BP 14: Cell mechanics I

Time: Wednesday 9:30–13:00 Location: H10

BP 14.1 Wed 9:30 H10

A large scan area AFM for measurements on large biological samples and on many cells — •Todor Krastev, David Grunwald, and Tilman E. Schäffer — Universität Tübingen, Institut für Angewandte Physik, Auf der Morgenstelle 10, 72076 Tübingen

The atomic force microscope (AFM) has become a robust and versatile tool for the investigation of mechanical properties of biological samples. However, the scan range of typical AFMs (100 μm in xy- and 10 μm in z-direction) is limiting its use on large samples. We present a Macro-AFM with a scan range of 25 mm in xy- and 0.25 mm in z-direction. The Macro-AFM allowed us to map the elastic modulus of a cross section of a whole mouse aorta in a single scan. Furthermore, we applied image detection techniques to fluorescently stained cells to facilitate automated, autonomous measurements of many cells. We demonstrate the robustness of the method by comparing the elastic modulus of cells in a control group to a group treated with cytochalasin D. By performing measurements on over 1000 single cells per group, we achieved a remarkably low statistical p-value.

BP 14.2 Wed 9:45 H10

Repetitive failures and success in particle retraction of macrophage filopodia — •Rebecca Michiels and Alexander Rohrbach — Lab for Bio- and Nano-Photonics, University of Freiburg, Georges-Koehler-Allee 102, 79110 Freiburg, Germany

Macrophages take up pathogens like viruses and bacteria in a process called phagocytosis. On their surface, macrophages express abundant filopodia, thin, needle-like protrusions, which they use to catch and retract pathogens, which are later engulfed. We investigate the adaptive mechanics of filopodia and analyze the biophysical principles governing the attachment and retraction of particles. To this end, we use a Photonic Force Microscope in which we combine DIC microscopy, fluorescence microscopy, optical tweezers and interferometric particle tracking. Filopodia retractions are induced by presenting optically trapped polystyrene beads to macrophage cells. The information gained from interferometric particle tracking is used to analyze the stiffness of the bond between cell and bead, the viscosity of the surroundings, the velocity of the retraction and the force-dependence of all these parameters. It can be shown that the strength of the attachment between cell and bead evolves dynamically during pulling. The experiments are complemented by fluorescence microscopy with live cells with labeled actin cytoskeleton. The characteristics of the movement of the bead are compared with the dynamics of the underlying actin retrograde flow. The results indicate that the bead retraction is mediated by a force-dependent coupling to the actin cytoskeleton.

BP 14.3 Wed 10:00 H10

Measuring cellular reactions upon particle approach on a broad bandwidth by photonic force microscopy — •Felix Jünger and Alexander Rohrbach — Department of Microsystems Engineering, Laboratory for Bio- and Nano-Photonics, University of Freiburg, Georges-Koehler-Allee 102, 79110 Freiburg, Germany

A particle diffusing in the vicinity of a living cell exerts broadband mechanical stimuli onto the cell membrane and influences the molecular processes of the cellular response. In this study, we demonstrate that molecular stimuli and the following cellular reorganization occur on different time scales.

We use photonic force microscopy (PFM) to approach optically trapped microbeads to the membrane of different biological cells and to track the bead's temporal fluctuations with nanometer precision and on a broad spectral bandwidth up to 2 MHz. The autocorrelation of the bead motion reveals the friction coefficient $\gamma(d)$, which changes significantly with the bead-membrane distance d. In addition, the frequency-resolved viscoelastic modulus $G(\omega,d)$ is obtained by analyzing the fluctuation data via the Kramers-Kronig relations.

Our results show that fluctuations of the incoming particle are slowed down and momentum transfer onto the cell is increased. Furthermore, we found evidence of a viscoelastic interaction of the bead with the cell coat, which is unique to HT29 cells and additionally regulates the momentum transfer to the cell surface and its mechanosensitive receptors.

BP 14.4 Wed 10:15 H10

Tuning Mechanics and Biochemical Recognition using Hyaluronic Acid Hydrogels — •Martin Schilling and Florian Rehfeldt — Third Institute of Physics - Biophysics, Georg-August-University, Göttingen, Germany

Many aspects of cell behavior are influenced by the mechanical properties of their microenvironment. To mimic the various elastic Young's moduli E of different in vivo environments of cells, it is necessary to design and mechanically characterize hydrogels for cell culture that are biocompatible and allow for a tunable elasticity.

Hyaluronic acid (HA), a polysaccharide consisting of disaccharide units, was chosen as base for the hydrogel system as it is biocompatible and not toxic for cells, thus allowing for 3D encapsulation.

Native HA hydrogels exhibit a visco-elasticity at the lower end of the physiologically relevant stiffness range. Here, we show that by chemical modification and subsequent covalent cross-linking, we can cover the required range from 0.1 kPa to 100 kPa. Additionally, altering the degree of modification of HA allows distinct and independent tuning of Young's modulus and biochemical recognition of HA by cells. Mixtures of both high and low modified HA are examined to combine both properties. The gelation kinetics of the resulting hydrogels are investigated by rheology using oscillatory shear tests both in the low and high strain (LAOS) regime. The resulting influence of mechanics and biochemistry of those HA substrates are investigated with hMSCs and RPM-MCs.

Invited Talk BP 14.5 Wed 10:30 H10

Physical determinants of phagocytic uptake and transport — •Holger Kress — Biological Physics Group, Department of Physics, University of Bayreuth, Germany

Phagocytosis is an essential part of the human immune system and an evolutionary highly conserved fundamental cellular process. Although a large number of molecules that are involved in phagocytosis are known already, a quantitative physical understanding of this intrinsically mechanical process is still lacking. Therefore we investigate physical determinants of phagocytic uptake and transport. We examine the cellular resolution limit for particle uptake by using holographic optical tweezers in combination with correlative light and electron microscopy to measure the ability of cells to discriminate between two spatially separated objects. These studies provide insights into the spreading of cell signaling during particle uptake. In addition, we investigate the influence of basic spatial factors for the transport of intracellular organelles and we show that not all phagosomes are transported directly from the cell periphery to the perinuclear region, but that they exhibit more complex transport characteristics which depend strongly on the size of the phagosomes. This transport behavior might be the foundation for a size-dependent cellular sorting mechanism for organelles. In addition, we quantify how the intracellular transport forces scale with the organelle size by using magnetic tweezers, which can provide cues for the number of motors involved in the transport of different-sized organelles.

15 minutes break.

BP 14.6 Wed 11:15 H10

Blood platelet formation - a biological Rayleigh-Plateau instability — •Christian Bächer and Stephan Gekle — Biofluid Simulation and Modeling, Bayreuth, Germany

Active stresses in the cell cortex, which can trigger changes in cell shape, are highly important for cell mechanics. Based on active gel theory and thin shell theory we incorporate active stresses in 3D simulations of elastic cell membranes in flows [1]. We combine the active force calculation with immersed boundary/lattice Boltzmann method to couple an active membrane to an external fluid.

Blood platelets are formed out of fragmenting protrusions of stem cells called megakaryocytes under presence of active stresses. Our simulations provide an explanation for this fragmentation: active stresses trigger a pearling instability of an elastic, biological cell membrane. This instability can be understood as a biological Rayleigh-Plateau instability with the active stress playing the same role as the surface tension of a liquid jet.

[1] C. Bächer, S.Gekle, J. Comput. Phys. (submitted), 2018

BP 14.7 Wed 11:30 H10

Numerical-experimental observation of shape bistability of red blood cells flowing in a microchannel — Achim Guckenberger[1], Alexander Kihm[2], Thomas John[2], Christian Wagner[2], and •Stephan Gekle[1] — [1]Biofluid Simulation and Modeling, Theoretische Physik VI, Universität Bayreuth — [2]Experimental Physics, Universität des Saarlandes

Red blood cells flowing through capillaries assume a wide variety of different shapes owing to their high deformability. In this work we construct the shape phase diagram of a single red blood cell with a physiological viscosity ratio flowing in a microchannel. We use both experimental in-vitro measurements as well as 3D numerical simulations to complement the respective other one. Two different major shapes are found, namely croissants and slippers. Notably, both shapes show coexistence at low (<1 mm/s) and high velocities (> 3mm/s) while in-between only croissants are stable. This pronounced bistability indicates that RBC shapes are not only determined by system parameters such as flow velocity or channel size, but also strongly depend on the initial conditions.

[1] Guckenberger et al. Soft Matter 14, 2032-2043 (2018)

BP 14.8 Wed 11:45 H10

Dynamic RT-DC: time-resolved mechanical single cell analysis at 100 cells / second — •Bob Fregin[1], Fabian Czerwinski[1], Konstanze Aurich[2], Doreen Biedenweg[2], Salvatore Girardo[3], Stefan Gross[4], and Oliver Otto[1] — [1]ZIK HIKE, Universität Greifswald, Greifswald, Germany — [2]Universitätsklinikum Greifswald, Greifswald, Germany — [3]Biotechnology Center, Technische Universität Dresden, Dresden, Germany — [4]DZHK, Universität Greifswald, Greifswald, Germany

Real-Time Deformability Cytometry (RT-DC) is a label-free technique for single cell mechanical analysis with high-throughput of up to 1,000 cells / second. Capturing the steady-state deformation at the end of a microfluidic channel RT-DC is currently limited to time-independent parameters like the elastic Young's modulus.

Here, we introduce an extension of RT-DC towards dynamic single cell measurements with the possibility to capture full viscoelastic properties of up to 100 cells / second. Cellular shape-changes along the entire length of the microfluidic channel are tracked in real-time and are subsequently analyzed by a Fourier decomposition. We demonstrate that this dynamic RT-DC allows for cell mechanical assays at the millisecond time scale fully independent of cell shape. We use this approach for the first comparison of peripheral blood cells based on their elastic Young's modulus as well as their viscosity.

BP 14.9 Wed 12:00 H10

The role of cell culture topology in cell mechanics: comparing 2D with 3D microenvironments — •Venkata AS Dabbiru[1], Muzaffar H Panhwar[1], Doreen Biedenweg[2], Fabian Czerwinski[1], Ricardo H Pires[1], and Oliver Otto[1] — [1]ZIK HIKE, University of Greifswald, Greifswald, Germany — [2]University Medicine Greifswald, Greifswald, Germany

Despite the widespread use of cell monolayers to culture cells in vitro, this 2D format does not recapitulate many of the physical cues present in the 3D environment that characterizes a tissue. In turn, these topological differences influence gene expression patterns and modulate the physiological behaviour of cells. However, the effect of topology on the mechanics of single cells has so far never been investigated systematically. Here, we apply real-time deformability cytometry (RT-DC) for the high-throughput mechanical phenotyping of single cells and cell spheroids. We compare HEK 293 cells obtained from planar monolayers (2D) and spheroidal (3D) formats and show that cells cultured in a 3D microenvironment have a larger Young's modulus when compared to those cultured in a 2D cell culture format. Furthermore, we show that cell confluency determines the average cell size but does not impact their mechanical properties.

BP 14.10 Wed 12:15 H10

High-throughput cell and tissue mechanics in virtual fluidic channels — •Muzaffar Hussain Panhwar[1], Venkata A.S. Dabbiru[1], Doreen Biedenweg[2], Ricardo H. Pires[1], Fabian Czerwinski[1], and Oliver Otto[1] — [1]ZIK HIKE, University of Greifswald, Greifswald, Germany — [2]University Medicine Greifswald, Greifswald, Germany

The biomechanical properties of cells and tissues are of utmost importance for a number of regulatory processes and complex functions, e.g., cell activation and migration. We use a novel development of real-time deformability cytometry (RT-DC) to access cell and spheroid mechanics of large samples. To accommodate the size range between cells and spheroids, we show that microfluidic geometries can be modified on-the-fly by polymer solutions creating virtual fluidic channels between two immiscible interfaces. After establishing virtual fluidic channels in the mesoscopic environment of a glass cuvette commonly used in flow cytometers, we explore their capability for high-throughput mechanical characterization. Performing RT-DC on an embryonic cell line cultured in 2D and comparing the results to several hundreds of spheroids as a 3D model system, a greater elastic modulus was found for single cells. These results might lead to a better understanding of tissue growth and could reveal insights into the mechanical dynamics of cell-cell interactions.

BP 14.11 Wed 12:30 H10

Stretching Adherent Cells with Light — •Tobias Neckernuss, Daniel Geiger, Jonas Pfeil, and Othmar Marti — Institute of Experimental Physics, Ulm University

In natural science and medicine mechanical properties of cells are important parameters. Over the years, countless techniques to assess all kinds of parameters have been developed for all kinds of cells. The most important ones are: stiffness, creep and relaxation constants. However, the investigation often relies on the interaction of a probe with the cells. Hence, the measured properties are always those of the joint system cell and probe. In 2001 Guck et al. demonstrated that it is possible to investigate the mechanical properties of suspended cells in a microfluidic channel with laser light. We present a setup to determine the mechanical properties of adherent cells in their physiological environment without having to alter their biochemistry or influence the measurement results by interaction of a probe with the cells. A setup for stretching and a method to detect the deformation of the cell have been developed together with the necessary data analysis algorithms. The deformation data are fitted to different kinds of viscoelastic models to describe the behavior of the cells with networks of springs and dashpods. The best models are selected by methods of information theory and the properties of 3T3 fibroblasts measured as cultured are compared to latrunculin treated ones.

BP 14.12 Wed 12:45 H10

A microfluidic method to sort capsules and cells according to their size and deformability — •Doriane Vesperini[1,2] and Anne Le Goff[1] — [1]Sorbonne universités, Université de technologie de Compiègne, CNRS, UMR 7 338 Biomécanique et Bioingénierie — [2]INM, Leibniz Institute for New Materials, Cytoskeletal fibers, (F. Lautenschläger Group)

Cell mechanical properties depend on their functions or pathologies such as cancer or infections. Sorting cells according to their stiffness is thus particularly interesting to help diagnosis. We propose a microfluidic device that consists of a half-cylinder obstacle located at the end of a rectangular straight channel. Upstream of the obstacle, a flow-focusing module centers cells on the obstacle. Downstream of the obstacle, a diffuser ends on 5 symmetrical outlets. Trajectories in the diffuser depend on several parameters, such as cell size, deformability and the capillary number Ca. The capillary number expresses the competition between elastic and viscous forces when the micro-object is squeezed onto the obstacle. Large and stiff micro-objects are more deflected than small and soft ones. We first demonstrate the efficiency of our device to sort micro-capsules according to their size at low flow strength and to their deformability at high flow strength. It is a passive, non-destructive, and sensitive system. We then downscale the microfluidic device in order to sort non-adherent cells.