BP 11: Bioimaging and Spectroscopy II

Time: Monday 15:00–17:15 Location: H43

BP 11.1 Mon 15:00 H43

Cellular Structures Resolved by X-Ray Diffraction with Micro- and Nanometer Beamsize: From Stem Cells to Cardiomyocytes — •Marten Bernhardt[1], Jan-David Nicolas[1], Marius Priebe[1], Markus Osterhoff[1], Carina Wollnik[2], Ana Diaz[3], Marina Eckermann[1], Florian Rehfeldt[2], and Tim Salditt[1] — [1]Institute for x-ray physics, Göttingen — [2]Third Institute of Physics - Biophysics, Göttingen — [3]Paul Scherrer Institut, Villigen

High resolution scanning small angle x-ray scattering (scanning SAXS) enables an access to local cellular structures on a mesoscopic scale. We have performed micro- and nanofocus SAXS recordings on naive human mesenchymal stem cells (hMSCs), neonatal rat cardiomyocytes and other differentiated cell lines and characterized their 2D-diffraction patterns: Results on freeze-dried samples reveal naive hMSCs to be rather weak scatterers with little anisotropic scattering behavior. In contrast, disassembled cells from neonatal rat tissue show a strong anisotropic diffraction signal, that enable us to track down filamentous structures by automated principal component analysis (PCA). These structures can be correlated to the visible light micrograph of fluorescently labeled actin. Successful wet chamber experiments provide a basis for future single cell recordings in chemically fixated and alive states.

BP 11.2 Mon 15:15 H43

Imaging proteins at the truly single molecule level — •Jean-Nicolas Longchamp[1], Stephan Rauschenbach[2], Sabine Abb[2], Conrad Escher[1], Tatiana Latychevskaia[1], Klaus Kern[2,3], and Hans-Werner Fink[1] — [1]Physics Department of the University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland — [2]Max Planck Institute for Solid State Research, Heisenbergstrasse 1, DE-70569 Stuttgart, Germany — [3]Institut de Physique de la Matière Condensée, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland

Imaging a single protein has been a long-standing dream for advancing structural biology and with this various fields in natural science. Here I will show that, sub-nanometer resolved images of individual folded proteins have been obtained for the first time ever. Electrospray ionization for the specific selection and sound deposition of individual proteins onto ultraclean freestanding graphene in an ultra-high vacuum environment and low-energy electron holography for the non-destructive imaging are combined in a novel experimental workflow.

BP 11.3 Mon 15:30 H43

Developing a modular sensor platform for intracellular environmental sensing — •Torsten Rendler[1], Jitka Slegerova[2], Ondrej Zemek[3], Jan Kotek[3], Andrea Zappe[1], Zhiqin Chu[1], Petr Cigler[2], and Jörg Wrachtrup[1] — [1]3. Physikalisches Institut, Universität Stuttgart, Deutschland — [2]Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo nam. 2, Prague 6, Czech Republic — [3]Department of Inorganic Chemistry, Faculty of Science, Charles University, Hlavova 8, Prague 2, Czech Republic

The development of nano-scaled sensors that can be embedded intracellular without minor modification on the local environments are important for various biological and medical applications. For this purpose we utilize the nitrogen vacancy (NV) center in nanodiamond providing close to single spin sensitivity and nanometer resolution at ambient conditions, providing at the same time long term stability and low cytotoxicity in biological systems. By combining the well-established clinical magnetic resonance imaging (MRI) agent namely Gadolinium with NV spin relaxometry in nanodiamonds, we developed a hybrid sensor that can be adjusted to monitor various physiological quantizes. As an example we demonstrate sensitivity to pH and changes in the redox potential at submicron length scales in a microfluidic channel. Furthermore we introduce our hybrid sensor system into cells and investigate it's response on the local cellular environment. The current work shall open a novel approach for studying subtle changes in physiological conditions, pointing to a new pathway for diagnosis and therapeutics at subcellular level.

15 min break

Invited Talk BP 11.4 Mon 16:00 H43

Chromophore Photophysics in Fluorescent Proteins of the GFP family — •Gerd Ulrich Nienhaus — Institute of Applied Physics, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany — Institute of Nanotechnology, Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany — Institute of Toxicology and Genetics, Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany — Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Genetically encoded fluorescent proteins (FPs) of the green fluorescent protein (GFP) family have become indispensable as marker tools for imaging live cells, tissues and entire organisms. Great efforts are ongoing in many labs to further optimize their photophysical properties by genetic engineering. The p-HBI chromophore of GFP (or a variant thereof in other FPs), which forms autocatalytically in the interior of the polypeptide chain, is exquisitely sensitive to the protein environment. By introducing amino acid modifications, its photophysical properties can be changed, e.g., for the purpose of color tuning. In photoactivatable FPs, chromophore properties can even be controlled by light irradiation, which is of key relevance for super-resolution optical imaging. Photoactivation may occur reversibly, by photoinduced cis-trans isomerization of the chromophore (photoswitching), or by permanent photochemical modifications (photoconversion). Here I shall discuss these photophysical effects in the context of the underlying mechanisms.

BP 11.5 Mon 16:30 H43

Axial super resolved cell dynamics by spectrally coded optical nanosectioning (SpecON) on biocompatible metal-dielectric substrates — Benjamin Schreiber[1], Hannah Heil[1], Martin Kamp[2], Kareem Elsayad[3], and •Katrin G. Heinze[1] — [1]Rudolf Virchow Center, University of Würzburg, Germany — [2]Technische Physik, University of Würzburg, Germany — [3]Advanced Microscopy, Campus Science Support Facilities, Vienna, Austria

Fluorescence microscopy is one major tool to visualize substructures of biological cells down to the single molecule level. While particular powerful in the lateral dimension high-resolution concepts are usually less effective in the axial dimension or compromise temporal resolution.

Here, we show how to overcome this problem by using biocompatible metal-dielectric coated substrates for live cell imaging. As fluorescent emitters can be treated as dipoles they can interact with surface plasmons in metallic interfaces and allows for distance dependent modulation of the emission properties. Thus, it is possible to record the distance-dependent spectral "fingerprint" to determine and visualize fluorophore axial distributions far beyond the Abbe-limit. Compared to respective fluorescence lifetime measurements, spectral changes of the fluorophore emission can be measured with higher temporal resolution. Here we monitor the super-resolved axial movements of living cells particular the dynamics of filopdia in melonama cells, and discuss future perspective for multidimensional imaging.

BP 11.6 Mon 16:45 H43

Non-contact atomic force microscopy of the purple membrane — •Alfred J. Weymouth[1], Katharina Pfeffer[1], Estefania Mulvihill[2], Daniel J. Müller[2], and Franz J. Giessibl[1] — [1]Department of Physics, University of Regensburg, Regensburg, Germany — [2]Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland

Atomic force microscopy is a unique tool for investigating biological samples. Images are normally acquired in tapping mode, in which the tip presses down on the sample with each oscillation. This is relatively non-destructive, requires only a small sample, and can resolve down to the nanometer-scale. (e.g. [1]) However, true non-contact techniques, such as frequency-modulation AFM, have been developed which allow atomic resolution before the tip presses down on the surface. Frequency-modulation AFM has acquired atomic contrast on surfaces in liquid environments. [2] We have started to explore the possibility of using non-contact AFM with a stiff cantilever (a qPlus sensor with k = 3515 N/m) to image biological membranes. As a first sample, we looked at the purple membrane. We present our first results of the membrane islands and atomic resolution of the mica substrate.

[1] M. Pfreundschuh, D. Martinez-Martin, E. Mulvihill, S. Wegmann

and D.J. Müller. Nat Protoc, 9, 1113 (2014)

[2] T. Fukuma, K. Kobayashi, K. Matsushige and H. Yamada. Appl Phys Lett, 87, 034101 (2005)

BP 11.7 Mon 17:00 H43

Seeing intermolecular interactions in morphology: AFM-IR of aggregated thin porphyrin films — •Timur Shaykhutdinov, Peter Kate, Simona Pop, Andreas Furchner, and Karsten Hinrichs — Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V., ISAS Berlin, Schwarzschildstr. 8, 12489 Berlin, Germany

A comprehensive understanding of hierarchical self-assembly of low-dimensional supramolecular systems is of fundamental importance for applications in the field of nanobiotechnology and bioengineering. Metalloporphyrins are extremely versatile molecular building blocks and serve as active components in a variety of biological systems. Their capability to self-organize over a wide range of length scales spanning from a few nanometers up to hundreds of micrometers is promising for the development of multifunctional biofilms.

Although the morphology of porphyrin aggregates has been studied extensively, their formation mechanisms have remained unclear up till now. In this work we show nanoscale IR spectroscopic evidence of different porphyrin stacking as the underlying cause of morphological patterns of aggregated thin porphyrin films.

Here we apply resonance-enhanced AFM-IR, a high-sensitivity non-destructive nanospectroscopic technique in the fingerprint region, and link intermolecular stacking interactions to nanostructured morphology.