BEMF News

Biological Electron Microscope Facility, Pacific Biomedical Research Center, University of Hawai‘i at Mānoa

BEMF News

Here at BEMF we are continuing to upgrade our equipment and software. We have recently purchased a new, semi automated, critical point dryer and an updated sputter coater for sample preparation.

Our Hitachi Field Emission Scanning Electron Microscope (SEM) is now equipped with the latest Soft Imaging System (SIS) software. Users will be able to perform sophisticated image analysis and multiple image alignment as well as create a personalized database for better organization and efficiency.

The BEMF is partially supported by a Biomedical Research Infrastructure Network award, RR-16467, from the National Center for Research Resources, National Institutes of Health

Responsible Approach to Fluorescent Microscopy

Before spending hours in the lab working out your protocol, get to know your sample. Many organic and inorganic materials have an inherent autofluorescence that could interfere with your primary objective. Certain fixatives, mounting media, and specimens may autofluoresce so it is important to include controls.

Controls are essential for good science yet you’d be surprised how many researchers skip this step and jump to their experimental slides, only to find out that their data were not real.

Try to identify established protocols for your experiment. If they exist, determine if their excitation and emission wavelengths correspond with the filter cubes and/or laser lines on your particular instrument(s).

For example, our Bio-Rad MRC1024 utilizes a krypton/argon laser with excitation lines at 488, 568 and 647nm. Corresponding emission filters include a 522, 568/585/or 605, and a 680nm.

1. Know your sample.
2. Does it autofluoresce?
3. Use controls.
4. Know your instruments.
5. Will the excitation and emission spectra of your fluorophore(s) correspond with your instrument’s filters?

See the full color version of this newsletter http://www.pbrc.hawaii.edu/bemf/bemfnews
Köhler Illumination

Köhler illumination, invented by August Kohler in 1893, is essential for achieving optimum resolution for photomicroscopy. This technique reduces glare and contrast artifacts by evenly illuminating the field of view for transmitted light microscopy.

Köhler illumination is achieved when the light path is properly aligned with your focused specimen.

Follow these easy steps and in 30 sec or less you’ll be on your way to optimum photomicroscopy.

1. Turn on base illumination
2. Focus your slide with the 10x objective
3. Fully close down the field diaphragm (Fig. A & C)
4. Focus the condenser until the edges of the field diaphragm are sharp and crisp (Fig. A & D)
5. Open the field diaphragm until the edges are nearly out of view
6. Center the field diaphragm with the two condenser centering screws (Fig. B & E)
7. Adjust the condenser iris aperture to achieve desired image contrast (Fig. B and F) or remove ocular and reduce iris by 20%
8. Adjust light intensity with the base illumination and/or neutral density filters
9. Repeat steps 2-8 each time you change objectives for truly optimum performance
Featured Research – Dr. John F. Scott* and the Glow Worms

* Associate Professor, Department of Biology, UH Hilo; Adjunct Associate Professor, Department of Cell & Molecular Biology, UH Manoa

Around the world, a nematode, *Caenorhabditis elegans*, has become the model organism of choice for many studies of multicellular animals. *C. elegans* is a free-living, non-pathogenic inhabitant of the soil which can be easily raised in the lab, grazing on a bacterial lawn grown on an agar substrate in petri dishes. The 1.3 mm worms develop through 4 larval stages with a generation time of about three and a half days. The adults are either males or self-fertile hermaphrodites; genetic crosses can be performed by mating the hermaphrodites with the males.

Every adult hermaphrodite contains precisely 959 cells; every male, 1031. The cell lineage charting the development of each of those cells from the fertilized egg is invariant from one individual to the next and has been completely elucidated. The worm is transparent. Many internal structures can be seen in living worms using differential interference contrast (DIC) microscopy, as in this 1000x image of the pharynx of an N2 worm taken using our Olympus BX-51.

The adult hermaphrodite’s nervous system is comprised of exactly 302 neurons. Each neuron has a unique morphology, connectivity, position, and lineage. The connections between those neurons and the 81 cells of the hermaphrodite’s musculature have been completely mapped by electron microscopy. Those studies found approximately 7,000 nerve cell synapses and gap junction connections in the entire hermaphrodite. Despite the structural simplicity of its nervous system, *C. elegans* displays specific behaviors and is capable of responding in a coordinated manner to external stimuli.

A sophisticated array of classical and molecular genetic tools are available for *C. elegans*. Its 97 Mb genome has been completely mapped and sequenced. Mutations resulting in a wide variety of uncoordinated movement phenotypes (*unc* mutants) have been characterized and provide a means with which to dissect functional aspects of the neuromuscular system. The functional alterations observed range from total paralysis to hyperactive movement, and include continuous twitching and various alterations in forward and backward movement patterns. Concomitant changes in the neuromuscular lineages and cell connections can be observed and investigated by various forms of light and electron microscopy. These techniques sometimes enable specific dysfunctions to be attributed to defects in particular cells.

In my lab, we screen mutagenized wild type (N-2) worms to isolate *unc* phenotypes and to search for novel uncoordinated mutations. For appropriate mutant strains, we compare neuron positions and connections in the mutants with wild type individuals using fluorescence microscopy with green fluorescent protein (GFP).

We have *C. elegans* GFP strains in our collection which express GFP globally throughout the nervous system and in specific classes of neurons (e.g., mechanosensory neurons). The images shown here are of strain OH441 obtained from the Caenorhabditis Genetics Center (CGC). GFP is expressed in all neurons of OH441’s wildtype nervous system from an integrated *unc-119::GFP* array. Many other neuron-specific GFP expression constructs are available from the CGC and other workers in the field. We introduce GFP expression into our *unc* mutant strains by traditional genetic crosses or, when necessary, by recombinant DNA technology.

High resolution three-dimensional images of living worms are obtained by optical sectioning and image analysis using confocal fluorescence microscopy. This 400x image of an OH441 worm was created from seven stacks of one hundred 1µm sections each, using the BEMF’s SIS multi-image alignment software.

On the online version, click for an example of the 3D visualization possible from an image stack containing 200 0.2µm sections in a rocking rotation of the head region of an OH441 worm.

Thus, *unc* mutations which are initially identified by their behavioral alterations can be characterized in terms of their genetics and their effects on nervous system connectivity. From these correlations we expect to gain new insights into how nervous system connectivity facilitates behavioral patterns in animals.

*This work was supported in part by Hawaii BRIN NIH Grant RR#16467 and a UH Hilo Research Council Seed Money Award.*
Online Resources

Can’t remember what the letters and numbers engraved on your objective lenses mean? Want to look up the excitation and emission wavelengths for a fluorophore? There are a number of Internet and World Wide Web resources available to you 24/7. Below are some of our favorite web sites.

Check out Molecular Expressions at http://micro.magnet.fsu.edu/ for everything you ever wanted to know about microscopy! The site began with beer shots (polarized microscopy of drying beer) and crystals of chemicals and evolved to include light microscopy, fluorescence and confocal microscopy, all kinds of optics, digital imaging and image processing. Includes many Java tutorials, special techniques, and photo galleries.

From Molecular Expressions there’s a link to Nikon’s Microscopy U: http://www.microscopyu.com, which has tutorials on light microscopy, phase, DIC, the focus and alignment of mercury and xenon arc lamps, and many other features.

The Olympus Microscopy Resource Center http://www.olympusmicro.com/ also is full of information. Specialized techniques include Hoffman modulation contrast, DIC, darkfield, phase contrast, polarized light microscopy, near-field scanning optical microscopy, and fluorescence and combined contrast and fluorescence techniques.

For looking at curves for excitation and emission of fluorophores, nothing beats http://www.omegafilters.com. Follow the link to the Curv-o-matic, and enter the fluorophore of interest.

In future issues we will add URLs for information on Listservers, more educational sites, commercial web sites, various national and international societies, university sites and government sites dealing with microscopy, digital imaging and image analysis. Stay in the loop!

Upcoming Conferences

National Society for Histotechnology
October 18-23, 2003, Louisville, KY, USA
Email: histo@nsh.org

Challenges in Biological Imaging: From Cells to Molecules
California Institute for Quantitative Biomedical Research (QB3 Institute). December 12-13, 2003, Lawrence Berkeley National Lab, Berkeley, CA, USA
WWW: http://www.qb3.org/

January 5 to 10, 2004, Fort Lauderdale, Florida, USA,
WWW: http://www unix.oit.umass.edu/~wc2004/

Microscopy By The Bay (ACMM18) - Australian Conference on Microscopy and Microanalysis 18
February 2 to 6, 2004, Geelong, Victoria, Australia,
WWW: http://www.deakin.edu.au/events/acmm18/

PITTCON 2004 - The Pittsburgh Conference 2004
March 7 to 12, 2004, Chicago, Illinois, USA,
WWW: http://www.pittcon.org/

SCANNING 2004
April 27 to 29, 2004, Hotel Washington, Washington, USA,
WWW: http://www.scanning.org/

Microscopy & Microanalysis 2004 - Sponsored by MSA and MAS
August 1-5, 2003, Savannah, Georgia, USA
WWW: http://www.msa.microscopy.com

Microscopy & Microanalysis 2005 - Sponsored by MSA and MAS
July 31-Aug 4, 2003, Honolulu, Hawaii, USA
WWW: http://www.msa.microscopy.com

Local Microscopy Society?

The Microscopy Society of America (MSA) was formed in 1942 primarily for electron microscopists. Over the years it has come to embrace all types of microscopy, microscopical techniques, and the various types of research and techniques associated with microscopy. The society has a formal journal, Microscopy and Microanalysis, and a large and well-attended annual meeting each August. Many areas of the United States have Local Affiliate Societies (LAS) which periodically have meetings, seminars, and workshops. These local societies foster exchange of ideas, techniques, and formation of collaborations. Our vision for a local microscopy society includes starting a seminar series and offering MSA-sponsored Mainland speakers, as well as organizing workshops. Depending on interest, we could hold quarterly, bi-annual or annual meetings. The Society could be as informal or formal as members desire. It could include having invited speakers, short presentations, long presentations, posters, student activities, workshops, or nothing at all except pupus and talk! It would be an ideal way to share tips and tricks and meet potential collaborators from the microscopy community. Interests could include EM, fluorescence, confocal, X-ray, microanalysis, materials, geological and biological microscopy applications, immunocytochemistry, histology and related topics.

There is, of course, an ulterior motive: there is pressure from the Microscopy Society of America to have a Local Affiliated Society involved in their annual meeting, and Microscopy and Microanalysis will be here in Hawaii in 2005. The pros are involvement in the meeting and a chance to design and sell fun souvenirs. Proceeds will help to fund the local society. The con is that you may have to work (we will be looking for volunteers!)

If you are interested in helping to form a society or just to show up for quarterly? biannual? annual? meetings, drop Tina a line at tina@pbrc.hawaii.edu and let her know if you are interested and what form you might like the society to take. All ideas and opinions welcome!

Microscopy & Microanalysis 2005 will be held here in Honolulu, Hawai‘i, July 31-August 4, 2005 at the Hawai‘i Convention Center. With 3000 expected participants, this will be the 63rd Annual Meeting of the Microscopy Society of America and the 39th Annual Meeting of the Microbeam Analysis Society.

Other participating Pacific Rim societies include the Committee of Asia-Pacific Societies for Electron Microscopy and other southeast Asian societies (CAPSEM), the Australian Microscopy and Microanalysis Society (AMMS), Microscopy New Zealand (MNZ), and the International Metallurgical Society (IMS). Besides a diverse scientific program covering all types of microscopy, it includes an extensive exhibit of instrumentation and support equipment.
WEDNESDAY
10/29/2003
10:00am - 3:30pm
Hawaii IMP Conf. Center
East-West Center
1777 East-West Road
Honolulu HI 96848
KOI ROOM

MICROSCOPY &
IMAGING SEMINAR

Nikon / Roper Sci. / Universal Imaging

Lectures/Presentations
10:00 Microscopy Contrasting Techniques
Kurt Neumann - Nikon Instruments
11:00 Configuring a Time Lapse Imaging System for Microscopy
Scott Monroe, Ph.D., Nikon Instruments
1:00 Confocal Capabilities for the Individual Lab
John Parsons - Nikon Instruments
2:00 Understanding Signal to Noise in Digital Cameras
Mike Meade - Roper Scientific

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