and observe more fluctuations in the same time interval. Spatial intensity distribution analysis (SpIDA) was developed to adapt analysis of the photon counting histogram (PCH) for laser scanning microscopy images to determine molecular brightnesses. We have extended SpIDA for multicolor analysis that allows for sensitive detection of heteromeric fluorescent species. Optical systems in FFS typically encode spectral information by using dichroic filters to direct emitted light onto two or more photodetectors. In contrast, our imaging system (Zeiss LSM 880) uses a diffraction grating to disperse emitted light onto an array of 32 PMTs. The increased spectral resolution afforded by this geometry has the potential to be powerful in FFS, however new challenges are introduced including intrinsic crosstalk between adjacent detectors due to their physical proximity. We introduce an approach for addressing these non-ideal detector effects. Multicolor SpIDA with non-ideal detector corrections is employed to study the interactions of D2-like dopamine receptors with signaling partners including G-proteins and G-protein coupled inwardly rectifying K^+ (GIRK) channels.

1713-Pos Board B622

Spatially Selective Dissection of Signal Transduction in Neurons Grown on NETRIN-1 Printed Nanoarrays via Segmented Fluorescence Fluctuation Analysis

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Axonal growth cones extend during neural development in response to precise distributions of extracellular cues. Deleted in colorectal cancer (DCC), a receptor for the chemotropic guidance cue netrin-1, directs F-actin reorganization, and is essential for mammalian neural development. To elucidate how the extracellular distribution of netrin-1 influences the distribution of DCC and F-actin within axonal growth cones, we patterned nanoarrays of substrate bound netrin-1 using lift-off nanocontact printing. The distribution of DCC and F-actin in embryonic rat cortical neuron growth cones was then imaged using total internal reflection fluorescence (TIRF) microscopy. Fluorescence fluctuation analysis via image cross-correlation spectroscopy (ICCS) was applied to extract the molecular density and aggregation state of DCC and F-actin, identifying the fraction of DCC and F-actin colocalizing with the patterned netrin-1 substrate. ICCS measurement of spatially segmented images based on the substrate nanodot patterns revealed distinct molecular distributions of F-actin and DCC in regions directly overlying the nanodots compared to over the reference surface surrounding the nanodots. Quantifiable variations between the populations of DCC and F-actin on and off the nanodots reveal specific responses to the printed protein substrate. We report that nanodots of substrate-bound netrin-1 locally recruit and aggregate DCC and direct F-actin organization. These effects were blocked by tetanus toxin, consistent with netrin-1 locally recruiting DCC to the plasma membrane via a VAMP2-dependent mechanism. Our findings demonstrate the utility of segmented ICCS image analysis, combined with precisely patterned immobilized ligands, to reveal local receptor distribution and signaling within specialized subcellular compartments.

1714-Pos Board B623

Open-Source Optical Projection Tomography of Large Organ Samples

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The study of biological mesoscopic samples (those with characteristic dimensions of 1-10 mm) benefits from techniques that can analyse and depict anatomical information on a whole organism level. Optical Projection Tomography (OPT) uses visible light to generate 3D imaging data of transparent mesoscopic objects (e.g. cleared mouse embryos) at micron-level resolution in both fluorescence and transmission. We present a low-cost OPT solution to image large transparent specimens (up to 17 x 14 mm) at a lateral resolution of ~23 um and provide an extensive open-source library for image calibration, reconstruction, and noise-reduction. In the development of treatment which induces alveolar cell proliferation and repair in the porcine pancreatic elastase (PPE) mouse model of emphysema, the use of OPT enables quantitative imaging of relevant proliferative cell lineage in cleared, large murine lung samples. The volume and distribution of alveolar cell clusters which have been labelled by immunofluorescence are measured in the intact lung of a mouse and visualized in 3D. Our design is easy to implement and is completely open-source, providing opportunities for further open microscopy developments for large organ imaging in model organisms.

1715-Pos Board B624

Flat-Field Illumination Microscopy for Large Field-of-View Quantitative Imaging

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Fluorescence imaging is a powerful tool for understanding of molecular mechanisms of cellular processes and functions. It is desirable to have a robust imaging method that enables us to obtain quantitative information. However, in laser-based fluorescence imaging, the nonuniform illumination distribution by the Gaussian-shaped beam results in severe problems for quantitative analysis of images. Additionally, it limits the field-of-view and leads to rapid photobleaching at the center of the beam. The solution for the uneven distribution is to have an even illumination beam. The method proposed is the reshaping of the Gaussian illumination profile into a Flattop illumination profile by using refractive optics. Our results showed that the flat-field illumination yielded far more leveled intensities and much wider field-of-view compared to Gaussian profile. We demonstrated epi- and TIRF illumination with multiple wavelengths. Our method is likely to combine with high-throughput imaging as well.

1716-Pos Board B625

Multicolor Two-Photon Fluorescence Lifetimes Microscopy by Wavelenght Mixing for Efficient and Simultaneus NADH and FAD Imaging Reveals Metabolic Shifts Associated to Cellular Differentiation and Oxidative Stress in Living Tissues

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Multiphoton imaging of metabolic coenzymes nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) is usually hampered by sequential dual wavelength excitation resulting in motion artifacts in dynamic tissues.

Here we implement multicolor two-photon Fluorescence Lifetime microscopy by combining wavelength mixing and efficient Fluorescence Lifetime microscopy (FLIM) to simultaneously and efficiently image NADH and FAD. Pulse trains from two laser beams are synchronized to give rise to two-color two-photon excited fluorescence through a virtual wavelength. We demonstrate simultaneously co-registered NADH and FAD images of comparable intensity, one shot ratiometric redox (FAD/(NADH+FAD)) imaging free of motion artifacts and simultaneous two-photon FLIM of NADH and FAD.

In living tissues we measure distinctive NADH and FAD lifetime gradients associated with cellular differentiation states and an increase in NADH and FAD lifetime associated to oxidative stress induced by UVA light exposure. We show that cellular heterogeneity in the ratiometric redox ratio (FAD/(NADH+FAD)) and FAD lifetime is different with respect to NADH lifetime distribution demonstrating that that these three parameters provide complementary information on the metabolic pathways and allow to identify unique metabolic cellular phenotypes.

1717-Pos Board B626

Long-Term Super-Resolution Imaging of Amyloid Structures using Transient Binding of Standard Amyloid Probes

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Oligomeric amyloid structures are crucial therapeutic and diagnostic targets in Alzheimer's disease and other amyloid diseases. However, these oligomers are too small to be resolved by conventional light microscopy. We have developed a new tool to image amyloid structures on a nanometer scale using standard amyloid dyes such as Thioflavin T (ThT), without the need for covalent labeling of the amyloid protein or staining via fluorescently labeled antibodies. Transient amyloid binding (TAB) imaging is compatible with epifluorescence and TIRF microscopies and uses 488 nm cw laser excitation to excite ThT molecules that are bound to amyloid structures. Dynamic binding and unbinding of ThT molecules generate photon bursts ('blinking') that are used for single fluorophore localization at nanometer resolution. Thus, photobleaching cannot degrade either the number or brightness of blinking events, which enables us to