BP 2: New Technologies

Time: Monday 10:15–13:00 Location: H43

Invited Talk BP 2.1 Mon 10:15 H43

Probing Cellular Events with Single Quantum Dot Imaging — •Maxime Dahan — Ecole normale supérieure, Paris, France

In the past years, experiments on membrane molecules have demonstrated the potential of single quantum dot (QD) tracking to decipher the dynamics of complex events and to study biochemical reactions at the single molecule level, directly in live cells. Here I will discuss the principles, methods and challenges of single QD tracking. In particular, I will present our current effort to go beyond membrane dynamics and make QD imaging a standard imaging technique in cell biology. First, I will discuss how QDs can be internalized into live cells, how their colloidal properties affect their intracellular behavior and how QDs can be targeted to specific biomolecules or organelles. Next, I will show the results of recent experiments on the motion of molecular motors kinesin and myosin V in the cytoplasm of live cells. These experiments give access to important parameters such as the velocity, the processivity or stepping characteristics of the motor, directly in its cellular environment. Finally, I will present the challenges that need to be met to improve the properties of QDs as biological probes and the strategies that we are implementing to prepare small fonctional nanoparticles with controlled valency using peptide-coated QDs. Overall, the combination of tracking measurements, single-molecule counting methods and emerging high-resolution imaging techniques offer exciting possibilities to probe the composition, structure and dynamics of supramolecular assemblies in live cells.

BP 2.2 Mon 10:45 H43

Near-fields in Fluorescence Microscopy - Absolute Determination of z-positions in the Nanometer Range — •Michael Berndt[1], Mike Lorenz[1], Jörg Enderlein[2], and Stefan Diez[1] — [1]Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden, Germany — [2]III. Institute of Physics, Georg-August-University, Göttingen, Germany

Typical processes in molecular biology take place on a nanometer range and are too small to be resolved by conventional light microscopy. To though reveal information from such systems, techniques are required which access information on the nanometer scale. Here, we present a novel near-field based method to precisely determine absolute heights on the length scale of 0-100 nm above surfaces. We make use of distance dependent quenching processes fluorophores undergo in the proximity to conductive interfaces and measure the influence on the fluorescence lifetime by wide-field fluorescence lifetime imaging microscopy [1]. We use theoretical computations based on the CPS theory to scale the determined lifetimes to absolute heights [2]. We apply our method to fluorescently labeled microtubules elevated to different heights by several spacer molecules. Hence we demonstrate a technique to measure molecule geometries with nanometer precision.

References: [1] M. Lorenz, RNA 15, 97-103 (2009), [2] R.R. Chance, A. Prock, R. Silbey, Advances in Chemical Physics XXXVII, 1-65 (1978)

BP 2.3 Mon 11:00 H43

Robust measurement of diffusion with scanning FCS — •Zdeněk Petrášek, Susan Derenko, and Petra Schwille — Biophysics group, Biotechnologisches Zentrum, Technische Universität Dresden, Tatzberg 47/49, 01307 Dresden, Germany

We present a simple modification of Fluorescence Correlation Spectroscopy (FCS), where the position of the measurement volume is not stationary, but is scanned along a circle of a diameter comparable to the volume size. The known scan radius serves as an internal spatial standard, meaning that the exact volume size need not be known to determine the diffusion coefficient. In this way the method gains robustness against optical distortions that may uncontrollably enlarge the measurement volume, or affect its shape.

The oscillations, introduced into the measured correlation curve by scanning, favourably constrain the fit of a model to the data. Therefore, a relatively narrow range of the autocorrelation is sufficient to obtain a stable fit. Consequently, the correlation values at short lag times, that may be influenced by complicated photophysics of the dye or suffer from a poor signal-to-noise ratio, or the values at long correlation times, that may be affected, for example, by depletion due to photobleaching, can be excluded from the analysis, contributing to the robustness of the method.

The implementation of scanning FCS based on a two-dimensional piezo scanner has been applied to studies both in bulk solution and on surfaces — supported lipid bilayers and giant unilamellar vesicles.

BP 2.4 Mon 11:15 H43

Which parameters influence resolution in optical subdiffraction imaging? — •Thorben Cordes[1,2], Carsten Forthmann[1], Christian Steinhauer[1], Jan Vogelsang[1], and Philip Tinnefeld[1] — [1]Applied Physics - Biophysics, LMU München, Amalienstr. 54, 80799 München, Germany — [2]Biological Physics Research Group, Clarendon Laboratory, University of Oxford, Parks Road, Oxford, OX1 3PU, United Kingdom

In this contribution we will discuss imaging resolution of recently developed superresolution approaches from two different perspectives: (i) At first we reconsider those factors that are actually responsible for the achievable resolution limit of all superresolution techniques - either using subsequent localization of single-fluorophores (STORM, PALM)[1] or targeted readout (STED, SSIM)[1] - by incorporating the photostability of the emitter. (ii) In a second step we experimentally investigate the dependence between resolvable fluorophore density and photophysical parameters that determine the actual imaging speed in a method termed Blink-Microscopy[2]. We therefore employed single-molecule cut-and-paste[3], a method that enables molecule-by-molecule assembly of structures with features below the diffraction limit. Then complex or regular structures with patterns below the diffraction limit were used as calibration structures to characterize how parameters, such as the ON/OFF-time ratio, influence resolution and imaging speed. References: [1] S. W. Hell, Nature Methods 6 (2009) 24-31. [2] C. Steinhauer, et al. JACS 130 (2008) 16840-16841. [3] S. K. Kufer, et al. Science 319 (2008) 594-596.

15 min. break

BP 2.5 Mon 11:45 H43

A Pore-Cavity-Pore Nanodevice to Trap and Optically Investigate Single Molecules — •Martin Langecker, Daniel Pedone, and Ulrich Rant — Walter Schottky Institut, TU München

Single engineered nanopores in solid state membranes have attracted broad attention in recent years as a tool to study single biological molecules like DNA or proteins. Here we introduce a novel solid-state device which comprises two stacked nanopores defining the in- and outlet of a pico liter cavity. This pore-cavity-pore (PCP) architecture allows for the electrical as well as optical examination of single molecules. The PCP device is fabricated by structuring nanopores into a sandwich SiN/Si/SiN wafer using e-beam lithography, wet chemical etching, and feedback controlled electrochemical etching steps. The in- and outlet nanopores of the fabricated PCP-devices are characterized by transmission electron microscopy, evidencing that the pore diameters may be controlled independently down to 10 nm. Through electric potential control we are able to inject and eject nano-objects into and out of the device. We present fluorescence experiments of single DNA molecules and nm-sized polystyrene beads inside the PCP device. We study the potential-dependent loading and unloading of the cavity with 40 nm fluorescently labeled beads and analyze the diffusion of single particles within the cavity by particle tracking. We find that the apparent diffusion coefficients inside the cavity deviate from values obtained for free diffusion in solution and correlate the deviation with the confinement effect of the cavity. Moreover, we present experiments showing the trapping and translocation of fluorescently labeled DNA.

BP 2.6 Mon 12:00 H43

Scanning Ion Conductance Microscopy vs. Atomic Force Microscopy in Cell Imaging — •Johannes Rheinlaender and Tilman E. Schäffer — Lehrstuhl für Angewandte Physik, Universität of Erlangen-Nürnberg, Staudtstr. 7, Bau A3, 91058 Erlangen, Deutschland

We performed a direct comparison of AFM and SICM by imaging the same fibroblast cell with both techniques in series. We thereby show the advantages and disadvantages of both techniques with respect to topography imaging of soft samples. The finite imaging force applied to the cell by the AFM tip causes vertical and lateral cell indenta-

tions, which we analyzed quantitatively. SICM imaging, on the other hand, is based on a non-contact imaging mechanism and provides true topography data. We show that thin, loosely-bound filopodia can be imaged with SICM at high resolution.
Rheinlaender, J. and T.E. Schäffer, J. Appl. Phys., 2009. 105(9): p. 094905

BP 2.7 Mon 12:15 H43

Quantitative biological imaging by ptychographic x-ray diffraction microscopy — •Klaus Giewekemeyer[1], Pierre Thibault[2], Sebastian Kalbfleisch[1], André Beerlink[1], Cameron M. Kewish[3], Martin Dierolf[2], Franz Pfeiffer[2], and Tim Salditt[1] — [1]Institut für Röntgenphysik, Georg-August-Universität Göttingen, Göttingen, Germany — [2]Department Physik (E17), Technische Universität München, Garching, Germany — [3]Paul Scherrer Institut, Villigen PSI, Switzerland

Mesoscopic structues with specific functions are abundant in many cellular systems and have been well characterized by electron microscopy in the past. However, the quantitative study of the three-dimensional structure and density of subcellular components remains a difficult problem.

In this contribution we show how these limitations could be overcome in the future by the application of recently introduced and now rapidly evolving coherent x-ray imaging techniques for quantitative biological imaging on the nanoscale. More specifically, we report on a recent scanning (ptychographic) diffraction experiment on unstained and unsliced freeze-dried cells of the bacterium *Deinococcus radiourans* using only a pinhole as beam defining optical element [1]. As a result quantitative density projections well below optical resolution have been achieved. [1] Giewekemeyer et al. PNAS (2009), in press.

BP 2.8 Mon 12:30 H43

The Nanofocus Endstation of the MiNaXS Beamline at 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-24098 Kiel — [2]DESY, Notkestraße 85, D-22603 Hamburg — [3]GKSS Forschungszentrum Geesthacht, Max-Planck-Straße 1, D-21502 Geesthacht

The former PETRA storage ring of DESY (Hamburg) was refurbished into PETRA III, one of the most brilliant x-ray sources worldwide. All beamlines of the new 3rd generation synchrotron radiation source are currently in their final state of completion.

The MIcro- and NAnofocus X-ray Scattering beamline (MiNaXS) is equipped with two endstations, out of which the farthest is designed to provide a high flux, monochromatic x-ray beam (8-25 keV) focused to a size of about 100nm * 100nm.

Due to the low divergent, sub-micron sized focus experiments with a superior spatial resolution and a flux sufficiently high to study both biological and synthetic materials will very soon become routinely available for nanodiffraction experiments at this endstation of MiNaXS.

This contribution presents the current status of the nanofocus endstation and future extensions. Along with the latest commissioning measurements exemplary and potential applications of nanofocused x-rays are shown. Their applicability to life and materials science is demonstrated on the basis of the availability of high flux density and coherence; both being key-features of the new PETRA III source.

BP 2.9 Mon 12:45 H43

Programmable Lab on a Chip System for single cell analysis — •Stefan Thalhammer[1] and Achim Wixforth[2] — [1]Helmholtz Zentrum München, Institut für Strahlenschutz, Ingolstädter Landstrasse 1, 85764 Neuherberg — [2]Universität Augsburg, Experimental Physiks I, Universitätsstrasse 1, 86159 Augsburg

The collection, selection, amplification and detection of minimum genetic samples became a part of everyday life in medical and biological laboratories, to analyze DNA-fragments of pathogens, patient samples and traces on crime scenes. Here, a multifunctional programmable Lab-on-a-Chip driven by nanofluidics and controlled by surface acoustic waves (SAW) is presented. This system combines serial DNA-isolation-, amplification- and array-detection-process on a modified glass-platform. The fluid actuation is controlled via SAW by interdigital transducers implemented in the chemical modified chip surface. The chemical surface modification allows fluid handling in the submicroliter range. Minute amount of sample material is extracted by laser-based microdissection out of e.g. histological sections at the single cell level. A few picogram of genetic material are isolated and transferred via a low-pressure transfer system (SPATS) onto the chip. Subsequently the genetic material inside single droplets, which behave like "virtual" beaker, is transported to the reaction and analysis centers on the chip surface via surface acoustic waves, mainly known as noise dumping filters in mobile phones. At these "biological reactors" the genetic material is processed, e.g. amplified via polymerase chain reaction methods, and genetically characterized.