**Biomaterial Interfaces**
**Room: Milo - Session BI-MoM**

**Nanobio Imaging**
**Moderator:** Sally L. McArthur, Swinburne University, Australia

8:40am **BI-MoM1 Multimodal Nano-Bio Imaging of Neuronal Cells and Tissues**, ***DaeWon Moon***, DGIST, Korea, Republic of Korea **INVITED**

Secondary ion mass spectrometry (SIMS) provides molecular specific information but for 2D imaging, SIMS needs specimens in general, to be frozen and dried for analysis in vacuum. For 3D imaging, specimens can be sputter profiled by recently developed gas cluster ions. To compensate the distortion due to cryosection and sputter profiling, we have been trying to develop a multimodal mass and non-linear optical imaging methodology of various cells and tissues for neuronal studies. In this presentation, multimodal SIMS and CARS imaging studies on neuron cells, olfactory bulb, and nematode *C. elegans* are reported.

For time-of-flight (TOF) secondary ion mass spectrometry (SIMS) imaging, 30 keV $Bi_3^+$ ions for 2D mass imaging and 30 keV $Ar_{1000}$ cluster ions for depth profiling were used to analyze various tissues such as *C. elegans*, and mouse olfactory bulb. As a complementary non-linear optical imaging, coherent Anti-Stokes Raman Scattering (CARS) were used to get 3D lipid imaging down to ~50 μm with 300 nm spatial resolution in-vitro or ex-vivo.

For *C. elegans*, lipid CARS imaging was obtained for live *C. elegans* but for SIMS imaging, *C. elegans* were dried with water and sputtered with 30 keV $Ar_{1000}$ cluster ions to get sectioned 2D SIMS images. Molecular specific SIMS imaging for lipids, neurotransmitters, and pheromones with complementary CARS lipid imaging were used to investigate the difference of molecular distributions in wild-type and various mutant *C. elegans*.

For a cryosectioned mouse olfactory bulb, SIMS imaging showed different distributions of lipid molecules and neurotransmitters which is consistent with the olfactory bulb structure of glomerulus, mitral cell layer, and granule cell layer. SIMS imaging to study the changes of neurotransmitters in an olfactory bulb upon odorant stimulus will be reported.

In conclusions, multimodal mass and non-linear optical imaging provides a practically useful platform to investigate cells and tissues for new biomedical understanding of neuronal systems. New challenges for non-cryo tissue section, plasma/fs laser based ambient mass spectrometry for live cell membrane mass imaging, and super-resolution CARS will be discussed.

9:20am **BI-MoM3 SPR Imaging Sensor for Visualization of Individual Cell Reactions and Clinical Diagnosis of Allergy**, ***Yuhki Yanase***, Hiroshima University, Japan **INVITED**

A technique to visualize individual living cell activation in a real time manner without any labeling is required in the fields of life sciences and medicine. Surface plasmon resonance (SPR) sensors detect the refractive index (RI) changes on the surface of sensor chips in label-free and on a real-time basis. We previously reported that SPR sensors could detect real-time large changes of RI in response to activation of living cells, such as mast cells, keratinocytes, basophils and B lymphocytes on a sensor chip without labeling, suggesting the potential of SPR as a new method for clinical diagnosis and drug screening. Thus, SPR sensor possesses great potential to reveal nano-scale living cell actions in evanescent field. However, conventional SPR sensors detect only an average RI changes in the presence of thousands of cells in an area of the sensor chip, and could offer only small number of sensing channels. Therefore, we developed SPR imaging (SPRI) sensor with a CMOS camera and an objective lens in order to visualize RI distribution of individual living cells and their changes upon stimuli

The sensor we developed is composed of a light source, P-polarizer, prism (RI=1.72), objective lens and CMOS camera. The SPRI sensor chips (RI=1.72, 20 mm × 20 mm × 1 mm) coated with gold thin film (1 nm Cr layer and 49 nm gold layer) by means of vapor deposition. The SPRI sensor we developed could detect reactions of individual rat basophilic leukemia (RBL-2H3) cells and mouse keratinocyte cells in response to specific or nonspecific stimuli. Moreover, the sensor could detect the reactions of individual human basophils isolated from patients in response to antigens. Furthermore, we also succeeded in distinguishing reactions of basophils activated by antigens from those of non-activated basophils spotted on an area.

The technique can visualize the effect of various stimuli, inhibitors and/or conditions on cell reactions as change of intracellular RI distribution at single cell levels. Establishment of the technique to rapidly isolate cells from patient blood should enable us to utilize SPRI system as a high throughput screening system in clinical diagnosis, such as type I allergy and drug hypersensitivity, and as a tool to reveal novel phenomena in evanescent fields around plasma membrane.

10:40am **BI-MoM7 SIMS of Cells and Tissues: Blasting Our Way to New Knowledge about Biology**, ***Lara Gamble***, *B. Bluestein, D.J. Graham*, University of Washington, USA **INVITED**

Imaging mass spectrometry can provide images of cells and tissues with chemical and molecular specificity. These chemically specific images could revolutionize our understanding of biological processes such as increasing our understanding of chemical changes in cells and tissues as a function of an applied stress or as a result of disease, and enable tracking the spatial distribution of metabolites and lipids. The mass spectral imaging capability of ToF-SIMS holds potential to achieve this goal with sub-cellular resolution. Chemistry of tumor microenvironments, lipid metabolomics relationship to cancer, delivery of nanoparticles to cells, and tissue repair could be visualized on a cellular and sub-cellular level. In this presentation, ToF-SIMS analysis of biological samples from 2D images of tissues to 3D images of nanoparticles in cells will be presented. Challenges with sample preparation for the ToF-SIMS environment and processing of the large amount of data will be discussed (including multivariate analysis of the ToF-SIMS image data). The advantage of combining ToF-SIMS images with optical images of the same samples (same slices and serial biopsy slices) will also be presented. This combination of images allows researchers to visualize a molecular map that correlates with specific biological features or functions.

11:20am **BI-MoM9 Probing the Determinants of Sphingolipid Distribution in the Plasma Membrane with SIMS**, ***Mary Kraft***, *J. Frisz*, University of Illinois at Urbana-Champaign, *P. Weber*, Lawrence Livermore National Laboratory, *R. Wilson*, University of Illinois at Urbana-Champaign, *J. Zimmerberg*, National Institutes of Health, *H. Klitzing*, University of Illinois at Urbana-Champaign

The plasma membrane is a selectively permeable lipid bilayer that separates cells from their surroundings. Numerous different lipid species, cholesterol, and a variety of different proteins form the plasma membranes of mammalian cells. One class of lipids, the sphingolipids, and their metabolites serve both as structural components in the plasma membranes of mammalian cells, and as bioactive signaling molecules that modulate fundamental cellular processes. Though segregation of the sphingolipids into distinct membrane domains is likely essential for cellular function, the sphingolipid distribution within the plasma membrane and the mechanisms that regulate it are poorly understood. To address this issue, we have combined metabolic labeling with stable isotopes and SIMS performed on a Cameca NanoSIMS 50 to image the distributions of stable isotope-labeled sphingolipids in the plasma membranes of fixed cells with ~100 nm lateral resolution. Using this approach, we previously discovered that the $^{15}$N-sphingolipids were enriched within distinct domains in the plasma membranes of fibroblast cells [1]. Here we report how we have used this approach to probe the mechanisms responsible for this sphingolipid organization. To determine whether the sphingolipid domains are dependent on molecular interactions with cholesterol or protein-based barriers that are established by the cytoskeleton and its associated membrane proteins, we used SIMS to image the $^{15}$N-sphingolipid distribution in the plasma membrane following cholesterol depletion and actin depolymerization. We also assessed whether these $^{15}$N-sphingolipid domains were co-localized with hemagglutinin, a specific membrane protein that is thought to have an affinity for sphingolipid-enriched membrane domains. Our results indicate that the sphingolipid organizations in the plasma membrane are dependent on the cytoskeleton, but not on favorable interactions with cholesterol or hemagglutinin.

[1] J. F. Frisz, K. Lou, H. A. Klitzing, W. P. Hanafin, V. Lizunov, R. L. Wilson, K. J. Carpenter, R. Kim, I. D. Hutcheon, J. Zimmerberg, P. K. Weber, M. L. Kraft, Proc. Natl. Acad. Sci. U.S.A., 2013, 110 (8), E613-E622.

11:40am **BI-MoM10 In-Situ TOF-SIMS and SFM Measurements Providing 3D Chemical Characterization of Inorganic and Organic Nanostructures**, *R. Moellers,* ION-TOF GmbH, Germany, *E. Niehuis,* ION-TOF GmbH, *F. Kollmer,* ION-TOF GmbH, Germany, *H. Arlinghaus,* ION-TOF GmbH, *R. Dianoux,* Nanoscan AG, Switzerland, *A. Scheidemann,* Nanoscan AG, ***Nathan Havercroft***, ION-TOF USA, Inc.

Information on the chemical composition, physical properties and the three dimensional structure of materials and devices at the nanometer scale is of major importance in nanoscience and nanotechnology. Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) is an extremely sensitive surface imaging technique which provides elemental as well as comprehensive molecular information on all types of solid surfaces. Depth profiling of multilayers with high depth resolution as well as three-dimensional analysis is performed using additional low energy sputter beams. However, the topography of the initial sample surface as well as the subsequent evolution of the topography by sputtering cannot be identified by the technique and lead to distortions of the detected depth distribution. Scanning Force Microscopy (SFM) provides the required complementary information on the surface topography with a resolution on the nanometer level.

We have combined the techniques ToF-SIMS and SFM in one UHV instrument. The TOF-SIMS analysis is performed using a new bismuth liquid metal cluster ion gun that can achieve a lateral resolution of 20 nm [1]. For the sputtering of inorganic materials the instrument is equipped with low energy oxygen and cesium beams. Sputtering of organic materials without radiation damage is performed by using large gas clusters with low energy/atom allowing molecular depth profiles of organic multilayers with a depth resolution of 5 nm as well as 3D analysis of organic nanostructures [2]. The SFM unit is mounted on a 3-axis high precision flexure stage scanner with a small out-of-plane motion for very accurate information on the surface topography. The SFM can be operated in contact mode as well as in a variety of dynamic modes to provide additional valuable information about the physical properties of the sample. In this paper we will present first results illustrating the strength of combined in-situ TOF-SIMS / SFM measurements and the potential for a wide range of applications.

References

[1] F. Kollmer, W. Paul, M. Krehl and E,. Niehuis, Surf. Interface Anal. 45, 312 (2013)

[2] E. Niehuis, R. Moellers, D. Rading, H.-G. Cramer, R. Kersting, Surf. Interface Anal. 45, (2013) 158

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