BP 7: Bioimaging and biospectroscopy II

Time: Tuesday 9:30–12:30 Location: H4

BP 7.1 Tue 9:30 H4

Strong cytoskeleton activity on millisecond timescales upon particle binding revealed by ROCS microscopy — Felix Jünger and •Alexander Rohrbach — Laboratory for Bio- and Nano-Photonics, Department of Microsystems Engineering, University of Freiburg, Germany

Cells change their shape within seconds, cellular protrusions even on subsecond timescales enabling various responses to stimuli of approaching particles. Typical responses are governed by a complex reorganization of the actin cortex, where single filaments and molecules act on even faster timescales. These dynamics have remained mostly invisible due to a superposition of slow and fast motions, but also due to a lack of adequate imaging technology. Whereas fluorescence techniques require too long integration times, novel coherent techniques such as ROCS microscopy can achieve sufficiently high spatiotemporal resolution. ROCS uses rotating back-scattered laser light from cellular structures and generates high-contrast images with 100 Hz and 150nm resolution, without fluorescence or bleaching. Here, we present an extension of ROCS microscopy that exploits the principles of dynamic light scattering for precise localization, visualization and quantification of the cytoskeleton activity of mouse macrophages. The local structural reorganization processes, encoded by dynamic speckle patterns, occur upon distinct mechanical stimuli, such as soft contacts with optically trapped beads. We find that a substantial amount of the near-membrane cytoskeleton activity takes place on millisecond timescales, which is much faster than reported ever before.

BP 7.2 Tue 9:45 H4

Imaging nanoscale aggregation of proteins ex vivo using the contrast in Förster resonance energy transfer obtained from 2D polarization fluorescence imaging (2D POLIM) — •Daniela Täuber[1], Adrian T. Press[2], Petra Martinac[2], Kay-Jovanna Benecke[2], Michael Bauer[2], Juanzi Shi[3], and Ivan G. Scheblykin[3] — [1]Biopolarisation, Leibniz-IPHT & Friedrich-Schiller-University Jena, Germany — [2]Anesthesiology and Intensive Care Medicine & Center for Sepsis Control and Care, Jena University Hospital — [3]Single Molecule Spectroscopy, Lund University, Sweden

Förster resonance energy transfer (FRET) is a well-established nanoruler suited to discriminate small aggregates of fluorescent molecules from just high concentration. Contrary to spectrally resolved two-color-FRET, polarization resolved fluorescence microscopy allows to determine homo-FRET between similar fluorophores. Conventional fluorescence anisotropy is restricted to isotropic samples, otherwise, the results depend on the choice of the lab frame. 2D POLIM was recently applied to study early protein aggregation of GFP-labeled human α-synuclein in models of Parkinson's disease ex vivo.[1] We used 2D POLIM to study f-actin aggregation in healthy and pathologic liver tissue, which is related to liver damage in systemic infection. A qualitative analysis showed variations of the FRET parameter, which can be compared to the pathologic condition of the sample. – [1] Camacho et al. 2D Polarization Imaging as a Low-Cost Fluorescence Method to Detect α-Synuclein Aggregation Ex Vivo in Models of Parkinson's Disease. Commun. Biol. 2018, 1, 157.

BP 7.3 Tue 10:00 H4

Photo-induced force microscopy (PiFM) and IR & Raman-spectroscopy on multicore magnetic nanoparticles (MCNPs) — •Nila Krishnakumar[1,2,3], Anika Strecker[1,3,4], Philip Biehl[2,5], Felix H. Schacher[2,5], Anne-Dorothea Müller[6], Anuradha Ramoji[1,7], Ute Neugebauer[1,2,5,7], Heidemarie Schmidt[1,2], and Daniela Täuber[1,2] — [1]Leibniz-IPHT, Jena, Germany — [2]Friedrich-Schiller-University Jena — [3]Abbe Center of Photonics, Jena — [4]Ernst-Abbe University of Applied Science, Jena — [5]Jena Center for Soft Matter — [6]Anfatec Instruments GmbH, Oelsnitz, Germany — [7]Center for Sepsis Control and Care, Jena University Hospital

MCNPs are promising candidates for theranostics of cancer and infectious diseases. PiFM is a new spectroscopy/imaging method which combines excitation in the mid infrared by quantum cascade lasers with detection using a conductive AFM tip. PiFM and IR & Raman-spectroscopy shall be used for detecting such MCNPs, and the results of the three spectroscopy/imaging methods compared.

BP 7.4 Tue 10:15 H4

3D depth profiling the interaction between an AFM tip and hydrated, native collagen fibrils in sheep tendon — •Martin Dehnert[1], Diana Voigt[1], Anke Bernstein[2], and Robert Magerle[1] — [1]Fakultät für Naturwissenschaften, TU Chemnitz, Germany — [2]Department Chirurgie, Universitätsklinikum Freiburg, Germany

Imaging with atomic force microscopy (AFM) the structure and the viscoelastic properties of hydrated, native tissue on the nanometer scale is challenging since the AFM tip interacts with a soft, compliant, partially fluid, and adhesive specimen. Furthermore, the tissue's water content needs to be maintained during AFM imaging. Here we study collagen fibrils in hydrated, native sheep tendon with AFM-based measurements of force–distance (FD) curves and amplitude–phase–distance (APD) curves. From this data, we reconstruct three-dimensional (3D) depth profiles of the tip–sample interaction. This allows for distinguishing the viscoelastic response of individual collagen fibrils within the tendon from the attractive capillary forces between the AFM tip and the viscous interfibrillar matrix. The 3D depth profiles reveal a large diversity in nanomechanical properties among individual collagen fibrils in both their viscoelastic response and the attractive tip–sample interaction. Furthermore, we obtain information about the local mechanical response of the interfibrillar matrix. We expect that this comprehensive nanomechanical characterization will contribute to a better understanding of tendon biomechanics on the nanometer scale.

BP 7.5 Tue 10:30 H4

Imaging biological samples with AFM using stiff qPlus sensors — •Korbinian Pürckhauer[1], Alfred J. Weymouth[1], Katharina Pfeffer[1], Estefania Mulvihill[2], Lars Kullmann[1], Michael P. Krahn[1,3], 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 ($\sim$ 0.1-10 N/m) and optical detection in a liquid environment. In this work we demonstrate the advantages of using stiffer sensors ($\sim$ 1 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. 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 molecular resolution images of a lipid bilayer and preliminary results on DNA Origami [2].

[1] Gross et al., Science 325, 1110 (2009). [2] Pürckhauer et al., Sci. Rep. 8, 9330 (2018).

BP 7.6 Tue 10:45 H4

Single particle tracking in 2+1 Dimensions using interferometric scattering Microscopy — •Philipp Kellner[1], Francesco Reina[2], Christoffer Lagerholm[2], and Christian Eggeling[1,2] — [1]Institute of Applied Optics and Biophysics, Philosophenweg 7, 07743 Jena — [2]Weatherall Institute of Molecular Medicine University of Oxford, Headley Way, Oxford

Directly recording Tracks of moving particles is essential for various parts of physics. In modern biophysics measuring the diffusion of lipids and proteins on biological membrane model systems is an example. Those measurements reveal not only information about the moving particle itself but also about heterogenities in the surrounding structures. Such observations require high spatial and temporal resolution. Novel interferometric scattering Microscopy (iScat) provides localization precision in the nm-range and time-resolution down to several microseconds. This talk will present the basic principles of iScat-Microscopy and underline its usability for biological questions. Further, optical improvement of the setup and the possiblity of detec-

tion using small labels in biological model membrane systems will be highlighted.

30 minutes break.

BP 7.7 Tue 11:30 H4

Quantitative 3d histology of Alzheimer's disease by holotomography — •Marina Eckermann[1], Mareike Töpperwien[1], Jasper Frohn[1], Thany Bui[1], Anna-Lena Robisch[1], Franziska Van der Meer[2], Christine Stadelmann-Nessler[2], and Tim Salditt[1] — [1]Institute for X-ray Physics, Göttingen University, Göttingen, Germany — [2]Institute for Neuropathology, University Medical Center, Göttingen, Germany

Towards quantitative 3d virtual histology of the human brain, we have recently achieved significant progress in reconstructing the neuronal architecture of human cerebella [1], combining propagation-based x-ray phase contrast imaging using laboratory μCT and synchrotron radiation [2]. In this way, we aim at a multi-scale workflow, to automatically identify nanometric pathological alterations from ccm-sized tissue blocks. In [3], nano- and μCT was used to image Aβ plaques in heavy metal stained murine tissue. In the present work, we investigate structural alterations from Alzheimer's disease (AD) in unstained, paraffin-embedded human hippocampus, hence based on native electron density variations. In control tissue data, we predominantly find intact neurons, characterized by a clear difference in electron density between the cytosol and the nucleus. Furthermore, affected neurons appear overall shrinky, with a perishing nucleus and increased density. Beyond that, we also observe diffuse objects of increased density in specific locations (analysis ongoing work).
[1] Töpperwien et al., PNAS 115, 27 (2018). **[2]** Töpperwien et al., Sci. Rep. 7, 42847 (2017). **[3]** Massimi et al., NeuroImage 184 (2019).

BP 7.8 Tue 11:45 H4

Nanoscale X-ray computed tomography for the 3D structural characterization of electrospun fibers — Adriana T González[1], Cristine S de Oliveira[1], Tobias Kürbitz[2,3], Christian E H Schmelzer[3], Ralf B Wehrspohn[1,3], and •Juliana Martins de S e Silva[1,3] — [1]Institute of Physics, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany — [2]Anhalt University of Applied Science, Köthen, Germany — [3]Fraunhofer Institute for Microstructure of Materials and Systems IMWS, Halle (Saale), Germany

Electrospinning is a well-established method for the production of fibrous non-woven biomaterials which are used in tissue engineering as biomimetic three-dimensional (3D) scaffolds to assist cell growth. The properties of these non-woven biomaterials, including their porous network, the size, and interconnectivity of the electrospun fibers are critical parameters for their successful application as scaffolds. As the conventional methods for characterizing these fibers have deficiencies, such as the use of toxic substances and incomplete visualization of the porous structure, here we explored phase-contrast nanoscale X-ray computed tomography (nano-CT) as an alternative method to image and characterize the 3D structure of electrospun gelatin-based fiber matrices. The 3D datasets obtained gave a visual insight into the morphology of the fibers. We observed changes in fiber thickness upon chemical cross-linking, resulting in declines of porosity of 23 % and of the surface area of 39 %. We show that phase-contrast nano-CT is a promising method for the fast, non-destructive and high-resolution 3D imaging and quantitative characterization of electrospun nanofibers.

BP 7.9 Tue 12:00 H4

Ratiometric fluorescence imaging and marker-free motion tracking of Langendorff-perfused beating rabbit hearts — •Vineesh Kappadan[1,2], Johannes Schröder-Schetelig[1,2], Ulrich Parlitz[1,2,3], Stefan Luther[1,2,3,4,5], and Jan Christoph[1,3] — [1]Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany — [2]Institute for Nonlinear Dynamics, Georg-August-Universität, Göttingen, Germany — [3]German Center for Cardiovascular Research (DZHK e.V.), partner site Göttingen, Germany — [4]Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Göttingen, Germany — [5]Department of Physics and Department of Bioengineering, Northeastern University, Boston, USA

Optical mapping based on fluorescence imaging is used for visualizing membrane voltage and Ca^{2+} concentration of isolated, Langendorff-perfused intact hearts. Despite recent progress in imaging of isolated beating hearts, accurate measurements of quantities such as the duration of action potentials are still challenging due to residual motion artifacts present in the fluorescence signal. Motion artifacts can be significantly reduced by the electromechanical uncoupler Blebbistatin, which may, however, affect properties like the action potential duration (APD). We show that marker free motion tracking combined with ratiometric fluorescence imaging techniques can be used to more reliably measure the APD from Langendorff-perfused, beating rabbit hearts and we use this technique to investigate the effects of Blebbistatin on the APD and restitution properties of the heart.

BP 7.10 Tue 12:15 H4

Label-free detection of individual nanosystems in liquids — •Larissa Kohler — Karlsruhe Institute of Technology

The label-free detection of nanosystems provides the opportunity to understand biomolecular dynamics and interactions without undesired modifications of the system. To achieve the high sensitivity required for studying individual solved nanosystem, we use signal enhancement in a fiber-based Fabry-Perot cavity with high finesse ($F \approx 10^5$), which is integrated in a microfluidic channel. The presence and dynamics of an individual nanoobject in the tight focus of the cavity mode ($w_0 \approx 1\,\mu m$) can be detected due to its induced frequency shift and decrease of amplitude of the signal. In this talk I will show results on the time-resolved measurement of the Brownian motion of individual silica nanoparticles and report the progress towards sensing of individual biomolecular nanosystems.