BP 35: Bioimaging and Biospectroscopy II

Time: Thursday 15:00–17:30 Location: HÜL 386

BP 35.1 Thu 15:00 HÜL 386

Molecule counts in complex oligomers with single-molecule localization microscopy — Tim Niklas Baldering[1], •Jakob Tómas Bullerjahn[2], Gerhard Hummer[2], Mike Heilemann[1], and Sebastian Malkusch[1] — [1]Institute of Physical and Theoretical Chemistry, Goethe-University Frankfurt, Frankfurt am Main, Germany — [2]Department of Theoretical Biophysics, Max Planck Institute of Biophysics, Frankfurt am Main, Germany

Single-molecule localization microscopy resolves nano-scale protein clusters in cells, and in addition can extract protein copy numbers from within these clusters. A powerful approach for such molecular counting is the analysis of fluorophore blinking using stochastic model functions. Here, we develop a theoretical model for quantitative analysis of photoactivated localization microscopy (PALM) data that accounts for the detection efficiency. By this, we are able to extract populations of different oligomers reliably and in complex mixtures. We demonstrate this approach analyzing simulated PALM data of a photoactivatable fluorescent protein. We generate simulations of blinking data of oligomers and of mixtures of oligomers, and show robust oligomer identification. In addition, we demonstrate this approach for experimental PALM data.

BP 35.2 Thu 15:15 HÜL 386

Confocal single molecule localisation microscopy for super-resolved fluorescence lifetime imaging — •Jan Christoph Thiele, Eugenia Butkevich, Oleksii Nevskyi, and Jörg Enderlein — Third Institute of Physics - Biophysics, Georg August University, Göttingen, Germany

Localisation based super-resolution microscopy techniques like dSTORM, PALM and PAINT usually rely on wide field or TIRF illumination and wide field detection. This allows for simultaneous acquisition of the whole field of view but comes with the limitations of a camera based detection. Instead, we use a confocal setup with a pulsed excitation, single photon detection and a fast laser scanner. We evaluate different dyes and conditions to achieve slow blinking kinetics and a high number of photons per switching event. Individual switching events could be localised utilising our confocal scanning approach and the corresponding super-resolved image could be reconstructed. The huge advantage of a single photon detection is that each localisation contains information about the fluorescence lifetime. This enables us to combine dSTORM with metal induced energy transfer (MIET), a distance dependant modulation of the lifetime of a fluorophore by a thin metal film. MIET enables axial localising single fluorophores with a precision below 5 nm. Our goal is to achieve a high, isotropic 3D-localisation accuracy by combining the high lateral precision of dSTORM with the high axial precision of MIET.

BP 35.3 Thu 15:30 HÜL 386

Super-resolution structured illumination microscopy of MreB dynamics and cell wall synthesis in B. subtilis — •Julian Roth, Johanna Mehl, and Alexander Rohrbach — Albert-Ludwigs-Universität, Freiburg

Total internal reflection fluorescent structured illumination microscopy (TIRF-SIM) is a unique approach combining high acquisition speeds with a two-fold increased lateral resolution at very high contrast. Our TIRF-SIM implementation enables simultaneous dual-color super-resolution imaging of dynamic, low fluorescent samples at several Hertz. The design is based on mechanical beam steering and phase shifting devices as well as a Michelson interferometer, thus avoiding diffractive elements. The fast TIRF-SIM setup is employed to gain a clearer view on the dynamics of cell wall synthesis machinery proteins in Bacillus subtilis, as we still lack fundamental knowledge of how bacteria build, expand and maintain their cell wall. The cytoskeletal proteins MreB, RodA and PbpH are essential components of the bacterial cell-shape generation system. By imaging these proteins with TIRF-M and TIRF-SIM, directional movement and non-continuous motion patterns could be analyzed in enhanced details. Based on a multi-motor transport model, a mechanistic Brownian dynamics simulation was developed that was able to reproduce measured transport quantities like velocity and number of transport pauses and direction reversals. These new insights support the model of MreB being transported by several motors, where PbpH and RodA are likely candidates for synthesis motors based on their measured and simulated motion patterns.

Invited Talk BP 35.4 Thu 15:45 HÜL 386

Super-resolution microscopy with DNA molecules — •Ralf Jungmann — LMU Munich — MPI of Biochemistry

Super-resolution fluorescence microscopy is a powerful tool for biological research. We use the transient binding of short fluorescently labeled oligonucleotides (DNA-PAINT) for easy-to-implement multiplexed super-resolution imaging that technically achieves sub-5-nm spatial resolution.

To translate this resolution to cellular imaging, we introduce Slow Off-rate Modified Aptamers (SOMAmers) as efficient and quantitative labeling reagents. We demonstrate the achievable image resolution and specificity by labeling and imaging of transmembrane as well as intracellular targets in fixed and live cell-specimen.

Apart from ever increasing spatial resolution, efficient multiplexing strategies for the simultaneous detection of hundreds of molecular species are still elusive. We introduce a new approach to multiplexed super-resolution microscopy by designing the blinking behavior of targets with engineered binding frequency and duration. We assay this kinetic barcoding approach in silico and in vitro using DNA origami structures, show the applicability for multiplexed RNA and protein detection in cells and finally experimentally demonstrate 124-plex super-resolution imaging within minutes.

15 min. coffee break

BP 35.5 Thu 16:30 HÜL 386

Phase-Contrast X-Ray Tomography of Marmoset Cochlea — •Jannis Justus Schaeper[1], Marius Reichardt[1], Marina Eckermann[1], Jasper Frohn[1], Christoph Kampshoff[2], Tobias Moser[3], and Tim Salditt[1] — [1]Institute for X-Ray Physics, Göttingen University — [2]Max-Planck-Institute for Experimental Medicine, Göttingen — [3]InnerEarLab, University Medical Center, Göttingen

The cochlea is the receptor organ in the inner ear that transduces sound into neuronal activity. Both fundamental aspects of signal transduction and neuro-physiology as well as biomedical research (implant technology, hearing loss and disorders) requires three-dimensional (3D) imaging techniques capable to quantify the micro-anatomy.

We present optimized 3D imaging of excised small-animal cochleae by phase-contrast x-ray tomography using highly brilliant synchrotron radiation, and show how this technique can complement classical histology and light sheet microscopy in a correlative imaging approach. Shape, volumes and densities of individual neurons can be assessed.

In view of age-related hearing loss we particularly aim at quantitatively evaluating the number of spiral ganglion neurones and hair cells in different age groups of marmoset models. We show how high contrast for soft tissue [1] can be achieved, in particular using our endstation GINIX at DESY [2]. Due to high contrast and little noise, automated segmentation becomes possible. The CT-images are compared to lightsheet microscopy data to infer structural changes induced by the clearing process. [1] M. Töpperwien et al., Sci. Rep. 8, 4922 (2018), [2] T. Salditt et al., J Synchrotron Radiat. 22 (2015), 867-878

BP 35.6 Thu 16:45 HÜL 386

A multisensory interface for exploring nanomechanical tissue properties with human senses — •Robert Magerle, Paul Zech, Andreas Otto, Martin Dehnert, and Alexandra Bendixen — Fakultät für Naturwissenschaften, TU Chemnitz

Tissues display a complex spatial structure and their mechanical properties remain largely unexplored on the nanometer scale. Here we present a multisensory interface that makes nanomechanical tissue properties accessible to human perception and cognition. With a haptic interface, we translate the 3D force fields measured with an atomic force microscope (AFM) on the nanometer scale into forces perceivable to humans. This allows human users to explore haptically the specimen's surface shape as well as its local nanomechanical properties while simultaneously employing multiple senses. We developed a generic hysteresis model that uses the force-vs.-distance data collected with the AFM to predict the force (output) of the haptic device for an arbitrary indentation trajectory (input). This allows the user to perceive the specimen's local elastic response as well as different types of

dissipative processes including viscoelasticity, elasto-capillary effects, adhesion hysteresis, and hysteresis due to capillary forces. The first samples studied include native (unfixed) hydrated tendon and living cancerous epithelial breast cells in culture medium.

BP 35.7 Thu 17:00 HÜL 386

Dissection of Plasmodium falciparum developmental stages with multiple imaging methods — •Katharina Preissinger[1,2], Beáta Vértessy[1,2], István Készmárki[3,4], and Miklós Kellermayer[5] — [1]Department of Applied Biotechnology and Food Sciences, BME, Budapest, Hungary — [2]Institute of Enzymology, Research Center for Natural Sciences, Budapest, Hungary — [3]Department of Physics, BME, Budapest, Hungary — [4]Department of Experimental Physics V, University of Augsburg, Germany — [5]Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary

Every year, more than 200 million people are infected with malaria. The protozoon is transmitted into the human body by a mosquito bite. In the blood stream, malaria parasites invade red blood cells (RBC), mature to rings and trophozoites, multiply to schizonts and then burst out of the cells, ready to invade further ones. The digestion of haemoglobin by all Plasmodium species results in the accumulation of a metabolic byproduct and in morphological changes of the RBC, alterating topology and mechanics, which are typically characterized with bright-field microscopy (BF).

To explore correlations of the Plasmodium-induced molecular, topographical and mechanical changes, we investigated infected RBC with atomic force microscopy (AFM), phase contrast and total internal reflection fluorescence (TIRF) microscopy. By combining these imaging methods, we could correlate the morphological changes of RBC with the Plasmodium falciparum developmental stages.

BP 35.8 Thu 17:15 HÜL 386

Thermal non-equilibrium drives concentration, salt and pH gradients — •Thomas Matreux, Dieter Braun, and Christof B. Mast — Systems Biophysics, Ludwig-Maximilians-Universität, München, Deutschland

The first steps in the emergence of life on Earth occurred on rocks and their constituent phases with a feedstock of simple molecules. Our aim is to combine this scenario with thermal non-equilibrium and bring together geomaterials, chemistry and microfluidics in a realistic environment.

The reaction chambers are sandwiched between highly heat conducting sapphire plates ensuring complete thermal control including possible thermal gradients. Microfluidic structures are made from FEP, which lets us focus on the interactions between the molecules. Ions leached from prebiotically plausible mineral samples are selectively accumulated by thermal gradients and permit enzymatic activity. Thermal non-equilibrium boundary conditions drive concentration gradients, enabling chemical reactions and generating and controlling pH gradients in a plausible prebiotic scenario. Local gradients driven by heat fluxes will offer unique opportunities to enable molecular selection and evolution at the origins of life.