**1013-Plat**
**Stem Cell Biophysics: Pre-differentiation Dynamics of Stress Fiber Polarization on Elastic Matrices**
**Andre E.X. Brown**[1], Florian Rehfeldt[1,2], Allison L. Zajac[1], Dennis E. Discher[1].
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]University of Göttingen, Göttingen, Germany.
Human mesenchymal stem cells (hMSCs) are mechanosensitive and specify their lineage based on the stiffness of their environment. That is, a purely mechanical cue is sufficient to cause cells on gels with an intermediate stiffness (Young's modulus E = 11 kPa) to adopt a spindle-like morphology characteristic of muscle cells and to up-regulate muscle markers such as MyoD. Inhibition of myosin II by blebistatin blocks this lineage specification. We analyzed the shape changes that occur at early times in cells cultured on soft to stiff (1, 11, and 34 kPa) gels using an automated image analysis algorithm to correlate morphological changes with stress fiber formation and orientation. While the total production of stress fibers increases monotonically with substrate stiffness similar to the trend in projected cell area, the orientation shows a maximum at intermediate stiffness, similar to the polarization. These early time changes are not due to changes in gene expression, which occur only after several days, but must instead have a more rapid biophysical basis. Both the myosin inhibition and correlation between stress fiber orientation and cell morphology suggest a critical role for these contractile structures in mechanosensing. To help dissect their function we used a multi-color hybrid fluorescence and atomic force microscope to locate specific stress fiber proteins such as actin and myosin and to correlate their presence with specific features in the high resolution AFM images. AFM also offers the possibility of determining the mechanics of the stress fibers themselves, and thus of integrating their properties into a more complete picture of the cell's mechanics and ultimately mechanosensitivity.

**1014-Plat**
**Contractile Force Generation Enhanced Tumor Cell Invasion, But Decreased Tumor Growth**
**Claudia T. Mierke**[1], Benjamin Frey[2], Martina Fellner[1], Martin Herrmann[3].
[1]Universität Erlangen, Erlangen, Germany, [2]Department of Internal Medicine III, Institute of Clinical Immunology and Rheumtology, University Hospital Erlangen, Erlangen, Germany, [3]Department of Internal Medicine III, Institute of Clinical Immunology and Rheumatology, University Hospital Erlangen, Erlangen, Germany.
The process of metastasis formation that includes cell invasion has been shown to cause malignant progression of tumors. The impact of cell mechanical properties on the malignancy of tumor cells has not been investigated systematically. Highly-invasive tumor cells expressed significantly higher amounts of the α5β1 integrin compared to weakly-invasive tumor cells. We hypothesized that the ability to generate contractile forces is a prerequisite for cell invasion and that α5β1-expression increased cell invasion through enhanced generation of these contractile forces. We analyzed whether α5β1-high or α5β1-low expressing breast carcinoma cells differ in their ability to invade into a 3-D collagen fiber matrix. Our results show that higher α5β1 expression increased cell invasiveness. We found that the increased 3-D motility of α5β1-high expressing cells depends on the integrin α5β1 activating traction generation. The increased invasiveness was inhibited by addition of myosin light chain kinase inhibitor ML-7 or by addition of the ROCK kinase inhibitor Y27632. Furthermore, we analyzed whether α5β1-high and α5β1-low cells formed tumors in mice. The tumor formation and growth is impaired in α5β1-high compared to α5β1-low cells. The integrin α5β1 acts as an enhancer of cell invasiveness where contractile forces are necessary to overcome the viscous drag, but as a suppressor of primary tumor formation and growth where increased motility is rather a hindrance for cell clustering to form tumors.

**1015-Plat**
**Pushing Off The Walls: A Mechanism Of Cell Motility In Confinement**
**Rhoda J. Hawkins**[1], Matthieu Piel[2], Ana-Maria Lennon-Dumenil[2], Jean-François Joanny[2], Jacques Prost[3], Raphaël Voituriez[1].
[1]Universite Pierre et Marie Curie, Paris, France, [2]Institut Curie, Paris, France, [3]Institut Curie/E.S.P.C.I, Paris, France.
Cell migration is involved in crucial processes in biology and physiopathology such as immune response and carcinogenesis. In these cases cells migrate through tissues composed of densely packed cells and/or extracellular matrix with a small mesh size. Despite extended studies of cell motility on 2D substrates it is not clear whether these results can be generalised to migration in confined environments such as tissues. We propose a novel, simple mechanism of cell motility, which relies mainly on the coupling of actin polymerization at the cell membrane to geometric confinement. We consider a polymerizing viscoelastic cytoskeletal gel confined in a narrow channel, and show analytically that spontaneous motion occurs. Interestingly, this mechanism does not require specific adhesion with the channel walls, and yields velocities potentially larger than the polymerization velocity of the gel. The contractile activity of myosin motors is not necessary to trigger motility in this mechanism, but is shown quantitatively to increase the velocity of motion. Our model qualitatively accounts for recent experiments that show that cells without specific adhesion proteins are motile only in confined environments while they are unable to move on a flat surface, and could help in understanding the mechanisms of cell migration in more complex confined geometries such as living tissues.

## Platform N: Membrane Dynamics & Bilayer Probes

**1016-Plat**
**High-Resolution Far-Field Fluorescence STED Microscopy Reveals Nanoscale Details of Molecular Membrane Dynamics**
**Christian Eggeling**, Christian Ringemann, Rebecca Medda, Birka Hein, Stefan W. Hell.
Max Planck Institute for Biophysical Chemistry, Goettingen, Germany.
Prominent problems in biology cannot be solved due to the limited resolution of conventional optical microscopy. For example, a whole range of membrane-associated processes are considered to be mediated through cholesterol-assisted interactions such as the formation of lipid nanodomains or 'rafts'. The direct and non-invasive observation of lipid or membrane protein dynamics in living cells, which are believed to occur on small spatial scales, is impeded by the resolution limit of >200nm of a conventional far-field fluorescence microscope or by the limited time resolution of single-particle tracking. We combine single-molecule based techniques such as fluorescence correlation spectroscopy (FCS) with stimulated emission depletion (STED) far-field microscopy to accesses a superior spatial and temporal resolution for observing the diffusion characteristics of molecules in the plasma membrane of living cells. Tuning the detection area between 250 nm and 30 nm in diameter, we directly reveal marked differences between different lipid or molecular classes. For example, sphingolipids or 'raft'-associated proteins are transiently trapped on the nanoscale in cholesterol-mediated molecular complexes. The presented direct detection of molecular dynamics in nanoscale areas of tunable size constitutes a powerful approach to study the dynamics of biomolecules in living cells.

**1017-Plat**
**3D Tracking of Antibody-Receptor Dynamics on RBL Cells.**
**Nathan P. Wells**, Guillaume A. Lessard, Peter M. Goodwin, James H. Werner.
Los Alamos National Lab, Los Alamos, NM, USA.
We have previously demonstrated the ability to track single quantum dots freely diffusing in three dimensions using a custom confocal microscope. The microscope is capable of both following the trajectory of a single (fluorescent) molecule and recording the time resolved (fluorescent) photon stream. With both positional and lifetime information, the microscope can provide fluorescent lifetime trajectories (FLT) analogous to fluorescent lifetime imaging (FLIM). Using this microscope we have recently begun to study the signal cascade process in the IgE-FcεRI antibody-receptor system in live RBL cells. We have demonstrated the ability to track fluorescently labeled IgE docked to the FcεRI receptors and observe 3D motion on the membrane. We are currently working to observe and track the endocytosis of the antibody receptor complex on a single molecule basis.

**1018-Plat**
**Physiological Membrane Tension Causes An Increase In Lipid Diffusion: A Single Molecule Fluorescence Study**
**Hari S. Muddana**, Ramachandra Rao Gullapalli, Tristan Tabouillot, Peter J. Butler.
Penn State University, State College, PA, USA.
The quantitative relationship between lipid bilayer tension and lipid dynamics is currently unknown. We used time-correlated single photon counting (TCSPC) and fluorescence correlation spectroscopy (FCS) to determine diffusion of DiI, a lipoid dye, in micropipette-aspirated model membranes. Fluid-phase giant unilamellar vesicles (GUVs) were prepared from DOPC lipid using electroformation and stained with nanomolar concentrations of DiIC12. When GUVs were stressed from 0.02 to 0.1 mN/m, the diffusion coefficient increased monotonically from $9 \times 10^{-8}$ to $13 \times 10^{-8}$ cm2/sec. Thus, for the first time, we show that physiological tensions on the order of those experienced when cells