BP 11: Novel Methods

Time: Tuesday 14:30–16:30 Location: ZEU 260

BP 11.1 Tue 14:30 ZEU 260

Fast Dynamics of Cellular Signals Studied With a Novel Miniaturized Multi-Channel Perfusion System — •Claus Fütterer[1], Luru Dai[2], Hans Fried[2], Arne Brombas[2], Arnd Baumann[2], Thomas Gensch[2], and Frank Müller[2] — [1]Forschungszentrum Jülich, IBN-4, 52425 Jülich — [2]Forschungszentrum Jülich, INB-1, 52425 Jülich

In order to study the dynamics of cellular signaling in single cells and ex vivo-tissues, we developed a novel miniaturized fluidic system that allows unparalleled fast and artefact-free solution exchange using multiple inflow channels. To this end, methods of microfabrication and numerical simulations have been involved.

HEK-293 cells were used that expressed octopamine receptors as well as the genetically encoded calcium sensor TN-L15, designed for calcium dependent Fluorescence Resonance Energy Transfer. Switching from octopamine free to octopamine containing solution leads to oscillations of the intracellular calcium concentrationof which the onset and fading out of these oscillations can be controlled with unparalleled temporal resolution.

Further we studied calcium signals in flatmounted retinae of transgenic mice that express TN-L15 in retinal ganglion cells. Cells were activated by switching the perfusion from normal extracellular solution to solution with high K+ concentration to enable the precise measurement of the intracellular calcium concentration. The high temporal resolution achieved in our set-up revealed differences in the response of individual ganglion cells that had not been detected before.

BP 11.2 Tue 14:45 ZEU 260

Giving biomechanics a spin: the Optical Cell Rotator — •Anatol Fritsch[1], Tobias Kiessling[1], Moritz Kreysing[2], Franziska Wetzel[1], and Josef Käs[1] — [1]University of Leipzig, Germany — [2]University of Cambridge, UK

In cell biophysics striking insights have often been connected to new developments of optical microscopy tools enabling a deeper look into the underlying physical principles of cells. We present our newly developed modified divergent dual-beam laser trap, which enables holding and controlled rotation of suspended cells and cell aggregates for high resolution tomographic imaging, called the Optical Cell Rotator.

Showing the possibilities of this technology, we studied the mechanical properties of cells in cell aggregates since it opens the possibility for a deeper understanding of cell-cell interactions in tissues. Malignant tumours are not only agglomerates of homogeneous cells, but rather complex structures containing diverse normal and pathological cells in different stages of aggressiveness. Recent investigations show that the biomechanical properties of benign cells differ from those of cancerous and metastatic cells. However, the optical deformability of primary lung and breast cancer cells compared to their corresponding cell lines show at the first sight an unexpected stiffening behaviour. To elucidate this finding we compare 3D and standard monolayer cultured cells by their mechanical properties with the Optical Stretcher enabling contact-free, whole cell elasticity measurements and the Optical Cell Rotator to connect the findings to the underlying cytoskeletal structure.

BP 11.3 Tue 15:00 ZEU 260

Periodic strain slows down osteoblast proliferation — •Matthias Maier[1], Pablo Fernandez[1], Ludwig Eichinger[2], and Andreas R. Bausch[1] — [1]E27 Zellbiophysik, Technische Universität München, D-85748 Garching, Germany — [2]Institut für Biochemie I, Universität Köln, D-50931 Köln

The quantitative study of mechanotransduction poses a major interdisciplinary challenge. The complex mechanical behaviour of cells demands systematic variation of key mechanical parameters such as strain rate, amplitude and stress, as well as control of adhesive conditions. At the same time the analysis of the cellular response must deal with biological complexity and heterogeneity. Here, we present an experimental setup which combines cell monolayer rheology with DNA microarray technology. By applying shear strain on over 10 million cells simultaneously, we obtain the large amounts of material needed for integral genomics or proteomics characterisation without compromising on a clean, well-defined mechanical perturbation. In a first application, we address the phenomenon that periodic shear at large amplitudes appears to influence osteoblast proliferation. Preliminary results with our setup followed by microarray analysis indeed reveal a down-regulation of genes involved in mitosis, most conspicuously anillin, an essential component of the contractile ring. We speculate on a direct mechanical effect of the external deformation on cytokinesis.

BP 11.4 Tue 15:15 ZEU 260

Label-free bioimaging of living human glioblastoma cells by confocal Raman microscopy — •Alexander M. Gigler[1], Katharina Klein[2], Guido Piontek[2], Thomas Aschenbrenner[3], Wolfram Bunk[3], Gregor Morfill[3], Jürgen Schlegel[2], and Robert W. Stark[1] — [1]Center for Nanoscience (CeNS) and Sect. Crystallography, LMU-München, D-80333 München — [2]Neuropathology, TU-München, Klinikum rechts der Isar, D-81675 München — [3]MPI for Extraterrestrial Physics, D-85748 Garching

Label-free imaging by confocal Raman spectroscopy is becoming a promising alternative to established methods for cell imaging requiring fixation and the use of fluorescent markers. With our setup we are able to image living cells at a high resolution in buffer solution (PBS). Different cellular compartments can be visualized and directly compared to immunofluorescence microscopy (IF). The comparison of Raman and IF image sets allows an assignment of organelles such as nucleus, endoplasmatic reticulum, and mitochondria. From the assigned areas we obtained average spectra of the compartments resulting in an individual spectral fingerprint for each specific region. These fingerprints can in turn be used to define spectral filters for mapping in an iterative procedure. Spectral maps of single cells provide the full set of biochemical information contained in the selected focal plane. To this end, we are using IF staining methods to verify our observations and assignments. On the long run, our aim is to identify specific molecular markers for therapeutic targeting and discriminate between cells of different lines or differentiation states based on spectral information.

BP 11.5 Tue 15:30 ZEU 260

Spatial chemical gradient measurements in microfluidic channels by arrays of nano-gap electrodes — •Enno Kätelhön[1,2], Marcel A. G. Zevenbergen[3], Edgar D. Goluch[3], Serge G. Lemay[3], Andreas Offenhäusser[1,2], and Bernhard Wolfrum[1,2] — [1]IBN-2, Forschungszentrum Jülich GmbH, Germany — [2]JARA- Fundamentals of Future Information Technology — [3]Kavli Institute of Nanoscience, Delft University of Technology, the Netherlands

In recent years, microfluidic devices have received growing attention along with the proceeding miniaturization of electrochemical sensors. In particular regarding biophysical applications, there is an increasing interest due to the potential to establish specific chemical environments inside of microfluidic systems. Since these systems feature a laminar flow trait, they allow setting up highly defined chemical gradient fields that are exclusively based on diffusive mixing. Thus, cell growth characteristics can be investigated concurrently within one experimental setup in different chemical environments.

We present a new method to evaluate the mixing gradient of redox- and non redox-active substances inside of a micro scaled flow. Our system features a set of interdigitated nano-electrode arrays that is incorporated into a PDMS microchannel. By this means, we can record cyclic voltammograms simultaneously at different locations inside of the channel as well as determine the concentration of the redox-active substance at specific spots. Owing to the nano scaled redox cycling approach, our method exhibits a high special resolution and a large current amplification.

BP 11.6 Tue 15:45 ZEU 260

Immunoassay based on long-range fluorescence quenching by gold nanoparticles — •Meike Kloster[1], Sergiy Mayilo[1], Fernando Stefani[1], Michael Wunderlich[1], Thomas A. Klar[1,2], Hans-Peter Josel[3], Dieter Heindl[3], Alfons Nichtl[3], Konrad Kürzinger[3], and Jochen Feldmann[1] — [1]Photonics and Optoelectronics Group, Department of Physics and CeNS, Ludwig-Maximilians-Universität München, Munich, Germany — [2]Institute of Physics and Institute of Micro- and Nanotechnologies, Technical University of Ilmenau, Ilmenau, Germany — [3]Roche Diagnostics GmbH, Penzberg, Germany

Förster energy transfer is a common tool for the detection of biomolecules. However, due to its short-range, the application is limited to small distances. Energy transfer from a dye molecule to a gold nanoparticle (AuNP) is effective over longer distances due to the larger cross-section of the particles and to radiative rate suppression [1]. Here we use the long-range fluorescence quenching by AuNPs to develop a novel immunoassay for a diagnostically relevant example: troponin T (TnT), an indicator of damage to the heart muscle. AuNPs and fluorescent dyes are functionalized with anti-TnT antibodies. In the presence of TnT, the AuNPs and the fluorophores are brought together by their specific interaction leading to fluorescence quenching. By using time-resolved spectroscopy, the contributions of direct energy transfer and radiative decay suppression to fluorescence quenching are quantified.

[1] E. Dulkeith et al., Nano Letters 5, 585 (2005)

BP 11.7 Tue 16:00 ZEU 260

SERS labels for red laser excitation: silica-encapsulated SAM on tunable gold/silver nanoshells — •Magdalena Gellner, Max Schütz, Bernd Küstner, and Sebastian Schlücker — Department of Physics, University of Osnabrück, 49076 Osnabrück

Silica-encapsulated self-assembled monolayers (SAMs) on tunable gold/silver nanoshells for use as surface-enhanced Raman scattering (SERS) labels in bioanalytical and biomedical applications with red laser excitation are presented. [1] This concept combines the spectroscopic advantages due to the maximum surface coverage and uniform molecular orientation of Raman reporter molecules within a SAM together with the high chemical and mechanical stability of a glass shell. The absorption, scattering and extinction spectra of various gold/silver nanoshells were calculated using Mie theory. Quantitative SERS efficiencies based on theoretical scattering intensities are compared with experimental findings. [2] Our improved SERS label design results in $\sim$ 180 times brighter SERS signals compared with existing approaches based on single gold nanospheres.[1] Using SERS-labeled antibodies, the selective localization of prostate-specific antigen (PSA) in the epithelium of prostate tissue specimens by immuno-SERS microscopy with red laser excitation is demonstrated.

[1] B. Küstner, M. Gellner, M. Schütz, F. Schöppler, A. Marx, P. Ströbel, P. Adam, C. Schmuck, S. Schlücker, Angew. Chem. Int. Ed., accepted

[2] M. Gellner, B. Küstner, S. Schlücker, Vib. Spectrosc., 2008, doi:10.1016/j.vibspec.2008.07.011

BP 11.8 Tue 16:15 ZEU 260

Impedance study of AlGaN/GaN HEMT structures in contact with electrolyte solutions — Michael Charpentier, •Hartmut Witte, Christian Warnke, Mathias Müller, Kay-Michael Günther, Armin Dadgar, and Alois Krost — Otto-von-Guericke-University-Magdeburg, Institute of Experimental Physics, 39016 Magdeburg

Planar multi-electrode-arrays (MEA) are widely spread for stimulation and recording of neuron network signals. Besides metal electrodes and Silicon, more and more group-III-nitride devices are used as substrates. For MEA applications the substrate impedance as one of the main signal transfer parameters has to be optimized. In this contribution we investigate the total impedance of AlGaN/GaN high electron mobility structures (HEMT) using impedance spectroscopy between 20 Hz and 2 MHz. The total impedance is composed of the contributions of the two dimensional electron gas (2DEG), the metal contacts and the horizontal and vertical layer material impedances. The impacts of these parts were studied by varying the layer arrangement and applied bias voltages, by using a MESA microstructuring, and by illumination of the samples. All variations significantly change the impedance spectra. Furthermore, samples with different total impedances show disparate signal behavior in contact with electrolyte solutions with varying pH-values and conductivities. Therefore, these investigations are useful for optimization of the device performance for different biosensor applications.