BP 12: Posters: Imaging

Time: Tuesday 9:30–12:30 Location: P1

BP 12.1 Tue 9:30 P1

Homo-Fret to Investigate the Oligomeric State of Proteins — •Franz-Josef Schmitt, Matthias Broser, Cornelia Junghans, and Thomas Friedrich — Institute of Chemistry, Biophysical Chemistry, TU Berlin, Straße des 17. Juni 135, D-10623 Berlin, Germany

The combination of various microscopic techniques allows to address complex problems in biophysics. We present a multi-parameter setup based on to a Nikon TI Eclipse microscope that combines time resolved fluorescence microscopy and simultaneous anisotropy microscopy by splitting the detector optically into two independent areas. All microscopic techniques (confocal, wide field, total internal reflection) can be done with a high photon throughput up to 2 Mio. photons/sec. with spectral and polarization resolution. Förster Resonance Energy Transfer (FRET) between identical fluorophores without the discrimination between donor and acceptor (Homo-FRET) allows the determination of the aggregation state of identical chromophors. Microscopic measurements of time resolved Homo-FRET were used to determine the aggregation state of a model system of fluorescence proteins (FKBP-GFP constructs) that can dimerize or form larger aggregates (up to pentamers) by adding a specific membrane-permeable agent. The technique has great application potential for the observation of the repair cycle of the D1 protein in the photosystem II.

BP 12.2 Tue 9:30 P1

Diffusion in Living Cells Determined by Multiparameter Imaging — •Franz-Josef Schmitt, Cornelia Junghans, Matthias Sturm, Marvin Schlischka, and Thomas Friedrich — Institute of Chemistry, Biophysical Chemistry, TU Berlin, Straße des 17. Juni 135, D-10623 Berlin, Germany

Multiparameter microscopy is close to becoming a standard technique in live cell imaging. The synergistic interplay between highly resolved fluorescence microscopy and photoswitchable fluorescence proteins lead to a brilliant new methodology that broke the diffraction barrier of optical microscopy. We show how the photoswitchable genetically expressed Dreiklang Protein is used to determine the local diffusion coefficients in the cell cytoplasm and the cell nucleus as well as the kinetics of the Dreiklang protein diffusion across membranes. The technique shows preferred pathways of proteins across the cell nucleus which are not visible by constantly fluorescing labels due to the strong overlap of the emission of single molecules that lead to homogeneous fluorescence patterns. The dynamics of selectively marked mitochondria is tracked and the mean square displacement is calculated showing regimes of hampered diffusion, free diffusion and active transport.

BP 12.3 Tue 9:30 P1

An insight into structural changes induced to cells by chemical fixatives using X-ray nano-diffraction — •Clémenet Hémonnot, Britta Weinhausen, Rita Graceffa, Robin Wilke, and Sarah Köster — Institute for X-Ray Physics, University of Göttingen, Germany

Various research methods have been developed to study the structure and composition of biological cells, the most prominent ones being fluorescence and electron microscopy. However, for most of these methods, sample preparation such as chemical or cryogenic fixation, staining or labeling, or tissue sectioning is prone to introduce artifacts. Thus, our aim was to use a label-free technique that probes cells in their native, aqueous environment at high resolution. We have applied X-ray nano-diffraction to image cells in microfluidic devices. High spatial resolution due to small wavelengths is combined with high penetration depth, enabling us to study entire cells. The microfluidic devices allow us to keep the cells alive as well as reducing the radiation damage by cooling the sample and by decreasing the concentration of free radicals through constant buffer or media flow. We have performed X-ray nano-diffraction experiments on fixed-hydrated and living eukaryotic cells. In particular, we have compared three widely employed chemical fixatives and analyzed the overall structure of the nucleus and cytoplasm. The different fixatives indeed introduce a different degree of structural changes on length scales on the order of 35 to 45 nm.

BP 12.4 Tue 9:30 P1

Assessing reptation motion with the WormTracker — Matthias Weiss and •Carsten Schade — Experimental physics 1, University of Bayreuth

The locomotion pattern of C. elegans (a small transparent roundworm) is complex and depends on the properties of the environment. To gain a better understanding of the locomotion mechanism of C. elegans, it is mandatory to take a closer look on the motion of individual worms. To get the exact movement pattern of C. elegans in different environments, we have constructed a worm imaging platform. This platform monitors in a time-resolved fashion the accurate shape and center of mass of worms during their movement.The tracking station allows single- and multi worm tracking. In the single tracking mode it is possible to track a single worm for any period of time. Therefore it is possible to track the complete lifetime of a single worm. In the multi tracking mode, it is feasible to track all worms on the plate and therefore to get comparative data from the movements of a worm ensemble in the same environment. The tracking software and the hardware are programmed with Matlab and LabView, i.e. it is easy to develop additional software packages for customized measurements.

BP 12.5 Tue 9:30 P1

Sub-diffraction imaging of cellular organelles — •Andreas Veres and Matthias Weiss — Experimental Physics I, University of Bayreuth, Bayreuth, Germany

Eukaryotic cells are highly compartmentalized. One of the most prominent organelles is the endoplasmic reticulum (ER) which assumes the morphology of a sponge-like web that spans throughout the cell. The full structure of the ER, however cannot be resolved by conventional light microscopy approaches as the typical mesh size is below the diffraction limit. To overcome this limitation, we have used SOFI (Superresolution optical fluctuation imaging) which provides a means to overcome the diffraction limit by exploiting fluorescence fluctuations of quantum dots. Based on our SOFI images we have determined the fractal dimensions of the ER and its porosity in cells at different stages.

BP 12.6 Tue 9:30 P1

Preparation of colloidal CdSe quantum dots for medical fluorescence labelling — •Svenja Herbertz[1], Lothar Houben[2], Kathrin Scheckenbach[3], and Thomas Heinzel[1] — [1]Solid State Physics Laboratory, Buildg. 25.23., Heinrich-Heine University Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf — [2]PGI-5, FZ Jülich, Wilhelm-Johnen-Str. , D-52425 Jülich — [3]HNO-Klinik, Buildg. 13.76, Heinrich-Heine University Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf

The size dependence of their energy bandgap allows producing quantum dots of various fluorescence wavelengths in one production process. We fabricated colloidal CdSe QDs with tunable emission via kinetic growth with TOP as stabilizer [1] and investigated the influence of various growth parameters on the fluorescence behavior. The produced QDs have a size-dependent fluorescence line with typical FWHM of about 40 nm. Via X-ray diffraction and HR-TEM nanocrystal diameters of about 2 to 6 nm were measured and a hexagonal wurtzite crystal structure was identified. The surface of the QDs as-fabricated was chemically modified to establish solubility in aqueous media. In the context of detecting drugs in cell cultures by fluorescence labelling with QDs, an agent of particular interest is the chemotherapeutic cisplatin. The interaction of cisplatin with QDs, for a start without a direct connection of the molecules, was investigated. It was found that the presence of cisplatin quenches the fluorescence intensity of QDs in water.

[1] E. M. Boatman et al., J. Chem. Educ. 82, 1697(2005).

BP 12.7 Tue 9:30 P1

Age related changes in human RPE cells, imaged by multicolor SIM microscopy. — •Florian Schock[1,2], Gerrit Best[1,2], Nil Celik[2], Alina Bakulina[6], Martin Hagmann[1,2], Rainer Heintzmann[4,5], Jürgen Hesser[6], Stefan Dithmar[2], and Christoph Cremer[1,3] — [1]Kirchhoff Institute for Physics, University of Heidelberg — [2]Department of Ophthalmology, University of Heidelberg — [3]Institute of Molecular Biology, Mainz — [4]Institute for Physical Chemistry, University of Jena — [5]Institute of Photonic Technology, Jena — [6]Institute for Medical Technology, Mannheim

In our society old-age diseases are becoming more frequent. One reason for these are non-degradable deposits of degradation products. These

are generated in normal cell-processes, but their accumulation during the human lifespan leads to dysfunction of cell-activity. This is believed to be the case in age related macula-degeneration, where the deposits form granules of the size of about 0.5μm to 3μm. Structured Illumination Microscopy is used to study auto-fluorescent proteins in this deposits, allowing us to separate and image them. In contrast to electron-microscopy, fluorescence microscopy has less stringent constraints to sample preparation and is generally less invasive. Additionally, it offers the possibility to separate different kinds of deposits by using multicolor excitation. This allows us to search for differences in the composition of the deposits. We analysed several histological samples by donors of different age. We present quantitative data to the relation between donor age and granula quantity. All work on human tissue was done according to the Declaration of Helsinki.

BP 12.8 Tue 9:30 P1

Aperture less Scanning Near-Field Optical Microscopy of Tobacco Mosaic Viruses and Intermediate Filament Desmin — •Niklas Biere[1], Alexander Harder[1], Mareike Dieding[1], Volker Walhorn[1], Sven Degenhard[2], Andreas Brodehl[3], Christina Wege[2], Hendrik Milting[3], and Dario Anselmetti[1] — [1]Experimental Biophysics and Applied Nanoscience, Bielefeld University — [2]Dpt. of Molecular Biology and Plant Virology, Institute of Biology, University of Stuttgart — [3]Heart and Diabetes Center NRW, Bad Oeynhausen

Aperture-less scanning near-field optical microscopy (aSNOM) makes use of both, high resolution topographic and fluorescence imaging. Atomic force microscopy (AFM) allows for a topographic resolution down to the atomic scale. Furthermore it can be operated in ambient conditions, liquids or in vacuum which makes it ideal for a large variety of different samples. The evanescent illumination of the AFM cantilever tip induces enhanced electromagnetic fields that are strongly confined to the tip apex. These can serve to excite and image single dye molecules. Here we report on our custom built aSNOM setup using commercially available monolithic silicon cantilevers. We investigated sparsely labelled tobacco mosaic viruses and the intermediate filament protein desmin. Both of which are complex structures composed of mixed building blocks. The simultaneous recording of topography and fluorescence data and their inherent alignment allows for the exact localization of distinct fluorescently labelled building blocks within the superordinate macromolecular structures.

BP 12.9 Tue 9:30 P1

Space- and time-resolved information of refraction index in a microresonator — •Tobias Menold[1,2], Michael Metzger[1], Alexander Konrad[1], Andreas Horrer[2], Sabrina Rau[1], Günther Gauglitz[1], Dai Zhang[1], Alfred J. Meixner[1], Dieter Kern[2], Marc Brecht[1,3], and Monika Fleischer[2] — [1]IPTC, University of Tübingen — [2]Institute for Applied Physics, University of Tübingen — [3]Zürcher Hochschule für Angewandte Wissenschaften, Institute of Applied Mathematics and Physics

The aim of our research is to determine the change of the refractive index within a Fabry Perot microresonator, consisting of a flat and a curved mirror in close distance (λ/2-region), in space and time using a standard colour CCD camera. Using white light illumination yields a position dependent transmission. If a flat and a convex mirror are used the transmission shows a spectrally well-defined Newton ring pattern. The possibility to sense small changes of the refractive index within the cavity depends on the resonator properties and the wavelength resolution of the CCD camera. The main issue is to extract the wavelength with high resolution out of the RGB values determined by the CCD camera. For this purpose we calibrated the CCD-chip using the transmission pattern of monochromatic light with a spectral range between 380 nm and 650 nm. Based on these images we were able to calculate the hue-value of HSV colour space. With that calibration it is possible to translate information from the CCD-camera into refraction index information. The calibration enables us to determine the refraction index within a microresonator with spatial and temporal resolution.

BP 12.10 Tue 9:30 P1

Probing quantum coherence in light-harvesting (LH1) complexes using FRET from a single nitrogen vacancy center — •Priyadharshini Balasubramanian[1], Anna Ermakova[1], Chris Schroeder[2], Liam McGuinness[1], Felipe Caycedo-Soler[2], Caroline Autenrieth[3], Susana Huelga[2], Martin Plenio[2], Robin Ghosh[3], and Fedor Jelezko[1] — [1]Institute for Quantum Optics, University of Ulm — [2]Institute for Theoretical Physics, University of Ulm — [3]Institute of Biology, University of Stuttgart

The formidable quantum efficiency of light harvesting complexes in photosynthetic organisms have been attributed to the long-lived quantum coherence effects. Experiments with ensemble of LH1 complex, have reported the observation of quantum coherence in the time scale of picoseconds range, but the results suffer from ensemble averaging over inhomogeneous distribution of site energy. Here we propose single molecule - Fluorescence Resonance Energy Transfer(FRET) experiment between a single NV center in diamond and the LH1 complexes, to probe the inter-ring coherence dynamics. The path of excitonic energy transfer(EET) between the rings will be measured by injecting excitation locally to a chromophore via a single NV center attached to an AFM tip, and the fluorescent emission is detected from a single red-shifted LH1 in the membrane. The distance traversed by the excitation energy in the membrane gives insight on the contribution of quantum coherence to the inter-ring energy transfer dynamics. Unraveling the role of coherent dynamics in the process of light-harvesting could provide inspiration for artificial photosynthesis.

BP 12.11 Tue 9:30 P1

Refractive index studies of biological cells and nuclei using digital holographic microscopy — •Mirjam Schürmann[1], Jana Scholze[1], Chii J. Chan[2], Paul Müller[1], Andrew E. Ekpenyong[1], Kevin J. Chalut[2], and Jochen Guck[1,2] — [1]Biotechnology Center, TU Dresden, Tatzberg 47/49, 03107 Dresden, Germany — [2]Cavendish Laboratory, University of Cambridge, Cambridge CB3 0HE, UK

In recent years, digital holographic microscopy (DHM) has increasingly been used in biophysical and cell biological studies for the determination of the refractive index of cells. This quantitative phase microscopy technique is non-invasive, in comparison to other traditional imaging techniques, which often require external labelling of biological samples. Previous work in our group on cell differentiation revealed a lineage-specific modulation in the cells' refractive index, suggesting the use of DHM as a useful tool for marker-free studies of cell differentiation. Recently we have also used DHM to study the optical properties of the cell nucleus, which may reflect its epigenetic nature. In contrast to other studies suggesting a high refractive index of cell nuclei compared to the cytoplasm, the present study reveals that the refractive index of isolated cell nuclei of a variety of cell types can be lower than the refractive index of the cell. In addition, nuclear refractive index was found to be highly sensitive to external salt concentrations. This characterisation of the optical properties of nuclei is important for the proper interpretation of laser trapping experiments of cells or the use of light scattering techniques in tissues, such as optical coherence tomography, for diagnostic purposes.

BP 12.12 Tue 9:30 P1

Towards a zone plate based ultra compact HHG driven XUV/soft X-ray scanning transmission microscope — •Christian Späth, Jürgen Schmidt, Huaihai Pan, Alexander Guggenmos, and Ulf Kleineberg — Ludwig-Maximilians-Universität München, Lehrstuhl für Experimentalphysik - Laserphysik, 85748 Garching, Germany

X-ray microscopy is an invaluable imaging method in many research areas with applications in physical, medical and biological problems as well as material science. Especially XUV/soft X-ray microscopy offers the great potential for investigating sensitive biological samples in their natural environment with low dose to reduce radiation damage and high spatial and energy resolution to address questions concerning sub cellular features or elemental composition. Here we report on our concept of an ultra compact microscope utilizing laser driven high harmonic radiation with ˜ 73 eV energy as a light source and a zone plate operated in transmission as the main focussing element combined with different detectors which enables us to run this system in scanning mode as a STXM but also in a modified version as a high resolution instrument in diffraction mode employing the CDI (coherent diffractive imaging) technique. Furthermore due to our pulsed light source the possibility of time-resolved microscopic analysis is given with a possible few-femtosecond temporal resolution.

BP 12.13 Tue 9:30 P1

Following the topography during abscission using SICM — •Ulrich Fromme[1], Natalie Elia[2], and Christph F. Schmidt[1] — [1]Drittes Physikalisches Institut, Fakultät für Physik, Georg-August-Universität, Göttingen — [2]Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University, Beer-Sheva, Israel

The last step in the division cycle of animal cells is abscission. It is defined as the cutting of the small intracellular bridge which remains as a connection between the two daughter cells after the cytoplasm has been divided. This bridge, the so-called midbody, is of an interesting structure, as it contains dense tubulin structures which originate from the cell division- and DNA-separation process. Due to the compactness of this structure, a complex system of membrane constriction and tubulin disassembly molecules are recruited for abscission. In this study we used Scanning-Ion-Conductance-Microscopy (SICM) to create topological movies of these structures in living MDCK cells. SICM can produce images with a resolution that is superior to standard optical methods without damaging or deforming the structures. This makes it possible to acquire high-resolution images and to observe the morphological changes of the intracellular bridge during the abscission process.