

Biological cells constantly communicate with their environment. Besides biochemical interactions, cells can also sense their mechanical micro-environment and external forces. Key players in the mechanosensing and -transduction processes are acto-myosin stress fibers that generate contractile forces.

To elucidate the mechanistic details of the physical interactions between cells and their surrounding, we use a dual optical trap to perform high-resolution measurements of cellular force fluctuations. Monitoring the displacement fluctuations of two fibronectin-coated beads attached to opposite sides of a cell and analyzing their correlated motions in conjunction with active probing of the cell with oscillating forces allows us to measure simultaneously the overall forces the cell generates and the fraction of that force transmitted to the environment. We present data of force fluctuations and cell stiffness of 3T3 fibroblasts obtained by such active and passive microrheology measurements. To distinguish non muscle myosin II - based activity from other effects, we used blebbistatin, a potent and specific inhibitor of non-muscle myosin II.

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Biomechanical Data Networks — ●TOBIAS R. KIESSLING, KENECHUKWU D. NNETU, ANATOL FRITSCH, ROLAND STANGE, and JOSEF A. KÄS — University of Leipzig, Institute of Experimental Physics I, Linnéstr. 5, 04103 Leipzig, Germany

The transition from benign tissue to malignant cancer is accompanied by various alterations of the cellular organization, amongst others of the cytoskeleton. This highly dynamic polymer network provides both, functional and mechanical stability to cells whereas small changes of the cytoskeletal composition are reflected in alterations of the mechanical properties of cells.

The Microfluidic Optical Cell Stretcher, built to monitor these cytoskeletal changes provides a fast and easy access to a range of physical parameters of thousands of cells. Methods derived from gene expression network analysis techniques will be discussed that help to reveal unbiased relations between measured physical properties and how these can be used to differentiate between benign and malignant cells without the need of any molecular marker.

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Quantification of hematopoietic stem cell and neutrophil chemotaxis using microstructured systems and ELISA — ●CHRISTINA LEINWEBER¹, RAINER SAFFRICH², ANTHONY D. HO², NICOLE NIEMEIER³, KATJA SCHMITZ³, MICHAEL GRUNZE^{1,3}, and AXEL ROSENHAHN^{1,3} — ¹Applied Physical Chemistry, University of Heidelberg — ²Department of Medicine V, University of Heidelberg — ³IFG/ITG, Karlsruhe Institute of Technology

The migration of hematopoietic stem cells (HSC) towards bone marrow, the so called homing process, plays an important role in modern leukemia therapy. HSC are supposed to be guided by a concentration gradient of chemokines which are expressed by marrow cells, the mesenchymal stromal cells (MSC). Therefore we investigate the chemotactic response and migration behavior of HSC using different in vitro chemotaxis assays with increasing intricacy, e.g. migration experiments in microwells, transwells and within microstructured systems. These chip systems allow studying single parameters, such as migration kinetics, thresholds, sensing sensitivity and swarm behaviour, by varying the geometry of the microchannel structures. In order to establish the methods, particularly the microstructures, we also used neutrophil granulocytes differentiated from HL-60 cell line as a model system. Additionally we performed ELISA experiments to analyze the expression of the chemokine SDF-1 by MSCs, as SDF-1 is already known to be involved in the signalling process and most likely controls HSC migration. We determined the SDF-1 concentration in dependence on expression time and on MSC culture media.

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A precise and rapid UV laser ablation system for developmental cell biology studies — ●FELIX OSWALD and STEPHAN GRILL — Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

We are presenting a state-of-the-art laser ablation system for use in cell biology studies. Diffraction-limited dissection of biological samples is achieved by coupling a pulsed ultraviolet laser (355 nm) to a conventional inverted microscope equipped with a confocal imaging system. With this setup, we can thus perform photo and plasma-induced ablation in areas up to 100 μm^2 and at high rates (500 Hz) by directing the beam with a fast mirror scanning system. Ablation experiments of the

myosin-actin cytoskeleton of *Caenorhabditis elegans* embryos demonstrates the versatility and power of such a nanoscalpel in probing the mechanical properties of cellular structures during development.

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4D-Tracking of pathogens by Digital In-line Holographic Microscopy — ●SEBASTIAN WEISSE¹, MATTHIAS HEYDT¹, NIKO HEDDERGOTT², MARKUS ENGSTLER², MICHAEL GRUNZE^{1,3}, and AXEL ROSENHAHN^{1,4} — ¹APC, University of Heidelberg — ²Zoology I, University of Würzburg — ³ITG, Karlsruhe Institute of Technology — ⁴IFG, Karlsruhe Institute of Technology

Digital Holographic Microscopy (DHM) using the in-line geometry is based on the original idea of Gabor's 'new microscopic principle'. An interference pattern containing the three dimensional information of the object encoded in phase and amplitude is recorded. Using computers, real space information about the object can be restored from these holograms applying a reconstruction algorithm. We built a portable, temperature-controlled holographic microscope to study the motion patterns of pathogenic microorganisms such as the blood parasite *Trypanosoma brucei*, the causative agent of African sleeping sickness under physiological conditions. The directed self-propulsion of Trypanosomes in the bloodstream of a mammalian host is essential for the clearing of immunoglobulins from the parasite's cell surface by hydrodynamic drag force. This mechanism is one of the parasite's strategies to evade the host's immune system and thus directly linked to pathogenesis. So far the motility studies on this unflagellated microorganism have only been carried out using standard 2D microscopy techniques. In our system parasites were tracked at varying temperatures and viscosities with high spatial and temporal resolution and the obtained 3D motion patterns statistically analyzed.

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High Resolution Growth Cone Actin Dynamics — ●MELANIE KNORR¹, TIMO BETZ², DANIEL KOCH³, and JOSEF KÄS¹ — ¹University of Leipzig — ²Institut Curie, Paris — ³Georgetown University, Washington D.C.

Neuronal growth is one of the fundamental processes in brain development and nerve regeneration. During growth, neuronal cells form long extensions, called neurites, which are guided toward their target sites by a motile structure at their tip, the so called growth cone. These growth cones are able to rearrange their cytoskeleton for directed growth, following very small guidance cues. Former research suggests amplification of these chemical signals via stochastic fluctuations of the leading edge of growth cones. Betz and Koch et al. already showed that the stochastic lamellipodium dynamics are determined by the interplay of actin polymerization, pushing the edge forward and molecular motor driven retrograde actin flow retracting the actin network. They identify switching of "on/off" states in actin polymerization as the main determinant of lamellipodial advancement. Further quantification of the suggested stochastic signal amplification, however, is limited by the spatial and temporal resolution of their imaging technique. Novel techniques and their realization are presented and discussed, able to detect the edge dynamics in higher temporal and spatial resolution.

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Vinculin regulates cell mechanical properties through src phosphorylation on its lipid anchor — ●NADINE LANG, GEROLD DIEZ, WOLFGANG GOLDMANN, and BEN FABRY — Biophysics Group, FAU Erlangen-Nürnberg, Germany

The focal adhesion protein vinculin links the actin cytoskeleton to integrin adhesion receptors. It has been reported that vinculin also binds to the lipid bilayer of the cell membrane. Vinculin with mutated or missing lipid binding regions leads to reduced focal adhesion turnover and decreased cell motility. We investigated whether this effect is directly caused by impaired lipid binding, or indirectly by mutations of residues on the lipid binding regions that are important for signaling. Vinculin has two lipid binding regions on its tail: one located on helix 3 has no phosphorylation sites, and another at the C-terminal (lipid anchor) which harbors a src-kinase regulated phosphorylation site at residue Y1065. Cells with mutations on helix 3 showed no change in stiffness (demonstrated by magnetic tweezer), in tractions (measured by traction microscopy) and in adhesion strength (determined by FN-coated bead detachment from the integrin receptor). In contrast, cells with missing lipid anchor or impaired lipid binding by mutating residues R1060 and K1061 showed strongly reduced stiffness, tractions and adhesion strength. Nearly identical behavior was observed if only the src phosphorylation site on the lipid anchor was mutated. These data show that