Nanoscale Science and Technology Division
Room 304 - Session NS1+AS+EM-MoA

Correlative Microscopy for Nanoscale Characterization

Moderators: Sidney Cohen, Weizmann Institute of Science, Israel, **Georg Fantner**, EPFL, Switzerland

1:40pm **NS1+AS+EM-MoA-1 Large Volume 3D Biological Imaging with Electron and Cryo-Super-Resolution Microscopy***, **Harald Hess,*** HHMI, Janelia **INVITED**

Volume or 3D electron microscopy continues to expand its potential for imaging ever larger biological entities while preserving a best compromise step edge isotropic resolution of 5-10 nm. This was driven by the challenge of imaging the entire fly brain in sufficient detail for extracting the circuitry of connectome. While the resolution is not of the standards of TEM's, such resolution is of unique value when it encompasses whole cells and complete tissues.We will review the capabilities of FIB-SEM, with ~100 micron sized volumes. Numerous examples can be browsed on openorganelle.com . A cryogenic protocol involving sample vitrification, cryogenic imaging by structured illumination or by photoactivated localization microscopy then followed by staining and resin embedding can then produce the sample suitable for further FIBSEM imaging. This effectively adds protein location information as a color to the 3D EM image. Likewise, several examples correlating specific proteins in the nucleus, on membranes, on and defining organelles and vesicles. Prospects of future challenges are discussed. We will also describe a system capable of imaging volumes approaching 1 mm^3 It is based on Ion Beam Etching and Milling with a Multi beam Scanning Electron MicroscopeIBEaM MSEM.

2:20pm **NS1+AS+EM-MoA-3 The Role of SnO_2 Processing on Ionic Migration in Multi-Halide Perovskites***, **Holland Hysmith,*** University of Tennessee Knoxville*; S. Park,* National Renewable Energy Laboratory*; A. Ievlev, Y. Liu,* Oak Ridge National Laboratory*; K. Zhu,* National Renewable Energy Laboratory*; M. Ahmadi,* University of Tennessee Knoxville*; J. Berry,* National Renewable Energy Laboratory*; O. Ovchinnikova,* Oak Ridge National Laboratory

Moving towards a future of efficient, accessible, and less carbon reliant energy devices has been at the forefront of energy research innovations for the past 30 years. Multi-halide perovskite (MHP) thin films have gained significant attention due to their flexibility of device applications and tunable capabilities for improving power conversion efficiency.[1] Many behavioral aspects to MHP's are thoroughly investigated: functionality of grain boundaries, recombination effects, ionic migration patterns, and hysteresis.[2-4]

Chemical Vapor Deposition (CVD) is a widely used technique for thin film coatings due to its ability for producing high volume batches of MHP's with larger grain sizes, fewer defects, and fewer grain boundary formations.[5-6] Additionally, nanoparticle processing has been applied to induce enlargement of grain boundaries, showcasing larger current signals than its MHP counterparts.[7] Therefore, how does common substrate processing techniques (i.e. CVD, nanoparticles, hybrid) influence the behavior of MHP phenomenon such as ion migration and grain boundary formation? Speculated as inducing ionic recombination and driving I-V hysteresis in MHP's, understanding how chemistry can be tuned to reduce such effects would be optimal.[8-9]

We demonstrate how a hybrid approach of CVD and nanoparticle SnO_2 substrate processing significantly improves the performance of $(FAPbI_3)_{0.97}(MAPbBr_3)_{0.03}$ perovskites in comparison to each technique utilized on its own. As shown in **Figure 1**, higher performing hybrid devices exhibit fused grain boundary formations, not seen in exclusive CVD or nanoparticle devices. Conductive Atomic Force Microscopy (c-AFM) was used to track fused boundary locations and differentiate them from topographic features. Such fusing behavior has been previously observed to showcase higher counts of current and reduce defects such has halide vacancies.[7]

In summary, to understand the chemistry behavior with respect to each device interface, Time of Flight Secondary Ionization Mass Spectrometry (ToF-SIMS) depth profiling was applied. Demonstrated in **Figure 2**, migration of K^+, Na^+, Ca^+, FA^+, MA^+ was found in hybrid devices, in addition to Ca^+ and Na^+ clustering on the perovskite/air layer. Salt clustering could be correlated to the fusing effect demonstrated in the surface morphology imaged in c-AFM. Presence of K^+ has shown to reduce defects driven by alkali iodides like NaI^- and Ca^+ can help with enlarging the bandgap layer in studies where Ca+ was used to replace Pb^+.[10-11] Furthermore, reduced separation between positive ion such as MA^+ and FA^+ from negative ions can decrease the potential responsible for I-V hysteresis.[12]

2:40pm **NS1+AS+EM-MoA-4 Nanoplastic Arrays – from Chaotic Measurements to New Order***, A. Madison, D. Westly, R. Ilic, C. Copeland, A. Pintar, C. Camp, J. Liddle,* ***Samuel M. Stavis,*** National Institute of Standards and Technology (NIST)

Nanoplastic particles are ubiquitous contaminants of the environment, and their unknown hazards are of deepening concern. Optical microspectroscopy is essential to elucidate the structure–property relationships of nanoplastic particles. However, a lack of standards that are fit for purpose limits the reliability of such measurements, resulting in a growing spate of chaotic reports. In particular, the default standard of a colloidal suspension has disadvantages, with sample preparation typically resulting in disordered arrays of nanoparticles with uncontrolled sizes on imaging substrates. Moreover, existing nanoplastic standards can have broad and asymmetric distributions of optical properties. This issue confounds inference of dimensional properties and requires further study.

Optical microspectroscopy often involves contrast from Rayleigh scattering, fluorescence emission, and Raman scattering to detect, quantify, and identify nanoplastic particles. Numerous issues limit accuracy, including optical responses that vary with nanoparticle dimensions and imaging systems that present aberration effects. These issues require standards that provide reference values of dimensional, optical, and positional properties. The latter issue is unexplored, motivating a new order of microscopy standards.

We introduce the concept of the nanoplastic array, addressing these issues. This prototype standard enables calibration, correction, and correlation of image data from multiple instruments, improving the accuracy of microspectroscopy measurements. To prove the concept, we fabricate nanoplastic arrays in nanoscale films of phenolic resin by electron-beam lithography, including both fluorescent dopants and sorbents to study optical properties that are indicators of chemical sorption and resulting hazards.

Our nanoplastic arrays feature three types of nanostructures. The simplest is a uniform film, enabling correction of non-uniform irradiance for the accurate analysis of fluorescence intensity, and providing reference spectra for Raman measurements. Building in complexity, arrays of uniform pillars provide reference dimensions and positions to correlate and calibrate multiple imaging modes. Finally, and most complex, variable pillar arrays facilitate measurements of optical properties as a function of dimensional properties, with fine gradations of pillar diameter enabling quantification of the limits of detection.

Nanoplastic arrays will enable new accuracy and reliability in optical microspectroscopy, advancing the quantitative study of nanoplastic contaminants to transform unknown hazards into known quantities.

3:00pm **NS1+AS+EM-MoA-5 Development of Nanoendoscopy-AFM for Visualizing Intracellular Nanostructures of Living Cells***, **Keisuke Miyazawa,*** Kanazawa University, Japan*; M. Penedo,* EPFL, Switzerland*; N. Okano, H. Furusho, T. Ichikawa, M. Shahidul Alam, K. Miyata,* Kanazawa University, Japan*; C. Nakamura,* AIST, Japan*; T. Fukuma,* Kanazawa University, Japan

Atomic force microscopy (AFM) is the only technique that allows label-free imaging of nanoscale biomolecular dynamics, playing a crucial role in solving biological questions that cannot be addressed by other major bioimaging tools (fluorescence or electron microscopy). However, such imaging is possible only for systems either extracted from cells or reconstructed on solid substrates. Thus, nanodynamics inside living cells largely remain inaccessible with the current nanoimaging techniques. Here, we overcome this limitation by the nanoendoscopy-AFM, where we fabricate a needle-like nanoprobe (diameter < 200 nm, length > 500 nm) made of Sillicon or Carbon, and insert it into a living cell directly in order to measure a force curve, and visualize 2D or 3D internal structures of living cells by the measured 3D force applied to the tip during three-dimensional tip scanning. By using this method, we measured the 3D force image of a human cancer cell (HeLa). The result clearly shows the nucleus in the living cell. In addition, our results using the developed nanoendoscopy-AFM showed undetectable changes by the previous methods such as actin fiber three-dimensional (3D) maps, and 2D nanodynamics of the membrane inner scaffold in the living cells. Unlike previous AFM methods, the nanoprobe directly accesses the target intracellular components, exploiting all the AFM capabilities, such as high-resolution imaging, nanomechanical mapping, and molecular recognition. These features of the nanoendoscopy-AFM should greatly expand the range of intracellular

structures observable in living cells, and contribute to the various life science research fields.

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