with biotin-MHC/peptide to bind the TCR with relatively high avidity, followed by streptavidin-quantum dots. They were then imaged on an emCCD-equipped microscope and kICS analysis was applied. Spatial intensity fluctuations in an image measured the clustering of receptors on the 100s of nm length scale. Changes in the intensity correlation function of the blinking QDs characterized clustering on the 10s of nm length scale. We also used kICS to measure changes in TCR diffusive transport. When T cells exhibited maximum activity 3-4 days after exposure to antigen, the degree of their TCR aggregation on both length scales was significantly higher than that of naïve cells, while TCR diffusion was a minimum. This new technology has powerful applications as it can be applied to just a few cells and we will show that it is able to detect changes in receptor organization of cells in vivo.

3958-Plat
The Pair-Correlation Approach to FCS
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Molecular diffusion and transport processes are fundamental in physical, chemical, biochemical and biological systems. Current approaches to measure molecular transport in cells and tissues based on perturbation methods like fluorescence recovery after photobleaching are invasive, fluctuation correlation methods are local and single particle tracking requires the observation of isolated particles for relatively long periods of time. We propose to detect molecular transport by measuring the time cross-correlation of fluctuations at a pair of locations in the sample. When the points are further than two times the size of the point spread function, the maximum of the correlation is proportional to the average time a molecule takes to move from a specific location to another. We demonstrate the method with simulations, using beads in solution and by measuring the diffusion of molecules in cellular membranes. The spatial pair cross-correlation method detects barriers to diffusion and heterogeneity of diffusion because the time of the correlation maximum is delayed in the presence of diffusion barriers. This non-invasive sensitive technique follows the same molecule over a large area producing a map of molecular flow and does not require isolated molecules thereby many molecules can be labeled at the same time and within the point spread function. Work supported in part by U54 GM064346 Cell Migration Consortium (MD and EG), NIH-P41 P41-RRO3155 (EG) and P50-GM076516 (EG).

3959-Plat
Spatially Resolved Fluorescence Fluctuation Spectroscopy (FFS) in Living Cells
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Fluorescence (cross-)correlation spectroscopy (FCS/FCCS) and generally fluorescence fluctuation spectroscopy (FFS) are confocal microscopy-based methods that allow to assess diffusion and transport properties as well as interactions of molecules (proteins, nucleic acids, compounds) in vitro and in vivo. Commercially available instrumentation enables routine measurements at one or few specific points inside living cells.
However, conventional FCS/FCCS experiments remain challenging because point measurements in a living cell are associated with large error caused by the heterogeneous environment of the cellular interior. Moreover, biological noise due to cell-to-cell variations of physical and biological parameters (e.g. intracellular viscosity, protein expression levels) induces further variations, which are difficult to separate from the measurement error. Currently, these problems are partially addressed by performing statistical data analysis of measurements from many different cells. However, it is desirable to obtain more reliable and robust data from single cells with spatial resolution. This requires a new approach allowing to perform simultaneous measurements and to circumvent the problems associated with confocal FFS: photobleaching, out-of-focus illumination and loss of spatial definition due to cell movements.
Here, we present a novel microscope that allows spatially resolved FFS measurements in 2D optical sections across cells. The setup is based on a single plane illumination microscope in which a thin diffraction-limited light sheet is used to illuminate a cross-section of the cell. The use of an electron-multiplying charge-coupled device (EM-CCD), placed perpendicular to the light sheet, with hundreds of single pixel detectors instead of an avalanche photodiode (a single pixel detector) enables to record on each pixel the incoming photons with single photon sensitivity and sub-millisecond time resolution. This is predicted to significantly reduce the error associated with single point measurements. It should also provide access to spatially resolved measurements of concentrations, interactions and mobilities.

3960-Plat
Multiplexed Measurement of Molecular Interactions using Hyper-Spectral Imaging and Multi-Parametric Detection
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A large number of molecules cooperate in an intricate network of interactions for the maintenance of the structural integrity, the metabolism and the function of the living cell. A challenge for engineering and physics in optical microscopy is to provide tools that could offer the highest spatio-temporal resolution with the capability to decode complex networks of molecular interactions by the development of technologies and methods that, at the same time, may provide cost-effective and user-friendly instruments.
We present our latest development of a novel architecture for a spectrograph that permits to characterize fluorescence emission (excitation and emission spectra, fluorescence anisotropy and fluorescence lifetime) in a quantitative and efficient manner. The novel system offers parallel acquisition with a single detector and, by the use of a novel solid-state detector (time-gated single-photon avalanche photodiodes) and a supercontinuum light source, it provides excellent versatility of use at comparatively low costs. We envisage that by the exploitation of Foerster resonance energy transfer between a number of fluorophores, this microscopy platform will be capable to probe multi-molecular interactions and to multiplex a variety of fluorescent biosensors.
Novel biophysical imaging techniques are fundamental for our research activities in cancer research: to probe the key molecular processes underlying genomic stability and for a better understanding of the molecular aspects of cancer.

3961-Plat
Analysis of Cdc-42 Mobility and Dimerization In Vivo by Higher Order Fluorescence Correlation Cumulants
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We demonstrate a new method for measurements of mobility dependent protein oligomerization using higher order fluorescence correlation cumulants. Fluorescence intensity distribution methods including fluorescence cumulant and moment analysis have been successfully used in recent years to analyze oligomerization phenomena. The extension of such methods to treat analysis at different binning times allows for the analysis of mobility dependent oligomerization. Nevertheless, the analysis of time binned distributions is mathematically complex and depends strongly on detector characteristics such as afterpulsing and dead time. Here we develop an equivalent method treating traditional correlation functions as bivariate fluorescence cumulants. Doing so brings the power of the cumulant analysis to bear on the measurement of mobility dependent oligomerization while maintaining the mathematical simplicity of the correlation functions that are the standard within fluorescence correlation spectroscopy. We use this technique to show that the low mobility pool of intracellular EGFP-cdc-42 in living yeast cells is on average a dimer while the high mobility pool is monomeric. Examination of mutant yeast strains suggests that the low mobility pool is associated with recycling vesicles, providing an explanation for both the slow diffusion as well as the oligomeric state of this species. This technique could be easily extended to other proteins in the yeast genome that demonstrate heterogeneous mobility.

3962-Plat
H33D Gen II: A New Photon Counting Camera for Single-Molecule Imaging and Spectroscopy
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We have developed a new generation of photon-counting camera consisting of a large area microchannel plate-based photomultiplier tube associated with a position-sensing anode. Our detector can record local count rates of approx 30 kHz and sustain global count rates of several MHz. We illustrate its capabilities by tracking single quantum dots in live cells with nanometer spatial resolution and sub-ms temporal resolutions over large areas and long durations.