BP 12: Posters: New Technologies

Time: Monday 17:15–20:00 Location: P3

BP 12.1 Mon 17:15 P3

Spectrally-resolved identification of fluorescent probes at low concentration — •Jens Wiedemann, Zdeněk Petrášek, and Petra Schwille — Biophysics group, Biotechnologisches Zentrum, Technische Universität Dresden, Tatzberg 47/49, 01307 Dresden, Germany

We present a simple technique for the detection and identification of a large number of spectrally distinct fluorescent probes at low concentration, based on the differences in their emission spectrum shape. The fluorescence originating from immobilized beads containing a low number (100s or less) of fluorescent molecules is dispersed by a prism and imaged by a CCD camera. A line illumination of the sample leads to a pseudo-image with one coordinate corresponding to the spatial dimension and the other coordinate to the emission wavelength. To image the whole field of view, the excitation line has to be scanned across the sample.

The beads present within the illuminated line are automatically identified and their spectrum compared with a set of reference spectra. We investigate both theoretically and experimentally the limits of the ability to correctly identify the spectrum, depending on the spectral overlap and the noise level. The possibility of resolving beads with spectral signatures created by a combination of different fluorophores, including different concentration ratios, and the effects of the interference of the bead autofluorescence, are being explored.

BP 12.2 Mon 17:15 P3

Biocompatibility of single crystalline $Fe_{70}Pd_{30}$ ferromagnetic shape memory films for cell actuation — •Mareike Zink[1], Yanhong Ma[2], and S. G. Mayr[2] — [1]Abteilung Physik der weichen Materie, Fakultät für Physik und Geowissenschaften, Universität Leipzig, Leipzig, Germany — [2]Leibniz-Institut für Oberflächenmodifizierung e.V., Translationszentrum für Regenerative Medizin, Fakultät für Physik und Geowissenschaften, Universität Leipzig, Germany

Ferromagnetic shape memory alloys (FSMAs) have received great attention as an exciting class of smart functional materials. They exhibit large reversible strains at moderate stresses with external controllability at constant temperatures which make them excellent candidates for biomedical actuation devices. FSMAs bear the significant potential for miniaturized devices for single cell actuation which is capable of yielding magnetically controllable shear strains and/or volume dilations. However, the biocompatibility of this material must first be well confirmed as it has not been done yet. Thus, our work focuses on the interaction of fibroblast cells with single crystalline $Fe_{70}Pd_{30}$ FSMA films on MgO substrates. Additionally, corrosion resistance of the films was obtained employing simulated body fluid (SBF) tests. Calcium-phosphate aggregates with granular microstructure were detected on the film surface after soaking in SBF. Cell viability and biocompatibility tests with NIH 3T3 cells revealed that the cells adhered and proliferated on the FSMA surface, whereas cells were smaller compared to cells on culture dish surfaces. Biocompatible polymer coatings on the $Fe_{70}Pd_{30}$ film can be employed to improve cell-substrate interactions.

BP 12.3 Mon 17:15 P3

Photobleichung höherer Ordnung - ein eng mit intrazellulärer Ablation verbundener Low-Density Plasma Prozess — •Stefan Kalies, Kai Kuetemeyer und Alexander Heisterkamp — Laser Zentrum Hannover e.V., Hollerithallee 8, D-30419 Hannover

Photobleichung bei nichtlinearer Anregung, wie in der Multiphotonenmikroskopie, ist im Gegensatz zur Photobleichung bei linearer Anregung kaum charakterisiert. In diesem Fall spielen Prozesse mit teilweise höherer Ordnung als der Anregungsordnung eine Rolle. Wir untersuchten diese Photobleichung höherer Ordnung per Multiphotonenmikroskopie in lebenden Zellen in vitro. Verwendet wurde das in den Zellen exprimierte, mobile "Enhanced Green Fluorescent Protein" (EGFP) sowie das immobile, extrinsische Fluorophor Hoechst. Die Abhängigkeit der Photobleichungsrate von der Leistung war für das Fluorophor EGFP kubischer und ab einer bestimmten Grenzwellenlänge biquadratischer Ordnung, während für Hoechst eine quadratische in eine kubische Ordnung überging. Es zeigte sich, dass die Bleichung mit der Bildung reaktiver Sauerstoffspezies korreliert. Aus den durchgeführten Untersuchungen lässt sich schließen, dass neben der sequentiellen Anregung in höhere ionische Zustände eine Multiphotonenionisation zur Photobleichung höherer Ordnung führen kann. Im Gegensatz zur linearen Photobleichung spielen Triplett-Zustände und molekularer Sauerstoff eine vernachlässigbare Rolle. Die Photobleichung höherer Ordnung zeigt starke Parallelen zur intrazellulären Ablation, die durch die Erzeugung eines Plasmas geringer Elektronendichte ("Low-Density Plasma") und reaktive Sauerstoffspezies erreicht werden kann.

BP 12.4 Mon 17:15 P3

Characterization and compensation of fs-Laser pulse broadening in a photonic crystal fiber for multi-photon endomicroscopy — •Tobias Ehmke, Sabine Donner, Alexander Krueger, and Alexander Heisterkamp — Laser Zentrum Hannover e.V., Hollerithallee 8, D-30419 Hannover

Multi-photon excitation microscopy is a fluorescence imaging technique which allows deep tissue imaging with short pulse infrared laser light. The optics of conventional multiphoton microscopes is too bulky for endo-microscopy and the rigid setup is unfavourable for many in-vivo applications. In order to gain flexibility and reduce size a fiber based probe for multiphoton endomicroscopy is under development. As a first step towards the endomicroscope problems associated with the propagation of short pulses in the optical fibers have to be addressed. Therefore, a setup consisting of a Ti:Sa laser emitting 140fs pulses, a prechirp unit, a double clad photonic crystal fiber and focussing optics are used. The dispersion and nonlinear effects in the fiber are studied with an autocorrelator and a spectrometer. The coupling efficiency of the fiber is determined to be over 60%. Without the prechirp unit pulse broadening into the ps-regime and a reduction of the spectral width is observed. The dispersion could be compensated by a grating compressor, generating a negative chirp for the pulse and is designed to put the pulse length back into the fs-regime behind the fiber. This is an important step towards the utilization of a fiber based multi-photon microscope for in-vivo applications.

BP 12.5 Mon 17:15 P3

Interdigitated micro-electrode arrays used as biosensors — •alireza mousavi[1,2], patriza lamberti[2], vincenzo tucci[2], and veit wagner[1] — [1]Jacobs University Bremen, Campus Ring 1, D-28759, Bremen, Germany — [2]University of Salerno, Dept. of Electrical and Information Engineering, 84084, Fisciano,Salerno, Italy

Detection of bio-molecules and pathogens by a rapid, sensitive and cost-effective method is of great importance in healthcare, food industry, water/environmental monitoring and for elimination of biosecurity threats. In this contribution various interdigitated microelectrode array designs are tested for their functionality as biosensors. Such electrode arrays have the advantage of low cost, low power consumption and miniaturized structure size. Test devices are fabricated either on silicon wafers or on low cost PET-foils. The adsorbates to be sensed are detected in two ways, i) by their specific dielectric signature via impedance measurements at different frequencies, and ii) by measuring the conductivity change in an additionally deposited, semiconducting surface layer patterned on top of the electrodes (field-effect transistor design). Both concepts allow label-free detection of adsorbed bio-molecules or cells. The experimental finding of the dependence on the geometry parameters of the interdigitated micro-electrode array and the electrical operation conditions are compared with predictions based on electrostatic and electroquasistatic theoretical finite-element analysis.

BP 12.6 Mon 17:15 P3

Microwave high-K based sensors for the analysis of liquids and molecules — •Eugen Hollmann, Roger Wördenweber, Thomas Grellmann, Kyrylo Greben, and Rolf Kutzner — Institute of Bio- and Nanosystems (IBN), Forschungszentrum Juelich, 52425

The aim of this work is the developement of microwave devises suitable for detection and alalysis of the properties of organic and biological solutions via microwave technology. Dielectric spectroscopy of liquids and molecules in liquid environment provides information about the mobility of molecules by probing its complex dielectric properties. The design of sensors is based on a number of thin film stripline topologies on different microwave suitable substrates (e.g., sapphire, LaAlO3)using high-k material for improvement of the devise sensitiv-

ity. Test measurements in a frequency regime up to 20GHz reveal the high resolution of this technology. Finally, it is demonstrated that the relaxation properties of water and aqueous solutions, alcohols and mixtures of alcohols with dipolar and non-polar solvents can be analyzed via these devices.

BP 12.7 Mon 17:15 P3

Combining Optical Trapping and Confocal Microscopy — •Constantin Spille, Florian Rehfeldt, and Christoph F. Schmidt — Drittes Physikalisches Institut, Georg-August-Universität Göttingen, Germany

Mechano-sensing and force transduction play an essential role in many cellular processes but the microscopic mechanisms are not yet understood. Acto-myosin stress fibers are key players in the physical response to the mechanical micro-environment. Optical trapping allows us to accurately measure forces exerted by the cell on trapped silica beads with pN accuracy and high time resolution. However, the cellular processes responsible for the forces cannot be resolved with a normal epifluorescence microscope due to the spherical shape of the cells when suspended in solution. We therefore built a dual optical trap into a commercial confocal microscope to be able to combine confocal scanning with optical trapping. We here discuss basic design considerations and show proof-of-principle data.

BP 12.8 Mon 17:15 P3

Using an ultrastable AFM to measure pN-forward forces of a growing neuronal Growth Cone — •Thomas Fuhs and Josef A. Käs — Universität Leipzig, Soft matter physics, Leipzig, Germany

We have already shown how to use an AFM to measure forward pushing protrusion forces of fast moving fish keratocytes on glass substrates at room temperature. But when trying this technique to measure slow moving neuronal growth cones at 37°C, one can no longer assume a drift-free system because of thermally induced artifacts. Therefore we incorporated an optical trap into our AFM-setup to measure, and correct for, the substrate's drift. Yet the scan head of the AFM does not allow using the forward scattered signal of the optical trap. To get position information nonetheless we use the backscattered light of our marker bead. With this we can still reduce the drift of the AFM scan head with respect to the substrate to less than 50 nm/h in all 3 dimensions. Using this stabilization we can realize the necessary observation times of 1h and even longer, while still being sensitive in the pN force range.

BP 12.9 Mon 17:15 P3

The FIRST project: Fragmentation cross sections for hadron therapy — •Christoph Schuy — GSI, on behalf of the FIRST collaboration

Motivation: The possibility to treat highly radio-resistant tumors while sparing OAR (organs-at-risk) has led to an increasing importance of hadron therapy. To further optimize treatment planning and benchmark Monte Carlo codes, a detailed characterization of the interaction of carbon ions in biological tissue and other relevant materials is necessary. The FIRST (Fragmentation of Ions Relevant for Space and Therapy) experiment, performed by an international collaboration (France, Germany, Italy, Spain), aims at studying nuclear fragmentation processes for therapy and space relevant ion beams and measure double-differential cross sections for high energy fragmentation reactions.

Methods: A complex detector array will be used to measure charge, mass, angular distribution and energy of all fragments plus high-energy neutrons in forward direction. The experimental setup consists of Aladin detectors (Aladin magnet, TP-MUSIC IV, ToF wall) and LAND (Large Area Neutron Detector) as well as newly designed detectors in the interaction region.

Outlook: The first experiment with 200MeV/u and 400MeV/u carbon beams on a carbon target is scheduled for late summer 2011 and will be performed in cave C at the GSI Helmholtz Centre for Heavy Ion Research in Darmstadt, Germany.

BP 12.10 Mon 17:15 P3

The Nature of Water at Material Interfaces — •Kai F. Hodeck[1], Kathrin M. Lange[1], Ulrich Schade[1], Andrei P. Sommer[2], Dan Zhu[2], Alexander Kothe[1], and Emad F. Aziz[1,3] — [1]Helmholtz-Zentrum Berlin für Materialien und Energie — [2]Universität Ulm — [3]Freie Universität Berlin

We studied the hydrogen bonding of water at the interface to sample materials of different polarity using soft X-ray absorption (XA) and Fourier transform infrared (FT-IR) spectroscopy. We show that the electronic structure of isolated water molecules in liquid solvents is neither like in the gas phase nor like in the bulk liquid phase, but rather like in ice [1]. Increasing the concentration gives rise to a solvent-specific clustering of the water molecules and the formation of distinct structures of the hydrogen bonding network: While in the polar acetonitrile environment, a shared solvation leads to string-like water structures, the less polar chloroform solvent facilitates an immediate phase separation, and a clustering of the water molecules. At the interface to the non-polar benzene solvent, a preferential orientation of the water molecules is found, which we interpret in terms of a formation of cage-like structures [1]. Upon going to the layering structure of water at extended hydrophobic interfaces we provide evidence for a strong, direct interaction with visible light as it is unknown for the bulk liquid [2, 3]. [1] K. Lange et al.: The Nature of the Hydrogen Bond of Water in Solvents of Different Polarities, The Journal of Physical Chemistry B; in print, DOI: 10.1021/jp109790z [2] A. Sommer et al.: Tuning Nanoscopic Water Layers with Laser Light. Langmuir, 24, 635

BP 12.11 Mon 17:15 P3

Electrical characterization of single nanopores in 30 nm thick silicon membranes — •Vedran Bandalo[1], Yael Liebes[2], Nurit Ashkenasy[2], and Marc Tornow[1] — [1]Institut für Halbleitertechnik, TU Braunschweig, Germany — [2]Ben-Gurion University of the Negev, Beer Sheva, Israel

We present the fabrication of silicon nanopore devices and their electrical characterization for DNA translocation measurements. The devices are based on Silicon-On-Insulator (SOI) substrates, where a 30-50 nm thick Si membrane is released using highly anisotropic reactive ion etching, followed by direct pore drilling using a novel FEBIE (focused electron beam induced etching) process. We measured the ionic conductance through individual pores in 100 mM KCl electrolyte solution at a typical noise level of about 3,7 pA RMS and 37 pA p-p, at 100 mV bias. The observed, approximately linear current-voltage characteristics with resistances in the range of 140 to 160 MΩ correspond to pore diameters of about 20 nm, according to a simple cylindrical shape model for the pore and in good agreement with the diameter measured by SEM. Furthermore, first results of the translocation of lambda-DNA through the Si nanopores, as well as novel concepts for integrating an Ag/AgCl electrode on-chip, in a closed cavity-like nanopore device, will be presented.

BP 12.12 Mon 17:15 P3

Interferometric nanoparticle tracking with few photons — •Dennis Müller and Rainer G. Ulbrich — IV. Physikalisches Institut, Georg-August-Universität Göttingen, Germany

We report interferometric tracking of nanoparticles with subwavelength accuracy in the limit of low light intensities. With only few photons contributing to the far-field interferogram the ultimate accuracy of a position measurement which can be achieved from phase reconstruction is limited by shot noise of the detected photons. By means of Monte Carlo simulations we have studied the precision of interferometric tracking as a function of the number of contributing photons and compared it with other tracking methods. The results were experimentally confirmed by tracking a single gold nanosphere, which was immobilized and moved in a controlled way by a piezo actuator.

BP 12.13 Mon 17:15 P3

Electron cryo-microscopy of biological specimens on ultrathin graphene-like conductive carbon nanomembranes — •Daniel Rhinow[1], Matthias Büenfeld[2], Nils-Eike Weber[2], André Beyer[2], Janet Vonck[1], Michael Schranz[3], Armin Gölzhäuser[2], Werner Kühlbrandt[1], Norbert Hampp[3], and Andrey Turchanin[2] — [1]Max-Planck-Institut für Biophysik, Max-von-Laue-Str. 3, 60438 Frankfurt — [2]Universität Bielefeld, Fakultät für Physik, 33615 Bielefeld — [3]Universität Marburg, Fachbereich Chemie, Hans-Meerwein-Str., 35032 Marburg

We have tested ultrathin carbon nanomembranes (CNM) comprising cross-linked biphenyl precursors as support films for transmission electron microscopy of biological specimens. Due to their low thickness of 1 nm CNM add virtually no phase contrast to the transmission pattern thus allowing background-free structural analysis of biological samples, which comprise mainly light elements. Furthermore, we have tested conductive carbon nanomembranes (cCNM) comprising

nanocrystalline graphene, obtained by thermal treatment of CNM, as supports for cryoEM of frozen-hydrated biological samples. cCNM are graphene-like substrates, which ideally match all requirements for cryoEM of electrically insulating biological specimens. To analyze the performance of cCNM support films we used purple membranes from Halobacterium salinarum and tobacco mosaic virus as test specimens.

BP 12.14 Mon 17:15 P3

Three-Focus-Fluorescence-Correlation-Spectroscopy (3fFCS) — •Lars Kreutzburg, Richard Börner, and Christian G. Hübner — Insitute of Physics, University of Lübeck, Ratzeburger Allee 160, 23562 Lübeck, Germany

Intracellular transport is achieved by the combination of diffusive and directed motion. FCS and its variants are well suited for the study of such processes. One of these variants presented by Dittrich and Schwille is the spatial-two-photon FCCS for one-dimensional flow measurements by shifting two detection volumes spatially in the precences of one large excitation volume. The later development of two-focus FCS (2fFCS) by Dertinger et al. allows for the determination of exact diffusion coefficients by splitting the optical pathways of two orthogonally polarized excitation beams via a DIC prism, which leads to two spatial shifted overlapping confocal volumes. Following these approaches, we propose a new variant, called three-focus FCS, which enables for the determination of the direction of directed motion of molecules by shifting three or more detection volumes relatively to one excitation spot. We will present a theoretical description of 3fFCS. Moreover, extensive simulations allow for the evaluation of the capabilities of this method. The simulations are compared with the first experimental results.

BP 12.15 Mon 17:15 P3

Detection of conformational states of proteins in *C. elegans in vivo* by confocal fluorescence anisotropy — •Volker Christoph Henschel[1], Alessandro Esposito[2], Eugenia Butekevich[1], Christoph Friedrich Schmidt[1], Fred Sylvester Wouters[3], and Dieter Robert Klopfenstein[1] — [1]Drittes Physikalisches Institut, Biophysics, Georg-August-University Göttingen, Göttingen, Germany — [2]MRC Cancer Cell Unit, Hutchison/MRC Research Centre, Cambridge, UK — [3]Laboratory of Cellular and Molecular Systems, Department of Neuro- and Sensory Physiology, Georg-August-University, Göttingen, Germany

The study of protein-protein interactions *in vivo* is often hindered by the limited acquisition speed of typical instrumentation used, for instance, for lifetime imaging microscopy. Anisotropy polarization is altered by the occurrence of Foerster Resonance Energy Transfer (FRET) and anisotropy imaging was shown to be comparatively fast and simple to implement. Here, we present the adaptation of a spinning disc confocal microscope for fluorescence anisotropy imaging that allowed to achieve in vivo imaging at high spatial and temporal resolution. We demonstrate the capabilities of this system and in-house developed analysis software by imaging living *Caenorhabditis elegans* expressing constitutive dimeric and monomeric proteins that were tagged with GFP.

BP 12.16 Mon 17:15 P3

Upgrading a Commercial Confocal Microscope to CW-STED Super-Resolution — •Til Driehorst, Florian Rehfeldt, and Christoph F. Schmidt — Drittes Physikalisches Institut, Georg-August-Universität Göttingen, Germany

Fluorescence microscopy is one of the most commonly used imaging techniques in the life sciences, particularly when investigating living organisms at the sub-cellular level. A major drawback is the diffraction-limited resolution. This limit has been overcome by several new methods such as stimulated emission depletion (STED) microscopy. Here we describe the upgrade of a Leica TCS SP5 X confocal microscope to super-resolution by implementing a custom-built STED system. Fluorophore excitation is done with a pulsed white light laser (WLL) source, while a 592 nm continuous wave (CW) laser is used for STED. The combination of a WLL source and 592 nm STED laser is well-suited for commonly used fluorescent markers such as the FITC and the yellow fluorescent protein (YFP) family.

BP 12.17 Mon 17:15 P3

Real-time 3-dimensional particle tracking with sub-nanometer accuracy at 5,000 frames per second — •Alexander Huhle, Sihwa Joo, Daniel Klaue, and Ralf Seidel — Biotechnologisches Zentrum, TU Dresden, Tatzberg 47/49, 01307 Dresden

Tracking the position of single spherical micrometer-sized particles in all 3 dimensions is crucial for modern force sensing techniques, such as optical and magnetic tweezers. It can be realized using either position sensitive devices in combination with focused laser illumination or a camera and wide-field illumination. While camera-based detection is very simple to implement and offers the attractive possibility to determine the positions of many particles in parallel, real-time tracking rates have so far been limited to several tens of frames per second due to the high computational effort of the employed software routines. Here we demonstrate 3 dimensional real-time tracking at 5,000 frames per second with sub-nanometer accuracy using a fast CMOS camera for image acquisition and employing GPU based computing. Computationally demanding parts of the tracking algorithm are carried out in the GPU that is specialized for highly parallelized execution. Tracking of the lateral particle positions is obtained by cross-correlating the image with its mirror image. The axial position is obtained from the radial intensity profile of the particles diffraction pattern when imaged in overfocus. High tracking rates are crucial to overcome the shot-noise limitations of camera-based detection at the second time scale and to resolve fast, dynamic processes.

BP 12.18 Mon 17:15 P3

Analytic solution for image analysis in localization microscopy with full accuracy — •Frederik Grüll, Manfred Kirchgessner, and Udo Kebschull — Kirchhoff Institute für Physics, Heidelberg University, Germany

In localization microscopy the resolution limit is improved by calculating the centroid of the image of each fluorescent point-like object. Current solutions obtain the center by fitting a Normal Distribution and optimize the maximum likelihood or least squares iteratively. Faster analytical approaches exist, but come with a reduced precision in noisy environments. We propose an algorithm that is based on maximum likelihood, but solves the problem analytically. Results show that we maintain full accuracy also for noisy images with a speedup of more than 100 compared to numerical fits, and provide an accurate error estimation for each localization. As a consequence image analysis for localization microscopy becomes real-time capable on standard computer hardware.