BP 34: Imaging

Time: Thursday 9:30–11:45 Location: ZEU 250

BP 34.1 Thu 9:30 ZEU 250

Fast Frame-Rate FLIM for Applications in Molecular Biology and Photosynthesis Research — •Franz-Josef Schmitt[1], Danilo Bronzi[2], Marco Vitali[1], Cornelia Junghans[1], Franco Zappa[2], and Thomas Friedrich[1] — [1]Institute of Chemistry, Biophysical Chemistry, TU Berlin, Straße des 17. Juni 135, D-10623 Berlin, Germany — [2]Dipartimento di Elettronica Informatica e Bioingegneria, Politecnico di Milano, Piazza Leonardo Da Vinci 25, I-20133 Milano, Italy

A monolithic 64x32 CMOS image sensor for fluorescence detection is presented. Each pixel consists of three 9-bit gated counters and a single-photon avalanche diode (SPAD). Thanks to their inherent digital nature, SPADs have a sensitivity limited only by photon shot noise and therefore single-photon imaging at very high-frame rate is enabled. Moreover, we used a sliding-time window scheme to achieve time-resolved photon detection for measuring the fluorescence lifetime with a temporal resolution down to 200 ps. The described sensor has been used for simultaneous imaging of the fluorescence amplitude and lifetime of a pH-sensitive dual-emission GFP fusion protein (deGFPph-Sens) expressed in chinese hamster ovary cells (CHO). This allows to monitor over time the pH distribution within individual compartments and organelles of living cells. Additionally, we present fluorescence induction images at the frame-rate of 1 kfps of dark adapted living cells of the blue alga Synechocystis sp. PCC 6803.

BP 34.2 Thu 9:45 ZEU 250

3D-Density Measurements of the Bacterium Deinococcus Radiodurans by Tomo-Ptychographic X-ray Imaging — •Robin N. Wilke[1], Marius Priebe[1], Matthias Bartels[1], Klaus Giewekemeyer[2], Ana Diaz[3], Malte Vassholz[1], and Tim Salditt[1] — [1]Institut für Röntgenphysik, Universität Göttingen, Germany — [2]European XFEL, Hamurg, Germany — [3]Paul Scherrer Institut, Villigen, Switzerland

D.radiodurans[1,2] is famous for its extraordinary resistance to high doses of ionizing radiation, which has been tentatively linked to the structural organization of DNA in the nucleoid[3,4]. Determination of the mesoscopic density in the nucleoid would help to test these ideas and eventually to discriminate different models of DNA packing.

Ptychographic phasing[5-9] is one promising (coherent) X-ray imaging technique for this particular problem and other biological applications. We present results of quantitative 3D resolved mass density maps of D.radiodurans by a combination of ptychography and tomography of unstained, unsliced and freeze-dried bacterial cells[8]. In addition, we show how ptychographic results can be enhanced by increasing the dynamic range of pixel detectors by introducing a Semi-Transparent Central Stop[9].

[1]Levin-Zaidman et al.,Science299,2003
[2]Eltsov&Dubochet,J.Bacteriol.187,2005
[3]Eltsov&Dubochet,J.Bacteriol.188,2006
[4]Minsky et al.,J.Bacteriol.188, 2006
[5]Rodenburg et al.,PRL98,2007.
[6]Thibault et al.,Science321,2008
[7]Giewekemeyer et al.,PNAS107,2010
[8]Wilke et al.,Opt.Expr.20,2012
[9]Wilke et. al.,ActaCryst.A69,2013

BP 34.3 Thu 10:00 ZEU 250

Imaging of biological cells with helium-ion microscopy — •André Beyer[1], Natalie Frese[1], Henning Vieker[1], Matthias Schürmann[2], Barbara Kaltschmidt[2], Christian Kaltschmidt[2], and Armin Gölzhäuser[1] — [1]Physics of Supramolecular Systems, University of Bielefeld, 33615 Bielefeld, Germany — [2]Cell Biology, University of Bielefeld, 33615 Bielefeld, Germany

Helium-ion microscopy (HIM) images are generated by scanning a beam of helium ions while recording the emitted secondary electrons. Advantages of HIM include the high resolution, high surface sensitivity as well as an efficient charge compensation mechanism, which allows imaging of insulating samples without the need for a conductive coating.

In this contribution, a HIM imaging study of biological cells is presented. This study focuses on neuronal differentiated inferior turbinate stem cells as well as mouse neurons which were prepared in different ways for imaging under the required vacuum conditions. Charging of specimens without conductive coating was effectively compensated by an electron flood gun. Therewith, extremely small features at cell surfaces were imaged with an estimated edge resolution of 1.5 nm. Indications of lipid rafts at the surface of all investigated cells will be discussed.

BP 34.4 Thu 10:15 ZEU 250

Suitability of the echo-time-shift method as laboratory standard for thermal ultrasound dosimetry — •Tina Fuhrmann[1], Olga Georg[2], Julian Haller[2], and Klaus-Vitold Jenderka[1] — [1]University of Applied Sciences, Merseburg, Germany — [2]Physikalisch-Technische Bundesanstalt (PTB), Braunschweig, Germany

Ultrasound therapy is a promising, non-invasive medical application. It is used e.g. for physiotherapy and lithotripsy for the destruction of kidney stones and has high potential for surgical and therapeutic applications like treatment of cancer, bone repair and treatment of stroke with high intensity focused ultrasound (HIFU). To further develop this technique, to ensure a save clinical application and repeatability of the treatment, laboratory dosimetry standards and quality control mechanisms for therapeutic devices are necessary.

Our approach is to measure temperature with a diagnostic ultrasound device by tracing the time-shift in the backscattered signal. This shift is mainly due to the temperature dependence of speed of sound. We evaluated the suitability of this echo-time shift method for laboratory dosimentry and quality control, especially its general suitability, uncertainties and limitations.

15 min. break

BP 34.5 Thu 10:45 ZEU 250

Optical tweezers based coherent sub-diffraction 3D imaging of helical bacteria and sensing of deformation forces — •Matthias Koch, Julian Roth und 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. The size of a bacterial cell or of small protrusions is often in the range of only a few tens to hundreds of nanometres and their shape may change rapidly. Therefore, advanced photonic measurement techniques are needed which are also capable of extracting forces and energetics on a broad temporal bandwidth. We show how an optical tweezers setup can be used to generate high contrast, 3D super-resolution movies of an only 200nm thin helical bacterium at rates up to 1kHz without any object staining [1]. Images are generated by analysing the interference pattern of the incident and the coherently scattered laser light in the back focal plane of a detection lens. Our system allows the simultaneous measurement and manipulation of fast shape changes and deformation forces generated by the cell. The bacterium itself uses a unique linear motor composed of cytoskeletal protein ribbons for propulsion/swimming. The subunits of these ribbons undergo subsequent conformational changes associated with force generation of the cell body. We show how this technique can be used to analyse the force an torque generation of the motor under different environmental situations.

[1] Koch, M. & A. Rohrbach (2012). Nature Photonics 6(10): 680-686

BP 34.6 Thu 11:00 ZEU 250

Scanning probe magnetic spin imaging of the protein complex ferritin — •Dominik Schmid-Lorch, Thomas Häberle, Andrea Zappe, Friedemann Reinhard, and Jörg Wrachtrup — 3. Physikalisches Institut und Forschungszentrum SCoPE, Universität Stuttgart, Germany

We present a novel technique to image nanoscale magnetic fields. It is based on the nitrogen-vacancy (NV) center, a color center in diamond, which can be used as a novel magnetic field sensor by monitoring the Zeeman-shift of its spin sublevels [1]. Mounted to the tip of an atomic-force microscope (AFM), this atomic-sized color center promises to map magnetic fields with a resolution in the atomic range [2-3]. Being sensitive enough to detect single electron and nuclear spins in its close environment, it could enable imaging and structure determination of

single biomolecules.

We will demonstrate imaging of magnetic resonance contrast agents with a resolution in the 10 nm range using this technique. Specifically, we have been able to image small ensembles of Ferritin, an iron storage protein complex [4], by detecting its spin noise with a scanning NV center probe. Beyond these results, we will present our progress towards imaging of single Ferritin complexes.

[1] G. Balasubramanian et al., Nature, Vol 455, 648-651 (2008)
[2] L. Rondin et al., Appl. Phys. Lett., Vol 100, 153118 (2012)
[3] P. Maletinsky et al., Nat. Nanotech., Vol 7, 320-4 (2012)
[4] M. Uchida et al., Magn. Reson. Med., Vol 60, 1073-1081 (2008)

BP 34.7 Thu 11:15 ZEU 250

The nitrogen vacancy in nanodiamonds as a bio-marker for resolving dynamics of bio molecules — •Torsten Rendler, Seoyoung Paik, Sany-Yun Lee, and Jörg Wrachtrup — 3. Physikalisches Institut, University of Stuttgart, Pfaffenwaldring 57, 70550 Stuttgart, Germany

Fluorescent probes are essential for bio imaging application. Therefore several different types of fluorescent markers like dye molecules, quantum dots and fluorescent nanodiamonds (fND) have been developed and studied for their purpose as bio-markers. Besides the superior long time stability of the fluorescent emissions, fNDs decorated with the nitrogen-vacancy (NV) color center exhibit another interesting property based on its electron spin system: The NV can probe both the orientation and strength of magnetic field in its environment [1]. This allows not only to locate the fND but also to detect its relative orientation to an external magnetic field in real time on a ms-timescale [2]. It also has been suggested that the rotational diffusion rate of nanodiamonds even in faster time scales are accessible [3]. These works motivate the development of experimental techniques to resolve the rotational diffusion of fNDs, which will also open the door for molecular motion tracing, like monitoring fast conformational changes of working bio molecules. As a preliminary test, we investigate the spin dynamics of NVs in freely rotating fNDs.

[1] Balasubramanian, G. et al., Nature 455, 648-651 (2008). [2] McGuinness, L. P. et al., Nat. Nanotech. 6, 358-363 (2011). [3] Maclaurin,D. et al.,PRL 108 240403(2012).

BP 34.8 Thu 11:30 ZEU 250

Detection of vancomycin resistances in enterococci using a combined dielectrophoresis-Raman setup and a three-level chemometric model — •Ulrich Schröder[1,2], Cora Assmann[2], Claudia Beleites[1], Uwe Glaser[1,2], Uwe Hübner[1], Wolfgang Pfister[4], Wolfgang Fritzsche[1], Jürgen Popp[1,2,3], and Ute Neugebauer[1,2] — [1]Institute of Photonic Technology, Jena, Germany — [2]Center for Sepsis Control and Care, Jena University Hospital, Germany — [3]Institute of Physical Chemistry and Abbe Center of Photonics, University Jena, Germany — [4]Institute of Medical Microbiology, Jena University Hospital, Germany

The rising resistances of pathogens towards antibiotics represent a significant problem in human health-care. In this context, enterococci have become one of the most challenging nosocomial problems. It is of utmost interest to detect these resistances early in time to initiate appropriate tailored antibiotic therapies. We present a combined DEP-Raman setup which traps bacteria directly from dilute suspension within micro sized regions so as to perform highly specific Raman spectroscopic analysis. The setup is used to analyze the response of sensitive and resistant enterococci with respect to the antibiotic vancomycin. In combination with a three-level chemometric model based on PLS and LDA we are able to detect the induced drug resistance within less than three hours. Compared to standard microbiological methods which take 24 hours and more our method holds the potential to reduce diagnosis time by orders of magnitude. Acknowledgement: Financial support of BMBF (FKZ 01EO1002) is highly acknowledged.