1657-Pos Board B567
Time-Resolved Observation of Neutrophile Migration Through Three-Dimensional Matrices
Stefan Münster, Louise M. Jawerth, Amy C. Rowat, David A. Weitz, Ben Fabry.
To reach a site of inflammation, neutrophiles exit blood vessels and migrate through the extracellular matrix (ECM). For this process, the dense protein meshwork of the ECM presents a major sterical barrier that a migrating cell has to negotiate. Here, we demonstrate that time-resolved, fluorescent confocal microscopy can be utilized to visualize the migration of activated neutrophiles through in vitro reconstituted ECM matrices along with the 3-dimensional structure of the matrix itself. We seed DMSO differentiated HL-60 cells on top of collagen type I or fibrin matrices of varying concentrations and activate them with 100nM fMLP. We collect time-series of 3D confocal image stacks of the cell body (stained with CMFDA), the nucleus (labeled with DRAQ5) and the surrounding matrices (labeled with TAMRA-SE). We determine the exact location of individual pores, their local size and the diameter of the connections of neighboring pores in the ECM matrix. By following the paths of migrating cells, we can then evaluate for each matrix concentration which pore size cells predominantly populate and correlate cell speed with pore size. Furthermore, using values reported for the the micromechanics of the matrices along with the local deformations of the ECM fibers, we can estimate the forces exerted by the cells during their migration.

1658-Pos Board B568
The Origins of Strain Stiffening in Stiff Biopolymer Networks
Louise Jawerth, Stefan Muenster, David Weitz.
The extracellular matrix proteins fibrin and collagen form biopolymer networks which are major constituents of tissues, tendons and blood clots. Their mechanical properties are important for proper function in the body. Previous work using atomic force microscopy (AFM) has characterized the mechanical properties of individual collagen and fibrin fibers, but there is currently no model which can predict bulk material properties from its constituent properties. Here, we study fibrin and collagen networks undergoing shear on a confocal microscope and compare this to bulk rheological measurements. We track individual fiber branchpoints as function of strain. We characterize the non-affinity of the motion and show that the low strain, linear regime corresponds to highly non-affine motion while the high strain, linear regime corresponds to affine motion. We also characterize individual fiber strain as a function of overall system strain and show that linear elastic beams are sufficient to describe the overall strain stiffening response measured in rheology.

1659-Pos Board B569
Actin Dynamics and Membrane Topography During T Lymphocyte Spreading
King Lam Hui, Brian Grooman, Chen Lu Wang, Arpita Upadhyaya.
Spreading of cells over substrates involves large scale physical rearrangements of the actin cytoskeleton and cell membrane and is of widespread physiological significance. When a T lymphocyte encounters an antigen presenting cell (APC) which presents antigen that the T cell recognizes, it spreads onto the surface of the target cell. This spreading results in signaling events and the subsequent formation of the immunological synapse. While early signaling events are known to play an important role in transcriptional response of the cell, the biophysical determinants of cell spreading kinetics are not well understood. In this study, a glass substrate coated with anti-CD3 antibodies was used to initiate the spreading response of T cells. The contact area between the cell and the substrate was used as a parameter to study the role of actin, myosin II, antigen density, membrane tension, RhoA and Rac signaling in determining the kinetics of the initial spreading response. We found that the contact area growth in time can be well described by a common physico-chemical mechanism and that the rate of spreading is independent of antibody concentration, myosin II activity and Rho signaling. We imaged the dynamics of the actin cytoskeleton using TIRF microscopy. Under certain conditions, we observed dramatic fluctuations of the edge velocity and the formation of membrane waves driven by actin polymerization at the cell substrate interface. Membrane deformations induced by such wavelike organization of the cytoskeleton may be a general phenomenon that underlies cell movement and cell-substrate interactions. Finally, we studied cell spreading on elastic substrates to investigate the possible roles of substrate rigidity on the spreading behavior, and the magnitude of traction forces exerted by the cell.

1660-Pos Board B570
Spreading Dynamics and Oscillatory Membrane Behavior of B Lymphocytes
Christina Ketchum, Chaohong Liu, Wenxia Song, Arpita Upadhyaya.
Formation of cellular junctions involves cytoskeleton driven membrane deformations that lead to the spreading of one cell over the surface of another. Mammalian B lymphocytes present B cell receptors, which upon contact with the specific antigen, trigger an activated spreading response on an antigen presenting cell or an antigen coated surface. As signaling events are initiated within a minute of contact, the rapid increase in contact area and accumulation of receptors during early spreading are critical for the immune response. The mechanisms that determine the kinetics of B cell spreading and receptor aggregation are not well understood. We have studied the spreading dynamics of mouse A20 B cells on antibody coated substrates as a model for B cell activated spreading. The adhesion area of the cell on the substrate was imaged using interference reflection microscopy. Concurrently, total internal reflection florescence was used to image the GFP-labeled actin cytoskeletal dynamics during the spreading processes. In order to test the robustness of this spreading behavior and study the effects of the physical environment we also initiated B cell spreading on fibronectin coated surfaces, antibody coated lipid bilayers, and gels of varying stiffness. A significant number of spreading cells were observed to exhibit weakly adhered, large amplitude membrane protrusions and retractions which were dependent on myosin II activity. A variety of inhibition experiments were further performed in order to elucidate the role of microtubules and signaling downstream of BCR ligation in this phenomenon. This dynamic membrane behavior may be a general mechanism for the B cells to sense the surrounding environment.

1661-Pos Board B571
Cell Motility on Varying Substrate Stiffness
Joshua Hoffman.
Endothelial cell motility has been widely studied, but most research observes single cell motion or collective cell migration on a glass substrate. Although previous findings are informative, there are few studies that observe collective cell migration on soft, physiological substrates. Here, we studied the effects of substrate stiffness on cell migration within a monolayer. To obtain substrates with different mechanical properties, polyacrylamide gels of varying stiffness were made. These gels were coated with the extracellular matrix protein fibronectin, and the surface was plated with a high density of human umbilical vein endothelial cells (HUVEC) or bovine aortic endothelial cells (BAEC). After the cells formed a complete monolayer, they were stained with Hoechst nuclear stain to visualize individual cell movement within the monolayer. We observed that the collective cell movement is slower as the substrate stiffness increases. Also, we noticed an interesting swirling motion within the monolayer, the dynamics of which were substrate-dependent. These results suggest that collective cell migration during tissue morphogenesis depends on the mechanical properties of the cellular environment, which often vary in diseased conditions.

1662-Pos Board B572
Swimming Motility of Monotrichous *Pseuomonas Aeruginosa*
Chen Qian, Chui Ching Wong, Sanjay Swarup, Keng-Hwee Chiam.
Flagella provide bacteria with the ability to move in the aquatic environment. For peritrichous species such as *E. coli* that possess flagella over their entire surface, a ''run and tumble'' model has been established that accounts for their complex motion. However, this mechanism cannot be applied to bacteria with single-flagellum (monotrichous). Furthermore, single degree of freedom of movement from one flagellum likely will restrict the ability of bacteria to change directions. This contradicts our experimental observation that *P. aeruginosa* frequently exhibit circular trajectories. While there have been some work on the near-surface hydrodynamics of monotrichous bacteria leading to circular trajectories, we have also observed such circular trajectories in bacteria swimming far away from the surface. To explain this phenomenon, we propose and test several hypotheses theoretically

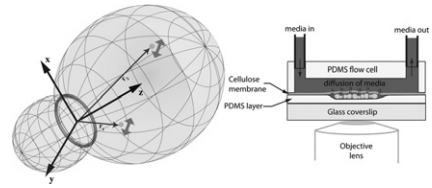
and experimentally. We show how the flexible body and the flexible hook connecting the helical flagellum and body may act together to generate the circular trajectories, and use modeling to show the range of curvatures allowed and compare this range to experiments. Finally, we discuss the implications of such circular trajectories in allowing monotrichous bacteria to undergo chemotaxis and interact with one another to aggregate.

1663-Pos Board B573
Effect of Nuclear Stiffness on Cell Translation In Vivo
Takamasa Harada, Dennis Discher.
Cell nucleus is the largest organelle and its stiffness is known to play an important role for cell migration in tissue. In this study, the effect of nuclear plasticity on cell translation is explored by controlling the cell stiffness by knocking down Lamin A/C, which is a major component of nuclear lamina, using RNA interference. After Lamin A/C is successfully down-regulated in A549 lung carcinoma cells, which are genetically modified to express far red fluorescent protein tdTomato, those cells are injected in NOD/SCID mice to investigate tumor progression and spreading of the cells in vivo by observation with Xenogen imaging system. Observation of tumor cell spreading in harvested tissues utilizing fluorescent microscopy is also done. This study qualitatively and quantitatively shows efficient spreading and proliferation of Lamin A/C down-regulated cells in comparison with control cells.

1664-Pos Board B574
Filament Depolymerization can Explain Chromosome Pulling During Bacterial Mitosis
Edward J. Banigan, Michael A. Gelbart, Zemer Gitai, Andrea J. Liu, Ned S. Wingreen.
Reliable chromosome segregation is crucial to all dividing cells, but the force-generating mechanisms underlying chromosome translocation in bacteria remain mysterious. Caulobacter crescentus utilizes a depolymerization-driven process in which a ParA protein structure grows from the new cell pole and binds to a ParB-decorated chromosome, and then retracts via disassembly, thus pulling the chromosome across the cell. This poses the question of how a depolymerizing structure can continuously and robustly exert forces as it is disassembled by the chromosome that it pulls. ParA binds to ParB, a protein that binds to the chromosome near the origin of replication (ori). ParB then disassembles ParA, and the ParB-decorated ori translocates across the cell as ParA retracts. In order to address the question of how depolymerization-driven motility can be sustained in steady-state and be robust to perturbations, we perform Brownian dynamics simulations of this system. We find that the interaction between ParB and ParA generates a steady-state ParA concentration gradient so that the concentration of ParA is higher in front of the chromosome than behind it. This suggests that the mechanism of translocation is "self-diffusiophoretic"; since the chromosome is attracted to ParA via ParB, it moves up the ParA concentration gradient, and thus across the cell. In addition, we find that the velocity of translocation is controlled by the product of a characteristic time scale for the chromosome to relax and the rate of disassembly of ParA. As long as this product is small compared to one, chromosome segregation is robust and proceeds at the speed of ParA disassembly. Our results provide a physical explanation of the mechanism of depolymerization-driven translocation in bacteria as well as predictions that can be tested by future experiments.

1665-Pos Board B575
Role of External Constraints on Single Cell Spreading on Microfabricated Substrates
Benoit Vianay, Jean-Jacques Meister.
Single cell spreading includes cell adhesion on extracellular matrix, mechanotransduction of external signal and cytoskeleton rearrangement. Involved in several crucial cellular processes like migration, differentiation or division, cell spreading is not a simple succession of events. Indeed, cellular elements involved during cell adhesion are also sensors that activate or inhibit signaling pathways. The actin cytoskeleton and adhesive site formations are continuously the results of protein assemblies and disassemblies influenced by signaling cross-talks. Integrins, the first signaling actor during adhesion, lead to the reorganization of the cell cytoskeleton and also the extra cellular matrix through actomyosin complexes which exert forces by contraction. The understanding of the whole spreading process remains difficult due to the complexity of the cellular response.
Micropatterning techniques are efficiently used to control and constraint cells spreading to specify and analyze their behaviors. The reproducibility of cytoskeleton and adhesive site organizations on micropatterned substrates reduces biological dispersion and allows to obtain statistical spatiotemporal measurements. Investigations on relevant cell adhesion actors through experimental measurements and modeling should point out links among them. The outcome of this work is to study relationships between cell spreading behaviors and extracellular matrix properties by monitoring precisely cytoskeleton and adhesive sites organizations.
We choose to simplify the cell environment by reducing the extracellular matrix to microfabricated substrates covered by a specified type of adhesive proteins. We thus apply two different external constraints: the geometry controlled by the adhesive pattern and the extracellular matrix biochemistry controlled by the type of protein functionalized on the substrate. Cell adhesion behaviors are therefore statistically quantified to study cellular mechanotransduction dependencies on controllable environmental conditions.

1666-Pos Board B576
Nonequilibrium Mechanics of the Mitotic Spindle Measured in Living Cells
Maria Kilfoil.
To carry out its life cycle and produce viable progeny through cell division, a cell must successfully coordinate and execute a number of complex nonequilibrium processes with high fidelity, in an environment dominated by thermal noise. One important example of such a process is the assembly of and maintenance of tension across the mitotic spindle, a nonequilibrium composite material including polymers and motor proteins that is responsible for organizing and separating the genetic material during cell division. The intrinsic microtubule dynamics and different motor proteins provide the forcing required for this dynamic process. We use high-resolution fluorescence confocal microscopy to observe and analyze the real space dynamics of the mitotic spindle in budding yeast and Drosophila melanogaster S2 cells. Centrosome trajectories are reconstructed from the three-dimensional fluorescence data and quantified in a coordinate system relevant to the cell division using specially-developed image analysis methods. The roles of specific motor proteins are isolated by altering their functionality through genetic and chemical means using a microfluidic device. We use the fluctuations in pre-anaphase centrosome positions to show how nonequilibrium motor activity controls the mechanical properties of an in vivo cytoskeletal network.



1667-Pos Board B577
Cell Edge Dynamics During Polarization
Mark E. Ambühl, Charles Brepsant, Jean-Jacques Meister, Ivo F. Sbalzarini, Alexander Verkhovsky.
Upon contact with a flat substrate, most eukaryotic cells first attach and spread radially, then break symmetry and start directed migration, a phenomenon known as polarization. We analyzed the dynamics of the cell edge during polarization using the model system of fish epidermal keratocytes, which exhibit consistent polarization and a simple, constant shape during migration. We have developed an automatic method based on a level set formalism to perform accurate cell edge segmentation from phase-contrast images and to obtain protrusion/retraction maps with high accuracy and resolution in space and time. Analysis of the resulting protrusion/retraction maps demonstrated that polarization was often preceded by a transient oscillatory state in which relatively large protruding regions were separated by retracting regions that traveled around the cell periphery as rotating ''blades'' or waves. Depending on cell size, three to five of these ''blades'' were observed per cell during the oscillatory phase. Convergence of the ''blades'' eventually led to a polarized state with only one blade (protrusion and retraction were hence segregated to opposite sides of the cell). We have extracted the parameters of the oscillations, such as wavelength, period, and propagation velocity, and characterized the dynamics of cell area, as well as perimeter and edge velocity during polarization. In order to investigate how the protrusion/retraction switch