BP 3: Bioimaging and biospectroscopy I

Time: Monday 9:30–12:45 Location: H11

BP 3.1 Mon 9:30 H11

Microviscosity of bacterial biofilm matrix characterized by quantitative fluorescence microscopy — •Valentin Dunsing, Tobias Irmscher, Stefanie Barbirz, and Salvatore Chiantia — Universität Potsdam, Institut für Biochemie u. Biologie, Potsdam, DE

Bacterial biofilms are surface-adherent communities of bacteria surrounded by an extracellular polymeric substance (EPS), which protects bacteria from antibiotics and pathogens. In this context, it remains unclear to which extent the EPS matrix imposes a physical barrier, e.g. to the transport of bacteriophages. To address this question, we have reconstituted the EPS of the bacterium *Pantoea stewartii* and investigated the diffusion properties of fluorescent particles using fluorescence correlation spectroscopy and single particle tracking. This approach allows to study the EPS spatial organization under various physicochemical conditions. We show that small probes diffuse freely in the EPS with diffusion coefficients similar to those measured in water. In contrast, large probes are drastically slowed down, showing anomalous subdiffusion. The degree of confinement increases with EPS concentration. At physiological concentrations, beads of the size of bacteriophages are up to 100-fold slowed down compared to the dynamics in aqueous solution. To overcome this physical barrier, bacteriophages are equipped with EPS degrading enzymes. We show that upon EPS degradation, strongly confined diffusion rapidly turns to free diffusion. Thus, our approach allows the investigation of dynamic changes of the biofilm microviscosity and shows that the EPS imposes a probe-size dependent diffusion barrier under physiological conditions.

BP 3.2 Mon 9:45 H11

Time resolved fluorescence spectroscopy of European Robin Cryptochrome 4. — •Anitta Rose Thomas[1], Jingjing Xu[2,3], Henrik Mouritsen[2,3], and Christoph Lienau[1] — [1]Institut for Physics,Carl Von Ossietzky University Oldenburg — [2]Institute of Biology and Environmental Sciences,Carl Von Ossietzky University Oldenburg — [3]Research Center for Neurosensory Sciences,Carl Von Ossietzky University Oldenburg

Cryptochrome proteins are special candidates for sensing the direction of the earth magnetic field due to the radical pair mechanism. While it is known that blue light absorption by the chromophore Flavin Adenine Dinucleotide(FAD), non- covalently bound to Cryptochrome, is initiating radical pair formation, it is challenging to probe the crucial chromophore-protein binding by all-optical means. Here, we study binding between FAD and European Robin Cryptochrome 4, the most likely candidate for avian magnetoreception, using time resolved fluorescence anisotropy measurement with 100 ps time resolution. The measurements show that the binding of FAD inside the Cryptochrome protein cage essentially locks the alignment of the optically excited transition dipole and effectively suppresses its rotational relaxation. The results give partial access to the electron transfer of the photo excited FAD chromophore which is faster than 100 ps.

BP 3.3 Mon 10:00 H11

Non-equilibrium dynamics of endoplasmic reticulum structures — •Konstantin Speckner, Lorenz Stadler, and Matthias Weiss — Experimental Physics 1, University of Bayreuth, Germany

Intracellular transport frequently shows anomalous characteristics, i.e. a sublinear increase of the mean-square displacement in time. Using single-particle tracking we have studied the subdiffusion of cellular organelle structures, with a particular emphasis on the impact of non-equilibrium driving forces imposed by cytoskeletal elements. In particular, we have analyzed the dynamics of tubular junctions in the endoplasmic reticulum (ER) network [1] and of ER membrane domains (ER exit sites, ERES) [2]. Our results demonstrate that both, ER junctions and ERES show a distinct subdiffusion with an anti-correlation of successive steps, reminiscent of fractional Brownian motion. Disrupting the microtubule cytoskeleton significantly altered the subdiffusive characteristics of both entities, highlighting that even subdiffusion in living cells is an actively driven process. While the motion pattern of ER junctions was seen to be directly dependent on the presence of microtubules, ERES were only indirectly affected. Our experimental data indicate that ER junctions move like monomer units of (semi)flexible polymers with the overall dynamics of the ER network being governed by fractons. ERES rather are mobile domains that perform a quasi-one-dimensional random walk on the shivering backbone of ER tubules.

[1] K. Speckner et al., Phys. Rev. E 98, 012406 (2018).
[2] L. Stadler et al., Biophys. J. 115(8), 1552 (2018).

BP 3.4 Mon 10:15 H11

Photoinduced processes of free bilins in solution: Femtosecond transient absorption spectroscopy on phycocyanobilin — •Maximilian Theiss[1], Tilman Lamparter[2], Maria Andrea Mroginski[3], Jörg Matysik[4], Chen Song[4], Wolfgang Gärtner[4], and Rolf Diller[1] — [1]TU Kaiserslautern, D-67663 Kaiserslautern — [2]KIT, D-76131 Karlsruhe — [3]TU Berlin, D-10623 Berlin — [4]Universität Leipzig, D-04103 Leipzig

Bilins are linear tetrapyrroles with rich photochemistry in solution (1,2). When bound to proteins they serve as chromophore in plant-phytochromes, bacterial sensor proteins and optogenetic systems (3). In the bound form protein-chromophore interaction restricts the potentially possible degrees of freedom (4). For a better understanding of the underlying mechanisms we study the primary photochemistry of the free bilin phycocyanobilin (PCB), employing fs transient absorption in the UV/Vis and mid-IR spectral region, complemented by quantum chemical calculations, static fluorescence and NMR measurements. In particular, PCB consists of different ground state species and shows photoinduced conformational changes (5) as well as alteration of protonation state. Additionally, broad IR continuum absorption bands in the transient absorption spectra indicate an ultrafast proton release reaction.

(1) Falk. (2012) The chemistry of linear oligopyrroles and bile pigments. SSBM. (2) Carreira-Blanco et al. (2016) PCCP 18:7148. (3) Gasser et al. (2014) PNAS 111.24:8803. (4) Singer et al. (2016) CPC 17:1288. (5) Dietzek et al. (2011) CPL 515:163.

BP 3.5 Mon 10:30 H11

High throughput real-time measurements and image analysis of suspended cells and particles — •Daniel Geiger, Tobias Neckernuss, Jonas Pfeil, and Othmar Marti — Institute of Experimental Physics, University of Ulm, Germany

Imaging of cells has proven to be a viable tool to determine properties like type or pathogenicity. Recent advances in microfluidics and lab-on-a-chip devices are based on the availability of detection systems to observe single cells with very high throughput. High-speed cameras in such applications have several drawbacks. The large amount of data needs buffering, which in turn requires offline data evaluation. Furthermore, data evaluation by standard computer architectures introduces unpredictable latency between measurement and data analysis. Hence, applications such as sorting are hardly possible by a system built of conventional camera and data processing.

We present a novel device that is based on an advanced imaging system combined with a field programmable gate array (FPGA) for control and data analysis. Due to specially developed algorithms the FPGA is able to analyze the data in real time with a fixed latency. Therefore, applications based on image analysis that require a fixed and reliable latency are feasible. Our measurement system is able to analyze up to eight regions of interest, each running at 5000 frames per second, simultaneously.

BP 3.6 Mon 10:45 H11

Light induced phycobiliprotein dynamics in *Halomicronema hongdechloris* adapted to far red light — •Franz-Josef Schmitt and Züleyha Yenice Campbell — Technische Universität Berlin, Sekr. PC 14, Straße des 17. Juni 135, 10623 Berlin

The phototrophic cyanobacterium *Halomicronema hongdechloris* contains chlorophyll *a* and *f* in photosystem II when it is grown under far red light conditions ($>$ 720 nm). In former studies we had shown that the phycobiliproteins (PBS) exhibit effcient excitation energy transfer (EET) to Chl *a* and Chl *f* within 200 ps if *H. hongdechloris* grown under far red light is illuminated with 630 nm. After adaption to far red light the PBS are localized in separated clusters of the cell. Short illumination with blue light (405 nm) leads to a mobilization of the PBS on the time scales of seconds. The PBS quickly appear completely decoupled from the photosystem II (PS II) for several seconds and subsequently recouple to the PS II with recovery of the EET from

PBS to PS II within seconds.

We assume that production of reactive oxygen species (ROS) leads to mobilization and recoupling of the PBP antenna complexes after the cells had been adapted to far red light conditions. In parallel high content of carotenoids is found in *H. hongdechloris* grown under far red light. We present a quantitative analysis of the PBP mobility in dependence of the applied light intensity and wavelength.

30 minutes break.

Invited Talk BP 3.7 Mon 11:30 H11

Cryo-Electron Tomography: Reconstruction Methods and Applications — •Achilleas Frangakis — Goethe Universitaet Frankfurt, Frankfurt, Germany

Correction of the contrast transfer function (CTF) of the microscope is a necessary step, in order to achieve high resolution from averaged electron microscopic images. Thereby, the CTF is first estimated and subsequently the electron micrograph is corrected, so that the negative oscillations of the CTF are equalized. Typically, the CTF correction is performed in 2D and the tilt-induced focus gradient is taken into account. Most often, the sample-thickness-induced focus gradient is ignored. Theoretical considerations, as well as implementation suggestions, for a 3D CTF correction that considers both gradients have been proposed before, although an implementation achieving a resolution improvement has been lacking, primarily due to computational reasons. Here, we present a comprehensive solution for a 3D CTF correction based on the Jensen-Kornberg scheme, which performs a slice-by-slice correction of the CTF within the tomographic reconstruction. We show that the computational requirements are comparable to those of 2D CTF correction. Using the examples of mitochondrial ribosomes and tobacco mosaic virus we demonstrate the improvement of the reconstruction quality with the 3D CTF correction, and the resolution gain on sub-tomogram averaging. More interestingly, for tomographic applications, the quality of the individual sub-tomograms before averaging increases significantly. We find that 3D CTF correction always produces equal or better results than 2D CTF correction.

BP 3.8 Mon 12:00 H11

Nanoscale dipole dynamics of protein membranes by Broadband Dielectric Microscopy — •G. Gramse[1,3], A. Schönhals[2], and F. Kienberger[3] — [1]JKU, Biophysics Institute, Linz, Austria — [2]BAM, Berlin, Germany — [3]Keysight Laboratories, Linz, Austria

The response of biological matter to electric fields is an intrinsic property in Biophysics which can be used to identify and characterize complex biological structures and sub-structures. At the same time, many physiological processes down to the cellular and sub-cellular level are based on electric and electrostatic interactions. Therefore quantitative investigation of dielectric properties at the nanoscale has gained major interest in recent years [1,2]. While dipoles at decreasing spatial scales within the biological structures relax with increasing frequencies, until now researchers could not address the frequency dependency of the dielectric permittivity and lacked a fundamental dimension for the understanding of many physical processes. We combined instrumentation for high frequency electrical characterization with the SPM and precise FEM based quantification procedures. The technique can be used to locally characterize biological micro-and nanoscale objects and allows for the first time quantitative nanoscale dielectric spectroscopy of bio-membranes in a broad frequency window from 3 kHz -10 GHz covering almost six orders of magnitude [3]. This allowed us to investigate the effect of surface water on the dipole dynamics in bR-membrane patches. The technique can be operated in dry and liquid environment. [1] Gramse G et al. 2013 Biophysical Journal 104 (p1257-62) [2] Biagi MC et al. 2016 ACS Nano 10, 1 [3] G. Gramse et al. Under Review

BP 3.9 Mon 12:15 H11

Machine learning approaches for optical microscopy in scattering media — •Johannes Seelig — caesar, MPG, Bonn

Light scattering hinders optical microscopy in many applications. For example in biological tissue such as the brain, only a small fraction of the entire sample can typically be accessed with diffraction limited resolution. Improved computational methods in machine learning open up novel opportunities to overcome these limitations. We will discuss the combination of approaches from adaptive optics, optical microscopy, and machine learning to improve optical imaging in various weakly and strongly scattering samples.

BP 3.10 Mon 12:30 H11

A multisensory interface for exploring nanomechanical tissue properties with human senses — •Robert Magerle[1], Stephen Barrass[2], Andreas Otto[1], Mónica Tamara Heredia Muñoz[1], Martin Dehnert[1], Thomas Baumann[1], and Alexandra Bendixen[1] — [1]TU Chemnitz, Chemnitz, Germany — [2]sonification.com, Canberra, Australia

With an atomic force microscope (AFM), the shape of a surface and its local mechanical properties can be measured in great detail on the nanometer scale. Understanding this complex and multidimensional data, however, is still in its infancy. Biological tissues in particular display a very complex spatial structure, and their mechanical properties remain largely unexplored on the nanometer scale. In the case of such complex data, analytical methods based on statistical data reduction have reached their limits. Here we present a new approach that fundamentally changes data analysis by making this complex data accessible to human perception and cognition. With a haptic interface, the force fields measured with an AFM are translated into forces perceivable to humans. Simultaneously, the surface shape and its local mechanical properties are visually and acoustically presented. This allows human users to interactively explore the forces measured on the nanometer scale, while simultaneously employing multiple senses. Humans are remarkably adept at discovering patterns within complex structures as well as deviations from these patterns. If we succeed in using this human ability for exploring nanomechanical tissue properties, this would offer the opportunity to discover new biomechanical phenomena.