1726-Pos Board B618
Imaging Proteins, Cells, and Tissues Dynamics during Embryogenesis with Two-Photon Light-Sheet Microscopy
Thai V. Truong, Daniel B. Holland, Vikas Trivedi, Scott E. Fraser.
California Institute of Technology, Pasadena, CA, USA.
Two-photon light sheet microscopy combines nonlinear excitation with the novel sheet-illumination, orthogonal to the detection direction, to achieve high penetration depth, high acquisition speed, and low photodamage, compared with conventional imaging techniques. These advantages allow unprecedented observation of the processes that govern embryogenesis, where the ability to image fast the dynamic three dimensional structure of the developing embryo, over extended periods of time, is critical. We present a selection of applications where two-photon light sheet microscopy is utilized to observe the dynamics of proteins, cells, and tissues, toward an understanding of the construction program of the developing embryos.

1727-Pos Board B619
Super-Resolution Localization of MAVS and RIG-I in MEF
Seongjin Park[1], Sultan Doganay[1], Zhijian J. Chen[2], Taekjip Ha[1].
[1]University of Illinois, Urbana, IL, USA, [2]University of Texas Southwestern Medical Center, Dallas, TX, USA.
Upon viral-infection, RIG-I-like receptor (RLR) recognizes cytoplasmic viral RNA and activates the mitochondrial antiviral-signaling protein (MAVS), which in turn activates the transcriptional factors to induce type I interferons (IFN). It has been reported that viral infection causes the majority of MAVS to form speckle-like aggregates on the outer membrane of mitochondria, while a fraction of recombinant MAVS form prion-like fibrils, and mitochondria themselves are redistributed closer to the nucleus. We designed a 3D two color super-resolution nanoscopy to investigate the morphology of MAVS fibrils and aggregates. As a reference for mitochondrial periphery, we imaged Tom20, a mitochondrial membrane protein, along with MAVS. We found that before infection MAVS proteins are uniformly distributed around mitochondria. However, after infection MAVS form aggregates on the mitochondrial membrane and constitute localized structures around the nucleus, while remaining on mitochondrial membrane. Our super-resolution approach allowed us to resolve the fine distribution of MAVS aggregates with respect to mitochondria. Moreover, we quantified the size distribution and morphology of MAVS clusters as a function of time after infection with high accuracy.

1728-Pos Board B620
Evaluating Ionic Conductances at the Single Vesicle Level using an Hybrid FRET Pair
Juan Pablo Vivar, Sebastian Brauchi.
Universidad Austral de Chile, Valdivia, Chile.
Introduction. In the chemical synapse, synaptic vesicles (SV) are in charge of neurotransmitter mobilization inside the cell. They are small sub-cellular structures defined by a lipid membrane containing different kinds of proteins including ion channels, transporters, and pumps all mediating ion exchange. This membrane can be considered an electric capacitor. The contribution of different conductances to the vesicular resting potential has been suggested but not described in detail, in part, due the limitations of the traditional techniques to access at this scale. We adapted a method initially used to follow fluctuations in plasma membrane potential, to evaluate changes in the electric potential of these subcellular structures. Material and methods. To monitor changes in the ionic permeabilities of SV we used a hybrid FRET pair: a GFP fused to a vesicular membrane protein and the hydrophobic ion dipicrylamine (DPA) which moves across the membrane in response to a change in the electrical potential, quenching the GFP emission. By this form, a change in membrane polarization is reinterpreted as a variation of the intensity of brightness. We setup the preparation of membrane sheets using a sonifier allowing full access to the intact docked vesicles. Single vesicle imaging was performed using a laser TIRF microscope coupled to a Hamamatsu Orca 12ER CCD camera. Results and Discussion. After loading DPA we perfused solutions with different ionic composition and record fluorescent fluctuations corresponding to the contribution of Na^+, K^+ and Cl^- ions to the vesicular resting potential.

1729-Pos Board B621
Illumination-Induced Changes in Action Potential during Optical Mapping in Langendorff-Perfused Rabbit Heart
Ruta Vosyliute[1], Arvydas Matiukas[2], Irma Martišienė[1], Jonas Jurevičius[1], Antanas Navalinskas[1], Rimantas Treinys[1], Regina Mačianskienė[1], Arkady M. Pertsov[1,2].
[1]Lithuanian University of Health Sciences, Kaunas, Lithuania, [2]SUNY Upstate Medical University, Syracuse, NY, USA.
Recent studies show that high-intensity laser illumination utilized in optical mapping studies can induce tissue heating and significantly affect action potential duration (APD). Here we investigate manifestations of this effect in Langendorff rabbit heart preparations during control and no-flow ischemia.
After isolation and cannulation the heart was immersed in a thermostat-controlled chamber at 37°C. Superfusion and perfusion were carried out with oxygenated Tyrode's solution at 20 ml/min and 10 ml/min, respectively. The tissue was stained with voltage-sensitive dye di-4-ANBDQBS. For excitation we used two lasers: a 670 nm laser with a holographic diffuser for constant broad-field illumination of the mapping area at with intensity of 1 mW/mm^2 and a 660 nm laser for which we used to probinge light-induced heating. The latter provided short-term (20s) spot-illumination of a small 5x5mm area with intensities of up to10 mW/mm^2. Fluorescence was recorded through a longpass 715nm filter using Andor iXon CCD camera at 500 Hz. Measurements were carried out during normal flow and global no-flow ischemia.
During normal perfusion high-intensity illumination induces APD shortening proportional to the light intensity, reaching ~10% at 10 mW/mm^2. The effect develops with the time constant $\tau \sim 5s$. Under ischemic conditions the effect develops slower ($\tau > 10s$) and is more pronounced. The heating effect superimposes onto ischemia-induced APD changes, causing additional shortening by up to 20%. The difference between the two cases can be explained by the presence/absence of perfusion, transporting away the heat produced inside the tissue. Our findings suggest that illumination-induced heating should be taken into consideration when designing optical mapping experiments with global and regional ischemia.
This research was funded by the European Social Fund under the Global Grant measure and NIH grant 5R03TW008039.

1730-Pos Board B622
Clustering of L-Type Cav1.2 Channels in Cardiomyocytes Visualized by a Custom-Built Fast Scanning STED Microscope
Yong Wu, Rong Lu, Xundong Wu, Pedro Felipe, Gardeazabal Rodriguez, Ligia Toro, Enrico Stefani.
University of California, Los Angeles, Los Angeles, CA, USA.
We directly visualize the cluster size distribution of the L-type Cav1.2 channels in isolated murine cardiomyocytes using super-resolution stimulated emission depletion (STED) microscopy and with antibody labeling. Cav1.2 channels play an essential role in excitation-contraction coupling of cardiac muscle by driving calcium ions into the cardiomyocytes. In fact, it has recently been shown that Cav1.2 channels can form functionally coupled small clusters to amplify the calcium ion influx. Such clustering of the Cav1.2 channels could not be directly observed due to the diffraction-limited resolution of conventional microscopes. We built a resonant scanning STED microscope achieving a lateral resolution of ~50 nm in a 60μm x 60 μm scanning area acquired at 16 nm per pixel. An 8 KHz resonant scanning mirror provides 16,000 lines per second imaging speed to shorten the exposure time in a single round of scanning, while between consecutive rounds of scanning, fluorophores may relax from the triplet states to the ground states rather than be photobleached. To keep up with the fast imaging speed, the output of the photomultiplier are sampled at 1.8 GHz to grab every detected photon. This microscope enabled us to clearly visualize the clusterized distribution of Cav1.2 channels immunolabeled by fluorescent dyes in isolated murine cardiomyocytes. We show in three different preparations that >80% of the Cav1.2 channels form larger clusters of size 100-150 nm along the T-tubules, and smaller clusters with a typical size of 50 nm (down to the resolution limit of our microscope) are inclined to be present between the T-tubules and on the periphery of the cells. These results provide additional evidence supporting the view that clustering enhances the functional role of Cav1.2 channels. Supported by NIH BRG RO1 HL088640.

1731-Pos Board B623
Volume Changes in Vesicles and Erythrocytes Probed by Microscopy and Spectroscopy at Variable Pressure
Alesia Antoine, Sang Hoon Park, Debopam Chakrabarti, **Alfons Schulte**.
University of Central Florida, Orlando, FL, USA.
High pressure can change the cell morphology and membrane fluidity. We combine high pressure microscopy and micro-spectroscopic probes to study pressure effects at the single cell level. In single erythrocytes large, reversible volume changes are observed over the pressure range from 0.1 to 200 MPa. To probe the role of the cell membrane we conduct high pressure studies on vesicles. Vesicles were extruded using 400 nm pores after being resolubilized in phosphate buffered saline (PBS). Giant vesicles were formed using extrusion, dehydration and rehydration techniques of the lipids 1,2-Dilauroyl-sn-glycero-3-phosphocholine (DLPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC.) using phosphate buffered saline to rehydrate the desiccated lipids. Vesicles are characterized by direct optical imaging and micro-Raman spectroscopy. The similarities and differences between these vesicles and erythrocytes with variations in pressure are investigated.