

Spring Symposium & Workshops @ Marine Biological Laboratory, Woods Hole

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Meeting Description:

Join NESM for our Spring Symposium & Workshops on May 2-3, 2013 at the Marine Biological Laboratory, Woods Hole, MA. The meeting is composed of Thursday afternoon workshops and Friday seminars. Registration closes 12:00 PM, April 22.

Meeting Costs (including a buffet lunch and an afternoon coffee break):

\$15 Workshops
\$115 Exhibiting Vendors (includes 6'x3' table and drape and regular member registration)
\$60 Regular Members
\$85 Regular Non-members (includes 2013-year membership)
\$30 Student Members
\$40 Student Non-members (includes 2013-year membership)
\$50 Retiree Members
\$60 Retiree Non-members (includes 2013-year membership)
Late Registration: Additional \$5

Meeting Schedule:

Workshops - Thursday, May 2

1:00PM - **Welcome:** Louie Kerr, *NESM Treasurer*

1:10PM - **Workshops Part I**

2:30PM - **Afternoon Break:** Coffee and refreshments

3:00PM - **Workshops Part II**

5:00PM - **Closing Remarks:** Louie Kerr, *NESM Treasurer*

Symposium - Friday, May 3

9:00AM - **Registration (Swope Center):** Coffee and refreshments

10:00AM - **Welcome (Meigs Room):** Fethah Kosar, *NESM President*

10:10AM - "Investigating neuronal signaling in dendrites, axons and synapses using fluorescence microscopy and organic voltage-sensitive dyes", Dejan Zecevic,

Ph.D., *Yale University School of Medicine*

10:50AM - “**Microscopic vibrational analysis in the world of art**”, Thomas Tague Jr., Ph.D., *Bruker Optics, Inc.*

11:30AM - **Vendor/Poster Session**

12:30PM - **Lunch (Swope Center)**

1:30PM - **Keynote: “From live cells to resin sections in 6 hours”**, Kent McDonald, Ph.D., *University of California, Berkeley*

2:30PM - **Afternoon Break:** Coffee and refreshments

3:00PM - “**Exploring functional interactions and dissociations between discrete brain regions during memory task performance in rats**”, Amy L. Griffin, Ph.D., *University of Delaware*

3:40PM - “**Application of EDS+EBSD to understand pipe corrosion in a power plant setting**”, Robert Brandom, *Bruker AXS*

4:20PM - **Closing Remarks:** NESM Board

Workshop Abstracts

Note: Workshops run concurrently.

High Pressure Freezing and Quick Freeze Substitution Methods – Kent McDonald, University of California, Berkeley

Capacity: 20

Biological tissue preparation for transmission electron microscopy has changed little over the last 50 years, and many of these techniques require multiple days in order to yield polymerized specimen blocks. This workshop will discuss the merits of high pressure freezing and demonstrate updated methods for quick freeze substitution and rapid resin infiltration/polymerization on cryoprotected-plunge frozen tissue samples. These refined methods require only 6 hours to process fresh tissue into polymerized blocks without compromising the quality of sample preservation.

Microanalysis with Optical and Infrared Microscopy – Thomas Tague Jr. & Fred Morris, Bruker Optics

Capacity: 6

This workshop will be focused on the principles of sample observation and characterization utilizing optical microscopy and infrared microscopy. Optical microscopy is traditionally the best tool for visual characterization allowing careful observation of the physical properties of the sample. Infrared spectroscopy, in general, is used to determine the sample chemical properties. Applications to art, pharmaceutical products, forensic evidence, and material science samples will be presented to demonstrate the capabilities of spectroscopic microanalysis as well as the limitations therein. Lastly, sample preparation and presentation for microanalysis will be demonstrated.

A demo unit of the LUMOS system will be available for hands on time. Feel free to bring samples to analyze.

Talk Abstracts & Bios

“Investigating neuronal signaling in dendrites, axons and synapses using fluorescence microscopy and organic voltage-sensitive dyes”, Dejan Zecevic, Ph.D., *Yale University School of Medicine*

Abstract: A central question in neuronal network analysis is how the interaction between individual neurons produces behavior and behavioral modifications. This task depends critically on how exactly signals are integrated by individual nerve cells functioning as complex operational units. Regional electrical properties of branching neuronal processes, which determine the input-output function of any neuron, are extraordinarily complex, dynamic, and, generally, impossible to predict in the absence of detailed measurements. To obtain such a measurement one would, ideally, be able to monitor subthreshold events at multiple sites as they travel from the sites of origin (synaptic contacts on distal dendrites) and summate at particular locations to influence action potential initiation. It became possible recently to carry out this type of measurement using high-resolution multisite recording of membrane potential changes with intracellular voltage-sensitive dyes. This approach, based on laser light excitation in wide-field epifluorescence microscopy mode, allows for the analysis of membrane potential signaling in all parts of the dendritic tree at single spine resolution as well as in axon collaterals and presynaptic axon terminals.

Bio: Dejan Zecevic is a Senior Research Scientist in the Department of Cellular and Molecular Physiology, Yale University School of Medicine. He received the Ph.D. in Biophysics from The University of Belgrade, Serbia and was trained in the laboratory of Dr. Lawrence Cohen who initiated the field of voltage-sensitive dye recording. Dejan is a pioneer of single neuron voltage-sensitive dye imaging techniques, a unique and cutting edge technology for monitoring membrane potential signals at multiple locations in individual neurons with sub-millisecond and sub-micrometer resolution.

“Microscopic vibrational analysis in the world of art”, Thomas Tague Jr., Ph.D., *Bruker Optics, Inc.*

Abstract: This seminar will be focused on the principles of sample observation and characterization of works of art utilizing optical microscopy and vibrational microscopy. Optical microscopy is traditionally the best tool for visual characterization, allowing careful observation of the physical properties of the sample. Vibrational (infrared and Raman) spectroscopy, in general, is used to determine the sample chemical properties. The highly complementary nature of these methods, coupled with a careful analysis of technique and provenance, allow the conservator to more carefully and accurately authenticate objects. Additionally, these same methods can be applied to carefully determine the chemical composition of objects facilitating the conservation process. The use of optical, infrared, and Raman microanalysis for the authentication of the lost Leonardo Da Vinci *Salvator Mundi* will be described. The conservation and restoration of ancient pictographs at the Hueco Tanks using these same methods will also be described.

Bio: Thomas Tague is the Applications Manager for Bruker Optics. He is also a member of the Advisory Boards of the Metropolitan Museum of Art in New York and EOS Photonics. Dr. Tague received his Ph.D. in 1992 from the University of Utah in Chemistry and his B.S., also in Chemistry, from the University of Texas at San Antonio in 1988. He is a member of the American Chemical Society, Society for Applied Spectroscopy, and the Microscopy Society of America. Tom has more than 45 publications and 5 patents.

Keynote: “From live cells to resin sections in 6 hours”, Kent McDonald, Ph.D., *University of California, Berkeley*

Abstract: Biological electron microscopists seem to be among the most conservative of people when it comes to changing their habits of specimen preparation. The response to

the question "Why do you use that particular method for preparing your samples?" is usually "We know it works and we don't want to change it", or, "This is the way we've always done it". Many of the common procedures found in the contemporary literature are essentially the same as those found in the literature from the mid-1960's or early 1970's. This is true for conventional benchtop processing and also for cryomethods that were developed in the 80's. About 2 years ago we began to do experiments to see if these "legacy" methods were really essential, or just habit. In particular, we wanted to see if we could reduce the turnaround time from live cell to resin sections from the usual times of days, or even a week or more, to something like a day or less. Because we are both convinced that cryofixation and processing methods produce the best results, we began our studies with freeze substitution, but then moved on to investigate resin infiltration and polymerization methods. An exception to some of these generalizations about long times is microwave processing, so we also wanted to see if that technology was really essential for short specimen preparation times. Freeze substitution (FS) is a process by which water in frozen cells is replaced (substituted) with a liquid organic solvent at low temperatures, usually in the -80 to -90 °C range. Automated freeze substitution devices costing tens of thousands of dollars are frequently used to control the time and temperature of warming the cells to higher temperatures, though some researchers prefer to use dry ice and a cooled metal block to achieve the same ends. Regardless of the method used it is common for FS to take 2-3 days and with plant samples the time can be twice as long. We have shown that it is possible to do freeze substitution in 2-3 hours using simple and inexpensive equipment. Some of these results will be presented representing a variety of cell and tissue types including plant leaves, bacteria, *C. elegans*, yeast, *Drosophila* embryos, and tissue culture cells. Details of how we currently set up and carry out the procedures will be presented.

After freeze substitution one still needs to infiltrate the samples with resin and polymerize that resin. Would it be possible to shorten those procedures also, but without use of an expensive research-grade microwave oven? The answer turns out to be yes, and in this presentation results will be presented from material that was rapidly frozen, freeze substituted, infiltrated with resin and polymerized in 6 hours or less. When writing up these results we discovered that rapid infiltration and polymerization schemes are not new. Hayat reviews some of these early studies that were published but somehow ignored or forgotten.

Our current work concerns methods for immunolabeling that are fast yet preserve cellular morphology in the absence of traditional fixatives such as aldehydes. Some preliminary results will be presented.

Bio: McDonald received his Ph.D. in Botany from the University of California, Berkeley in 1972. Following a year teaching at UCLA, he took a Post-doctoral position with J.D. Pickett-Heaps at the University of Colorado, Boulder to study the ultrastructure of mitosis in algae. In 1975, he joined the laboratory of J.R. McIntosh in Boulder to re-focus his research on the cell biology of mitosis. This led him back to Berkeley in 1979 to work on correlative light and electron microscopy of PtK cells and diatoms in the lab of W. Zacheus Cande. In 1987 he returned to Boulder to join the High Voltage EM Lab begun by Keith Porter and subsequently being run by J.R. McIntosh. It was at this time that the importance of cryofixation by high pressure freezing became a compelling interest that persists to this day. In 1993 he moved back to Berkeley to assume the Directorship of the campus Electron Microscope Laboratory, a teaching and research resource for UC Berkeley and other Bay Area institutions. His current research interest is improving the turnaround times for basic specimen processing procedures for biological electron microscopy.

"Exploring functional interactions and dissociations between discrete brain regions during memory task performance in rats", Amy L. Griffin, Ph.D., *University of Delaware*

Abstract: The hippocampus has been shown to be crucial for memory, but also plays a larger role in a system of brain structures that allow for memory-guided behavior. Of particular interest is the medial prefrontal cortex, which receives direct anatomical projections from hippocampus and is thought to use retrieved memories to construct a plan of action during task performance and to aid in memory consolidation following learning. Utilizing the technique of in vivo neurophysiology in conjunction with inactivation of discrete brain regions, we are uncovering the nature of the interplay between key structures known to be involved in learning and memory processes.

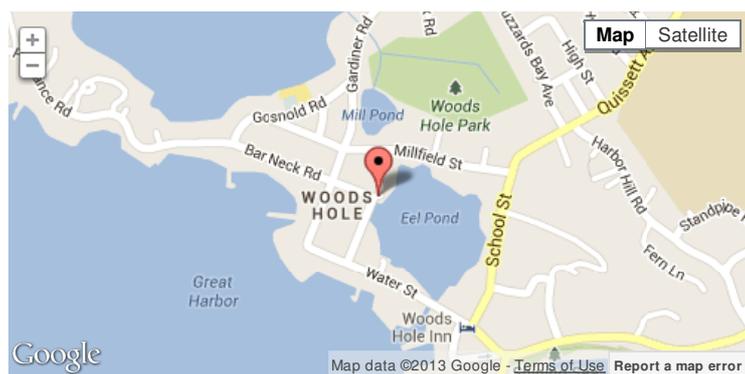
Bio: Dr. Amy Griffin received her Ph.D. in 2003 from Miami University, where she studied neural activity patterns underlying rabbit classical conditioning under the direction of Stephen Berry. For her post-doctoral work, Dr. Griffin had a joint appointment in the laboratories of Michael Hasselmo and Howard Eichenbaum in the Center for Memory and Brain at Boston University recording populations of single hippocampal neurons during memory task performance in rats, supported through an NRSA postdoctoral fellowship from the National Institutes of Mental Health. In 2007, Dr. Griffin joined the University of Delaware Psychology Department as an assistant professor. The focus of her research program is to explore the neural mechanisms of memory using two main approaches: investigating the effects of discrete brain region inactivation on memory task performance and recording populations of single neurons in freely moving rats during memory tasks. Together, these approaches are aimed at discovering the neural mechanisms driving memory-guided behavior.

"Application of EDS+EBSD to understand pipe corrosion in a power plant setting", Robert Brandom, *Bruker AXS*

Abstract: Scanning electron microscopes provide the electron beam for a number of useful analytical techniques including energy-dispersive x-ray spectroscopy (EDS) and electron backscatter diffraction (EBSD). In this study, a steam pipe from a coal-fired power plant was examined to determine the chemistry and phases present in the corroded layer of the pipe; however, EDS alone could not determine the phases. EBSD data, acquired simultaneously with the EDS data, helped to identify the phases thanks to their chemistry and crystallography.

Bio: Robert is a sales engineer for Bruker AXS in the northeastern US, working with customers that use SEM/TEM/FIB/microprobe techniques that include EDS, EBSD, WDS, XRF-EM (x-ray source on the SEM) and micro-CT on the SEM, and bench-top micro-XRF systems. He has an M.S. in geology from the University of Missouri-Rolla, where he focused on mineral exploration, ore microscopy and mineral liberation microscopy. He also has an MBA from the University of Minnesota. He was previously an SEM applications engineer for Leica and US marketing manager for LEO Electron Microscopy, and CD-SEM product manager for Schlumberger.

Location:



Swope Center
250 MBL Street
Woods Hole, MA 02543
Phone: 508-548-3705

Parking:

Free parking facilities are located on site. Please register first in the Swope Center (15 on map) to receive your parking pass. [Campus Map](#)

Housing:

Housing accommodations are available via the MBL for Thursday night. To arrange for housing, please contact [Liz McCarthy](#) at 508-289-7214.

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Banner - Blair Rossetti, Marine Biological Laboratory
