Nanometer-scale Science and Technology Division
Room 203A - Session NS+MN+PC+SS-WeA

IoT Session: Bio at the Nanoscale

Moderators: Juraj Topolancik, Roche Sequencing Solutions, Liya Yu, NIST Center for Nanoscale Science and Technology

3:00pm **NS+MN+PC+SS-WeA-3 Nanoscale Label-free Imaging of Protein Molecules via Photo-induced Force Microscopy***, D Nowak,* ***Sung Park,*** Molecular Vista

Nanoscale real space imaging of biological and biomaterial surface is not straightforward even with advances in microscopy techniques. Photo-induced Force Microscopy (PiFM) [1] combines infrared (IR) absorption spectroscopy and atomic force microscopy (AFM) via illumination of the tip-sample junction with tunable IR laser light and mechanical detection of forces acting on the tip in response to absorption of light by the sample. By mapping the IR absorption of the sample as a function of IR wavelength and position, nm-scale resolution is achieved in displaying the locations of heterogeneous materials on the surface of a sample. For protein molecules, amide I and II bands are readily accessible via tunable quantum cascade laser and provide ways to interrogate the molecule's local chemical environment. PiFM can provide both high resolution spectral imaging at a fixed wavenumber and full PiFM spectrum (analogue to FTIR spectrum) with a spectral resolution of 1 cm^{-1} and spatial resolution of sub-10 nm. Results on collagen molecules, individual icosahedral protein cages, and nanoparticle/protein systems will be presented.

[1] D. Nowak et al., Sci. Adv. **2**, e150157 (2016).

3:20pm **NS+MN+PC+SS-WeA-4 Evaluating Reaction-diffusion Immunoassays via High-resolution Imaging Techniques***, Imanda Jayawardena,* University of Queensland, Australia*; S Corrie,* Monash University, Australia*; L Grondahl,* University of Queensland, Australia

Immunodiffusion is a simple assay used for the determination of a target protein concentration in a biological sample using a distance-based measurement. The assay allows the sample containing the antigen of interest to combine with an antiserum in a gel-based substrate leading to the formation of a ring-shaped precipitate ('precipitin ring'), the size of which is proportional to target protein concentration.[1] Using the malarial antigen HRP2 and antisera from immunized rabbits as the model system, for the first time, we are investigating transforming the current immunodiffusion assays into a more rapid and sensitive format.

The traditional assay substrate, agarose, is a severely diffusion limited system. The porosity of the gel is a key determinant of diffusion properties and is an essential parameter required for the study and modification of the assay. However, the scientific data on agarose pore size determination is based on artefact laden microscopy images of agarose hydrogels. Thus, we have performed an in-depth investigation on best imaging techniques for accurate pore size determination. The precipitin ring structure is a band of antigen-antibody precipitate, in significant contrast with the surrounding gel substrate. A brief preliminary investigation has been reported by Fedorov et al. on precipitin ring structure[2], and we have extended this work by applying microscopic imaging techniques.

For imaging hydrogels, high pressure frozen gels were subjected to cryo-SEM[3], and was established as the most accurate technique to study the native structure of the gel. Atomic force microscopy was found to complement cryo-SEM data while CLSM due to its limited resolution was found to be inadequate for the imaging of hydrogels. For imaging the protein-rich precipitin ring structure, high pressure frozen ring sections were subjected to cryo-SEM, however, more meaningful insight on the ring structure was obtained in this instance through CLSM studies.

Herein, we aim to present our work described above on imaging agarose hydrogels for accurate pore size determination and imaging precipitin ring structures associated with immunodiffusion assays.

1. Mancini, G.; Carbonara, A. t.; Heremans, J. *Immunochemistry* **1965**

2. Fedorov, A. A.; Kurochkin, V. E.; Martynov, A. I.; Petrov, R. V. *Journal of Theoretical Biology* **2010**

3**.** Aston, R.; Sewell, K.; Klein, T.; Lawrie, G.; Grøndahl, L. *European Polymer Journal* **2016**

4:20pm **NS+MN+PC+SS-WeA-7 The Last Nanometer – Hydration Structure of DNA and Solid Surfaces Probed by Ultra-High Resolution AFM***, Uri Sivan, K Kuchuk, I Schlesinger,* Technion - Israel Institute of Technology, Israel **INVITED**

Recent advancements in atomic force microscopy facilitate atomic-resolution three-dimensional mapping of hydration layers next to macromolecules and solid surfaces. These maps provide unprecedented information on the way water molecules organize and bind these objects. Since the hydration structure governs the energetics of solvation and interactions between objects immersed in solution, the new data are invaluable when trying to resolve fundamental questions such as identification of molecular binding sites and interaction mechanisms.

After a short presentation of our home-built microscope, characterized by sub 0.1 Å noise level, the talk will focus on two representative studies. The first one will disclose our recent finding that in solutions in contact with atmosphere, hydrophobic surfaces are generically coated with a dense layer of adsorbed gas molecules. This layer renders the hydrophobic interaction a certain universality, regardless of the underlying surface. The second study will present our recent success in obtaining ultra-high resolution images of DNA and 3d maps of its hydration structure. This study shows that labile water molecules concentrate along the DNA grooves, in agreement with known position of DNA binding sites.

5:00pm **NS+MN+PC+SS-WeA-9 Open-hardware, High-speed Atomic Force Microscopy using Photothermal Off-resonance Tapping***, Georg Fantner,* École Polytechnique Fédéral de Lausanne, Switzerland **INVITED**

Self-assembly of protein complexes is at the core of many fundamental biological processes. To reach a comprehensive understanding of the underlying protein self-assembly reactions, high spatial and temporal resolution must be attained. This is complicated by the need to not interfere with the reaction during the measurement. Since self-assemblies are often governed by weak interactions, they are especially difficult to monitor with high-speed atomic force microscopy due to the non-negligible tip-sample interaction forces involved in current methods. Here we develop a high-speed atomic force microscopy technique, photothermal off-resonance tapping (PORT), which is gentle enough to monitor self-assembly reactions driven by weak interactions. Using photothermal actuation on ultra-small HS-AFM cantilevers we perform force-distance curves at two orders of magnitude higher rates than in conventional off-resonance methods. From the time-domain tip sample interaction we extract tip-sample force curves to quantify the *"static"*forces due to the cantilever deflection, and the *"impact"* forces due to the rapid deceleration of the cantilever tip upon impact. Experimental characterization of the tip-sample forces in HS-tapping mode-AFM and PORT revealed that imaging forces in PORT are less than 1/5thof those exerted in conventional HS-AFM.

One of the key enabling factors for PORT is the real time control of the cantilever position using photothermal excitation. This requires low level, control of the feedback architecture and optimized AFM instrumentation. I will discuss the relevant components developed in my laboratory and explain how we share them with the broader scientific community using an open-hardware scheme.

We apply PORT to dissect the self-assembly reaction of SAS-6 proteins, which form a nine-fold radially symmetric ring-containing structure that seeds formation of the centriole organelle present in all eukaryotic cells. Using machine learning algorithms we traced hundreds of molecules over time to extract reaction kinetics from single molecule interactions. These measurements show that 9-fold closed SAS-6 rings are under pre-tension in their natural state. Due to the high temporal and force resolution provided by PORT, we found that, contrary to the current belief, more than one assembly route exists to reach the nine fold symmetry. These observation resets our current thinking about the assembly kinetics of this crucial step in cell replication.

5:40pm **NS+MN+PC+SS-WeA-11 Development of Multimodal Chemical Nano-Imaging for *in situ* Investigations of Microbial Systems***, A Bhattarai, B O'Callahan, P El Khoury,* ***Scott Lea,*** Pacific Northwest National Laboratory*; K Park, E Muller, M Raschke,* University of Colorado Boulder

Existing genomic and biochemical methods cannot directly probe the physical connections involved in microbial metabolic processes over relevant length scales, spanning the nano-meso-micrometer spatial regimes. Determining the location and function of such biomolecules would aid in identifying the mechanisms governing microbial interactions. We are addressing these technical and conceptual gaps by developing a single multimodal chemical imaging platform that can interrogate

biomolecules in living systems using three complementary label-free, nanoscale, ultrasensitive chemical imaging techniques:

Infrared scattering scanning near-field optical microscopy (IR *s*-SNOM)

Tip-enhanced Raman nano-spectroscopy (TERS)

Multimodal hyperspectral optical nano-spectroscopy.

We have built and developed these imaging modalities independently prior to integration into a single, multimodal chemical nanoscope. As part of our benchmarking experiments, we performed TERS measurements targeting prototypical systems and constructs and demonstrated <1 nanometer precision in ambient TERS chemical imaging measurements.[1] We also established an overall broader scope of TERS[2] and illustrated that TERS is not restricted to nanoscale chemical imaging, but can also be used to probe different aspects of local fields confined to a few nanometers. Our new setup, equipped with a hyperspectral imager, enables hyperspectral fluorescence, optical absorption, dark-field scattering, Raman scattering, and topographic imaging. Recently, we used this capability to visualize pigments in lipid monolayers and within a single live *T. lutea* cell in solution.[3] For IR *s*-SNOM, we are working on developing an AFM capable of bottom illumination and collection of IR light to support measurements in aqueous environments. The approach would use a piezoelectric scanner mounted ZnSe prism to enable evanescent wave illumination and collection of scattered IR light. We are also benchmarking the IR *s*-SNOM with the TERS and hyperspectral imaging modalities on a number of model biological systems including bacteria, collagen, and cytochromes.

This unique AFM-based instrument could be used to investigate a wide range of biomolecules through their characteristic electronic and vibrational signatures, over the nano-meso-micrometer scales. This platform will not only enable recording chemical images of single microbial cells at the subcellular level, but it will also enable mapping entire microbial communities with chemical selectivity.

1. Bhattarai A and El-Khoury PZ (2017) *Chem Commun***53**(53): 7310-7313.

2. Bhattarai A *et al*. (2017) *Nano Lett***17**(11): 7131-7137.

3. Novikova IV *et al*. (2017) *Chem Phys***498-499**: 25-32.

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