# BP 34: Posters: New Technologies

Time: Thursday 17:15–20:00 Location: Poster B2

BP 34.1 Thu 17:15 Poster B2

**A novel efficient and fast method to reconstruct free energy landscapes** — •Jens Smiatek and Andreas Heuer — Westfälische Wilhelms-Universität Münster, Institut für Physikalische Chemie, 48149 Münster, Germany

We present a novel method to efficiently explore and calculate free energy landscapes inspired by the Well-tempered Metadynamics algorithm [1]. The technique which is called "Weighted Histogram Metadynamics on a grid" is grid-based and allows a very fast computation in contrast to Well-tempered Metadynamics and further methods. The underlying free energy landscape is reconstructed on a grid as an estimate for a biasing potential. An exact histogram reweighting scheme is finally applied to compute the free energy landscape and for the demand of thermodynamic consistency. Furthermore by filling the free energy minima our method allows a rapid decrease in simulation time in the calculation of rare events. In addition, the calculated free energy landscape is not restricted to the actual choice of collective variables and can in principle be extended on the fly to all variables of interest. As an example for our method, we present the free energy landscape of the alanine dipeptide in solution for several collective variables.

[1] Barducci A., Bussi M. and Parrinello M., Phys. Rev. Lett. 100, 020603 (2008)

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**BioRef - a time-of-flight reflectometer for soft matter applications at HZB** — •Markus Strobl[1,2], Roland Steitz[2], Martin Kreuzer[1,2], Reiner Dahint[1], and Michael Grunze[1] — [1]Universität Heidelberg — [2]Helmholtz Zentrum Berlin

BioRef, a time-of-flight reflectometer at Helmholtz Zentrum Berlin (HZB) is currently under construction. Combined with an in-situ infrared spectrometer it will be optimised for soft matter applications at solid-liquid interfaces. A flexible double-chopper set-up together with a wavelength band chopper will enable the selection of well defined wavelength bands at different defined wavelength resolutions in order to optimize measurements with regard to the given application [1]. A state-of-the-art 2D position sensitive 3He detector will be used for the reflectivity measurements in horizontal scattering geometry. The time-of-flight mode is also chosen to realise the investigation of dynamic interface processes under shear and flow conditions. A choice of different wavelength bandwidths with at the same time constant wavelength resolution enables to focus on defined features in the reflection curve depending on the requirements of the specific measurements utilizing the highest possible efficiency. A q-range spanning 3 orders of magnitude and reflectivity measurements over more than 6 orders of magnitude are envisaged and their feasibility is supported by Monte Carlo simulations [2].

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**Phase estimation from interferograms with few photons** — •Dennis Müller[1], Thomas Hotz[2], and Rainer G. Ulbrich[1] — [1]IV. Physikalisches Institut, Georg-August Universität Göttingen, Germany — [2]Institut für Mathematische Stochastik, 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. We have studied the precision of such a phase estimation, based on the maximum likelihood method, for different experimental configurations. Its dependence on the form of the interference pattern, the number and relative position of detection channels, and the total number of detected photons has been analyzed.

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**The Optical Cell Rotator: Image propagation through fibers for contact-free rotation of cells** — •Michael Schmidberger, Moritz Kreysing, and Jochen Guck — Cavendish Laboratory, Department of Physics, University of Cambridge

The Optical Cell Rotator (OCR) is a dual-beam laser trap that allows to hold and orient living cells stable in 3D. Unlike earlier versions of fiber-based traps, OCR uses fibers supporting more than one optical mode. Combined with adaptive optics, this allows for the generation of non-trivial trapping geometries while still having all the advantages of fibers.

We provide detailed analysis of the problems one faces when trying to propagate images through optical fibers. We then present solutions specifically interesting for optical trapping applications and show how these results can be used to rotate living cells stepwise through the focal plane of practically any optical microscope. Finally, we discuss how this feedback independent rotation mechanism can serve as basis for already established, but so far impractical tomographic imaging techniques.

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**STED microscopy: High-resolution imaging of dynamic processes** — •Christian Osseforth[1], Jeffrey Moffitt[2], and Jens Michaelis[1] — [1]Ludwig-Maximilians Universität München, Department Chemie und Biochemie, Butenandtstr.11, 81377 München — [2]FAS Center for Systems Biology, Harvard University, Cambridge, MA 02138

Stimulated emission depletion microscopy has been shown to overcome the diffraction limit of normal confocal fluorescence microscopes by resolving structures down to 19 nm in x and y [1]. While for a long time the application of STED microscopy has been hindered due to the necessity of using complicated laser systems, the recent development of commercially available supercontinuum lasers have significantly lowered the cost and complexity of operating such a setup in a lab environment [2]. Here we present our current STED microscope setup using the aforementioned compact laser source in conjunction with a highspeed scanning stage. This allows for observing dynamic processes in vitro and in vivo with nanoscale resolution. Restrictions in scanning speed are set by the repetition rate of the laser source (1 MHz at the moment) but are thought to improve as the demand for fast, high power super-continuum lasers rises. We will discuss general design considerations as well as practical considerations for building a STED system.

[1] Wildanger D et al.; J. Microsc. 2009; 236(1):35-43
[2] Wildanger D et al.; Opt Express 2008; 16(13): 9614-9621

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**Combined SERS/AFM microscopy on single gold nanoparticle clusters** — •Dennis Steinigeweg, Mohammad Salehi, Magdalena Gellner, Max Schütz, and Sebastian Schlücker — Fachbereich Physik, Universität Osnabrück, Barbarastr. 7, 49069 Osnabrück

Surface-enhanced Raman scattering (SERS) is an ultrasensitive technique of Raman spectroscopy for molecules on or near metallic nanostructures that support localized surface plasmon resonances. Single noble metal nanoparticle clusters exhibit extremely high and very localized near-field enhancements in the junctions between adjacent particles ("hot spots").

We employ SERS from self-assembled organic monolayers (SAM) on the surface of single gold nanoparticle clusters for probing their optical/plasmonic properties. Atomic force microscopy (AFM) on the same objects provides the corresponding topographic information.

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**New Carbohydrate-based Protein Sensor Realized with Cantilever Arrays** — •Kathrin Gruber[1], Tim Horlacher[2], Peter H. Seeberger[2], and Bianca A. Hermann[1] — [1]CeNS and Walther-Meissner-Institute, Walther-Meissner-Str. 8, 85748 Garching, Germany — [2]Max Planck Institute of Colloids and Interfaces, Department of Biomolecular Systems, Arnimallee 22, 14195 Berlin, Germany

Cantilever based detection opens new means for the label-free analysis of biomolecular interactions in real-time and up to eight channels. In the static operation mode, the biomolecular interaction is transduced into a deflection of a micrometer thin silicon beam that can be read-out with nanometer precision via optical beam deflection. Owing to recent advances in carbohydrate sequencing and synthesis, glycomics is catching up fast to the more established fields of genomics and proteomics. Measuring carbohydrate interactions is key to understand carbohydrate function requiring the development of reliable, sensitive and selective sensor surface chemistries. Cantilever array have

been successfully used in gene fishing, single base pair recognition, and antigen-antibody assays. We design a purely carbohydrate based sensing layer to single out the central glycoconjugate recognition. Using a reference sensor to account for non-specific binding, we detect carbohydrate-protein interactions down to nanomolar concentrations. We verify the selective binding of proteins to carbohydrate functionalized cantilevers by a competitive inhibition assay. Our results pave the way for carbohydrate based cantilever sensors as a robust, scalable and label-free method to study medically relevant carbohydrate-protein interactions.

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**Magnetic Tweezers Setup for Single Molecule Experiments** — •Carolin Rademacher, Sebastian Horstmeier, Christoph Pelargus, Andy Sischka, and Dario Anselmetti — University of Bielefeld, Department of Physics, Experimental Biophysics and Applied Nanosciences

Besides atomic force microscopy and optical tweezers magnetic tweezers are a powerful tool for micromanipulations and single molecule force spectroscopy. We introduce a magnetic tweezers setup, that is based on a multipole-alignment operating with electromagnets to accomplish drag- and rotation-experiments with individual magnetic beads and allow operation at low mechanical vibrations. Since a wide range of applications is wanted, the pole pieces of the setup are manufactured through contact-lithography and electro-deposition [1], so that alterations are easy to accomplish in form and size. In the future we want to apply the magnetic tweezers to study rotating motor proteins under in vitro conditions. Here we present and discuss our setup and show the first data of our calibration experiments.

[1] A. H. B. de Vries, B. E. Krenn, R. van Driel, and J. S. Kanger. Patterned Electroplating of Micrometer scale Magnetic Structures on glass substrate. Journal of Microelectromechanical Systems (J-MEMS), 13:391 - 395, 2004.

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**Single nanoparticle detection and the use of holographic optical tweezers for object manipulation in micro-fluidic devices** — Julia S. Gebauer and •Lennart Treuel — Universität Duisburg-Essen, Essen, Germany

A Single NanoParticle Sensor (SNPS) has been developed on the basis of a modified optical tweezers approach and is used to count single NPs in micro-channels with high time resolution. The direction of a particle passing through the focus point can be determined by a suitable signal evaluation.

Laser pumps generated by optical tweezers can be used to establish controlled flow conditions in micro-fluidic devices. The flows generated by this approach are used to purposefully pump nano- and microobjects in selected directions. The utilisation and characterisation of these micro pumps will be presented in this work. The combination of the ability to selectively pump nano- and micro objects with the single nanoparticle counter described above is expected to strongly enhance the use optical tweezers and their derivatives in new Lab-on-a-chip developments.

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**Atomic scale magnetometry using single defects in diamond** — •Thomas Wolf[1], Helmut Rathgen[1], Rolf Reuter[1], Gopalakrishnan Balasubramanian[1], Fedor Jelezko[1], Dirk Bald[2], and Jörg Wrachtrup[1] — [1]3. Physikalisches Institut, Universität Stuttgart, Germany — [2]Structural Biology Group, Vrije Universiteit Amsterdam, Netherlands

Diamonds contain natural defect centers in their lattice structure known as color centers. Electron spin states in these centers (e.g. the Nitrogen-Vacancy or NV-center) can be changed and measured with optical techniques at room temperature. Using magnetic fields and magnetic field gradients the centers can be located spatially on the nanometer scale and allow for directional analysis. The potential for sub-nm precision by using magnetic resonance techniques has been shown by our group and collaborators.

Diamonds with diameters of a few nanometers containing a NV-center are comparatively cheap and can be produced in large scale. By chemical treatment functionalization of these can be achieved.

Using small nanosized diamond crystals containing a NV-center we intend to use diamond as non-toxic biological marker for a new magnetic resonance imaging technique having potential to overcome the classical resolution limit of light microscopy under physiological conditions.

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**A hazzardfree fabrication process for arbitrarily shaped microparticles** — •Lukas Bogunovic, Jan Regtmeier, and Dario Anselmetti — Experimental Biophysics and Applied Nanoscience, Physics Faculty, Bielefeld University, Bielefeld

The use of micro- and nanoparticles impacts applications in biotechnological, chemical and physical sciences like the manipulation and transport of objects in microenvironments or as model migrants in microfluidic systems [1,2]. However, most of the commercially available particles are spherical. Therefore their field of application with respect to shape dependent phenomena is strongly limited.
Here, we propose a novel simple and inexpensive method for the production of arbitrarily shaped microparticles with a manufacturing spreading better than 2.5%. Comparable methods essentially need hazardous acids like hydrofluoric acid (HF) or complicated processing setups which are therefore delicate to handle. Our process involves structuring of the particles in SU-8, which can be doped with tracers like magnetite or fluorescent dyes. Afterwards they are released from their substrate into a surfactant solution with a treatment in an ultrasonic bath.

[1] I. Safarik, M. Safarikova, *Chemical Papers*, **63**, 497-505, 2009
[2] J. Hanes et al., *Advanced drug delivery reviews*, **28**, 97-119, 1997

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**Measuring rotational diffusion of proteins by fluorescence correlation spectroscopy** — •Anastasia Loman, Ingo Gregor, and Joerg Enderlein — Third Institute of Physics "Biophysics", Georg-August-Universität, Göttingen

Translational and rotational diffusion are thermally driven processes which depend on molecular parameters as size and shape but also on interaction between molecule and solvent environment. Fluorescence correlation spectroscopy (FCS) is a well known technique to measure translational diffusion coefficients of fluorescent molecules thus monitoring intramolecular changes and intermolecular interactions.

Here we apply fluorescence correlation to measure rotational diffusion. In contrast to conventional fluorescence anisotropy measurements, a correlation based method will work also when the rotational diffusion time is much longer than the fluorescence decay time. Thus, the method is ideally suited to study the rotational diffusion of macromolecules in aqueous solutions having rotational diffusion times of dozen to hundred nanoseconds. By using a pulsed interleaved excitation scheme with crossed excitation polarization, we are able to maximize the temporal dynamics of the measured correlation curve as caused by rotational diffusion. The method is exemplified on sizing the large globular proteins such as amylase, ovalbumin and human serum albumin.

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**Fluorescence spectroscopic studies of protein conformational dynamics** — •Phillip Kroehn — Drittes Physikalisches Institut, Georg August Universität Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen

Proteinfolding is the physical process by which a polypeptidechain folds into its functional three dimensional structure from a random coil.

The WW-domain is the smallest betasheet known. It consists of a lightly hydrophobic core and takes part in protein-protein interactions by binding of proline rich regions. By singlemolecule FRET studies we want to analyse the folding/unfolding dynamics of the WW-domain and its intermediate states. The unfolding of the protein will be accomplished by chemical or thermal denaturation.

To use smFRET we are going to label the WW-domain specifically with fluorophoric dyes by employing the orthogonal system. This new technique allows us to use a stopcodon as a codon for an unnatural aminoacid, like para-acetylphenylalanine, whereby it is placed in the polypeptidechain. We are able to specifically label these unnatural aminoacid in the protein.

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**Fabrication of hybrid nano-micro-fluidic channels with extreme aspect ratios** — •Eugenie Fredrich, Martina Everwand, Jörg Käsewieter, Dario Anselmetti, and Jan Regtmeier — Experimental Biophysics & Applied Nanoscience, Bielefeld University, Universitätsstraße 25, 33615 Bielefeld, Germany

In the micro- and nanofluidic analysis of single molecules, often com-

plex 3D geometries with high aspect ratios are required. Among the basic preconditions for the production of the structures, stability is the most fundamental one, but hard to realize with soft lithography. Furthermore, a spatial accuracy within the range of a few nanometers is especially required for nanofluidics.

In the novel method presented here, contact lithography with the photoresist SU8 is employed to create negative relief structures as small as 670 nm. The microfluidic device is molded with a polydimethylsiloxane (PDMS) bilayer. The first thin layer consists of h-PDMS, which accounts for the stability of the fabricated structures, whereas the second thicker layer is made of the more elastic Sylgard-184 PDMS allowing for flexibility and experimental handling. The resulting channels have a width of 200 $\mu$m and free hanging barrier structures forming a flow-through gap of 670 nm, i.e. an aspect ratio of about 300:1.

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**Applications for fluorescence lifetime imaging: Fast genomic characterization and lifetime measurements on zero-mode waveguide** — •Mira Prior, Ingo Gregor, and Jörg Enderlein — Third Institute of Physics "Biophysics", Georg-August-University Göttingen

Previous studies showed that the lifetime of the DNA-intercalating dimeric cyanine dye TOTO depends on the insertion of the dye into AT or GC base pairs of the nucleic acid sequence. Utilizing this characteristic we want to develop a fast and simple method of characterizing unknown double-stranded DNA. With time-correlated single-photon counting (TCSPC) and fluorescence lifetime imaging (FLIM) we want to directly determine the lifetime of the dye YOYO in the DNA-strand depending on the insertion into an AT or GC base pair. A second application for FLIM is the detection of lifetimes of a dye on a zero-mode waveguide. The zero-mode waveguide consists of a circular aperture in an aluminum layer on a microscope slide. The detection volume is confined by the small aperture, which allows single-molecule measurements of high dye concentration in the micromolar range. TCSPC and FLIM allow us to determine the lifetime of the dye ATTO647 in dependence of the aperture diameter. The principle of the analysis of the detected photons and the identification of the single-molecules is based on a multi-exponential curve fitting and a maximum likelihood principle for the DNA-analysis. We want to analyze whether the distribution of lifetimes on the length of the ds-DNA strand correlates with the sequence of this DNA.

BP 34.16 Thu 17:15 Poster B2

**Towards solid state nanopores with single walled carbon nanotube contacts** — Camille Raillon[1], Sudhir Husale[1], •Matthias Heise[2], Juri Allerdings[2], Christoph Strunk[2], and Aleksandra Radenovic[1] — [1]LBEN, IBI EPFL Lausanne 1015 Switzerland — [2]Institut für experimentelle und angewandte Physik, 93040 Regensburg

We integrate nanoelectrodes with solid state nanopores for detection of passing molecules, e.g., DNA. To control the translocation speed we use optical tweezers [1], to increase spatial resolution of the sensor electrodes made from single walled carbon nanotubes (SWNT) comparable in thickness and distance to a single nucleotide are desirable. We used dielectrophoresis (DEP) method to attach nanotubes in the lithographically defined nanogaps. DEP is well known method and can potentially be used as an efficient trapping tool in the fabrication of such molecular devices. When an electric field is applied, we have observed that density of SWNTs in the nanogap can be tuned with the applied voltage ($\tilde{}$ 0.1 V to 0.5 V). Nanogaps $<$ 10nm have been achieved in this way. In a complementary way we grow SWNT from e-beam predefined catalyst particles deposited on our Si3N4 membranes. We then drill a hole through the membrane using the focused beam of a transmission electron microscop (TEM) at a position where a nanotube was grown. By this we cut the tube in half, resulting in a nanopore with a pair of SWNT nanoelectrodes. [1] E. H. Trepagnier et al., Nano Letters 7, 2824 (2007).