
# Friday, May 3, 2013

## Symposium Schedule:

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

10:00AM - **Welcome (Meigs Room):** Fettah 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 (Lillie Auditorium): "From live cells to resin sections in 6 hours"**, Kent McDonald, Ph.D., *University of California, Berkeley*

2:30PM - **Afternoon Break (Sponsored by Xradia):** 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*

## 30TH ANNUAL SPRING SYMPOSIUM

# CORPORATE VENDOR EXHIBIT

We extend our deepest gratitude and thanks to all our exhibiting vendors for their financial support. A portion of the cost for a table display is used to reduce registration costs for students and retirees.

Advanced Microscopy Techniques, Corp.

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# 30TH ANNUAL SPRING SYMPOSIUM
# ABSTRACTS & BIOS

### 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*

**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.

### 10:50AM – "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.

## 1:50PM – 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.

## 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*

**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.

## 3:40PM – "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.

# 30TH ANNUAL SPRING SYMPOSIUM
# POSTER EXHIBIT

### Cuttlefish skin papilla morphology suggests a muscular hydrostatic function for rapid changeability

J.J. Allen, G.R. Bell, A.M. Kuzirian, R.T. Hanlon, *Brown University* and *Marine Biological Laboratory*

Coleoid cephalopods adaptively change their body patterns (color, contrast, locomotion, posture, and texture) for camouflage and signaling. Benthic octopuses and cuttlefish possess the capability, unique in the animal kingdom, to dramatically and quickly change their skin from smooth and flat to rugose and three-dimensional. The organs responsible for this physical change are the skin papillae, whose biomechanics have not been investigated. In this study, small dorsal papillae from cuttlefish (Sepia officinalis) were preserved in their retracted or extended state, and examined with a variety of histological techniques including brightfield, confocal, and scanning electron microscopy. Analyses revealed that papillae are composed of an extensive network of dermal erector muscles, some of which are arranged in concentric rings while others extend across each papilla's diameter. Like cephalopod arms, tentacles, and suckers, skin papillae appear to function as muscular hydrostats. The collective action of dermal erector muscles provides both movement and structural support in the absence of rigid supporting elements. Specifically, concentric circular dermal erector muscles near the papilla's base contract and push the overlying tissue upward and away from the mantle surface, while horizontally arranged dermal erector muscles pull the papilla's perimeter toward its center and determine its shape. Each papilla has a white tip, which is produced by structural light reflectors (leucophores and iridophores) that lie between the papilla's muscular core and the skin layer that contains the pigmented chromatophores. In extended papillae, the connective tissue layer appeared thinner above the papilla's apex than in surrounding areas. This result suggests that papilla extension might create tension in the overlying connective tissue and chromatophore layers, storing energy for elastic retraction. Numerous, thin subepidermal muscles form a meshwork between the chromatophore layer and the epidermis and putatively provide active papillary retraction.

### Dissecting the Mechanisms of Katanin-Mediated Microtubule Severing and Depolymerization

M.E. Bailey, J.D. Diaz, J.L. Ross, *UMass Amherst*

Microtubules are important for forming networks in cells from mitotic spindles and cilia to neuronal networks. Microtubule networks are regulated by a variety of proteins including microtubule-severing enzymes, which regulate the length of microtubule filaments through severing and depolymerization. Katanin, the first-discovered microtubule severing enzyme, is a AAA+ enzyme that oligomerizes into hexamers and uses ATP hydrolysis to sever microtubules. It has been suggested that katanin targets to microtubule defects, and we showed that Katanin targets interfaces between microtubules made with dislocation defects. To further dissect the severing and depolymerization abilities of katanin, we performed in vitro biophysical assays using four different types of microtubules with different known defects to distinguish where katanin may be targeting. Our results show that there is preferential severing of microtubules with fewer protofilaments and that the c-terminal tail is required for severing activity. However, microtubules without any c-terminal tails are still depolymerized and do so at a faster rate, suggesting that katanin utilizes two different mechanisms to drive depolymerization and severing of microtubules.

**Microtubule Motor Traffic Jams**

L. Conway, D.Wood, E. Tüzel, J.L. Ross, *UMass Amherst*

Intracellular transport of cargo particles is performed by multiple motors working in concert. Although vesicular cargos appear to have a fixed and small number of motor teams, soluble proteins have been demonstrated to transiently self-assemble into small complexes that can be transported by microtubule motors in the process of slow axonal transport. To investigate the motility of self-assembled cargos in crowded environments, we performed in vitro motility reconstitution experiments with high-resolution particle tracking. Motility is reconstituted by allowing quantum dot cargos to associate to motors on cytoskeletal filaments during the transport process, using kinesin motors and microtubules as a model system. Although the other motors on the filament act as traffic to hinder forward motion, this pool of bound motors also enables the run length and attachment time of the cargo to increase, enhancing overall cargo transport. High motor density on the filaments and the self-assembled cargo results in reduced velocity, increased pausing, and short reversals of the cargo. These results suggest that cellular self-assembled cargos may overcome traffic jams and obstacles through transient and weak associations of multiple motors.

**NESM would like to extend a special thanks to Xradia for sponsoring the 1st Place Poster Prize.**

# 30th Annual Spring Symposium
# Notes