living cells at an unparalleled spatial and temporal resolution (50-100 nm, 4-8 min for a 30 μm scan). Furthermore, we were also able to map the elasticity of a reconstituted actin network, which has not been achieved before. In combination with optical techniques this opens up a unique simultaneous view of the mechanics of the living cell and the mechanical properties of its relevant molecular components.
Elasticity maps of a live HUVEC cell (30 μm scan)

1906-Pos
What is Measured By Passive Microbead Rheology?
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It is often claimed that the dynamic modulus G* of a viscoelastic medium can be measured by following the trajectory of a small bead subject to Brownian motion. In the pioneering manuscript that introduced the idea [T. Mason and D. Weitz, Physical Review Letters 74, 1250 (1995)], this equivalence between the autocorrelation function and G* was assumed. Later work claimed that a correspondence could be proven, but to our knowledge, the proof has never been shown. We use here an analytic solution of the forces on a sphere undergoing arbitrary displacement in an arbitrary viscoelastic medium combined with the fluctuation-dissipation theorem to derive what is actually measured in the microbead rheology experiment. We find that a convolution of G* is indeed measured in the followed autocorrelation function. Under certain restrictions the autocorrelation function is a direct measurement of the dynamic modulus as is typically used. We examine experimental data published in the literature and are unable to find any data where the restrictions do not hold. Nonetheless, the results suggest that the technique could also be used at higher frequencies, if proper analysis is made of the data.

1907-Pos
Intracellular Diffusion in Fission Yeast Cells Depends on Cell Cycle Stage
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During the cell cycle, rearrangements of the cytoskeletal network play an essential role, in particular for the success of cell division. In order to quantify the influence of cytoskeletal rearrangements on the viscoelastic properties of the intracellular space, we studied the diffusion of endogenous lipid granules within single fission yeast cells in the different stages of the cell cycle. The position of the granules was tracked with optical tweezers at nanometer and sub-millisecond resolution and the data were analyzed with a power spectral analysis. We found that the majority of the lipid granules underwent subdiffusive motion during all stages of the cell cycle, i.e. the mean squared displacement of the granule is $2Dt^{\alpha}$ with $\alpha<1$. With our experiments we have shown that α is significantly smaller during interphase than during any stage of mitotic cell division and, surprisingly, we did not find significant differences of α in the different stages of cell division. These results indicate that the cytoplasm is more elastic during interphase than during cell division and that its elasticity is relatively constant during the stages of cell division.

1908-Pos
A Comparison of Single-Cell Elasticity of Osteogenic Cells Measured with the Optical Stretcher and Holographic Microscope
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While it has been known for some time that bone mass is remodeled in response to mechanical stress, the identity of the primary mechanosensor has yet to be clearly established. To determine if cellular elasticity may play a role in the cell's ability to detect a pressure variation, we have used the optical stretcher to measure the elasticity of individual osteogenic cells. To determine cell elasticity from measurements of cellular deformation, the optical pressure on the cell surface is computed using a ray optics model which assumes a value for the index of refraction of the cell. Previously we have estimated this value from measurements of other eukaryotic cells, but the optical pressure varies significantly with small changes in refractive index. Therefore, digital holographic microscopy is used to improve estimates of this critical parameter. We consider the overall impact that a spatial variation in the index of refraction can have on the determination of the optical stress, and compare single-cell elasticity measurements of red blood cells, 2T3 osteoblast and MLO-Y4 osteocyte cells.
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Microtubule Motors-Kinesin-1

1909-Pos
An Atomic-Level Engine that Accounts for Kinesin Motility and Catalysis
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Kinesin motor proteins convert the energy of ATP hydrolysis into stepping movement along microtubules. In this process, the microtubule can be considered as kinesin's regulatory partner, responsible for activating the enzyme's functional behavior. In the absence of atomic resolution structures describing the kinesin-microtubule complex, the mechanism of this activation has remained unknown. We use cryo-electron microscopy to derive atomic models describing the complete, microtubule-attached, kinetic cycle of a kinesin motor. The resolution of our reconstructions (~8Å) enabled us to unambiguously build crystallographically-determined conformations of kinesin's key subcomponents into the density maps. The resulting models reveal novel arrangements of kinesin's nucleotide-sensing switch loops and of its microtubule binding element known as the switch II helix. Based on these models, we present a detailed molecular mechanism accounting for kinesin's force generation cycle. In this mechanism, the switch loops control a seesaw-like movement of the catalytic domain relative to the switch II helix, which remains fixed on the microtubule surface. Microtubules couple the seesaw movement to ATP binding by stabilizing the formation of extra coils at the N terminus of the switch II helix, which interact directly with the switch loops. Tilting of the seesaw to assume the ATP-bound orientation in turn elicits a power stroke by the motor domain's force-delivering element known as the neck linker. This sequence of events accounts for the essential mechanics of kinesin's force-delivery cycle, and also yields a new model for the catalytically active conformation of kinesin's ancestral relative, myosin.

1910-Pos
Free Energy Changes During Kinesin's Force-Generating Substep
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We have previously suggested that Kinesin-1 generates force by transient folding between the N-terminal cover strand and the C-terminal neck linker domains into a beta-sheet, the so-called cover-neck bundle (CNB). Once formed, the CNB has a conformational bias sufficient to move the neck linker forward. Replica exchange molecular dynamics simulations have been performed to elucidate the energetics of CNB formation with and without load. Without load, the CNB state is weakly favorable compared to non-CNB states by 0.85 kcal/mol at 300 K, which is in agreement with a previous experimental value based on electron paramagnetic resonance, 0.72 kcal/mol (Rice et al, Biophys. J. 84:1844 (2003)), although the identity of the states involved is not certain. In non-CNB states the mobile neck linker points mostly forward in the ATP-like conformation of the motor head, so there is relatively little conformational difference with the CNB-state. By contrast, when a 10-pN rearward load is applied at the end of the beta9 part of the neck linker, a new local energy minimum appears for a rearward-pointing state. Compared to the CNB state, the free energy of the rearward-pointing state is higher by 2.96 kcal/mol at 300 K. This indicates that the CNB readily forms under applied load and thus is able to move the neck linker forward. The significance of these results for the mechanism by which kinesin-1 walks on microtubules will be discussed.

1911-Pos
Neck-Linker Length is a Critical Determinant of Kinesin Processivity
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The kinesin neck-linker domain is a key mechanical element underlying processive kinesin motility. Not only is neck-linker docking thought to be the dominant conformational change in the kinesin hydrolysis cycle, chemomechanical communication between the two head domains must necessarily be transmitted through the two neck-linker domains and their shared coil-coil. Hence, the length of the neck-linker is expected to have a strong influence on kinesin run length, a quantitative measure of processivity. Across different kinesin families, motors with longer neck-linker domains, such as Kinesin-2 are generally less processive than Kinesin-1, which has the shortest neck-linker domain among N-terminal kinesins. However, there is disagreement in the literature as to whether artificially extending the Kinesin-1 neck-linker alters the motor run length. Using single molecule TIRF analysis to visualize GFP-tagged motors in 80 mM PIPES buffer, we find that lengthening the Kinesin-1 neck-linker by three amino acids results in a five-fold reduction in run length. Consistent