Fluorescence Spectroscopy I

1041-Pos Board B827
Studying Spatial Gradients of Signaling Proteins in Mitotic Spindles with Time-Integrated Multipoint Moment Analysis
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The organization of the mitotic spindle is orchestrated by the activities of multiple signaling proteins, such as the GTPase Ran. It has been proposed that the Ran pathway produces a cascade of events which gives rise to spatial gradients in the behavior of soluble proteins, which in turn produce spatial gradients in microtubule behaviors important for spindle assembly. Previous experiments have directly demonstrated the existence of gradients around the spindle in the upstream components of the Ran pathway, but it is still unclear if there are significant gradients in the downstream soluble components in this pathway. We recently developed a method, TIMMA, time-integrated multipoint moment analysis, a multipoint form of fluorescence fluctuation spectroscopy capable of quantitatively measuring the concentration, diffusion coefficient, and molecular brightness of soluble proteins throughout live cells. We are using TIMMA to characterize the behaviors of the upstream and downstream components of the Ran pathway in live mitotic cell to test the validity of the Ran gradient model.

1042-Pos Board B828
Evaluation of High-Content Screening Fluorescence Correlation Spectroscopic (HCS-FCS) Data
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High-content screening fluorescence correlation spectroscopy (HCS-FCS) determines diffusion properties, local concentrations and molecular interactions of biologically relevant proteins in thousands of individual S. cerevisiae cells. We automated one color auto-correlation fluorescence spectroscopy to investigate the properties of the yeast proteome. We also used two-color cross-correlation for interaction screens.

For the proteome wide one-color yeast screen, we investigated more than 4000 of the approximately 6000 proteins known in S. cerevisiae. This resulted in over 250,000 measurements on more than 500,000 individual cells. This amount of data required automated tools for data quality control and analysis. Our pipeline started with the evaluation of transmitted light images of the measured cells. We extracted features from these images and trained a support vector machine (SVM) to classify them into healthy yeast cells and samples we wanted to exclude from further analysis. A similar approach enabled us to classify raw fluctuation data taken from the remaining cells and the auto-correlation curves we derived from them. As training sets, we used measured and simulated data. To analyze the correlation curves obtained from the fluctuation data, we fitted different diffusion models. We used mixed-effects models to extract averaged fit parameters for the same protein measured in multiple cells. To select the most appropriate diffusion model we used Akaikes ’An Information Criterion’ (AIC).

This approach not only allows the analysis of large data-sets as they occur in our HCS-FCS experiments and camera based FCS described in literature, but has the additional advantage of reducing the human bias.

1043-Pos Board B829
Dual-Color Single Plane Illumination Fluorescence Correlation Spectroscopy (SPIM-FCS) using a Single Photon Detector and Hardware Based Image Processing
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We are interested in the movement and the interaction of fluorescently labeled molecules inside living cells. Such mobility measurements at low intensities can be carried out very well using single photon avalanche diodes (SPAD) which allow count rates in the MHz regime.

Here we present a custom built single plane illumination microscope (SPIM) equipped with a new 128x512 SPAD pixel array as an image sensor and a full frame time resolution of <10μs. This is at least one order of magnitude better than current setups using electron multiplying CCD cameras (EMCCD). Using a dual-view device we can simultaneously image two spectral channels of the sample onto different regions of the sensor.

The underlying multi-tau correlation algorithm is implemented directly in custom hardware. This is done using reconfigurable logic chips, so called field-programmable gate arrays (FPGA). Our current implementation can correlate 32x32 pixels with a resolution of 1μs in real time.

1044-Pos Board B830
Nanopores for 60 nm Resolution of Membrane Organization and Dynamics
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Dynamic nanoscale domains in the plasma membrane may be responsible for a variety of cellular signaling processes via membrane receptor clustering and lipid phase partitioning. However, current experimental approaches are limited in spatial and/or temporal resolution to address many membrane domain hypotheses. We have developed a new approach utilizing an array of nanopores to examine membrane organization and dynamics with near-field optical fluorescence microscopy without incorporating a scanning probe or disturbing the membrane. These nanopores are glass-filled, cylindrical pores (> 50 nm diameter) in a thin aluminum film on a fused silica support, and they provide a planar surface for unperturbed cell adherence and growth. A nanopore confines the transmitted excitation light to a sub-diffraction limited spot directly above aperture, providing a 40-fold decrease in the illuminated area as compared to diffraction-limited illumination of the plasma membrane. Otherwise conventional microscopy excitation sources and fluorescent probes are used to enable fluorescence correlation spectroscopy (FCS) with 60 nm and 1 μs resolution.

Further, these apertures provide two key benefits for FCS in addition to improved resolution: ensured alignment of numerous illumination spots for cross-correlations and ensured focusing of illumination on the cellular membrane as opposed to focusing within the cytoplasm. Chromatic aberrations and slight laser misalignment that may complicate far-field, two-color FCS are less of concern with nanopores because the illumination profile is determined by the aperture directly. This technique has been applied to both model and living cell membranes to examine the diffusion of lipids and proteins in nanoscale dimensions. In particular, we will present results demonstrating the effectiveness of this technique to observe diffusion and interactions of membrane proteins and lipids that change in response to cross-linking-mediated activation of a selected component with consequent changes in the membrane environment.

1045-Pos Board B831
Distribution Characterization with Fluorescence Cumulant Analysis
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Fluorescence correlation spectroscopy (FCS) is a powerful tool for biophysical research, capable of monitoring concentrations and molecular interactions on a femtoliter scale. However, it is difficult in practice to resolve multiple molecular species using FCS unless there is a large difference in molecular weight. Several techniques have been developed to combat this problem, including photon counting histograms (PCH), fluorescence intensity distribution analysis (FIDA), and fluorescence cumulant analysis (FCA). These are capable of resolving multiple components on the basis of differences in molecular brightness, but have typically focused on analysis of single species with a small number of species. Fluorescence cumulant analysis can be extended to determine the parameters of brightness distributions containing many components, yielding a set of simple algebraic relations between the factorial cumulants of a measured fluorescence intensity series and the moments of the brightness distribution. Using PCH in conjunction with FCA, the best-fit distribution may be distinguished from several candidate models. We present simulations and experiments testing the applicability of this analysis. This technique may be useful in studies where the molecules of interest form a variety of multimers or aggregates.