BP 20: Poster VIII

Bioimaging and Biospectroscopy (BP 18.1 – BP 18.26)

Time: Tuesday 14:00–16:00 Location: P2/4OG

BP 20.1 Tue 14:00 P2/4OG

High-throughput scanning SAXS of desmin-expressing cells — •Chiara Cassini[1], Manfred Burghammer[2], Harald Herrmann[3], and Sarah Köster[1] — [1]IRP, Georg-August-Universität Göttingen, Germany — [2]ESRF, Grenoble, France — [3]DKFZ, Heidelberg, Germany

Desmin is the main intermediate filament (IF) protein in muscle cells. Recently, a large number of mutations in the desmin gene have been discovered to be pathogenic. In order to assess the structures formed in cells by normal and mutant desmin, a high resolution method, capable of retrieving structural information at sub-cellular length scales, without the need for slicing the cells, is preferable. Thus, we used scanning small angle X-ray scattering (SAXS) on three different cell lines generated from IF-free mouse fibroblasts: one expressing wild type desmin, one expressing R406W-desmin, and the IF-free mother cell clone itself. The cells were grown on silicon nitride windows and measured in freeze-dried state. Each window contained tens to hundreds of cells. Each cell scan used to take minutes to hours; recently, we were able to employ a special fast scanning mode that allowed us to image an entire window in about 8 hours only. This approach ensured the collection of a statistically significant pool of data in a reasonable time span. The large quantity of data thus collected was treated with a combination of semi-automated segmentation of the dark field images and parallel computations. In the end, we were able to carry out a statistically relevant comparison of local structure-related parameters of the three cell lines, such as anisotropy and orientation.

BP 20.2 Tue 14:00 P2/4OG

Studying molecular interactions with a combination of microfluidics and FFS — •Eleonora Perego and Sarah Köster — IRP, Georg-August-Universität Göttingen, Germany

Assembly and aggregation of biomolecules into larger complexes are fundamental processes in living organisms. Ordered protein assembly is vital for the organism, as for example the assembly of the cytoskeletal filaments, however sometimes disordered aggregates, which can be toxic as fro example alpha-synuclein fibrils, are also produced. It is fundamental to study both the ordered assembly and the disordered aggregation with high spatial resolution (on a single molecule level) and good temporal precision (in the order of ms) to gain a complete knowledge of these reactions. Here, we combine fluorescence fluctuation spectroscopy (FFS), employed to measure the interactions, with microfluidics to access the temporal information. We focus on studying the ordered assembly of vimentin, an intermediate filament (IF) protein which is part of the cytoskeleton, using a multi-layer microfluidic device that prevents the protein from coming in contact with the channel walls. This type of device also provides a controlled diffusive mixing of assembly buffer and protein solution. Employing FFS in these devices enables us to precisely measure the labelling stoichiometry of the assembling protein, which allow us to follow the very first time steps of vimentin assembly. Our results show that the combination of microfluidics and FFS provides a suitable approach for studying the aggregation of biomolecules in real time, which is important for understanding cellular behavior.

BP 20.3 Tue 14:00 P2/4OG

Imaging single glycans — •Xu Wu[1], Martina Delbianco[2], Kelvin Anggara[1], Stephan Rauschenbach[1,3], Sabine Abb[1], Peter Seeberger[2], and Klaus Kern[1] — [1]Max-Planck-Institut für Festkörperforschung, Stuttgart — [2]Max-Planck-Institut für Kolloid und Grenzflächenforschung, Potsdam — [3]Chemistry Research Laboratory, Department of Chemistry, University of Oxford

Imaging biomolecules guides the understanding of their diverse structures and functions [1]. Real space imaging at sub-nanometer resolution by cryo-electron microscopy has provided key insights into proteins and their assemblies. Direct molecular imaging of glycans, the predominant biopolymers on earth with a plethora of structural and biological functions, is currently not possible. Inherent glycan complexity and backbone flexibility requires single molecule approaches for real space imaging. Glycan characterization using mass spectrometry and nuclear magnetic resonance provides insights into size, sequence, branching, and connectivity but rely on structure reconstruction from indirect information. Here, we show direct low temperature scanning tunneling microscopy (STM) imaging of single glycan molecules that are isolated by mass-selective, soft-landing electrospray ion-beam deposition [2]. Sub-nanometer resolution allows for the visualization of glycan connectivity and discrimination between regio-isomers. Direct glycan imaging is an important step towards a better understanding of the structure of carbohydrates.

[1] Unwin, P. N. T, et. al. J. Mol. Biol. 94, 425-440 (1975). [2] Rauschenbach, S., et. al. Annu. Rev. Anal. Chem. 9, 473-498 (2016).

BP 20.4 Tue 14:00 P2/4OG

Asymmetries & gradients during early C. elegans embryogenesis — •Rebecca Benelli, Philipp Struntz, Dirk Hofmann, and Matthias Weiss — Universität Bayreuth

To enable differentiation of cells and to facilitate cell organization the establishment of gradients is crucial in early embryogenesis. We have used the model organism C. elegans and a custom built light-sheet microscope to study the formation of protein and organelle gradients in three dimensions over time. Due to the low phototoxicity and reduced bleaching induced by this selective illumination long term observations without developmental perturbations are made possible. The focus of the current study is on evolution until the first cell division, which, next to the different sized daugther cells, is characterized by a lot of accompanying asymmetries. We study the protein concentration of two vital proteins in early development with respect to their axial as well as radial distribution. Also, two organelles with opposing gradients are investigated. Since diffusion plays a vital role in the establishment of gradients a new multiplexed diffusion measurement technique (SPIM-FCS) is used to quantify changes in diffusive behavior of proteins in space and time.

BP 20.5 Tue 14:00 P2/4OG

The relative densities of cell cytoplasm, nucleoplasm, and nucleoli are robustly conserved during cell cycle and drug perturbations — •Kyoohyun Kim and Jochen Guck — Max Planck Institute for the Science of Light, 91058 Erlangen, Germany

The cell nucleus is an essential cellular compartment as the location of gene expression and DNA replication. While the large amount of its chromatin confined in the finite nuclear space could install the picture of a particularly dense organelle surrounded by less dense cytoplasm, recent studies have begun to report the opposite. However, the generality of this observation has so far not been tested. Here, we used combined optical diffraction tomography (ODT) and epi-fluorescence microscopy to systematically quantify the mass densities of cytoplasm, nucleoplasm, and nucleoli of HeLa-FUCCI and RPE-FUCCI cells being challenged by various perturbations. We found that the nucleoplasm maintains a lower mass density than cytoplasm, but lower than nucleoli, during cell cycle progression by scaling its volume to match the increase of dry mass during cell growth. Moreover, actin and microtubule depolymerization and changing chromatin condensation altered volume, shape and dry mass of the various cellular compartments, while the relative distribution of mass densities was still robustly conserved. Our findings suggest that cells regulate relative mass densities across membrane-bound and membrane-less compartments, likely by different as of yet unknown mechanisms. This surprising robustness of mass densities contributes to the increasing importance of physical properties of biological cells in current biological research.

BP 20.6 Tue 14:00 P2/4OG

Biocompatible fluorescence modulating nanocoatings for sharper and faster screening applications — •Deyan Nestorov[1], Julia Fender[2], Kristina Lorenz[2], and Katrin G. Heinze[1] — [1]Rudolf Virchow Center, University of Würzburg, 97080 Würzburg, Germany — [2]Institute of Pharmacology and Toxicology, University of Würzburg, 97078 Würzburg, Germany

Biocompatible metal-dielectric nanocoatings have been shown to act as versatile fluorescence modulators. They can be customized to different spectral ranges and biological architectures of interest. Here, we explore such coatings for dual-color high-throughput screening (HTS)

applications highlighting ligand-receptor interactions. Culturing adherent cells directly on nanocoated substrates allows to enhance both the signal-to-noise ratio of the detected fluorescence from the fluorescenctly tagged membrane proteins as well as the Förster resonance energy transfer (FRET) efficiency as a measure of the protein conformational changes upon ligand binding. Our simulations show that the signal contrast could be enhanced by a factor of 2.7, and thus the screening rate accelerated by a factor of 1.6. We plan to experimentally establish such an approach for high-throughput ligand screening of G-Protein coupled receptors which comprise over 30% of all drug targets. A major advantage of this approach is that it could be directly integrated into common setups with ease and thus offers new perspectives for pharmacological research.

BP 20.7 Tue 14:00 P2/4OG

Metal Induced Energy Transfer for Structural Analysis of Focal Adhesions via Actin Stress Fiber Angles — •Lydia Rebehn, Fabian Port, and Kay-E. Gottschalk — Institute of Experimental Physics, Ulm University, Ulm, Germany

Focal adhesion (FA) structures physically anchor cells to the extracellular matrix and facilitate cell interactions with their environment. They are composed of different protein components assembled in a highly regulated conformation. Intracellularly, FAs couple with actin to transfer external forces into internal mechanical and biochemical signals. Their structure is difficult to resolve since the components interact in the nanometer range [1],requiring localization of molecular components with nanometer-range resolution in x-, y-, and z- axes. Analyzing the actin stress fibers of cells in 2D culture can give information on the FA attachment to the surface based on angle analysis. The technique we use is Metal Induced Energy Transfer (MIET) [2]. We have provided an initial analysis of the angles between actin stress fibers and a 2D substrate, demonstrating the worth of MIET for cellular structure analyses near the basal membrane with nanometer accuracy.

References:

[1] Kanchanawong, P., Shtengel, G., Pasapera, A. M., Ramko, E. B., Davidson, M. W., Hess, H. F., & Waterman, C. M.,Nature, 468(7323), 580-584 (2010)

[2] Chizhik, A. I., Rother, J., Gregor, I., Janshoff, A., Enderlein, J., Nature Photonics, advance on(January), 1-8 (2014)

BP 20.8 Tue 14:00 P2/4OG

Investigating nanoplasmonic membranes as cellular strain sensors — •Peter Kolb and Kay-E. Gottschalk — Institute of Experimental Physics, Ulm University, Ulm, Germany

Metallic nanoparticles (NPs) display specific electromagnetic resonances, known as localized surface plasmon resonances (LSPR). These LSPRs strongly depend on the size and geometry of the NPs [1], as well as the local environment of the particles. Coupling between closely spaced NPs creates resonance dependence on their inter-particle distances. Since a change in this distance leads to a shift of the LSPR, nanoplasmonic membranes can be used as strain sensors [2]. The combination of gold NPs and polydimethylsiloxane (PDMS) substrate offers a biocompatible membrane that can be used for in vitro studies. Combining electron beam lithography, electron beam evaporation, dry lift-off, and reactive ion etching, we produce gold NP arrays on PDMS membranes. These nanoplasmonic membranes are analysed via scanning electron microscopy, atomic force microscopy, and spectroscopy. Supported by electromagnetic simulations, we investigate their properties and suitability as cellular strain sensors.

References:

[1] Chen, Yiqin, et al., Reliable fabrication of nanostructures without an adhesion layer using dry lift-off. Nanotechnology 26.40 (2015): 405301.

[2] Maurer, Thomas et al., The beginnings of plasmomechanics: towards plasmonic strain sensors. Frontiers of Materials Science 9.2 (2015): 170-177.

BP 20.9 Tue 14:00 P2/4OG

Investigation of Actin near the Basal Membrane of Living Cells using Metal Induced Energy Transfer — •Fabian Port, Ulla Nolte, Peter Kolb, Carolin Grandy, and Kay-E. Gottschalk — Institute of Experimental Physics, Ulm University, Germany

Focal adhesions function as anchoring points to the extracellular matrix, and also enable cells to sense and exert forces on their environment [1]. Focal adhesions are complex structures consisting of a multitude of different proteins. Despite the important role of the focal adhesion complex in cellular adhesion, its structure and dynamics remain difficult to resolve [2]. Knowing the exact position of the proteins in the focal adhesion complex in live cells is necessary to understand their working principles. For a detailed analysis of the focal adhesions dynamic architecture, we require a method to measure small distances that may be applied over a variable time scale. To meet this challenge, we use Metal Induced Energy Transfer (MIET) [3] to resolve protein positions at the nanoscale level in live cells. Using MIET, we analyse the dynamics of focal adhesion associated actin with ultra high resolution.

[1] Geiger, B. et al., Nature Reviews. Molecular Cell Biology, 10(1), 21-33 (2009)

[2] Kanchanawong, P. et al., Nature, 468(7323), 580-584 (2010)

[3] Chizhik, A. I. et al., & Enderlein, J., Nature photonics, advance on(January), 1-8 (2014)

BP 20.10 Tue 14:00 P2/4OG

Micropatterning on Gold Surfaces for Biophysical Applications — •Carolin Grandy, Peter Kolb, Fabian Port, and Kay-E. Gottschalk — Institute of Experimental Physics, Ulm University, Ulm, Germany

Cells change their shape based on their environment and are highly sensitive to mechanical and geometric factors. Micropatterning is a simple method to manipulate and control cell shape [1]. Our primary goal was to develop a reproducible method to manipulate and normalize cell shape on gold with micropatterning for quantitative super-resolution imaging. We use methoxy polyethylene glycol (PEG) thiol to create a self-assembled monolayer on gold, due to the strong bonds formed between thiol groups and gold [2], and the protein repellent nature of PEG. This monolayer can be oxidised through a photomask with deep UV-light [1]. The oxidised PEG pattern is no longer protein repellent, and can be coated with extracellular matrix proteins and seeded with cells. The surfaces we developed and analyzed can be used in a wide range of biophysical applications, including metal induced energy transfer and surface enhanced raman scattering.

References:

[1] M Thery, Micropatterning as a tool to decipher cell morphogenesis and functions, Journal of Cell Science 2010 123 (24), 4201-4213.

[2] R.G. Nuzzo et al, Fundamental studies of the chemisorption of organosulfur compounds on gold(111). Implications for Molecular self-assembly on gold surfaces, Journal of the American Chemical Society 1987 109 (3), 733-740.

BP 20.11 Tue 14:00 P2/4OG

Surface functionalization of Nanodiamonds — •Svenja Maurer, Frederike Erb, Karolina Zeh, and Kay-E. Gottschalk — Institute of Experimental Physics, Ulm University, Germany

Fluorescent Nanodiamonds (FNDs), due to their properties, find application in various areas and can be used for fluorescence bioimaging. FNDs contain photostable nitrogen vacancy centers. Their fluorescence shows neither blinking nor photobleaching. The NV-center absorbs green light and emits photons in the near-infrared range. FNDs can be used in vitro as well as in vivo due to their biocompatibility. The fluorescence characteristics can be influenced by the environment such as an external magnetic field and thus FNDs can be used in versatile nanosensors [1]. Various surface modifications of FNDs are possible and necessary for different experiments. We present techniques to functionalize the FND's surface [2].

[1] Hsiao, Wesley Wei-Wen, et al. "Fluorescent nanodiamond: a versatile tool for long-term cell tracking, super-resolution imaging, and nanoscale temperature sensing." Accounts of chemical research 49.3 (2016): 400-407.

[2] Krüger, Anke, et al. "Surface functionalisation of detonation diamond suitable for biological applications." Journal of Materials Chemistry 16.24 (2006): 2322-2328

BP 20.12 Tue 14:00 P2/4OG

Fluorescent nanodiamond as a detector for magnetic field fluctuations — •Frederike Erb and Kay-E. Gottschalk — Institute of Experimental Physics, Ulm University, Germany

Fluorescent nanodiamonds (FNDs) offer various new imaging and metrology approaches, especially in the life sciences. Nanodiamonds containing nitrogen-vacancy centers (NV-centers) as fluorophores emit light in the near-infrared window of bioimaging. Their luminescence properties depend on the environment and thus FNDs cannot only be used for bioimaging but also find an application as part of various

nanosensors. A nanodiamond sensor can be smaller than 50 nm in diameter and read-out optically without contact. As they are biocompatible and non cytotoxic, they can be used for many experiments in biological samples.

We present experiments using the NV-center in nanodiamond as a magnetic field detector. Gd^{3+} ions in the surrounding of the nanodiamond introduce magnetic field fluctuations, which affect the NV's spin relaxation time T_1 [1]. Reading-out this T_1-Time with a commercial confocal microscope gives a measure of the Gd^{3+} concentration in the sample.

References:

[1] Kaufmann, S. et al. (2013): Detection of atomic spin labels in a lipid bilayer using a single-spin nanodiamond probe. In: Proceedings of the National Academy of Sciences 110 (27), S. 10894-10898.

BP 20.13 Tue 14:00 P2/4OG

Nanomechanics of collagen fibrils in native tendon — •Martin Dehnert[1], Anke Bernstein[2], and Robert Magerle[1] — [1]Fakultät für Naturwissenschaften, Technische Universität Chemnitz, Germany — [2]G.E.R.N. Tissue Replacement, Regeneration & Neogenesis, Department of Orthopedics and Trauma Surgery, Medical Center and Faculty of Medicine, Albert-Ludwigs-University of Freiburg, Germany

We use atomic force microscopy to determine the three-dimensional depth profiles of the local nanomechanical properties of collagen fibrils and their embedding interfibrillar matrix in native (unfixed), hydrated tendon. AFM imaging in air with controlled humidity preserves the tissue's native water content and allows for high-resolution imaging the assembly of collagen fibrils beneath an approximately 5 to 10-nm-thick layer of the fluid components of the interfibrillar matrix. We collect pointwise force–distance (FD) data from which we construct 3D depth profiles of the local tip–sample interaction forces. We observe diversity in the nanomechanical properties of individual collagen fibrils in their adhesive as well as their repulsive, viscoelastic mechanical response. The contact points between adjacent collagen fibrils are discovered to be twice as stiff as the fibrils. Furthermore, the 3D depth profiles of the tip–sample interaction allow for accurate measurements of the fibrils' shape within the interfibrillar matrix. With a newly developed force–distance measuring scheme, we separate the viscoelastic deformation from the elastic response of the hydrated collagen. This sheds new light on the role of interfibrillar bonds, the mechanical properties of the interfibrillar matrix, and the biomechanics of native tendon.

BP 20.14 Tue 14:00 P2/4OG

Femtosecond laser cell surgery and wound healing on Drosophila embryos — •Elena Ramela Ciobotea, Ruby van Dijk, Bastian Zielinski, Mostafa Aakhte, Cristian Sarpe, Arne Senftleben, H.Arno J. Müller, and Thomas Baumert — Heinrich-Plett-Straße 40, 34132 Kassel, Germany

In this work we combine fluorescence microscopy and fs laser tissue ablation on a living Drosophila melanogaster embryo to investigate the wound closure in different stages of development. In contrast to typical larger scale UV laser damage, single cells or membranes can be targeted. Pulses from an 800nm Ti:Sapphire high energy oscillator were selected by the Pockels cell and coupled into a Leica confocal fluorescent microscope with a 63x oil immersion objective (NA: 1.32). 3h old embryos were dechorionated, washed, mounted in halocarbon oil between a microscope slide and a cover slip and imaged with a 488 nm Ar laser for up to 30 minutes after fs treatment. Images were assembled and analyzed using ImageJ software and a preliminary LabVIEW program. Firsts experiments were performed on embryos with cell nucleus marker and plasma membrane. Enhancement of the setup lead to well defined control of the energy deposition on the target tissue by varying exposure time and burst sequence with the Pockels cell, as well as optimizing temporal pulse length of the ultrashort laser pulses. Recent experiments on Myosin-GFP-labeled embryo with ablation in different stages show a larger migration of the myosin towards the damage over time for early stages in comparison with late stages of development.

BP 20.15 Tue 14:00 P2/4OG

Development of a fast real time sub-pixel accurate multi particle tracking system — •Jonas Pfeil, Daniel Geiger, Tobias Neckernuss, and Othmar Marti — Institute of Experimental Physics, Ulm University, Ulm, Germany

A multitude of functions in the human body depend on the rheological behaviour of the tissue and hence of the single cells involved. To determine the microscopical cell properties there is a need for microrheological measurement devices. An established technique is the passive and active microrheology. Small beads with known properties embedded in the probed material are tracked by an imaging system. By comparison with theoretical models, mechanical properties of the cells can be computed.

We present a new implementation of a system for particle tracking which can track up to 8 particles with sub pixel precision at high speeds of up to 10,000 frames per second. We will discuss the setup, the implemented method for subpixel tracking and the hardware consisting of an FPGA and a CMOS sensor.

In addition we will show a fast and fixed latency algorithms for the approximative multi-parameter fitting of 2D surfaces.

BP 20.16 Tue 14:00 P2/4OG

Local membrane height dynamics of live cells — •Max Ulbrich[1], Christian Völkner[1], Regina Lange[1], Heiko Lemcke[2], Robert David[2], Martina Grüning[3], Barbara Nebe[3], Ingo Barke[1], and Sylvia Speller[1] — [1]Institute of Physics, Physics of Surfaces & Interfaces, University of Rostock, 18059 Rostock — [2]University Medical Center, Cardial Regeneration, University of Rostock, 18057 Rostock — [3]University Medical Center, Dept. of Cell Biology, University of Rostock, 18057 Rostock

Cellular membrane fluctuations are considered for monitoring physiologic and pharmacologic effects [1]. Scanning Ion Conductance Microscopy (SICM) is a nanoprobing method to acquire morphologies on live cells. We operate the nanopipette-probe on fixed lateral locations and record SICM time traces in order to assess membrane fluctuations and cell activities with regard to processes [2]. Height variations of live osteoblasts and cardiomyocytes are analyzed in time and frequency domain. Cardiomyocytes show a pronounced frequency response behavior due to the electromechanical action, which can get modulated upon change of location or environment. Osteoblasts show rather strict 1/f behavior, with varying amplitude among different cells or cell surface regions. Close inspection points towards low frequency modulations in the course of migration. We discuss possible correlations with cell activity obtained by supporting optical microscopy.

[1] B Rappaz, et al, Blood Cells Mol. Dis. 42 (2009) 228

[2] S-O Kim, et al, Nano Convergence (2017) 4:5

BP 20.17 Tue 14:00 P2/4OG

High throughput sorting in droplet-based microfluidics — •Patricia Schwilling[1], Tobias Neckernuss[1,2], Daniel Geiger[1,2], Jonas Pfeil[1,2], Ralf Schuster[1], Lisa Kwapich[1], and Othmar Marti[1] — [1]Institute of Experimental Physics, Ulm University — [2]Sensific GmbH

Droplet-based microfluidics offer a method to meet the ever-increasing demand for cost-efficient high-throughput analysis and experiments by fabricating up to thousands of nanoliter-sized water-in-oil droplets per second. Each droplet serves as a tiny reaction chamber in which for example specific biochemical reactions may take place. One field, among others, that utilizes this technique is single-cell RNA sequencing by encapsulating functionalized beads with cells to capture the cells' RNA for subsequent sequence analysis. It is therefore inevitable to encapsulate single cells with single beads to ensure the highest resolution. Here, we present a setup to eliminate droplets containing more than one cell together with more than one bead using dielectrophoretic force and ODIN - a high-performance optical sensing system for real-time counting, analysis, and sorting. The setup is further capable to sort biological and synthetic particles and complex structures in a continuous flow based on their respective mechanical or phenotypical properties.

BP 20.18 Tue 14:00 P2/4OG

Wavelength and pressure dependent measurement of the retinal radiation exposure during diaphanoscopic illumination — •Nicole Sieber[1], Philipp Kölbl[1], Christian Lingenfelder[2], Kathrin Stucke-Straub[3], Sebastian Kupferschmid[4], and Martin Hessling[1] — [1]Institute of Medical Engineering and Mechatronics, Ulm University of Applied Sciences, 89081 Ulm, Germany — [2]Pharmpur GmbH, 86343 Koenigsbrunn, Germany — [3]Department of Mathematics, Natural and Economic Sciences, Ulm University of Applied Sciences, 89081 Ulm, Germany — [4]Clinic of Ophthalmology, Bundeswehrkrankenhaus Ulm, 89081 Ulm, Germany

In vitreoretinal surgery the imaging of the ocular fundus is of great interest. Therefore, sufficient bright illumination of the intraocular space is necessary. However, excessive irradiance can cause irreversible photochemical and thermal damage to the retina. In the international standard DIN EN ISO 15007-2: 2014 limit values for the irradiance of the retina are given. These values must not be exceeded during surgery.

In fact, the actual intraocular irradiance caused by ophthalmological illumination systems has never been determined. In this study the retinal irradiances during diaphanoscopic illumination through the porcine eye wall is investigated. The irradiance is wavelength and pressure dependent if the illuminator is pressed against the eyeball. The irradiance is measured spectrally resolved for different pressures inside eyes to assess the photochemical and thermal retinal hazard. These data also permit calculating the appropriate application time of the illumination system during eye surgery.

BP 20.19 Tue 14:00 P2/4OG

First steps toward reversible protein immobilization on conductive carbon nanomaterials — •Lara Jorde[1], Zehao Li[2,3], Jacob Piehler[2], Changjiang You[2], and Carola Meyer[1] — [1]Department of Physics, University of Osnabrück, Germany — [2]Department of Biology, University of Osnabrück, Germany — [3]College of Life Science, Beijing University of Chemical Technology, China

The surface immobilization of biomolecules is indispensable for most characterization techniques and therefore a goal in structure analysis. Fundamental understanding of the effects of immobilization on proteins functionalized to these conductive carbon nanomaterials thus enables the development of new types of electronic biosensors. Graphene is used as a calibration material for the CNT functionalization. Here, we achieved the Graphene-functionalization by a pyrene-linker compound binding to Green Fluorescent Protein (GFP) exemplary for a wide range of proteins. Reflectance interference- and total internal reflectance fluorescence spectroscopy reveals a specific and reversible protein binding on graphene with well-defined binding kinetics. Fluorescence quenching caused by graphene is determined by fluorescence lifetime imaging microscopy, confirming a tight yet reversible immobilization of the GFP-fluorophore on graphene. Challenges with regard to the application of this functionalization route and characterization techniques on CNTs are discussed, which have great transport properties for a later analysis of different protein configurations that allow for one-dimensional alignment and high sensitivity of sensing.

BP 20.20 Tue 14:00 P2/4OG

Single molecule localization microscopy in front of a tuned mirror — •Hannah S. Heil[1], Marie-Christine Dabauvalle[2], Sven Höfling[3], Martin Kamp[3], Markus Sauer[2], and Katrin G. Heinze[1] — [1]Rudolf Virchow Center, University of Würzburg — [2]Biocenter, University of Würzburg — [3]Technische Physik, University of Würzburg, Würzburg, Germany

Single-molecule localization microscopy (SMLM) methods have evolved as powerful tools to image cellular structures with virtually molecular resolution. We have demonstrated that higher photon yield at lower background on biocompatible metal-dielectric coatings substantially improves the SMLM performance, and significantly increases the localization precision and, thus, the image resolution (Heil HS et al., Light Sci Appl 7 (99), 2018).

The strength of the approach is that - except for the coated cover glass - no special microscope setup is required. We show that biocompatible metal-dielectric nanostructures fabricated on microscopy coverslips improve the resolution of direct stochastic optical reconstruction microscopy (dSTORM). The enhanced signal-to-noise ratio induced by the metal-dielectric coating sharpens the localization precision, and exceeds Widefield and Total Internal Reflection Fluorescence (TIRF) dSTORM performance without the need for a special TIRF objective lens in a much simpler setup. The resolution improvement is spectrally and spatially tunable as experimentally demonstrated for dual-color SMLM in cells and allows to access additional spatial information in the axial dimension.

BP 20.21 Tue 14:00 P2/4OG

The Structure of Red Blood Cells' Aggregates — •Mehrnaz M. Babaki[1,2] and Minne Paul Lettinga[1,2] — [1]ICS-3 Soft Condensed Matter, Forschungszentrum Jülich GmbH, Jülich, Germany — [2]Laboratory for Soft Matter and Biophysics, KU Leuven, Leuven, Belgium

Red Blood Cells (RBCs) aggregate in blood plasma due to presence of proteins like fibrinogen, immunoglobulin M and C-reactive protein. The characteristic face-to-face morphology of RBC's aggregates is similar to stacks of coins, which is referred to as rouleaux. The first step in understating rouleaux formation is the aggregation of two RBCs, which is called doublet. The formation and shape of a doublet is governed by bending and shear elasticity and adhesion energy of RBCs.

We induce aggregation of RBCs by adding different type of particles to RBCs dispersed in a density matched buffer. The ideal long rage attraction is induced by rod-like fd-viruses. Rode-like fd-viruses with a high length to diameter ratio are used as a depletant agent. The interaction is tuned by varying the concentration of the fd-virus. We employ ultra-fast confocal microscope to image the aggregates of RBCs to investigate the 3D shape of doublets.

By increasing the concentration of fd-virus, we observe a transition between line contacted doublets, where RBCs do not deform but touch along a circle, to doublets, where individual RBCs deform and are in full contact. The full surface contacted doublets can have different shapes which we developed a fingerprint to distinguish between these shapes.

BP 20.22 Tue 14:00 P2/4OG

Divergence-free smoothing for 3D direct traction force microscopy — •Johannes Blumberg[1,2] and Ulrich Schwarz[1,2] — [1]Institute for Theoretical Physics, Heidelberg University — [2]BioQuant, Heidelberg University

In traction force microscopy (TFM), the mechanical forces of cells adhering to an elastic substrate are estimated from the substrate displacements as measured by the movement of fiducial markers. Usually this estimate is obtained by minimizing the mean squared distance between experimentally observed and predicted displacements (inverse TFM). In direct TFM, in contrast, the stress tensor and the surface tractions are calculated directly and locally from the deformation field using the underlying material law. This procedure makes it easier to estimate not only tangential, but also normal forces, and to deal with non-planar substrates. However, it is not clear how accurate direct TFM performs compared with inverse TFM. We develop a new method to estimating the local inaccuracy, based on the divergence-freeness of the stress tensor in an equilibrium setup. This improves the usability of this method and possibly allows traction force microscopy to be used in a wider range of settings.

BP 20.23 Tue 14:00 P2/4OG

XPS evaluation of enzymatically oxidized nanocellulose from tunicate biomass — •Philipp Moritz[1,2], Oliver Höfft[1], Anthi Karnaouri[3], Paul Christakopoulos[3], Ulrika Rova[3], Georgia Sourkouni[2], and Wolfgang Maus-Friedrichs[1,2] — [1]Institute of Energy Research and Physical Technologies, Clausthal University of Technology, 38678 Clausthal-Zellerfeld — [2]Clausthal Centre of Material Technology, Clausthal University of Technology, 38678 Clausthal-Zellerfeld — [3]Department of Civil, Environmental and Natural Resources Engineering, Luleå University of Technology, 97187 Luleå

The use of highly crystalline oxidized nanocellulose has recently increased considerably, particularly in biomedicine. The exact physicochemical and mechanical properties of cellulose nanocrystals depend primarily on their origin and the manufacturing process.

The aim of our experiments is to obtain highly crystalline and oxidized nanocellulose from the invertebrate tunicate species Ciona intestinalis. A novel process consisting of an organosolv pre-treatment and a subsequent enzyme treatment was developed for this purpose. The biocatalyst lytic polysaccharide monooxygenase (LPMO) is known to oxidatively cleave the glycosidic bond of cellulose without reducing its crystallinity. To evaluate the oxidizing effect of the novel pretreatments, X-ray photoelectron spectroscopy (XPS) was used.

Compared to the untreated raw nanocellulose, XPS indicates a significant increase of the oxidized "C=O/O-C-O" and "O-C=O" species achieved by the novel physicochemical pre-treatment. An additional in-situ enzyme treatment leads to further oxidation of the material.

BP 20.24 Tue 14:00 P2/4OG

Point Spread Function engineering in real time for iSCAT — •Vivien WALTER and Mark WALLACE — Department of Chemistry, King's College London, London, United Kingdom

Interferometric scattering (iSCAT) microscopy extends the limits of traditional optical microscopy by imaging the interference pattern between light scattered by an object and a reference beam reflected from a surface. Efficient subtraction of the large reference signal and its magnitude relative to the scattering of the object is key to high-speed high-sensitivity imaging. However, subtraction of the reference signal in complex biological samples requires more sophisticated processing to detect single biomolecules.

Optical processing of images is commonly used in microscopy to optimise the collected signals by increasing contrast or decreasing noise. Fourier plane processing is a common method to select specific image

properties and while powerful the application of optical processing to iSCAT has typically been limited to static apodising filters. Adaptive Fourier filtering can be performed using Spatial Light Modulators (SLM), computer controlled high resolution devices capable of applying any type of filter in real-time.

We demonstrate here the application of real time SLM processing applied in iSCAT microscopy to obtain a 6-fold increase of the signal-to-noise ratio. We investigate a range of optical processing methods, and demonstrate that this optical pre-processing reduces the molecular weight threshold of detectable label-free proteins and polymers.

BP 20.25 Tue 14:00 P2/4OG

Development of a flat-top laser excitation for TIRF microscopy-based single molecule FRET experiments — •Wubulikasimu Yibulayin and David Dulin — Junior Research Group 2, Interdisciplinary Center for Clinical Research, Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Erlangen, Germany.

Total internal reflection fluorescence microscopy (TIRFM) is a widely used technique for single molecule fluorescence spectroscopy studies, in particular in combination with single molecule fluorescence resonance energy transfer (smFRET). The advent of large detector scientific CMOS camera has enabled high throughput observation of single molecule using TIRFM. However, the Gaussian distribution of intensity in the excitation laser renders the illumination inherently inhomogeneous, whereas a flat-top intensity distribution would be ideal. Several approaches have been developed to convert the Gaussian laser excitation beam into a flat-top beam, such as injecting the excitation laser(s) into a mechanically vibrated multimode fiber (MMF). Here, we have assembled a custom two-colors TIRF microscope with the excitation lasers (532 nm and 640 nm) are injected into a MMF. To remove the speckle added by the MMF to the laser intensity profile, we couple to the MMF a piezo element that vibrates at high frequency, enabling a uniform flat-top illumination, while maintaining the TIR conditions. This all-custom microscope will provide a platform for hybrid force-fluorescence spectroscopy measurements, e.g. TIRFM-magnetic tweezers.

BP 20.26 Tue 14:00 P2/4OG

Low-budget High-resolution Fluorescence Microscope — •Leon Claassen[1], Lukas Lechler[1], Florian Schwarz[1], and Jens Pflaum[1,2] — [1]Experimental Physics VI, Julius Maximilian University of Würzburg, 97074 Würzburg — [2]ZAE Bayern, Würzburg

Non-destructive optical methods are vital for the determination of material properties in various fields of application. In particular, fluorescence microscopy has become an established technique which can be used for analyzing solid state properties such as the optical band-gap of semiconductors or in biology to identify and spatially resolve labeled constituents in cells or cellular processes. However, fluorescent microscope setups are usually very expensive and thus, financially hardly viable for schools or technical classes. Here, we present a low-cost laser-scanning version of such a setup based on two DVD-scanners driven by an Arduino controller and slightly modified optics, which offers a resolution up to 15 μm within a scanning range of 5 x 5 cm^2. We demonstrate the characteristics of the relevant electronic and optical components and show first results obtained by operating the microscope in a neat reflection mode. Thereafter, the necessary conversions to run the setup in fluorescent mode are presented together with preliminary studies on fluorescent organic single crystals as well as labeled biological structures. Based on these achievements we will discuss future optimizations to improve the performance and, in particular, the lateral resolution of the setup down to the micrometer length scale. The University of Würzburg is acknowledged for financial support within the Fund for Innovative Projects in Teaching.