SDU


# IMAGING 2020 WORKSHOP

## STATE-OF-THE-ART METHODS FOR MATERIALS AND BIOLOGICAL SCIENCES: FROM OPTICAL TO ION MICROSCOPY

## Abstracts

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# IMAGING AND ANALYTIC POSSIBILITIES IN THE HELIUM ION MICROSCOPE

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Helium Ion Microscopy (HIM) utilizes a Gas Field Ion Source (GFIS) to create a Helium or Neon ion beam with a diameter better than 0.5nm and 1.8 nm, respectively. The method is well known for its high resolution imaging and nano-fabrication capabilities which it is able to provide not only for conducting but also insulating samples without the need for a conductive coating. The latter specimens are typically found in the fields of biosciences, MEMS/NEMS technology, catalyst research and many others. The availability of He and Ne ions with either low or moderate sputter yields, allow direct write nano-structuring with a precision below 10 nm in the HIM [1, 2]. However, the existing GFIS based focused ion beam (FIB) tools suffer from the lack of a well-integrated analytic method that can enrich the highly detailed morphological images with materials contrast. While HIM technology is relatively young several efforts have been made to add such an analytic capability to the technique. So far, ionoluminescence [1, 3], backscattering spectrometry (BS) [1, 4], and secondary ion mass spectrometry (SIMS) using a magnetic sector [5] or time of flight (TOF) setup have been demonstrated [4]. I will present results obtained using the above mentioned methods beginning with ionoluminescence and its application to various materials systems. The method is in particular suited for the analysis of various defects present in the sample and the behaviour of defects under ion beam irradiation. In the second part of the talk I will present our newly developed TOF-BS and TOF-SIMS setup which allow to obtain information on the composition of the sample. They both utilize the same cost efficient and minimal invasive pulsing scheme for the primary ion beam. The lateral resolution reached for TOF-BS is approximately 50 nm while for TOF-SIMS a value of 8nm could be reached. First images will be presented and the performance of the TOF-SIMS spectrometer will be discussed.

**References**

[1] Gregor Hlawacek and Armin Gölzhäuser, editors. *Helium Ion Microscopy*. NanoScience and Technology. Springer International Publishing, Cham, 2016.

[2] Gregor Hlawacek, Vasilisa Veligura, Raoul van Gastel, and Bene Poelsema. *Helium ion microscopy*.J. Vac. Sci. Technol. B Microelectron. Nanom. Struct., 32(2):020801, March 2014.

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[3] Vasilisa Veligura, Gregor Hlawacek, Raoul van Gastel, Harold J.W. Zandvliet, and Bene Poelsema. *Investigation of ionoluminescence of semiconductor materials using helium ion microscopy*. J. Lumin.,157:321–326, January 2015.

[4] Nico Klingner, René Heller, Gregor Hlawacek, J. von Borany, John A. Notte, Jason Huang, and Stefan Facsko. *Nanometer scale elemental analysis in the helium ion microscope using time of flight spectrometry*. Ultramicroscopy, 162: 91–97, March 2016.

[5] Tom Wirtz, David Dowsett, and Patrick Philipp. *SIMS on the Helium Ion Microscope: A Powerful Tool for High-Resolution High-Sensitivity Nano-Analytics.* In Gregor Hlawacek and Armin Gölzhäuser, editors, *Helium Ion Microsc.*, chapter 13, pages 297–323. Springer International, Switzerland, 2016.

# SIMS ON THE HELIUM ION MICROSCOPE: A UNIQUE TOOL FOR HIGHEST RESOLUTION NANO-ANALYTICS AND CORRELATIVE MICROSCOPY

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The Helium Ion Microscope (HIM) is known as a powerful tool for high resolution ion microscopy and ion nano-fabrication. In 2015, we first presented a Secondary Ion Mass Spectrometry (SIMS) system which we specifically developed for the HIM in order to provide as well nano-analytical capabilities [1]. SIMS is based on the generation and identification of characteristic secondary ions by irradiation with a primary ion beam. It is an extremely powerful technique for analysing surfaces owing in particular to its excellent sensitivity (detection limits down to the ppb/major and trace elements are detectable), high dynamic range (a same signal can be followed over several orders of magnitude), and ability to differentiate between isotopes. Our SIMS system is based on (i) specifically designed secondary ion extraction optics coupled with post-acceleration transfer optics, providing maximized extraction efficiency while keeping a finely focused primary ion beam for highest lateral resolution, (ii) a compact floating double focusing magnetic sector mass spectrometer allowing operation in the DC mode at full
transmission (and hence avoiding duty cycles like in TOF systems that either lead to very long acquisition times or, for a same acquisition time, intrinsically limits the sensitivity) and (iii) a specific detection system allowing the detection of several masses in parallel.

We have demonstrated that our instrument is capable of producing (i) mass spectra with high mass resolution, (ii) very local depth profiles and (iii) elemental SIMS maps with lateral resolutions down to 12 nm [1-5]. Furthermore, by combining high resolution SE images with elemental and isotopic ratio maps from SIMS, in-situ correlative imaging can be performed [2,3,6]. This approach allows SE images of exactly the same zone analysed with SIMS to be acquired easily and rapidly, followed by a fusion between the SE and SIMS data sets. Moreover, the depth profiling capability of the SIMS add-on allows it to follow the chemical composition in real time during nano-patterning in the HIM for applications such as end-pointing.

Here, we will review the instrument layout as well as its performance and present a number of examples taken from various fields of materials science (battery materials, solar cells, micro-electronics, coatings, multilayers) to show the powerful analytical capabilities and correlative microscopy possibilities of the tool.

**References**

[1] T. Wirtz, P. Philipp, J.-N. Audinot, D. Dowsett, S. Eswara, Nanotechnology 26 (2015) 434001.

[2] T. Wirtz, D. Dowsett, P. Philipp, Helium Ion Microscopy, edited by G. Hlawacek, A. Gölzhäuser, Springer, 2017.

[3] D. Dowsett, T. Wirtz, Anal. Chem. 89 (2017) 8957-8965.

[4] P. Gratia, G. Grancini, J.-N. Audinot, X. Jeanbourquin, E. Mosconi, I. Zimmermann, D. Dowsett, Y. Lee, M. Grätzel, F. De Angelis, K.Sivula, T. Wirtz, M. K. Nazeeruddin, J. Am. Chem. Soc. 138 (49) (2016) 15821–15824.

[5] P. Gratia, I. Zimmermann, P. Schouwink, J.-H. Yum, J.-N. Audinot, K. Sivula, T. Wirtz, M. K. Nazeeruddin, ACS Energy Lett. 2 (2017) 2686-2693.

[6] F. Vollnhals, J.-N. Audinot, T. Wirtz, M. Mercier-Bonin, I. Fourquaux, B. Schroeppel, U. Kraushaar, V. Lev-Ram, M. H. Ellisman, S. Eswara, Anal. Chem. 89 (2017) 10702-10710.

## HELIUM ION AND OPTICAL MICROSCOPY OF SPIDER SILK

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Spider silk has many properties that may be of industrial use as the tensile strength of spider silk is comparable to that of alloy steel and the silk is about half as strong as for example Kevlar but has the advantage of being spun at room temperature. The aim of this project is to characterize Major Ampullate silk (MAS) and Minor Ampullate silk (MiS) spider silk fibers from the orb web weaving spider Nephila Madagascariensis by determining the biochemical, nano- and microscopic structures within the silk and couple these to the macroscopic properties such as tensile strength and elasticity. Using Coherent Anti-Stokes Raman Scattering (CARS) and fluorescence microscopy the lipids and proteins of the fiber were analyzed and visualized revealing the overall structure of the fiber. To image the nanoscopic structures within the silk, He Ion Microscopy was applied. By surface sputtering it was possible to etch away the most outer layers in order to visualize the inner protein arrangements with no special sample preparation. It was found for the first time without cryofreezing that the protein core consists of fibrils arranged parallel to each other and to the long axis of the fiber.

## IMAGING OF BIOLOGICAL CELLS WITH HELIUM-ION MICROSCOPY

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This HIM imaging study of biological cells focuses on neuronal differentiated inferior turbinate stem cells as well as mouse neurons which were prepared in different ways for imaging under the required vacuum conditions. Charging of specimens without conductive coating was effectively compensated by an electron flood gun. Therewith, extremely small features at cell surfaces were imaged with an estimated edge resolution of 1.5 nm. Indications of lipid rafts at the surface of all investigated cells will be discussed.

## OPERANDO STUDIES OF BATTERY MATERIALS

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Correlating material properties to the structure at multiple length scales lies at the core of material science. This can only be achieved through combination of a series of characterization techniques, which yield information about different length scales ranging from the atomic-, over the nano- and micro- to the macro-scale and often combines direct probing as in imaging with indirect probing as in diffraction. Furthermore, to truly probe the dynamic behavior of the materials, information needs to be collected while the materials are operating, i.e. via *operando* or *in situ* methods.
For battery materials, understanding the nature of the structural transitions that occur in the electrodes as the batteries are charged and discharged are of vital importance, as these transitions determine the potential, efficiency, reversibility and stability of the system. This talk will show how *operando* synchrotron radiation powder X-ray diffraction (SR-PXD) in combination with imaging through transmission and scanning electron microscopy (TEM and SEM) and micro X-ray fluorescence can yield unprecedented knowledge about battery electrode materials on multiple length scale.

## PAIR CORRELATION ANALYSIS OF FIXED PALM AND POWER SPECTRAL POINT ANALYSIS OF LIVE PALM APPLIED ON AQP3

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The hormone Arginine Vasopressin (AVP) increases urine concentration via an increase in cAMP, leading to insertion of AQP2 containing vesicles into the apical plasma membrane. We previously found that a short-term increase in cAMP leads to an increase in lateral diffusion of AQP3, revealing short-term regulation. To further study if AQP3 is regulated at the nanoscale level, we first combined single molecule detection using Photoactivatable Localization Microscopy (PALM) imaging of fixed cells with pair correlation (PC). This showed that AQP3 organize in nano-domains smaller than 60 nm and upon stimulation mimicking vasopressin, changed organization to 60 – 200 nm sized nano-domains. Thus, PC-PALM revealed regulation at the nanometer resolution.
Furthermore, we performed live-PALM of AQP3 upon cAMP stimulation and have done analysis by power spectral analysis. This is the first time live-PALM data is analyzed by power spectra analysis. The analysis was done by first identifying isolated spots and fitting with a two-dimensional point-spread function. The localization errors were found theoretically and the diffusion coefficient for each trajectory was calculated using a covariance-based estimator. To demonstrate that the considered

molecules were indeed freely diffusing with identical diffusion coefficients, we calculated the power-spectrum of each trajectory. The power-spectral values were rescaled with their expectation values given theoretically as a function of the averaged diffusion coefficient and the localization errors. The power spectral analysis did not show increased diffusion coefficient upon cAMP stimulation compared to the controls.

Thus, fixed PALM revealed that AQP3 changed nanoorganization in the plasma membrane upon stimulation mimicking vasopressin; from an even distribution to an organization in nanoclusters. This indicates short-term hormone regulation of AQP3 at the nanoscale level, which may be important in urine concentration. PC-PALM may be used to reveal so far undetectable protein regulation at the nanoscale. We furthermore did live-PALM and showed an increase in diffusion after cAMP stimulation using power spectral analysis.

## ZEISS X-RAY MICROSCOPY - 3D AND 4D IMAGING OF PRACTICAL VOLUME SAMPLES

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X-ray tomography has emerged as a new powerful imaging technique that obtains 3D structural information from opaque samples under a variety of conditions and environments. It has rapidly become an accepted laboratory technique offering quantitative information in both the materials sciences and life sciences. We present ways in which non-destructive 3D volumetric information, obtained via laboratory nanoscale and sub-micron X-ray microscopy (XRM) are increasingly used to probe scientific questions as a complement to Electron- and Light-based microscopy methods. These correlative methods, relating to XRM, provide an opportunity to study materials evolution at multiple length scales in 3D and utilize this information to inform or guide postmortem analysis to be most efficient.

# IMAGING POLYMORPHIC, TEXTURED SQUARAINE THIN FILMS AND FILM BLENDS

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The dihydroxy anilino squaraine with isobutyl side chains SQIB crystallizes into at least two polymorphic structures: a monoclinic and an orthorhombic phase. Spin-casted thin films show distinct birefringent morphological features that can be correlated with the polymorphic phases. The formation of the features is controlled by admixing the fullerene PC60BM into the solution used for spincoating as well as by subsequent thermal annealing. The absorbance spectra show characteristic signatures of molecular H- and J-aggregates featuring an additional Davydov splitting. The in-plane orientation of the transition dipoles for the Davydov components is deduced from polarized light microscopy and related to the electrostatic response of the thin film probed by Kelvin Probe Force Microscopy (KPFM).