BP 11: Bioimaging and Biopspectroscopy I

Time: Tuesday 9:30–13:00 Location: H 1028

Invited Talk BP 11.1 Tue 9:30 H 1028

Cryo-Electron Tomography: Method Development and Application on Cell-Cell Junctions and Nuclear Exploration — •Achilleas Frangakis — BMLS, Goethe University, Frankfurt

Cryo-electron microscopy is the major technique used in my laboratory and my talk will focus on two applications: The first involves the understanding of the macromolecular supra-organisation in the nucleus. Within cryo-electron tomograms we could visualize the complete ribosome biogenesis in a frozen hydrated state, from which the structure of the elongating RNA Polymerase I was solved at 25 Å resolution. Subsequent cryo-EM single particle analysis of the isolated RNA Polymerase I led to a structure at 3.8 Å resolution that unravelled how the RNA Polymerase I is allosterically controlled.

The second involves the analysis and the structure of cell-cell junctions that are of major importance for tissue homeostasis and are heavily involved in disease and signaling. We studied the adherens junctions and the slit diaphragm of the kidney but also the interaction of Mycoplasmas to the host cells.

Ultimately my talk should highlight our efforts towards visualizing interactions of macromolecular machineries within the unperturbed cellular context.

BP 11.2 Tue 10:00 H 1028

Microstructural analysis of the walls of termite nests using X-ray micro-tomography — •Kamaljit Singh[1], Bagus P. Muljadi[2], Ali Q. Raeini[1], Veerle Vandeginste[2], Martin J. Blunt[1], Christian Jost[3], Guy Theraulaz[3], and Pierre Degond[1] — [1]Imperial College London, UK — [2]The University of Nottingham, UK — [3]Centre de Recherches sur la Cognition Animale, CNRS, Toulouse, France

Termite nests have long been investigated for thermoregulation and ventilation by self-sustaining CO2 exchange to the outer atmosphere. Although the outer walls of termite nests are believed to be porous, and have been hypothesized as a source of gas exchange, the morphological features of the walls, and their role in controlling ventilation and heat conduction are unknown. We have investigated the microstructure of the outer and inner walls of the Trinervitermes geminatus termite nests (from Senegal and Guinea) in three dimensions using high-resolution X-ray micro-tomography. In the Senegal nest, we observe inter-connected network of larger and smaller pores. By contrast, the walls of the Guinea nest contain only the inter-connected larger pores. The smaller pores do not form, due to larger fraction of clay in the nest. From the 3D flow field simulations, we show that the presence of larger inter-connected pores in both nest materials enhances the permeability and CO2 diffusion across the outer walls. Moreover, the network of larger pores help in draining the water from the nest walls after rainy periods, therefore, re-establishing the ventilation of the nest as well as providing structural stability to the nest.

BP 11.3 Tue 10:15 H 1028

High resolution imaging of the drug delivery into stratum corneum of human skin probed with scanning near-field optical microscopy — •P. Patoka[1], G. Ulrich[1,2], K. Yamamoto[1], A. Klossek[1], F. Rancan[3], A. Vogt[3], U. Blume-Peytavi[3], P. Schrade[4], S. Bachmann[4], G. Ulm[2], B. Kästner[2], and E. Rühl[1] — [1]Physical Chemistry, Freie Universität Berlin, Takustr. 3, 14195 Berlin — [2]Physikalisch-Technische Bundesanstalt (PTB), Abbestr. 2-12, 12587 Berlin — [3]Klinisches Forschungszentrum für Haut- und Haarforschung, Charité Universitätsmedizin, 10117 Berlin — [4]Abteilung für Elektronenmikroskopie at CVK, 13353 Berlin

Understanding the mechanism of topical drug delivery into human skin requires the use of multiple techniques. Among those techniques label free methods are of special interest, avoiding drug-labels or skin-label interactions. Scanning near-field optical microscopy can be used to obtain detailed information on the correlation of the local drug distribution with highly resolved topographical information. Recent results from optical near-field microscopy imaging, investigating the penetration of the anti-inflammatory drug dexamethasone in human skin, are reported.

After resonant excitation of dexamethasone by a quantum cascade laser, operating in the mid-infrared regime, the penetration of dexamethasone in the stratum corneum is visualized. Imaging with high spatial resolution of <10 nm gives access to detailed information of the local drug distribution within the lipid matrix of the stratum corneum and its substructures. By using this technique also the presence of natural corticosteroids within the stratum corneum and ceramides is revealed. These measurements can be correlated with recent results obtained from X-ray microscopy and high resolution electron micrographs allowing us to reach an improved understanding of the drug penetration in human skin using label-free spectromicroscopy.

BP 11.4 Tue 10:30 H 1028

Observing the Plasmonic Photothermal Effect on Individual BaF3-Cells using Targeted Gold Nanostructures — •Phillip Witthöft, Lisa Prisner, and Alf Mews — Universität Hamburg, Institut für Physikalische Chemie, Hamburg, Deutschland

The exploitation of the plasmonic photothermal effect of gold structures such as gold nanorods for photothermal therapy in cancerous tissue is of great interest for the scientific community. While a wide array of specific and non-specific applications has been developed in the past, further understanding of this process and the parameters involved at the cellular level is of utmost importance for the development of these systems. In our project, we investigate the photothermal effect of gold nanorods in detail on the single cell level. We have developed a method to irradiate only an individual cell with the desired wavelength to induce a plasmonic photothermal effect and successfully observe the reaction of one individual cell to the temperature rise in real time by plotting the color saturation of the cell over time in the presence of trypan blue. We use a targeted delivery system facilitated by the interaction of the Interleukin-6 receptor with the Interleukin-6 specific aptamer AIR-3A and compare the efficiency for plasmonic photothermal therapy of these targeted nanostructures to the efficiency of non-targeted PEG-coated nanostructures. We were able to observe that cells incubated with targeted nanostructures already show their maximum color saturation after half of the time compared to non-targeted nanostructures. Based on our observations we discuss the mechanism and specificity of the photothermal effect on the single cell level.

BP 11.5 Tue 10:45 H 1028

NV based modular sensor platform for intracellular environmental sensing — Jan Vavra[1,4], Ivan Rehor[2], •Torsten Rendler[3], Mona Jani[4], Jan Bednar[6,7], Michael M. Baksh[5], Andrea Zappe[3], Joerg Wrachtrup[3], M. G. Finn[5], and Petr Cigler[4] — [1]Department of Chemistry, University of Oslo, Norway — [2]Debye Institute for Nanomaterials Science, University of Utrecht, Netherlands — [3]3th Physical Institut, University of Stuttgart, Germany — [4]Academy of Sciences, Institute of Organic Chemistry and Biochemistry, Czech Republic — [5]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, United States — [6]CNRS, University Grenoble Alpes, France — [7]Fac Med 1, Charles University Prague, Czech Republic

Monitoring intracellular concentration of chemical moietis is important for biomedical applications. We utilize the so called nitrogen vacancy color center (NV) in nanodiamonds (NDs) as a sensor working under physiological conditions. NVs itself are photostable and allow to sense for example magnetic fields or temperature in their direct vicinity. NDs have been shown to be of low toxicity and therefore form the perfect host for intracellular sensing. To enable chemical sensing we developed a hybrid sensor platform. A polymer is crafted to the ND surface hosting Gd(III) complexes that alter the NV spin lattice relaxation time T_1. By triggering the release of Gd(III) by a chemical species, the change in T_1 can be used to measure for example pH. In the current work we concentrate on the development and description of a new sensor by coating fluorescent nanodiamonds with a supported lipid bilayer.

15 min. break

BP 11.6 Tue 11:15 H 1028

Wide-Field Nuclear Magnetic Resonance Imaging using Nitrogen-Vacancy Centers in Diamond — Florestan Ziem, •Marwa Garsi, Helmut Fedder, and Jörg Wrachtrup — 3. Physikalisches Institut, Universität Stuttgart

Electron and nuclear magnetic resonance are essential tools in the life and material sciences. Significant advances in high resolution, high sensitivity sensing at sub-cellular length scales have been shown using nitrogen-vacancy (NV) centers in diamond, promising label-free imaging and single molecule analysis. E.g. by controlling and detecting the electronic state of individual NV centers, single molecule detection [1] and nanoscale NMR with resolution of chemical shift [2] have been demonstrated. Here, we show our recent progress towards transferring these techniques to wide-field imaging using ensembles of NV centers and multiplexed quantum state detection on a CCD camera. One of our key achievements is the homogenous manipulation of all NV centers over a large area. For this, we use optimal control algorithms to shape the driving microwave pulses to accomplish parallel orchestration of NV centers in a field of view of 60 x 60 μm^2. By performing nuclear magnetic wide-field imaging on solid state thin films on the diamond surface, we demonstrate an optical resolution of ∼300 nm and B-field sensitivity of 100 nT μm$^{3/2}$ Hz$^{-1/2}$. Our results pave the way towards rapid magnetic resonance imaging with sub diffraction limited optical resolution, with the ultimate goal to understand fundamental processes at the level of single cells and organelles. [1] Lovchinsky et al, Science 351, 836 (2016). [2] Aslam et al, Science 357, 67 (2017).

BP 11.7 Tue 11:30 H 1028

CellMOUSE: A novel high throughput real-time measurement method for suspended cells and particles — •Tobias Neckernuss, Daniel Geiger, Jonas Pfeil, Markus Sporer, Stefan Reich, and Othmar Marti — Institute of Experimental Physics, Ulm University

The optical measurement of cells has proven to be a viable tool in biology and in clinical applications. It is used to distinguish different cell types as well as healthy, mutated and dead cells based on parameters like size, shape and morphology. Common methods, based on video microscopy or light scattering, are either limited in throughput, analysis speed or information content.

We present a new optical measurement device, the so called CellMOUSE, that is able to measure suspended cells and particles in real-time with very high throughput of more than 500 cells per second. Measurement quantities like speed, size, morphology and shape of the cell are obtained immediately after passing the detector. In contrast to other techniques, CellMOUSE measures and evaluates each cell individually and the result is not based on the statistics of an ensemble. This is, for instance, important for cell sorting applications. Furthermore, the measurement does not require buffering so that continuous screening of cell properties over an unlimited time span is possible.

BP 11.8 Tue 11:45 H 1028

Theoretical simulation of biomarkers for *in vivo* MRI of extracellular pH — •Simone Köcher[1,3], Stephan Düwel[1,2], Christian Hundshammer[1,2], Franz Schilling[2], Steffen J. Glaser[1], Josef Granwehr[3], and Christoph Scheurer[1] — [1]Department of Chemistry, Technische Universität München, Garching, Germany — [2]Department of Nuclear Medicine, Klinikum rechts der Isar, Technische Universität München, Garching, Germany — [3]IEK-9 - Fundamental Electrochemistry, Forschungszentrum Jülich, Jülich, Germany

Up to now, there are no techniques available to routinely measure extracellular pH in the clinic. Pathological deviations from the systemic pH are often caused by cancer, inflammation, infection, and other diseases. Hyperpolarized [1,5-$^{13}C_2$]zymonic acid (ZA) was recently introduced as a novel MRI biomarker for dissolution dynamic polarization (DNP) measurements of extracellular pH with good resolution. Systematic, time-consuming, experimental screening for promising biomarker molecules can be facilitated by theoretical *ab initio* calculations of chemical shifts and pK_a values. We introduce a theoretical screening approach for pH-sensitive biomarkers and point out the important technical aspects. For ZA, the calculations show good accuracy in the prediction of the pH-dependent ^{13}C chemical shift sufficient for a theoretical pre-screening of potential biomarker molecules.

BP 11.9 Tue 12:00 H 1028

Imaging in Biologically-Relevant Environments with AFM Using Stiff qPlus Sensors — •Korbinian Pürckhauer[1], Alfred J. Weymouth[1], Katharina Pfeffer[1], Lars Kullmann[1], Estefania Mulvihill[2], Michael P. Krahn[3], Daniel J. Müller[2], and Franz J. Giessibl[1] — [1]University of Regensburg, Regensburg, Germany — [2]ETH Zürich, Basel, Switzerland — [3]University Hospital of Münster, Münster, Germany

High-resolution imaging of soft biological samples with atomic force microscopy (AFM) is challenging because they need to be imaged with very low forces to prevent deformation. Typically, AFM of those samples is performed with soft silicon cantilevers ($k \approx 0.1 - 10\,\mathrm{N/m}$) and optical detection in a liquid environment.

In this work we demonstrate the advantages of using stiffer sensors ($k \approx 1\,\mathrm{kN/m}$) which were used to obtain unprecedented spacial resolution of molecules in vacuum at low temperatures [1]. In liquid environments, the high stiffness of the qPlus sensor allows us to use small amplitudes in a non-contact mode and obtain high quality factors [2]. The samples are immersed in aqueous solution in a liquid cell and we use qPlus sensors with long tips, only submerging the tip apex.

Atomic resolution of muscovite mica was achieved in various solutions. To prove that we can non-destructively image soft biological samples with stiff sensors, we show images of lipid membranes and finally molecular resolution images of a lipid bilayer.[3]

[1] Gross et al., Science 325, 1110 (2009). [2] Ichii et al., Jpn. J. Appl. Phys. 51, 08KB08 (2012). [3] Pürckhauer et al., submitted.

BP 11.10 Tue 12:15 H 1028

Simulation of FRET Dyes Allows Direct Comparison Against Experimental Data — •Ines Reinartz[1], Claude Sinner[1], and Alexander Schug[1,2] — [1]Karlsruhe Institute of Technology, Karlsruhe, Germany — [2]John von Neumann Institute for Computing, Forschungszentrum Jülich, Jülich, Germany

Single molecule Förster Resonance Energy Transfer (smFRET) experiments provide valuable insight into protein dynamics. Akin to a molecular ruler, different protein conformations can be observed by measuring the energy transfer depending on the distance between selected residues labeled with dyes. Besides this distance, the energy transfer is also dependent on the mutual orientation of the dyes. Both can be gained from atomistic simulations.

We develop a coarse-grained simulation technique with few parameters while maintaining full protein flexibility and including all heavy atoms. The computational efficiency of these simulation protocols allows for simulating large systems and heterogeneous ensembles as found in, e.g., protein folding.

The FRET efficiency histograms we gain from our simulations are directly comparable to experimental measurements. With access to distances and orientations from atomically resolved trajectories, we want to improve the planning and interpretation of smFRET measurements. As an example, we compare distributions from 2-color and 3-color FRET experiments and simulations for ClyA in monomer and protomer conformation, as well as folded and unfolded ensembles of different systems.

BP 11.11 Tue 12:30 H 1028

Optical Mapping of Contracting Hearts — •Johannes Schröder-Schetelig[1], Jan Christoph[1,2], and Stefan Luther[1,2] — [1]Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany — [2]German Center for Cardiovascular Research (DZHK e.V.), Göttingen, Germany

Optical mapping of isolated, intact hearts or myocardial cell cultures using fluorescent dyes has become a very well established tool in cardiac research. However, the method as such can be very sensitive to movement of the sample, resulting in severe motion artifacts in the recorded optical signals. In order to prevent this, in the past either the contraction had to be suppressed or more sophisticated strategies like ratiometric imaging had to be applied.

Here, we present a new method, which combines marker-free 2D video tracking techniques with panoramic optical mapping of freely beating and contracting Langendorff-perfused hearts using multiple calibrated cameras. We find that it is possible to accurately track and reconstruct the 3D deformation of the cardiac surface. The tracking is achieved without the need for additional landmarks or special patterned lighting schemes. By projecting the fluorescence videos directly onto the deforming mesh geometry, motion artifacts become significantly reduced. This opens up a new way, where the contraction of a heart is not considered a disturbing limitation any longer, but is now a property which can be measured and studied.

BP 11.12 Tue 12:45 H 1028

Synchronization-based Reconstruction of Cardiac Electrical Wave Dynamics from Mechanical Deformation using High-speed 4D Ultrasound — •Jan Christoph, Jan Lebert, Ulrich Parlitz, and Stefan Luther — Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany

Heart rhythm disorders such as ventricular or atrial fibrillation are

determined by turbulent electrical vortex wave activity, which occupies the heart muscle and causes irregular contractions and inefficient beating of the heart. Both cardiologists and basic scientists are highly interested in obtaining visualizations of this highly dynamic and complex wave structure and its 3D spatio-temporal organization throughout the heart muscle, to be able to develop strategies for its efficient and reliable termination during ablation or defibrillation therapies. In recent work, we have shown that high-resolution 4D ultrasound can be used to image vortex-like mechanical structures in the fibrillating heart and that it is possible to reconstruct an electromechanical vortex filament structure that characterizes the spatio-temporal organization of ventricular fibrillation within the heart wall. Here, we show that in addition to imaging the mechanical activity, it is possible to reconstruct the electrical wave dynamics, which caused the deformations of the tissue, and which remains invisible in the imaging experiments, numerically. Using a synchronization-based approach, we demonstrate that even complex electrical vortex wave patterns, such as spiral waves or spiral wave chaos, can be reconstructed in elastic excitable media from the analysis of the deformation mechanics.