Wednesday Morning, October 31, 2012

Applied Surface Science
Room: 20 - Session AS-WeM

Surface Analysis of Biological Materials Using Vibrational & Non Linear Optical Spectroscopy Techniques (8:00-10:00 am) / 3D Imaging & Nanochemical Analysis - Part 1 (10:40 am-12:00 pm)

Moderator: R.P. Richter, CIC biomaGUNE & MPI for Intelligent Systems, Spain, D. Roy, National Physical Laboratory, UK, V.S. Smentkowski, General Electric Global Research Center

8:00am **AS-WeM1 Fibril Formation within the Extracellular Matrix, from Preventing Bacterial Infections to Artificial Tissue Generation**, ***P. Koelsch***, University of Washington **INVITED**

The ability to probe an interface beneath a layer of living cells *in vitro* without the need for labeling and fixation has the potential to unlock key questions in cell biology and biointerfacial phenomena. In particular fibril formation within the first steps of cell adhesion has been has been identified to play a key role for cell-implant interactions, for microbial biofilm formation on industrial surfaces, or for understanding basic phenomena in the context bacterial infections mediated through fibrillar assemblies. In this contribution we show how the technique of second-harmonic-generation microscopy and sum-frequency-generation spectroscopy can be utilized to detect ordered structures within tissue and at interphases between substrates and living, adherent cells. These were detected within the first steps of cell adhesion in real-time and *in vitro* with no labeling and/or fixation required.

References:

[1] Diesner, M.-O.; Welle, A.; Kazanci, M.; Kaiser, P.; Spatz, J.; Koelsch, P., In vitro observation of dynamic ordering processes in the extracellular matrix of living, adherent cells. *Biointerphases* **2011,** 6, (4), 171-179.

[2] Diesner, M. O.; Howell, C.; Kurz, V.; Verreault, D.; Koelsch, P., In Vitro Characterization of Surface Properties Through Living Cells. *J. Phys. Chem. Lett.* **2010,** 1, (15), 2339-2342.

[3] Howell, C.; Diesner, M. O.; Grunze, M.; Koelsch, P., Probing the Extracellular Matrix with Sum-Frequency-Generation Spectroscopy. *Langmuir* **2008,** 24, (24), 13819-13821.

8:40am **AS-WeM3** ***In Situ*** **Monitoring of SDS Adsorption on Positively Charged Surfaces**, ***S.-H. Song***, *P. Koelsch, T. Weidner,* University of Washington, *M.S. Wagner,* The Procter & Gamble Company, *D.G. Castner*, University of Washington

Surfactants are important compounds used in many industrial applications, with sodium dodecyl sulfate (SDS) being one of the most widely used surfactant. This study uses vibrational sum-frequency-generation (SFG) spectroscopy and surface plasmon resonance (SPR) analysis to investigate molecular ordering and orientation within SDS films formed on positively charged surfaces. Substrates with different charge density and polarity include CaF_2 at different pH values and chemically modified CaF_2 and Au surfaces prepared by RF glow discharge plasma deposition of allylamine (AAm) films and heptylamine (HApp), respectively. SFG spectra were recorded in various spectral regionsfor SDS concentrations ranging from μM to mM. At 0.2 mM SDS concentration, the intensity of CH and OH peaks decreased to background levels independently of the substrate. Previous studies have suggested that the SFG intensity minimum at 0.2 mM is due to neutralization effects of the positively charged (CaF_2) surface by the anionic charged head group of SDS.[1] In our studies, we found out that (i) the loss of SFG signal occurring at 0.2 mM is independent of surface charge density and (ii) SFG spectral intensities of lower concentrations vary significantly, whereas above 0.2 mM signals become reproducible. Therefore, in analogy to the behavior observed for alkane thiols on gold,[2] we interpret the loss of signal to a loss in order induced by a transition from a striped phase to a stand-up phase. As the number density of adsorbed SDS molecules increases above 0.2 mM, a second minimum in SFG intensity can be observed for all substrates, but at concentrations that are substrate dependent. Here we propose a model in which a monolayer is built up, but with opposing head group orientations (towards the substrate and the solution phase). This is supported by (i) SPR data showing a saturating number density towards SDS monolayer coverage at concentrations around the critical micelle concentration and above, (ii) a minimum for methyl vibrations related to an equal number in downward and upward orientations in the monolayer, and (iii) SFG spectral analysis for the polar SO_3^- band revealed a band structure with two contributions of positive and negative phases. This can be associated to spectral shifts in close proximity to the substrate and opposing headgroup orientations (towards the substrate and the solution).

1. Becraft, K. A.; Moore, F. G.; Richmond, G. L., *Journal of Physical Chemistry B* **2003,** 107, (16), 3675-3678.

2. Schreiber, F., *Progress in Surface Science* **2000,** 65, (5-8), 151-256.

9:00am **AS-WeM4 Enhanced Infrared Spectroscopy and Near-Field Microscopy with Infrared Antennas**, ***T. Taubner***, RWTH Aachen University, Germany **INVITED**

Infrared (IR) spectroscopy allows for the investigation of chemical, structural or electronic properties of a sample material by directly probing molecular, crystal lattice or charge carrier oscillations. Combined with scattering-type near-field optical microscopy (s-SNOM), which relies on the scattering of light at a sharp metallic tip, it is possible to obtain such spectroscopic information in images with strongly subwavelength spatial resolution [1-4] of typically about 20-30 nm. Currently, the main limitation of this technique comprises of the low signals that demand tunable laser sources and restrict the spectral range of operation.

Recently, new broadband IR light sources enabled s-SNOM near-field spectroscopy on different polar crystals [5], semiconductor nanostructures [6] as well as biominerals [7]. The majority of these experiments has been performed on samples which provide a resonant optical interaction between the sample and the probing tip, thus resulting in comparably strong signals. For the detection of weak molecular vibrations like polymers and proteins however, the SNOM signals either have to be enhanced or stronger IR light sources have to be developed.

Here we present a way to enhance the near-field probing process by suitable substrates [8], increasing both signals and contrasts in infrared s-SNOM when probing thin sample layers. In a next step, we investigate the use of resonant nanostructures ("infrared antennas", [9,10]) to enhance s-SNOM sensitivity even further. Additionally, we compare enhanced near-field spectra with the corresponding far-field spectra obtained by diffraction-limited FTIR-Microscopy. We will also present our lasted results obtained with a new powerful broadband IR laser source that is currently developed at the Fraunhofer ILT.

References

[1] T. Taubner, R. Hillenbrand, F. Keilmann, *APL* **85**, 5064 (2004).

[2] R. Hillenbrand, T. Taubner, F. Keilmann, *Nature* **418**, 159 (2002).

[3] A. Huber et al., *Nano Letters* **7**, 774 (2006).

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[5] S. Amarie, T. Ganz & F. Keilmann, *Optics Express* **17**, 21794 (2009).

[6] F. Huth et al., *Nature Materials* **10**, 352 (2011).

[7] S. Amarie et al., *Beilstein J. Nanotechnol.***3**, 312 (2012).

[8] J. Aizpurua et al., *Optics Express* **16**, 1529 (2008).

[9] F. Neubrech et al., *Physical Review Letters* **101**, 157403 (2008).

[10] R. Adato et al., *PNAS* **106**, 19227 (2009).

9:40am **AS-WeM6 FT-IR Spectrochemical Imaging: Applications with Focal Plane Array and Multiple Beam Synchrotron Radiation Source**, ***M. Unger***, *E. Mattson, J. Sedlmaier, Z. Alavi, R. Dsouza, B. Manandar, C. Hirschmugl*, University of Wisconsin Milwaukee

FT-IR spectrochemical imaging, which combines the chemical specificity of mid-infrared spectroscopy with spatial specificity, is an important demonstration of label-free molecular imaging. Mid-infrared optical frequencies are resonant with the vibrational frequencies of functional groups, thus an absorption spectrum is a "molecular fingerprint" of the material at every pixel. Each spectrum can be correlated with known material properties to extract chemical information. Synchrotron based FT-IR spectrochemical imaging, as recently implemented at the Synchrotron Radiation Center in Stoughton, WI, demonstrates the new capability to achieve diffraction limited chemical imaging across the entire mid-infrared region, simultaneously, with high signal to noise ratio.

IRENI [1] (Infrared Environmental Imaging) extracts a large swath of radiation (320 hor. × 25 vert. $mrads^2$) to homogeneously illuminate a commercial IR microscope equipped with an infrared Focal Plane Array (FPA) detector. Wide field images are collected. IRENI rapidly generates high quality, high spatial resolution data. The relevant advantages (spatial oversampling, speed, sensitivity and signal to noise ratio) will be presented and demonstrated using examples from a variety of disciplines, including

formalin fixed [1] and flash frozen tissue samples [2], live cells, fixed cells, paint cross sections and polymer fibers will be presented.

* This work has been done with support from NSF (MRI-DMR-0619759 and CHE-1112433) and the Synchrotron Radiation Center, which is also supported by NSF (DMR-0537588) and UW-Milwaukee and UW-Madison.

[1] M.J. Nasse, et al. "High resolution Fourier-transform infrared chemical imaging with multiple synchrotron beams", Nature Methods, 8, (2011) 413-416

[2] M.Z. Kastyak-Ibrahim, et al. "Biochemical label-free tissue imaging with subcellular –resolution synchrotron FTIR with Focal Plane Array Detector," NeuroImage 60, (2012) 376-383.

10:40am **AS-WeM9 3D Analysis using X-ray Computed Tomography**, ***S.R. Stock***, Northwestern University **INVITED**

X-ray Computed Tomography (CT) can be performed on meter-sized objects or micrometer wide samples, and introduction of commercial x-ray tube-based instruments and dedicated synchrotron systems has produced an explosion of studies spanning the sciences and engineering disciplines. Using one of several numerical reconstruction methods, CT combines x-ray projections (radiographs) into a cross-sectional map, generally in 3D, of the x-ray absorption of the specimen. Other modalities (x-ray phase contrast, scattering, etc.) can also be used for reconstruction. The noninvasive interrogation allows the specimen to be studied multiple times during its evolution or to be returned undamaged to the museum collection.

This talk briefly introduces the fundamentals of x-ray CT and the different approaches to data acquisition and reconstruction. Most of the examples will focus on microCT, that is, reconstructions with isotropic volume elements (voxels) from 1-50 micrometers on edge. The first example is quantification of crack opening in a metal sample as a function of 3D position and applied load. In the second example, microCT data forms the basis of finite elements (FE) modeling of response of a spine of the sea urchin *Diadema setosum* to different applied loads. The third example illustrates local tomography, where data for high resolution reconstruction is only collected over a portion of the sample cross-section. Differences in x-ray phase contrast instead of x-ray absorption can be used as the basis for reconstruction; and the examples show how differences in polymers and soft tissues can be imaged using this approach. Intensity diffracted from the different phases within a specimen provide the basis for reconstructing the distribution of crystallographic phases; one example is SiC fibers in an Al matrix.

11:20am **AS-WeM11 High Spatial Resolution 2D and 3D TOF-SIMS Analysis using Cluster Ion Beams**, ***F. Kollmer***, *S. Kayser*, ION-TOF GmbH, Germany, *N. Havercroft*, ION-TOF USA, Inc., *D. Rading, R. Moellers, W. Paul, E. Niehuis*, ION-TOF GmbH, Germany

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is a very sensitive surface analytical technique. It provides detailed elemental and molecular information about surfaces, thin layers, interfaces and full three-dimensional analysis of a sample. A major improvement especially for the analysis of molecular surfaces on a small scale has been the introduction of cluster ion beams that increases the sensitivity by orders of magnitude [1].

In recent years Bi clusters have become a standard primary ion species for all TOF-SIMS imaging applications providing a lateral resolution of down to 80 nm. Recent developments allow pushing the performance further towards the physical limits of the technique. Under optimized conditions we will present a lateral resolution of less than 20 nm by applying Bi_3 clusters on a certified reference material [2] and real world samples.

Usually for TOF-SIMS depth profiling and 3D-analysis, a dual beam approach is accomplished with dedicated analysis and sputter beams. However, the analysis of structures at greater depth (> 10 µm) needs long sputter times and the build-up of surface roughness at the crater bottom limits the achievable spatial resolution. In order to overcome these limitations we used a combined SIMS/FIB setup for the analysis of inorganic samples. Hereby, the Bi cluster beam is used to FIB mill a crater into a sample and the vertical crater wall is subsequently analysed by TOF-SIMS. We will present 2D and 3D data of reference samples, as well as, real world samples analysed by this approach.

The challenge of three dimensional analysis of molecular surfaces is to maintain the molecular structure of the exposed surface while removing the covering material. In this respect the development of new sputter ion sources using massive Ar clusters allows the preservation of molecular information under high dose sputtering [3]. This has enabled TOF-SIMS to do depth profiling and 3D analysis of organic materials. In our contribution we will present 3D analysis of an OLED display device using an Ar gas cluster ion beam for sputtering in combination with a Bi cluster beam for analysis.

[1] F. Kollmer, *Appl. Surf. Sci.* 231-232, **2004,** 153–158

[2] M. Senoner, W. E. S. Unger, *Surf. Interface Anal. 39*, **2007**, 16-25

[3] S. Ninomiya, K. Ichiki, H. Yamada, Y. Nakata, T. Seki, T. Aoki, J. Matsuo, Rapid Communications in Mass Spectrometry **2009**, 23, 1601-1606

11:40am **AS-WeM12 An Evolution of TOF-SIMS for Biological Analysis: From 2D Imaging to 3D FIB-TOF Tomography**, ***G.L. Fisher***, *J.S. Hammond, S.R. Bryan*, Physical Electronics

TOF-SIMS has become an important tool for 2D and 3D imaging mass spectrometry of biological and complex material specimens due to it's unique capability to detect molecular and elemental ions at a spatial resolution of ≤ 300 nm, a mass resolution of ~ 15,000 m/Δm, and without the sample treatments or labeling required by e.g. MALDI or fluorescence microscopy. Among the advantages of TOF-SIMS are ~ 2 nm sampling depth, parallel detection and collection of the entire mass spectrum at every image pixel, and sensitivities in the ppm to ppb range. The ability to image surfaces having a large degree of topography while maintaining artifact-free chemical imaging is also highly desired; the resulting elemental and molecular images provide important information regarding the composition of biointerfaces, tissues and cells, and of materials such as oxide fuel cells and OLEDs.

Characterization of specimens to a depth of several microns below the sample surface has become somewhat routine with the use of a sputter ion beam to remove multiple layers of atoms and molecules between analysis (chemical imaging) cycles. Nevertheless, there are practical limitations to the use of ion beam sputtering for probing both organic and inorganic specimens beyond the surface region. Among the difficulties and limitations is the fact that the various matrix components sputter at different rates, called preferential or differential sputtering, which results in a distortion or complete loss of the true 3D chemical distribution as a function of depth. Many specimens also contain void spaces that are impossible to preserve in 3D images obtained by sputter depth profiling.

An alternative approach to achieve 3D chemical imaging of chemically complex specimens is to utilize *in situ* FIB milling and sectioning in conjunction with TOF-SIMS chemical imaging… what we have called FIB-TOF tomography. With FIB milling, the interior of a specimen is revealed to depths of more than 100 µm. 3D chemical imaging with a z-dimension of greater than 10 µm in tomographic increments of ≤ 0.5 µm may be achieved within a reasonable analysis time. The advantage of the FIB-TOF approach is that artifacts caused by sputter depth profiling such as differential sputtering and accumulated ion beam damage are avoided. 3D imaging by FIB-TOF tomography will be illustrated first with organic / inorganic composite materials. Applications in biological and clinical cancer research will also be presented with an emphasis on the conditions required to achieve FIB-TOF tomography.

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