THE KAVLI Institute for Brain and Mind

ABSTRACTS

Imaging the Brain: From Molecules to Circuits and Beyond

Friday, December 8, 2017

Chaired by Mark Ellisman (UC San Diego) & Jennifer Lippincott-Schwartz (Howard Hughes Medical Institute)

Sponsored by The Kavli Institute for Brain and Mind (KIBM)
Co-Directors: Rusty Gage (Salk Institute) & Nick Spitzer (UC San Diego)

SALK INSTITUTE FOR BIOLOGICAL STUDIES UC San Diego

Building the Brain: Dynamic in vivo Imaging of Synapse Assembly and Function

Daniel Colón-Ramos, Yale University

The human brain consists of an estimated 100 billion neurons and over 100 trillion synapses—there are more neurons in a single human brain than stars in the Milky Way galaxy. The capacity of a neuron to find its correct postsynaptic targets in this complex environment is critical for the formation of the precise circuits which underlie behavior. How neurons find the correct targets and how the correct wiring of the brain influences behavior are fundamental questions in neuroscience. To address them in a genetically tractable system we established WormGUIDES (Global Understanding in Dynamic Embryonic Systems), a novel resource that will result in the creation of the first cell biological atlas of embryogenesis and neurodevelopment for any animal. WormGUIDES will map, in 4D, all nuclear position and neurodevelopmental decisions for every neuron in the nematode, *C. elegans*, and from zygote until hatching. Foundational to WormGUIDES is a novel paradigm that links cutting edge microscopy, computational biology, developmental genetics, and neuroscience to continuously visualize, identify and image all neurons throughout *C. elegans* embryonic development. *C. elegans* is the only animal with a known wiring connectivity map, and the tools created through WormGUIDES will enable examination of how this connectome emerges during development. The impact of WormGUIDES could be transformative, providing a comprehensive resource that will enable examination of currently inaccessible aspects of neurodevelopment, and an understanding of how molecular signals can simultaneously, but precisely, coordinate wiring of neural circuits.

Revealing Secrets Hiding in Plain Sight

Mark Ellisman, UC San Diego

A grand goal in brain research is to understand how the interplay of structural, chemical, and electrical signals in and between neurons, glia, and the vasculature give rise to normal and abnormal functioning of nervous systems. New technologies are hastening progress as biologists make use of an increasingly powerful arsenal of tools and technologies for obtaining data, from the level of molecules to whole organs. This talk will highlight projects in which development and application of new contrasting methods and imaging tools have allowed us to observe otherwise complex or hidden relationships between cellular, subcellular, and molecular constituents of cells, particularly those comprising the brain.

Reverse Engineering the Fly Brain: Getting the Circuit Diagram

Harald Hess, Howard Hughes Medical Institute

If the brain functions like a computer processer, then deciphering its circuit should give us insight as to how it works. In fact, modern tools used in the semiconductor industry scan, inspect, or clone the wiring and billions of transistors used in a computer chip. With several hundred million synapses connecting all the nerve cells, a simple fly brain has the complexity close to that of modern processors and should be amenable to reverse engineering. The imaging challenges are however very different with the brain being a highly varying interconnected 3D structure while semiconductor circuits are self-similar with largely 2D layouts. This has driven the development of new 3DI electron microscopies that can resolve the requisite nanometer detail while spanning 3D distances approaching a millimeter. We will present such data for the case of the fruit fly brain, discuss the barrier of automated wiring reconstruction, and explore how such connectivity information might clarify brain function.

High-Speed Volumetric Imaging of Brain Activity

Na Ji, UC Berkeley and Howard Hughes Medical Institute

To understand computation in the brain, one needs to understand the input-output relationships for neural circuits and the anatomical and functional relationships between individual neurons therein. Optical microscopy has

emerged as an ideal tool in this quest, as it is capable of recording the activity of neurons distributed over millimeter dimensions with sub-micron spatial resolution. I will describe how we use concepts in astronomy and optics to develop next-generation microscopy methods for imaging neural circuits at higher resolution, greater depth, and faster speed. To understand computation in the brain, one needs to understand the input-output relationships for neural circuits and the anatomical and functional relationships between individual neurons therein. Optical microscopy has emerged as an ideal tool in this quest, as it is capable of recording the activity of neurons distributed over millimeter dimensions with sub-micron spatial resolution. I will describe how we use concepts in astronomy and optics to develop next-generation microscopy methods for imaging neural circuits at higher resolution, greater depth, and faster speed.

Imaging Exocytosis and Endocytosis at Synapses Using Electron Microscopy

Erik Jorgensen, University of Utah

To understand the rapid membrane dynamics or cells or synapses requires observations at high spatial and temporal resolution. “Flash-and-freeze” electron microscopy combines optogenetics with electron microscopy to capture millisecond changes in synaptic morphology during neurotransmission. These experiments indicate that membrane is internalized as quickly as 30 ms after exocytosis. Because of its rapid speed, we call this process “ultrafast endocytosis.” The internalized membrane then fuses to form a synaptic endosome which is resolved into synaptic vesicles with a t-1/2 of 5 seconds. I will discuss improvements to the instrumentation that will facilitate resolution of membrane fusion, which particularly difficult since exocytosis occurs within 500 microsceonds of stimulation.

Whole-Animal Imaging with High Spatio-Temporal Resolution

Philipp Keller, Howard Hughes Medical Institute

Light-sheet fluorescence microscopy has emerged as a powerful imaging technique that provides exceptionally high imaging speed and high spatial resolution while minimizing the amount of light energy used to interrogate the specimen. This combination of capabilities makes light-sheet microscopy indispensable for developmental and functional *in vivo* imaging of complex biological systems with high spatio-temporal resolution.

We are developing advanced implementations of light-sheet microscopy, such as our SiMView, hs-SiMView, and IsoView microscopes for simultaneous multi-view imaging of large living specimens, and are further enhancing these instruments by adaptive imaging techniques for improving spatial resolution and automating complex imaging experiments. We are using these methods to systematically reconstruct whole-embryo development in multiple model systems (fruit fly, zebrafish, and mouse) at the single-cell level and to perform high-resolution functional imaging of the entire early nervous system.

Complementing these imaging techniques, we are developing strategies for automated, efficient and robust image processing of the resulting large-scale microscopy data sets, including methods for multi-view data processing, cell segmentation, and cell tracking. This combined experimental and computational framework allows us to quantitatively analyze neuronal activity across the nervous system of behaving animals, systematically extract developmental lineages and their interrelationships at the system level, and link this developmental building plan to emerging functional properties of the early nervous system.

Navigating the Cellular Landscape with New Imaging Technologies

Jennifer Lippincott-Schwartz, Howard Hughes Medical Institute

New imaging techniques are helping to reveal the complex dynamics of cells, including how multi-scale structural relationships form and self-adjust to changing environmental conditions. In this talk, I will discuss emerging fluorescent technologies that are increasing spatio-temporal resolution and permitting simultaneous multispectral imaging of multiple cellular components. Using these tools, it is now possible to begin constructing an “organelle interactome” describing the interrelationships of different cellular organelles as they carry out critical functions. The same tools are also revealing new properties of the cell’s largest organelle, the endoplasmic reticulum, and how disruptions of its normal function due to genetic mutations may contribute to important diseases.

Genetically Encoded Tools for Brain Analysis

Atsushi Miyawaki, RIKEN Brain Science Institute

In a signal transduction diagram, arrows are generally used to link molecules to show enzymatic reactions and intermolecular interactions. To obtain an exhaustive understanding of a signal transduction system, however, the diagram must contain three axes in the space and the time base, because all events are regulated ingeniously in space and time. The scale over time and space is ignored in biochemical approaches in which electrophoresis is

applied to a specimen prepared by grinding millions of cells. A farseeing article entitled, “Fluorescence Imaging Creates a Window on the Cell,” was written by Roger Tsien in 1994, which appeared in *Chemical & Engineering News*. He advocated employing the so-called real-time and single-cell imaging technique to fully appreciate cell-to-cell heterogeneity. He also had steadfastly pursued the creation of a reliable gate that would enable researchers to better understand the “feelings” of individual cells.

Over the past two decades, various genetically encoded probes have been generated principally using fluorescent proteins, and are used to investigate the function of specific signaling mechanisms in synaptic transmission, integration, and plasticity. I will discuss how the probes have advanced our understanding of the spatio-temporal regulation of biological functions inside cells, neurons, embryos, and brains. I will speculate on how these approaches will continue to improve due to the various features of fluorescent proteins that serve as the interface between light and life.

Due to recent remarkable progress in gene transfer techniques, including electroporation, virus-mediated gene transfer, and germline transmission of transgenes, the experimental animals to be studied are not limited to mice but extended to primates. Newly emerging genetically encoded tools will surely stimulate the imagination of many neuroscientists, and this is expected to spark an upsurge in the demand for them.

Optical Tools for Unraveling Whole-Brain Neuronal Circuit Dynamics Underlying Behavior

Alipasha Vaziri, The Rockefeller University

The combination of optogenetics and high speed functional imaging are providing new opportunities to understand how the collective dynamics of neurons in functional networks leads to behavior.

While traditional imaging modalities based on two-photon imaging have relied on the manipulations of light in the spatial domain, multi-photon microscopy via femtosecond optical pulses can also provide a new degree of freedom via the pulse spectrum that can be used to “sculpt” the spatial localization of light within the sample. This has been exemplified in the technique of temporal of focusing through which a decoupling of the axial from the lateral confinement of light can be achieved. Using this technique in combination with genetically encoded calcium (Ca2+) indicators, we have demonstrated near-simultaneous recording of whole-brain neuronal activity in *C. elegans* at single cell resolution. More recently we developed a variant light sculpting microscopy that has enabled unbiased single- and dual-plane high-speed (up to 160 Hz) Ca2+ imaging in the mouse cortex as well as *in vivo* volumetric calcium imaging of a mouse cortical column (0.5 mm×0.5 mm×0.5 mm) at single-cell resolution and fast volume rates (3–6 Hz). This has enabled *in vivo* recording of calcium dynamics of several thousand neurons across cortical layers and in the hippocampus of awake behaving mice.

Light-field microscopy in combination with 3D deconvolution and other more sophisticated mathematical signal demixing strategies is another highly scalable approach for high-speed volumetric Ca2+ imaging. Using this technique termed, Seeded Iterative Demixing (SID), we have recently demonstrated video-rate recoding of neuronal activity within a volume of 0.6mm×0.6 mm×0.2 mm located as deep as 380μm in the scattering mouse as well as whole-brain imaging of larval zebrafish during sensory stimulation. These tools combined with high-speed optogenetic control of neuronal circuits, advanced statistics tools and mathematical modeling and will be crucial to move from an anatomical wiring map towards a dynamic map of neuronal circuits.