NetNotes

Edited by Thomas E. Phillips
University of Missouri
phillipst@missouri.edu

Selected postings from the Microscopy Listserver from January 1, 2012 to February 28, 2012. Complete listings and subscription information can be obtained at http://www.microscopy.com. Postings may have been edited to conserve space or for clarity.

Specimen Preparation:

flat embedding large-size tissue in LR White

I have a faculty member who wants to embed brain tissue pieces that are 100–200 microns thick, 2 cm in length and 1 to 2 cm in width in LR White. I have Aclar film and have located PTFE flat molds with large enough cavities that may work. Before I proceed, I would like to hear from anybody who may have experience in doing this kind of embedding. Any advice is appreciated. I am especially interested in knowing if the Aclar film will really work well enough to seal the mold and prevent air from interfering with the polymerization. Also would it be best to first use UV polymerization in a cold chamber or can I go directly to the embedding oven at 65°C? Could variable wattage microwave polymerization be used without submerging the mold underwater? As always thanks in advance for the help. ***Tom Bargar tbargar@unmc.edu Tue Jan 29***

Do you have access to an oven with vacuum? If so then just connect to a nitrogen source and alternate pulling a vacuum and the flooding with nitrogen to drive out the oxygen. Then leave the oven at 60°C to polymerize. Only leave vacuum low, at about 5 lb, so as not to force resin to creep up the molds. Debby Sherman dsherman@purdue.edu Tue Jan 29

LR White can be tricky, but we have a method that seems odd but always works. In our experience it is better to not fill all the PTFE mold spaces with samples. We have to orientate Drosophila retina and larvae very strategically, so we place our samples into the center 6 wells, overfill the wells including two extra empty ones on either side of the samples. Press the Aclar film over the samples in the middle first then gently lay out the film toward both sides so that the resin pours over into the left-over empty well spaces toward the ends. The very end well spaces will not polymerize very well, but the ones with the samples will turn out very well, at least for us. We then place the mold into a 60°C oven overnight to two days, sometimes three depending on the weather. So far we have been able to thick section and do fluorescence staining with no problem. Lita Duraine duraine@bcm.edu Tue Jan 29

My thanks to everyone who responded to my request on flat embedding a large piece of tissue (2 cm long, 1 to 1.5 cm wide and 100 microns thick) in LR White. So the embedding certainly looks feasible. Related to this project, the faculty member has asked me if it is possible to section the resulting block in 10-micron thick sections? This is for confocal imaging apparently. So he would like to get a section that is 10 microns thick and approx. 2 cm long and 1 to 1.5 cm wide and I presume heat fixed to a glass slide. Now the thickest I have ever cut is 2 microns on my Diatome Jumbo Histo diamond and less than 8 mm wide. Is there anyone out there with experience in cutting a section like this? I feel the LR White would be too brittle to go as thick as 10 microns without cracking, but I really don't know. Can the block be sectioned on a standard histology microtome, the way you section a paraffin block? Any and all advice would be appreciated. Tom Bargar tbargar@unmc.edu Thu Jan 31

Do you have the option of embedding some of the tissue in another resin that would not be suitable for TEM? If so than I would suggest embedding in JB-4 resin which can be cut with larger glass knives (and also disposable metal blades). Usually this is done on a JB-4 microtome and uses wider glass that is cut with a special knife breaker. These pieces of equipment may not be available to you. However, it may be possible to use a standard paraffin microtome to cut sections of this size in this resin. Perhaps others can comment on that since I have not tried it. Ultrastructure of tissue embedded in JB-4 resin is of much higher quality than that embedded in paraffin. It also can still be stained with many of the aqueous stains used for LM. Debra Sherman dsherman@purdue.edu Thu Jan 31

It doesn't make sense to me to epoxy embed and cut 10 µm sections for confocal examination. For confocal I suggest cutting the large tissue at 50 µm then process and image with a confocal. Most confocals can image at a depth of at least 50 µm. A multi-photon confocal can image deeper ~200–300 µm. Otherwise, if the epoxy preparation is important, cut 2 µm sections and use a widefield microscope; confocal is not really needed since you already have thin sections. The individual section images can be aligned post-microscopy. However, considering the problems of section folding and wrinkling that I have encountered with 4 mm × 4 mm × 1.5 mm epoxy sections, larger sections may present even more headaches and frustration. Larry Ackerman larry.ackerman@ucsf.edu Thu Jan 31

I haven't used Spurr's for many years. I use Eponate 812 or EMbed-Epon equivalents. I put a drop of water on a glass slide then transfer a section from the knife boat to the drop of the water with a Minutien pin (very fine needle) glued on a wood applicator stick. Usually I put two or three sections on a drop but for larger sections one per drop. The slide then goes on a hot plate set at a temperature so the water drop dries slowly ~3–4 minutes—sometimes, just allowing the water to air dry at room temperature works better. Then I stain the sections with Toluidine Blue for 10–20 seconds and rinse with water. I am usually just looking for an area that I wish to thin section and do not use the 1–2 µm sections for microscopy. Another possibility to minimize wrinkles is to stretch the sections in the knife boat—with a heat pen or solvent vapors (chloroform vapors are not healthy). Larry Ackerman larry.ackerman@ucsf.edu Mon Feb 4

Some time ago was a posted query regarding embedding large tissues in LR White. As an alternative to flat embedding molds, I suggest using polyethylene Wheaton Snap Caps for LR White embedding. They are available from Fisher in 22 mm diameter (06-450-201) and also in larger diameters. To drive out inherent moisture in the caps, store them in the oven prior to use. Once the sample is embedded in LR White within the caps, place these directly in a 60°C oven for polymerization, without any cover, with the media directly exposed to the atmosphere within the oven. Importantly, together with the samples, place either a 500 mL beaker of dry ice or a small, uncovered thermos of liquid nitrogen. The sublimation of CO_2 or evaporation of nitrogen displaces enough of the atmosphere within the oven cavity for

 doi:10.1017/S1551929513000394

https://doi.org/10.1017/S1551929513000394 Published online by Cambridge University Press

Flash&Go™

ARM200F TEM with Cold FEG

GaN [211] HAADF at 200 kV

ABF, HAADF and EELS $Ca_3Co_4O_9$ (110)
Data courtesy of Dr. Robert Klie, University of Illinois at Chicago

Unrivaled flash speed
Unrivaled raw data

Visualization of hydrogen atomic columns in YH_2 by ABF imaging (Y = red, H = green) *Data courtesy of Ryo Ishikawa and Dr. Eiji Abe (The University of Tokyo)*

Visit www.jeolusa.com/FlashandGo

Atomic Resolution S/ TEM

JEOL
Another Extreme Imaging Solution

Global Solutions Provider for Advanced Technology
www.jeolusa.com • salesinfo@jeol.com
978-535-5900

https://doi.org/10.1017/S1551929513000394 Published online by Cambridge University Press

https://doi.org/10.1017/S1551929513000394 Published online by Cambridge University Press

quality polymerization of the media. There may be a thin gooey layer at the top of the caps but this can be wiped clean with ethanol. The media separates easily from the caps! Including an Aclar film in the bottom of the cap works well for a crystal-clear view of the embedded sample. Doug Keene drk@shcc.org Fri Feb 22

Specimen Preparation:

water-solvent swap

I have nanoparticles in what I suspect to be dirty water and would like to transfer them into a clean solvent for drop-casting. Is it possible to evaporate the water and then add the appropriate solvent or am I completely oblivious to some well-known nanoparticle in solution rule? ***Marissa Libbee mlibbee@gmail.com Sat Jan 12***

Evaporating the water will only concentrate the impurities and will likely aggregate the nanoparticles as well. You need to look at methods using filtration or centrifugation. However, I would start by looking at the method used to prepare the nanoparticles initially to see if there are ways to prevent the contamination in the first place. Debra Sherman dsherman@purdue.edu Sat Jan 12

There's a lot to consider when contemplating cleaning up nanoparticles in solution. Do the nanoparticles have any form of functionalization? A peptide group? A surfactant? Anything? Or do they just have an appropriate counter ion in the solution that keeps them from flocculating/agglomerating? The answer there will determine your next step, and whether or not you can easily consider changing solvents. Assuming your particles have something (surfactant or otherwise) to prevent agglomeration, then you can consider a centrifugation/wash/re-suspend/(sonication?)/centrifugation routine. Depending on the weight/size of your nanoparticles, you can also consider using dialysis tubing, as long as there is a significant enough weight difference between your impurity and your nanoparticles. That will also determine the diffusing species and direction. Once you get past those questions, then you can start to consider whether or not you can even suspend the nanoparticles in your desired solvent. John jpapalia@papalia.net Sat Jan 12

No—drying could change your particles irreversibly and cause substantial aggregation. I recommend using dialysis against purified water. If the things you want to remove from the suspension are dissolved, then the dialysis will result in the diffusion of the dissolved species from the suspension to the surrounding purified water. Check out the experimental section of this paper, and you will find details about how to perform dialysis against purified water in order to remove dissolved species from a suspension of nanoparticles and water. Effect of Ionic Strength on the Kinetics of Crystal Growth by Oriented Aggregation, Nathan D. Burrows, Christopher R. H. Hale, and R. Lee Penn (2012) *Crystal Growth and Design*, DOI: 10.1021/cg3004849. If you have a few more details about your nanoparticles, I'd be happy to chat about sample prep options. We have worked with a range of nanoparticles in aqueous systems, ranging from very "dirty" suspensions of natural materials in natural waters to nanoparticles harvested from aqueous reactors to very clean systems that have been dialyzed against purified water. Lee Penn rleepenn@hotmail.com Jan 14

What is the "dirt"? If it is sediments, then decant the less dirty supernatant. If what's decanted has dirt bigger than the nanos, then filter with forethought. If the filtrate has dissolved material then use dialysis that won't pass the nanos to reduce the volume holistically and simultaneously concentrating the nanos. You might also use differential centrifugation. All of the above depends on the size of the nanos, and how they behave, and whether you actually know they are present. Fred Monson fmonson@wcupa.edu Wed Jan 16

Specimen Preparation:

metal coating depth into pores

Is it possible to coat a porous sample (~80 μm pore size) in a way that allows deposition of the coating material (e.g., Pt) into the pores? ***Soya Gamsey sgamsey@hotmail.com Wed Jan 23***

If you are considering sputter coating, there is a method I have used but not for pores as small as you have. Place the sample in the sputter coater and pump it to attain the best possible vacuum. Do not bleed gas into the system but try to force it to coat; automated systems may not let you do this old systems will. The theory is that with very little gas you have more straight line deposition, the only chance of putting the coat inside the pores. Steve Chapman protrain@emcourses.com Wed Jan 23

Sputter and evaporative coating methods are line of sight to the source. This means if you want to coat into a porous surface, you have to play games with the geometry. The most common way would be with a sample spinner, wherein the sample is doubly rotated on an eccentric path, usually while tilted. This will only allow you to coat those pores with line of sight to the source from any possible orientation. Jacob Kabel jkabel@mail.ubc.ca Wed Jan 23

I'm going to defer to others for the specifics on this, but wouldn't an osmium tetroxide treatment work for getting into the porous surface cavities? I know it's not exactly the most environmentally friendly solution, but would that work better than the sputter coating methods? Justin A. Kraft kraftpiano@gmail.com Wed Jan 23

Time of coat depends upon your coater; perhaps try 1-minute coats. Tilt 45° in one direction for the first coat, then 45° in the opposite direction for the second. For the final coat move to the best vacuum as described. In my experience the higher vacuum coat is important because the object of sputter coating is to cause the metal to be deviated away from line of site by the gas. The better vacuum increases the mean free path of the metal and that is the only way to penetrate holes. An interesting experiment for the doubters is to take a nut (as in nuts and bolts) about 1.16 inch hole diameter and see which technique puts metal through the hole. Steve Chapman protrain@emcourses.com Wed Jan 23

This is a perfect sample for low vacuum or low voltage imaging if you have those capabilities. Also, you can try coating with carbon using a vacuum evaporator. In this case the carbon is projected down on the sample with minimal direction change. Thus chances are better to get some down into the pores. However, do keep in mind that you need to evaporate in a good vacuum to get the finest coating. This along with metal shadowing to more effectively cover the surface of your sample may be adequate to minimize charge accumulation. Debra Sherman dsherman@purdue.edu Wed Jan 23

Educational Outreach:

electron microscopy

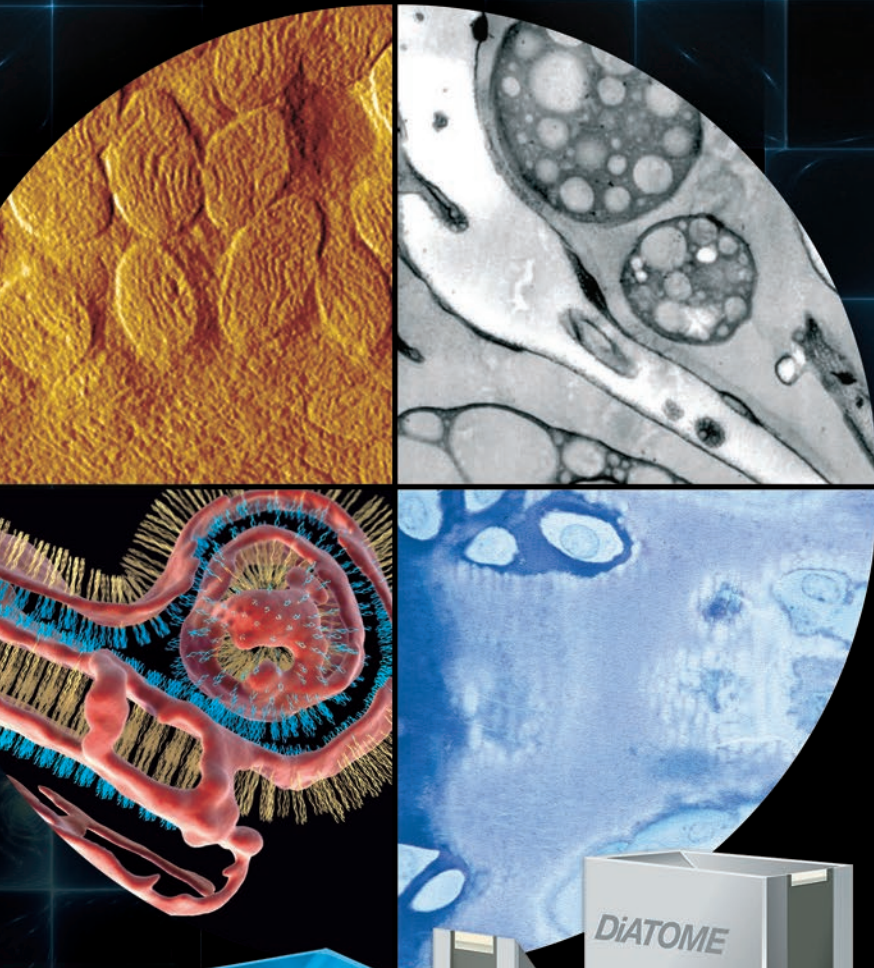
The NUANCE Center at Northwestern University is going to be producing a new web series called "What's it made of?" It will be a short, semi-educational show mainly featuring SEM and EDS to examine normal, everyday objects. We'd love to use this as a fun educational outreach to show the uninitiated the types of things that we can do in the world of electron microscopy. So please share with all your friends and whoever. The first episode has been posted and examines the sparkly ink on the $20 bill. http://youtu.be/z3qHg1QOAk4. ***Eric Jay Miller eric-miller@northwestern.edu Fri Feb 8***

SEM:

chamber contamination

Our laboratory recently purchased a "specially" commissioned SEM. The idea was that instrument was as oil free as possible, so it was

the highest quality... the most precise sectioning... incomparable durability



Free Customer Service

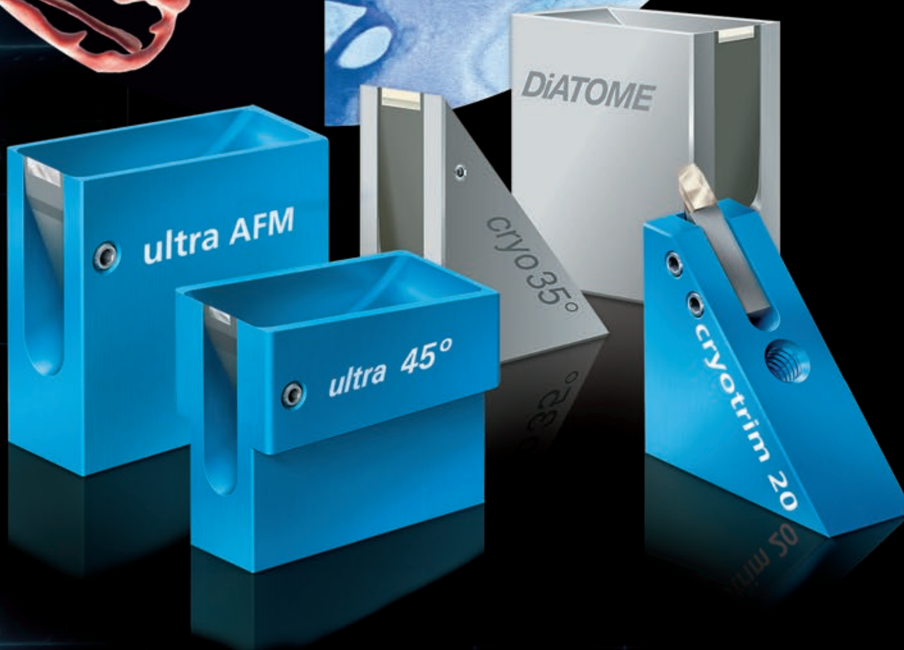
Sectioning tests with biological and material research specimens of all kinds. We send you the sections along with the surfaced sample, a report on the results obtained and a recommendation of a suitable knife. Complete discretion when working with proprietary samples.

Re-sharpening and Reworking Service

A re-sharpened Diatome diamond knife demonstrates the same high quality as a new knife. Even knives purchased in previous years can continue to be re-sharpened. The knives can be reworked into another type of knife for no extra charge, e.g. ultra to cryo or 45° to 35°.

Exchange Service

Whenever you exchange a knife we offer you a new Diatome knife at an advantageous price.



40 years of development, manufacturing, and customer service

DiATOME

diamond knives

ultra 45° • cryo • histo • ultra 35° • STATIC LINE II
cryo-P • cryo immuno • ultra sonic
cryotrim 45 and 25 ultra • AFM & cryo AFM • cryo 25°

DiATOME US • P.O. Box 550 • 1560 Industry Rd. • Hatfield, Pa 19440
Tel: (215) 412-8390 • Fax: (215) 412-8450 • email: sgkcck@aol.com • www.emsdiasum.com

https://doi.org/10.1017/S1551929513000394 Published online by Cambridge University Press

https://doi.org/10.1017/S1551929513000394 Published online by Cambridge University Press

equipped with a turbomolecular pump and a scroll pump. To our dismay, there is a substantial contamination: the clearly visible infamous "dark squares". They are currently negotiating with the manufacturer but in the mean time we need to assess its quantity and type. We are thinking of preparing some polished sample and collect a set of images at different integration times and make a plot of darkness vs. time. The question is however, what would be an acceptable "darkening" rate? Does anyone know if there exists some sort of standardized protocol? Can anyone recommend a procedure? Some recommendations in dealing with the manufacturer are also welcome. Their first reaction was—It is from your samples! Fortunately, it was very easy to show this was not the case. Now they are improvising all sorts of "cleanings." ***Uchechukwu Mba uchechukwu_mba@yahoo.com Wed Jan 16***

You should be able to scan an area for several minutes, without seeing build-up. See the NIST paper (Evactron.com). Short of a UHV solution, it is not surprising to have contamination in a SEM or FIB. There are so many hydrocarbons diffusing out of crevices and off surfaces from machined parts and plastics in the instrument, especially when it is newer. If you have budget, an in-situ cleaning device like the GV-10x or Evactron is extremely helpful. Larry Scipioni les@zsgenetics.com Wed Jan 16

For a protocol you might look at: Conru and LaBerge (1975) Oil Contamination with SEM Operated in Spot Scan Mode. *J Phys E Sci Instr* 8:136–138. Tony Havics ph2@sprynet.com Wed Jan 16

Do contact XEI Scientific Inc., in Redwood, Ca. They sell devices that plug into SEMs to decontaminate them of hydrocarbons. You can find them at http://www.evactron.com/. Ian Holton ian@acutance.co.uk Wed Jan 16

I remember reading something in an old Kurt Lesker catalogue stating that if you use a turbomolecular pump like a diffusion pump, with isolation valving so that the pump can run continuously, it will be very clean. On the other hand, if you run it the way many do, stopping it and bringing it to atmosphere every time you vent the chamber, you will get oils creeping up through the turbo pump and contaminating the chamber. This explained to me why SEMs that I worked on where the diffusion pump had been replaced with a turbo were very clean and Amrays (in particular) that had turbos and a single vent valve were so terribly dirty. Is your turbo in a system with roughing, backing and high vacuum isolation valves, or just a single vent valve? If your turbo is mag-lev, then it is most likely isolated and runs all the time due to the limited life of the crash bearings. Otherwise, you might have the "simple" system, and it will most likely be dirty. Ken Converse kenconverse@qualityimages.biz Wed Jan 16

XEDS:

unidentified extra peaks

I have an odd question that I am hoping the more experienced microanalytical folks can help me with. I think we are seeing some type of "Escape" peak using an SDD XEDS system, but these are not the 1.740 keV Silicon escape peaks I am used to. They are much closer to the primary peak, vary in energy displacement from the primary peaks, and seem to be proportionally larger than Silicon escape peaks from Si\Li detectors. Examples: 0.372 keV below Ti Ka (@ 4.508 keV), 0.610 keV below Ni Ka (@ 7.471 keV), and 0.646 keV Cu Ka (@ 8.040 keV). They are not "system peaks" as they track with the larger primary peaks as we change samples. I suspect that they are "escape artifacts" of SDD´s and obviously I do not spend enough time reading MicroNews *and the microanalysis literature to be aware of them. Anyone want to help us out, please? We are working with a JEOL-2100, LaB_6 at 200 kV, and a Bruker Quantax 200 SDD. Using a beryllium holder. Count rates of 0.7–2.0 kcps (TEM remember?), but have tested with same results at higher 10–60 kcps. The Bruker software does not provide any identification markers. As they are a significant size, if they are an escape artifact of SDD's, their absence from the primary peak will significantly effect the quant calculations. Is this correct?* ***Richard E. Edelmann edelmare@miamioh.edu Thu Feb 21***

I wonder if it might be some sort of incomplete charge collection or else a calibration error. We ran into incomplete charge collection on a broken Ge detector. It broke after it warmed up and we got a shadowing of the spectrum downscale from where it was supposed to be. As I recall, we found peaks at about 60% of the energy of the main peaks. Yours seems to be consistently around 92% of the energy of the main peak (i.e., ~8% downscale). I mention calibration error in case different portions of the detector feed into different preamps. (Does anyone do that?) Maybe one segment is out of calibration. I hope your system is under warranty. I would guess you need to get Bruker in to evaluate the detector and/or its setup. Maybe it is just a calibration issue. I would be interested in seeing some of the data if you could provide a screen shot or an MSA copy of a spectrum. Warren Straszheim wesaia@iastate.edu Thu Feb 21

Not a calibration error, as the peaks above and below all align where they should. I hope it is not a "shadowing" as you suggest. Image at: http://www.cami.muohio.edu/xeds/Weirdpeak1.jpg. Richard E. Edelmann edelmare@miamioh.edu Thu Feb 21

Actually, Warren's idea is compelling, even though I have not heard of this before. The energy ratio of the "shadow" peak to the characteristic peak is constant at 0.918+–0.01. Sounds like there are two calibrations acting here with different gains. Ken Livi klivi@jhu.edu Thu Feb 21

I vote for the "new artifact from new SDD" as explained by Ritchie et al in their 2011 *Microscopy and Microanalysis* article (vol 17, pp 903–910): Compton Scattering Artifacts in Electron Excited X-Ray Spectra Measured with a Silicon Drift Detector by N.W.M. Ritchie, D.E. Newbury, and A.P. Lindstrom. I am using it in my electron microprobe class this semester, so have a link to it, if you need it. www.geology.wisc.edu/~johnf/g777/777MMarticles2.html. John Fournelle johnf@geology.wisc.edu Fri Feb 22

Here is a question for Richard . . . When you said "Examples: 0.372 keV below Ti Ka (@ 4.508 keV), 0.610 keV below Ni Ka (@ 7.471 keV), and 0.646 keV Cu Ka (@ 8.040 keV)." These were examples from different samples, each having a Ni or Cu as the major element? Question for John Fournelle. . . . Can you give more specifics about the new artifact? I'm interested. Ken Livi Fri Feb 22

We found our unidentified peaks. They were caused by our over working of our XEDS system. Thanks to the good folks at Bruker. Shutting the system down and allowing it to cool for an hour then restarting cleared the "extra peaks." With respects to Dr. Ritchie et al., like with any new technology new "quirks" (aka "Features") will arise, and it takes experience to learn the pitfalls to watch out for, and once again the microscopy list comes through in letting us all learn a little more. The one thing I do wish to make clear, as we all should realize that in naming specific vendors in postings in this list, it is done so that the specifics of a situation are known, and never should be taken as an off-handed criticism of a vendor. I for one very much appreciate vendors who attend and post responses to the list. I think we all benefit from open discussions. Richard E. Edelmann edelmare@miamioh.edu Mon Feb 25

ESEM:

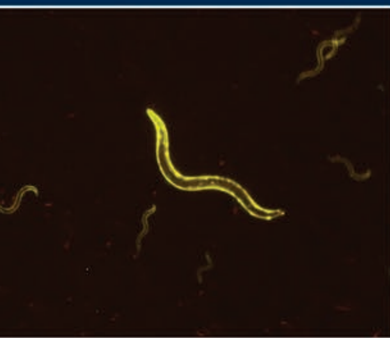
reading beam current

I am using a Philips FEI XL30 ESEM machine. I was wondering if it was possible to (1) measure the current that is striking the sample (not the filament) and (2) control the intensity of the beam current aside from changing the spot size (e.g., specify a beam with current of

get more glow for your dough...

EMS IS PROUD TO INTRODUCE...

NIGHTSEA™ FLUORESCENCE VIEWING SYSTEMS



Fluorescing YFP-transgenic *C. elegans* photographed using the NIGHTSEA Stereo Microscope Fluorescence Adapter. Photograph © NIGHTSEA/Charles Mazel

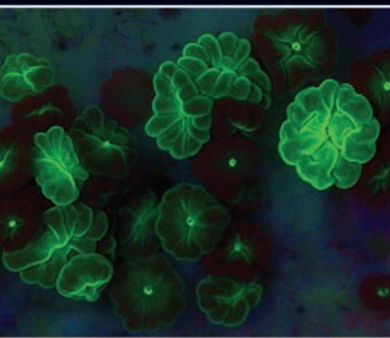
Fluorescence has become the tool of choice for studying many animal models on upright and inverted research stands.

New technology from NIGHTSEA™ now extends fluorescence to standard routine stereo microscopes, where its specificity and sensitivity provide an ideal assist for life science applications.

THIS SIMPLE SYSTEM IS EXCELLENT FOR:

- Quick screening of your fluorescent genotypes – *Drosophila*, zebrafish, *C. elegans*, . . .
- Genotype sorting
- Fluorescence-aided dissection, injection, or micromanipulation
- Freeing up your research-grade fluorescence microscopes for more demanding work
- New faculty start-up budgets
- Bringing fluorescence into the teaching laboratory

GFP-tagged *Drosophila* larva. Photograph © NIGHTSEA/ Charles Mazel



Coral Polyps — Fluorescence. © Wade Cooper

please contact us for more information

NIGHTSEA™ Stereo Microscope Fluorescence Adapter

Adapt your existing lab stereo microscopes for fluorescence

NIGHTSEA™ Fluorescence Excitation Flashlights

Rapid screening of your fluorescent transgenic experiments

NIGHTSEA DFP-1™ Dual Fluorescent Protein Flashlight

NIGHTSEA BlueStar™

NIGHTSEA™ Barrier Filter Glasses

Multiple styles available

Electron Microscopy Sciences

P.O. Box 550 • 1560 Industry Rd.
Hatfield, Pa 19440

Tel: (215) 412-8400
Fax: (215) 412-8450
email: sgkcck@aol.com

www.emsdiasum.com

https://doi.org/10.1017/S1551929513000394 Published online by Cambridge University Press

1 nA instead of spot size 1). My ultimate goal is to attain a beam with a current on the order of nanoamps. ***Gang qiu gangqiu0@gmail.com Mon Jan 14***

Remembering back to when we did e-beam lithography on that microscope, there was a BNC connector on the door. We would plug our ammeter into this connector, and it seems that we had to disconnect a wire that grounded the stage inside the chamber. Disconnecting the wire would disable the touch sensor, so we had to be extra careful about not crashing the pole piece. Jonathan Abbott forzaabbott@gmail.com Mon Jan 14

Your request is quite normal, except for the part about doing it without changing the "spot size." You will probably need a Faraday cup and a nano-ammeter to measure the current reaching your sample. I would like to think you could find such a meter without much trouble at Drexel. I don't know where you plug that into an XL30. I suppose it is on the front door, but others could say for sure. A Faraday cup may be as simple as small, deep hole drilled into a piece of carbon. If you want to get fancy, you can glue a used aperture over the top so that the electrons that enter the hole stay in the hole and get absorbed. In that case, sample current = beam current. Now about "spot size"—I learned on SEMs that didn't have a "spot size" setting. They had condenser lens controls without any particular numbering. You adjusted the lens one way to choke the beam down for more resolution and the other way for more beam current. Of course, one of the side effects was that the size of the beam spot on the sample changed a little. I usually didn't worry about how much since I was working at lower magnifications (by today's standards). I choked it down for high resolution microscopy while maintaining a decent signal-to-noise ratio in my image. I increased it as necessary to get enough current for a decent count rate for x-ray analysis. The setting varied some depending on my needs and how much time I had. Of course, you can also change the objective aperture to minimize spot size for a given beam current. You'll have to experiment a little to see what gives you the sharpest image at 1 nA. So again, what is your concern about the spot size? The three SEMs I work with now have spot sizes that range up to 7, 60, or 255. They are quite arbitrary and non-linear in their numbering. You should probably go through an exercise to see what your numbers correlate to in terms of incident beam size. I think your beam diameter will probably be no more than a few nm even at hefty currents. Hopefully you can focus better than many of our users so that you really do attain the optimum effect of that spot size. My work tends to concentrate on microanalysis and the beam diameter is much smaller than the interaction volume diameter. I can safely ignore the beam diameter and just call it zero. Interaction volume is the main determinant of x-ray resolution. So now I spend a fair amount of time determining the interaction volume as a function of voltage in an effort to keep my interaction within the phase of interest while still exciting lines that I can resolve in the EDS spectra. Of course, you may have a different issue at hand, maybe e-beam lithography, but I think you will still find that the interaction volume is more the determining factor. Set the condenser lens (spot size) where you need to at the voltage you need to. Warren Straszheim wesaia@iastate.edu Mon Jan 14

You have had a good deal of advice on this problem but may I address the control of the beam current? There are three variables 1) Emission current from the gun—bias setting or filament position—move the filament toward the cathode aperture for a higher current or increase the emission current control. 2) The setting of the first condenser lens—stronger lens for a lower current. 3) The size of the beam defining aperture—larger aperture for more current. From my knowledge there are hardly any, if any instruments that do directly link the beam current with control of the condenser system. Whilst the manufacturers will often guess the current they will be placing on the specimen (their readout figure) this guess is only true for their idea of where the above three variables are set; their guess is very much an approximation! The only true indication of beam current is the Faraday Cup! Steve Chapman protrain@emcourses.com Tue Jan 15

I believe that there is a basic limiting factor and that is electron current density. This is pretty much determined by the source, and with thermionic sources, when you increase the emission current you increase the area from which electrons are emitted, all else being equal, you have a larger spot. When you increase your condenser lens current, you get a smaller spot, but with many fewer electrons. The same is true when you change final aperture size. In the end, there will be essentially a fixed spot size for a given beam current when everything is focused. If the spot size you want is larger than the nominal spot size for the current you need, you can just defocus the beam. If you need a smaller spot for a given current, you need a different electron source that will yield a higher current density. Given all the variables in setting up a beam (kV, emission current, condenser current, "objective" current, working distance, aperture sizes), the only way to have a known current is to measure it using a Faraday cup and pico-ammeter, as explained in other posts. You may be able to establish some "standard settings" that will readily put you in the ball park, but if the current is critical, it must be measured. Anyone doing quantitative x-ray analysis or small scale e-beam lithography will confirm this. Ken Converse kenconverse@qualityimages.biz Wed Jan 16

Cryo-Ultramicrotomy:
trimming high pressure freezing cups

We need to take sequential sections down through a high pressure freezing (HPF) frozen sample and doubt that we will be able to get the sample out of the cup. This means we will probably have to trim away the cup from around the sample. We know this can be done (in fact, we are trimming away gold with an old beat-up diamond as I write this), but doing it in a time frame that makes it affordable and doesn't destroy the knife in the process is the problem. A couple potential approaches: 1) pre-trimming the block with a Dremel or similar tool and leaving a small amount of gold intact around the sample cup area before doing HPF. Would this still provide an adequate seal during the actual freezing? 2) Doing the trimming of the metal in a cryostat with a steel knife after freezing the sample, then transferring into the cryo-ultramicrotome for the final sections. Does anyone have any thoughts on these or other approaches? To repeat, we hope to get the cup-trimming part down to a manageable amount of time, without trashing every (expensive) knife we have. ***Randy Tindall tindallr@missouri.edu Mon Feb 11***

I doubt a cryostat would be cold enough to prevent crystallization of the of vitrified ice. If a diamond can cut the gold, have you tried a glass knife? Alternatively, I think the cryo-ultramicrotome has a tool for this! It is a metal rod with a sharp end that fits in the knife holder—take a peek in your goodies box. You could have extras made at the machine shop. Thomas E. Phillips phillipst@missouri.edu Mon Feb 11

As Tom Phillips suggests, there are options in the cryo-ultramicrotome. We do trimming of frozen roots with attached soil with a tungsten-coated glass knife, which would probably work for you. Also, could you make/buy the freezing cups in a softer metal or other material? Rosemary White rosemary.white@csiro.au Mon Feb 11

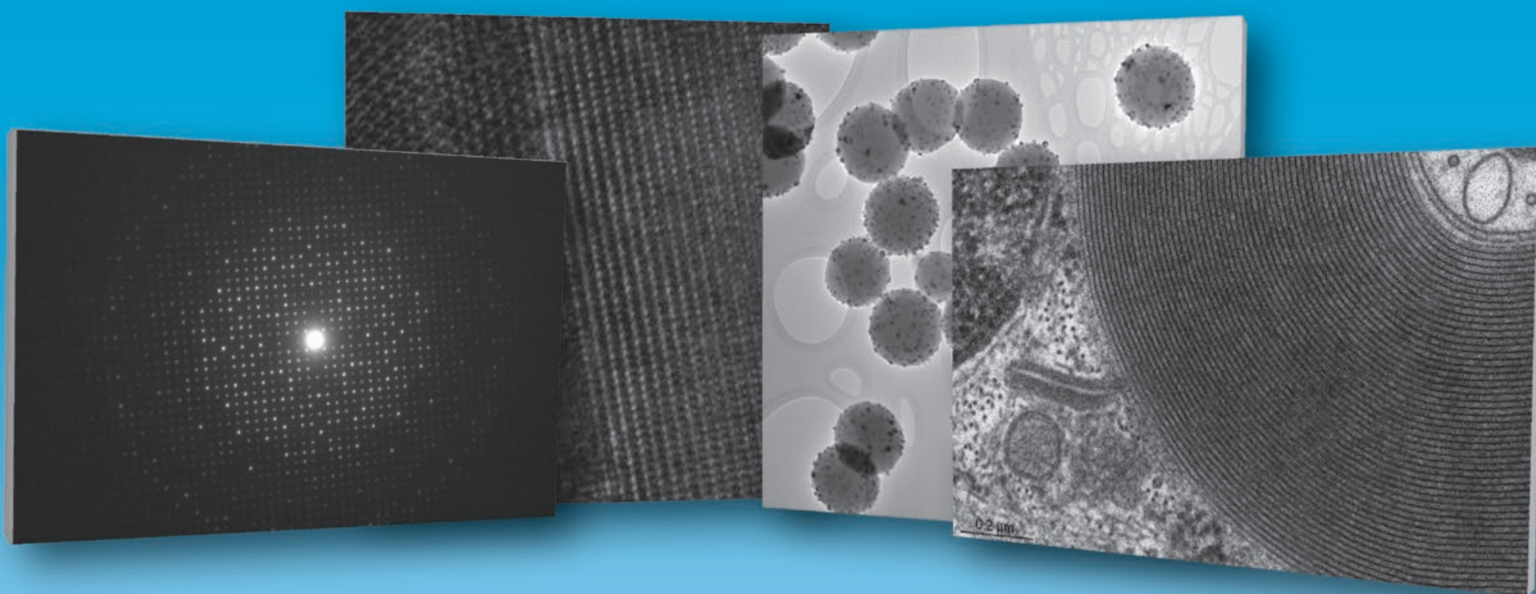
Instrumentation:
welding tungsten filaments to posts

We are working with an x-ray CT machine that burns through filaments on a weekly basis; not from misuse, it's just the nature of the beast. We are looking into the feasibility of spot welding our own

https://doi.org/10.1017/S1551929513000394 Published online by Cambridge University Press

ORIUS®: Smart TEM imaging

True pixel resolution — maximize information
Fast and robust — easy set-up and focus
Automation software — increase productivity
TRADE-IN OPTIONS — *upgrade your old camera*



TEM IMAGE COMPETITION 2013

Do you have a stunning image of your sample you acquired on a Gatan camera or energy filter? How about winning a $1,000 USD Apple e-gift card for that fantastic image! For complete instructions and online registration, please go to:

www.gatan.com/competition.php

Images, left to right:

- Electron diffraction pattern from KNbO oxide recorded with a bottom mount ORIUS®SC1000 CCD camera on a TEM at 200 kV. Image courtesy of Daliang Zhang, Berzelii Centre EXSELENT on Porous Materials, Stockholm University, Sweden.
- Image of $SrRuO_3$/5nmPZT/$SrRuO_3$ thin film on $SrTiO_3$ (001) recorded with a Gatan ORIUS®SC200B camera on a TEM at 200 kV. The sample was final-polished with a Gatan PIPS™II at 300 Volts.
- Pd/SiO_2 catalyst recorded with ORIUS®SC600 at TEM magnification of 9900x and 200 kV. Image courtesy of Peter Crozier, J.M. Cowley Center for High Resolution Electron Microscopy, Arizona State University, USA.
- Image of myelinated nerve recorded with ORIUS®SC1000W (35mm mount) CCD camera on a TEM at 80 kV, at 85 kx magnification. Image courtesy of Kenneth L. Tiekotter, University of Portland, USA.

TEM Digital Imaging

www.gatan.com/products/digital_imaging

www.gatan.com

Enhancing Electron Microscopy

ANALYTICAL TEM | DIGITAL IMAGING | SPECIMEN PREPARATION | TEM SPECIMEN HOLDERS | SEM PRODUCTS | SOFTWARE

https://doi.org/10.1017/S1551929513000394 Published online by Cambridge University Press

https://doi.org/10.1017/S1551929513000394 Published online by Cambridge University Press

tungsten filaments to the posts that stick out of the ceramic block inside the electron gun. We've located a business that will sell us filaments of the correct diameter, and bent in the same hair-pin shape as what we are currently using. We've also found on campus a lab with a tiny spot-welder, and they're willing to let us use it. Having never tried anything of the sort, I have some questions: 1) The business that sells the filaments has two grades: lamp-grade tungsten and a "3RW" alloy made with 3% Re. They said the Re-bearing alloy lasts longer. I figure we'll try it. Is there any reason not to? (Using SEM/EDS I couldn't tell whether or not there was trace rhenium in our old filaments, as the peaks would be right next to tungsten's, and buried in the shoulder.) 2) The lab with the spot welder said they just used some "general" electrode tips, and thought they should work OK, but that it might depend on the composition of the posts. The posts are some kind of tungsten iron chromium alloy. Does this raise any warning flags? (I don't want to wreck their welder.) 3) I am accustomed to cleaning the gun, and replacing/centering filaments for an SEM, but have always ordered filaments that came already pre-attached to the ceramic block. I couldn't find anything with the right dimensions from standard SEM suppliers. So, we plan to re-use the old blocks, but they have a noticeable build-up of I'm guessing mostly evaporated tungsten. Should I worry about cleaning this stuff off? Any advice people might have about making their own filaments would be greatly appreciated. As an undergrad, I had a resourceful professor who made his own filaments, which gave me the idea. I also remember him sticking a paperclip (in place of whatever was supposed be there) in a busted Pirani vacuum gage. Come to think of it, he also came to lecture once with giant bandages on his hand, from trying to pull something out of a running lawn mower. ***Karl Peterson krpeters@mtu.edu Tue Feb 26***

We fabricated our own filaments for the electron microscopes in our student lab for many years. It is not particularly difficult to do, once you work out the system, and the results are very satisfactory—and it saves quite a bit of money. You should have no trouble spot welding tungsten filaments to the posts on your filament disks. Mostly, it takes a steady hand and some care to get the filaments properly centered. Most spot welders have copper rods for contacts, because they have high electrical and thermal conductivities and therefore don't stick to most metals in the welding process. You will need to experiment a bit to get the proper time and current values to get a good weld without burning through the wire. The tungsten alloy containing Re will probably give you a bit longer life, and I see no reason for not using it. It probably is not absolutely necessary to clean the deposit off the ceramic base, but you can probably do it easily with a mildly alkaline solution, maybe Alconox, or sodium carbonate, or dilute sodium hydroxide. Wilbur C. Bigelow bigelow@umich.edu Tue Feb 26

I would be inclined to clean the bases with NaOH just to avoid any bypass currents. The pins should be of a particular alloy to match the coefficient of expansion (CoE) of the particular ceramic being used. As examples, look up Invar and Kovar. The first has a very low CoE and the second has a CoE that matches borosilicate glass so it can be used for feed-throughs in vacuum tubes, hot cathode ion gauges, etc. Whatever the alloy is, it probably won't create any problems for you with the spot welding. Ken Converse kenconverse@qualityimages.biz Wed Feb 27

Instrumentation:
mounting Nikon 1 series camera

I am very interested in hearing from anyone who has mounted a Nikon 1 series compact system camera on a light microscope. I am considering trying a Nikon V1 camera and a cheap LCD monitor via HDMI for a low-budget imaging system on an old Leitz 160 mm tube length microscope. I have a trinocular head (38 mm photo port), and an adapter for it to C-mount (with no optics in it). Is this adapter the 1x, which I understand is suitable for covering a 1" sensor? If so, all I need is a c-mount to 1 series mount adapter, and I should be ready to go. ***Ben ben.micklem@pharm.ox.ac.uk Thu Jan 3***

I've done this with a Canon camera. These are a bit easier because the phototube can be purchased, and Canon has built in features for tethering the camera to the computer, live focus, etc. However, it looks like you're on the right track. The sensor has to be 160 mm from the back of the objectives. Otherwise, the focus point will change for the objective when a specimen is focused by eye. It's best to devise a means to slide the camera up and down along 2 tubes (a wider diameter tube and a narrower diameter tube), or to order a focusing tube from Thorlabs. The quick and dirty way is to use one tube at a narrower diameter and a larger diameter "sleeve." Have a machine shop drill threaded holes into the sleeve for thumb screws (or, if you're like me, you do it yourself with a drill press and hand threading device). It may be a bit difficult to get two different sized tubes that are meant to fit to each other, but you can try Thorlabs or Edmund Optics. Mount your camera, focus by eye, and then, while the camera is giving you a live view, adjust the outer sleeve up or down until the image is in focus. Then tighten the thumb screws against the inner tube at that position. The image may not fill the sensor. Who cares. I suspect you will have plenty of pixel coverage, even for Nyquist rates. Simply crop the images after these are acquired, best done via an automated routine in Photoshop or other imaging program. In that way, the crop will always remain the same. Anyway, you'll likely get darkening at the edges (vignetting), some barrel distortion, out-of-focus areas, etc., so even if the entire sensor is filled, you'll still want to exclude image edges. If it goes the other way and the sensor is overfilled, then you will have to drop a

Microscopist and Facility Manager
Center for Electron Microscopy

The responsibilities of this position include the management, operation, and maintenance of the microscopes in the laboratory. The incumbent will train and assist faculty, student and outside users. This position will supervise an Assistant Manager, an accounting assistant and a laboratory technician, as well a graduate assistants and student workers. The Facility Manager will develop strong ties with other research laboratories in both academic and industrial settings in the region.

For a detailed job description and instructions on how to apply, please visit our website:

http://www.pdx.edu/hr/sites/www.pdx.edu.hr/-files/RSP%20CEM%20-Manager%20CEMN%20Laboratory%20D94200.pdf

Portland State UNIVERSITY

https://doi.org/10.1017/S1551929513000394 Published online by Cambridge University Press

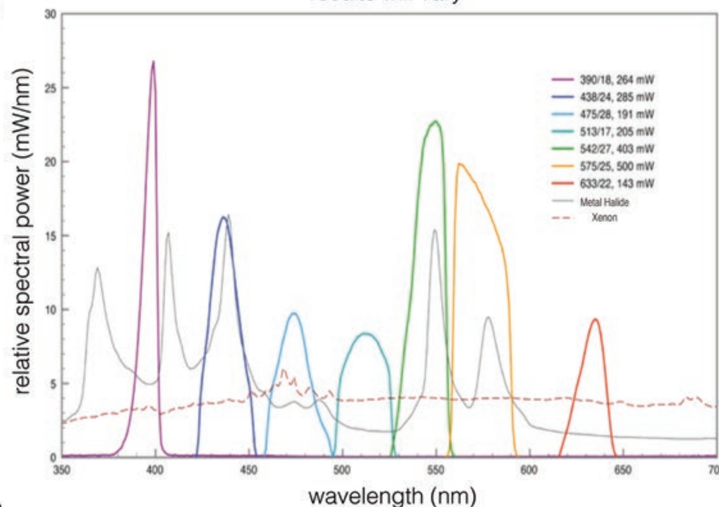
a powerful, multi-color, solid-state illuminator

why buy a lamp when you can have a light engine?

solid state sources, more power than an Arc Lamp *and*

- White light and narrow band outputs
- Six independent sources within UV-Vis-NIR
- User exchangeable filters
- Spectral and power stability
- Illumination uniformity
- Electronic shuttering
- Microsecond switching times
- Minimal heat generation
- Computer control of independent outputs
- Long life > 20,000 hours, 36 month warranty
- Off-the-shelf and OEM configurations
- Custom OEM requests encouraged
- Optical design services available, please inquire

specific outputs are a function of instrument parameters
results will vary



Lumencor, Inc. 14964 NW Greenbrier Parkway, Beaverton, OR 97006 USA **E info@lumencor.com** **www.lumencor.com**

New Plasma Cleaning Solutions from XEI Scientific, Inc.
INTRODUCING:

Both the Evactron CombiClean & SoftClean products:

Clean gently with oxygen radicals and no sputter etching.
Clean samples and TEM Holders
Works with TEM Sample Holder of all major manufacturers.
Provide Sample Storage
Includes a 5-year warranty.

The Evactron® CombiClean System™

The CombiClean System provides seamless control of multiple Plasma Radical Sources (PRS) for cleaning SEMs, and FIBs. Its design includes an intregrated SoftClean Chamber for sample cleaning and storage and an external PRS for cleaning of instrument chamber. Change from instrument cleaning to sample cleaning with the flip of a switch. Compatible with both scroll and rotary vane pumps.

The Evactron® SoftClean™ Chamber

The Evactron SoftClean Chamber is pictured here with PRS, which may be purchased separately.

The SoftClean Chamber is an affordably priced add-on solution for existing Evactron users allowing pre-cleaning of samples before examination in the microscope.

Over 1,400 systems sold. Contact us today for more information.
www.Evactron.com 1-800.500.0133 sales@evactron.com

lens into the tube. If you could find out the focal length of the tube lens in that model of Leica, then you could calculate coverage of the sensor (there's a lens after the objective along the light path). Or, you can turn off all the lights and hold a piece of paper where the sensor should be to get the diameter of the virtual image when focused. Jerry Sedgewick jerrysedgewick@gmail.com Wed Jan 9

Back in January I posted a question about using a Nikon 1 series inter-changeable lens, small sensor camera for microscopy. These cameras have a 1″ sensor (the recent thread on CCD sizes reminded me to get back to the list with my experiences), which is ideal for this application. I bought a Nikon V1, which is heavily discounted at the moment, less than 30% of the new RRP: £250 with lens. We also bought a c-mount to Nikon 1 series adapter, IR remote release, fast SD card and card reader, mini-HDMI to HDMI cable, and a cheap full HD monitor with HDMI input (total cost under £140). So for under £400 GBP we have a complete imaging system. Some photos of the set-up: http://booking.mrc.ox.ac.uk/NikonV1_microscope_mount.jpg. We fitted this to a Leitz Dialux microscope (160 mm tube length) with a 1x c-mount adapter on the trinocular head's photo port. The C-mount adapter gives perfect focus registration with the image in the oculars. The shutter is fired by IR remote release. After turning on the camera, it takes three button presses to engage the mode where it waits for the IR signal. All other settings (e.g., exposure mode, white balance and ISO) are saved when the camera is turned off. The camera will only work in fully manual exposure (the lenses normally communicate electronically with the body, and without this, the camera isn't very helpful). The live view on the monitor doesn't show the effect of changing the exposure; it is always auto-gained. It does show white balance effects though. Having said that, as it will do a quick review of the shot after it is taken, and changing the shutter speed is just a matter of nudging a lever on the back of the camera, it only takes a few shots to get the right exposure. We are using the live view on a 20″ LCD via HDMI, which has very fast frame rate and is lag-free for focusing and searching. The camera does show the imperfections in the peri-plan optics of the low-end EF objectives, but cropping to the central region gets rid of the soft edges. E.g. This example taken with the 10× 0.25 NA EF objective: http://booking.mrc.ox.ac.uk/exampleV1image.jpg The camera can also take impressive full HD movies at 60 fps, so would be useful for teaching purposes. Hope this mini-review is of use to someone looking for color bright-field imaging on a low budget. Ben Micklem ben.micklem@pharm.ox.ac.uk Wed Feb 13

SEM:

wood

*Can I insert wood in electron scanning microscope quanta 200? **Hadeel hadeelzh@yahoo.com Tue Feb 12***

There should be no problem examining wood in the Quanta as far as the SEM is concerned. The question will be what changes take place in the sample and if they are acceptable. You might need to use environmental mode if it is essential to keep the moisture content the same. Otherwise, you could use variable pressure mode to eliminate charging without coating. What are you looking for in your sample that you want to use SEM in the first place? Warren Straszheim wesaia@iastate.edu Wed Feb 13

MT

ONLY ONE COMPANY SETS THE GOLD STANDARD IN SAMPLE PREPARATION

The Desk V HP, a sputtering system that can also be equipped with carbon evaporation, delivers breakthrough electron microscopy sample preparation.

DENTON VACUUM
BARRIERS BECOME BREAKTHROUGHS

Visit our new website
www.dentonvacuum.com/mt

https://doi.org/10.1017/S1551929513000394 Published online by Cambridge University Press