## INDUSTRY NEWS

systems designed with improved scan head optics to boost optical performance to 400nm. The C1-Plus also includes faster image acquisition with bi-directional scanning, support for X and Y scan axes rotation and new laser options for improved control over laser illumination intensity. Also available for the C1-Plus are directly modulated 408nm, 440nm and 638nm diode lasers, as well as a new diode pumped solid state laser at 561nm. Continuously variable attenuation for each laser line, either manually or through EZC1 software by Acousto-Optical Modulators (AOM), improves live cell imaging and FRAP and FRET applications. In addition, Nikon's new EX-type three laser table can accommodate all of the new lasers plus a filter exchanger for selecting either the 488nm or 514nm line of a multiline Argon ion laser. EZC1 v2.2 software controls the C1 System and operates in either a Windows 2000 or Windows XP-Professional environment. The EZC1 software easily integrates new C1-Plus hardware, enabling many new C1-Plus capabilities including bi-directional scanning for faster frame rates used in live cell imaging applications. The software controls AOM's and directly modulated diode lasers for using FRAP and other applications requiring precise and automated control of laser illumination intensity. Enhanced animated rendering easily animates a single cell slice from a 4-dimension X, Y, Z, and Time acquisition, or animates a projection from the same data, to detect movement within a cell over time. Additionally, a new merge function allows a separately acquired differential interference contrast (DIC) image to be merged with a projection of a stack acquired to match the DIC section thickness making it possible to map the precise location of a specific florescence dye within the cell. For more information visit the Nikon website at www.nikonusa.com. Product related inquiries can be directed to Nikon Instruments at 800-52-NIKON

Technical Manufacturing Corporation (TMC) designs and manufactures **custom acoustic enclosures** specifically for precision instruments. Designed to prevent background acoustic noise from disturbing delicate measurements and micro-manufacturing processes, these enclosures are used in OEM applications such as scanning-probe microscopes, interferometers, and other sensitive metrology instruments. The integrated steel and stainless steel systems incorporate TMC's proprietary acoustic control techniques to ensure optimal equipment performance at the difficult-to-isolate frequencies including those below the human hearing threshold. Unlike the more familiar anechoic panel constructions, which target frequencies at which human hearing is most sensitive, TMC enclosures are specifically designed to provide maximum attenuation in the 5-to-50Hz. frequency range at which precision instrument are most sensitive. Contact: Steve Ryan, TMC V. P., Marketing, 978-532-6330, email: sryan@techmfg.com



## NETNOTES

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**Selected postings from the MSA Microscopy Listserver (listserver@msa.microscopy.com) from 6/9/04 to 8/10/04. Postings may have been edited to conserve space or for clarity.**

### IMMUNOCYTOCHEMISTRY – Colloidal gold stability

*I am not very happy with my current supplier of secondary gold immunoreagents. I would appreciate input regarding potential new suppliers, as well as general comments about the shelf-life, stability, sensitivity of your favorite immunoreagent brand. From: Peter Rohloff <rohloff@uiuc.edu> 09 Jun 2004*

Immunogold reagents are a peculiar lot. The characteristics of the gold particle itself is dependant on which reducing agent is used. Unfortunately, the most common method involves the reduction of chloroauric acid with citrate-tannic acid. There is a stochiometric relationship between the ratio of the chemicals used and diameter of the gold particle produced. This results in tremendous affinity for cytoskeletal components. Tannic acid is a mordant often used to preserve microtubules. However, if the reducing agent is sodium borohydride, one can circumvent this affinity. You may wish to ask potential suppliers which reduction method they employ. In addition, it is paramount that one uses teleostan fish gelatin as a stabilizer instead of BSA. (Reference: Birrel et al., "Pitfalls of Immunogold Labeling," J. Histochem. Cytochem. 35(8):843-53; 1987). Michael C. Peters <petersmc@stanford.edu> 09 Jun 2004 20:40

### IMMUNOCYTOCHEMISTRY – Control for ABC reaction

*I am looking for any suggestions regarding the visualization of the neural tracer cholera toxin subunit B, CTB, in tissue sections. The CTB was injected into either the eye or the cortex and allowed to transport for 2 days. The tissue was fixed and sliced and now I am attempting to label the CTB with DAB. The CTB has biotin conjugated to it already. Since it already has biotin, I have tried proceeding with an ABC (avidin-biotin complex) reaction and then DAB. Twice, varying the time in ABC, the reaction has not worked. Besides CTB concentration and injection issues as possible causes of the failure, I am trying to determine if the visualization reaction I am trying to perform is correct. If anybody can make any suggestions for visualizing something already tagged with biotin, I would appreciate it. Anita McCauley <mccaulak@wfu.edu> 26 Jul 2004*

Take a 1-3 µl droplet of your biotinylated probe and spot it on a 0.5 cm wide strip of nitrocellulose paper (get it from somebody who does western blots). Block the rest of the sites on the paper by incubation in 1% BSA for several hours. Incubate in your streptavidin-HRP or ABC kit and then develop. You can do these steps in a microcentrifuge tube or small glass test tube. It should show a brown spot if it is working. Tom Phillips <phillipst@missouri.edu> 27 Jul 2004

### LM - Photographing Protozoa

*I am photographing protozoa and am looking for a way to slow them down or even kill them for digital photomicroscopy. I am using methyl cellulose to slow them down but I'd like to find a way to incapacitate them without their cellular structure disintegrating. Michael Reese Much <muchphoto@earthlink.net> 15 Jun 2004*

# NETNOTES

Below are some articles from Miscape that address slowing down protozoa and vital staining of live protozoa. Many of the classic formula for slowing various wee creatures call for narcotics that we considered difficult to get. If you work in a facility that is licensed to use controlled substances, fine, follow the approved procedures. Carbon dioxide bubbled in the media slows and kills many organisms. Physical compression in thick media also helps. If you can't find a compressing cell, I have been looking for an excuse to make some for my own use and this might get me off high center. Matching the chemical to the desired action, usually relaxation at death, or as the processes slows, is what is desired and sometimes this can be achieved by using a weaker, slower acting solution of the chemical. If you can find a proven protocol for a given chemical, it is far better to use this, than to waste time developing your own. Far better to walk on the shoulders of giants than to wander the wilderness alone doing the same work over and over again. On slowing and killing protozoa: http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/artmay02/rhmicrotech.html. Vital staining: http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/artfeb00/rhvital.html. Gordon Couger <gcc@couger.com> 15 Jun 2004

*Editors Note: From a conversation with Peter Ingram at Duke University Medical Center:* "We have worked on protozoa for years....and the way you slow them down without killing them is to suspend them in a 5-10% solution of hydroxyethylcellulose; quite "legal" and available from any chemical supply house at minimal cost."

**TEM – Formvar support films**

*I have been trying to make Formvar support grids by methods that have always produced good results in years past. Now, however, the Formvar refuses to leave the glass slides as I lower them into a container of water. Has anyone experienced this? Can you give me a clue? My procedure now and in the past is to dip clean slides in a solution (w/v) of 0.25% Formvar in dichloroethane, air dry, then scrape bottom and sides of the slide with a razor blade. William Perreault <william.j.perreault@lawrence.edu> 19 Jun 2004*

What I have found that helps ever so much with Formvar is to take a Kimwipe (a brand of tissue paper--probably lens paper would work too) and rub it vigorously over the surface of the slide and then dip it into the Formvar and cast as usual. I guess this charges the surface with a little static electricity, but whatever the mechanism, this seems to work a treat. Tobias Baskin <baskin@bio.umass.edu> 20 Jun 2004

After scraping an outline of the film to be cast with a clean razor blade, I breathe on the coated slide and the vapor of my breath makes the film look frosty. Then I quickly dip the slide at a 45 degree angle into a bowl of water and the film floats off. If you don't want to blow on the slide, I have held the slide over a beaker of hot water (recently boiled) and the water vapor likewise makes the film on the slide look frosty. As an aside, I no longer use Formvar or Parlodian, but prefer Butvar which, in my hands, makes very stable films even during the humidity of summer in Iowa. I don't even bother to carbon coat my films, which I routinely cast on 1x2 copper or nickel slot grids. Dean Abel <dean-abel@uiowa.edu> 20 Jun 2004

I had similar problems, not with plain Formvar, but with holey Formvar, and the solution was to put a layer of Apiezon L onto the slide. Two ways that worked were to put a small dab on the slide and spread it with a finger and thumb on opposite sides of the slide or to dissolve the Apiezon in a hydrocarbon solvent and dip the slide as one would

into the Formvar. The former method has advantages for holey films in that it causes the holes to line up in the direction that one rubbed one's finger; the latter method should give a more uniform film--although I never measured this--so is more suitable for plain Formvar films. I also dissolved 0.25 g of Alconox in 1 L water, heated the solution to 50 C and used the warm solution to float off the Formvar. Bill Tivol <tivol@caltech.edu> 22 Jun 2004

**TEM - Wenelt Cleaning**

*We normally clean the Wehnelt of our TEM with trichlorofluoroethane and alumina powder. The process takes some time. Does anybody know if there is any other way to do it faster? Jafar Al-Sharab <jafarhan@rci.rutgers.edu> 23 Jul 2004*

Are you using W or LaB6 filaments? The former requires an abrasive and I've used Pol and Wenol, which are pastes and may be faster than trichlorfluoroethane and $Al_2O_3$. $NH_4OH$ will dissolve LaB6 without abrasive, and it is a lot less work than using abrasive. Since $NH_4OH$ is easier to remove than $Al_2O_3$, the total process should be quicker even though you will have to leave the Wehnelt in the $NH_4OH$ for longer than it takes to polish it with abrasive; furthermore, there is no chance of leaving scratches with $NH_4OH$. Bill Tivol <tivol@caltech.edu> 23 Jul 2004

My advisor, Will Bigelow, taught me over 30 years ago that if you want to clean stainless steel, use stainless steel cleaner. Revere Ware stainless steel cleaner is still available in Home Depot and is aqueous, so it leaves no oily residue to outgas, and the Wehnelt ends up clean enough to eat off of. John Mardinly <john.mardinly@intel.com> 23 Jul 2004

Three separate pieces of advice: 1. Get a spare Wehnelt, clean it and store in a hermetically sealed plastic bag. Use the pre-cleaned Wehnelt to service the instrument and clean the used one at your convenience. 2. To ease cleaning, soak the used Wehnelt overnight in diluted solution of the cleaner used for surgical SST instruments, like Alconox or similar. Rinse thoroughly in deionized water and then clean or polish as you do usually. Concentration for the soaking solution will have to be found empirically, as it depends on the particular cleaner you use and contaminants. 3. Use high quality deionized water instead of tap water to dilute your cleaner. 4. "Handbook of Electron Tube and Vacuum Techniques" by Fred Rosebury, AVS Classics series, lists on page 15 a few acid-based recipes for SST cleaning. SS-3, based on HF and HNO3 at room temperature should dissolve any contaminants, including tungsten, quite rapidly, but I would rather prefer to use milder cleaner and some polishing then deal with HF. Valery Ray <vray@partbeamsystech.com> 24 Jul 2004

I sonicate mine in a concentrated ammonia solution (about 30% of the lab 'concentrated') for about 10 minutes. This dissolves much of the deposit and softens the rest. Then I polish with household silver cleaner ('Silvo' brand). This is a slightly abrasive runny cream. I have been told that the one for silver is less abrasive than the one for brass ('Brasso'). Then I wash it under the hot tap, rinse with methanol, and dry it with tissues. Takes about 30 minutes. Ritchie Sims <r.sims@auckland.ac.nz> 26 Jul 2004

We used to use cleaning paste until a service engineer showed me an easier method. Now I sonicate in about 5% Decon 90, rinse and sonicate in acetone. David Patton <David.Patton@uwe.ac.uk> 26 Jul 2004

If you use abrasive polishing, here is a labor saving hint. Wooden shaft cotton swabs will fit a 3/32" collet for a "Dremel tool" (small rotary grinder). Cut the swab so that only a very short piece of shaft protrudes from the collet or it will break off. Wear safety glasses. I use Pol or Wenol with the "high speed rotary Q-tip" and it is very effective. Hint 2: To clean the ID of the hole, break (or cut) the swab shaft to a taper and use only the tapered wooden shaft and some polishing compound. Woody White <nwwhite@bwxt.com> 26 Jul 2004

One method I was taught years ago by a service tech was that any plated parts could be cleaned by immersion in plain household ammonia, (not the sudsy type) or a 5% solution of ammonium hydroxide. Depending on how contaminated the cap is, it will take 5 to 20 minutes. The plus side of this method is--a quick rinse in distilled water followed by alcohol or acetone and the cap is ready for use. Mannie Steglich <msteglic@mdanderson.org> 24 July 2004 There are two parts to an ideal cleaning procedure. 1. Wet cleaning is by far the best route, no cotton, no particles, no fiddling, just liquids doing the job. 2. Having a solvent for the media that you wish to remove (in this case ammonia is a solvent for tungsten). The cathode assembly is placed, aperture face upwards, in a beaker of stock ammonia solution diluted 3 parts ammonia to one part water. The stock solution is normally about 40% ammonia. After a maximum of 15 minutes in the ultrasonic cleaner the beaker is placed under running water and thoroughly flushed through. Care should be taken to ensure that none of the clamping or alignment screws have fallen out of the cathode assembly and could be flushed away! The cathode is then washed with alcohol before being dried with a hair drier. A new filament is fitted and centered. The assembly is checked for cleanliness by observing with a 20x lens or binocular microscope prior to re-installation in the microscope. Total time for this procedure is less than 25 minutes. Safety: Great care was taken not to allow the ammonia solution to make contact with the skin or eyes of the operator. When flushing the solution through with water its flow should be set so as not to splash the solution over the operator prior to placing the beaker under the flow. Steve Chapman <protrain@emcourses.com> 26 Jul 2004

**SEM – Making 3D images**

*Is there a way to make SEM pictures 3D? I know that tilting is involved but what degree? Is it better to colorize and use the red/blue glasses or is just tilting and using the stereo glasses best? Paula Sicurello <psicurel@odu.edu> 23 Jun 2004*

The technique you are referring to is called "Stereo imaging" or similar. Basically you acquire 2 images with a specified tilt difference. What is best depends on what you want to do with the images. Display on a computer monitor: If you don't have a special monitor that allows the use of polarized glasses, you have two options: 1) create an anaglyphic image (red/blue or green/blue), and use the appropriate glasses. This will give you a good 3D display on the monitor, although the image appears yellowish. 2) Switch between the two stereo images repeatedly. If done correctly, it will also provide a sense of depth without using any glasses, but the image is not fixed (seems to be oscillating). The stereo angle for these types of images should be in the range of 6-10 degrees. If you go much beyond that, the brain cannot fuse the two images anymore. This also depends on the 3-dimensionality of the sample. For printing you can go the anaglyphic route (see 1 above), or you can mount two prints at appropriate distances and use special 3D glasses for looking at the prints. These glasses make sure that each eye sees only the print that it is supposed to see, and you get again a 3D impression, which can also be in color. The anaglyphic images have the advantage that they are independent of distance from the print.

The stereo glasses require a specified geometric arrangement, which includes the distance from the prints. Finally, you can also use the stereo images to calculate a 3D surface profile of your sample. Once you have done that, you can use 3D display software to rotate and look at the 3D object and actually measure in 3 dimensions. In all cases you need to "adjust" the images, *i.e.*, pick one point that is at the identical position on both images. If you don't do that, there may be a lateral shift between the images that can destroy the 3D appearance. Mike Bode <mb@soft-imaging.com> 23 Jun 2004

Tilting is crucial to making an anaglyph (or enabling the viewer to see a 3D image when using red/green or red/blue glasses) of SEM images. In short what you are doing is mimicking what your two eyes do when you look at an object. Your eyes are tilted at slight angles relative to each other. The images seen thorough both eyes are combined in your brain to produce an image with 3D information. If you only have one eye than you cannot see in 3D unless shadows help fool your brain in interpreting the image. You can do the same thing with an SEM image. You need to take two images with one tilted relative to the other. The amount of tilt depends on the degree of roughness of the sample which is in turn somewhat related to magnification, but usually 7 degrees is a good starting point. What is important is to keep the center of the image stable for both images. This is easily done by putting a piece of transparent film (such as from a clear sheet protector) on the viewing screen and using a grease pencil to mark relevant features near the center of the image. Then move the stage to line up the second image. Any adjustment in focus should be done by adjusting working distance. If you use your focus knob you will be changing the strength of your final (objective) lens which in turn affects the rotational path of the electrons in the beam. Modern microscopes have software to easily overlay the images to create a red/blue or red/green image which, when viewed thorough the appropriate glasses, will give you your 3D image. You can also take the two images and combine them in PhotoShop, as well as other programs, to make the final anaglyph. If you need more complete instructions, e-mail me. I can send the article that appeared in Microscopy Today, Vol #99-1 (Jan. 1999). The book by Bozzola & Russell also contains a discussion of making stereo images Debby Sherman <dsherman@purdue.edu> 23 Jun 2004

*Editor's Note: See the article by the Greenhuts, this issue.*

**SEM – Etching titanium oxide**

*Question: Does anyone know of a good titanium oxide etch? I am trying to sort out areas of titanium oxide from clean titanium. Chemical or plasma options are available to me. Stephanie McCracken <stephanie.l.mccracken@medtronic.com> 13 July 2004*

Have you tried plasma treatment with $CF_4$ only? Normally, it is recommended to use $CF_4$ with about 10% $O_2$, but since you don't want to oxidize the Ti metal, try just the $CF_4$. Or you might want to try $CF_4$+$O_2$ and then switch to $CF_4$ only to finish. Scott D. Walck <walck@ppg.com> 14 Jul 2004

**MICROSCOPY – Testing water quality**

*Is there a simple test available for testing deionized water quality? Sara Goldston <cogold13@yahoo.com> 23 Jun 2004*

There are three concerns for water purity. The crudest is whether anything is growing in it; since not a lot will grow in a saturated NaCl solution, you can see that the microbe test isn't very stringent. The presence of ions increases the conductivity, so having a conductance meter will tell you how free of ions your water is. There can be non-ionic solutes, however, that do not increase the conductivity; so while conductivity is the usual measure of water purity, there can still be dissolved material. In fact, the output of ion exchange resins--used in many water-purification systems--can contain non-ionic material generated by the resin itself. For really pure water, the output of the deionized system can be distilled. There may even then be impurities, but deionized, distilled water is usually adequate. I don't know any tests better than conductivity for water purity. Bill Tivol <tivol@caltech.edu> 22 Jun 2004

**IMAGE INTERPRETATION: Shadow direction in EM and planetary exploration**

*I have recently seen some planetary exploration photographs which remind me of features seen in polymers under SEM / TEM. If you look at the following two: (a) Olympus Mons Caldera on Mars: http://nssdc.gsfc.nasa.gov/imgcat/html/object_page/vo1_890a68.html, and (b) Craters on Phoebe from Cassini: http://antwrp.gsfc.nasa.gov/apod/ap040614.html. Don't you think they've got the shadow direction wrongly oriented? The Phoebe craters look like tents and the caldera appears to be sticking up. Not the sort of mistake an electron microscopist would make!? Robert H. Olley <r.h.olley@reading.ac.uk> 15 Jun 2004*

You are quite correct that the direction of the shadow changes the perception of height so that depressions appear raised and mounds appear as craters; this is a fairly well-known visual phenomenon. The advantage of a laptop is that one can hold the screen upside down and see the proper perspective. As to whether "they've got the shadow direction wrongly oriented", one disadvantage of ultramacroscopy is that the lighting conditions are fixed, and, until we can figure out how either to move the sun or provide a new one, we have to take what is given. Of course, the images could be rotated by 180 degrees before they're posted, but that would interchange the north and south poles, which goes against astronomical convention. Bill Tivol <tivol@caltech.edu> 15 Jun 2004