Thursday, October 20, 2011

Center for Biological Imaging
&
Center for Nanoscale Systems

Harvard University, Cambridge, MA

Meeting Schedule:

1:30 pm **Workshop Registration:** Center for Biological Imaging (CBI), Room 2058

2:00 pm **Workshops:** BioSEM, BioTEM, CARS, Super Resolution SI/PAL-M, and X-Ray MicroCT – *see workshop descriptions for locations*

4:00 pm **Meeting Registration and Tour of CBI:** CBI, Room 2058 – Refreshments Served in Room 1079

5:20 pm **Welcome (Biolabs Building 1080):** Richard Schalek, *NESM President*

5:30 pm **"Imaging the Connectome"**, Jeff Lichtman, Ph.D., Harvard University

6:30 pm **Dinner**

7:45 pm **"Super Resolution Light Microscopy: Structured Illumination and PAL-M"**, Bernhard Goetze, Ph.D., Zeiss

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Workshops

BioSEM: Variable Pressure and Environmental SEM Workshop – Adam Graham

Center for Nanoscale Systems, 11 Oxford St, LISE B20A, Cambridge MA 02138

In this workshop we will work with uncoated and unprocessed samples to demonstrate variable pressure on a Zeiss EVO SEM located in the Center for Nanoscale Systems (CNS) at Harvard University. This application is ideal for investigating native plant tissues and biological samples. We will also spend some time working in WET mode which allows us to image hydrated samples keeping the working chamber at 40-100% humidity. This capability has many interesting applications for both biological and material samples.

BioTEM: Energy Filtered Transmission Electron Microscopy (EFTEM) Workshop – Carolyn Marks

Center for Nanoscale Systems, 11 Oxford St, LISE B20B, Cambridge MA 02138

Energy filtering provides a way to achieve contrast in TEM samples composed exclusively of light elements. This workshop will cover the basics of working with an energy filtering system to increase contrast in biological and polymer samples. Attendees are encouraged to bring a prepared grid of a low contrast sample for examination. We will be using the Zeiss Libra 120 EFTEM housed within the Center for Nanoscale Systems (CNS) at Harvard University.

CARS Imaging Workshop – Dr. Arthur McClelland

Center for Nanoscale Systems, 11 Oxford St, LISE G04, Cambridge MA 02138

Coherent Anti-Stokes Raman Scattering (CARS) imaging is a label-free optical imaging technique based on the Raman response of the sample. By using the anti-Stokes signal spectral interference from auto fluorescence that commonly plagues biological samples is suppressed. This nonlinear optical technique also allows the mapping of 3D distributions of small molecules in a sample. The workshop will cover the basics of CARS imaging and introduce users to the CARS imaging setup at Harvard's Center for Nanoscale Systems (CNS).

Super Resolution: SI and PAL-M Imaging Workshop – Dr. Bernhard Goetze

Center for Biological Imaging, 16 Divinity Ave, Biological Labs Room 2052, Cambridge MA 02138

Structured Illumination (SI) and Photo-Activated Localization Microscopy (PAL-M) are two of the super resolution light microscopy techniques that have recently been developed and commercialized. SI has a resolution of ~100nm. PAL-M has a resolution of 20-40nm. This workshop will introduce these two super resolution techniques and introduce participants to the Zeiss Elyra system at Harvard's Center for Biological Imaging (CBI). The CBI is an open user facility at Harvard University.

X-Ray Imaging and MicroCT Workshop – Dr. Turgut Fettah Kosar

Center for Nanoscale Systems, 11 Oxford St, LISE G27, Cambridge MA 02138

X-Ray imaging has come a long way since the first radiograph taken by Wilhelm Conrad Röntgen in 1895. Over the last century, the techniques advanced from taking low-resolution two-dimensional (2D) projections of mostly biological samples to high-resolution imaging and three-dimensional (3D) reconstruction of volumes of a broad range of objects and artifacts. This 3D recreation of scanned sample volumes is commonly referred to as computed tomography (CT). A typical X-ray CT system can non-destructively produce both 2D and 3D images of samples made of almost any material. There are now stand-alone laboratory systems available on the market that are capable of achieving 50nm spatial resolution without the need of a very bulky and expensive synchrotron X-ray source. The applications of these techniques are very diverse, spanning fields such as medicine, materials science, mechanical and aeronautical engineering, microelectronics, geology, anthropology, museum studies, biological sciences, and many others.

This short workshop will provide an introduction to the basic theory and practice of X-ray imaging and CT at micrometer length scales, also known as X-ray microCT. We will go through the basics of using X-rays to image internal features of samples and creating 3D computer images. The workshop will be held on the X-Tek HMX ST 225 microCT system located in the Center for Nanoscale Systems (CNS) at Harvard University. No prior knowledge of X-ray imaging is required.

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Talks & Bios

5:30 pm – **Imaging the Connectome**

Dr. Jeff Lichtman, Harvard University

Abstract:
Connectional maps of the brain may have value in developing models of both how the brain works and how it fails when subsets of neurons or synapses are missing or misconnected. Such maps might also provide detailed information about how brain circuits develop and age. I am eager to obtain such maps in neonatal animals because of a longstanding interest in the ways neuromuscular circuitry is modified during early postnatal life as axonal input to muscle fibers is pruned. Work in my laboratory has focused on obtaining complete wiring diagrams ("connectomes") of the projections of motor neuron axons in young and adult muscles. Each data set is large and typically made up of hundreds of confocal microscopy stacks of images which tile the 3dimensional volume of a muscle. As a first step to analyze these data sets we developed computer assisted segmentation approaches and to make this task easier, have developed second generation "Brainbow" transgenic mice that in essence segment each axon by a unique fluorescent spectral hue. Once the axons are segmented, we have been able to graph the connectivity matrices that result. This effort has led to new insights into the developmental processes which help the mammalian nervous system mold itself based on experience. Analysis of these complete muscle connectomes show a striking single axis gradient of connectovity that we think is related to the ordered ranking of neural activity in axons (the "size principle" of Henneman). In brain however, as opposed to muscle, the high density of neuropil is overwhelming, which has precluded using the confocal optical approaches that have worked in the peripheral nervous system because there are too many neural processes in each optical section. We have thus developed of lossless automated physical sectioning strategy that generates thousands of ultra thin (~25 nm) sections on a firm plastic tape. We have developed a thin-section scanning electron microscopy approach to visualize these sections at 3nm lateral resolution. This method makes large scale serial microscopic analysis of brain volumes more routine. We are now focused on developing an automated pipeline to trace out neural circuits in brains using this technique.

Bio:
Jeff Lichtman is Jeremy R. Knowles Professor of Molecular and Cellular Biology at Harvard. He received an AB from Bowdoin (1973), and an M.D. and Ph.D. from Washington University (1980) where he worked for 30 years before moving to Cambridge in 2004. He is a member of the newly established Center for Brain Science. Lichtman's research interest revolves around the question of how mammalian brain circuits are physically altered by experiences, especially in early life. He has focused on the dramatic re-wiring of neural connections that takes place in early postnatal development when animals are doing most of their learning. This work has required development of techniques such as "Brainbow"

transgenic mice to visualize neural connections and monitor how they are altered over time. Recently his efforts have focused on developing new electron microscopy methods to map the entire wiring diagram of the developing and adult brain. This "connectomics" approach has as one of its aims uncovering the ways information is stored in neural networks.

7:45 pm – **Super Resolution Light Microscopy: Structured Illumination and PAL-M**

Dr. Bernhard Goetze, Zeiss

Abstract:
This talk will give an overview of two super resolution technologies (SIM and PAL-M). The focus of this talk is both on the technological side as well as the applications which can be done with those approaches. SIM (Structured Illumination Microscopy) is a technology where we use overlaying patterns and the resulting Moire Fringes to double the resolution of a light microscope (90-120nm). PAL-M (Photoactivation Light Microscopy) uses the sub-pixel precise localization of single fluorescent molecules to achieve resolutions down to 20nm with a conventional light microscope.

Bio:
Bernhard Goetze studied Biology at the University of Hohenheim / Stuttgart with a focus on Olfaction and Physiology for his Masters. He obtained his Ph.D. from the University of Tuebingen where he did his thesis at the Max Planck Institute of Developmental Biology about RNA binding proteins and local protein synthesis at synapses in hippocampal neurons. Bernhard joined Zeiss Germany in 2006 as a product manager for confocal microscopes, confocal software and super resolution microscopes. Since June 2010 Bernhard heads the group of embedded consultants of Carl Zeiss Microscopy LLC in the US and works at the CBI at Harvard University.

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