Nanoparticle-Liquid Interfaces Focus Topic
Room: 201 B - Session NL+AS+BI+SA-FrM

Emerging Methods to Identify and Measure Nanomaterials in Biological Environments

Moderator: G. Ceccone, European Commission, Joint Research Centre, IHCP, Italy

8:20am **NL+AS+BI+SA-FrM1 3D Views of Hydrated Biological Cells with Soft X-ray Tomography**, ***C.A. Larabell***, University of California, San Francisco **INVITED**

SXT is similar in concept to the well-established medical diagnostic technique, computed axial tomography (CAT), except SXT is capable of imaging with a spatial resolution of 50 nm, or better. We examine whole, hydrated cells (between 10-15 μm thick), eliminating the need for time-consuming and potentially artifact-inducing embedding and sectioning procedures. Cells are rapidly frozen then imaged using photons with energies between the K shell absorption edges of carbon (284 eV, λ=4.4 nm) and oxygen (543 eV, λ=2.3 nm). In this energy range, photons readily penetrate the aqueous environment while encountering significant absorption from carbon- and nitrogen-containing organic material. Consequently organic material absorbs approximately an order of magnitude more strongly than water, producing a quantifiable natural contrast image of cellular structures. By collecting images from multiple angles through 360 degrees of rotation, SXT reconstructions yield information at isotropic resolution.

Images are formed using unique optics called zone plates (ZP). An X-ray ZP optic consists of a number of concentric nanostructured metal rings, or zones, formed on a thin X-ray transmissive silicon nitride membrane. The width of the outermost ring determines the spatial resolution of the ZP lens, whereas the thickness of the rings determines the focusing efficiency. In our microscope, we use a condenser ZP lens with an overall diameter of 1 cm and an outer zone width of 50 nm. The high-resolution objective ZP lens has a diameter of 63 μm, 618 zones, a focal length of 650 μm at 2.4 nm wavelength, and an outer zone width of 50 nm.

Because SXT is fast (~ 5 min per tomographic data set), we can examine large numbers of cells. Since organic material absorbs approximately an order of magnitude more strongly than water, a unique and quantifiable natural contrast image of cellular structures is generated. X-ray absorption follows Beer's Law, therefore the absorption of photons is linear and a function of the biochemical composition at each point in the cell. As a result, a linear absorption coefficient (LAC) value of each voxel can be calculated. For example, lipid drops with high concentrations of carbon are more highly absorbing (LAC=0.7 mm^{-1}) than fluid-filled vesicles (LAC=0.2 mm^{-1}). We can determine the position of specific molecules by overlaying fluorescence microscopy signals on cell structures obtained with x-ray imaging. In addition, we can directly determine the locations and numbers of metal probes throughout the cell.

9:20am **NL+AS+BI+SA-FrM4 Ultrathin Electron Transparent Membranes as a Platform for Scanning Electron and Photoelectron Imaging and Spectroscopy of Fully Hydrated Nanoparticles**, ***X.M. Ma***, *J. Geisler-Lee*, Southern Illinois University Carbondale, *M. Amati, L. Gregoratti*, Sincrotrone Trieste, Italy, *S. Guenther*, Technical University Muenchen, Germany, *M. Kiskinova*, Sincrotrone Trieste, Italy, *A. Kolmakov*, Southern Illinois University Carbondale

The increased use of engineered nanoparticles (ENPs) in biomedical applications and their inevitable release into the environment has prompted considerable need to study of their uptake, accumulation and transport inside biological tissue and in plants. This is particularly true for addressing the ENPs fate on a cellular level which inevitably requires the microscopy approach. For long time optical microscopy with the resolution in the order of 100 nm was the major tool available. Better resolution can be readily achieved with traditional transmission (TEM) or scanning (SEM) electron microscopy. However, it requires histological sample treatments such as fixation, staining, dehydration, freezing etc which excludes *in vivo (in situ)* modes of observations and can alter their native morphology, functionality and living cycles. Different from standard environmental SEM, where the near sample pressure is limited by ca few tens of Torr, we are actively working on fabrication and tests of electron transparent membranes for ambient pressure electron spectromicroscopy and its application to fully hydrated samples for phytotoxicity, and materials research. Such enclosed environmental cells, equipped with 50-100 nm windows transparent for 10-20 keV electrons, can maintain the sample at atmospheric pressure and/or fully hydrated. This approach is beneficial compared with dry methods since *in vivo* SEM/TEM observations at nanoscale can be performed. Using this methodology, we were able to image the uptake of silver (Ag) NPs by living Arabidopsis roots on a cellular level. It was shown that NPs with the sizes larger than 20 nm accumulate preferably on the surface of the cellular walls and do not to traverse the plant cell membrane.

Recent developments in high yield fabrication and handling protocols of ultrathin (~1 nm) membranes, such as graphene or graphene oxide sheets with thicknesses comparable to the effective attenuation length (EAL) of 200-1000 eV electrons opened the opportunity to perform traditional XPS (X-ray Photoelectron Spectroscopy) and AES (Auger Electron Spectroscopy) at the interfaces between the membrane and fully hydrated samples. Using model water solutions and NPs, we report here on major design principles of such cells as well on first spectral demonstrations, advantages and limitations of this new technique.

9:40am **NL+AS+BI+SA-FrM5 Small-angle X-ray Scattering Investigation of Functional Materials at Inorganic-Marcromolecular Interfaces**, ***T.W. van Buuren***, *T.M. Willey, J.R.I. Lee, I.C. Tran, M. Bagge-Hansen*, Lawrence Livermore National Laboratory

Development in nanoscale engineering has enabled bioelectronics that can mimic and/or interact with the biological systems. Lipid bilayer-functionalized Si nanowires are considered as a promising candidate for the construction of bioelectrochemical devices. These biomimetic lipid bilayers serve as a general host matrix for bio-functional components such as membrane proteins. Though meaningful technological advancement of these materials has been made, critical questions about their structural and chemical composition remain. Small angle x-ray scattering (SAXS) experiments are used to investigate the structure of the lipid bilayers on Si nanowires, which provide information on the overall 1-D bilayer structure, the effect of substrate curvature on the lipid packing and local self-organization. The SAXS derived lateral-averaged characterizations are then corroborated with local arrangements of lipid bilayers on Si nanowires revealed by Scanning Transmission X-ray Spectroscopy (STXM). The results provide insights into a number of unresolved questions that are crucial for the comprehensive understanding this class of materials.

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