intercellular lipid raft heterogeneity we used fluorescence polarization microscopy, displaying local enrichment of GPI-anchored proteins as a complementary approach. Furthermore, we utilized the environment-sensitive fluorescence lifetime of NBD-PC to further study membrane order on a single-cell level. We surmise, that the cell-to-cell variability detected is representative of a context-dependence of cellular plasma membrane organization. Cell synchronization and cell cycle analysis were conducted to elucidate a potential correlation of plasma membrane condition and the position of the cells in the cell cycle.
Considering the high spatial and temporal dynamics of plasma membrane lipid rafts and the sophisticated methods that are necessary to study them it is conceivable, that the additional complexity of the membrane organization described here has not been detected in previous investigations. Nevertheless, it might be representative of cellular mechanisms that enables adoption to environmental demands or proliferative progression.

984-Plat
Protein Micropatterning in Live Cells: A Tool for Creating Membrane Domains with Raft-Like Properties
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Microstructured surfaces provide a unique and versatile platform to study cellular processes associated with the plasma membrane. In a recent study we presented a micropatterning assay to monitor protein-protein interactions in the live cell plasma membrane and used it to characterize the interaction between CD4, a major co-receptor in T cell signalling, and Lck, a protein tyrosine kinase essential for early T cell signaling (Schwarzenbacher, et al., Nat Methods, 2008). For this assay, cells are plated onto microstructured surfaces partly covered with ligands (antibodies) targeted against membrane proteins (bait), and the co-localization with a fluorescently labeled protein of interest (prey) is monitored.
Here, we employ this technique to probe indirect protein-protein interactions, namely of the lipid raft-associated GPI(glycosylphosphatidylinositol)-anchored protein CD59 and a GPI-anchored GFP. Interestingly, antibody-mediated micropatterning of CD59 leads to a colocalization of GPI-GFP (and vice versa). The mechanism of this can be envisioned as follows: By patterning of one GPI-anchored protein, a certain membrane microenvironment is created and the other GPI-anchored protein preferentially localizes into this environment. We employ different compounds that have been reported to act as 'raft' or 'non-raft' markers to further characterize the nature of the generated membrane patterns.

985-Plat
Selective Detection of Phospholipids in Model Cell Membranes by AFM Stylus Modified with Probe Protein
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We detected sphingomyelin (SM), a ubiquitous kind of phospholipids, contained in model cell membranes, using AFM tips linked with lysenin, a probe protein that specifically binds SM [1]. The model membranes, containing phosphatidylcholines (DOPC or DPPC) and cholesterol as well as palmitoyl sphingomielin (PSM), were deposited on mica as double layers in an aqueous buffer solution. Smooth bilayers of pure PSM and PSM+cholesterol were discerned with tips without lysenin. The PSM+DOPC and PSM+DOPC+cholesterol areally were separated into two phases in the sub-micron scale. By lysenin-conjugated tips, on nearly 90% of the force-curve cycles, no attractive force was observed. In the rest 10% cases, we observed fairly constant adhesion forces ~ 170 pN for the bilayers containing PSM. This force is less than one tenth of the force to remove a phospholipid molecule out of bilayer [2], and therefore recognized as the lysenin single-molecular bonding force with PSM. Lysenin tips were active for averagely 1000 approaches to PSM containing membranes. Some tips were never active. We plotted the distribution of adhesive force over the two phases seen on the PSM+DOPC(+cholesterol) bilayers. In one of two phases, the adhesive forces ~ 170 pN were observed, and in the other phase, adhesive forces were not measured. The phase condensation of PSM was apparently demonstrated by the distribution of force curves. The bilayer lipid phase containing PSM and cholesterol corresponds to the ''raft'' structure on the real cell membranes, anticipated to be instrumental in cellular signaling as well as virus infection. Our present technique will be applicable for direct observation of the raft on real live cell membranes.
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[2] R. M. Sullan et al., *Langmuir***25** (2009) 7471.

986-Plat
Competition between Line Tension and Curvature Stabilizes Modulated Phase Patterns on the Surface of Giant Unilamellar Vesicles. a Simulation Study
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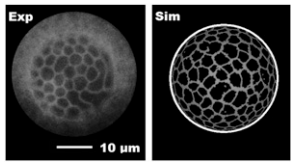
When prepared in the liquid-liquid coexistence region, the four component lipid system distearoyl-phosphatidylcholine (DSPC)/dioleoyl-phosphatidylcholine (DOPC)/palmitoyl,oleoyl-phosphatidylcholine (POPC)/Cholesterol with certain ratios of DOPC and POPC shows striking modulated phase patterns on the surface of giant unilamellar vesicles (GUVs). In this simulation study we show that the morphology of these patterns can be explained by the competition of line tension (which tends to favor large round domains) and curvature, as specified by the Helfrich energy functional. In this study we use a Monte-Carlo simulation on the surface of a GUV to determine the equilibrium shape and phase morphology. We find that the patterns arising from these competing interactions very closely approximate those observed, the patterned morphologies represent thermodynamically stable configurations, and that the geometric nature of these patterns is closely tied to the relative and absolute values of the model parameters.



987-Plat
Nanoscale Electrostatic Domains Induced by Cholesterol in Model Lipid Membranes Relate to Amyloid Binding and Toxicity
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Kelvin Probe Force Microscopy (KPFM) is a type of scanning probe microscopy which specifically addresses electrostatic properties of materials and has a great potential to bring new discoveries in biomedical research, but currently has limited biological applications.
In this work, we report one of a very few applications of Kelvin probe force microscopy (KPFM) to study complex structures of lipid films and lipid-protein interactions. Molecular arrangement of lipids and proteins gives rise to complex film morphology as well as distinct electrical surface potentials, which may rule many biological processes and diseases. Using Frequency Modulated - KPFM (FM-KPFM) we discovered an intriguing nanoscale electrostatic effect of cholesterol that may be crucial for understanding the mechanism of amyloid toxicity in relation to Alzheimer's disease (1). Earlier we observed similar electrostatic domains induced by cholesterol in pulmonary surfactant (2,3). Here we show that this electrostatic effect of cholesterol is not specific to only pulmonary surfactant films, but is also present in model lipid systems and plays an important role in amyloid-lipid interactions. We postulate that this previously unknown nanoscale electrostatic effect of cholesterol is a fundamental property, which may greatly influence the interactions of lipid membranes with other charged molecules.
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3.B.Moores, F.Hane, L.M.Eng, Z.Leonenko, Kelvin probe force microscopy in application to biomolecular films: Frequency modulation, amplitude modulation, and lift mode, Ultramicroscopy, 2010, 110(6), 708-711.

988-Plat
Unexpected Effects of Cholesterol on Membrane Permeability
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Understanding and predicting the permeability of lipid membranes, based on the membrane composition and the properties of the permeating solute, is one of the central goals in membrane biology. One important component of lipid membranes is cholesterol, that is abundant in animal cells in concentrations ranging from 20 to 50 mol%. Here we apply systematic molecular dynamics simulations to study the partitioning of solutes between water and cholesterol-containing membranes. We derive potentials of mean force for six different solutes permeating across 25 different lipid membranes containing

five different types of phospholipid plus a cholesterol content varying from 0 to 50 mol%.
Surprisingly, cholesterol decreases solute partitioning into the lipid tail region of the membranes much more strongly than expected from experiments on macroscopic membranes, suggesting that a laterally inhomogeneous cholesterol concentration and permeability may be required to explain experimental findings. The simulations indicate that the cost of breaking van der Waals interactions between the lipid tails, rather than the area per lipid, account for the reduced partitioning in cholesterol-containing membranes.
In addition, we find that the lipid head groups constitute the main barrier against permeation of bulky apolar solutes, and that cholesterol is able to reduce this barrier and thereby increase the permeability. We present new experimental data, confirming these computational predictions.
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Platform: Biosensing and Imaging

989-Plat
Seeing the Unseen in Cell Machinery Single Living Cells by Label-Free Spectroscopic Imaging
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Biomolecules such as DNA, carbohydrates, lipids, metabolites and drugs play essential roles in the biochemistry of cells. However, monitoring the spatiotemporal dynamics of these molecules represents a formidable challenge. Label-free microscopy using spectroscopic signals as contrast opens a new window for watching biomolecules and nanomaterials inside living cells and human body. I will present our most recent advances in both development and applications of label-free imaging platforms, including deep tissue imaging by listening to harmonic molecular vibration, study of DNA replication in living cells by coherent Raman microscopy, and transient absorption imaging of nanomaterials.

990-Plat
Wide-Field Magnetic Imaging using Nitrogen-Vacancy Color Centers in Diamond
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We present recent work on developing a wide-field magnetic imaging system using the nitrogen-vacancy (NV) color center in diamond. The NV centers can function as a robust and bio-compatible magnetic sensor at room temperature. Optical detection of the spin state of NV centers allows magnetic field imaging with sub-micron spatial resolution. We use this imaging system to study biomagnetism. Techniques to further improve the magnetic field sensitivity and the spatial resolution are also discussed.

991-Plat
Listening to Cells: A Non-Contact Optoacoustic Nanoprobe
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Cells have a complex composition that yields an intricate rheological behavior, appealing for measurements over a wide frequency range. However the existing techniques cannot exceed the kHz range, and rely for most on injected or contacting functionalized microprobes. Here, we report on an innovative non-contact optoacoustic probing of the mechanical properties of single cells at GHz acoustic frequencies under physiological conditions.
We culture cells on a biocompatible Ti6Al4V metal alloy. Low-energy femtosecond laser pulses are focused at the cell-Ti6Al4V interface to a sub-µm spot. The ensuing ultrafast thermal dilatation of the metal launches a high-frequency sound pulse in the cell. Acoustic propagation is measured remotely with an ultrafast laser probe through Brillouin light scattering. This all-optical non-invasive technique offers a broad frequency range, extending up to 1 THz, a sub-µm lateral resolution and a nanometer in-depth resolution.
For illustration, we here concentrate on the cell nucleus. Using the laser-generated GHz acoustic waves, we probe the stiffness and viscosity of the nuclei of various cell types. We demonstrate for the first time that, at GHz frequencies, the nucleus stiffness tends to a unique value, much larger than that observed at kHz frequencies. We demonstrate that this universal stiffness reflects the compressional dynamics of the nucleus components.
We also show that the evolution of the mechanical properties of the nucleus during cell differentiation is correlated with a specific gene expression pattern. In the frame of soft glass rheology, we project the GHz mechanical properties of the differentiated nuclei to the kHz range. Comparison with the kHz data obtained by alternative techniques suggests the existence of a new absorption process appearing at GHz frequencies.
This innovative technique defines a new class of experiments to enlighten cell mechanics in physiological conditions at a subcell scale.

992-Plat
High-Resolution Fluorescence Measurements Correlated to Cellular Traction Forces
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Many cellular mechanosensory functions rely on molecular interactions related to cellular force exertion. Despite the rich biochemical knowledge in this field, the current mechanistic picture - that can be enhanced by high-resolution optical microscopy techniques - is still in its infancy. Here, we describe a simple yet universal approach to combine high-resolution optical microscopy techniques with traction force measurements. Etching 50 µm high spacers next to a micropillar array and inverting the array onto a thin coverslip before imaging enables the use of a short working distance objective. In our setup we use a spinning-disk confocal on an inverted microscope with a 100X, NA 1.4 objective. Our deflection detection resolution per micropillar is below 30 nm, corresponding to a force resolution of 500 pN. We validate our technique by imaging fixed and live mouse fibroblasts with dye-conjugated fibronectin micro-contact printed on the micropillars and immunostaining for paxillin and actin. We observe forces of up to 15 nN on individual pillars using a precise force-deflection relationship calibrated using scanning electron microscopy, tensile testing and finite element analysis. We quantified focal adhesion growth with increasing force of 4 ± 1 nN/µm^2 and co-orientation of focal adhesion elongation and force direction within 2 ± 24 degrees. Furthermore, the cells remain viable during overnight live-cell imaging and stay adhered to the substrate. Our novel approach opens up the combined application of traction force measurements and super-resolution optical microscopy techniques.

993-Plat
Lipid Hop Diffusion on the Plasma Membrane - a STED-FCS Investigation
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Currently two of the most important - as well as controversial - proposed membrane-organizing principles are the ''lipid rafts'' hypothesis, proposed by K. Simons, and the ''picket-fence'' model, proposed by A. Kusumi. To date, both hypotheses encounter obstacles for full acceptance due to technical limitations on the available superresolution and single molecule techniques and limitations on the correspondent probes utilized by such techniques applied to living cells.
Trying to elucidate the lipid hop diffusion dilemma, which would be the underlying principle for the ''picket-fence'' model, we planned a series of STED-FCS experiments to probe the diffusion of phospholipids on the membrane of different cell types. The superresolution of STED microscopy allows FCS experiments to probe areas comparable in size to the compartments created by the actin cytoskeleton, arguably one of the major structures responsible for lipid and protein segregation in the cell membrane.
Using STED-FCS, we were able to detect phospholipid hop diffusion in two of the cell lines studied (NRK and IA32) and free diffusion was observed for the other cell lines under consideration (Ptk2, Vero, Hela). Different treatments to deplete the actin cytoskeleton on IA32 and NRK cells resulted in the extinction or hindrance of phospholipid hop diffusion. The same result was observed in