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differentiation stages, GFP-PLP transfected oligodendrocyte cells, OLN-93, and Giant Unilamellar Vesicles, (GUVs) prepared from myelin lipids.
Here, we show the different distribution pattern of specific proteins, such as PLP and MBP, as well as lipids, such as GalC and SGC, in OLN-93 cells and primary oligodendrocytes. We also mapped the diffusion behavior of GFP-PLP in plasma membrane and subcellular compartments of OLN 93 cells by FCS. Furthermore, the spatial distribution of MBP, PLP, GalC and SGC varies along the different stages of differentiation of primary oligodendrocytes. In GUVs, we show the effect of GalC and SGC on lipid organization and domain assembly.
By integrating confocal microscopy and FCS data from live cells and model membranes, we shed some light on the relation between myelin lipid-lipid and lipid-protein interactions and myelin assembly and function, in order to better understand neurological diseases.

Imaging and Optical Microscopy I

750-Pos Board B550
Optimal Acquisition and Analysis of Images for PALM and STORM
Alexander R. Small, Edward Shore, Forrest Hippensteel, Nahom Yirga.
Superresolution microscopy techniques like PALM and STORM enable fluorescence imaging subwavelength resolution. Fluorophores are switched on and off, with a sparse subset of the fluorophores emitting light at any given time. Consequently, the fluorophores form non-overlapping blurs in the image plane, enabling localization of molecules with subwavelength resolution limited only by noise in photon detection. We used a combination of theoretical, statistical, and computational techniques to determine the fundamental limits of performance. Using a kinetic model of fluorophore activation and bleaching, we are able to prove the existence of an optimal image acquisition scheme, that maximizes the number of single-molecule (i.e. no over-lapping blurs) within a given time constraint. In this scheme, the error rate (defined as the ratio of the number of multi-molecule overlap images to the number of images of single molecules) is constant. Interestingly, at fast acquisition speeds, the scheme is actually very robust: Deviations from the optimal scheme decrease the number of good images, but decrease the number of bad images (overlaps) to partially compensate. We also developed a formalism for benchmarking algorithms that correct errors by removing overlap images. Surprisingly, only a handful of performance parameters matter for image quality, opening up the possibility of designing fast error correction algorithms based on simple principles. Finally, to optimize the localization procedure, we have developed a rapid approximation to the Gaussian Mask technique for least squares fits. Our algorithm uses a simple expansion to significantly reduce the number of function evaluations used in fitting. The results are similar to those obtained when the Gaussian Mask algorithm is applied to an image that has undergone noise filtering. This suggests the possibility of doing very fast molecule localization on images represented in a basis where they are sparse.

751-Pos Board B551
Application of Regularized Richardson-Lucy Algorithm for Deconvolution of Confocal Microscopy Images
Martin Laasmaa, Marko Vendelin, Pearu Peterson.
While confocal microscopes have considerably smaller contribution of out-of-focus light than widefield microscopes, the confocal images can still be enhanced by deconvolution if the optical and data acquisition effects are accounted for.
Several deconvolution algorithms have been proposed for 3D microscopy. In this work we analyze the Richardson-Lucy iterative algorithm that is derived for Poisson noise and combined with total variation (TV) regularization. The influence of TV regularization on deconvolution process is determined by one parameter. However, the choice of regularization parameters is often unknown while it has considerable effect on the result of deconvolution process. The aims of this work were: to find good estimates of regularization parameter from the input; to develop an open source software package that would allow testing different deconvolution algorithms and that would be easy to use in practice. For that, we derived a formula to estimate this regularization parameter automatically from the images as the algorithm progresses. To assess the effectiveness of this algorithm, synthetic images were composed on the basis of confocal images of rat cardiomyocytes. From the analysis of deconvolved results, we have determined under which conditions our estimation of TV regularization parameter gives good results. The estimated TV regularization parameter can be monitored during deconvolution process and used as a stopping criterion. As a result, we propose a practical method to deconvolve confocal microscope images that uses estimated regularization parameter depending on the input image.
We applied the deconvolution algorithm to study mitochondrial organization in rat cardiomyocytes. An open source software for deconvolving 3D images is available in http://sysbio.ioc.ee/software/.

752-Pos Board B552
Point Spread Diffraction Patterns and Super-Resolution Particle Localization
Stephen M. Anthony, Anish V. Abraham, Jerry Chao, Sripad Ram, E. Sally Ward, Raimund J. Ober.
Sub-diffraction limit localization of molecules within cells depends upon fitting the observed photon distribution to the point spread function. As such, accurate knowledge of the point spread function is important to super-resolution microscopy and critical to determining the trajectories and dynamics of molecules within cells. While simple geometrical optics serves well to represent light propagation on the macroscopic level, more elaborate wave representations are necessary to describe light propagation within a few wavelengths of focal points, such as single fluorophores imaged by microscope objectives. As a result, numerous theoretical approximations to experimental point spread functions exist as the exact theoretical point spread function is unknown. Further consideration must be given that for realistic in vivo experiments, frequently events of interest will not entirely match the design conditions of the microscope; most events of interest will not be perfectly in focus, nor will the index of refraction within the cell necessarily match that of the medium. Additionally, a number of imaging modes explicitly rely upon out-of-focus images for three dimensional localization. Here, through serial sectioning microscopy, we explore the experimental point spread function in comparison with various models, determining which models are robust and provide accurate sub-diffraction limit localization for realistic data.

753-Pos Board B553
Multiparameter Fluorescence Image Spectroscopy
Ralf Kuehnemuth, Heike Hornen, Suren Felekyan, Stefanie Weidtkamp-Peters, Elisabeth Knust, Claus A.M. Seidel.
In multiparameter fluorescence image spectroscopy (MFIS) simultaneously all information observable in a confocal fluorescence experiment is recorded photon by photon. Subsequent analysis of the primary fluorescence data (polarization, color, arrival time and spatial origin of the photons) enables e.g. calculation of fluorescence correlation / cross correlation spectra (FCS / FCCS), Förster resonance energy transfer (FRET) efficiency, fluorescence lifetime and anisotropy. Display of the results in more dimensional histograms to combine the fluorescence parameters of interest leads to species selective images and thus maximizes the selectivity of fluorescence microscopic methods. This is particularly useful for identification of low-abundance species and/or examination of evolving or unstable samples, where sequential or long time measurements are not feasible. Moreover it facilitates a statistical relevant data analysis of the pixel information, which makes an efficient quantitation of heterogeneities possible. Some aspects of MFIS and its power will be demonstrated in typical experiments.
The expression of fluorescent proteins fused to a protein of interest in cultured cells or whole organisms like drosophila embryos has become a well established tool to investigate e.g. the dynamic behavior, complex formation and interaction of these proteins under physiological conditions. However the detected fluorescence signal emerging from most biological samples is affected by autofluorescence. Here we use MFIS to characterize autofluorescence by looking at different fluorescence parameters like fluorescence lifetime and spectral properties. Subsequently we can filter pixel-wise for the contribution of autofluorescence to the detected fluorescence signal. The multiparameter approach significantly helps to increase the robustness of the analysis. It also supports the economic use of photon information and thus allows one to keep the expression levels of fluorescent proteins as low as possible to preserve physiological conditions as much as possible.

754-Pos Board B554
Protein Domain Organization in the Nuclear Pore Complex Studied by Fluorescence Anisotropy
Alexa L. Mattheyses, Martin Kampmann, Claire E. Atkinson, Sanford M. Simon.
Fluorescence anisotropy measurements can yield information about the mobility, orientation and proximity of fluorophores and can be used to study the dynamics of proteins in vivo. The nuclear pore complex (NPC) is a large macromolecular complex, the size and complexity of which present experimental challenges. The dynamics of nucleoporins (nups) were examined using fluorescence polarization microscopy. Using a theoretical framework which exploits the symmetry of the NPC and its organization in the nuclear envelope we can resolve the order and disorder of individual protein domains within the complex. Specific domains of individual nups were tagged with GFP and