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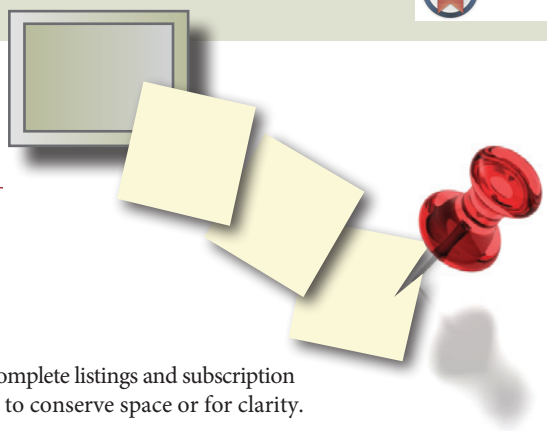

NetNotes

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Selected postings from the Microscopy Listserver from October 27, 2016 to December 31, 2016. 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:
embedding blood clots

Has anyone embedded a vein or artery with a blood clot attached successfully? The blood clot seems to disappear. I am guessing the embedding is dissolving it. ***Susan Van Horn susan.vanhorn@stonybrook.edu Thu Oct 27***

Generally, I guess the treatment may have been too harsh with regard to perhaps the dissection procedure. How was the vessel razor-blade trimmed prior to fixation? "Full" fixation and perhaps conservative / mild dehydration? I would not believe 'your' blood clot would simply disappear. Long ago I did TEM and SEM studies / specimen preparations on (small and big) vessels (Arteria thoracica interna) which had to be evaluated for blood clot / thrombus formation after use of an ultrasonic aspirator/dissector device. (cf: https://www.researchgate.net/publication/200468940 Effects of ultrasound treatment of the arteria thoracica (A.mammaria) interna (ATI) during preparation for coronary artery bypass surgery (CABG): a correlative light microscopic, scanning and transmission electron microscopic study.) I don't know if you are processing in a tissue processor where you will have problems finding the smallest pieces of tissue or substrate. If you process manually in small snap-cap vials made from glass, you perhaps will see such disrupted tissue material more easily. Blood clots aren't easily/rapidly fixed by the usual procedure, so you might prolong your primary fixation (use buffered formaldehyde-glutaraldehyde fixative) at least twice as long as usual or longer (use of a specimen rotator recommended!). You can also use (low molecular weight; e.g. 4%, 8%) tannic acid (sometimes called gallic acid) in combination either with the primary fixative. You could also use acrolein if that is available for you (Caution: Hazard) in combination with the primary fixative. Cf. also perhaps https://www.ncbi.nlm.nih.gov/pubmed/7013112 (just as only one special literature reference) Wolfgang Muss wij.muss@aon.at Fri Oct 28

Specimen Preparation:
gas-filled storage container

I'm looking for a smallish storage container for SEM tubes and mounted specimens that I can fill/purge with gas. This is opposite of a vacuum container. Something less than a cubic foot would be a good size. Any ideas or sources? ***Gary Gaugler gary@microtechnics.com Sat Dec 3***

A small gas-purged box is very easy and quick to make. Pick an "airtight" or "weather resistant" storage box of your liking in your local hardware store or online. Make sure the box has a breather valve to discharge excess pressure. Pick a fitting or quick-disconnect and some 1/4" or 6mm tubing at the same place. Drill a hole in the box, attach the tube through the fitting or quick-disconnect, and connect to the purge gas supply. Most airtight boxes would come with a breather valve already installed, but, if not then you can get a valve on E-Bay or order here: http://www.agmcontainer.com/breather_valves Valery Ray vray@partbeamsystech.com Sat Dec 3

I repurposed an old glass desiccator that had a pump-out port in the lid as a low-O_2 storage unit. Basically, I stuck a Styrofoam coffee cup inside and filled it with LN_2 and left the pumping port open to vent the boil-off. I figure that the N_2 is generally going to be colder than the residual atmosphere and force the warmer air out the port. When the LN_2 has evaporated, I just close the valve. We had some people put SEM mounts that were in the air-tight storage tubes in the desiccator. I'm not sure how much good that did! Henk Colijn colijn.1@osu.edu Sat Dec 3

Take a look at a gas-ported desiccator. I have no affiliation with any supplier. https://www.belart.com/suggested-search/product/desiccators/gas-ported.html Richard Ross richard.ross@allisontransmission.com Mon Dec 5

Lab Management:
electronic lab notebooks

Anyone using electronic lab notebooks? Any comments/remarks on pros/cons? ***Jon Krupp jkrupp@deltacollege.edu Mon Nov 28***

After 40+ years of paper notebooks I converted to a full electronic notebook about 4 years ago. I use an iPad Pro with a program called NotesPlus (Apple Store). You can type via keyboard, draw or write using a stylus, as well as capture photos and store them all in the notebook. It can import PDFs, and for me, importantly, it can export the notebook or pages to a PDF file on a server where you then archive and/or share with colleagues. Fits my operation mode beautifully as I can store the experimental note with the data all in an archive. The program is extremely cheap < $20, but it only runs on an iPad. Disclaimer: I have no commercial connections with either companies, but wish I did. Nestor Zaluzec anl.nestor.zaluzec@gmail.com Tue Nov 29

LM:
resolution calculator for Android devices

I'd like to draw your attention to a new tool for microscopists I've developed for Android devices, "Resolution". After entering magnification, immersion medium, lambda, and NA the App will calculate resolution (actual and theoretical), axial resolution and fluorescence brightness of your objective. Each objective entered can be easily saved and restored. You can select your camera, binning and additional magnification to determine if you're sampling at Nyquist frequency. All information generated can be easily shared via e-mail or MMS with the share button. The App is free to download and compatible with phones or tablets running Android 4.0 or above. I hope you find it useful! Please use the link below on your Android device to install: https://play.google.com/store/apps/details?id=com.Barlowax.resolutionfragments **Andrew L. Barlow andybarlow100@hotmail.com Thu Dec 8**

Instrumentation:
Pirani gauge

On our Edwards Scancoat Six (now about 16 years old), the Pirani gauge is faulty and needs to be replaced. Or does anybody know how to

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repair? We would need the W wire — can it be replaced? Easy? Is it W? We have an Edwards PRE10K (D02428000) — and we asked Edwards and they told us that this Pirani is not available anymore. Does anybody have one for sale? Does anybody know another type which would fit into the Scancoat Six? ***Reinhard Rachel reinhard.rachel@biologie.uni-regensburg.de Tue Nov 15***

To my knowledge, after Edwards dropped this kind of equipment from their portfolio, the Indian company HHV continued to market these instruments and sell spare parts for them. I once heard rumors that HHV may have been where this Edwards equipment was built. If this were true, then contacting HHV would be the best approach for you. There webpage states: "We hold an exclusive license from Edwards to manufacture and supply the well-known ScanCoat Six, Auto306, Auto500 and forensic deposition systems under the HHV brand and have added our own range of systems to our product line-up." (http://www.hhvltd.com/). Marco Möller mmoller@cicbiomagune.es Tue Nov 15

If you can dismantle the gauge and get to the filament you can try sonicating in mild HCl solution. I do this routinely with my Penning gauge on my FESEM when it gets coated with carbon. Michael Delannoy mdelann1@jhmi.edu Tue Nov 15

Pirani can be repaired if you really wanted to do it, you can DIY after reading some of books and papers on vacuum instrumentation and techniques, or contact some of the places that routinely repair vacuum instrumentation. Examples would be Duniway Stockroom (duniway.com), or VGM Inc. (vgminc.net), or Scientific Instrument Services (sisweb.com) - I am sure there are also plenty of places capable of repairing Pirani in Europe as well, Google is your friend. Replacement PRE10K also available elsewhere: there are four of these gauges listed on E*Bay, also check Labsource (labsoruce.come) and PLC Center (plccenter.com). Valery Ray vray@partbeamsystech.com Tue Nov 15

I might suggest checking with Duniway Stockroom Co. They sell and service all kinds of vacuum gauges, and may be able to help you. (www.duniway.com) Wilbur C. Bigelow bigelow@umich.edu Tue Nov 15

TEM:
grid orientation

When a grid with a protein polymer is placed in the scope, should it be placed filament side up, or filament side down for the sharpest digital pictures? The samples are small biopolymers in different conformations. They are negatively stained with ethanolic 2% uranyl acetate. ***Vickie Kimler vakimler@oakland.edu Mon Nov 28***

I was taught, a long time ago, that the sample should be "placed filament side up". Did you consider using carbon film grids for your samples? Negative staining on thin carbon support film should result in better signal-to-noise ratio ("sharpest digital picture"). Oldřich Benada benada@biomed.cas.cz Tue Nov 29

TEM:
imaging magnetite particles

Is it safe for a TEM microscope to image a holey C film dispersed with thousands of Fe_3O_4 magnetite particles (100 nm)? The specimen is prepared in the usual way as many other nanoparticles specimens. Will the particles fly to the pole pieces? The user said the particles are iron oxide and are superparamagnetic (and have no permanent magnetization). I don't quite understand these terms, but by diffraction I identified magnetite Fe_3O_4. If it's not safe, what solutions are there to get the job done? I would be very grateful if you can share with me your experience and comments. ***Z Zhou z.zhou@lboro.ac.uk Wed Nov 30***

I know it's alarming, but yes, it is in general safe. I have looked at magnetite nanoparticles at both HP and at OSU in a JEOL 2500 and Titan TEM respectively without serious issues. They key is they need to be nanoparticles. I believe Steve Chapman and Protrain wrote a nice reply to a similar question 4 or 5 years ago. In that reply Steve mentioned that the Van der Waals force is so large on a nanoparticle they really stick well to the carbon film! I agree, I have seldom seen one fly up. However, as a precaution, I blow a duster can or dry nitrogen gas over my grids before putting any nanoparticles in the TEM (suggestion from Debby Sherman of Purdue). This works very well to dislodge any nanoparticles that are not well adhered. Then as you magnify up on the nanoparticles, halt the analysis if they begin to move! Pete Eschbach peter.eschbach@oregonstate.edu Wed Nov 30

Adding to the nice comments that Peter made, you are able to reduce the level of magnetic interference with any "uncomfortable" material, by simply extending the objective lens focal length through adjusting your eucentric stage to its lowest position. Cranking the z' so that you are turning your focus controls anticlockwise will reduce the active objective lens current. The magnification will drop, as will the level of resolution achievable. For those who want a little more resolution the trick is to take the z' the other way, shortening the focal length, with the increase in the operating lens current serving to reduce the aberrations and increase resolving power. Steve Chapman protrain@emcourses.com Thu Dec 1

SEM:
Position changes when change sample height

I have a problem about using an FEI SEM. After I focused and linked Z, the sample position will always change a lot when I raise or drop the sample. Basically the area in the image will go to the opposite direction with the sample. Why would this happen? I don't have much knowledge about SEM theory so, any advice is appreciated. ***Jason 13qw9@queensu.ca Sun Dec 11***

It does sounds like there is quite a bit of movement in the image. If so, it may be that the stage *Z* axis isn't parallel to the beam axis. First determine in which direction the image is shifting. On most of the FEI scopes I've worked with, the stage *Y* axis is vertical and *X* axis is horizontal on the screen. If the image shift is along the vertical (*Y*) axis, adjust your sample tilt several degrees plus and minus. One direction should increase the shift and the other decrease it. If the shift is along the *X* axis, visually examine the stage from the side and see if you can see anything that is cocked. I would recommend discretion when fiddling with the stage since the bearings and other components are matched for submicron precision. This may be worth a service call. Henk Colijn colijn.1@osu.edu Mon Dec 12

When you say change or drop the specimen I assume you mean a *Z* position adjustment? The "problem" that you see with a change in *Z* is quite normal. The vertical motion of the stage is through a thread and the take up of any slack in that thread results in specimen movement in *X* and *Y* directions. With an electrically driven stage, manufacturers have the option of providing anti-backlash adjustments to make *X* and *Y* movements more positive. I do not know a manufacturer that has anti-backlash compensation in the *Z* direction. Steve Chapman protrain@emcourses.com Tue Dec 13

SEM:
Peltier cooling stage

We are trying to purchase a Peltier cooling stage for SEM samples that need to be frozen like: milk, ice cream, fruits and similar food industry items. The stage that has been offered to us achieves -50°C to +70°C. I was wondering, for our applications, if this temperature range is enough? Or should we go for other cooling stages with -180°C? ***Sun researchers4u@gmail.com Fri Dec 23***

As you consider the cooling stage, you should think about the vapor pressure of what you are inserting into your chamber. I assume that you have a high-vacuum SEM and that the primary vapor you are concerned with is water. The tables of water vapor pressure as a function of temperature should be readily available online. You want

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to ensure that the partial pressure of water is much below (orders of magnitude) the chamber pressure of your scope. Also, you need to take into account the thermal conductivity of the sample you are examining. Remember that the temperature of the exposed surface can be well above the cooling temperature of the stage itself. Remember that the beam will also cause local heating of the sample. In my TEM, I have seen ice evaporate under the beam when the stage temperature was reading <-100°C. Henk Colijn colijn.1@osu.edu Fri Dec 23

I will second Henk's comments that the beam will lead to localized increased temperatures. You will have to play around with the conditions to get what you want. We have a Peltier stage on our Quanta. I'm not sure that ours has as much as a -50°C to 70°C range. I think our cooler allows 25°C deviations up or down from ambient and that is relative to a temperature conditioned water bath. I am not sure you would need the -180°C model. I would ask you to consider the full process of your experiments. How will you introduce your samples? Do you have a cryo transfer system? How will you prepare your samples for examination? That could be in-situ but probably elsewhere. Do you have an SEM capable of low-vacuum or environmental mode? Henk, you spoke of keeping the vapor pressure of the water in the sample far below the pressure of the chamber. Are you dealing with a high-vacuum-only instrument? Couldn't that still result in problems? If the partial pressure of water in your vacuum is below the vapor pressure of water at the sample temperature, the water in the sample would still sublime. Granted, if the temperature is very low, that should be a slow process. Conversely, if the partial pressure of water in your chamber is higher that the vapor pressure of water at sample temperature, then you would have frost build up on the sample through deposition. It will be a tricky business. Warren Straszheim wesaia@iastate.edu Fri Dec 23

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