AKB 28 Single Molecule Probes

Time: Friday 12:00–13:00 Room: ZEU 255

AKB 28.1 Fri 12:00 ZEU 255

Optical trapping and tracking: novel approaches in cell biophysics — •Alexander Rohrbach[1,2] and Holger Kress[2] — [1]Institute of Microsystem Technology (IMTEK), University of Freiburg, Georges-Köhler-Allee 102, 79110 Freiburg — [2]European Molecular Biology Laboratory (EMBL), Meyerhofstrasse 1, 69117 Heidelberg

Optical micromanipulation has open new possibilities for investigating infrequent events. Especially optical traps allow increasing the interaction probability between interacting partners. Energy fluctuations and diffusion are maintained inside the optical trap, enabling a natural dynamic interaction, which is not given e.g. in AFM experiments. However, only a fast and precise three-dimensional detection system allows measuring the broad spectrum of interaction dynamics. Although holographic traps enable fascinating possibilities of optical manipulation, particle tracking and thus measurements are strongly limited in speed and precision. This drawback can be overcome with e.g. scanning optical traps. In this talk I demonstrate, how fluctuation dominated processes in cell biology can be controlled and measured with nanometer precision and at a rate of 1 kHz to 1 Mhz. On the one hand, the uptake, binding and intracellular transport of particles to/in macrophages are investigated. On the other hand, a complete helical bacterium (a 200 nm thin spiroplasm) is oriented and tracked interferometrically in a scanning optical trap. This allows new insights into the complex flexing and rotation dynamics of this simplest form of life.

AKB 28.2 Fri 12:15 ZEU 255

Theoretical analysis of single-molecule force spectroscopy experiments: heterogeneity of chemical bonds — •Peter Reimann and Martin Raible — Universitaet Bielefeld

We show that the standard theoretical framework in single-molecule force spectroscopy by Evans and Ritchie [Biophys. J. 72, 1541 (1997)] has to be extended in order to consistently describe the experimental findings. The basic new concept is to take into account heterogeneity of the chemical bonds, resulting in excellent agreement between theory and experiment.

AKB 28.3 Fri 12:30 ZEU 255

A Combined Setup for Single Molecule Manipulation and Optical Spectroscopy — •Volker Walhorn, Rainer Eckel, Christoph Pelargus, Joerg Martini, Dario Anselmetti, and Robert Ros — Experimental Biophysics, Physics Department, Bielefeld University, Germany

Atomic force microscopy (AFM) as well as fluorescence microscopy (FM) are both intensively used techniques to elucidate interactions, dynamics and structures of single biomolecules. Synchronous fluorescence- and atomic force microscopy allow diverse new approaches in single molecule techniques. We present our novel setup capable of simultaneously performing total internal reflection fluorescence microscopy (TIRFM) and atomic force microscopy/spectroscopy. For topographical or force readout a home-built AFM head is mounted on an inverted optical microscope equipped with a TIRFM objective, significantly reducing background noise. Spectral information is obtained by excitation with an Ar+ laser and fluorescence detection with a high speed CCD camera. Temporal and spatial correlation of topographical, force and fluorescence data can yield important information of the dynamics and molecular mechanisms of guest-host-interactions as well as protein folding pathways.

AKB 28.4 Fri 12:45 ZEU 255

Metal coated full body glass tips as high resolution probes for SNOM fluorescence imaging — •Heinrich Gotthard Frey[1], Carsten Bolwien[2], Robert Ros[1], and Dario Anselmetti[1] — [1]Experimentelle Biophysik und angewandte Nanowissenschaften, Fakultät für Physik, Universität Bielefeld, Universitätsstraße 25, 33615 Bielefeld — [2]Frauenhofer-Institut für Physikalische Messtechnik IPM, Heidenhofstraße 8, 79110 Freiburg

For biological applications, scanning near-field optical microscopy of single fluorescent dye molecules require probes which combine high near-field intensities with high optical and topographical resolution.

Such probes can be realised by full body glass tips completely covered by a thin metal layer. In order to achieve strong fields at the tip apex, it is important to illuminate the tip under an inclination angle with the polarisation parallel to the inclination plane. Optimised values for metal layer thickness and inclination angle have been investigated by multiple multipole simulation showing that the achievable optical resolution is roughly given by the tip radius.

These probes have been tested by imaging single fluorescent dye molecules. They show fluorescence patterns with one or two peaks, which can be explained by means of the electrical field distribution at the tip apex. For an aluminium coated tip with 25 nm tip radius, the fluorescence patterns have peaks with a full width half maximum of about 15 nm.

This optical probe is especially suited for cantilever probes, where the inclined illumination can easily be realised.