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SAMPLE PREPARATION - osmium ignition

As a cautionary tale I relate an incident that happened in our lab recently. Someone placed 0.25 g of osmium tetroxide crystals into a dry, clean 50ml polypropylene Falcon tube and it immediately burst into flames. The inside of the tube turned black and the crystals disappeared. Luckily, she was working in the chemical hood so no harm was done. As I have been working with osmium tetroxide for many years with no problem, it was a surprise to me that this chemical could burst into flames so easily. Can anyone offer any insight on why this might happen? One reason why this may not have happened to me is that I always follow the good advice of adding solid to liquid. Paul Webster <pwebster@hei.org> 08 Nov 2007

OsO_4 is a strong oxidizing agent, and polypropylene will burn, so I am not too surprised. The wisdom of adding solid to liquid is exemplified here, since in that case, the concentration of OsO_4 is lessened, and furthermore the mass of water will counteract any temperature increase caused when the polypropylene is oxidized. Both effects slow down the oxidation reaction. Bill Tivol <tivol@caltech.edu> 08 Nov 2007

I've always first rinsed the ampoule in hot water and then distilled water. The osmium tetroxide crystals would melt and then I'd allow them to recrystallize along the sides of the ampoule. Next we'd place the ampoule inside a glass-stoppered reagent bottle (100 ml) and shake the bottle vigorously to break the ampoule. Any solutions, water, buffers, etc. would be added to the reagent bottle. Lastly we'd sonicate the solution for at least 30 minutes. Method of Bill Wergin and the Wisconsin gang. Always with safety precautions: gloves, eye protection, fume hood, etc. No problems. Bruce F. Ingber <bingber@srrc.ars.usda.gov> 09 Nov 2007

I really hate to have the glass fragments in my osmium stocks. I pour a few mls of liquid nitrogen into a 50 ml disposable beaker and then add the osmium ampoule. The difference in contraction rates between the glass and osmium crystal results in it popping of the glass and no longer being sticky. I then snap the top off the ampoule and pour the crystals into a water filled bottle. If you use a stir bar to mix, it goes in solution within an hour. This is easy to see since there are no glass bits that look like osmium crystals. Naturally we do all of the above steps in the fume hood. You don't want to add liquid nitrogen to an open or cracked vial since it could expand rapidly and explode but if you add the ampoule to only a few mls, it rapidly turns to gas and after 2 minutes or so, you can remove the still cold but no longer nitrogen immersed vial. Tom Phillips <phillipst@missouri.edu> 09 Nov 2007

SAMPLE PREPARATION – wood for TEM

I tried to prepare wood (Fagus and Pinus) for transmission electron microscopy by embedding in Epon and LR White and got terrible problems in sectioning. The blocks seemed okay, they were not soft. We got sections, but they immersed in the trough and did not stretch. The result was awful. I do not understand what went wrong, maybe, poor infiltration? Is there anybody who has experience with wooden material? Anne Heller <heller@uni-hohenheim.de> 03 Dec 2007

Coincidentally, I just finished a study of tension wood in sweet gum. I did immunogold-silver and didn't take it to the TEM, but the sections looked pretty good. Because we wanted optimal immunoreactivity, we used LR White (not Epon). Here's what I did: Using a chisel, a wafer of wood about 1x1 cm square (parallel to the surface of the trunk) and about 3-5 mm thick was removed from a living tree. The wafer was immediately immersed in water and split into "matchsticks", approximately 2-3 mm per side, by inserting a razor ~1 mm into the top edge of the wafer and splitting it along the grain (not cutting). These "matchsticks" were then put into glutaraldehyde and, using a brand-new razor blade, cut into 1-2 mm thick slices. So you have squares of wood, about the size of a TEM grid, about 1-2 mm thick, with the long edge of the cells running top to bottom through the thinnest dimension. By splitting the wood (and not just cutting it), you are assured that the orientation of the tracheids are parallel to the long axis of the matchstick. So when you cut the matchsticks into wafers, the tracheids are wide open to your solutions, thus facilitating the infiltration of the wood specimen. The rational here is that if these cells were conducting water before you removed them from the tree, they should conduct alcohol and resin, too. The samples were fixed for 24 hours at room temp and then dehydrated in 25%, 50%, 75% (2 hours each) and absolute ethanol overnight. They were then infiltrated with LR white resin with increasing concentrations (25%, 50%, 75%, neat - 24 hours each step), also at room temperature. Specimens in neat resin were placed onto a shaking platform for 48 hours. Slices were placed into cylindrical polyethylene capsules and oriented with the faces of the wafers facing the bottom of the capsule and polymerized at 55° C for 2 hours. We cut mostly 0.55 micron sections. Other notes: you'll want to be really slow with the block trimming - this resin-wood material is extremely tough. Take really thin slices with a new blade. These blocks are also really hard on the diamond knife. I'll send you a picture to back up my protocol. My sweetgum samples didn't require this, but if your wood is really dry (*i.e.* it is full of air, won't sink in your solutions, etc.), you might have to vacuum it to get the air out and your solutions in. I'd vacuum the wafers - the air has the smallest distance to travel. I got this "matchstick" technique from Clair et al, 2005: Precautions for the structural analysis of the gelatinous layer in tension wood. IAWA Journal 26: 189-195. Andrew Bowling <andrew.bowling@ars.usda.gov> 03 Dec 2007

SAMPLE PREPARATION - perchloric acid hazards

Does anyone know whether it is safe to store the mixture of 20% perchloric acid and 80% methanol at room temperature? Qingfeng Xing <qxing@ameslab.gov> 11 Dec 2007

Fifty years ago I did a lot of work polishing the alloys used then in the manufacture of jet engines. These alloys were so corrosion-resistant that about the only thing strong enough to electrolytically polish them were the various solutions based on perchloric acid. Therefore, I worked with these solutions a lot. Basically, perchloric acid becomes an explosive when it becomes heated, when it is concentrated, and in contact with an easily oxidizable material (such as cotton, paper, most organic solvents, most kinds of cloth, *etc.*). Therefore it is imperative to avoid these conditions! If the hot, concentrated stuff comes in contact with such materials it can produce a very violent explosion. In one instance a graduate student filtered a small amount of a perchlorate salt out of a solution, then placed the damp filter paper that carried the salt in a drying oven. Luckily, he left the room before the stuff let loose, whereupon it drove the door of the oven across the room and embedded it in the opposite cinder block wall. (However, we routinely used boiling perchloric acid to digest mineral samples in certain analytical procedures—no organic or oxidizable material present). In the work I did, we always kept the polishing solution cooled to below 10°C during the electrolysis process. For solutions made with acetic anhydride we kept the temperature at the point where crystals of the anhydride just started to form in it. We stirred the solutions vigorously during the electrolysis process to prevent localized heat build-up at the electrode surfaces. And we worked inside a large stainless steel or polyethylene pan with a half-inch of water in the bottom, which we washed thoroughly after an experiment was completed so that no residue of the solution remained around to become concentrated by evaporation. Before a perchloric acid solution actually explodes it will start to develop a brownish-red color. Therefore, we always kept a large beaker full of ice water sitting by our polishing apparatus, which we would have poured into the polishing solution to dilute and cool it if this ever happened, and we kept the door of the lab open so that we could then leave in a hurry. We stored our stock solutions in glass bottles with glass stoppers. It is important to avoid bottles with ordinary caps made of organic materials. We always rinsed the bottles thoroughly with water, to remove any traces of solution that might have dribbled down the sides of them during solution transfer. With these precautions we stored perchloric acid solutions of all

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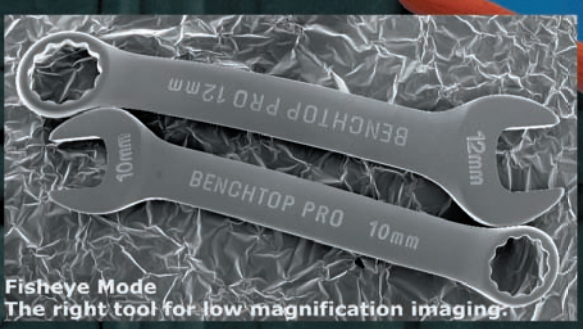

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kinds for many months with no problems. Perchloric acid is a very useful reagent if you use it with proper precautions and due respect. Wilbur C. Bigelow <bigelow@umich.edu> 11 Dec 2007

Great summary, Wil. If you don't mind, I would like to send this to a customer that I have been talking to about electropolishing and one of the electrolytes that are recommended is perchloric solutions. I would like to add another point. One of the safety things that you missed is that you should work in a perchloric acid rated hood. These hoods are designed so that they can be periodically washed down in the exhaust area. In the Number 5 book in the Philips TEM series, which is about TEM sample preparation, they also have a small section on the safety precautions for perchloric acid solutions as well as others. Scott D. Walck <walck@southbaytech.com> 11 Dec 2007

To elaborate slightly on one of the points about perchloric hazards: many metal perchlorates are explosive when dry (including nickel perchlorate). So even if an organic substance is not present, there can still be serious hazards. When using a perchloric-based electrolyte to prepare superalloys for microscopy, one should take care to avoid letting used electrolyte evaporate to leave a dry deposit. Also, perchloric fumes may react with metal ductwork some distance away from the fume hood, producing explosive salts. Remodeling workers have been blinded or maimed simply by tapping on a fume hood duct which was not perchloric-rated, but had been used for perchloric-acid work. If you've not had experience with this stuff, it's wise to take some time for reading and self-education before working with perchloric! Roy Arrowood <arrowood@utep.edu> 12 Dec 2007

SAMPLE PREPARATION - differential polymer staining

One of our graduate students is working on a block copolymer system (NIPAM/PDMA). The polymers are relatively similar in structure, however they differ in that one contains a secondary amine and the other a tertiary amine. We are looking for a heavy metal stain (and/or staining conditions/protocols) that is likely to preferentially attach to one of these groups. Neil Coombs <ncoombs@chem.utoronto.ca> 25 Oct 2007

I don't have an answer to the chemical question but instead suggest trying AFM phase imaging instead of TEM. Atomic Force Microscopy has been very successful in getting image contrast between different polymer domains without staining. The mechanical interaction of the AFM probe with the sample surface senses local differences in stiffness and/or adhesion in order to create contrast. The AFM can scan as-produced exterior surfaces of test samples and on ultramicrotomed blocks in order to see inside the bulk. There is no need to create a good thin section, just a smooth block face. Examples of phase images can be found at: www.asmicro.com/Applications/phase.htm <donc@asmicro.com> 25 Oct 2007

MICROTOMY - alternative ultramicrotome knives

I am trying to find a reasonably inexpensive way for students to practice using our ultramicrotome, without the potential for damaging the diamond knives. I have not had any luck making glass knives using tools we have available, and the knife cutters are quite expensive. In addition, the whole process of gluing the boat onto the knife, or using tape and nail polish, seems a bit cumbersome. I'm not so concerned with whether the sections are electron-thin at this point, only that we can develop the method. I was hoping it would be possible to buy cheap steel knives that could fit into the knife holder for our RMC ultramicrotome. This would seem natural, since we already use razor blades to trim the blocks. We just need some way to mount the blades, but I can't locate any type of steel knife that is designed to fit in the ultramicrotome holder. The other possibilities I have come across are sapphire and tungsten carbide knives. The tungsten carbide knives at least come in a triangular shape, so we just need some tape and nail polish to make the boat. We can always switch to the diamond knife when we need ultra-thin sections. I would be grateful for any suggestions someone may have. I thought there might be some type of boat available that can hold razor blades. Maybe the only option is to be more protective of the diamond knives. We are mainly cutting polymeric materials embedded in resin. Phil Ahrenkiel <phil.ahrenkiel@sdsmt.edu> 13 Nov 2007

I understand your problem. To be honest, your best bet for students really is a glass knife. It does take some practice, but is tremendously cheaper than having someone destroy a several thousand-dollar diamond knife. On the other hand, I have often wondered why some creative person does not sell "premade glass knives" with the trough attached. Perhaps someone will contact you in this regard. I believe this would be your best solution. What sort of tools are you using to make the glass knives? If you are using standard glazier's tools (scoring wheel, glazier pliers), then I can understand your problem since it takes a lot of practice. Also, you should be using special glass strips designed for making ultramicrotomy knives rather than trying to break large pieces of plate glass. You might check out eBay, since I see items like glass knife makers and even ultramicrotomes selling very inexpensively ($200 and $500, respectively). Maybe some kind soul would donate a knife maker to you. Alternatives: I don't know how much they cost, but tungsten carbide knives are not exactly throw away knives. Razor blades are OK for rough trimming but would quickly dull when cutting resins. Sapphire knives are not cheap and they are easily damaged and cannot be resharpened. John Bozzola <bozzola@siu.edu> 13 Nov 2007

I grew up on the old Keith Porter "Free Break" method of making glass knives. If you start with a square of glass 1/4 to 1/2" thick. and 4" square, you can make a small score (1/4") halfway along one edge, perpendicular to the edge, and then cause a break with curved glaziers pliers. It may be hard to find the curved pliers, but you might be able to get them from EF Fullham. If not, you can attach toothpicks to a standard set of glaziers pliers such that one side has a single toothpick in the center, and the other has two that flank the center. You can then squeeze very gently, and watch the crack progress across the glass if you are lucky. This process can be continued until you have 1" "squares". A diagonal score, followed by more squeezing will often give you a good knife. If the whole tape/boat business is too much trouble, you can place a large bead of wax along the edge of the knife. Make sure that the glass is clean first. Remarkably, this will hold sufficient water for sections to float off. I can't send a diagram through the server, but I could probably put something together that I can send you directly. Let me know. Joel <jbs@temple.edu> 14 Nov 2007

Regarding selling pre-made glass knives, I'm sure you are aware of the (somewhat true) urban legends about glass being a liquid and the edge flowing over time - I'm sure this isn't going to be an issue at the level of training students being discussed, but it is going to be hard for some people to turn loose of that one.... One thing I have observed, may be related to our local indoor environment or could be more general, but glass that I have broken more than a few days before loses it's edge wetting properties. I've never seen it mentioned anywhere. I have used our Harrick Plasma Cleaner for 10sec on older knives (bare, or with tape or waxed-metal troughs already attached) to make them hydrophilic again. Dale Callaham <dac@research.umass.edu> 14 Nov 2007

The edge flow legend is largely just that. Over the years, I found that one could prepare a batch of knives several weeks to months in advance and still use them successfully. I never said anything about it since it seemed to go against the grain and seemed to be a bit lazy, rather than efficient. Recently, Herb Hagler officially stated the following: "The old mythical tale that glass knives must be made fresh just is not true. If care is taken in the making of high-quality glass knives, they may be stored for many months to years and used repeatedly until they become unusable after 5 to 15 or more uses for thin sectioning." This quote is on page 70 of his chapter "Ultramicrotomy for Biological Electron Microscopy" in: Electron Microscopy Methods and Protocols (2nd Ed), Edited by John Kuo. Humana Press. ISBN 13: 978-1-58829-573-6. In other words, the glass knives are treated like a diamond knife, in many ways, when cutting ultrathin sections. They are not so long lasting when cutting thicker sections, however. Your observation about the loss of wetability of the knife edge is exactly what I have seen and the major reason why I was forced to make knives within a couple days of use. In fact, I could actually see a whitish film on the surface of the knife and assumed this was condensate of laboratory fumes and organics from the paraffin used to seal the trough -- or maybe even from the adhesives when tape was used to seal the trough. Your idea to use a plasma cleaner is really neat! Thanks for sharing your observations. John Bozzola <bozzola@

siu.edu> 14 Nov 2007

I was able to buy curved glazier's shears and a scriber at a store that sells glass and tools to artists who work with stained glass. Don Chernoff <donc@asmicro.com> 14 Nov 2007

I totally agree with you, a good knife maker and (good) glass is the way to go. Consistent good knives at a reasonable price as long as one is following the instructions for the knife maker! And the handling of the knife! As always: "It's the fool behind the tool". Markus F. Meyenhofer <micro@superlink.net> 14 Nov 2007

IMAGE ANALYSIS - stitching high-resolution microscope images

I am working on an independent study project to analyze the differences in lignin quantities between the roots and the stems cut from the four cardinal directions from different tree genera. I have taken high resolution micrographs of sections that I cut and stained, but am having trouble finding an adequate solution to stitching the photos together. I have toyed with Hugin and PTAssembler, which both utilize the open-source Panorama Tools library, Autopano, and Enblend. Yet, I am either not using these tools correctly, or they are not adequate for what I want to do. I have also heard that ImageJ might also be decent tool to use with the TrakEM2 plugin. On average, I have approximately 20-40 800x600 pixel uncompressed TIFF images being stitched in a grid fashion, which make up the entire image of the section. My question is whether you are familiar with these tools and their abilities. Are you aware if they could perform such a task? Do you have any suggestions as far as where to look for information on this topic? Ryan Cook <cookrn@muohio.edu> 16 Nov 2007

The newest version of Photoshop has a nice feature that stitches together images. I have been using it extensively with LM brightfield immunofluorescence (4-14 images each about 1.3 MB). Only problem is that it won't process groups of images in batches. You have to select each set which is annoying when you have 20 sets of images. But it works well. Tom Phillips <phillipst@missouri.edu> 16 Nov 2007

Photoshop CS would do if you have enough overlapping features (or you have to manually stitch some as the algorithm might fail). We used to stitch routinely 100s of images if illumination was correctly properly. If any distortion is present in your images, you might try Autostitch. But I do not remember if it takes Tiff files. Xuejun Xue-jun Sun <xjsun@ualberta.ca> 17 Nov 2007

When I am not taking pictures with a TEM I am taking them with a camera. I have been doing a number of panoramas lately that are combined from multiple images in multiple rows. So far my largest image is over 900MB and it was stitched together from three rows of seven images, each being about 20MB, 4200 p X 2800 p each. Photoshop CS3 has really improved the stitching process over the earlier versions. And yes, you do have to have some overlap in the images. I have been stitching each row separately and then I stitch all of the rows together. With smaller images like what you have it might be worth a try to see if PS can do it all at one time. I expected to have to do a lot of tweaking but if you have a proper overlap that the program can recognize the program can handle it automatically. Of course, this doesn't go very fast and I have hung up my computer numerous times but it does get the job done. Norm Olson <nholson@ucsd.edu> 17 Nov 2007

Try PTGui software (http://www.ptgui.com) (at least their demo version which is free for one month). If your images do not overlap sufficiently, you can manually choose corresponding features which simultaneously appear in two adjacent images. It is easy to do if you correctly arrange yours images in the right order using the "Source images" function. Prof. Jean-Paul Baïlon <jean-paul.bailon@polymtl.ca> 17 Nov 2007

Autostitch has long been a favorite for photographers stitching panoramas. The demo works with JPEGs only, but it should be easy to convert for a trial. The demo is available here: http://www.cs.ubc.ca/~mbrown/autostitch/autostitch.html . If it is satisfactory the same webpage refers to several softwares that have licensed the algorithm. Michael Shaffer 17 Nov 2007

Our lab has been using ImageJ with the plug-in MosaicJ. MosaicJ is a semi-automated method; you give the program an initial guess by placing images in rough alignment, then let the program automatically translate (and rotate, if necessary) the images to assemble the final mosaic. Output quality is very high. All ImageJ types are supported for input. On a dual-core 3GHz Pentium 4 with 4 GB RAM it takes about 10 minutes to align 10 - 15 individual 1392 x 1040 pixel RGB TIFFs. MosaicJ has an excellent help page: http://bigwww.epfl.ch/thevenaz/mosaicj/ I've written a few notes on installation of MosaicJ: http://staff.washington.edu/takenomm/trainingdocs/analysis/mosaic.html Marc Takeno <takenomm@u.washington.edu> 20 Nov 2007

IMMUNOCYTOCHEMISTRY – adherent cells

I have been asked to do immuno-EM on adherent cells. The PI also provided pellets of the same cells, as controls for the antibody reaction: the cells in the pellets are transfected with the antigen. The rub is that what the PI is really interested in is the interaction of the adherent cells with B cells which have been added to the culture. He is especially interested in looking for the antigen in the fine pseudopod-like projections/connections that form between the 2 cell types. Hence, the cells must remain as an intact monolayer. I've had good luck with the pellets in LR White. What can I do about the monolayers? Most of the immuno resins won't polymerize in the presence of air/oxygen. I've tried making an Aclar sandwich, but that was a mess. Should I try an Epon-like resin and then etch the sections? I know that someone out there will have faced this in the past. Leona Cohen-Gould 17 Oct 2007

It is possible to flat embed your cells in the tissue culture dish. All one needs to do is exclude the air. What I have done is to grow the cells in 35 mm dishes. Fix and dehydrate as normal. Fill the dish to over-full and then put the cover on the dish upside-down. If you put the cover on so that one side is down and then you angle the cover down into place so that there is no bubble under the cover. I have also found that the LR White reacts with some tissue culture dish plastic. What has worked is to coat the inside of the tissue culture dish and the top of the cover with sterile, molten 1.5% agar and 0.5% gelatin. I put some in the dish, swirl around until all surfaces are coated, pour out the excess and let dry. The cells do not stick as well, but what I have worked with have been fine. Let me know if you want a formal protocol. David Elliot <elliott@arizona.edu> 17 Oct 2007

You can also do cells on coverslips (glass or Thermanox) for flat embedding; the blocks are easier (I think) to retrieve once polymerized, plus you can get away from having to treat the polystyrene dishes to "protect" them from being damaged by the resin. I do resin infiltrations in glass coverslip "Coplin" jars - I'm not sure of their real name, but they are really short Coplin-like jars that hold 22 x 22 mm coverslips instead of slides. Smaller coverslips can be done in glass scintillation vials or regular old EM snap-cap vials. A zillion ways to embed: old JB-4 style molds with a bit of Saran wrap, Aclar film (since you already have that), or other transparent cover work very well if you are doing UV polymerization. I used to use the JB-4 chucks as covers when I was doing thermal polymerization in these molds. Another option: several companies sell molds that will work for UV polymerization of coffin blocks if you can work with cells grown on small Thermanox strips, and I know of one company that may make slide-casting molds out of UV-transparent stuff for you if you ask nicely. In a pinch, I've used aluminum weighboats with Saran wrap sort of floated on top for bigger samples/coverslips—this uses a lot of resin, but it works. I also tried the Aclar sandwich trick and was not thrilled with the mess that resulted. Tamara Howard <thoward@unm.edu> 18 Oct 2007

We also like Thermanox round coverslips for growing cells since they do not react with any media or solvent that is generally used for EM. We embed them in Wheaton snap caps, which are also impervious to embedding media and the smaller coverslips (abut 11 mm) fit well in them. If polymerization is to be done at 60°C, a 1000 ml beaker of dry ice can be included in the polymerization oven to insure a CO_2 atmosphere in which the media will polymerize with no problems. I expect something similar could be done with UV polymerization.

IMMUNOCYTOCHEMISTRY - pre-embedding tissue cultures

I have a very interesting co-culture system for myelination using DRG neurons that are non-GFP and myelinating with cells that are GFP. We have done extensive immunofluorescence experiments using the confocal to try and

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answer one curious problem. In some cases with certain GFP cells, myelination occurs but the GFP protein seems to be squeezed out of the wraps so that the fluorescence is minimal. A question has been raised if the cells that are doing the myelinating are truly the GFP cells or a contaminating non-GFP cell from the DRG neurons. I am considering doing a pre-embedding immunolocalization for GFP protein using ultra small gold. Then further processing the sample and doing silver enhancement on the sections on Nickel grids. Has anyone done a technique similar to this? If so can you provide any tips or advice? Joanne <jbabiarz@rci.rutgers.edu> 29 Nov 2007

Have you thought to localize GFP on the surface of sections cut from your tissue? We've tried two antibodies that work well: RDI (Cat# GRN-FP3abg) goat anti-GFP or Abcam (ab290) rabbit anti-GFP. Either work diluted 1:50 in Tris for 120 minutes. We used tissue embedded in LR White following fixation in 4% paraformaldehyde/1% glutaraldehyde in Dulbecco's media for 30 minutes on ice, rinsed twice for 15 minutes in media, then immersed in 0.1 M glycine in Tris-HCl, pH 7.4 for 60 minutes. Tissues were then dehydrated in an ice cold ethanol series to 90%, then infiltrated in 1:1; 1:2; 1:3 90% ethanol:LR White media for 45 minutes each on ice, then in two changes of ice cold LR White for 60 minutes each, then a final change of LR White at ambient temperature for 60 minutes. Polymerization was at 60C excluding oxygen. This technique may work a little better than an *en bloc* procedure since you will not need to permeabilize your tissue, nor will you need to silver/gold enhance. Doug Keene <drk@shcc.org> 29 Nov 2007

TEM: Objective aperture vs. EFTEM

We hope to do tomography on 0.5 micron thick cryo samples. I am wondering how much, in terms of resolution, we would gain using energy filtering over using the small 10 or 20 micron objective aperture. Is there any way the diffraction aperture could be brought in to add resolution? The TEM platform is a Tecnai F20. Bob Harris <bharris@uoguelph.ca> 02 Nov 2007

0.5 µm cryospecimens are pretty thick for a 300 kV instrument, so I'd be doubtful that you'd get good resolution at 200 kV. A small objective aperture will increase contrast at the cost of resolution, but that may not be too much of a cost, if the resolution is poor anyway. The diffraction aperture is an area-selecting aperture in imaging mode, so it would be of no benefit. Bill Tivol <tivol@caltech.edu> 02 Nov 2007

TEM – alignment problem

I may be having a mental block on this one, but I can't remember the alignment parameter that would address the following problem: In our TEM, especially at high magnifications, using the coarse focus control causes the beam to oscillate away from the screen center. The image of the specimen does not move significantly so I am not talking about alignment with the voltage or current centering. The condenser and objective apertures are aligned, as verified by sweeping the beam with the brightness control. In short, the beam moves around during focusing, but the specimen's image does not. The problem is that we constantly have to re-center the beam during high magnification work to maintain illumination. Any thoughts from the Collective on what I'm missing? Randy Tindall <tindallr@missouri.edu> 13 Nov 2007

Have you recently cleaned the specimen holder? I had a problem with the beam moving a long time ago and someone suggested that I clean the specimen holder and after that the problem went away. Maybe it will work for you. Patricia Stranen Connelly <connellyps@nhlbi.nih.gov> 13 Nov 2007

This could be a charging issue - if so, cleaning the specimen rod would sort it. And a plea to Listers: I have a problem with the focus of our Philips CM10: When the image is static using the wobbler, the image looks distinctly out of focus (though it looks OK with a holey carbon film), and is greatly improved by ignoring the wobble when focusing the image. Can anybody advise if there is an optimum under focus adjustment available on the CM10 (as there was/is with JEOL TEM's), or a means of adjusting (or reconnecting) the wobbler to co-relate with the image focus. Any advice gratefully received. Alastair McKinnon <a.d.mckinnon@abdn.ac.uk> 13 Nov 2007

I would consider the beam shift (or tilt too) and its compensators. Some of the old TEMs even have beam compensators (X and Y) on the upper column, such as old JEM TEMs, which compensate the beam shifts during changing the focus of objective-lens in a large range, such as through focus series. Long Li <longli_tem@hotmail.com> 03 Nov 2007

With regard to your optimum under focus adjustment, one needs to rely upon the manufacturer to provide such a facility, not all do! In the JEOL, depending upon your magnification the instrument, when set up under wobbler focus, gave the wobbler/true focus and then stepped underfocus by a set amount when the focus control was released. If we did the job by eye we would probably set the optimal under focus (OUF) of a lower than JEOL set it, but it was a good start. I train people to set wobbler focus and then to watch the focal change as they move underfocus until they see what is in their mind their own OUF. Take images in steps either side of this point to see "on a print" what people in the lab agree is the best micrograph - your OUF! Plotting graphs relating to OUF for a particular material (organelle density) makes life a good deal easier if a novice is using the instrument - set wobbler focus and from the graph set the OUF for that organelle density - easy. Be aware that OUF changes with, kV, magnification, organelle density, section thickness Steve Chapman <protrain@emcourses.com> 14 Nov 2007

I have until now missed this topic but may I add data to help with the understanding? Lenses have fields that overlap, we do not want them to do so but they do! This problem is most noticeable when the final condenser lens picks up a focal change due to the stronger objective field impinging on the condenser field as an offset. In the past, manufacturers have approached this problem in a number of ways. JEOL placed an equal and opposite deflection on their "balancing coils" so that we did not see the effect. Hitachi used a top hat objective pole piece to attempt to reduce the field overlap. Philips always balanced the illumination movement by adjusting the relationships of the upper and lower pole pieces. I do not know which route is taken with modern instruments but I am sure the problem is dialed out in some way. My worry is why you have just noticed the problem. I would guess there may be another "problem" that has emphasized the condenser movement. I would ask if your current objective focal length (lens current) or condenser lens currents are normal, or do you have a malfunction in the objective area, lens current or deflection system. This would be typical of such a fault in that the lenses are not running at their correct values? Let me know the cure as we may be able to help others that will suffer in the future. Steve Chapman <protrain@emcourses.com> 14 Nov 2007

TEM - lattice fringes

I'm flying blind here. I have a question from a user about something I don't know about. He looks at nanoparticles. Recently he showed me a picture of some of his particles and asked about the location of the lattice lines. The particles are Ti, about 2 nm in size on a plain carbon film. He looks at them at a HRTEM lab someplace else. His pictures show lots of background grain, but you can make out some areas that are denser and we assume that these are his particles. Associated with many of the particles are a series of parallel lines that look like lattice fringes, but they appear to be offset from the particle. His question is why do these lines appear to be offset from the main image of the particle? I have a picture if you need to see it. Any ideas? Jonathan Krupp <jmkrupp@ucsc.edu> 13 Nov 2007

Focus - as you adjust the objective focus, you should see the lines move relative to the particle. There will also be changes in contrast. Probably haven't got the explanation exactly right but my understanding is that you are seeing delocalization arising from the spherical aberration of the objective lens. That is, because the diffracted beams contributing to the lattice image are following different, off-axis paths through the objective, the spherical aberration of the lens brings then to focus in a different plane to the undiffracted beam. Different diffracted beams follow different paths, so different sets of planes will have different relationships to the particle. As you change the objective lens, essentially, you moving the image plane along the optic axis - the diffracted beams, making up the lattice images will therefore appear to move relative to the particle. If you image the sample in an aberration corrected TEM with Cs~=0, then the lattice image will be exactly coincident with the particle. Larry Stoter < larry@cymru666.plus.com> 13 Nov 2007

TEM - micelle solutions

Every now and then, someone asks me to look at a solution of just surfactant or bile salt micelles (i.e., sodium taurocholate/lecithin or Polysorbate 80). They give me literature citing scattering studies that show these micelles should be of some "TEM appropriate" size. They usually request cryo TEM as the sample prep method, since they don't want to perturb the system by introducing contrast agents. What inevitably happens is that I prepare the solution for cryo TEM (vitrify solution on Lacey Carbon grid and image) and see nothing. So here are my questions: 1. Does anyone work with micelle systems such as these, or is it a lost cause due to inherent low contrast, or reasons I'm not aware of? I've done some preliminary literature searches, and found nothing specifically on TEM imaging of surfactants or bile salt micelles alone in solution (which may have already answered my question). 2. If so, what type of sample prep or contrast agents do you use? Any information is much appreciated. Jessica Cervantes <cervantes@bendres.com> 30 Oct 2007

I think that your vitreous ice cryoTEM approach is the best choice to image micelles. Several years ago we were studying gallstone formation and attempted to visualize by cryoTEM the earliest stages of cholesterol nucleation using a supersaturated model bile composed of cholesterol, lecithin, and taurocholate in an aqueous solution with 0.15M NaCl. We were able to see various types of unilamellar and multilamellar vesicles in a background of micelles. Our homemade holey carbon films were relatively thick because we were trying to capture particles expected to be much larger than micelles. You might want to consider very thin carbon layers. I think we were single side blotting to concentrate the particles; there may be some trial and error in working out the blotting times You can take a look at the micrographs in the paper in Biophysical Journal Vol. 76, pp.1436-1451. There are more technical details and possibly some useful references. Donald Gantz <gantz@bu.edu> 30 Oct 2007

TEM - ice contamination

May I have your opinions about a test method for ice contamination. It has been suggested to use following way to test whether there is an ice contamination or not during the cryoTEM session: Cooling down a plain carbon film grid in LN2. Cold transfer it into microscope and focus the beam at lower magnification (5000X). It should be possible to see a burning area on the carbon film and then bring the temperature up. This area would disappear during the temperature increase so this would suggest that there is an ice contamination. Your options are highly appreciated. Peiyi Wang <p.wang@sheffield.ac.uk> 07 Nov 2007

Better yet, if you have a Gatan holder: Put the grid in the holder at room temperature. Put it in the scope and then cool the holder down while it is in the scope. When it is cold, let it sit in the scope for a while. When it has been there for a while, go to a higher magnification and put the beam on the carbon with the beam expanded to just the size of the viewing screen. Let it sit for a while, and then go down in magnification. If there is contamination, you will see a round foot print of the beam used at the higher magnification on the lower magnification image you see on the screen. The suggestion you have below would also work but if contamination is slow or very little, the contamination would be gone fast after the beam exposes the area before > you see burning. Angel Paredes <angel.paredes@uth.tmc.edu

I might not have stated very clearly the purpose of this test. The suggestion of this method is to be used to find out whether there is ice contamination during the cold transfer or not. In other words, can we determine where the ice contamination came from? Is there any when we just open the gun valves by this method without knowing previous condition of the carbon film? Peiyi Wang <p.wang@sheffield.ac.uk> 07 Nov 2007

The sort of ice that comes from a poor transfer is not subtle. It is large multi-micron sized boulders (high contrast) of crystalline ice. It is obvious if this sort of contamination is present. The sort of ice that slowly builds up because of a poor vacuum is what David and Angel are testing for. Bob Grassucci <bob.grassucci@wadsworth.org> 07 Nov 2007

TEM – electrical interferences

As we are in works for our new TEM, we have to watch closely to avoid that the relative clean environment we have found won't be cramped by a bad electrical wiring or something else like that. So my question is on uninterrupted power supplies (UPS). What about EM interferences generated by a 10 kVA UPS. Is that a concern for HR TEM/STEM, HR-SEM, etc, or are the level too low, and the frequency generated too high to be a source of problem ? The specifications I've read gives only characteristics in a 150 kHz - 30 MHz, but nothing in the ~0 - 20 kHz. Of course, distance is the easiest way to limit the effects of possible interferences radiated by the UPS itself, but we don't want to be on the safe side, and, for example, put the UPS too far away... and catch again interferences from the environment by the long cable needed. What is the feedback from UPS users? Until now, we don't have UPS in use, on the EM equipment. Jacques Faerber <jacques.faerber@ipcms.u-strasbg.fr> 06 Nov 2007

We have a UPS on our FEI Polara TEM. It and the electronics are in an equipment room adjacent to the scope room, and there is some shielding in the wall separating the two rooms. The input goes through both autotransformers and the UPS, then to the electronics and the HT supply and scope. We were concerned that both the autotransformers and the UPS have large coils that could be magnetic field sources, but we have not had any problem with fields at the scope. Bill Tivol <tivol@caltech.edu> 06 Nov 2007

EDX - plants and seeds

I really need to hear a discussion from the experts on the practicality of detecting the elemental content in plants and seeds. A plant scientist here uses conventional methods to determine elemental and mineral content of his plants, but would like to know if it is possible to use EDX to confirm these values, not to quantify, just qualify relative values. Is there a particular software package designed to detect or be more sensitive to the elemental content in plants...seeds, leaf, etc. He is particularly interested in FE, Mg, Mn, Zn. Winnie Westbrook <ewestbrook@vsu.edu> 02 Nov 2007

Many years ago I did EDX on T pallidosa pollen and on germinating pine seeds. I was able to see differences in composition at different points in the specimen and at different stages in the case of the pine seeds. It definitely is possible--even straight-forward-- to take EDX spectra of plant materials with properly prepared specimens using either SEM or TEM. Whether one can detect the elements you list, however, depends on their concentration in the specimens. EDX is sensitive only to elements that constitute a reasonably large fraction of 1% or more of the specimen. The exact sensitivity depends on the element, whether there are interferences from nearby peaks, the microscope parameters, and other factors. I would be surprised if the elements you list are present in sufficient concentrations in a ~1 μm^2 area of a section or ~1 μm^3 volume that would be examined in TEM or SEM respectively, but I don't know enough about plant compositions to be authoritative about this. Bill Tivol <tivol@caltech.edu> ol 02 Nov 2007

EDX - biological sample

Does anyone have suggestions about how to get more accurate EDX quantitative analysis results on biology samples? A friend of mine asked about the practical procedure, but I just have material science background. Huisheng Jiao <huisheng.jiao@gmail.com> 31 Oct 2007

Biological samples do not provide a flat polished surface. Therefore, there is no quantitative analysis, only qualitative. The only real exception I can think of is if you're using an analytical TEM with thin sections rather than an SEM. In terms of improving your qualitative signal, lower kVs often help, depending upon what elements you're looking for. Ideally, you want the beam kV to be 2 to 3 times the x-ray keV you are looking for. Since most heavier elements also have low energy lines (L & M), low kV doesn't necessarily preclude looking for heavier elements, although it's not without its own issues. Ken Converse <kenconverse@qualityimages.biz> 01 Nov 2007

Cheng Huang and colleagues have been doing quantitative EDX on biological samples for 20 or so years. This can be done if the sample is frozen, a flat surface planed using a cryomicrotome, then very carefully subliming any ice off the sample once in the microscope (on a cryostage), withdrawing back into the cryotransfer unit, coating, then doing EDX. You can quantitate with the sample far from the nosepiece, to reduce any remaining topographical artifacts, and if you use as standards the ion of interest frozen in a carbon slurry matching the biological material as closely as possible. To avoid interference from Au lines, Cheng et al usually coat with Al for light element analysis, which is done at 15 kV. Rosemary White <rosemary.white@csiro.au> 02 Nov 2007