compatibility, (iii) one-wavelength switching, (iv) labeling densities and (iv) video-rate imaging with subdiffraction resolution.
(1) Heilemann et al., Angew. Chemie, 47, 6172-6176 (2008)
(2) Heilemann et al., Angew. Chemie, 48, 6903-6908 (2009)
(3) Heilemann et al., Laser & Photonics Review, 3, 180-202 (2009)

941-Pos
Optimizing Super Resolution Microscopy
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Super-resolution microscopy is often done by imaging isolated fluorescent probes as diffraction-limited spots with objective-type total internal fluorescence (TIRF) microscopy. The centers of these spots are commonly, sub-optimally located by least-squares fitting a 2D Gaussian to each spot's intensity distribution. Here we give the optimal localization procedure based on the true point spread function (PSF) known from wave optics. From a single focused image of a fluorophore molecule with fixed or time-resolved spatial orientation, we estimate the fluorophore's position and orientation using maximum likelihood estimation. We achieve the highest possible precision, given by Fisher's information limit. In the same manner, optimal localization is demonstrated for isotropic distributions of dipoles, e.g. fluorescent beads, excited by the evanescent wave produced in TIRF. Using this as a baseline, we compare a number of estimators and demonstrate that (i) for a 2D Gaussian, the unweighted least-squares fitting squanders one third of the available information, and weighted least-squares fitting is unreliable; (ii) a popular formula for the localization error of a 2D Gaussian fit exaggerates its precision beyond Fisher's information limit; (iii) maximum likelihood fitting of a 2D Gaussian is, on the other hand, practically optimal. We also present new, reliable formulae for the precisions of the various localization methods.

942-Pos
Fluctuation Analysis with the Spinning Disk Confocal Microscope
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Analysis of the fluctuations in time and space of confocal images has the potential to provide information about molecular diffusion and molecular interactions directly in live cells. Fluctuation image analysis has been commonly done in the laser scanning microscope. In the slow regime, when the fluctuations are slower than the frame rate, the time correlation between the same pixel in different frames of an image stack provides all the information about diffusion and brightness. In the fast regime, for example for molecules diffusing in the cytoplasm, the frame rate is too slow to follow the fluctuations due to diffusion. In the raster scan confocal microscope, these fluctuations are detectable because of the correlation of the intensity with the next pixel in the same line or in the next line. In fluctuation spectroscopy an important parameters is the sampling time that must be shorter that the time of the decay of the fluctuation. In the spinning disk confocal microscope, the sampling time at each pixel is very short. However, in the normal data acquisition protocol of the spinning disk microscope the intensity at one pixel is averaged with the intensity at the same pixel after the disk has performed several rotations. In this work we triggered the camera acquisition so that each pixel is visited only once per frame acquired. While we are observing fluctuations due to fast moving bright particles, the fluctuations due to dim particles seem to be buried in the noise of the system. We are investigating the origin of this extra noise and developing methods to characterize it so that it can be properly subtracted.
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943-Pos
A Simple System for Long-Term 3D Tracking of Quantum Dot Probes in Live Cells
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The intracellular signaling function of G-protein coupled receptors and tyrosine kinase receptors is governed in part by their transport dynamics on the cell surface and within the cell cytosol. Fluorescent nanocrystal quantum dot (QD) probes are especially well suited for investigation of the spatial dynamics of these membrane receptor proteins, including their membrane diffusion, internalization, and intracellular transport. The bright and relatively photostable fluorescence of QDs have enabled dynamic tracking of single or discrete groups of receptors in live cells, but a major limitation in obtaining long-term information on receptor dynamics is that QD probes can only be observed for as long as they remain within the depth of field of the microscope. Because receptors often diffuse or are actively transported out of the focal imaging plane, measurements of QD trajectories are limited in time and z location.
We have overcome this limitation by implementing a 3D tracking system to track receptors for extended durations. Our 3D tracking system is simple and flexible, requiring only an epifluorescence microscope, computer control of a piezo-driven stage and an EMCCD camera. We demonstrate 100-200 nm z-position accuracy over a 10 micron depth for 10s of minutes, with temporal resolution of 7.2s per (x,y,z) coordinate. These capabilities allow measurement of QD probe positions over whole cells for durations relevant to the long-term signaling dynamics of membrane protein receptors. We will present the application of this system to measuring the spatial dynamics of QD-membrane receptor probes for long durations in live cells.

944-Pos
High-Pressure Microscopy for Modulating the Structure and Function of Biomolecules
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Protein hydration is an important factor for structural formation and enzyme activity. To study the effects of hydration, it is desirable to monitor protein structures or biological activities by modulating the intermolecular interaction between protein and water molecules. Application of pressure is one of the powerful methods for enabling the modulation of protein hydration. Here, we report a novel microscopy for visualizing the pressure-induced changes in the structure and function of biomolecules. We have developed a high-pressure chamber (Nickel alloy, hasteloy C276, 60 à- 50 à- 20 mm), which was available up to 2,000 bar. The chamber was mounted on a commercially available microscope equipped with a long working distance objective lens. The microscope apparatus enabled to observe bright-field, phase-contrast, dark-field and epi-fluorescent images at high-pressure conditions. We studied the effects of pressure on the structure and function of cytoskeletal proteins using the kinesin-microtubule complex as a model system [1]. Under high-pressure conditions, taxol-stabilized microtubules were shortened from both ends at the same speed. The sliding velocity of kinesin motors was reversibly changed by pressure and reached the half-maximal value at ~1000 bar. Further analysis showed that the pressure mainly affects the stepping motion, but not the ATP binding reaction. The application of pressure is thought to enhance the structural fluctuation and/or association of water molecules with the exposed regions of the kinesin head and microtubule. These pressure-induced effects could prevent the kinesin motors from completing the stepping motion.
[1] Nishiyama *et al.*, *Biophys J.* **96**(3) 1142-1150 (2009).

945-Pos
Particle Image Cross Correlation Spectroscopy (PICCS)
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In vivo studies of the dynamics of single molecules and particles have produced a wealth of biological insights. The diffusion behavior of a membrane receptor, for example, reveals the structure of the plasma membrane. In recent years we have developed an analysis technique to quantify single molecule translational movement on a nm length and ms time scale (Particle Image Correlation Spectroscopy (PICS), Semrau, Schmidt., Biophys. J., 2007).
The insight gained from experiments on a single molecular species is however limited. No biomolecule operates on its own and often it is the very interaction between different types of molecules which is biologically most relevant. To that end we further developed PICS for experiments with two differently labeled molecular species. Particle Image Cross-Correlation Spectroscopy (PICCS) allows us to unambiguously measure molecule colocalization, even at large molecule densities and down to a length scale of 10 nm. To demonstrate the method's power we studied the intracellular transport of the morphogen Dpp enclosed in endosomes. Dpp establishes a gradient in the wing imaginal disk of fruit fly larvae, providing positional information to cells. Using PICCS we found that 52% of apical Dpp is in early endosomes and that early endosomes contain 1.9 times as much Dpp as other endosomes. Our data suggests that Dpp resides shorter in early endosomes compared to late/recycling endosomes.
PICCS makes it also possible to push the limits of the time scales on which molecular movement can be measured. By labeling one molecule with two spectrally resolvable fluorophores we can follow the dynamics of the molecule on a 100 µs time scale.
To summarize, PICCS opens up a whole new range of *in vivo* single molecule experiments: Molecular correlations and dynamics can be measured with unprecedented accuracy and temporal resolution.