

Sunday Afternoon, October 28, 2012

Biomaterials Plenary Session

Room: 23 - Session BP+AS-SuA

Biomaterials Plenary - Bioimaging: In Vacuo, In Vitro, In Vivo

Moderator: M.R. Alexander, University of Nottingham, UK

4:00pm **BP+AS-SuA1 NanoBio Imaging for New Biomedical Applications**, *D.W. Moon*, Korea Research Institute of Standards and Science **INVITED**

Surface and interface analysis techniques have been mainly developed to meet the demands on atomic scale characterization from semiconductor industries. KRIS has been trying to meet the surface and interface analysis challenges from semiconductor industries and furthermore to extend the application scope to biomedical areas. In this presentation, I'd like to report our recent activities of nanobio imaging for new biomedical applications such as 1) Coherent Anti-Stokes Raman Scattering (CARS) for atherosclerotic plaque imaging 2) Time-of-flight secondary ion mass spectrometry (TOF-SIMS) for mass imaging of collagen fibrils, atherosclerotic plaques, and cancer tissues and 3) Surface Plasmon Resonance Imaging Ellipsometry for cell adhesion, migration, and infiltration dynamics for HUVEC, CASMC, and T cells 4) TOF-medium energy ion scattering spectroscopy (TOF-MEIS) for nanothin films and nanoparticles such as CdSe/ZnS quantum dots and calcium hydroxyapatite nano-size biominerals. Future challenges of nanobio imaging for biomedical applications will be discussed.

4:40pm **BP+AS-SuA3 3-D View into Cells by X-ray Nano-Tomography**, *G. Schneider, P. Guttman, S. Werner, K. Henzler, S. Rehbein*, Helmholtz-Zentrum Berlin für Materialien und Energie GmbH, Germany **INVITED**

X-ray imaging offers a new 3-D view into cells. With its ability to penetrate whole hydrated cells it is ideally suited for pairing fluorescence light microscopy and nanoscale X-ray tomography. The HZB TXM at the undulator U41 provides a spectral resolution of 10.000 and a spatial resolution of 11 nm. For high resolution tomography, we adopted a tilt stage originally developed for electron tomography. The stage is able to tilt samples up to $\pm 80^\circ$. Such a large tilt of flat sample holders is impossible with TXM at bending magnet sources because they require a monochromator pinhole to be positioned close to the specimen. In our TXM, the holder geometry is no longer restricted to glass tubes. Conventional fluorescence images are diffraction-limited to ~ 200 nm, whereas current TXM achieve a ten-fold improvement in resolution. Since fluorescence and X-ray microscopy permit analysis of whole cells, it is possible to investigate the same cell in both microscopes by correlative microscopy. These correlative studies are ideally suited to X-ray microscopy because of its ability to image cells in 3D. In the talk, we present the cryo TXM and selected applications. In particular, we will show the internal structures of mammalian cells, i.e. plasma membrane, nuclear membrane, nuclear pores, nucleoli, endoplasmic reticulum, vesicles, lysosomes and mitochondria. It is now also possible to resolve internal organellar structures, such as mitochondrial cristae, the double nuclear membrane and lysosomal inclusions. In addition, we discuss ways towards 10 nm 3D imaging of cells. Keywords: X-ray microscopy, tomography, cell organelles, correlative microscopy

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4. G. Schneider, P. Guttman, S. Rehbein, S. Werner, R. Follath, J. Struct. Biol. 177 (2012), 212-223

5:20pm **BP+AS-SuA5 Nanoscopy with Focused Light**, *S.W. Hell*, Max-Planck-Institut für Biophysikalische Chemie, Germany **INVITED**

In STED microscopy¹, fluorescent features are switched off by the STED beam, which confines the fluorophores to the ground state everywhere in the focal region except at a subdiffraction area of extent. In RESOLFT microscopy,^{2,3} the principles of STED have been expanded to fluorescence

on-off-switching at low intensities I , by resorting to molecular switching mechanisms that entail low switching thresholds I_s . An I_s lower by many orders of magnitude is provided by reversibly switching the fluorophore to a long-lived dark (triplet) state² or between a long-lived 'fluorescence activated' and 'deactivated' state.^{2,5} These alternative switching mechanisms entail an I_s that is several orders of magnitude lower than in STED. In imaging applications, STED/RESOLFT enables fast recordings and the application to living cells, tissues, and even living animals.^{6,7}

Starting from the basic principles of nanoscopy we will discuss recent developments^{8,9} with particular attention to RESOLFT and the recent nanoscale imaging of the brain of living mice⁷ by STED.

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