BP 28: Posters: Imaging

Time: Thursday 17:30–19:30 Location: Poster A

BP 28.1 Thu 17:30 Poster A

Morphological analysis of MDCK Epithelial tissue on different substrates — •Sara Kaliman[1], Christina Jayachandran[2], Ana-Sunčana Smith[1], and Florian Rehfeldt[2] — [1]Institute for Theoretical Physics and Cluster of Excellence: EAM, FA University Erlangen, Germany — [2]3rd institute of Physics-Biophysics, University of Göttingen, Germany

Significant progress in comprehending the morphology and the internal organization of single cells has been achieved over the last decade. However, cell agglomerates and tissues are understood to a much lesser extent. Here we study the positioning and the orientations of the cell nuclei with respect to the cell body, as well as the organization and the correlations between the cells in two-dimensional epithelial tissues. For this purpose, epithelial Madin-Darby canine kidney (MDCK) cells are grown on collagen coated polyacrylamide gels of various elastic moduli (1-34 kPa) and on stiff glass substrates. Upon fixation and double staining, all cell-lines were imaged in fluorescence, the results of which were processed by a self-made MATLAB program. We find a similar organization in tissues with equivalent local density, irrespective of the substrate elasticity, even though the dynamics of the tissue growth depends on the underlaying matrix. In all cases, we find little deviations from random distributions. Furthermore, favorable comparison between true cell body and the Voronoi construction based on the nuclei shape suggests that the intracellular interactions dominate those of cells with the substrate. Consequently, the morphology of cells in tissues differ significantly from that of individual cells.

BP 28.2 Thu 17:30 Poster A

Fluorescence spectral coding for identification of molecules at low concentration — •Zdeněk Petrášek, Jens Wiedemann, and Petra Schwille — Biotechnologisches Zentrum, TU Dresden, Tatzberg 47/49, 01309 Dresden, Germany

We investigate how a combination of 2-3 distinct fluorescent dyes could be used to produce multiple spectral codes for unique species identification. As an experimental model we use non-fluorescent beads to which different fluorescent molecules are attached. The emission from immobilized beads is dispersed by a prism and imaged by a camera.

We study how the noise level influences the ability to resolve two different spectra, using both simulations and experiments. The effects of the spectral overlap and of the number of spectral channels were explored. Introducing convenient expressions for the noise level in the spectrum and the difference between two spectra leads to a simple relationship between the spectral difference (which can be calculated from noiseless reference spectra) and the resolvability, defined as a maximum noise level required to guarantee a given success identification rate. Experiments show that the bead autofluorescence, if not exceeding the probe fluorescence, does not prevent correct identification of the spectral code. Initial results of simulations further suggest that finding the bead in the noisy image may be more difficult than identifying its spectral code, once the bead is found.

BP 28.3 Thu 17:30 Poster A

3-Dimensional Characterization of Fibroblasts with Enhanced Differential Interference Contrast Microscopy — •Malte Ohmstede, Erik Bernitt, and Hans-Günther Döbereiner — Institut für Biophysik, Universität Bremen

Circular dorsal-ruffles, a phenomenon found on the dorsal surface of fibroblast cells, are not yet well understood, actin based membrane protrusions. Algorithms for characterization of three-dimensional properties and dynamics have been developed for evaluation of optical sectioning data, which is gained with differential interference contrast. Due to the low optical density of fibroblast specimens, differential interference contrast is used. Optical sectioning data allows for evaluation of living specimens, thus enabling characterization of dorsal ruffle dynamics. Differential interference contrast, however, complicates evaluation of image data due to gradient-like image properties. Complications with image segmentation, i.e. separating image foreground and background, have been resolved by application of a two-dimensional Hilbert Transform on image data to gain bright-field-like images from gradient sources. Further processing was done by executing morphological filters and skeletonization on the binary images gained by segmentation. Contour data gained by skeletonization was then extracted for further evaluation and processing.

BP 28.4 Thu 17:30 Poster A

Measuring the local refractive index of cells with a laser trap based interferometer — •Kai Bodensiek, Paula Sánchez, Yvonne Kretzer, and Iwan Schaap — III Physikalisches Institut, Faculty of Physics, Georg-August Universität, Götingen, Germany

To be able to measure the spatial distribution of the refractive index in individual living cells we constructed a micrometer sized Fabry-Pérot interferometer that was placed in a microscope sample chamber. One mirror of the interferometer is formed by the microscope coverslip and the second movable mirror was positioned with a laser trap in a direction perpendicular to the coverslip. The local cellular refractive index is extracted by simultaneously measuring the local height of the cell and the cell induced phase shift of the interference signal. Because the instrument is implemented in a diffraction-limited optical microscope we can study the local organization of the cell and its nucleus.

BP 28.5 Thu 17:30 Poster A

Sample preparation and delivery for bio-imaging at the European XFEL Facility — •Joachim Schulz — European XFEL GmbH, Hamburg, Germany

Recent success in femtosecond x-ray protein nano-crystallography [Nature 470, p.73 (2011)] and imaging of mimivirus particles [Nature 470, p.78 (2011)] demonstrate the prospects of free-electron lasers for biophysics.

At the European XFEL GmbH in Hamburg (Germany) we design and build a 3.4 kilometres long x-ray free-electron laser. This facility will produce femtosecond x-ray pulses with wavelengths below an Angstrom. The expected brilliance of the facility will be considerably higher compared with other x-ray FELs and the repetition rate of 27.000 pulses per second will be unique. One of the six end-stations of this laser facility will be optimized for imaging experiments on biological samples. The European XFEL Facility will start operation in 2015.

One of the challenges for biological imaging techniques at free-electron laser sources is the preparation and delivering of specimen into the vacuum of the x-ray laser. Lab space and equipment will have to be provided close to the end-stations. The bio-samples have to be efficiently delivered to the beam and hit by the x-ray pulses. To this end methods have to be developed to keep the samples in the natural state and to avoid too much contamination of the vacuum. In this presentation I will show first considerations concerning the sample delivery system for bio-samples at the European XFEL Facility.

BP 28.6 Thu 17:30 Poster A

Nanotomography of bovine tendon — •Stefan Kubitza[1], Stephanie Röper[1], Anke Bernstein[2], and Robert Magerle[1] — [1]Chemische Physik, TU Chemnitz, D-09107 Chemnitz — [2]Department für Orthopädie und Unfallchirugie, Muskuloskelettales Forschungslabor, Universitätsklinikum Freiburg, 79106 Freiburg

Collagen is the most abundant fibrous protein in mammals. It can be found in various types of biological tissue, for example in tooth, bone and tendon. We use atomic force microscopy (AFM) based nanotomography for imaging the nanoscaled structure of this biological material. The specimen is ablated layer-by-layer by wet chemical etching and imaged with tapping mode AFM after each etching step. From resulting series of AFM images the three-dimensional structure is reconstructed. In our experiments we focus on native bovine tendon (collagen type I) and report about the suitability of different etching solutions for nanotomography imaging.

BP 28.7 Thu 17:30 Poster A

Photoemission electron microscopy of magnetotactic bacteria — •Christoph Keutner[1], Ulf Berges[1], Claus M. Schneider[2], and Carsten Westphal[1] — [1]DELTA / Experimentelle Physik I, TU Dortmund, Maria-Goeppert-Mayer-Str. 2, D-44221 Dortmund — [2]PGI-6, Forschungszentrum Jülich, D-52425 Jülich

In their natural environment magnetotactic bacteria (MTB) respond to earth's magnetic field by aligning their direction of motion parallel or antiparallel to the magnetic field. This sensitivity to the magnetic field is caused by a magnetic particle chain contained within the MTB's

body, the so called magnetosome chain. Presently, there are first considerations of applying this phenomenon within a biologic propulsion and steering system for nanorobots [1]. Therefore the magnetic properties of these magnetosomes are of particular interest.

In this work first experiments of imaging MTB with photoemission electron microscopy (PEEM) were performed, here the bacteria *Magnetospirillum magnetotacticum* [2]. In order to prepare the MTB in a UHV compatible way, several methods were tested. The best results were obtained with a lyophilized specimen applied on a Si-wafer. So far, first images of *M. magnetotacticum* could be recorded. In order to achieve a higher resolution and to image the internal magnetosome chain, further modifications and experiments are ongoing.

[1] S. Martel, M. Mohammadi, O. Felfoul, Z. Lu and P. Pouponneau, Int. J. Rob. Res. **28**, 571 (2009)
[2] R. Blakemore, Science **190**, 377 (1975)

BP 28.8 Thu 17:30 Poster A

Multifocus Fluorescence Correlation Spectroscopy using a programmable phase modulator — •Thomas Kuckert, Zdeněk Petrášek, and Petra Schwille — Biotechnologisches Zentrum, TU Dresden, Tatzberg 47/49, 01307 Dresden, Germany

Fluorescence Correlation Spectroscopy (FCS) has been used for studying molecular dynamics and interactions in many fields, ranging from physics, chemistry to biology. Many applications, especially those within living cells or tissues where the measurement time is limited, would benefit from the ability to perform measurements at different positions simultaneously. This requires both excitation localized within a number of well-defined volumes, and separate detection of fluorescence from within these volumes. Here we present an ongoing project the goal of which is to construct such a flexible multifocus FCS setup. Previously, multiple excitation spots have been realized by diffractive optical elements designed specifically for a given spot pattern, without the possibility to adapt the pattern to the sample. We use a programmable phase modulator (PPP), which allows flexible formation of a variable number of foci at arbitrary positions. Initially, avalanche photodiodes will be used for detection, to assess the quality of foci produced by the PPP. Eventually, fast EM-CCD is intended for simultaneous detection from all foci to reach full flexibility.

BP 28.9 Thu 17:30 Poster A

Interferometric Particle Tracking — •Dennis Müller and Rainer G. Ulbrich — IV. Physikalisches Institut, Georg-August-Universität Göttingen, Germany

We report interferometric tracking of gold nanoparticles with spatial resolution in the nanometer range and sub-millisecond temporal resolution. This technique combines a dark-field microscope with laser illumination with a Mach-Zehnder-like interferometer which follows the camera exit of the microscope. In the interferometer scattered light from the object particle is superimposed with scattered light from a reference particle which has been immobilized in the object plane. Readout is done by avalanche photodiodes. The phase of the resulting interferogram is a very sensitive measure for displacements of the object particle relative to the reference particle. This configuration eliminates effectively the effect of microscope drift on the measurement and makes this technique a promising candidate for applications like motor protein tracking experiments.

BP 28.10 Thu 17:30 Poster A

Approaches for improved dual color Photoactivated Localization Microscopy — •Paolo Annibale, Mattia Greco, Marco Scarselli, and Aleksandra Radenovic — LBEN, IBI EPFL Lausanne 1015 Switzerland

Dual color PhotoActivated Localization Microscopy (PALM) presents unique challenges due to the specific photo-physical behavior of the fluorescent proteins used as a tags and to the asynchronous nature of the photoactivation and localization of the molecules belonging to each fluorophore pair. We systematically investigate the effect of molecular photoblinking and fluorescence dark times on a typical dual color PALM experiment. This allows us to propose a method to identify potential photoblinking-originated artifacts by screening for concomitant spatial and temporal clusters, a prerequisite for the correct localization and co-localization of oligomeric sub-diffraction limit cellular structures [1]. Using a setup axially stabilized to better than 5 nm we compare the performance of three different dual color fluorescent protein pairs using as positive controls fusion constructs imaged both in-vitro and on the plasma membrane of HeLa cells. We present an application of these methods to the imaging of a pair of plasma membrane proteins involved in cell signaling.

[1] P. Annibale et al. Nature Methods 8 (2011) 527-528

BP 28.11 Thu 17:30 Poster A

Mechanical Properties of Primary Cilia — •Christopher Battle and Christoph F. Schmidt — 3te Physikalisches Institut, Georg-August Universitaet, Goettingen, Germany

Recent studies have shown that the primary cilium, long thought to be a vestigial cellular appendage with no function, is involved in a multitude of sensory functions. One example, interesting from both a biophysical and medical standpoint, is the primary cilium of kidney epithelial cells, which acts as a mechanosensitive flow sensor. Genetic defects in ciliary function can cause, e.g., polycystic kidney disease (PKD). The material properties of these non-motile, microtubule-based 9+0 cilia, and the way they are anchored to the cell cytoskeleton, are important to know if one wants to understand the mechano-electrochemical response of these cells, which is mediated by their cilia. We have constructed two optical-trapping microscopes for this purpose, one upright instrument with access for patch-clamping pipettes using back-scatterered force and displacement detection, and one inverted microscope with optimized DIC imaging capabilities in conjunction with a double optical trap. With these instruments we can probe the activation of ciliated MDCK cells in confluent monolayers and the mechanical properties of the cilium.