Applied Surface Science
Room 2005 - Session AS+BI-WeA

Imaging and Characterization of Biological Materials

Moderator: L.J. Gamble, University of Washington

2:00pm **AS+BI-WeA1 Imaging Biomolecules at Surfaces**, ***R.M.A. Heeren,*** *E.R. Amstalden, A.F.M. Altelaar, M. Froesch, L.A McDonnell,* FOM-Institute for Atomic and Molecular Physics, The Netherlands **INVITED**

Mass spectrometry is one of the technologies that enable the investigation of the spatial organization of biomolecules at complex surfaces. The potential of imaging MS as a biomedical imaging technique is evident. Direct biomarker visualization on tissue is only one of a few key applications. Imaging mass spectrometry is currently undergoing rapid developments in areas spanning the entire technology chain required to generate a mass resolved chemical image. New detection technology and novel imaging approaches improve speed, sensitivity and spatial resolution of imaging MS. In this contribution we will discuss several technological and methodological aspects of imaging mass spectrometry using a set of selected applications in biomedical imaging. Various approaches exist that use different incarnations of imaging mass spectrometry ranging from protein profiling to high resolution imaging using MALDI and SIMS. In our studies, a novel stigmatic or microscope mode imaging MS strategy is employed that allows for the rapid generation of high resolution, large field-of-view mass resolved images of cells and tissue. This mass microscope is combined with tissue digestion strategies that aid in the identification of larger proteins found in tissue section. One of these strategies involves the so-called molecular scanner, a technique where proteins are electro-blotted from the tissue through a membrane containing immobilized proteolytic enzymes. While the proteins pass through the membrane they are digested into smaller proteolytic peptides that are subsequently captured on a PVDF membrane. This technology enhances the detection sensitivity as multiple peptides are generated from a single protein molecule. The advantages of this high resolution imaging approach, using the molecular scanner will be demonstrated on cervical tissue sections obtained in the framework of a biomarker discovery study for cervical cancer.

2:40pm **AS+BI-WeA3 Acquisition of Chemical Information from Cell Samples Using TOF-SIMS Imaging**, *D. Breitenstein,* TASCON GmbH, Germany*; C. Rommel, J. Wegener,* University of Münster, Germany*; R. Moellers, E. Niehuis,* IONTOF GmbH, Germany*;* ***B. Hagenhoff,*** TASCON GmbH, Germany

Our ongoing studies focus on the mass spectrometric imaging for cells, an emerging area in TOF-SIMS research. In a joint research effort we want to elucidate the transport mechanism for drugs through the blood-liquor barrier. Being a vacuum technique TOF-SIMS faces some challenges for the analysis of epithelial cells. A standard approach to maintain the cellular integrity of cells during the experiments is freeze fracturing or cryomicrotomy. However, these techniques increase the experimental effort significantly. As we were not interested in the lateral distribution of elemental species like Na of K but small and middle sized molecules we concentrated on different fixation techniques instead. Mainly, paraformaldehyd and glutardialdehyd were used. In order to visualize the three-dimensional molecular structure of the cells C@sub 60@ sputtering was combined with imaging using Bi@sub 3@ primary ions. In order to compare the results with a standard analytical technique in biochemistry, additionally cells were treated with fluorescent dyes. The intact fluorophores could be detected successfully in the TOF-SIMS images. The obtained mass resolved images for the fluorescent dyes were compared successfully with the widely accepted technique of confocal laser scanning microscopy (CLSM), proving the validity of the chosen mass spectrometric approach. This work was supported by the German Federal Ministry for Education and Research (Grand: 0312002B).

3:00pm **AS+BI-WeA4 Complementary Application of SIMS and CARS for Biochemical Imaging of Cells and Tissues**, ***D.W. Moon,*** Korea Research Institute of Standards and Science, Korea*; T.G. Lee, E.S. Lee, J.Y. Lee,* Korea Research Institute of Standards and Science*; J.W. Shim,* AmorePacific Corporation*; J.W. Kim, K.W. Kim,* Seoul National University, Korea

There have been significant progresses in analysis of biomolecules on surfaces using surface analysis tools such as XPS, SIMS, SPR, and FT-IR. Major demands of biosurface analysis come from DNA chips, protein chips, and surface modification for tissue engineering. However, cell physiologists and medical and pharmaceutical scientists prefer in-vitro analyses of biomolecules in live single cells and tissues in spite of technical difficulties to biochemical assays. In this presentation, we report our recent studies on 2D or 3D label-free biochemical imaging of various cells and tissues such as liver, skin, retina, and hair based on complementary use of coherent anti-Stokes Raman scattering (CARS) and SIMS. TOF-SIMS measurements are based on cluster ion bombardment such as Au3, Bi3, and C60 and CARS measurements are optimized for the C-H vibration of biomolecules in live cells and tissues. CARS showed clear C-H chemical bond specific images with 300 nm spatial resolution and 1 μm depth resolution down to 100 μm depth, revealing detailed tissue structures in the sub-cellular level without any damage problems. SIMS showed much more surface sensitive and specific biomolecular mass images with some depth profiling capabilities. The present status and the future prospect of complementary use of CARS and SIMS with sensitivity and selectivity improvement based on a broad band spectrum and cluster bombardment, respectively will be discussed for practical applications in disease diagnostics and cell and tissue based drug screening.

3:20pm **AS+BI-WeA5 Desorption Electrospray Ionization: Fundamentals and Applications in Surface Analysis and Biological Imaging**, ***R.G. Cooks,*** *Z. Takats, J.M. Wiseman, D. Ifa, N. Talaty, A. Jackson, A. Venter,* Purdue University **INVITED**

This talk concerns ambient ionization using desorption electrospray ionization (DESI) and the related methods. These procedures allow direct analysis of biological samples, including proteins and lipids, on surfaces or in tissue without sample preparation. Biological fluids can also be examined directly, or by adsorption on a matrix like paper. DESI is suitable for characterization of both large and small molecules and it combines features of electrospray ionization (ESI) with those of the family of desorption ionization (DI) methods. It allows organic molecules present on surfaces to be analyzed by mass spectrometry without requiring that the sample be introduced into the vacuum system of the mass spectrometer. DESI has high sensitivity, is virtually instantaneous in response time, and there is little or no sample preparation. The sample is sprayed with charged microdroplets of water or a simple organic solvent. The sample remains fully accessible to observation as well as additional physical and chemical processing during the analysis. Applications to metabolomics, to high throughput analysis of pharmaceutical preparations, to drugs and drug metabolites in blood, serum and other biological fluids, are described. Tissue imaging is demonstrated with lipids being used as biomarkers to search for disease and in-vivo sampling of living tissue surfaces is described. Laser doppler anemometry is used to characterize the DESI mechanism. It is shown that at least two major processes are involved. One involves transfer of molecules from the surface to the droplet projectiles, the other involves proton or other charge transfer from the slow-moving projectile to the sample molecule. Variations on the DESI method in which reactive compounds are included in the spray solvent allow recognition of specific functional groups.

4:00pm **AS+BI-WeA7 Characterization of Bacterial Spores using Nano-Secondary Ion Mass Spectrometry (NanoSIMS)**, ***S. Ghosal,*** *S.J. Fallon,* Lawrence Livermore National Laboratory*; T. Leighton, K. Wheeler,* Children's Hospital Oakland Research Institute*; I.D. Hutcheon, P.K. Weber,* Lawrence Livermore National Laboratory

Bacterial spores are elementally zoned at the nanometer scale. This zonation may be controlled by spore physiology, physical factors, and elemental diffusion. Here we present a recently developed nanometer-scale secondary ion mass spectrometry (NanoSIMS) technique that allows the direct visualization and quantification of elemental concentration gradients within spores. By using NanoSIMS depth profile analysis together with sample preparation techniques such as focused ion beam (FIB) sectioning, we are able to probe the three dimensional elemental distribution within individual Bacillus thuringiensis israelensis (Bti) spores with nanometer scale resolution (~10 nm depth and 50 nm lateral). Our results show the expected distributions for physiologically controlled elements (Ca, P) and provide baseline data other elements (e.g., Li, F, Cl, S). We also demonstrate cation and anion mobility in spores under hydrous conditions. Our results suggest a permeation mechanism by which elements diffuse into and out of the spore along hydration pathways on rather short time scales. Additional studies are in progress to define the rates and mechanisms controlling ion mobility in spores. @FootnoteText@ @footnote@This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract W-7405-Eng-4.

4:20pm **AS+BI-WeA8 Vacuum Ultraviolet Postionization for Mass Spectrometry of Small Molecule Analytes in Bacterial Biofilms**, *L. Hanley,* ***P.D. Edirisinghe****, M. Zhou,* University of Illinois at Chicago*; K.A. Skinner-Nemec, C.S. Giometti,* Argonne National Laboratory*; J.F. Moore,* MassThink*; J.E. Hunt, W.F. Calaway, M.J. Pellin,* Argonne National Laboratory

Mass spectrometric analysis and imaging of intact microbial biofilms are difficult with established methods. A new experimental strategy is discussed for analyzing small molecule analytes within intact biofioms: laser desorption followed by postionization with 7.87 eV radiation of molecular analytes whose ionization potentials have been lowered by chemical derivatization with an aromatic tag.@footnote 1@,@footnote 2@ Postionization mass spectrometry with derivatization is developed on small peptides with aromatic or native tags such as a tryptophan residue. The new method is then applied to the detection of a quorum sensing peptide in a Bacillus subtilis bacterial biofilm. Finally, detection of an antibiotic is demonstrated by direct 7.87 eV postionization, without derivatization. These mass spectrometric methods show promise for the study of antibiotic resistance in microbial biofilms as well as other studies of small molecule analytes within complex biological matrices. @FootnoteText@ @footnote 1@P.D. Edirisinghe et al., Anal. Chem. 76 (2004) 4267.@footnote 2@L. Hanley et al., Appl. Surf. Sci. (2006), in press.

4:40pm **AS+BI-WeA9 Chemical and Biological Differentiation of Human Breast Cancer Cell Types Using Time-of-Flight Secondary Ion Mass Spectrometry**, *K. Kulp, E. Berman, M. Knize, J. Felton, E. Nelson, J. Montgomery,* Lawrence Livermore National Laboratory*; L. Wu, D. Shattuck,* University of California, Davis*;* ***K. Wu****,* Lawrence Livermore National Laboratory

We use time-of-flight secondary ion mass spectrometry (TOF-SIMS) to image and classify individual cells on the basis of their characteristic mass spectra. Using statistical data reduction on the large data sets generated during TOF-SIMS analysis, similar biological materials can be differentiated on the basis of a combination of small changes in protein expression, metabolic activity and cell structure. We apply this technique to image and differentiate three carcinoma-derived human breast cancer cell lines (MCF-7, T47D, and MDA-MB-231). In homogenized cells, we show the ability to differentiate the cell types as well as cellular compartments (cytosol, nuclear, and membrane). These studies illustrate the capacity of TOF-SIMS to characterize individual cells by chemical composition, which could ultimately be applied to detect and identify single aberrant cells within a normal cell population. Ultimately, we anticipate characterizing rare chemical changes that may provide clues to single cell progression within carcinogenic and metastatic pathways.

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