2012-Pos Board B742
Automated Synapse Detection and Validation by Correlated Array Tomography and Scanning Electron Microscopy
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Array tomography (AT) is a method for mapping the expression patterns of many proteins at high resolution in three dimensions. Using ultrathin serial tissue sections, multiple cycles of immunohistochemistry, optical imaging, and 3D reconstruction, AT can map the expression of multiple proteins at sub-micrometer resolution across large tissue volumes. Using antibodies to synaptic proteins, AT has been used to reconstruct synaptic architecture in brain tissue, and we have developed automated routines for counting synapses. To validate our algorithms and immunostaining, we examined AT samples by both light and electron microscopy in order to correlate synaptic protein immunoreactivity and ultrastructure. We cut a 30-section array of 70 nm serial sections of Lowicryl-embedded mouse hippocampus and ran two cycles of immunostaining and light microscopy. It was then stained with heavy metals and a subregion of six serial sections imaged by field-emission scanning electron microscopy (SEM). After deconvolving, stitching, aligning and merging AT and SEM data, we manually scanned the SEM volume and detected 98 synapses, largely by the presence of electron-dense post-synaptic densities. We then overlaid fluorescence signals from anti-PSD-95 and anti-synapsin antibodies (Cell Signaling) and compared them to the ultrastructurally-defined synapses. Finally, we ran light-level data through our automated synapse-detection algorithm which detects the co-localization of PSD-95 and synapsin local maxima. To determine false negatives, we examined how many SEM-identified synapses were detected by the algorithm. An optimized algorithm detected 72% of SEM-identified excitatory synapses. False-negatives were caused by failure of either immunostaining (12%) or the algorithm (16%). To determine false positives, we examined how many algorithm-detected synapses corresponded to SEM-identified synapses. 81% were validated, while 19% were false-positives often due to the close-packing of multiple synapses. We expect to extend these analyses to other markers and algorithms.

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Structural and Dynamic Study of Caveolin-1 Membrane Microdomains by Single Molecule Imaging
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Within the plasma membrane, the protein caveolin-1 (cav-1) forms various diffraction limited microdomains that have yet to been precisely characterized in terms of structure and dynamics. Using single molecule and super-resolution imaging techniques we study the membrane distribution and the dynamics of caveolae and cav-1 scaffolds at the nanometer scale in both fixed and live cells. Cav-1 knock out MEF cells were rescued with cav-1 fusions to SNAP, ptagRFP or mCherry tags co-expressed at equimolar amounts with a cav-1-split-GFP fusion for 3D super-resolution imaging and quantitative posttranslational modifications of individual cav-1 proteins with single molecule sensitivity. At the plasma membrane, 3D super-resolution imaging by dSTORM and PALM revealed the expected ~100 nm globular shape of caveolae as well as single layer cav-1 domains with structures matching the expected size of cav-1 scaffolds. We also show that, in live cells, individual cav-1 can be posttranslationally targeted with synthetic peptides using split-GFP as both a targeting platform and an optical reporter for nanometer precision modifications of cav-1 microdomains. Towards a selective and high precision posttranslational destabilization of cav-1 microdomains using the cell's own machinery, we designed synthetic peptides that specifically induce the ubiquitination of split-GFP via the VHL ubiquitin pathway.
By combining structural, dynamic and perturbation studies using molecular tools and ultrasensitive single molecule imaging techniques, we start to unveil the molecular organization of cav-1 microdomains and their dynamic interplay at the plasma membrane of eukaryotic cell.

2014-Pos Board B744
Improved Super-Resolution Imaging in Heavy Water
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Advanced fluorescence microscopy techniques including single-molecule and super-resolution imaging require bright and photostable dyes that can be selectively targeted to biomolecules. There is therefore an ongoing interest in the development of improved chromophores for biology, especially ones that absorb and emit in the near-infrared. Single fluorophore brightness is a key parameter in localization-based super-resolution imaging techniques such as (F)PALM, (d)STORM, or GSDIM: the brighter the emitter, the more precisely it can be localized and the better the resolution is in the final image. Nonetheless, with few exceptions, most commercially available fluorophores excitable beyond 630 nm have poor fluorescence quantum yields with values around 0.3 or lower limiting their brightness.
We introduce a simple, cost-effective, and biocompatible method to enhance the fluorescence quantum yield of several commercially available oxazine (ATTO655, ATTO680, ATTO700) and cyanine (Cy5, Alexa Fluor 647, Cy5.5, Alexa Fluor 700, Cy7) fluorophores [1, 2]. We demonstrate that for all oxazines and one cyanine, the fluorescence quantum yield more than doubles in heavy water (D2O) compared to water (H2O). This increase leads to the detection of twice as many photons per molecule and to a corresponding improvement in the mean localization precision, which enables higher resolution in biological subdiffraction imaging. We demonstrate that super-resolution imaging in D2O can be used to resolve diffraction-limited subcellular structures such as filopodia or microtubules and by performing quantitative morphological analysis of ligand-induced clustering of arrestin proteins upon G protein-coupled receptor stimulation [3].
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Precise Measurement of the Relative Position of RNA Dimers within Virus-Like Particles using 2-Color 3D Super-Resolution Fluorescence Microscopy
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A long-standing question in retrovirus biology is how RNA genomes are spatially organized within virions. Here, we explore the relative 3D spatial distribution of RNA dimers (1) within ~130-nm diameter virus-like particles (VLPs) with single-RNA sensitivity (2) using the double-helix microscope. The double-helix microscope, a modification of the standard epifluorescence microscope, is capable of precise and accurate three-dimensional (3D) localization of fixed fluorescent single molecules throughout a 2 μm-thick focal volume (3). Furthermore, the use of a sophisticated registration algorithm, based upon a locally weighted quadratic transformation function, enables accurate co-localization of yellow and red fluorescent emitters with <10 nm registration error in 3D (4). We routinely localize fluorescently tagged RNA coat protein-clusters (via Bgl- and MS2-based fluorescent protein labeling) to a 3D precision of 10-15 nm within the VLPs. By systematically varying the location of these coat protein-clusters along the length of the RNAs, we elucidate the spatial organization of packed RNA dimers inside these VLPs.
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2016-Pos Board B746
Characterizing Membrane Protein Interactions in Vivo by Multiparameter Fluorescence Image Spectroscopy
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Förster resonance energy transfer (FRET) due to its sensitivity of distance has been widely used to investigate the structure and interaction of biomolecules. Multiparameter fluorescence image spectroscopy (MFIS)[1] provides particular advantages to FRET imaging because all the parameters are monitored simultaneously with picosecond accuracy that allows for a comprehensive analysis on the biological system[2].

However, the decreased donor's fluorescence lifetime in FRET can have two reasons (1) the conformational change or (2) change in fraction of interacting proteins. Here we present a comprehensive analysis method to extract all the information from low number of photons typical for fluorescence imaging. Using a 3-species model, we analyzed the sub-ensemble data and found the decay pattern of each species, and then the decay patterns were applied to obtain pixel-wise species fractions for the entire image.
The method was applied to study the different protein interactions *in planta*. The studied protein interactions are listed below classified by the signaling pathway they belong to:
1. CLAVATA pathway: CLV1, CLV2 and CORYNE (CRN).
2. Flagellin pathway: brassinosteroid-insensitive1-associated kinase1 (BAK1) and flagellin-sensitive 2 (FLS2).
We were able to monitor the formation of FLS2-BAK1 receptor complexes over time after infiltrating the ligand flg22. Utilizing this method, we also show that the complex of CLV1, CLV2 and CRN is already assembled before ligand-binding, but that CLV3-binding to CLV1 could induce changes in the receptor complex formation and conformation in certain region of the cell membrane.
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2017-Pos Board B747
Visualization of Stimulation Dependent Localization of Single Endogenous mRNAs to Dendritic Spines
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RNA localization and local protein synthesis plays an essential role in synaptic plasticity as well as learning and memory. However it has not been possible to image the dynamics of mRNAs under the influence of synaptic signaling in a live neuron. Here, we develop a fluorescence microscopy method to visualize endogenous mRNAs with single molecule sensitivity and at the same time to deliver synaptic stimulation at a single synapse. To visualize endogenous mRNA, we used a transgenic mouse in which 24xMS2 binding sites had been knocked into the 3' untranslated region (3' UTR) of the essential β-actin gene. We introduced fluorescent protein tagged MS2 coat proteins in the cultured primary hippocampal neuron to label the endogenous β-actin mRNA. We built a microscope that is able to stimulate a single dendritic spine with photo-uncaging of neural transmitters. With this system, we verified that repeated synaptic stimulation by photo-uncaging resulted in structure plasticity and long term potentiation of a single spine. We investigated the dynamics of β-actin mRNA under the influence of synaptic stimulation. We found that single β-actin mRNAs localized to the base of the stimulated spines. Subsequent neuropharmacology study revealed that this stimulation dependent mRNA localization phenotype depends on the synaptic signaling through NMDA receptor. This project is supported by the grant GM84364 from National Institute of Health to RHS.

2018-Pos Board B748
3D Tissue Reconstruction by using Deep Tissue Fluorescence Imaging System and FLIM
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We have demonstrated a new detection method used in two-photon fluorescence microscope called the DIVER (Deep Imaging via Enhanced-Photon Recovery) with enhanced capabilities for deep tissue imaging. The microscope uses a large area photo-detector to collect scattered emission photons directly from the wide area of a specimen. This detection scheme allows for increasing the imaging depth in tissues by about 6 folds due to a unique detector design and its increased photons collection efficiency compared to conventional methods. The DIVER system is also capable of performing Fluorescence Lifetime Imaging (FLIM), a very powerful tool to segregate various features in cells and tissues. The combination of deep tissue imaging and fluorescence lifetime provides contrasted images based on physiological parameters at depths that are not achievable by conventional microscopes. The DIVER potentially can be useful for imaging of various tissue samples, in particular for human skin cancer diagnostics, providing 3D cellular resolved images. We present the principle of operation of the microscope and show images obtained in unstained tissues in the heart, liver, kidney, lungs and other organs of mice. As an example of SHG in biological tissue, we have imaged the medial thigh muscle of a mouse obtained from the upper region of the leg. The muscle, roughly 3-4 mm thick, multiple z-stack images were acquired at field of view ranging from 400-900 μm, we can see the organization of the sarcomeres as well as the striations present in each sarcomere. We also have taken z-stack of the mouse heart where upon 3D reconstruction we can differentiate the different layers of the heart muscle as well as the ventricle.
This work is supported in part by NIH grants P50 GM076516 and P41 GM103540 and the Keck grant KF 50242.

2019-Pos Board B749
Polarization and Scattering Consequences in Advanced Optical Microscopy of Biological Samples through Mueller Matrix Signature
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Recent advances in Optical Microscopy are mainly related to fluorescence super resolution methods. Since the request for challenging applications on cell aggregates (i.e., tumor spheroids) or tissues/organs and small organisms (i.e. zebrafish) is growing, scattering is a very important issue in image analysis. As well, polarization properties of the excitation/absorption and emission properties can provide further information on the single molecule organization within the sample. I am hereby proposing, as done on confocal microscopy [1,2], an improvement of advanced methods, including SHG imaging, utilizing Mueller calculus and differential polarization/scattering imaging [3], and exploiting optically active biological structures [4] with particular interest in chiral objects [5]. Fluorescence and SHG data can be enriched by Mueller matrix signature. Since it was demonstrated the possibility of getting ultrastructural information about chromatin-DNA organization by means of circular intensity differential light scattering [6], the Mueller matrix integrated approach allows moving forward label free imaging. A Mueller Matrix polarimetry [7] integrated architecture will be discussed based on photoelastic modulation (vs. Pockels cell) in a polarization generator along the excitation pathway duplicated in the detection one and a classical electrodynamics model of data interpretation [8].
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2020-Pos Board B750
Optical Volume Measurement of Beating Cardiac Myocytes using Quantitative Phase Imaging
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This paper describes recent research in developing a high-resolution optical microscope capable of real-time quantitative phase imaging to study morphological changes such as relative optical volume and optical thickness in cellular structures. A pixelated wire grid polarizer mask bonded to a CCD camera sensor enables simultaneous measurement of multiple phase contrast interference patterns. These data are processed in parallel to obtain phase, optical path difference (OPD), optical thickness (OT), optical volume (OV), simulated DIC (gradient), simulated dark field (gradient magnitude), and 3D typographic movies.
Results will be presented of quantitative measurements of optical volume changes in beating rat cardiac myocytes before and after treatment with IPHC (isoproterenol hydrochloride). Full phase and topographic data were obtained at 15 frames/sec. Live cells were prepared and grown on either #1 coverslips or highly reflective slides. Cells cultures were placed into a