band around 3100 cm$^{-1}$. Thus I did measurements down to 2800 cm$^{-1}$ as shown in Figure 1b. Unexpectedly the positive signal did not decay to zero but kept positive until 2800 cm$^{-1}$ that seems too low to be assigned to an OH stretch.

The spectra in Figure 1 were all obtained with use of quartz as a reference. It is well-known that quartz is generally not employed as an optical window for IR spectroscopy because of possible IR resonance due to impurity. Therefore it is not perfectly safe to use quartz as a reference in SFG spectroscopy. Rather it is safer to use D$_2$O as a reference, because the contamination of H$_2$O in a D$_2$O sample can be very well controlled under the detection limit by SFG and D$_2$O is virtually nonresonant above 2800 cm$^{-1}$. Figure 2a and b show the Im $\chi^{(2)}$ spectra of the water (H$_2$O) surface with D$_2$O used as a reference.2,4 Very clearly Im $\chi^{(2)}$ below 3100 cm$^{-1}$ is zero within the experimental uncertainty, indicating that the small positive band around 3100 cm$^{-1}$ once assigned to ice-like structure etc. is an artifact. Shen and Tian's group still believes that quartz is the best reference, and therefore their latest Im $\chi^{(2)}$ spectrum in Figure 2c (black open circles) was obtained with use of quartz as a reference rather than D$_2$O.5 However, note that the small
positive band around 3100 cm\(^{-1}\) in Figure 2c is now much smaller than in Figure 1c even without changing a reference. They also reported the Im \(\chi^{(2)}\) spectrum of the D\(_2\)O surface (green solid circles in Figure 2c) with use of quartz as a reference. Because Im \(\chi^{(2)}\) of D\(_2\)O is almost equal to that of H\(_2\)O below 3100 cm\(^{-1}\) in their data, finally they also admitted that the small positive band around 3100 cm\(^{-1}\) has nothing to do with vibrational resonance.

In HD-SFG spectroscopy, it is of utmost importance to choose a good reference. We must remember that quartz alone did not allow us to recognize the positive band around 3100 cm\(^{-1}\) as a physicochemically irrelevant artifact, and that the artifact misled the research community of SFG spectroscopy\(^6\) until D\(_2\)O was introduced in 2015.\(^2,\,4\)

**Figure 1.** Im \(\chi^{(2)}\) spectra of the H\(_2\)O surface with quartz used as a reference.

**Figure 2.** Im \(\chi^{(2)}\) spectra of the H\(_2\)O surface with D\(_2\)O (a, b) and quartz (c) used as a reference.

Silica/water interface is one of the most fundamental mineral oxide/water interfaces, and a number of investigations have been carried out for unveiling the interface structure so far. Heterodyne-detected vibrational sum frequency generation (HD-VSFG) spectroscopy is a very powerful tool to selectively obtain vibrational spectra of the interfacial molecules [1], and we successfully applied it to “buried” solid/liquid interfaces very recently [2]. HD-VSFG is a nonlinear spectroscopy providing second-order susceptibility $\chi^{(2)}$, and only the anisotropically oriented interfacial molecules are probed. Thus, for uncharged interfaces, the thickness probed by this technique is considered to be typically as thin as 1 nm. However, the silica/aqueous interface is negatively charged at pH $> 2$ because of deprotonation of the surface silanol (SiOH $\rightarrow$ SiO$^-$ + H$^+$), and hence the water molecules in the electric double layer (EDL) get oriented due to the electric field created by SiO$^-$. For such charged interfaces, HD-VSFG spectroscopy probes not only the topmost water but also water in the EDL which extends to the bulk with a substantial depth. Therefore, for clarifying the topmost water structure at charged interfaces, it is desired to extract the topmost water spectrum from the experimentally observed spectra. In this study, we demonstrated decomposition of the observed $\chi^{(2)}$ spectrum of the charged silica/aqueous interface into the topmost and the EDL water components. The decomposition was achieved by the analysis of the ionic strength dependence of the spectra, with no need of any theoretical models about EDL. The obtained decomposed spectra provide clear information about the topmost water structure at the interface.

Figure 1(a) shows the imaginary part of $\chi^{(2)}$ (Im$\chi^{(2)}$) spectra of the silica/aqueous interface with various ionic strengths at pH 12. In Figure 1(a), all the spectra appear with the positive sign in the entire OH stretch region, indicating that the interfacial water molecules are net-oriented with their hydrogen atoms up toward the silica surface (“H-up” orientation). This observation is consistent with our previous work [2]. As explained above, these spectra contain the contributions of both of the topmost water and water in the EDL. In fact, the spectral intensity substantially decreased with increasing the ionic strength, which is interpreted as compression of the EDL due to the screening of the electric field by the counter cation. Especially in the spectrum measured with very high ionic strength (2000 mM), the contribution of the water in the EDL is considered to be practically negligible.

To further discuss the spectral change in Figure 1(a), we subtracted the “2000 mM” spectrum from the spectra of the lower ionic strength. As shown in Figure 1(b), the
spectral shape of the difference spectra is insensitive to the ionic strength. This suggests that the experimentally observed spectra shown in Figure 1(a) can be considered to be linear combinations of two components: a component with a fixed amplitude and a fixed shape, and the component varying its amplitude but having a fixed shape. In other words, the topmost water spectrum, which was observed as “2000 mM” spectrum, is insensitive to the ionic strength, whereas the spectral component due to the water in the EDL changes its amplitude but does not change its spectral shape with the change of the ionic strength. Therefore, it is safely confirmed that the “2000 mM” spectrum is the spectrum of the topmost water (“topmost water spectrum”) and the difference spectrum is that of water in the EDL region (“EDL spectrum”).

The decomposed two spectra are compared in Figure 1(c). The EDL spectrum is similar to the bulk Raman spectrum, indicating that the water molecules in the EDL are bulk-like. This observation is consistent with the previous studies [3, 4]. On the other hand, the topmost water spectrum is clearly different from the bulk spectrum, showing a positive broad band in the low-frequency region and a positive small band around 3600 cm\(^{-1}\). The red-shifted broad band is ascribable to the water molecules which form hydrogen-bonds to the negatively charged silanlates. The high-frequency band indicates that some of water molecules form weaker hydrogen-bonds with silica than that in bulk. This band is assignable to the water which interacts with bridged oxygen of the silica (Si-O-Si).

**Figure 1.** (a) \(\text{Im}^{(2)}\) spectra of charged silica/aqueous interfaces at pH 12 with ionic strength from 10 to 2000 mM. (b) The difference spectra (see text). Broken lines in (b) are those after normalization. (c) The obtained decomposed spectra of the topmost and EDL water. A sketch of interfacial water structure is depicted on the right side.

Cation and water molecules adsorbed on gold film electrode investigated by attenuated total reflection surface enhanced infrared absorption spectroscopy

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Surface enhanced infrared absorption spectroscopy (SEIRAS) is a versatile tool to characterize submonolayer adsorbates on electrode surface. In particular, attenuated total reflection (ATR) geometry is crucial to elucidate surface species while suppressing seriously disturbing absorption of water [1]. Indeed, ATR-SEIRAS has been used to investigate various anions like sulphate or perchlorate, as well as neutral molecules such as thiols [2] and biomolecules [3] on gold (Au) and Pt electrodes. However, there are scarce reports on adsorption of hydrated cations with IR spectroscopy except those concerning the effect of cations on adsorption of underlaid anions and water at potentials above the potential of zero charge (pzc) of Pt electrode [4]. Here, we report adsorption of hydrated cations and water molecules on Au electrode at potentials below the pzc (around +0.5 V, vs. Ag/AgCl). We also discuss the enhancement mechanism of SEIRA based on localized surface plasmon (LSP) [5], and lightning rod effect of gold nanostructures.

An Au film with a thickness of 30 nm was evaporated in vacuum (10^{-6}-10^{-7} Torr) onto a Si hemi-cylindrical prism, which was assembled with a home-built glass cell containing electrolyte solutions, reference and counter electrodes. An FT-IR spectrometer (BioRad FTS- 6000), a variable angle ATR facility (Seagull, Harrick) and a potentiostat (EG &G, 263A) were used for ATR-SEIRA measurements of Au film electrode at various potentials. In the ATR-IR measurements, incident angle of 60° was employed on the basis of calculations using the effective medium theory and preliminary measurements of thiol monolayer samples. A finite difference time domain (FDTD) method was used to evaluate the LSP resonance and enhanced IR absorption spectra from adsorbates on gold nanorods with various aspect ratios and long axis length. For instance, an Au nanorod with a long axis length of 1 μm and a short axis width of 50 nm gave the LSP resonance at mid-IR region (2-4 μm), which is primarily determined by the long axis length instead of the aspect ratio of nanorod. Promising enhancement of 20-30 in IR absorption was calculated for rectangular Au nanorods (a size of 40×20×20 nm) arrayed on a Si prism.

First, we empirically confirmed that our Au film electrodes have exclusive orientation of (111) surface by X-ray diffraction measurements, and give a sharp redox peak at ~0.9 V in cyclic voltammogram attributed to the order-disorder phase transition of sulphate species, as well as a pair of peaks at ~0.6 V corresponding to adsorption/desorption of sulphate species. In addition, pronounced absorption bands at ~3450, and 1650 cm^{-1} were observed for O-H stretching and H-O-H bending modes of water molecules, which adsorbed with sulphate species on Au electrode at potentials above the pzc as evidenced by a stretching band of S-O at 1160-1200 cm^{-1}. Thus, our Au film electrodes provided marked SEIRA effect with sufficiently large (111) surfaces. We applied the Au film electrodes to investigate adsorption of hydrated cations at potentials below the pzc at various pH. We found that hydronium ions preferentially adsorb on Au electrode compared to sodium ions at acidic pH in ATR-SEIRA spectra. Indeed, IR absorption of hydronium ions was predominantly observed at ~3550 cm^{-1} even in solutions containing the same amount of hydronium ions.
and sodium ions. In contrast, hydrated sodium ions provided pronounced absorption peaks at ~3480 and 1660 cm⁻¹ at potentials below the pzc in neutral and basic pH solutions, where the amount of sodium ions is much larger than that of hydronium ions (Fig. 1). Interestingly, the peak position of an O-H stretching mode up-shifted with increasing a potential with a slope of ~18 cm⁻¹/V between 0.0 and +0.4 V, indicating the water molecules directly adsorb on Au electrode. We confirmed that cation adsorption is responsible for this water band using ammonium sulphate, which gave the H-N-H bending peak at ~1440 cm⁻¹ as well as water bands below the pzc.

Second, we observed the stronger absorption of adsorbed water hydrating to the cations with larger electrostatic parameters \( \xi (=z/r, \text{ where } z: \text{ charge and } r: \text{ size of cations}, \text{ Fig. 2}) \). This is presumably attributed to larger amount of water molecules hydrating to adsorbed cations with higher \( \xi \) due to higher attractive potential [6]. Such water molecules showed almost the same peak position at ~3450 cm⁻¹, indicating hydrogen bonding in hydrating water molecules is markedly and similarly suppressed.

Third, we investigated water molecules adsorbed on hydrophobic layer at Au electrode. Different halide ions of Cl⁻, Br⁻ and I⁻ adsorbed on Au electrode at potentials above ~0.0 V, providing similar O-H stretching band at ~3600-3700 cm⁻¹. It is known that halide ions densely adsorb on Au electrode, to which most of negative charge of halide is transferred. Thus, the O-H stretching bands appeared at 3500-3700 cm⁻¹ are attributed to water molecules adsorbed outside the hydrophobic halogen layer on Au electrode. Interestingly, similar water bands were observed at 3620 cm⁻¹ on hydrophobic monolayer of thiophenol on Au electrode.


![Fig. 1 ATR-SEIR spectra at Au electrode in 0.2 M Na₂SO₄ solutions (pH=5.9).](image1)

![Fig. 2 ATR-SEIR spectra of water molecules coadsorbed with metal ions (\( \xi \)) on Au electrode (-0.5 V).](image2)
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Parallel Session 20

Spectroscopic Studies of Small Molecules and Gas-Phase Species
This talk will describe recent results from the Zwier group on the single-conformation infrared spectroscopy of mass-selected, cryocooled ions in the gas phase. A schematic diagram of the apparatus is shown in Figure 1.\textsuperscript{1,2} It consists of a nano-ESI source, linear quadrupole ion traps for mass selection, and a cryocooled ion trap where the ions are collisionally cooled to temperatures of 10 K. The UV spectrum is recorded as a photofragment signal by interrogating the cryocooled ions with the output of a doubled dye laser or OPO, creating photofragments that are subsequently detected following selective ejection of the parent ions in a third quadrupole ion trap (q3). Single-conformation IR spectra are then recorded by fixing the UV laser on a transition due to a single conformer of the ion, and preceding the UV pulse with an IR pulse. When the IR laser is resonant with an IR transition of the ion, absorption removes population from the ground state being monitored by the UV laser, creating a dip in the UV photofragment signal.

The single-conformer infrared spectra of the D-Pro and L-Pro diastereomers of the protonated pentapeptides YADPAA and YAPPA, and their corresponding Gly-containing counterparts, YAPGA and YGPAA, will be described. The spectra show striking sensitivity to the chirality of the Pro residue, and to the additional flexibility imparted to the peptide by the presence of a Gly residue in place of Ala.

In [YA\textsuperscript{L}PGA+H]\textsuperscript{+}, conformers arising from two unique conformational families are observed. We use this prototypical circumstance to demonstrate the technique of IR-induced population transfer spectroscopy, a method first introduced on neutrals\textsuperscript{3} and recently implemented by Rizzo and co-workers\textsuperscript{4} on ions. By introducing a delay between IR excitation and UV detection, the IR-excited ions can isomerize and re-cool, leading to a transfer of population following conformational isomerization. The gains and dips in IR signal can be used to determine the fractional abundances of the ions in the cryocooled ion trap, as demonstrated in Figure 2.

**Figure 1.** Schematic diagram of the apparatus for single-conformation, cryocooled ion spectroscopy.

**Figure 2.** (A) IR Population transfer spectra of the two conformers of protonated YG$^+$PAA. (B) Weighted sum of the population transfer spectra, from which the fractional abundances can be deduced.
Gas Phase Ion Pair Structure of Ionic Liquids Via Vibrational Spectroscopy.

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State-of-the-art on-orbit propulsion systems are based on hydrazine or one of its derivatives reacting with either a liquid oxidizer or a solid catalyst. These systems are mature, reliable, and quite efficient. However, they are also extremely difficult (i.e., expensive) to handle on the ground because of the inherent toxicity of hydrazines. Furthermore, because mission lifetimes are often driven by propellant expenditure and mission cost is driven largely by weight, of which propellant is one contribution, any increase in propulsion efficiency would be a welcome advance in the space community.

Over the last decade, ionic liquids (ILs) have become a subject of interest as a potential hydrazine replacement, in large part because their near-zero vapor pressure leads them to be considered environmentally-friendly as well as suitable for use in space. In general, their unusual properties and nearly infinite variety suggest potential solutions to research problems in a wide number of fields, but these same features also make the search for the ideal IL for any particular application to be a difficult one. Small structural changes can give rise to large, and often counterintuitive, changes in macroscopic properties. The most well-known example is probably 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([emim][Tf2N], structure in Figure 1), in which replacement of the C2 hydrogen with a methyl group triples the viscosity[1]. Indeed, [emim][Tf2N] has become perhaps the most studied and debated ionic liquid, exhibiting an unusual infrared spectrum that has proven exceedingly difficult to disentangle. Attempts to characterize the nature of intermolecular bonding in the [emim][Tf2N] liquid – in particular the existence of any inter-ion hydrogen bonding – were confounded by a complex spectrum that reflected the possibility of multiple conformations and Fermi resonance, as well as nonintuitive responses to deuteration.[2] In order to develop ILs as space propellants, or indeed for any other application, the community must build a detailed understanding of structure-property relationships.

Our entry in this field, and the focus of this talk, is the jet-cooled spectroscopy of the gas-phase ion pair of [emim][Tf2N]. We entrain the exceedingly small vapor pressure of heated [emim][Tf2N] into a supersonic expansion of helium, then probe the vibrational spectroscopy of the resultant ion pair using typical ultraviolet-infrared two-laser time-of-flight mass spectrometry techniques. The resulting spectra, though not conformationally resolved, offer insight into the ion pair structure by comparison to very high level (MP2/aug-cc-pVTZ) calculations.[3] Our attempts to achieve a more complete understanding of the spectrum by deuterating the C2 hydrogen were met with some of the same complexities observed in the liquid and in the bare [emim] cation,[4] leading us to reinterpret the original infrared spectrum in terms of a rather wide conformational space of the ion pair.[5] These spectra also highlight the inherent difficulties of attempting to
make structural assignments on the basis of C-H stretches due to the rampant anharmonic coupling to overtones and combination bands.

Figure 1. Stick structure of [emim][Tf2N] showing the numbering of the carbon atoms in the [emim] ring. The C2 position is particularly critical to understanding this system’s microscopic structure and macroscopic properties.

Quantum State-Resolved Studies of Gas/Surface Reaction Dynamics by Vibrational Spectroscopies

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We use vibrational spectroscopies and infrared lasers for quantum state-resolved studies of methane chemisorption and state-to-state scattering from Ni and Pt surfaces [1]. Methane dissociation is the rate limiting step in the steam reforming process used by the chemical industry to convert natural gas into a mixture of H₂ and CO known as synthesis gas. We prepare surface incident methane molecules in specific ro-vibrational quantum states by state-selective infrared laser excitation via rapid adiabatic passage [2] in a molecular beam. The state prepared molecules then collide with a clean single crystal transition metal surface in ultrahigh vacuum and both reactive and non-reactive processes are monitored by infrared spectroscopic techniques.

Surface bound methyl species as products of the dissociative chemisorption of methane are detected on the platinum surface by Reflection Absorption Infrared Spectroscopy (RAIRS). RAIRS allows for real-time and in-situ monitoring of the uptake of chemisorbed methyl species enabling quantum state-resolved measurements of reactive sticking coefficients. RAIRS is also used to study the vibrationally bond selective dissociation [3] of partially deuterated methanes demonstrating that a single quantum of C-H stretch excitation of the incident methane is sufficient to achieve bond-selective chemisorption (Figure 1). Furthermore, RAIRS allows for site specific detection of reaction products used to measure separately the dissociation probability of methane on steps and terraces sites on Pt(211).

Non-reactive, inelastic energy transfer is probed by combining infrared laser tagging of scattered molecules with bolometric detection (Figure 2). These first methane state-to-state scattering experiments yield state-resolved information about rotation and vibrational energy transfer between the incident molecule and the solid surface.

Our state-resolved experiments provide clear evidence for mode- and bond-specificity as well as steric effects in chemisorption reactions and show that methane dissociation cannot be described by statistical rate theory but requires a dynamical treatment including all internal vibrational and rotational degrees of freedom of the dissociating molecule. The detailed reactivity and state-to-state scattering data from our measurements are used as stringent tests in the development of a predictive understanding by first principles theory [3-5] of these industrially important gas/surface reactions.
Figure 1. Bond selective chemisorption of CH\textsubscript{3}D on Pt(111) detected by RAIRS. Top: Without state-specific laser preparation, both C-H and C-D cleavage occurs with statistical branching ratio. Middle: Excitation of the incident CH\textsubscript{3}D to the antisymmetric C-H stretch mode \(\nu_4\) make the dissociation completely bond selective.

Figure 2. Quantum-state-resolved detection of CH\textsubscript{4} scattered from a Ni(111) surface using infrared laser lagging in combination with a cryogenic bolometer.

References
Transient Infrared Spectra of Reaction Intermediates Detected with a Step-Scan Fourier-Transform Spectrometer in Reactions of Criegee Intermediate CH$_2$OO with HCOOH and SO$_2$

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Criegee intermediates were proposed to be produced in ozonolysis reactions of alkenes, which are responsible for the non-photolytic production of OH, formation of H$_2$SO$_4$, organic acids, and aerosols in the atmosphere. These intermediates were detected directly in the gas phase only recently when a new reaction scheme using UV photolysis of CH$_2$I$_2$ in O$_2$ to generate the simplest Criegee intermediate CH$_2$OO was employed [1]. The direct methods of production/detection have stimulated active research on Criegee intermediates; our understanding of related important reactions in the atmosphere is becoming clarified [2]. The reaction of CH$_2$OO with water was reported to be the most important channel for the loss of CH$_2$OO in the atmosphere. Two additional reactions CH$_2$OO + SO$_2$ and CH$_2$OO + HCOOH were reported to be important because of their large rate coefficients, $(3.4-4.1)\times10^{-11}$ [1] and $(1.1\pm0.1)\times10^{-10}$ cm$^3$ molecule$^{-1}$ s$^{-1}$ [3], respectively. Even though these reactions are rapid in the atmosphere, their impacts to atmospheric chemistry depend on the nature of the products. However, the intermediates and products of these reactions were little investigated in laboratories; only theoretical studies have been reported [4–6].

We employed a step-scan Fourier-transform spectrometer coupled with a multipass absorption cell to monitor time-resolved IR absorption of transient species produced upon UV irradiation of a flowing mixture. The reaction intermediates were identified according to quantum-chemically predicted reaction schemes, vibrational wavenumbers, IR intensities, and simulated rotational contours.

Upon 308-nm irradiation of a flowing mixture of CH$_2$I$_2$/O$_2$/N$_2$/SO$_2$, four bands of CH$_2$OO were observed, as reported previously [7]. At an initial reaction period, five new bands appeared at 1350, 1220, 1100, 940, and 880 cm$^{-1}$. They are tentatively assigned to a cyclic adduct 1,3,2-dioxathietane-2,2-dioxide (Fig. 1A) rather than an exo heteroozonide ring adduct (Fig. 1B). The band observed at 1391.5 cm$^{-1}$ increased in a later period and is assigned to the degenerate $\nu_3$ stretching mode of SO$_3$, which is the major product of the reaction CH$_2$OO + SO$_2$. The rotational contour of this band agrees satisfactorily with that simulated according to rotational parameters of SO$_3$ predicted with the B3LYP/aug-cc-pVTZ method (Fig. 1C). We did not observe the products HCOOH (+ SO$_2$) from the minor channel; an upper limit of 5% for the branching ratio of this channel was estimated.

Upon irradiation of a flowing mixture of CH$_2$I$_2$/O$_2$/N$_2$/HCOOH at 308 nm, six new bands appeared at 887, 925, 1025, 1115, 1169.5, 1341.5, and 1760 cm$^{-1}$. They are assigned to the P5 conformer of the adduct hydroperoxymethyl formate (HPMF), but some contribution
from the P6 conformer cannot be excluded. The observed vibrational wavenumbers and IR intensities agree with those predicted with the B3LYP/aug-cc-pVTZ method, with anharmonic vibrational wavenumbers 860, 901, 1060, 1157, 1346, and 1751 cm\(^{-1}\). The previously reported product formic acid anhydride was unobserved; it might be due to heterogeneous reactions. The rate coefficient for formation of HPMF was determined to be 6.4\(\times\)10\(^{-11}\) cm\(^3\) molecule\(^{-1}\) s\(^{-1}\), slightly smaller than the previous report of (1.1\(\pm\)0.1)\(\times\)10\(^{-10}\) cm\(^3\) molecule\(^{-1}\) s\(^{-1}\) determined from the decay of CH\(_2\)OO [5].

![Figure 1](image)

**Figure 1.** Transient adducts (A) 1,3,2-dioxathietane-2,2-dioxide and (B) exo heteroozonide ring of the reaction CH\(_2\)OO + SO\(_2\). (C) Spectra of the end-product SO\(_3\). Upper trace (black circles) is experimental data and lower trace is the comparison with simulation (red). (D) Comparison of the literature (top) and experimental (middle) spectra of HPMF with that simulated for conformer P5 (bottom).

Methyl mercaptan, CH$_3$SH, is an important sulphur-bearing molecule of atmospheric and astrophysical interest [1]. As with its CH$_3$OH relative, internal rotation introduces substantial complexity into the vibrational spectra in the form of torsional splittings. However, in contrast to methanol, there has been little previous high-resolution study of CH$_3$SH and the torsional patterns of its vibrational modes have been largely unexplored, apart from the C-S stretch [2]. In the present work, Fourier transform spectra of the other three lower infrared vibrational bands of CH$_3$SH, shown in Fig. 1, have been investigated at 0.001 cm$^{-1}$ resolution employing synchrotron radiation at the Canadian Light Source.

The relative band strengths and structures are remarkably different from those of CH$_3$OH, with the CSH bend being very weak and both the in-plane and out-of-plane CH$_3$ rocks being strong with intensities comparable to the C-S stretch. The CSH bend has parallel $a$-type character with no detectable $b$-type component. The in-plane CH$_3$ rock is of mixed $a/b$ character, whereas the out-of-plane rock is a purely $c$-type perpendicular band. The band origin regions have rich torsion-rotation Q sub-branch structure, as shown in Fig. 2.

Figure 1. FTIR spectrum of the four lower vibrational bands of methyl mercaptan.

Figure 2. $Q$ branches in the origin regions of the in- and out-of-plane CH$_3$-rocking bands.
To establish the torsion-rotation labeling for the observed transitions, we used Loomis-Wood plots to identify series of related lines, Excel spreadsheet difference tables to extrapolate to higher \( J \) rotational quantum number, and ground-state combination difference (GSCD) checks implemented in the spreadsheets to confirm our proposed assignments. Our sub-band assignments extend up to about \( K = 10 \) for all the modes and are well-determined from the GSCDs, particularly for the \( \text{a/b} \) in-plane rock for which \( \Delta K = 0, +1 \) and \( -1 \) lines are all observed. We then obtained upper-state energy term values by adding ground-state energies to the observed line wavenumbers, fitted these to \( J(J + 1) \) power-series expansions to obtain substate \( J = 0 \) origins, and subtracted the \( K \)-rotational energy to obtain torsional substate origins, illustrated in Fig. 3. For the CSH bend, the origins follow the normal oscillatory torsional pattern as a function of \( K \) with a fitted amplitude of \( 0.362 \text{ cm}^{-1} \), as compared to \( 0.653 \text{ cm}^{-1} \) for the ground state and \( 0.801 \text{ cm}^{-1} \) for the C-S stretching mode [2]. The torsional energy curves for the out-of-plane rock are also well-behaved but are inverted, with an amplitude of \( 1.33 \text{ cm}^{-1} \). In contrast, the origins for the in-plane rock do not display a clear oscillatory structure but are scattered over a range of about \( 2 \text{ cm}^{-1} \), with indications of some significant perturbations.

![Figure 3. \( K \)-reduced torsional substate origins (in cm\(^{-1}\)) for the CSH-bending and out-of-plane CH\(_3\)-rocking modes of CH\(_3\)SH. Torsional symmetry labels are circles for \( A \) species, squares for \( E_1 \) and triangles for \( E_2 \). The lines represent Fourier fits to the origins.](image)

The amplitudes of the torsional curves are sensitive to the ratio of the torsional barrier height, \( V_3 \), to the reduced torsional \( a \)-inertial constant \( F \). If we assume \( F \) to be constant for all modes, the observed amplitudes from our curves would correspond to \( V_3 \) values of 443.93, 419.95 and 515.45 cm\(^{-1}\) for the ground, C-S stretch and CSH bend states, respectively, showing a significant variation. The out-of-plane rock amplitude would give a nominal \( V_3 \) of 363.82 cm\(^{-1}\) but the significance of this value is not entirely clear for a state with inverted curves. The torsional splittings also affect the apparent vibrational frequencies of the modes. Here, we find values of 801.5503, 1074.0 and 957.0436 cm\(^{-1}\) for the CSH bend and in-plane and out-of-plane rocks, respectively.


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Parallel Session 21

Vibrational Imaging 3
Raman microscopy for molecular imaging of biological samples

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Raman spectroscopy has been utilized as a powerful technique for material analysis in various research fields. However, the technique has not been applied in biological research due to the requirement of a long measurement time, and the analytical capability of Raman spectroscopy has not been fully utilized for biological or medical studies. To improve the image acquisition speed, we introduced the line illumination technique in laser scanning Raman microscopy [1, 2]. Sample illumination by a line-shaped focus allows us to detect Raman spectra in parallel from different points in the sample, which significantly decreases the time to measure a two-dimensional or three-dimensional distribution of Raman spectra in the sample. The line illumination can also be combined with the structured illumination technique to improve the spatial resolution [3].

Fig. 1A shows the Raman images of a living HeLa cell obtained by the Raman microscope developed. The Raman images were reconstructed by the intensities of Raman scattering detected at 746, 1685, and 2855 cm\(^{-1}\), which are assigned to the vibrational modes in cytochrome c seen mainly in mitochondria, phenylalanine included commonly in various proteins, and lipid molecules (CH\(_2\) stretching), respectively. The intracellular structures, such as mitochondria and lipid droplets, and the cell body were clearly observed. The image acquisition time was about 15 minutes for 400 x 120 pixels with the exposure time of 5 s/line. By optimizing the exposure condition, we can also observe relatively slow biological events, such as cell division and the cytochrome c release during apoptosis (Fig. 1B) [4]. We have also applied the developed technique to visualize the change in the composition of intracellular molecules during cell differentiation [5], osteoblast mineralization [6], and T-cell activation [7]. The technique has also been combined with anti-Stokes fluorescence imaging in order to bridge the fluorescence-based and Raman-based biological studies [8].

We also proposed a Raman imaging technique for imaging small molecules. Fluorescence imaging of small molecules has been difficult since the size of fluorescence molecules are typically larger than the target molecules and fluorescence labeling changes the chemical property of the target. We used alkyne as a tag for small molecules and detect the tag by Raman microscopy. An alkyne is a triple bond between two carbon atoms and can be detected by Raman scattering separately from endogenous molecules since alkyne shows a Raman peak at the wavenumber region where endogenous molecules do not provide Raman peaks. We demonstrated the alkyne-tagged Raman imaging in observations of deoxyuridine (Fig. 1C) [9], semi ubiquinone in a living cell [10], and sphingomyelin in an artificial lipid monolayer [11].
Figure 1. (A) high-resolution Raman imaging of a living HeLa cell. (B) time-lapse Raman observation of an apoptotic HeLa cell. (C) Raman-tag imaging of EdU in a living HeLa cell.

Chemical Nano-Imaging with Tip-Enhanced Vibrational Spectroscopy

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Many organic materials in electronic biological systems gain functionality through molecular coupling and heterogeneous morphologies at functional interfaces, with multi-length scale structural order and multi-timescale dynamical interactions. To gain the desired nanometer spatial resolution with simultaneous spectroscopic specificity we combine scanning probe microscopy with vibrational spectroscopies using both tip-enhanced Raman (TERS) and IR scattering scanning near-field optical microscopy (IR s-SNOM). We use TERS and IR s-SNOM to probe at the homogeneous sample size limit by virtue of the nanometer spatial near-field localization. Together with the associated enhanced near-field light-matter interaction provides domain-level structural information and even single-molecule sensitivity. We present several recent advances in near-field microscopy applied to nanoscale chemical identification, imaging the local environment, and observations of dynamical fluctuations at the single molecule limit [1-5].

Figure 1. Tip-enhanced/tip-scattering vibrational Raman and IR nano-spectroscopy and imaging: a) schematic showing tip scattered light with measured vibrational spectrum and nanoscale image [1]. b) SINS and IR s-SNOM measure crystallinity and image orientational defects in a thin film molecular material [2-3]. c) TERS time series spectra measure intramolecular vibrational redistribution and single molecule spectra diffusion [5].

IR s-SNOM has become a powerful tool for nanoscale chemical identification of organic materials through a combination of single-wavelength imaging and broadband nanoscale spectroscopy (Fig. 1A). [2]. We show an extension of infrared nano-spectroscopy to investigate the structure-function relationship of materials beyond basic chemical identity, using the structural sensitivity of vibrational modes combined with nanoscale spatial resolution. The sensitivity to bond orientation and packing gives access to measures of disorder, structural orientation, and polymorphism. From the symmetry-selective probing of vibrational normal modes we measure nanoscale maps of crystallographic structure in molecular materials through characteristic normal modes using IR s-SNOM. We observe nanoscale defects through spatial variation in the molecular orientation of otherwise morphologically well-ordered thin films [3].

Through vibrational solvatochromism, IR s-SNOM gives insight into coupled electronic structure, local electric fields, and charge transfer. Vibrational line shapes change with
modification to their local chemical environment. Specific marker resonances serve as sensitive probes of highly heterogeneous and coupled molecular systems. Using a combination of multispectral s-SNOM imaging with computational image analysis, we map spatial inhomogeneity in long chain block copolymer heterostructures that form disordered quasi-lamellar structures, with significant mixing across the interface. Solvatochromism of the carbonyl mode measures heterogeneity in the local environment within and between domains map on the natural length scales of disorder [4].

Disorder and multi-timescale fluctuations continue to play a role in material properties down to the single molecule limit due to intra-molecular and system-bath coupling. TERS has enabled identification of single molecules through their vibrational fingerprints, yet a time-averaged or spatially averaged picture of even single molecules blurs molecular motions or vibrational dynamics [5]. We use high spectral resolution TERS at variable and cryogenic temperatures in order to quantify ultrafast vibrational dephasing through temperature dependent broadening of intrinsic linewidths. At cryogenic temperatures, individual hopping events slow to the timescale of seconds, and we observe uncorrelated jumps and spectral diffusion in time series spectra as shown in Fig. 1C. Through statistical correlation analysis of fluctuations of individual modes, can determine instantaneous changes in molecular orientation as well as heterogeneity due to a changing local environment. These results expand TERS from a tool for molecular fingerprinting to a powerful probe of single molecule dynamical processes [5].

Beyond these specific examples of determining composition, structure, and coupling in molecular systems, I will demonstrate the general applicability of vibrational nanospectroscopy and -imaging for the study of heterogeneity in a wide range of molecular and bio systems as well as plasmonic/photonic materials. I will discuss the ongoing extension into the time domain for ultrafast nano-imaging as well as developments for probing under in-situ and environmental conditions. I will conclude with a broader perspective of the range of novel near-field nano-imaging modalities, complementing other advanced vibrational spectroscopies.

References:
CARS spectroscopic imaging of living cell and tissues using a supercontinuum light source

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In vivo and in situ visualization of biological tissue without staining and molecular labeling are of considerable importance in biological and medical science. Optical coherence tomography and confocal microscopy are widely used in ophthalmologic diagnosis for in situ imaging. Both techniques are capable of capturing micrometer-resolution, three-dimensional images from optical scattering media such as tissue. However, they provide limited image contrast and poor molecular specificity due to their contrast mechanisms, which rely on spatial variations of refractive indices. Recently, multiphoton microscopy (MPM) has attracted much attention for unique imaging capability with molecular specificity. MPM is based on the nonlinear interactions between molecules and photons. With the use of the white-light laser source, we can extend MPM to spectroscopic MPM. In the present study, development of spectroscopic MPM and its application to tissue imaging are described.

Figure 1 shows the experimental setup[1]. The master laser source for our home-build spectroscopic MPM system is a sub-nanosecond cw Q-switched Nd:YAG laser. The fundamental 1064-nm laser beam is firstly divided into two. One is used as an $\omega_1$ laser beam, and the other is introduced into a photonic crystal fiber. This fiber converts the 1064-nm-centered narrow spectral line to white-light supercontinuum (SC). The broadband SC is used as the $\omega_2$ beam. Two beams are superimposed and introduced into a microscope. The sample is placed upon a piezo electric stage for raster scanning. The multimodal signals are guided into two spectrometers, and detected by two CCD cameras.

Figure 2 summarizes the results of label-free spectroscopic imaging of rat retina (frozen section) using our home-build spectroscopic MPM[1]. Figure 2a shows a H&E stained image, which was obtained from the same sample for label-free

*Figure 1. Experimental setup for spectroscopic MPM.
imaging but a different slice. Figure 2b shows spectral profiles of the signal in UV-visible region, where second harmonic generation (SHG) (upper) and third harmonic generation (THG) (lower) are detected. Figures 2c and 1d show the typical spectra and images of coherent anti-Stokes Raman scattering (CARS). The positions x and y correspond to the photoreceptor layer and the outer nuclear layer, where the signal due to lipid and DNA is mainly detected. Based on the multiple nonlinear optical processes, we can reconstruct label-free molecular spectroscopic images, which clearly captures the layered structure of retina. In particular, we have succeeded in detecting rootlets of connecting cilia in the retinal photoreceptor, which was observed at the center row of the SHG image (d)[2].

**Figure 2.** Label-free imaging of retina. H&E stained image(a); spectral profiles of the signal in UV-visible(b), and near-infrared(c); label-free images of SHG, THG, and CARS(d)

Hyperspectral imaging based on surface enhanced hyper-Raman spectroscopy

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Combination of surface enhanced Raman (SERS) and surface enhanced hyper-Raman scattering (SEHRS) could be a promising tool in micro-spectroscopic applications, because it allows to collect complementary chemical and structural information in the vicinity of plasmonic nanoparticles with extremely high electromagnetic enhancement \cite{1,2}. Depending on the molecular symmetry, SEHRS may probe IR active modes or additional “silent” modes, which are seen neither in Raman nor in IR spectra. In addition, the inherently low multi-photon cross sections in SEHRS can easily be overcome in local optical fields of metal nanostructures by exploiting the nonlinear scaling of the signal with the intensity of the excitation field \cite{1}. As a result, the cross sections can be enhanced to be up to $10^{-46}$ cm$^4$ s \cite{1}. Furthermore, two-photon microscopy can offer advantages due to the lower photon energy excitation, deeper penetration and better spatial resolution, while the detection stays in visible spectral range where the cost-effective Si-based detectors work. Because, technically, SERS and SEHRS can be detected within one micro-spectroscopic setup, it is obvious to combine these two complementary methods to map the spatial distribution of spectrally almost identical molecules. Applying multivariate signal processing techniques (e.g., principal component analysis, PCA) for one- and two-photon excited SERS spectra, the sensitivity in the analysis of distributions of structurally very similar analyte molecules can be substantially increased \cite{3}.

Here, we investigated the SEHRS and SERS spectra and the spatial distributions of dye molecules, crystal violet (CV) and malachite green (MG), on immobilized plasmonic surfaces (illustrated on Fig 1.). The first sample type consisted of two connected regions a CV and CV-MG-mixture, while the second sample type an MG and CV-MG–mixture. The quasi-simultaneously obtained one- and two photon excited SERS spectra were recorded by scanning many small, microscopic areas in regions containing both, the pure respective dye and a mixture of both dyes, as well as at the distinct non-border regions on both sides on a macroscopic (millimeter) scaled slide. The chemical structures of the two dye molecules only differ in one dimethylamino group, hence their SEHRS and SERS spectra show high similarity. To exploit each small spectral variation between the spectra of the studied dyes and their mixtures, we successfully employ PCA to map the distribution of the molecules. The presented hyperspectral mapping technique constitutes a novel method for multiplex imaging of complex biological systems. As the first real demonstration of this SEHRS and SERS based hyperspectral imaging, we demonstrate
that the SEHRS fingerprints in mapping mode can be also collected from live macrophage cells after incubation with nanoparticles tagged with different molecules. Application of combined SEHRS and SERS based hyperspectral imaging in live cells can be utilized e.g., in microenvironmental studies or the investigation of metabolic states.

**Figure 1.** Illustration of the combined surface enhanced Raman and hyper-Raman hyperspectral imaging to map the distribution of dye molecules. After excitation at 532 and 1064 nm, respectively, we obtained the SERS and SEHRS fingerprint of structurally almost identical analyze molecules and identified their spatial distribution using PCA.

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Concentration Imaging of Acid and Alkaline Solutions in Microfluidic Channel

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Micro-chemical analyses/syntheses using microfluidic chips have been intensively studied because of their great practical advantages: efficient chemical reaction, mixing control, and reduction of reagent amount. It is important and necessary for the analyses/syntheses to measure and control the concentrations of target solute molecules/ions in liquid solutions flowing in microfluidic channels. However, conventional techniques using fluorescent dyes cannot measure the concentrations of multiple solutes simultaneously. In this study, we thus used a near-infrared (NIR) absorption imaging method [1, 2] to visualize and measure the concentrations of aqueous solutions of acid and alkali. This method is based on the characteristics of the ν₁ + ν₃ absorption band of water at around a wavelength of 1440 nm. No fluorescent dye is thereby needed.

The imaging system consisted of a halogen lamp, filter wheel with three narrow bandpass filters, InGaAs camera, and optics [1, 2]. The wavelengths of the narrow bandpass filters were chosen by analyzing variations in the ν₁ + ν₃ absorption band against variations in the concentrations of HCl, NaOH, and NaCl. Then a linear regression model for converting the absorbances into the concentrations was constructed.

Figure 1 shows the images of concentrations of HCl, NaOH, and NaCl when the aqueous solutions of HCl and NaOH flowed through a Y-shaped glass fluidic channel with a width of 6.2 mm and a depth of 0.2 mm. In Fig. 1 (a) and (b), the two solutions of HCl and NaOH are clearly distinguished and their interface where the diffusion and reaction occurred is observed. In Fig. 1 (c), the generation of NaCl by the neutralization reaction is seen at the interface along the flow direction. The differences between the three images indicate the validity of the method of this study. Figure 1 (d) shows the transverse profiles of the concentrations of HCl, NaOH, and NaCl. The concentration profiles are stoichiometrically consistent with each other.
Figure 1. Concentration images of (a) HCl, (b) NaOH, and (c) NaCl at the merging region of Y-channel when the aqueous solutions of HCl (2 mol/L) and NaOH (2 mol/L) flowed and merged. (d) Transverse profiles of the concentrations along the C-D lines indicated in the images.

Tuesday June 13 2017

Parallel Session 22

Energy, Catalysis, & Molecular Electronics
Raman Spectroscopy of Buried Nanometric Films in Molecular Electronic Devices

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Electrochemical deposition of aromatic organic molecules by reduction of diazonium reagents enables formation of molecular layers with sufficient integrity for use in molecular electronic junctions of interest to microelectronics. Raman spectroscopy permits structural characterization of 1-20 nm thick organic layers during device fabrication and in-situ during operation in electronic circuits\(^1,2\). In some cases, successive deposition of silver islands and nanoparticles provides electromagnetic field enhancement, with the resulting SERS effect permitting observation of a broader range of molecular structures. Raman monitoring of both single component and multilayer structures will be described, with the general devices structure shown in figure 1.

Figure 1. Carbon-based molecular junction containing fluorene oligomers between conducting carbon contacts. Top contact may be transparent to permit spectroscopic characterization by Raman and UV-vis absorption.

Electronic phenomena in the completed devices include rectification\(^2\), photocurrents\(^3\), and light emission\(^4\). Raman of intact, operating devices has established the integrity of the molecular layer and also structural changes during device operation\(^5\). An example of a rectifying organic bilayer containing anthraquinone (AQ) and nitroazobenzene (NAB) is shown in figure 2, following successive deposition of AQ and NAB on a carbon film containing Ag nanoparticles.
Figure 2. SERS spectra of a two-component layer of AQ/NAB (A), and one-component layers of NAB (B) and AQ (C). D. is a difference spectrum calculated by subtracting spectrum (B) from spectrum (A), i.e. \((I_{AQ/NAB}) - (I_{NAB})\). All spectra are averages of 100 accumulations with 1 second CCD exposures using a laser point focus (4.33 mW at the sample).

Generation of Electricity at Graphene Interface Governed by Underlying Surface
Dipole-Induced Ion Adsorption

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Aqueous droplet moving along graphene surface can produce electricity. This interesting
phenomenon provided environment-friendly means to harvest energy from graphene
interface in contact with sea wave or rain droplets. However, microscopically, the nature
of charge adsorption at the graphene interface is still unclear. Here, utilizing sum-
frequency spectroscopy in combined with measurement of electrical power generation,
the origin of charge adsorption on graphene was investigated. It was found that the direct
ion-graphene interaction is negligibly small, contrary to the early speculation, but the
ordered surface dipole from the supporting substrate, such as PET, is responsible for ion
adsorption at the interface. Graphene serves as a conductive layer with mild screening of
Coulomb interaction when aqueous droplet slips over the surface. These results pave the
way for optimization of energy harvesting efficiency of graphene-based device.
Vibrational Sum Frequency Generation Spectroscopy of Oligothiophene Thin Films at Early Stages of Film Growth

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Among organic electronic materials, conjugated oligothiophenes offer a unique balance of processability and performance that has made them the subject of intense study for several decades [1, 2]. Polycrystalline films of α-sexithiophene (6T) can be vapor deposited as thin film samples without degradation, producing samples that are homogeneous and have high charge carrier mobilities. Of particular relevance to the current study, it has also been shown that the 6T structure varies significantly with evaporation rate, substrate type, and temperature. In this study we use the surface specificity of vibrational sum frequency generation spectroscopy (VSFG) to monitor 6T molecular structure during the early stages of film growth. The spectroscopic data are combined with topographical information via atomic force microscopy (AFM) to obtain a more complete picture of 6T order and orientation as it grows on glass substrates.

Figure 1. AFM images of A) 5 nm, B) 20 nm, and C) 50 nm 6T thin films on glass demonstrating the Stranski-Krastanov growth mechanism.

The topographies of 6T films change in peculiar ways during the early stages of deposition. AFM images of 5, 20, and 50 nm show that the outer surfaces change from having small (hundreds of nm) to large (microns) grains. The images suggest a Stranski-Krastanov (SK) growth mechanism where the first few layers of the material are deposited in a layer-by-layer fashion but later change to simultaneous multilayer growth (formation of 3D grains) [3]. This has been previously shown to be the case for oligothiophene films grown at room temperature [4]. The accompanying VSFG spectra for these films show amplitude in the C=C symmetric (M) and asymmetric (L) stretching modes at 1460 cm⁻¹ and 1490 cm⁻¹, respectively. The transition dipoles of these vibrations lie along nearly orthogonal molecular axes [5], as shown in the overlaid structure in Figure 2. The ssp and sps VSFG spectra in Figure 2 qualitatively show that 6T at the material interfaces orients with its long axis at an angle giving it some amplitude for both in- and out-of-plane polarization combinations.
Figure 2. VSFG spectra collected from 5, 20, and 50 nm thin films of 6T in the A) ssp and B) sps polarization combinations. In both frames the red, blue, and maroon data points are the 5, 20, and 50 nm thicknesses, respectively. Top inset shows the molecular structure of 6T and the assigned symmetric (M) and asymmetric (L) modes with their relative orientation on the molecular coordinates.

A closer examination of these changes was then achieved by preparing a 6T sample with a thickness gradient so that a range of thicknesses could be studied on a single sample. The VSFG spectra showed a gradual change over a relatively large range of sample thicknesses. The data at these thicknesses could be fit globally using identical second-order susceptibilities, implying that the spectral differences were due solely to changes in film thickness rather than molecular structure. On the other hand, at sub-monolayer coverages, the VSFG spectra changed sharply, showing that the structure at these thicknesses was altered from the multilayer range.

Measuring Chemical Composition and Optical Properties at the Nanoscale with AFM Probes: Application Photovoltaics.

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Measuring optical and chemical properties at the nanoscale is important for engineering materials in photovoltaics to sensing and other applications. Photo Thermal Induced Resonance (PTIR)\textsuperscript{1,2} is a novel, technique that employs an AFM tip as a local detector to locally transduce the thermal expansion of the sample induced by light absorption into large cantilever oscillations (Fig 1). By leveraging lasers tunable from 500 nm to 16000 nm our PTIR setup\textsuperscript{3,4} yields absorption spectra (electronic or vibrational) and maps with a wavelength-independent resolution as high as 20 nm, extending PTIR to the visible range for the first time.

![Diagram of PTIR measurement](image)

**Figure 1.** (A) Illustration of the PTIR measurement: if the sample absorbs the laser pulses (blue discs in purple cones) it rapidly expands deflecting the AFM cantilever which is monitored by a four-quadrant detector. (B) The maximum peak to peak deflection during the cantilever ring down is proportional to the absorbed energy.

In the first part of the talk I will discuss the PTIR working principles. Later I will present some of our recent work on organic-inorganic perovskite solar cells. Perovskites attract interest in photovoltaic application because they combine the high efficiency
typical of inorganic semiconductors with low material cost and ease of fabrication. However, knowledge of how the local material properties, such as the chemical composition, the bandgap and the defect density are related to perovskite devices operation is still incomplete. By proving access to the local chemical composition and bandgap PTIR provides unique information to characterize and engineer these materials.

I will show that PTIR can be used for:

1) Map the distribution of the constituent ions in perovskite lateral solar cells and provide the first direct evidence of ion electromigration in these devices.5
2) Map the distribution of chloride ions in situ in mixed Cl-I perovskites as a function of annealing6
3) Study material decomposition due to thermal degradation in situ for different material compositions6,7
4) Demonstrate that organic-inorganic perovskite crystals are ferroelastic.8

(8) Strelcov, E.; Dong, Q.; Li, T.; Chae, J.; Shao, Y.; Deng, Y.; Gruverman, A.; Huang, J.; Centrone, A. Science Advances, accepted.
Nowadays, the main interest of electrochemical research is the characterization of fuel cells and their chemical reactions in a very detailed way. Recent developments during the last years allow the combination of electrochemical measurements with high sensitive spectroscopic methods, e.g. Raman spectroscopy. We investigated the oxygen reduction reaction (ORR) focusing on the immobilization method for an efficient and valid setup. The development of the performance of artificial ORR catalysts aims to achieve an energy-efficiency that resembles natural biocatalysts. The most promising candidates are synthesized biomimetic catalysts inspired by nature. The presented Hangman (Figure 1, B) was synthesized to investigate the influence of a second coordination sphere (the hanging group) on a catalytic reaction at the porphyrin [1]. The catalytic activity of this molecule was shown already in solution [2]. In our studies, this molecule was immobilized on the surface of a rotating disc electrode (RDE) to investigate its catalytic behaviour with spectro-electrochemical methods [3]. The main interest was to discover if the immobilization method itself influences the activity of porphyrin complexes on surfaces. For that purpose, the Hangman complex was immobilized on surfaces using four different approaches. Three drycast immobilization methods were established with different thicknesses of catalyst on the surface (Figure 1, C). The fourth method was the incubation cast (coordination of the catalyst as a monolayer) that leads to a rather fragile system, however it allows unbiased predictions of the catalytic activity. All four methods show different redox behaviours correlating linearly to the amount of catalyst and consequently to the number of multilayers on the surface. Although the monolayer seems to suit best to characterize the unbiased catalyst properties, it is not feasible owing to a high signal loss induced by laser light applied during the observation of the turnover processes by Raman spectroscopy. Also, the electrochemical measurement did not result in a valid voltammogram due to insufficient current detection. It turns out that a certain amount of catalyst has to be immobilized on the surface to obtain a significant signal keeping the drawbacks of a multilayer formation in mind. The characterization of these multilayer systems show that different species are formed which are typical for iron based heme systems. The Hangman molecule forms a µ-oxo-dimer (reduced species positioned at 1347 cm⁻¹, Figure 1, A) although either the large mesityl groups located at the porphyrin should prevent this kind of dimer formation. The dimer species is directly influenced by the immobilization method and thereby its probability increases with the number of multilayers.
Figure 1. Resonance Raman Spectra of drycast methods: Hangman (red, blue, black) and dimer reference spectra (brown, pink); measurements were done in air saturated pH 7 phosphate buffer (A), structure of the Hangman porphyrin (B), schematic illustration of all four drycast methods (C).

Tuesday June 13 2017

Parallel Session 23

Aqueous Surfaces 3
Molecular Insight into the Adsorption of Surfactants at Solid/Liquid Interfaces as revealed by Linear and Non-Linear Vibrational Spectroscopy Techniques

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The adsorption of surfactants onto hydrophilic surfaces from aqueous solutions has been studied intensively for more than half a century. Different models for surfactant adsorption have been proposed as experimental techniques reveal new additional information regarding their adsorption process. [1,2] Here we make use of Vibrational Sum Frequency Spectroscopy (VSFS) and Total Internal Reflection Raman (TIR-Raman) spectroscopy to specifically study the adsorption of ammonium perfluorononanoate (APFN) at the buried calcium fluoride/solution interface. These complementary techniques allow determining the adsorbed amount and provide molecular structural information such as average tilt and orientation, ion binding, and the presence of asymmetric aggregates of both surfactant and water molecules located in the interfacial region. [3]

Figure 1. Sketch of the (A) TIR Raman and (B) VSFS experimental setup. (C) Molecular structure of the fluorosurfactant APFN.

Targeting the vibrations associated to the surfactant's carboxylate head group and fluorocarbon chain, allowed extracting information regarding molecular structural changes along the adsorption process. Moreover, variations in the surface charge were inferred from the stretching modes of interfacial water molecules. The experimental results indicate that APFN forms an electrostatically bound hydrophobic monolayer with the fluorocarbons chains exposed to water, already at a concentration 20 times below the critical micelle concentration. At that point, the positive surface charge becomes fully
neutralized by the negatively charged surfactant species. Thereupon, APFN adsorption persists due to van der Waals forces, resulting in the formation of a double layer and accumulation of the negative charge on the calcium fluoride surface. The results are further discussed in the context of the adsorption behaviour observed for other surfactants on hydrophilic surfaces.

Figure 2. (A) TIR Raman spectra at different polarization combinations (Px,Py,Sx,Sy) of APFN adsorbed to CaF2 above the critical micellar concentration. (B) VSF spectra collected in the PPP polarization combination at selected APFN concentrations.

Second-Order Vibrational Lineshapes from Charged Interfaces
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The influence of the surface potential on the lineshapes of second-order spectra, which yield microscopic information about interfaces, has remained enigmatic until now. Here, we reveal considerable potential-dependent contributions and demonstrate how to account for them when seeking molecular information from charged interfaces using second-order spectroscopy. This work is based on the following documents:

Monitoring pH-dependent specific ion effects on structural changes of the silica/water electrical double layer using sum frequency generation

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The effect of pH and electrolyte composition can greatly affect the structure of water at the silica/water interface. Many studies have concluded that pH can drastically affect dissolution rates of silica. In addition, it has been found that alkaline and alkaline earth cations dramatically increase the rate of dissolution of silica and quartz, with possible mechanisms of dissolution related to cation hydration and imposed changes in water orientation at the silica/aqueous interface[1, 2]. Several studies have investigated specific cation effects on silica within the context of the Hofmeister series, which is directly related to ion size and hydration. However, there are only a limited number of studies that report on the pH-dependence of SIEs with regards to cation adsorption on silica [3-5]. Furthermore, there is currently no experimental evidence for how the water structure behaves within the electrical double layer (EDL) at the silica/water interface as a result of pH-dependent SIEs. In this work, vibrational sum frequency generation (SFG) was used to selectively probe water order within the silica/aqueous EDL in the presence of different monovalent cations as function of pH.

**Figure 1.** Peak fitting results for ssp SFG water spectra in 0.5 M CsCl and 0.5 M LiCl as a function of pH. A/T for the 3200 cm⁻¹ peak is shown in a) and for the peak at ~3400 cm⁻¹ in b). The color scale indicates corresponding postitive (blue) and negative (red) surface potentials for the OHP (a) and 0-plane (b).

In this work, a direct Hofmeister series of cation adsorption was observed from pH 7-10, where large, weakly hydrated ions (Cs⁺ and K⁺) adsorbed more strongly than small, strongly hydrated ions (Na⁺ and Li⁺), with increasing ion adsorption causing a decrease in SFG intensity. However, above pH 10 an inversion in the Hofmeister trends was observed, with Li⁺ being more strongly adsorbed than Cs⁺. In addition, the two peaks within the water spectrum exhibited different pH-dependent behaviors, providing evidence of two populations of water within the EDL: one near the surface within the
compact layer (3400 cm\(^{-1}\)) and one further from the surface in the diffuse layer (3200 cm\(^{-1}\)) (Figure 1), which as been suggested previously[6, 7]. It was postulated that differences between the pH-dependent trends of these two peaks was due to different orienting forces, with the water contributing to the 3200 cm\(^{-1}\) peak being primarily affected by the outer Helmholtz potential and water in the 3400 cm\(^{-1}\) peak being largely affected by the negative charge at the 0-plane. A minimum in the 3200 cm\(^{-1}\) peak intensity was observed at pH 7-8 (Figure 1). This suggested either overcharging of surface, with the minimum corresponding to an outer Helmholtz potential of zero, or the presence of another interfering water population with an opposite orientation to that in the bulk, possibly related to ion hydration. It is also possible that a combination of both scenarios was occurring. A diagram depicting changes in ion adsorption at different pH ranges, overcharging at the outer Helmholtz plane and the presence of an asymmetric hydration sphere around the cation is presented in Scheme 1.

### Scheme 1

Schematic of a) Cs\(^+\) and b) Li\(^+\) ions at silica/water interface and corresponding alignment of water. Water thought to be within the hydration sphere of the cation is shown in blue.

Evolution of anatase surface active sites probed by in situ sum-frequency phonon spectroscopy

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Low-coordinated lattice sites play key roles in oxide surface reactions; however, it remains challenging to probe such sites in situ under surface reactions, especially outside the ultrahigh vacuum. Using optical sum-frequency generation \cite{1}, we investigated such sites on titanium dioxides (TiO\textsubscript{2}) by probing the stemming surface phonon modes. We identified a highly localized surface phonon mode on the benchmark photo-catalyst surface, anatase (101), associated with surface active sites at low-coordinated titanium ions and conjoint oxygen vacancies. This phonon serves as a spectroscopic signature for direct, in situ monitoring of such sites under reaction conditions. By monitoring the mode evolution in situ under the uv irradiation, we found, in sharp contrast to that in the vacuum, the stability of surface active sites are strongly regulated by ubiquitous environmental molecules such as methanol and water; even weakly associated molecules, like hydrogen and nitrogen, showed an appreciable effect. Our finding signifies not only the urgency of in situ surface characterization under real atmosphere, but also calls for a more comprehensive view on heterogeneous catalysis: instead of considering only a surface with reactants on it, the active role played by the ambient gases must be seriously taken into account \cite{2}. 

![Diagram of surface active sites and molecules](image.png)
**Figure 1.** Sum-frequency spectroscopy reveals the rich interplay between surface active sites and ambient gas molecules.

Molecular Interactions and Hydration States of Ultrathin Functional Films at the Solid–Liquid Interface

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Structure and function of polymer and protein films in their native aqueous environment are driven by molecular interactions. Classical infrared spectroscopy can, in principle, probe those interactions, but quantitative band interpretations are usually hindered by strong solvent absorption. Optical effects such as band-shape distortions, baseline drifts due to thin-film interference, and effective-medium effects from film hydration furthermore exacerbate a straightforward quantification.

We significantly improve the infrared analysis of ultrathin films in aqueous environments by employing in-situ infrared-spectroscopic ellipsometry (IR-SE) combined with rigorous optical modeling [1]. This powerful analytical approach avoids otherwise typical misinterpretations of the aforementioned spectral features and enables the simultaneous quantification of chemical composition, hydration states, structure, and molecular interactions.

We apply IR-SE to study thin films and covalently end-grafted, nanometer-thin brushes of poly(N-isopropylacrylamide). This thermoresponsive polymer is an ideal model system for studying interactions of proteins at solid–liquid interfaces. Quantitative analyses (Figure 1) are based on a dielectric layer model that accounts for film swelling and deswelling, hydration of hydrophilic amide and hydrophobic isopropyl side groups, as well as molecular interactions of the polymer's amide moieties. We thereby quantify the hydration and structure dependence of intra- and intermolecular C=O⋯H–N and C=O⋯H$_2$O hydrogen bonds (Figure 2), elucidating their role in the brush's temperature-induced phase separation.

The presented method is directly applicable to functional and biorelated films like polymer and polypeptide layers, which is of topical interest for interface studies, such as membrane processes and protein unfolding.
Figure 1. (A) Measured and fitted IR-SE spectra of PNIPAAm films in aqueous, dry, and humid ambient. (B) In-situ set-up for probing solid–liquid interfaces. (C) Examples of molecular interactions as identified and fitted via optical modeling of IR-SE data.

Figure 2. Fitted evolution of various interacting C=O species with film hydration.

Tuesday June 13 2017

Parallel Session 24

Chemometrics and Data Analysis
Molecular Interactions and Hydration States of Ultrathin Functional Films at the Solid–Liquid Interface

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New perspectives and methods for acquiring, analyzing and post-processing of 3D Raman imaging data sets.

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The number of papers published per year on Raman imaging and Raman mapping have doubled in the last five years. Much of this growth may be attributed to an increase of the ease of use of confocal Raman microscopes as well as facilitated data evaluation of the obtained Raman images. This in turn made this technique available to a broader range of researchers. Unfortunately the simplification of the control of confocal Raman microscopes often was accompanied by lower performance than physically possible.

This contribution introduces new concepts to facilitate the use of confocal Raman microscopes in terms of general operation, for the measurements on rough surfaces and in terms of data evaluation whilst maintaining highest throughput and resolution.

For the general operation of a confocal Raman microscope there has been for a long time a discrepancy in the performance and the ease of use of the microscopic part when compared to high-end optical microscopes. To overcome this discrepancy a new control concept for the operation of the sample positioning, motorized objective selection, auto-illumination and auto-focusing was developed. This introduces an intuitive tactile interface facilitating the orientation on the sample in order to locate the area of interest and to make full use of the high-end optical microscope included in for example the alpha300 or apyron microscope series.

Rough surfaces can pose a challenge for systems with high confocality. Some of these systems can obtain depth resolutions of <800nm FWHM and thus a change in the sample height of only a few hundred nm will resolute in a dramatic change of the signal intensity. To overcome this, the TrueSurface module was introduced already in 2010 [1]. This technique allows the non-contact recording of the surface topography and the consecutive measurement of a confocal Raman image while tracing the topography measured before. This technology allowed for many measurements which before this introduction would not have been possible [2,3]. Its limitations were however, the two step process, which took some additional measurement time and the fact that with this method changes in a sample topography as a function of time as for example the swelling or shrinking of a sample could not be compensated. For this reason a new generation of the TrueSurface module has been developed allowing the simultaneous closed loop compensation of surface topography while performing confocal Raman microscopy. Fig. 1 shows the result of a confocal Raman image obtained once without using the TrueSurface compensation and once with the TrueSurface module active. The cross sections reveal that the height change in the FWHM of the signal is within the diffraction limited depth resolution underlining the performance of the system. Further examples underlining the performance of this technology will additionally be presented.
For the evaluation of confocal Raman images many outstanding algorithms have been developed in the past such as cluster analysis or non-negative matrix factorization. Whilst they can be used to extract most of the information from the given dataset they lack to some degree intuitiveness and ease of use. To overcome this, the True Component Analysis was developed which guides the user in an intuitive way to the extraction of the information from the data set. It employs an image based evaluation to a least square fit while clearly visualizing the real spectral information contained within the dataset. The power of this data evaluation method will be demonstrated in live data evaluation of measured datasets.

Figure 1. Confocal Raman Measurement on a Si lens. [a]: Raman image without topography correction; [b]: Raman image with topography correction; [c]: Topography; [d]: Cross sections from a-c

Raman spectroscopy coupled with a novel use of MCR-ALS algorithm unravels the metabolic switch of breast cancer cells towards organ-specific metastasis progression

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Raman spectroscopy (RS) is a very promising tool to reveal biochemical changes that occur in cancer processes at cellular level. However, analyzing clinical samples, RS needs improvements to deconvolve from the spectra pure molecular contributions. We compared the strengths of Multivariate Curve Resolution (MCR) [1] versus Principal Component Analysis (PCA) to deconvolve meaningful molecular components from a set of mix spectra. We exploited the capability of MCR algorithm to easily include initial estimates and constrains. We demonstrate the ability of MCR to deconvolve from the RS undesired background signals that can be subtracted obtaining clearer cancer cell spectra. We used two triple negative breast cancer cells, MDA-MB 231 and MDA-MB 435, to illustrate the RS insights that infer into the metabolic changes required to specific metastasis progression. Our results show that increase amino acids and decrease mitochondria signals are attributes of bone metastatic cells, in contrast to lung metastasis tropism that is characterized by high phosphatidylserine and Cytochrome C levels. Therefore, we propose a method based in MCR algorithm to reveal unique biochemical insights in the molecular progression of cancer cells composition using RS.

Figure 1. Original Raman spectra (A) and Raman spectra after the subtraction of quartz and water signals (B) using MCR loadings. More features are visible after background subtraction that will lead to a better future MCR analysis.

We propose a new methodology to extract the useful molecular information encoded in RS of biological samples. By using the flexible MCR-ALS algorithm meaningful molecular components could be extracted, thanks to the previously subtraction of background signals.
that perturbed the inherent cell RS. This methodology opens up new possibilities for the clinical diagnosis use of RS, since it permits to remove signals from substrates or chemicals used in cytological techniques. It also provides a rapid, reliable and label free method to disentangle, in cancer cytology, the biochemical components involved in metastasis progression, as new tool to improve prognosis and early prevention.

Figure 2. A) MCR analysis from cell Raman spectra of MDA-MB-231, B02, MDA-MB-435P and 435B cell lines after the subtraction of Quartz and water signals. Three components were deconvolved. MCR loadings (left) and scores (right) of Component 2 are plotted. Component 2 has bands of aminoacids and is associated with a higher bone tropism. B) MCR analysis of MDA-MB-231 P, B02 (the high bone metastatic cell variant) and B02-PRDX2 that over-expressed PRDX2 and have in vivo low bone metastasis ability [2]. Three components were deconvolved. This component is found to be inversely associated to bone tropism.

We apply this methodology to study the molecular differences between primary breast cancer cell lines and their metastatic variants in bone (Fig. 2). We identified biochemically meaningful molecular components that have a role on the metastatic ability of breast cancer cells. Specifically, the aminoacid component increase whereas cytochrome C and lipids bands decrease in bone metastatic cell variants (B02) with regard to their correspondent parental cell lines (231P and 345P). Then, a ratio of these three components might be suggested to validate this data in clinical samples.


Stand-off hyperspectral imaging – towards mobile, high throughput remote chemical identification and quantification using chemometrics

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Stand-off Raman spectroscopy is a versatile tool for a variety of applications, such as safety investigations, forensics or geosciences. During the project “OPTIX” (FP7) we showed that the detection of small amounts of explosives and hazardous chemicals at distance of 100 m is possible even through opaque containers. Here, we present a different approach to remote Raman sensing employing hyperspectral Raman spectroscopy. The classical grating as a dispersion element is exchanged in favor of a liquid crystal tunable filter (LCTF), which allows for direct imaging of a selected Raman shift onto the intensified CCD (iCCD). This maintains a high degree of local information and allows for higher throughput, as whole areas can be imaged at once. Measurements with our first prototype, still equipped with a water-cooled, flashlamp-pumped excitation laser, as well as first measurements with a diode pumped, air cooled, small laser are shown. The capability of remote chemical analysis incorporating chemometrics on a reference sample consisting of different polymers will be presented.

Stand-off Raman spectroscopy, where the instrumentation is physically separated from the sample under investigation, can be extremely advantageous for analysis of dangerous, fragile or inaccessible samples. It is mostly used in safety applications, i.e. detection of explosives [1] or geosciences, where the remote detection of minerals on planetary surfaces is desirable [2].

Figure 1. (A) Experimental configuration illustrating the laser, the telescope as the collection optic, the Rayleigh filter [F], the LCTF as the dispersive element and the camera. (B) and (C) depict rendered CAD drawings of the experimental setup.
Usually, a pulsed laser point is targeted on the surface of interest, the backscattered photons are collected using different forms of telescopes and directed towards a spectrometer. However, when large surface areas must be scanned, it is advantageous widening the laser spot and using a square detection array to directly image the area under surveillance. Different kinds of filter can be used to discriminate different Raman shifts, i.e. acousto-optical filters [3] or, as in this study, LCTFs [4].

![Figure 2](image)

**Figure 2.** (A) Intensity distribution at 2900 cm\(^{-1}\) (B) Collection of figures comparing spectra obtained by binning 10 pixels on a specific part of the image representing the respective polymer and spectra obtained with a Raman microscope (Horiba LabRAM 800 HR).

The presented prototype can collect Raman spectra from a sample at several meters’ distance (15 m for this study), which are directly loaded into Epina ImageLab [5], a chemometric analysis program for imaging purposes. A test sample consisting of four different polymers was measured and different chemometric methods were applied to increase signal-to-noise ratios and to classify the results. Furthermore, a compact, air-cooled, diode-pumped laser with high repetition rates (~10 kHz) was tested to facilitate a mobile prototype with minimal electrical as well as physical requirements. The results are compared to a stationary, water-cooled and flashlamp-pumped laser.

Optical Spectroscopy for Agricultural Applications in Low-Resource Settings: Pilot Studies on Soil Analysis and Mycotoxin Detection

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Agricultural productivity has a profound impact on quality of life in low-resource countries. Investing in technological innovations and building sustainable markets around inventions is a key pathway to boosting agricultural outputs and alleviating poverty. Timely and reliable information is crucial when it comes to making recommendations for the farmers or evaluating the quality of the crops for market. Meanwhile, the ability to offer analytical services at reasonable costs is important. Unfortunately, conventional laboratory technologies are usually costly and require skilled labor. Optical spectroscopy techniques, including Raman, mid infrared (MIR), and near infrared (NIR), probe molecular structures that are relevant to the chemistry of biological samples like plant, soil, and body tissues. Recent advances in photonic hardware have enabled low-cost, portable spectroscopic devices to become viable options for real-time and onsite chemical analysis for both agriculture and health [1, 2, 3]. In combination with advanced chemometrics and machine learning techniques, optical spectroscopy has demonstrated satisfactory analytical performance, and regulated bodies have approved such techniques for chemical analysis [4, 5]. At Intellectual Ventures Laboratory, we investigated the utility of several low cost Raman, NIR, and MIR devices towards practical applications in developing countries. Specifically, our goal is to come up with low-cost spectroscopic tools that can provide soil nutrient analysis for smallholder farmers, verify fertilizer authenticity, and detect mycotoxin contaminations in harvested grains (See Fig 1).

Figure 1. (A) Examples of existing commercial low cost spectrometers – NIR from Young Green Energy (top) and Raman from Snowy Range (bottom). (B) Raw and (C) preprocessed spectral data of fertilizers from the two devices in (A).
A common pitfall when dealing with real-world spectroscopic applications is the limited availability of standard samples. Based on our collected spectral dataset, we discuss how pilot studies of smaller sample sizes can benefit from statistical learning methods such as cross-validation and bagging, which we used to evaluate model performance and improve prediction accuracy. For spectral data handling, we tested the effect of different combinations of spectral pre-processing methods, e.g., normalization, baseline removal, and transformation, on regression model performance. We also investigated ways to automatically optimize Savitzky-Golay filter setup through a grid search. We applied principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) to classify common types of fertilizers (Fig 2A). For quantitative analysis of soil nutrients, we evaluated PLS regression (Fig 2B) and multi-layer perceptron neural networks. The results obtained in our experiments helps us to figure out the suitability of certain techniques for targeted applications and data analytics procedures, which should be of interest to a broad spectrum of audiences.

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REFERENCES
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