BP 13: Imaging

Time: Tuesday 9:30–12:00 Location: H44

Topical Talk BP 13.1 Tue 9:30 H44

Ultrasensitive detection, microscopy, tracking, and manipulation of nano-objects — •Vahid Sandoghdar — Max Planck Institute for the Science of Light, Erlangen, Germany

The advent of fluorescence microscopy and spectroscopy in the 1990s ushered in single molecule detection as a powerful tool for a wide range of studies, ranging from biophysics to quantum optics. Since then a number of techniques have pushed the limits of spatial resolution and detection sensitivity for the visualization of matter down to the single molecule level. In our laboratories, we have approached these issues in two different ways. First, we have developed extinction detection and spectroscopy for investigating nonfluorescent single nano-objects such as metallic nanoparticles, viruses, quantum dots, and organic molecules [1-6]. In particular, I will present measurements of single virus motion and its interaction with receptor lipids [4], studies of nanoparticle fast motion on artificial membranes, and very recent detection of unlabeled single proteins [7]. In the second approach, we exploit cryogenic single molecule detection to push localization microscopy to the angstrom precision level [8].

[1] K. Lindfors, et al, Phys. Rev. Lett. 93, 037401 (2004). [2] P. Kukura, et al, Nano Lett. 9, 926 (2009). [3] P. Kukura, et al, Nature Methods 6, 923 (2009). [4] P. Kukura, et al, J. Phys. Chem. Lett. 1, 3323 (2010). [5] M. Celebrano, et al, Nature Photonics 5, 95 (2011). [6] M. Krishnan, et al, Nature 467, 692 (2010). [7] M. Piliarik and V. Sandoghdar, in preparation. [8] S. Weisenburger, et al, in preparation.

BP 13.2 Tue 10:00 H44

Probing Transcription Factor DNA Binding at the Single Molecule Level in Live Mammalian Cells — •J. Christof M. Gebhardt[1,4], David M. Suter[1,4], Rahul Roy[1,2], Ziqing W. Zhao[1], Alec Chapman[1], Srinjan Basu[1,3], Tom Maniatis[3], and X. Sunney Xie[1] — [1]Harvard University, Cambridge MA, USA — [2]Indian Institute of Science, Bangalore, India — [3]Columbia University Medical Center, New York NY, USA — [4]equal contribution

Imaging single fluorescent proteins in living mammalian cells is challenging due to out-of-focus fluorescence excitation by common microscopy schemes. We report the development of a novel fluorescence microscopy method, reflected light sheet microscopy (RLSM), which allows selective plane illumination throughout the nucleus of living mammalian cells, for reducing out-of-focus fluorescence signal. Generation of a thin light sheet parallel to the imaging plane and close to the sample surface is achieved by reflecting an elliptical laser beam incident from the top by 45° with a small mirror. The thin light sheet allows for an increased signal-to-background ratio superior to previous illumination schemes and enables imaging of single fluorescent proteins with up to 100 Hz time resolution. We demonstrate the sensitivity of RLSM by measuring the DNA-bound fraction of glucocorticoid receptor (GR) and determine the residence times on DNA of various oligomerization states and mutants of GR and estrogen receptor (ER), enabling us to resolve different modes of DNA binding of GR. Finally, we demonstrate two-color single molecule imaging by observing the spatio-temporal co-localization of interacting protein pairs.

BP 13.3 Tue 10:15 H44

STED Microscope with Spiral Phase Contrast — •Marcel Andreas Lauterbach[1], Marc Guillon[1], Asma Soltani[2], and Valentina Emiliani[1] — [1]University Paris Descartes, Paris Sorbonne Cité, Neurophysiology and New Microscopies Laboratory, Paris, France — [2]University Paris Descartes, Paris Sorbonne Cité, Laboratory of Membrane Dynamics and Neurological Diseases, Paris, France

We present a STED (STimulated Emission Depletion) microscope with phase contrast imaging capabilities. Phase contrast permits the visualization of weak phase objects without any labeling. It is implemented without extra optical elements in the STED light path, which would compromise STED imaging capabilities. Scanning phase contrast allows for registration with the fluorescence images and principally for simultaneous recording of phase contrast and STED images.

Phase contrast images of objects as thin as a few nanometers in optical path mismatch are obtained. The method provides a second imaging channel in a STED microscope for phase contrast imaging even in index-matched mounted cell cultures. This allows for correlation of morphological structures with high-resolution fluorescence images.

BP 13.4 Tue 10:30 H44

Object-adapted optical trapping and shape-tracking of helical bacteria — •Matthias Koch and Alexander Rohrbach — Lab for Bio- and Nano-Photonics, University of Freiburg, Georges-Koehler-Allee 102, 79110 Freiburg, Germany

Simple living cells such as bacteria are often regarded as model systems in order to analyse basic cellular reactions. Therefore, advanced photonic measurement techniques are needed which are also capable of extracting forces and energetics on a broad temporal bandwidth.

We show how the trapping potential of an optical tweezers setup can be adapted to the shape of a tiny elongated helical bacterium (Spiroplasma melliferum - SM) in order to hold and orient it in the focal plane of a microscope [1]. Further, the coherently scattered laser light is used to analyse its fast and complex cellular shape changes with nm precision at rates up to 1 kHz. By localizing each slope of the only 200nm thin bacterium we generate high contrast, super-resolution movies in three dimensions - without any object staining.

To demonstrate this method, we analysed SM bacteria and show how temporal changes in the transition between different energetic modes during external disturbances can be identified and imaged in 3D. As a response to an external perturbation, the minute long death of the bacterium is recorded and analysed by decaying energy fluctuations, which represents a novel approach in bacteriology.

[1] Koch, M. and A. Rohrbach (2012). "Object-adapted optical trapping and shape-tracking of energy-switching helical bacteria." Nature Photonics 6(10): 680-686.

BP 13.5 Tue 10:45 H44

Subsurface Imaging of Cells Using Atomic Force Acoustic Microscopy at GHz Frequencies — •Matthias Büchsenschütz-Göbeler[1], Jan Rother[3], Andreas Janshoff[3], Walter Arnold[2], and Konrad Samwer[1] — [1]I. Physikalisches Institut, Universität Göttingen — [2]Department of Material Science and Materials Technology, Saarland University — [3]Institut für Physikalische Chemie, Universität Göttingen

We describe a technique to image subsurface structures in living biological cells (e.g. Madin Darbin canine kidney cells, type II) using Atomic Force Acoustic Microscopy operating at 1 GHz. The cells are insonified with 1 GHz ultrasonic waves which are amplitude modulated at a fraction or multiple frequency of cantilever contact-resonance [1]. The transmitted signals are demodulated by the nonlinear tip-surface interaction, enabling one to image the inner structure of the cell based on their ultrasonic scattering power. The latter one is determined by the ultrasonic frequency, the acoustic mismatch between the elastic properties of the host material (cytoplasm) and the subsurface objects to be visualized (e.g. nucleus), by their geometry and by diffraction effects. Interference fringes can be seen in both the amplitude and phase images. Besides images, we will present an interpretation of the contrast mechanism for imaging. Financial support by the DFG SFB 937 is thankfully acknowledged.

[1] Imaging of Subsurface Structures Using Atomic Force Acoustic Microscopy at GHz Frequencies, S. Hu and C. Su, and W. Arnold, J. Applied Phys. 109, 084324 (2011)

BP 13.6 Tue 11:00 H44

Magnetic spin imaging at ambient conditions — Steffen Steinert[1], •Florestan Ziem[1], Andrea Zappe[1], Nicolas Götz[1], Liam Hall[2], Lloyd Hollenberg[2], and Jörg Wrachtrup[1] — [1]3. Physikalisches Institut, Universität Stuttgart, 70569 Stuttgart, Germany — [2]School of Physics, University of Melbourne, Victoria 3010, Australia

A variety of magnetic imaging and sensing methods exist, including NMR, SQUIDs, atomic vapors and magnetic resonance force microscopy. While NMR is a standard technique for imaging in living organisms and the other techniques achieve sensitivities down to single spins, currently no technique combines few spin sensitivity, submicron spatial resolution and ambient operational conditions. Negatively charged nitrogen-vacancy centers (NVs) in diamond are promising candidates to fill this gap. Since the spin state of the atomic-sized defect can be optically polarized and read out, NV centers allow for high resolution magnetic sensing via Zeeman-shift, spin precession and relaxometry. We employ an ensemble of implanted NVs for wide-field

magnetic sensing and imaging. Applications to microfluidic spin detection and magnetic spin imaging with diffraction limited resolution are presented. Several spin species are analyzed in regard to their possible application as magnetic sensing markers.

BP 13.7 Tue 11:15 H44

Motion induced oscillations of *Physarum polycephalum* detected by AlGaN-GaN High Electron Mobility Transistors — •Thomas Lippelt[1,2], Hartmut Witte[1], Marcus J. B. Hauser[2], and Alois Krost[1] — [1]Otto-von-Guericke-Universität Magdeburg, Inst. Exp. Phys., Abt. Halbleiterepitaxie — [2]Otto-von-Guericke-Universität Magdeburg, Inst. Exp. Phys., Abt. Biophysik

Due to the high sensitivity and biocompatibility of planar AlGaN/GaN High Electron Mobility Transistors (HEMTs) arrangements, such a device may be used as a biosensor in vast, living systems like amoebal cells, for sensing cellactivity. The scope of AlGaN/GaN HEMT structures for in situ detection of cell movement and growth of extended, living organisms like slime molds was studied in this contribution. As a object of investigation, we chose the *Physarum polycephalum*, a slime mold which represents one giant single cell with remarkable abilities to build network-like structures. While migrating over the HEMT sensing area, the slime mold was video monitored and the source-drain-impedance at 10 kHz has been recorded. By correlating the gray values of the video pictures and the source-drain-impedance it was found that the periodic cell movements affect the source-drain-impedance and cause oscillations with characteristic cycle periods from 100s to 140s.

BP 13.8 Tue 11:30 H44

Observing lipid diffusion in membranes with microsecond time and nanometer spatial resolution — •Susann Spindler[1], Chia-Lung Hsieh[2], Jens Ehrig[1], and Vahid Sandoghdar[1] — [1]MPI for the science of light, Erlangen, Germany — [2]Institute of Atomic and Molecular Sciences, Academia Sinica, Taiwan

Lipid membranes play an important role in biological cells not only by defining the boundaries of the cell and cell organelles, but also by taking an active part in membrane trafficking and signaling. Remarkably, while a great deal is known about lipid membranes, a large number of fundamental questions remains open. These are for example issues concerning the local nanoscopic heterogeneity of the membrane induced via lipid–lipid and lipid–protein interactions. While this nano-organisation is believed to be crucial for the high functionality of the cell membrane, it is still far from being well understood. To address this issue, a high spatial and temporal resolution is needed.

In our laboratory, we have developed a powerful approach for single-particle-tracking based on interferometric scattering (iSCAT) microscopy, which meets these requirements. By attaching small gold nanoparticles of 20 nm diameter to lipid molecules and detecting their weak linear scattering signal by iSCAT, we are able to localize the position of the molecules with nanometer-accuracy within microseconds. This allows us to detect even very small deviations from free diffusion, opening the door to studying membrane dynamics with unprecedented clarity. We will present experimental data for a variety of systematic studies.

BP 13.9 Tue 11:45 H44

Quantifying Lipid and Protein Diffusion in Black Lipid Membranes — •Kerstin Weiss and Jörg Enderlein — Drittes Physikalisches Institut, Georg-August-Universität Göttingen, Göttingen, Germany

Lipid diffusion is crucial in many biological processes. However, accurately determining diffusion coefficients in membranes remains difficult. We used dual-focus fluorescence correlation spectroscopy (2f-FCS) to measure lipid diffusion in black lipid membranes (BLMs), i.e. lipid bilayers spanned over a pore. To generate these bilayers a commercially available setup is employed. We first tested the effect of different ionic strengths in neutral and negatively charged bilayers. Moreover, we investigated the influence of mono- vs. divalent ions. While monovalent ions have no effect on lipid diffusion in both systems, addition of divalent ions severely decreased the lipid*s diffusion coefficient in negatively charged BLMs. Furthermore, the investigated the validity of the Saffman-Delbrück model which is used to describe protein diffusion in lipid membranes.