NetNotes

Edited by Thomas E. Phillips
University of Missouri

phillipst@missouri.edu

Selected postings from the Microscopy Listserver from November 1, 2012 to December 31, 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:
CO_2 regulator

I'd like to put a regulator on the Denton critical point dryer (CPD) CO_2 tank so I know how much I have left before I start a run. There seem to be plenty of dual regulators with the low side >120 psi output, but if you go to dual with high pressure on both dials they become expensive: http://store.cyberweld.com/smhiprre820s.html. Can I run this CPD with the lower pressure output? ***Joe Uknalis joseph.uknalis@ars.usda.gov Tue Dec 11***

You need the full cylinder pressure, 800 psi, to have liquid CO_2 for you CPD. The only way I know of estimating when the tank is going to run out of liquid CO_2 is by weighing the cylinder. The pressure inside the tank does not go down when you are using the liquid; it will only go down when you have used all the liquid and only have CO_2 gas in the cylinder. John Nailon j.nailon@uq.edu.au Tue Dec 11

I wouldn't bother. You're using a siphon CO_2 tank, yes? So you're withdrawing liquid CO_2 from the tank, which means you won't see any pressure drop until you've run out of liquid CO_2, in which case it's too late. The best way to know how much CO_2 you have left is to have the tank on a scale. The supply company should put in a specific weight of CO_2, usually 65 pounds, and there is (supposed to be) a tare weight on the tank. When you've used say 64 pounds, change tanks. If you're not using a siphon CO_2, change tanks and get one. Food grade is best (less contamination like oils). Phil Oshel oshel1pe@cmich.edu Wed Dec 12

Specimen Preparation:
samples in SDS

This may or may not be a trivial question. I have been given some samples of particles suspended in water with a little SDS/SLS (sodium dodecyl sulphate) surfactant thoughtfully added to keep them from settling. The general plan is to catch some particles on a carbon grid as normal, but I would like a little advice to avoid disaster, since I only have a few ml of suspension to try things out with. So—has anybody made a powder sample from a solution with SDS in? Does it result in a horrible contaminating organic mess on the sample or is it a perfectly reasonable thing to pipette straight from such a liquid onto the grids just as you would for, e.g., isopropanol? If it would be best to remove the SDS before making powder samples, does anybody have a better method than pouring the suspension through a filter paper and washing the solid off the paper with a more sensible liquid? ***Jo Sharp j.sharp@sheffield.ac.uk Fri Nov 23***

Are the particles charged? You might want to look into coating grids with a water soluble poly-amino acid or other simple polymer with opposite charge; e.g., gold particles (−) easily bind to grids coated with poly-L-lysine (+) in a wide pH range. Sulphate would certainly interfere somewhat, but it might be worth a try. The procedure is very simple and gives neat results with the least possible contamination. If you need more info, contact me off list, please. Jan Leunissen leunissen@aurion.nl Fri Nov 23

A reasonably good TEM sample could be prepared from 2–3 microliter of suspension. If your nanoparticles are heavy and dense enough (i.e. an inorganic staff) and their content is not extremely low you will be able to identify them on an amorphous carbon support even with SDS present in the sample. From my experience, under primarily drying (you may do that in any vacuum chamber pumped with rotary pump) a liquid structure is broken and the surfactant is spontaneously separated from other components of dispersion. At high content, it may solidify aside as semi-transparent body on top of your grid so that you may remove it from the grid by tweezers. At lower content, SDS may crystallize in micron-scale surface crystallites on a grid apart from nanoparticles. At very low content you will observe 3D amorphous low contrast features on a grid still apart from your nanoparticles. So, you see that SDS will most likely not disturb your imaging except of one sad case of expected organic nanoparticles in dispersion. In this case I would never dry the dispersion at all, but go to any cryo-EM technique preserving the structure of liquid. Otherwise you would most likely lose the true morphology and size of your organic nanoparticles. Inna Popov innap@savion.huji.ac.il Tue Nov 27

Specimen Preparation:
starch inclusions

Just wondering if anyone has ever come up with a decent method for TEM imaging of starch inclusions in plant tissue, like soybean, without having the field full of holes where starches saw their opportunity to escape. I expect not, but hope blooms eternal. ***Randy Tindall tindallr@missouri.edu Wed Dec 12***

Starch is difficult to embed, no doubt about it. My best results are by embedding in Spurr's resin (infiltrate slowly). Howard Berg rhberg@danforthcenter.org Thu Dec 13

Specimen Preparation:
LR White

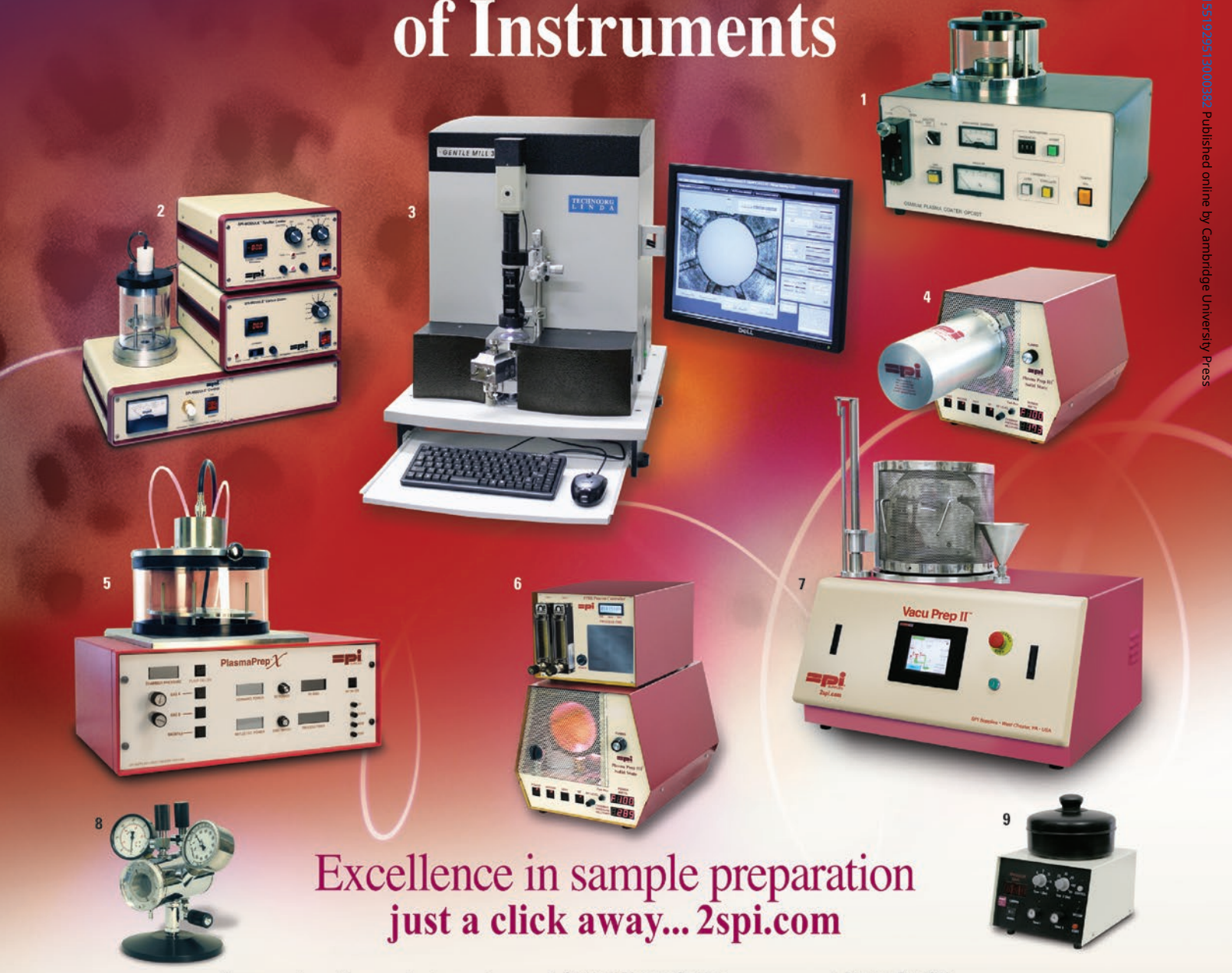
I have a faculty member who would like to know if it is possible to embed an entire mouse brain in LR White and then section it. Has anyone out there tried this? ***Tom Bargar tbargar@unmc.edu Mon Dec 17***

How would you propose sectioning it? Your knives must be a lot wider than mine. I doubt you could cut it on a paraffin-style metal knife microtome. Tom Phillips phillipst@missouri.edu Mon Dec 17

But you could do it using JB-4 resin. However, that does require a different type of microtome. You can use glass knives that are much wider than those used with an ultramicrotome. Section thickness will be in the 6–10 micron range but quality of ultrastructure is far superior to paraffin and many of the stains used for paraffin will work with that resin. Debby Sherman dsherman@purdue.edu Mon Dec 17

I think it is not impossible. It can be done if you can break glass knives that size. Diamond knives are limited to 8mm (cutting edge length/histo knives), as far as I know. I myself used 10 mm glass knives to dissect whole specimens of krill (*Meganyctiphanes norvegica*)

The SPI Supplies Family of Instruments



Excellence in sample preparation
just a click away... 2spi.com

Your results will never be better than your sample preparation. See how SPI Supplies can help you deliver the highest quality results for all your SEM/EDS, TEM and FESEM applications.

1. **Osmium Plasma Coaters** for FESEM Applications
2. **SPI-MODULE™** Sputter/Carbon Coater Module
3. **Gentle Mill™** Ion Milling System
4. **Plasma Prep™ III** Solid State Plasma Cleaner for cleaning TEM holders
5. **Plasma Prep™ X** Parallel Plate Plasma Etcher
6. **Plasma Prep™ III** Plasma Etcher with **PPIII Process Controller**
7. **Vacu Prep™ II** Turbo Pump Evaporation System
8. **SPI-DRY™** Critical Point Dryer
9. **Precision Spin Coater** Spin coater

SPI SUPPLIES®

SPI Supplies Division of **STRUCTURE PROBE, Inc.**

P.O. Box 656 • West Chester, PA 19381-0656 USA
Phone: 1-610-436-5400 • 1-800-2424-SPI (USA and Canada) • Fax: 1-610-436-5755 • 2spi.com • E-mail: sales@2spi.com

embedded in LR White. Semi-thin sectioning as well as ultrathin sectioning worked well, but glass knives are quite "soft," so I used about 100 for an animal of about 5 cm length. And my sections where quite small compared to a mouse brain (>5 × 5 mm). If the sections get too large, it is difficult to transfer them from the water bath to the staining trays. Torsten Fregin torsten.fregin@zoologie.uni-hamburg.de Tue Dec 18

I did not but 0.35 sec Googling gives this: http://www.nature.com/nmeth/journal/v9/n12/full/nmeth.2213.html. http://www.ihcworld.com/smf/index.php?topic=2889.0. http://www.microscopy-analysis.com/news/researchers-prepare-whole-mouse-brain-sem. It seems that the faculty member will not be the first one. Stephane Nizets nizets2@yahoo.com Tue Dec 18

Follow-up to my message: I had a look at the wonderful work of Prof. Denk in Germany. Embedding, imaging and cutting the whole brain are perhaps just the easiest steps! Because if you analyze the sections by EM, you'll end up with a huge amount of data. If you read his paper: http://ac.els-cdn.com/S0959438811002133/1-s2.0-S0959438811002133-main.pdf?_tid=c01a10c4-48fb-11e2-acb8-00000aab0f26&acdnat=1355825867_58990577c5690b1c13c0a396312cb658 you'll see that just 1 mm^3 of brain by EM gives 1 Petabyte of data!! The work hours to analyze the data are best described in tens of thousands. In some dark corner of my memory I seem to remember that a group of scientists designed a computer game where the players had to delimit axons in EM pictures. This is a way to distribute the work hours around the world. The person responsible for the analysis should better enjoy these Christmas holidays because they may well be the last ones for a long time! Stephane Nizets nizets2@yahoo.com Tue Dec 18

You're right! A group of scientists from Harvard, MIT and Max Planck Institute put their heads together and developed a computer game, which launched on the public domain last week, called "Eye-Wire," that allows budding scientists to help trace the neural network of the nerves from the eye to the brain. Here's the link to their announcement: http://blog.eyewire.org/eyewire-the-official-launch/?goback=.gde_80899_member_194622760 and the link to the game: https://eyewire.org/ Ed Haller ehaller@health.usf.edu Tue Dec 18

Specimen Preparation:
etching stainless steel

I'm a neophyte in metallographic crystallography. I have a user looking for grain structure size changes in stainless steel 316. Samples are some wires embedded in phenolic resin for polishing. They polished pretty well. Following a recommendation, we have etched with a solution of 4 g picric acid in 100 mL ethanol. But we are not seeing the grain structure in reflected light microscopy; moving to SEM later today. Is this a good etchant for SS314? How long would you recommend etching for? Any other suggestions or recommendations? ***Richard E. Edelmann edelmare@miamioh.edu Tue Dec 18***

Stainless is a pain! What makes it stainless is what makes it hard to etch. . . . Unless the grain size is very small—if you don't see the grains on the optical microscope, you won't on the SEM. Also, if you haven't done many polished samples for the SEM you'll find you need to kick up the contrast much more than usual. There isn't much contrast (better optically!). As long as the grain size is large enough, SEM is not required for grain size determination in stainless. But for either equipment it needs to be etched! We do the following: For polishing: We usually finish polishing with Buehler MasterPrep after 1 micron diamond. It's expensive but effective. It's 0.05 micron alumina in a proprietary solution. Wear gloves and safety glasses, it's slightly corrosive. This polish is not required but I have found it is worth the money to reduce times that my students spend struggling in the lab. Warning—for some alloys, it will remove inclusions (like oxide inclusions) so if you are interested in the inclusions, use of the Buehler MasterPrep may not be worthwhile. For etching: The best etch is generally electrolytic but I'm always willing to try something else first since I'm not skilled at electrolytic. Simple picric in ethanol is an etch for carbon, and low-alloy steels, and will not work for stainless. There is a picric-HCL-ethanol etch that works for martensitic stainless steels (called Villella's) but I've never found it to work for austenitics. The alloying level is much higher in austenitics so they are a different world. What I find almost always works on stainless (both cast and wrought) is one of the very, very nasty "regia" etchants including (from George Vander Voorts's Metallography Principles and Practice): 1) 1 part HNO_3, 1 part HCl, 1 part water (General purpose etch for most stainless steels. Stir solution during etching (20°C) for uniform, stain-free results. Outlines constituents, reveals grain structure.) 2) 4 parts HCL, 3 parts HNO_3, 4 parts water (Use same procedure as number 1). 3) 15 mL HCL, 5 ML HNO_3 (agua regia. For austenitic grades. Use fresh. Use at 20°C for about 5 sec. Attacks sigma, outlines carbides. After 20 s, sigma completely dissolved. Reveals grain boundaries. Do not store etchant! 4) 3 parts HCL, 1 part HNO_3, 1 part glycerol (Glyceregia. For austenitic grades. Reveals grain structure, outlines sigma and carbides). Warning! These etchants are nasty and you should use a fume hood with complete coverage with safety gear. I do not store any of these etchants as the book states for some of the similar compositions—Do not store. Many, many, years ago, as an undergraduate we had an etchant that was stored explode so I've always paid attention whenever that is stated in a book. Comments: I put the etchant in a beaker and dip the sample in with tongs and (while wearing all the safety gear!) gently move the sample around. Otherwise you get bubbles from the etchant that form and leave unetched, perfect circles all over your sample. Very irritating. Leave plenty of room in the beaker so that etchant does not splash out when you dip and stir. Add enough etchant so the mounted sample is covered, even when you raise it slightly to gently stir. It's better to have the sample face up so you can see it. It will become cloudy when it is etched. This may be difficult to see if the diameter of the wire is small. Wait for it to get a bit cloudy and then check it on the microscope. When etching is complete, you should see all the grain boundaries. You don't want to over-etch or the sample becomes too three-dimensional for optical microscopy. You can etch—check on scope—etch—check on scope, etc. You don't have to re-polish if you under-etch. You do have to re-polish if you over-etch. Usually the last polishing step will remove the over-etch. You do know that dry picric is explosive, right? It's shock-sensitive and quite dangerous. I used it for years without knowing this and fortunately, never had it explode. Use your MSDS! Also, if the picric fumes build up in a hood they can crystallize and become explosive. We have a picric hood that washes down to help with this problem. In general, I avoid using the picric unless necessary. I keep it, submerged in water, but only use it when I have to. Sorry for the safety lecture but I am a worrier! Good luck and feel free to contact me off line with questions if you don't find this or the other recommendations to work. Robin Foley (old metallurgist that still etches many different cast and wrought stainless alloys on a regular basis!) rfoley@uab.edu Thu Dec 20

Per George Vander Voort: "Fortunately, there have been no documented cases of explosions from picric acid in laboratories, according to Phifer [1]. If it is wet with water, it is not an explosive hazard and any attempt to blow it up by a bomb squad will only result in picric acid being spread all over the immediate area. The concern has always been in finding an old bottle that has dried up producing dehydrated picric acid, and if it has a metal cap, rather than a plastic

cap. In such a case, shock-sensitive metallic picrates may have formed at the cap-bottle interface. The solution is to have a robot pick it up and re-hydrate the picric acid after opening the bottle under water. If the cap is plastic, and the acid has dried out, friction from opening the cap could cause detonation. The solution here is to place the bottle in a large bucket or tank of water and allow water to dissolve any dried picric acid on the cap threads. Leave the bottle in the water for a few days until some water can be seen inside the bottle. Then, while under water, open the lid and re-hydrate the picric acid. Obviously, the wise lab manager checks the picric acid bottle periodically (which can vary with lab usage of picric in etchants) to make sure that the picric acid remains wet. Today, bottles are sold with at least 30% water content. A good practice is to keep a log of when the bottle has been checked and when water is added. Also, use only plastic or glass spatulas to remove picric from the bottle and add it to the etchant. Do not use metal spatulas and clean the cap and threads on the bottle and on the cap with a wet paper towel. If you have copper piping, do not dispose of picric acid by pouring it down the drain as explosive metallic salts could form." My opinion (having created some shock sensitive peroxides, and managed them [and others'] as well): If it's dry, keep it dry. Not a significant risk. If it's wet, don't let it dry and saltate or react with metals. And again, it will not be a significant risk. I have both forms and they are kept that way dry/dry, wet/wet. I have handled dry picric a lot over the years and never had an issue, as long as it hadn't dehydrated from solution. Postscript—A fusion prep of picric forms beautiful crystals (under polarized light) that often show grain boundary migration. Tony Havics ph2@sprynet.com Thu Dec 20

As others have suggested, etching stainless steel can be tricky. Surface preparation is critical, especially for small wire with cold worked structures. The Vandervoort book is a great reference with many options. The *ASM Handbooks on Metallography* (Volume 9, I think) are also excellent, and one or more editions of this set will likely be in your library. One issue that you might have with these samples, since you are a neophyte in this area, is recognizing when you have revealed the true structure. A small wire may have such highly elongated grains that all you may see with a perfect preparation and etch is a jumble of lines generally parallel to the wire. Anyway, back to the etchant question. For type 316 stainless steel, picric acid solutions are not going to work nor are Kalling's or Marble's reagents likely to be very successful. Even the classic electrolytic etch with 10% oxalic acid for austentic stainless alloys can be less than satisfactory for T316. Here are some options for you: 1) If you want general grain structure, electrolytic etching at about 6 VDC in 10% ammonium persulfate in water will usually give a more uniform and reliable etch than oxalic acid for T316. 2) An immersion etch for general structure (if you don't or can't do electrolytic) is a solution of 12.5 g of $CuCl_2$ in 350 mL of concentrated hydrochloric acid mixed at a 3:1 ratio with concentrated nitric acid (the $CuCl_2$ and HCl can be mixed and stored, but only mix in the nitric just before etching). Immerse or swab gently for a few seconds. This etch is sensitive to surface conditions; a good polish is essential and etch immediately after polishing. (The stainless steel polished surface will passivate quickly in ambient air, which inhibits etching.) 3) If you want only to see the grain boundaries, try electrolytic etching with 60% nitric acid in water at 1.1 VDC (30 to 120 s). Larry D. Hanke hanke@mee-inc.com Sun Dec 23

Specimen Preparation:

problem with Spurr's resin

We have been having problem with the Spurr's embedding: It always has a soft center area of the tissue even when the size of the tissue is small. The softness makes even thick (1 μm) sections difficult to cut. I am wondering if the problem comes from a chemical in the set since there are a few labs with the same problem here. ***Gina Zhang gzhang@u.arizona.edu Thu Dec 20***

My first thought was that your tissue is not completely dehydrated. How big are the tissue pieces? As I'm sure you know, ideally the pieces should be about a half-millimeter across for good infiltration at normal times. If they are bigger, you could extend the dehydration steps to include a couple more 100% acetone or propylene oxide steps, and then add a couple more infiltration steps and/or increase infiltration times. Plant tissue is often harder to infiltrate than animal tissue. Adjust your schedules accordingly. Randy Tindall tindallr@missouri.edu Thu Dec 20

I agree with Randy. It looks like you have a problem with resin infiltration or maybe with dehydration. I would increase incubation time and the number of changes of resin/propylene oxide, resin, and maybe alcohol. For my tough specimens, usually bone, infiltration with resin can take up to three days. I would not suspect chemicals when seeing soft spots only in the center area of specimens. Vladimir M. Dusevich dusevichv@umkc.edu Thu Dec 20

Fully agree with Randy and Vladimir. It is not very likely that there is anything the matter with the embedding reagents. Logic should tell us that if there is a heterogeneity pattern over the specimen, i.e., defined areas well embedded and sectioning well, other areas flawed, then it is a procedure/specimen issue. When you say: "a few labs have the same problem here"—Do they embed the same tissue as well? Have you tried the Spurr's from their lab which would seem the thing to do. Jan Leusnissen leunissen@aurion.nl Thu Dec 20

And to embed large parts of plant seeds—infiltration for a month or so . . . time to take a break and leave your tissue sitting in resin! Rosemary White rosemary.white@csiro.au Thu Dec 20

Image Processing:

reading metadata

I am wondering if anyone knows of a program that can extract the metadata from TIFF files saved by an FEI Helios Nanolab 650. There is an incredible amount of data that can be read by right-clicking into "Properties" on the TIFF file (when using the FEI support PC), but I cannot seem to find a program that can read this data offline. I figure it might be somehow proprietary, but thought I would email the list in hopes that someone has some experience with this. ***Josh Taillon jtaillon@umd.edu Sat Nov 3***

Try using Graphicconverter, if you work MAC-based. If it cannot read the metadata, you can ask Thorsten Lemke at http://www.lemkesoft.com/ to do a special add-on for you to read out the data into an EXCEL sheet or something like that. Stefan Diller stefan.diller@t-online.de Sat Nov 3

I usually open the FEI tiff file using the Notepad or other text viewer. You will see at the start a lot of gibberish, but going to the end of the file you will see all the metadata. You will need to figure out what they mean, but most of them are pretty straightforward. Carlos Kazuo Inoki carlos.inoki@lnls.br Sat Nov 3

Our Bio-formats project is designed to open proprietary microscopy formats. This library can be used in many programs including ImageJ; see http://loci.wisc.edu/software/bio-formats. We currently support 127 formats and are trying to add support for electron microscopy formats include FEI tiff. See: http://loci.wisc.edu/bio-formats-format/fei-tiff. Kevin W. Eliceiri eliceiri@wisc.edu Sat Nov 3

Anyone can also read the metadata from most tiff files using any simple text reader such as Notepad. Open the file with notepad and scroll down to the bottom of the file. You will see metadata similar to the following: [Beam] HV=20000 Spot=5 StigmatorX=0.000550687 StigmatorY=–0.000959411 BeamShiftX=0 BeamShiftY=0 ScanRotation=0 ImageMode=Normal Beam=EBeam Scan=EScan. Joe Neilly joe.p.neilly@abbott.com Mon Nov 5

While it is possible to use a text editor (Notepad, Notepad++, TextPad) to find TIFF metadata, the flexibility of the TIFF format doesn't always make it easy. The metadata can be stored anywhere, the beginning, the end or the middle of the file. However tools like http://meta-extractor.sourceforge.net/documentation.htm or http://www.awaresystems.be/imaging/tiff/astifftagviewer.html can reliably extract metadata from TIFF files either using a friendly GUI or an automatable command line interface. Usually the meta data you want will be associated with the "ImageDescription" tag (code 270 or 0x010E) although there are numerous other tags that could contain useful information. (See http://www.awaresystems.be/imaging/tiff/tifftags/baseline.html). Some vendors even implement custom tags and register these with Adobe for special data types like x-ray spectrum data. (See http://partners.adobe.com/public/developer/tiff/index_reg.html.) Nicholas W. M. Ritchie nicholas.ritchie@nist.gov Tue Nov 6

Thank you for the wide range of responses. I just wanted to give a summary of what I have learned so that it may be useful for other people. The FEI metadata fields are definitely accessible just by opening the TIFF file as a text file in your favorite text editor. This is more than sufficient if all you're doing is checking a number quickly (Thank you to everyone that pointed this out to me). Using the AsTiffTagViewer (http://www.awaresystems.be/imaging/tiff/astifftagviewer.html) (Thank you, Nicholas Ritchie), I was able to find that the codes are stored as ASCII text in tag number 34682 (for the FEI Helios, at least). This is a non-standard tag, but is freely readable using Tiff libraries. The most useful website I found was http://www.farsight-toolkit.org/wiki/FARSIGHT_Tutorials/Bio-Formats. Thank you Kevin, from Wisconsin for pointing me in the right direction. This is a simple tutorial that will lead you through installing the Bio-formats command line tools. They have developed a plugin to read specifically from FEI Tiffs, and the 'showinf' command will cleanly display all the custom FEI tags that are present in the file. Using this, you can then "grep" or search for whatever particular information you need. I think this will be the solution I use going forward. Thank you everyone for all of your help. I certainly learned a lot. Joshua Taillon jtaillon@umd.edu Wed Nov 7

Image Analysis:

crystallography vector problem

Recently, in the middle of the night I found myself, not counting sheep, but trying to solve in my head the following: For a three-dimensional lattice with primitive base vectors **a**, **b**, **c**, *find a vector perpendicular to the bc plane and with a length equal to the spacing of those planes. I have no idea why I wanted to know this; perhaps I needed it in the dream from which I awoke. (Note that the vector sought is not the reciprocal lattice vector* **a*** *since that has a length that is not the plane spacing but the reciprocal of the plane spacing.) The result I got was (derivation below):* $(\mathbf{a}\cdot\mathbf{b}\times\mathbf{c})\mathbf{b}\times\mathbf{c}/(\mathbf{b}\times\mathbf{c})\cdot(\mathbf{b}\times\mathbf{c})$ *Now this expression is more complicated than I anticipated. So my question is: can anyone tell me of a simpler expression? P.S. It did not help me get back to sleep. Derivation: We need a vector perpendicular to the bc plane.* $\mathbf{b}\times\mathbf{c}$ *is such a vector. Thus, a unit vector in that direction is* $\mathbf{b}\times\mathbf{c}/|\mathbf{b}\times\mathbf{c}|$. *The length of the vector needs to be the plane spacing which is the projection of the vector* **a** *onto this unit vector, namely* $\mathbf{a}\cdot\mathbf{b}\times\mathbf{c}/|\mathbf{b}\times\mathbf{c}|$. *The final result is obtained by multiplying the unit vector in the appropriate direction by the (scalar) length of the vector to give the result above.* ***Alwyn Eades jae5@lehigh.edu Sat Oct 13***

A while back I posted a question about writing an expression for the vector distance between planes in a crystal. I found the expression $(\mathbf{a}\cdot\mathbf{b}\times\mathbf{c})\mathbf{b}\times\mathbf{c}/(\mathbf{b}\times\mathbf{c})\cdot(\mathbf{b}\times\mathbf{c})$ and asked if anyone could tell me of a simpler expression? I thought I would do an update because a couple people have sent me messages to say that there is no simpler result, but Phil Ahrenkiel of South Dakota School of Mines and Technology sent me the beautiful and simple result $\mathbf{a}^*/(\mathbf{a}^*\cdot\mathbf{a}^*)$ where $\mathbf{a}^*$ is the reciprocal lattice vector for the crystal. Alwyn Eades jae5@lehigh.edu Thu Oct 25

LM:

cleaning a CCD camera

I am afraid I am not able to solve a seemingly basic problem. We have an inverted Zeiss light microscope with a CCD camera (PixelLink) just above the oculars. There is a lens (a ring) between the camera and the microscope. We have three fixed halos on the background of images and I can't find the origin of the dust particles causing the halos. The halo appears as if the dust were near focus. Here are the tests I did in order to diagnose the problem: When I turn the camera with the ring attached (both turn together in relation to the microscope), the halos don't move even a bit. The dust must be part of the camera/ring, not the microscope. If I detach the camera from the microscope (with the ring attached), the background (when I direct the camera to the light coming from the window) is perfectly clean. The dust is not part of the camera/ring but is part of the microscope! It makes no sense! Or perhaps the ambient light is not enough to give a clear picture? I have a bright uniform background. One additional note: if I make the ring loose and I slowly shake the camera/ring a bit, the halos shake also a bit on the picture. I already cleaned the lens/ring and the fine glass in front of the camera CCD with lens paper and ethanol. Any comment welcome. ***Stephane Nizets nizets2@yahoo.com Mon Oct 15***

The light coming through the microscope is coming at the camera/coupling optics from a small range of angles, making the dust cast sharp shadows on the sensor. Off the microscope the light comes into the camera/coupling optics from a wide range of angles, and the shadows are softened to the point of being invisible. The same happens with dust on DSLR sensors—often dust is not visible until you stop down a lens to f/11 or so. You could try performing the same test in a dark room with a small high-powered LED torch located a few feet away. When you say you cleaned the glass in front of the CCD, do you mean an IR (hot mirror) filter in front of the sensor? If so, there could be dust on the internal side of the hot mirror (if there is a significant gap between that and the sensor). Does the hot mirror have a retaining ring allowing its removal? Ben Micklem ben.micklem@pharm.ox.ac.uk Mon Oct 15

LM:

artifact of a video

I asked the question before but I didn't give enough information about the problem. So I'm going to explain it again. I'm using a Zeiss Axiovert 200M inverted microscope to record the process of a cylindrical fiber being pulled from a viscous solution. The process uses a syringe needle to perform the pulling so it is not possible to use a cover-slide. As well, I use a 40× objective (for 400× final magnification) so I'm sure that only an inverted microscope can be used because the liquid layer is about 1–2 mm thick. There's always an artifact in the form of a black/white spot (either really black or very bright depending on the focus) at the location where the viscous solution is lifted up by the fiber and forms a "convex" structure. I'm not aware of any way to use epi-illumination on an inverted microscope. I do use phase contrast to visualize the fiber formation. Does anyone know what caused the black/white spot? ***Lingling Xu xulingling723@gmail.com Fri Dec 14***

It is caused exactly by that "convex" structure you are observing. Its surface is at another angle, forms a type of lens on its own, so it refracts the light differently and you see: (i) black because the light refracted by the convex structure does not enter the objective; or

https://doi.org/10.1017/S1551929513000382 Published online by Cambridge University Press

(ii) see white because the convex structure refracts the light in the focal point of the objective. That is my understanding from the point of my basic knowledge of optics. Maybe someone else can explain it better, or correct me. And I am not sure if that would be named an artifact. Josif Mircheski jmircheski@us.es Fri Dec 14

Since the phase contrast mechanism is based on differences in density, it is not very surprising that with a gel, a fiber and a syringe needle you get a strong contrast. Did you try to observe your material in brightfield mode? Hopefully your fiber is still visible in brightfield! If your Axiovert 200M has fluorescence capability, you may try to label your fiber and your gels with two different colors. Stephane Nizets nizets2@yahoo.com Tue Dec 18

Instrumentation:

water cooling

Good time for a discussion around water cooling (temperature is under 0°C outside). We have started our TEM (FEI G20) some 8 years ago, the cooling loop is completely isolated from light and it was filled with tap water and an anticontaminant called Thermoclean. The temperature is set on 17°C but we had some periods of total inactivity so the water came to room temperature for several months. Since then I never changed the water, just added some more when needed. I did a microbiological test every year and it was always negative, until this year (minimal contamination, but still something). I wondered if there is a standard accepted procedure for the water cooling: is there a need to change the whole water after some time? Do you use an anti-contaminant and which one and how? I also have a colleague who is running another machine with a water cooling set on 38°C, which would be perfect to use as a bioreactor but he does not agree. He tried tap water with Thermoclean but he got a strong contamination after 2 months. I must say that the Thermoclean is now 8 years old and although it is still young for a whisky, it may be too old and it lost its activity so I advised my colleague to buy a new one. Anyway, I would still be grateful if someone could give me a hint for a product which inhibits microbial growth at 38°C in a water cooling loop. ***Stephane Nizets nizets2@yahoo.com Mon Dec 10***

This from the FEI Technical Service Group: We advise to use Thermoclean DC, Please contact the supplier at support support@bioanalytic.de (http://www.bioanalytic.de/waterbath-stabilizers.en.html) for more information if interested. In reading your question I would at least add new Thermoclean DC every year. I had enclosed two PDF's but the listserver stripped those. For Titan we can use the MSB procedure; for Tecnai we do not know yet but if it is a Tecnai with single chiller (i.e., without UT or Lorentz lens coils which can be recognized by having an additional cooler) this can also be used. In the document it is mentioned that it can be used up to 85 degrees but please contact the supplier for the dose and how often this needs to be replaced. Hope you find this information useful. I should add that actually (other than the comments you make about people currently being on the beach, etc.) there is a real variation worldwide regarding what the best treatment is depending on local water quality, acidity, cleanliness, additives etc. so you will find that different labs have different procedures. FEI tried to standardize the treatment protocol, but because of variations in water, acidity particularly, this is difficult to do. Jan Ringnalda jan.ringnalda@fei.com Thu Dec 13

I'm new to this lab and the recent PM we had identified we had cooling water contamination and leaks in the FEI Tecnai. The service engineer said it is up to the lab to maintain the cooling system to the point of changing the water on a regular basis. Within the EM itself it is FEI's problem. So now we are instituting an annual schedule for that. Tim Morken timothy.morken@ucsfmedctr.org Thu Dec 13

TEM:

imaging of carbon nanotubes

Could I get some help on microscope set-up and sample prep for carbon nanotubes (CNTs)? A researcher wants to look at microwave effects on single and multi-walled CNTs (shape defects, end defects). I was going to use thin holey carbon support films at 75 kV, but have seen nice images of CNTs at 200 kV. Any suggestions? Also, do CNTs generate enough contrast on their own, or could negative staining be used to help improve the fine surface detail required to observe or amplify the subtle defects I am supposed to see. Thanks in advance for any suggestions, before I begin imaging. ***Mark Grimson mark.grimson@ttu.edu Fri Nov 2***

I have, in the past, done extensive TEM work on both single wall NT and multi-wall NT of carbon. I was able to very successfully use a 300 kV FEG TEM beam (at 300 kV and also at 200 kV) to get a good contrast on CNTs (without any negative staining). A lot of HRTEM was performed with great results. Of course, the single wall NTs are more challenging as they tend to damage (deform and eventually decompose) under the beam faster than the MWNTs. I however was able to get HRTEM on single wall NTs by allowing exposure under the electron bean for the least amount of time and also by using low dose methods. I used grids with holey carbon or holey carbon/Formvar support films. I would put the CNTs in a vial with appropriate suspension, very lightly sonicate and use a micro-pipette to put a drop on the grid. Let it naturally dry for a little bit and overnight it in a desiccator, with a clean vacuum pump attached, before taking it to the TEM. In cases where suspension does not carry any oil in it or it is simply alcohol, a natural dry for an hour or two is good enough before TEM. Zia ur Rahman zia.rahman-1@nasa.gov Fri Nov 2

TEM:

HR lattice image or artifact?

I recently tilted a very thin foil of aluminum to the ⟨110⟩ zone axis, carefully aligned the instrument, inserted a large objective aperture, and attempted lattice imaging of fine precipitates. Much to my surprise, I obtained lattice images of the Al ⟨110⟩ zone. A Fourier transform of the HR lattice image, using my image-space magnification calibration, shows spacings of 0.2335 and 0.2034 nm in a clear ⟨110⟩-shaped diffractogram. Looking up the lattice spacings (UIUC WebEMAPS website), I find values of 0.2338 and 0.2025 nm for the (111) and (002) planes in aluminum, so this makes me think I'm seeing a true Al ⟨110⟩ lattice image. However, I'm using a CM200FEG with SuperTwin lens, which should give Cs = 1.2 mm and Cc = 1.2 mm. Using the equation for point resolution in De Graef's book (P. 601), and plugging in Cs = 1.2e-3 m and lambda = 2.508e-12 m, I calculate ps ~ 0.237 nm, which makes me think I shouldn't be able to see these planes—and that neglects Cc and delta-E, so resolution ought to be even worse. Am I misunderstanding point resolution, and need to calculate a different (more forgiving) resolution function? Or am I imaging some sort of artifact (surface oxide?) that is convincingly similar to my real information? ***Chad Parish parishcm@ornl.gov Mon Nov 5***

Since you are using a FEG source, the contrast transfer function (CTF) doesn't damp very quickly. Normally point resolution is quoted as the Scherzer resolution, i.e., at the first zero of the CTF at a particular defocus. In LaB_6 TEMs, the source adds a damping function to the CTF so you usually don't see much beyond that 1st zero. With a FEG, you have much less damping of the CTF and the oscillations of the CTF start to affect your image. Remember that the CTF indicates information transfer through the scope, so if the CTF is non-zero you are passing information. If the particular spatial frequencies are between the 1st and 2nd zeros of the CTF, you will see those frequencies in your image. We've seen 1.5A information on a 200 kV SuperTwin

FEG by adjusting the objective defocus so that the CTF passes those frequencies. Interpretation should be done very carefully on 2nd and 3rd zone images. Henk Colijn colijn.1@osu.edu Mon Nov 5

You might get some insights by downloading Max Sidorov's "CTF Explore= r" at: http://www.maxsidorov.com/ctfexplorer/. You will be able to see how resolution beyond the point resolution can be achieved with a FEG. The only catch in real microscopy is that the waves carrying resolution beyond the point limit may be out of phase with waves carrying resolution at different frequencies, and the resulting images may not be directly interpretable representations of the structure of you specimen. Also if you study John Spence's classic text on HRTEM you will have a good grasp of what is going on here. John Mardinly john.mardinly@asu.edu Tue Nov 6

Many thanks to the people who replied, both on- and off-list, to my query. The answer appears to be, if I am understanding and summarizing correctly, that point resolution (~0.24 nm for a CM200FEG-ST) is a less appropriate measure than information limit (~0.14 nm). The FEG source results in a slower damping of the transfer function, so although the Al lattice spacings are past the first zero of the function, the transfer of information is generally not zero at those spacings, so the information makes it to the camera. Several people cautioned me that since we're beyond the first pass band I should exercise extreme caution in interpretation of the image information. However, since the interest of the experiment was to see if precipitates aligned along given lattice directions, and was not structure determination, I think I am safe in that regard. I was able to convince myself with the following simple MATLAB script tested under Version 7.10.0.499 (R2010a)—clear all; close all; pack; memory chi = @(u,df,l,Cs) pi.*df.*l.*u.*u+0.5.*pi.*Cs.*l.*l.*l.*u.*u.*u.*u; %transfer function l=2.508e-3; %wavelength in nm Cs=1.2e6; % Cs in nm u=0.01:0.01:6; % spacing in inverse nm dfSCH=-1.2*(Cs*l)^0.5; % Scherzer defocus plot(u,sin(chi(u,dfSCH,l,Cs))) hold all u111=1/.2338; u200=1/.2025; plot(u111,sin(chi(u111,dfSCH,l,Cs)),'o') plot(u200,sin(chi(u200,dfSCH,l,Cs)),'*'). Chad Parish parishcm@ornl.gov Thu Nov 8

TEM:

adding a digital camera

I know that Hitachi H7000 is hardly a new instrument but ours is well maintained and fully operational so we need to consider how best to manage photography in the future. At present we use Kodak 4489 cut film and it produces excellent results, which are then scanned using a flatbed scanner. However there are clearly long term issues about the future of the film, there are the general problems with darkroom processing and chemicals and the inevitable delay in producing results or indeed the cost of staff time if we rush them through. We are aware of the basic types of digital capture available. In our case 35 mm port (wide field capture) or under the film camera (high resolution capture) using something capable of 2k × 2k would be suitable. We mostly image biological tissues and negative stains so the 35 mm port would seem sensible. It would help us greatly if anyone with an H7000 or similar with a retro fitted digital camera system would be willing to share their experiences with us. In particular, we would want to know what manufacturer and type, why they chose it, how easy it was to have fitted and how it's worked out. Any info would greatly help us in our decision making. ***Malcolm Haswell malcolm.haswell@sunderland.ac.uk Wed Nov 21***

As you may know, we run a series of courses known as Protrain Portfolio where students have teaching data, test specimens and a series of practical exercises to complete. These courses go very well when the "student" uses a below screen digital capture system, but often when using a 35 mm port system the resolution is insufficient to resolve the subtle changes we are trying to have the students recognize. Remember the 35 mm port systems have a much lower magnification than the below screen systems. But on visiting these customers we find we are able to resolve the subtle changes on the screen that were not visible on their 35 mm port camera. I do not know the resolution of the cameras we are taking about, but it worries me that if people want to see the data they view on the screen the resolution of these cameras is extremely important, less so for below screen systems. I should add that some of these customers have been satisfied with their normal results when I, to be honest, have not! Steve Chapman protrain@emcourses.com Wed Nov 21

I too have a Hitachi H-7000 TEM (~25 years old) that has been well maintained. I replaced the film camera with a Gatan MeganScan 4K × 4K digital camera about 10 years ago and I never used the film again. I have been happy with the Gatan camera, which uses optical fiber (higher sensitivity), instead of glass lenses. It offers high resolution and the software (Digital Micrograph) is easy to use. The problem with an old TEM is that the camera does not 'talk' to the microscope and you have to input the magnification manually. The camera is slow in today's standard, and I do not believe Gatan still produces this model anymore. Zhaojie Zhang zzhang@uwyo.edu Wed Nov 21

We own an old Zeiss EM902, which is equipped with a Gatan 694 slow scan camera (1k × 1k). This one is bottom mounted and we use the viewing screen to examine the specimen and search for a good position to take a shot. The search mode of the cam is quite slow (not as slow as our 4k × 4k Ultrascan) and does not replace the viewing screen for this job. There is no connection to the microscope to read out date. But you can activate a dialog that asks for the magnification just before you take a picture. I think Gatan is quite expensive, but if you can find a used one, the price can be fine. The software is only expensive if you need special plugins for microscope control or EELS. Michael Epping m.epping@arcor.de Wed Nov 21

I have a Hitachi 7100 TEM (basically a 7000 with a motorized stage) and had a Gatan 7 megapixel bottom mount camera installed about 5 years ago. The installation went smoothly and we have been very happy with the high-resolution images, which have been used in a number of publications. The digital micrograph software is easy to use and no one in our lab has any desire to go back to film. We do mostly bacterial and viral imaging so the bottom mount camera made sense for us. Tony Greco tgreco@marine.usf.edu Mon Nov 26

SEM:

sample suggestions

I would really like to get my hands on some micro biological samples suitable for SEM. The kinds of things I am thinking about are radiolarians, forams, coccoliths, diatoms, that kind of thing. Any ideas about sources of raw materials I can get to provide beginning SEM students with a surprise at the microscope? ***Jonathan Krupp jkrupp@deltacollege.edu Wed Nov 7***

Diatomaceous earth would be good. Soft, sedimentary rock made of diatoms, used for filters, mild abrasives (some toothpastes, although I can't think of a brand off the top of my head), and the like. Also, have a chat with the geologists in your area—lots of the limestones in California are composed mostly of forams, ostracods, and suchlike critters. Along with dragging a plankton net through SF bay. Phil Oshel oshel1pe@cmich.edu Wed Nov 7

I have a few environmental scientists from down the hall who image diatoms on our SEM. I believe they have small glass diatom "traps" they use to collect samples, though I'm fairly sure you can pick up almost any stone in a healthy stream and scrape some off. As far as sample prep goes, they acidify the diatoms to eliminate biological material, then drip a slurry onto an Al stub and dry the sample. It seems to work pretty well and I've been able to get great images of a number of different genera. Rob Dean deanr@dickinson.edu Wed Nov 7

If you are looking for diatoms, why not run over to your local home improvement or swimming pool supplier and pick up some diatomaceous earth. It is often used as filtering material for swimming pools and there are now some eco-friendly insecticides that use diatomaceous earth. (Do a net search.) You will have to suspend the material to separate the diatoms from the rest of the "earth," but it is a cheap source of diatoms! Henk Colijn colijn.1@osu.edu Wed Nov 7

I did just that on several occasions. I was able to go to a local pool supply place and just sweep about a square foot of the floor near the diatomaceous earth stack and got enough sample to make several stubs. Justin Kraft kraftpiano@gmail.com Wed Nov 7

Check out this website so the students can compare their findings. http://www.ucl.ac.uk/GeolSci/micropal/diatom.html. Lita Duraine duraine@bcm.edu Wed Nov 7

And there's also kitty litter—I think this is still mostly diatomaceous earth. Rosemary White rosemary.white@csiro.au Wed Nov 7

SEM:
low-temperature cathodoluminescence

Does anyone have any experience with this? We have a Gatan CL system on a Hitachi 3400 and we were curious how beneficial a cooling system would be. Would a Peltier stage be cold enough? Would an LN_2 cold finger be too cold? ***Eric Jay Miller eric-miller@northwestern.edu Tue Nov 13***

We have the same system, and a Deben cold stage. Only goes down to –30°C (high vacuum), but so far it's been all we need. Mostly used for mineralogical samples. It will be interesting if anyone needs colder temperatures. Phil Oshel oshel1pe@cmich.edu Tue Nov 13

MT

https://doi.org/10.1017/S1551929513000382 Published online by Cambridge University Press