BP 34: New Technologies

Time: Friday 10:15–13:00 Location: ZEU 260

Invited Talk BP 34.1 Fri 10:15 ZEU 260

Super-resolution fluorescence imaging of cellular structure and dynamics — •Markus Sauer, Sebastian van de Linde, Teresa Klein, Anna Löschberger, Thorge Holm, and Sven Proppert — Biotechnology and Biophysics, Julius-Maximilians-University Würzburg, Germany

We introduce direct stochastic optical reconstruction microscopy (dSTORM), a new general approach for multicolor super-resolution fluorescence imaging based on reversible photoswitching of standard small organic fluorophores. Photoswitching of organic rhodamine and oxazine fluorophores, i.e. the reversible transition from a fluorescent to a non-fluorescent state in aqueous buffers exploits the formation of long-lived radical anions through reaction with thiol compounds and repopulation of the singlet ground state by reaction with molecular oxygen. We unravel the underlying switching mechanism, investigate the importance of labeling strategies and densities, and demonstrate super-resolution imaging with different commercially available organic fluorophores with high spatial and temporal resolution. Finally, we demonstrate that dSTORM in combination with suited chemical tags can be advantageously used for dynamic live cell imaging at resolutions ~ 20 nm.

BP 34.2 Fri 10:45 ZEU 260

Nanoscale Localisation of Adhesion Receptors and Binding Domains of Matrix Proteins Using Electric Field Microscopy — •Christina Müller[1], Dimitar Stamov[1], Carsten Werner[1,2], and Tilo Pompe[1] — [1]Leibniz Institute of Polymer Research Dresden, 01069 Dresden, Germany — [2]Center for Regenerative Therapies Dresden, 01307 Dresden, Germany

Biomaterial interfaces constitute the intersection of living tissues and artificial scaffolds. In order to reveal exogenous cues in guiding cell behaviour a precise localisation of adhesion receptors and their ligands at the extracellular matrix (ECM) at biomaterial interfaces is needed. Although several techniques allow for the determination of spatial protein distributions, most of them require specialised conditions (electron microscopy) or lack high resolution (optical microscopy). We applied scanning force microscopy to provide at the same time nanoscale resolution and easy access to biological samples at material interfaces. Using electric force microscopy and immunogold labelling we revealed the localisation of cell adhesion receptors, i.e. integrins, at ECMs reorganised by adherent cells. The modulated anchorage of the adhesion ligands by the polymer support lead to different reorganisation patterns of fibronectin fibrils at the adhesion sites together with differences in the distribution and density of integrins. By probing specific domains at fibronectin fibrils, we additionally found them in a stretched state caused by the involved receptor forces. The versatility and straightforward implementation suggests the used method to further characterise different sets of ECM structures and corresponding receptors.

BP 34.3 Fri 11:00 ZEU 260

Distance measurements in the nanometer range by in-cell EPR — •Malte Drescher — Emmy-Noether-Gruppe Physikalische Chemie, Universität Konstanz, 78457 Konstanz

In the past years, Electron paramagnetic resonance (EPR)spectroscopy has witnessed tremendous methodological and instrumental developments. These new methods have strong impact on biostructural research. Diamagnetic material such as most biomacromolecules (DNA, proteins, etc.)can be investigated by site-directed spin-labeling. Of particular interest are Double Electron Electron Resonance (DEER) techniques giving access to inter- and intramolecular distance distributions in the nanometer range. Dynamics can be monitored on a scale from pico- to microseconds. The utilization of pulsed EPR methods is especially useful for addressing structural features in complex systems.

In particular, our current effort to go beyond in-vitro approaches and to in-cell EPR will be introduced. Distance measurements by in-cell EPR using a spin-labeled DNA model system will be demonstrated.

[1] S. Domingo Köhler, A. Weber, S. P. Howard, W. Welte, and M. Drescher, Protein Science 19 (2010) 625-630 [2] M. Robotta, P. Braun, B. van Rooijen, V. Subramaniam, M. Huber, and M. Drescher, ChemPhysChem (2010) accepted [3] V. Singh, M. Azarkh, T. Exner, J. Hartig, M. Drescher Angewandte Chemie Int. Ed. 48 (2009) 9728-9730

BP 34.4 Fri 11:15 ZEU 260

Optical manipulation of neuronal networks bursting dynamics — •Ghazaleh Afshar[1,3], Ahmed El Hady[1,2,3], Theo Geisel[1,3,4,5], Walter Stuehmer[2,3,4], and Fred Wolf[1,3,4,5] — [1]Max Planck Institute for Dynamics and Self organization, Göttingen, Germany — [2]Max Planck Institute of Experimental Medicine, Göttingen, Germany — [3]Bernstein Focus for Neurotechnology, Göttingen, Germany — [4]Bernstein Center for Computational Neuroscience, Göttingen, Germany — [5]Faculty of Physics, Georg August University, Göttingen, Germany

Cultures of channelrhodopsin-2 transfected hippocampal neurons allow simultaneous optical stimulation and electrical recording from neuronal networks. As has been previously reported, after maturation, these networks develop an electrical activity that is characterized by synchronized bursts. In this work, we study the influence of whole field blue light illumination on burst dynamics of these cultures. During stimulation the mean firing rate is significantly different than before and after stimulation. Moreover, the mean spike rate during burst is significantly higher during stimulation. After turning off the stimulus, a silent period follows and then the network gradually switches into an ongoing state of bursting activity with even higher mean spike rate during stimulation. In fact, the mean duration of burst decreases during and after stimulation compared to non-perturbed spontaneous activity. We conclude that light stimulation can be used to persistently influence bursting dynamics in biological neuronal networks.

15 min. break

BP 34.5 Fri 11:45 ZEU 260

Dip-Pen Nanolithography and Polymer-Pen Lithography for Bio-Medical Applications — •Falko Brinkmann[1,2,3,4], Sylwia Sekula[1], Michael Hirtz[1], and Harald Fuchs[1,2,3] — [1]Institut für Nanotechnologie, Karlsruher Institut für Technologie, 76128 Karlsruhe — [2]Physikalisches Institut, Westfälische Wilhelms-Universität Münster, 48149 Münster — [3]Center for Nanotechnology (CeNTech), 48149 Münster — [4]Institut für Tumorbiologie, Universitätsklinikum Hamburg-Eppendorf, 20246 Hamburg

Dip-Pen Nanolithography (DPN) is a versatile tool for the fabrication of arbitrary patterns on a wide range of surfaces. It uses the tip of an atomic force microscope (AFM) as a miniature quell-pen which is dipped into inks like silanes or phospholipids. Its capability to pattern different functional materials on the same surface with 1D or 2D tip arrays simultaneously (multiplexing) leads to wide interest in biology and medicine. Feature sizes range from less than 100 nm to the several micrometers.

Polymer-Pen Lithography (PPL) is based on a polydimethysiloxane (PDMS) stamp with millions of tips. Compared to silicon tip arrays, polymer pens are inexpensive and are able to deposit inks with higher throughput. Multiplexed dot-arrays can be accomplished in nanometer resolution over many square centimeters within a few minutes.

Both DPN and PPL can be used to fabricate functional nano- and micropatterns for bio-medical studies like protein-binding, virus-detection and cell-adhesion with potential application in biosensors and medical lab-on-chip devices.

BP 34.6 Fri 12:00 ZEU 260

Combined 3D structural and molecular imaging using optical coherence tomography and laser scanning microscopy — •Maria Gaertner[1], Peter Cimalla[1], Lilla Knels[2], Sven Meissner[1], Wolfgang M. Kuebler[3], and Edmund Koch[1] — [1]TU Dresden, Faculty of Medicine Carl Gustav Carus, Clinical Sensoring and Monitoring, Dresden, Germany — [2]TU Dresden, Faculty of Medicine Carl Gustav Carus, Department of Anatomy, Dresden, Germany — [3]Institute for Physiology, Charité Berlin, Germany and and Department of Surgery, University of Toronto, Ontario

Since the early 1990s, optical coherence tomography (OCT) has an emergent impact on biomedical and biophysical research. As a non-invasive optical technique, it provides three-dimensional, contactless, high-resolution (μm) imaging of tissue substances with penetration depths of up to several millimeter. Exploiting its abilities, in vivo histological studies become feasible without extraction of biological tissue. The sample's morphology can easily be obtained within a few

milliseconds. Apart from all its benefits, the lack of molecular specific interactions limits this method to a mere coarse investigation of tissue architecture. Utilizing laser scanning microscopy, the detailed molecular structure of biological samples can be obtained via specifically binding dyes to the substance of interest. In this study, we present a combined setup for simultaneous OCT and confocal fluorescence microscopy, allowing fast three-dimensional imaging of lung morphology and detection of elastic fiber distributions arising from the biomolecule elastin within lung tissue.

BP 34.7 Fri 12:15 ZEU 260

Enhancing the penetration depth in biological matter using Microscopy with Self-Reconstructing Beams — •Cristian Gohn-Kreuz and Alexander Rohrbach — University of Freiburg, Laboratory for Bio- and Nano-Photonics, 79110 Freiburg, Germany

Microscopy with Self-Reconstructing Beams (MISERB) is an imaging technique derived from the concept of light-sheet based microscopy. In this technique optical sectioning, i.e. the avoidance of out-of-focus light, is achieved by creating a thin sheet of light within a fluorescently labeled sample, while detecting the emitted light in a direction perpendicular to the illumination axis. In contrast to regular light sheet based microscopy, where the light sheet is either created by a cylindrical lens or a laterally scanned Gaussian beam, the light sheet in MISERB is created by a laterally scanned Bessel beam [1]. This beam belongs to the class of self-reconstructing beams. It recuperates its beam profile a short distance behind a scattering object. Due to this fact Bessel beams can provide a higher penetration depth than Gaussian beams in dense biological media like e.g. human skin. The penetration depth and image quality deep inside an object however is still limited by scattering artifacts. These artifacts result from the fact that the original beam profile gets distorted while propagating through thick scattering media. In this work we will investigate the controlled reduction of scattering artifacts by individually matching the illumination beam to the sample under consideration, thus enhancing the penetration depth in thick scattering media. [1] F. O. Fahrbach, P. Simon, and A. Rohrbach, Nature Photonics 4, 780-785 (2010)

BP 34.8 Fri 12:30 ZEU 260

Nanofocus Endstation of MiNaXS Beamline @ PETRA III — •Christina Krywka[1], Stephan Roth[2], Ralph Döhrmann[2], and Martin Müller[3] — [1]Christian-Albrechts-Universität zu Kiel, Institut für Experimentelle und Angewandte Physik, Leibnizstraße 19, D-24118 Kiel — [2]DESY, Notkestraße 85, D-22607 Hamburg — [3]Helmholtz-Zentrum Geesthacht, Institut für Werkstoffforschung, Max-Planck-Straße 1, D-21502 Geesthacht

PETRA III, located on the site of DESY (Hamburg) is the world's most brilliant synchrotron radiation source. The nanofocus endstation of its MiNaXS-Beamline (Micro- and Nanofocus X-ray Scattering) is currently on the verge of entering into user-dedicated mode.

MiNaXS was designed to provide a high flux, low divergence monochromatic x-ray beam (8-23 keV) and it's nanofocus endstation is targetting to deliver a nanofocused beam with a focal spot size in the order of 100nm * 100nm and a high coherence option dedicated for diffraction experiments at both biological and synthetic materials. Experiments can be performed in both wide-angle and small-angle x-ray scattering geometry (WAXS and SAXS) and this combined with the targetted spatial resolution being so far unique.

The very first commissioning experiments were successfully performed in November and December 2010 at the nanofocus endstation, rendering the MiNaXS beamline operational at both of it's endstations. This contribution presents the current status of the nanofocus endstation, the results of the commissioning experiments as well as the planned future extensions.

BP 34.9 Fri 12:45 ZEU 260

Evolutionary algorithms used to study fluorescence decay curves of photosystem II core complexes from thermosynechococcus elongatus — •Joachim Börner[1], Franz-Josef Schmitt[1], Athina Zouni[2], Hans Joachim Eichler[1], and Gernot Renger[2] — [1]Institute of Optics and Atomic Physics, Berlin Institute of Technology — [2]Max-Volmer-Laboratory of Biophysical Chemistry, Berlin Institute of Technology

The character of kinetic limitation of the exciton trapping in Photosystem II (PS II) is not yet clarified. It is a matter of discussion whether the excitation energy transfer (EET) from core antennas to the reaction center or the succeeding electron transfer (ET) is the rate limiting step of the overall process. In order to address this problem we investigated core complexes from Thermosynechococcus elongatus by using the technique of time correlated single photon counting with picosecond resolution. The obtained fluorescence decay curves were analyzed within the framework of a structure based model with a set of coupled differential equations. Self-designed evolutionary algorithms were used to achieve a satisfying fit of the experimental data by simulated decay curves. The algorithm allows the determination of transition rates of EET and ET processes in PS II and the stability and variance of these rates by random modifications of an initial set. Our results revealed that the EET from the core antennas to the reaction center is most likely limiting the overall exciton trapping with typical transfer time constants in the order of some tens of picoseconds whereas the charge separation (electron transfer) is accomplished within a few ps only.