BP 10: Posters: Imaging

Time: Monday 17:30–19:30 Location: Poster B2

BP 10.1 Mon 17:30 Poster B2

Distinguishing immature and mature HIV-1 particles by superresolution optical fluorescence microscopy — •Viola Mönkemöller[1], Benjamin Dale[2], Wolfgang Hübner[1], Benjamin Chen[2], and Thomas Huser[1] — [1]Biomolecular Photonics, Universität Bielefeld, Germany — [2]Immunology Institute, Mount Sinai School of Medicine New York, NY, USA

The human immunodeficiency virus (HIV) is the agent of the global epidemic of the immune disease AIDS (acquired immunodeficiency syndrome). Therefore further investigations on the viral structure and molecular mechanisms are of particular importance in the search for medical therapies. HIV-1 is assembled in infected cells and buds out as an immature particle. The uncleaved major structural polyprotein Gag defines the immature non-infectious state of the virus. HIV-1 has to undergo maturation to become infectious. During the maturation process Gag is cleaved which leads to a different structure in the infectious HIV-particle. We are interested in characterizing the immature and mature virus particles by superresolution fluorescence microscopy. The size of HIV-1 particles is approx. 130nm in diameter and is therefore below the diffraction limit of conventional fluorescence light microscopy. Techniques such as PALM (Photoactivated Localization Microscopy) and STORM (Stochastic Optical Reconstruction Microscopy) should allow us to resolve structures down to 20nm spatial resolution.

We plan to utilize this and other superresolution approaches to track the position and state of individual HIV virions in 4D, which will be very useful for future studies on HIV infection mechanisms.

BP 10.2 Mon 17:30 Poster B2

Monitoring early embryogenesis with single-plane illumination microscopy — •Philipp Struntz, Rolf Fickentscher, and Matthias Weiss — Experimental Physics I, University of Bayreuth, Germany

Single-plane illumination microscopy (SPIM) is a fluorescence-imaging technique that combines rapid widefield detection with optical sectioning. Due to a reduced phototoxicity induced by bleaching, SPIM is capable of providing a long-term, three-dimensional time-resolved in vivo imaging of specimen. We have built and automated a SPIM-setup specifically designed for imaging eggs of the nematode Caenorhabditis elegans in the early stages of embryogenesis. With the obtained time-lapse images it is possible to track cell positions within the developing embryo to get information about the spatiotemporal development of tissues. Based on this, we have designed a simple model that is capable of explaining experimental observations during the first stage of embryogenesis.

BP 10.3 Mon 17:30 Poster B2

On the role of Sec16 in the self-organization of exit sites in the endoplasmic reticulum — •Julia Hoffmann and Matthias Weiss — Experimental Physics I, University of Bayreuth, Germany

The endoplasmic reticulum (ER) is the first station of newly synthesized proteins in eukaryotic cells. The export of properly folded proteins occurs almost exclusively at specialized membrane domains, so-called ER exit sites (ERES). Protein export at ERES but also the self-organization of ERES themselves are still poorly understood. Sec16 is a large peripheral protein that plays an important role in both processes. However, its precise function has remained elusive so far. Here we present an investigation of Sec16 dynamics in vivo by means of quantitative fluorescence microscopy techniques. As a result, we have found that Sec16 rapidly cycles between an ER-bound and a cytoplasmic state (ratio about 1:1) while being mobile in both states. Moreover, our data indicate a role of Sec16 as a metastable platform for the self-assembly of ERES.

BP 10.4 Mon 17:30 Poster B2

PyCorrFit - a versatile tool for FCS data analysis — •Paul Müller — Biotechnology Center of the TU Dresden, Germany

We present the general-purpose fluorescence correlation spectroscopy (FCS) evaluation software PyCorrFit. PyCorrFit supports various commercially available FCS setups (e.g. ConfoCor3, Zeiss), and offers several built-in model functions, exploiting a wide range of applications. The program comes with several features for data manipulation, permits the import of external model functions that do not require prior programming skills, and can be expanded to fit the needs of the more versed user. We demonstrate the implementation of PyCorrFit in current research, such as diffusional measurements in lipid monolayers or protein affinity assays in free solution. PyCorrFit is free, open-source and available at http://fcstools.dyndns.org/pycorrfit.

BP 10.5 Mon 17:30 Poster B2

SOFI of GABA-B neurotransmitter receptors in hippocampal neurons elucidates intracellular receptor trafficking and assembly — •Anja Huss[1], Omar Ramírez[2], Felipe Santibáñez[2], Andrés Couve[2], Steffen Härtel[2], and Jörg Enderlein[1] — [1]III. Institute of Physics: Biophysics, Georg-August-University Göttingen, Germany — [2]Institute of Biomedical Sciences, Faculty of Medicine and Nucleus of Neural Morphogenesis (NEMO), Universidad de Chile, Santiago, Chile

The synaptic efficacy of neurons depends on the availability of neurotransmitter receptors and is controlled by their intracellular trafficking routes. GABA-B receptors (GABA-BRs) are heteromeric proteins found at the plasma membrane of dendritic postsynaptic sites. Detailed insights of trafficking routes and thus the assembly of the subunits are still missing.

To address this question we have studied the distribution and colocalization of the GABA-BR subunits in the plasma membrane and in the intracellular compartments in hippocampal neurons with dual-color, 3D Stochastic Optical Fluctuation Imaging (SOFI). SOFI is a fluorescence imaging modality which yields super-resolved spatial resolution, 3D-sectioning and high image contrast.

BP 10.6 Mon 17:30 Poster B2

Researches on iron containing bacteria with PEEM — •Christoph Keutner[1], Ulf Berges[1], Philipp Espeter[1], Alex von Bohlen[2], David J. Keavney[3], Claus M. Schneider[4], and Carsten Westphal[1] — [1]DELTA/Experimentelle Physik I, TU Dortmund — [2]ISAS Dortmund — [3]APS, Argonne National Laboratory — [4]PGI-6, FZ Jülich

Members of the polyphyletic group of magnetotactic bacteria (MTB) are forming chains of membrane-encapsulated particles by the natural process of biomineralisation. These so-called magnetosome chains consist of greigite or iron oxides, and are used for an oriented navigation along the Earth's magnetic field. This is very important for the bacteria since they seek the oxic-anoxic transition zone which is their optimal living environment. In technological, medical, and environmental applications these magnetosomes are considered as a perspective material due to their narrow size and shape distribution.

In this work experiments on imaging the MTB species *Magnetospirillum magnetotacticum* with photoemission electron microscopy (PEEM) were continued. Now, an improved preparation procedure yielded a significantly higher MTB concentration on the sample surface. At the same time the residuals of the culture medium could drastically be decreased by this new procedure. We present first photoelectron emission microscopy data recorded at the Advanced Photon Source, Chicago (USA). The measurements show an element-specific detailed structure of the bacteria, including a clear signature of the iron oxides within the bacteria.

BP 10.7 Mon 17:30 Poster B2

Wide-field magnetometry imaging using nitrogen-vacancy centers — Steffen Steinert[1], •Nicolas Götz[1], Florestan Ziem[1], Andrea Zappe[1], Liam Hall[2], Lloyd Hollenberg[2], and Jörg Wrachtrup[1] — [1]3 Physikalisches Institut, Universität Stuttgart, 70569 Stuttgart Germany — [2]2School of Physics, University of Melbourne, Victoria 3010, Australia

Imaging of weak electromagnetic signals with sub-cellular resolution under life sustaining conditions is of great importance in the context of life science, but could not be achieved yet. A diamond doped with a shallow layer of nitrogen-vacancy centers (NV) potentially fulfills this requirements. The spin state of a single negatively charged NV center can be optically polarized and read out. Measuring the local Zeeman shift and spin state lifetime of several NVs enables to determine the magnetic fields in the vicinity of the Diamond. Using a wide-field microscopy technique, we can simultaneously capture the signal of a 60*m

x 60*m area of the diamond surface on a CCD camera with diffraction limited resolution. Here we present the investigation of different magnetic species as marker for magnetic cell imaging via microfluidic detection. We also evaluate the feasibility of label-free imaging using this technique.

BP 10.8 Mon 17:30 Poster B2

Non-Invasive Imaging and Quality Assessment of Artificial Cartilage — •Lena Nolte[1], Iwan Schie[2], and Thomas Huser[1,2] — [1]Universität Bielefeld — [2]University of California, Davis

More than five million people in Germany are currently suffering from osteoarthritis. Due to the daily stress placed in our joints the cartilage between the bones wears off. Once the cartilage is damaged it cannot recover by itself which leads to little treatment options. New approaches in tissue engineering provide promising results in the development of artificial cartilage that could be transplanted to a patient. Nevertheless, the quality of this artificial cartilage must be assessed in order to prevent further issues for the patient. The challenge, however, is to investigate the cartilage at a microscopic scale without influencing it as otherwise with fluorescence microscopy. Here, we developed a combination of several optical techniques to analyze the cartilage without special preparation or labeling. We demonstrate how cartilage cells (chondrocytes) can be visualized by coherent anti-Stokes Raman scattering (CARS), and the extracellular matrix (ECM) using Second Harmonic Generation (SHG). Images of a size of up to 1.5 cm x 7.5 cm with a spatial resolution down to 1 μm can be reconstructed. Furthermore, spontaneous Raman scattering can be applied to obtain Raman spectra from specific points of interest. This provides molecular information about the cartilage composition within these specific regions. We believe this novel multiphoton microscopy system is a powerful device which enables us to expose the similarity and differences between natural and artificial cartilage.

BP 10.9 Mon 17:30 Poster B2

High Harmonic Generation For Coherent Diffractive Imaging — •Sergey Zayko[1], Eike Mönnich[1], Murat Sivis[1], Tobias Mey[3], Dong-du Mai[2], Klaus Mann[3], Tim Salditt[2], and Claus Ropers[1] — [1]Materials Physics Institute and Courant Research Centre, University of Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen — [2]Institute fo X-ray Physics. University of Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen — [3]Laser-Laboratorium Göttingen e.V. (LLG) Hans-Adolf-Krebs-Weg 1 37077 Göttingen

Light sources based on high harmonic generation (HHG) open up the possibility to achieve coherent ultra-short high energy pulses up to the soft x-ray regime in a tabletop setup. Here, we present an implementation and characterization of a compact HHG setup to perform coherent diffractive imaging (CDI) and pump-probe spectroscopy with femtosecond temporal resolution. Coherent pulses up to the 51st harmonic order corresponding to 80 eV photon energy have been obtained.

The degree of spatial coherence and the optical wavefront of high harmonics were characterized. As a proof-of-concept, a test pattern with 170 nm feature size structured by focused ion beam milling was imaged by CDI at a wavelength of 32 nm.

BP 10.10 Mon 17:30 Poster B2

Chasing the next level in molecular sensitivity — •Henning Hachmeister, Michael Stührenberg, Lena Nolte, Christian Pilger, Gerd Wiebusch, and Thomas Huser — Biomolecular Photonics, Universität Bielefeld, Germany

Fluorescence microscopy is a well-established, molecularly specific imaging technique in the life sciences which continues to gain popularity due to the ongoing development of novel fluorescent probes and superresolution imaging techniques. The use of fluorescent probes, however, is potentially detrimental to biological activity and requires careful controls. Rapid photobleaching and the toxicity of many of these probes greatly limit their use in physiological studies of cells.

A new way to image the structure of cells is the use of vibrational spectroscopy as a microscopic imaging technique. In vibrational spectroscopy, vibrations of molecular bonds inside a molecule are excited using laser beams. Within the last decade the nonlinear optical imaging of molecular modes by coherent anti-Stokes Raman scattering (CARS) has been developed to the point where it can now be routinely use to image e.g. lipid structures within cells within the need for fluorescent probes.

Here, we demonstrate a versatile, new technique called doubly-resonant CARS (DR-CARS) which can enhance weak CARS signals by up to 1000x to enable the detection and analysis of other molecular bonds besides aliphatic C-H groups.

BP 10.11 Mon 17:30 Poster B2

Broadening the applications of vibrational spectroscopy in microscopy with super resolution techniques — •Christian Pilger, Henning Hachmeister, Michael Stührenberg, Lena Nolte, Gerd Wiebusch, and Thomas Huser — Biomolecular Photonics, Universität Bielefeld, Germany

In vibrational spectroscopy coherent anti-Stokes Raman scattering (CARS) is an established method for imaging biological samples on the microscopic scale. This process results in the specific excitation of molecular bonds. Utilizing a four-wave mixing process the Raman signal is strongly enhanced, and can now be used e.g. for imaging inner cell structures.

In comparison to fluorescence microscopy, CARS requires no organic fluorophores to highlight cellular structures of interest. Thereby the biological structures and processes are unaltered and the signal is persistent.

This type of laser scanning microscopy is, however, still limited in its spatial resolution by the diffraction limit. We are exploring a number of potential ways that will enable us to overcome the diffraction limit in nonlinear optical microscopy. Ultimately, we hope to be able to image individual viral particles in vivo during their entire life cycle from the assembly of new virions to the infection of new cells.

BP 10.12 Mon 17:30 Poster B2

Stimulated Raman Scattering for noninvasive live imaging — •Michael Stührenberg, Henning Hachmeister, Lena Nolte, and Thomas Huser — Biomolecular Photonics, Universität Bielefeld, Germany

A common method for the visualisation of biological molecules is fluorescence microscopy. By using a fluorescent dye, the molecules of interest can be made visible. Fluorescence microscopy covers a wide range of techniques and continues to gain popularity in respect to i.e. superresolution and 3D live cell imaging. Nevertheless, these techniques have some major disadvantages. Amongst other things there is no guarantee that the probes won't affect certain characteristics of the molecule, such as motility or binding capability. Therefor, the use of fluorescent probes requires careful controls in order for it to not be detrimental to biological processes. To overcome these drawbacks, we demonstrate a rapid noninvasive imaging technique based on Raman scattering. In Stimulated Raman Scattering (SRS) the molecule of interest is excited to a specific vibrational mode. This is achieved by the application of 2 different confocal laserbeams overlapping in time and space. Through excitation in a vibrational mode, one beam loses intensity, while the other one experiences, through induced stimulation, an intensity gain. The probability for this is small compared to a fluorescent signal and requires a lock-in based detection system. Yet this results in a nearly background free noninvasive imaging technique, which can be tuned to a wide range of molecules. The ultimate goal is live imaging for which we apply a homebuilt lock-in amplifier with a high temporal resolution.

BP 10.13 Mon 17:30 Poster B2

Sample preparation and delivery at the European XFEL Facility — •Sadia Bari[1], Charlotte Uetrecht[1,2], and Joachim Schulz[1] — [1]European XFEL GmbH, Notkestraße 85, 22607 Hamburg, Germany — [2]Molecular Biophysics, Uppsala University, Husargatan 3, 75124 Uppsala, Sweden

The ultrashort, high-intensity X-rays of the European XFEL will enable new science with a wide range of potential samples delivered in various forms into diverse environments. Providing the delivery methods and target systems that allow such a variety of samples to be studied using the fast repetition rate and high peak intensity of the European XFEL is the job of the Sample Environment group.

Sample delivery methods that are planned and in preparation will be introduced and discussed; for example a liquid jet primarily used in nano-crystallography and an aerodynamic lens optimised for single particle imaging of e.g. aerosols and viruses. But also the options for fixed targets and their challenges like fast sample exchange, control of temperature and sample orientation will be presented.

BP 10.14 Mon 17:30 Poster B2

Scanning X-Ray Nano-Diffraction on Hydrated Cells in Microfluidic Devices — •Britta Weinhausen, Christian Dammann, Oliva Saldanha, Robin Wilke, Christian Olendrowitz, Jens-Friedrich Nolting, Tim Salditt, and Sarah Köster — Institute

for X-Ray Physics, University of Göttingen, Germany

The combination of X-ray scattering techniques with sample chambers based on microfluidics allows for studying biological samples in a well-controlled and adaptable environment. However, bio- as well as X-ray compatibility of the materials, the device geometry and the sample environment is a challenging issue during device fabrication. Especially when using high-flux synchrotron radiation sources, material degradation or strong background scattering is observed for most materials that are commonly used for microfluidic experiments in combination with visible light microscopy.

We develop a novel type of X-ray compatible microfluidic device, which is based on silicon nitride membranes as window material and allows for studies on hydrated biological samples. As sample we choose adherent eukaryotic SK8K18-2 cells, exhibiting a pronounced keratin network structure. Scanning nano-diffraction experiments using hard X-rays are performed on fixed (hydrated) as well as living cells in these flow chambers at different synchrotron set-ups. Different contrast mechanisms are employed to generate real-space images of the cells with a resolution on the order of the beam size.

BP 10.15 Mon 17:30 Poster B2

New Developments in Laboratory SAXS Instruments — •Bastian Arlt[1] and Andreas Keilbach[2] — [1]Anton Paar Germany GmbH, Hellmuth-Hirth-Straße 6, D-73760 Ostfildern — [2]Anton Paar GmbH, Anton-Paar-Straße 20, A-8054 Graz

Surfactants, dispersions, polymer or protein solutions and (micro-) emulsions are intensively investigated systems in current science. Consequently, an essential point is the careful characterization of these systems in-situ. The small angle X-ray scattering (SAXS) technique offers precise and fast measurements to investigate parameters such as size, shape, interaction effects of particles in solution. Thus, SAXS is a complementary method to TEM, AFM, or NMR techniques. SAXS measurements are performed at synchrotron facilities or, thanks to recent developments, using laboratory instruments which have become an excellent alternative.

We are going to present the latest developments and trends in the field of laboratory SAXS instruments. Thanks to high-flux X-ray sources, short exposure times are possible. Additionally, Anton Paar has explored novel techniques in sample positioning which are commonly known from synchrotron measuring stations and allow extending the available detection range and resolving smallest dimensions.

BP 10.16 Mon 17:30 Poster B2

Raman spectroscopic characterization of sepsis relevant bacteria appearing in urinary tract infections on a dielectrophoresis chip — •U.-Ch. Schröder[1,2], C. Assmann[1], A. Ramoji[1], U. Glaser[1,2], U. Hübner[2], Ch. Leiterer[2], A. Csáki[2], W. Fritzsche[2], M. Bauer[1], J. Popp[1,2,3], and U. Neugebauer[1,2] — [1]Center for Sepsis Control and Care, Jena University Hospital — [2]Institute of Photonic Technology Jena e.V. — [3]Institute for Physical Chemistry and Abbe Center of Photonics, University Jena

Sepsis reflects a dysregulated response of the immune system due to the invasion of pathogens characterized by high mortality rates. Due to the requirement for overnight cultivation steps the standard microbiological diagnostic methods are too slow for an early start of tailored therapy. To make things worse bacteria are getting more and more resistant towards antibiotics. We have combined direct dielectrophoretic capturing of bacteria in dilute suspensions above a quadrupol microelectrode array with using micro-Raman spectroscopy to achieve a novel rapid, highly specific, label-free and culture-independent lab on chip method. As a proof-of-principle study two commonly participating bacterial strains in urinary tract infections, Escherichia coli and Enterococcus faecalis, are respectively captured, and are classified with respect to their molecular signature. To demonstrate rapid antibiotic susceptibility testing, the differentiation of antibiotic treated and non-treated Enterococcus faecalis strains has been carried out. With the help of multivariate statistical analysis a robust classification model has been established. Acknowledgement: BMBF (FKZ 01EO1002)

BP 10.17 Mon 17:30 Poster B2

Characterization of Caco-2 cells via confocal Rama Microscopy — •Susanne Kimeswenger[1,2], Krisztina Vincze-Minya[1], Mohammad-Reza Lornejad-Schäfer[2], Klaus Schröder[2], and Sabine Hild[1] — [1]Institute of Polymer Science, Johannes Kepler University, Altenbergerstr. 69, 4040 Linz, Austria — [2]Biomed-zet Life Science GmbH, Industriezeile 36/I, 4020 Linz, Austria

Eucaryotic cells consist of 60-70 % water, the rest is bioorganic material, which is highly heterogenous: lipids, peptides, carbohydrates and nucleic acids. The distribution of these components varies in different cell types and cell states. Over the past few years Raman spectroscopy got more and more interesting for the characterization of cells, as Raman spectroscopy is a non-destructive, non-contact in vitro method to analyze cells providing information about their viability, differentiation status and tumorigenicity. The human carcinoma cell line Caco-2 has the special characteristic to showing a small intestine enterocyte phenotype when cultured for 21 days. Caco-2 cells seeded on different substrates (silicium, calcium fluoride) were tested. Confocal Raman microscopy reveals the differences between vital and fixated cells. As correlation of Raman spectra and different cell components in fixated cells was not possible, characterization of Caco-2 cells was performed in vital cell status; the identification of Raman spectra of cell components was promising. Furthermore we have revealed differences between differentiated and proliferated Caco-2 cells regarding to their Raman spectra, reflecting differences in bio-molecular composition.