SIM1 G0901
nismicro@stanford.edu

Neuroscience Microscopy Service

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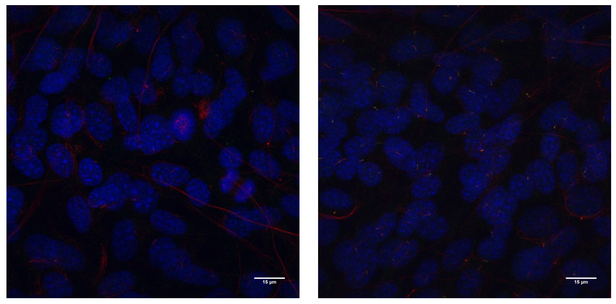
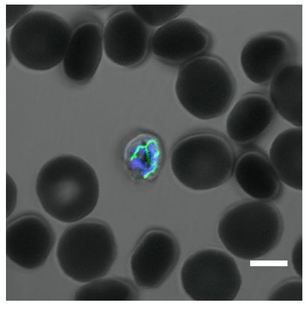
nisms.stanford.edu

Confocal

Zeiss LSM510 and LSM710, each fully equipped for live-cell imaging



Smoothened protein (green) accumulates in the primary cilium (acetylated tubulin, red) upon Hedgehog pathway activation with ShhN (right panel) in contrast to cells with no Hedgehog pathway activity (left panel). Images are of NIH-3T3 cells, maximum intensity projection from a 72 µm z-stack. Scale bar = 15 µm. James Kim, Phil Beachy lab



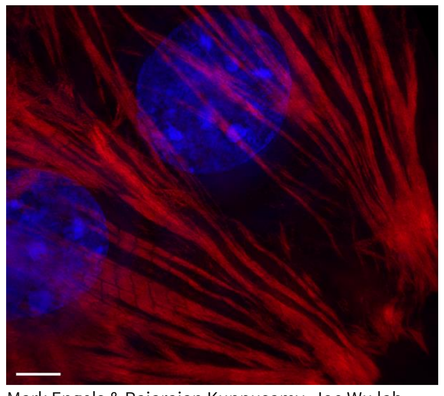
Plasmodium falciparum, one of the causative agents of malaria, is shown within a human red blood cell in this single plane from a confocal z-stack (left). As the parasite prepares for schizongony, it replicates its DNA (shown in blue) but has not yet divided. The apicoplast (green), an non-photosynthetic plastid organelle essential to the parasite, branches and must associate with each nuclei to be incorporated in the daughter cells upon division. These images are helping us to define how the apicoplast grows and develops through the life cycle of the parasite as well as determine what cellular events are required for each stage of apicoplast development. Scale bar = 5 µm.

The branching structure of the apicoplast is shown in this 3D rendering of a SIM super-resolution z-stack. These images are helping us to resolve details of the apicoplast three-dimensional structure otherwise unavailable by conventional microscopy. We hope to how perturbations of the cell cycle affect the morphology of the apicoplast. Scale bar = 5 µm. Katherine Amberg-Johnson and Jolyn Gisselberg, Ellen Yeh lab

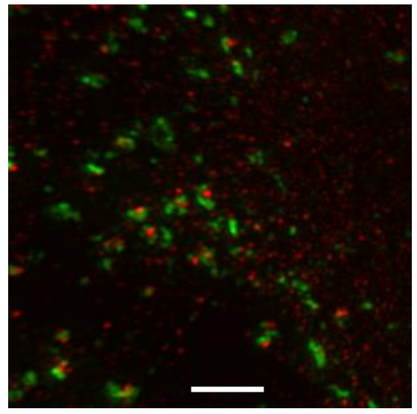
Super-Resolution

GE/Applied Precision OMX V4 with SIM /fast widefield /TIRF imaging featuring both structured illumination *and* localization super-resolution modes

Structured illumination super-resolution image of peri-somal synapses in cultured hippocampal neurons. Bassoon (presynaptic) in green and homer (post-synaptic) in red. Scale bar = 2 µm.

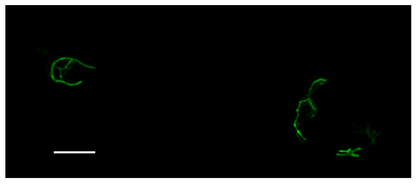


Mark Engels & Rajarajan Kuppusamy, Joe Wu lab



Sergio Leal-Ortiz, Craig Garner lab

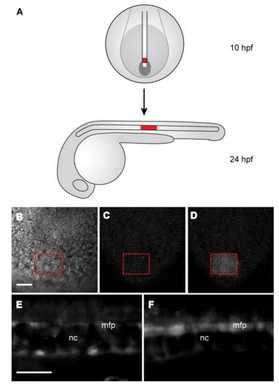
Embryonic stem cell-derived cardiac myocyte with troponin (red) and nuclear DNA (blue). Scale bar = 5 µm.



Two-Photon

An *in vivo* and a tissue slice rig, each equipped for simultaneous 2P imaging and 2P photoactivation

2-photon (2P) photoactivation of caged no-tail (*ntl*) morpholino alters cell fate in a spatially-defined volume of cells in a zebrafish embryo.



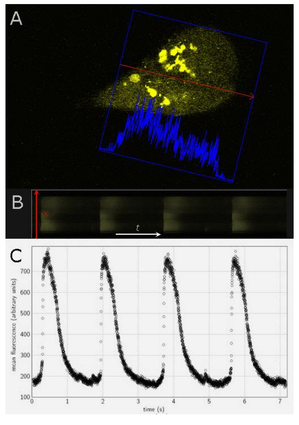
A) Schematic diagrams showing location of targeted cells (red rectangle, dorsal view) in the posterior axial mesoderm, irradiated at 10 hours post-fertilization (hpf). These cells normally differentiate into vacuolated notochord cells by 24 hpf (red rectangle, lateral view). B-D) 2P photoactivation protocol. Embryos are injected with a photoactivatable coumarin tracer dye and either an inactive caged oligomer or caged *ntl* morpholino. B) Dodt gradient contrast image of zebrafish posterior axial mesoderm, 10 hpf. Red rectangle shows area to be irradiated. C) Prior to photoactivation, no fluorescence is observed with 820 nm 2P excitation. D) After 2P irradiation for 2 min. at 750 nm (65 mW at back focal plane of the objective), photoactivated coumarin tracer fluorescence is observed in the irradiated area with 820 nm 2P excitation. E) At 24 hpf, the control shows photoactivated tracer fluorescence primarily in vacuolated notochord cells (nc), and little fluorescence in medial floor plate cells (mfp). F) In contrast, the caged *ntl*-injected embryo shows a significant increase in labeled mfp cells, and a corresponding decrease in labeled nc cells. Scale bars = 50 µm.

Shawn Ouyang and IlyaShestopalov, James Chen lab

Image Analysis

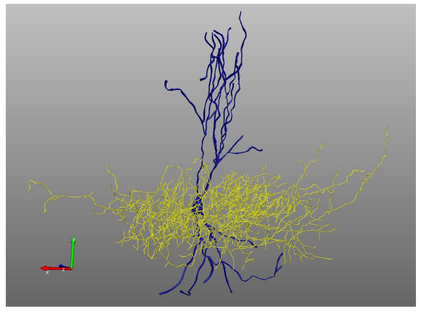
Two high-end workstations featuring **Neurolucida** for tracing neurons, **Imaris** for 3D visualization and analysis, **Matlab** and **Fiji/ImageJ** for image analysis

Spontaneous Calcium Transients in iPSC-derived Cardiac Myocytes: fast line-scanning with LSM510 confocal

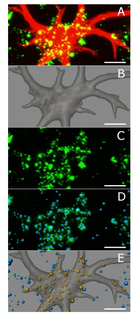


Panel A shows a single cultured cardiac myoctye, loaded with Fluo4 AM. The overlay in red shows the single line that was scanned to monitor changes in calcium levels over time. Panel B shows a series of 3700 sequential line scans with time increasing to the right. Panel C shows the transient influxes of calcium as the cultured myocyte spontaneously beats. Each point is the average background-subtracted Fluo4 fluorescence intensity of a single line scan. Scan line length = 70 µm.

Masayuki Yazawa, Ricardo Dolmetsch lab

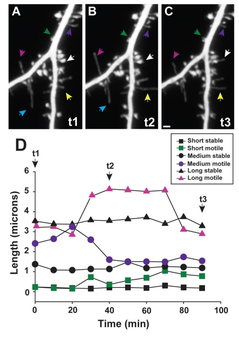


This biocytin filled neuron (at left) is a fast-spiking, parvalbumin-expressing GABAergic basket cell in the CA1 region of the mouse hippocampus. Dendrites (blue) of this cell type characteristically span through multiple hippocampal layers while their dense axonal arbor (yellow) innervates hundreds of cells in the pyramidal layer. Scale bars = 42µm. Csaba Földy, Thomas Südhof lab



Phagocytosis of neural debris by glial cells. Panel A shows the glial cells (red) and neural debris (green—phagocytosed debris tends to appear yellow). Panel B: cell surfaces were defined using smoothing and thresholding operations. Panels C–D: volume of neural debris was quantified by segmenting the green channel (C) into discrete spheres (D, in blue), using intensity thresholding and size criteria. Panel E: selection of debris spots that were either touching or enclosed by the cell surface (in orange). Scale bars = 10 µm. Won-Suk Chung, Ben Barres lab

Spine motility in the apical tuft of cortical L5 neurons in P27 YFP+ mouse (YFP-H line).



(A, B, C) Maximal intensity projection images from 2P Z-stacks acquired at three time points (t1 = 0 min, t2 = 40 min, t3 = 90 min). Some spines only retracted (blue and purple arrow) or extended (green arrow), some exhibited both retraction and extension (pink and yellow arrows), and in some spine-head shape change was followed by the formation of a new protrusion (white arrow). (D) Time courses showing changes in length of protrusions. Colored symbols in (D) represent the change in length of spines depicted in (A, B, C). Scale bar = 1 µm.

Maja Djurisic and George Vidal, Carla Shatz lab