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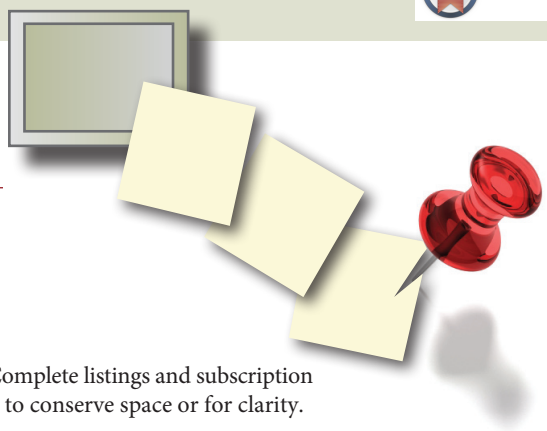

NetNotes

Edited by Thomas E. Phillips
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Selected postings from the Microscopy Listserver from January 1, 2018 to February 28, 2018. 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
pigments for microtomy

I am wondering if anyone one has any insight on how to prepare pigments or powders to be embedded for microtomy. I have only worked with biological tissue and do not know if materials samples need to be infiltrated the way tissue samples do. In addition, if so is there a different technique or different embedding resin needed for an infiltration process? I cannot find any information anywhere! ***Janine Hernandez janine.hernandez182@gmail.com Mon Jan 29***

Powders and pigments likely do not require encapsulation. It is usually much easier to place them on a supporting grid directly for imaging. This of course assumes that the size of the powder or pigment is small enough for electron transmission. Often it is worth it just to check in case it is possible; which makes sample prep easy. There are a few methods that could be used to prepare these- one that I have had some success with fine powders in the past is as follows: place an amount of the powder into isopropanol, ultrasonicate to break up agglomerations, dip a holy-carbon grid into the liquid, pull it out, and allowing the grid to air dry. If there was a small enough amount, but enough to be finely dispersed in the alcohol, you will pick up some on the grid. This grid, when dried, can then be imaged with the powders suspended on the holy-carbon. It may take a few trials to get enough from the alcohol suspension. Another method that has worked for me in the past (assuming the powder is well separated) is a very light dusting of the powder mid-air above the grid (allowing some of the powder to fall down onto the grid on the table). This one is less likely to obtain separated powders that are small enough to image, but I have seen some success with it as well in the past. Use of a clean fine artist paint brush to flick the powder into the air above the grid may work here. Please be careful not to allow the alcohol with the powder or the powder in air to get on your skin or to be breathed in. Some solvents (acetone notably) allows chemicals to enter through the skin, and we are learning that nanopowders are not healthy for you to breathe in as well. I hope this is helpful. You will likely get a number of great answers from the list-serve and I'm looking forward to learning from their answers also! Wishing you a grand time exploring small worlds! Allen J. Hall ajhall@prairienanotech.com Mon Jan 29

Specimen Preparation
vacuum desiccator sample storage

*We are looking for a compact storage solution to keep membrane boxes under vacuum and/or inert atmosphere. I am considering the containers offered by SPI (*https://www.2spi.com/item/01602-ab/spidry-sample-preservers/*) and I am also aware of Ted Pella's bell jars, which are a bit too large for our purposes. Does anyone have recommendations for other containers we can consider?* ***Steven Spurgeon steven.spurgeon@pnnl.gov Mon Feb 12***

I have been using this model for storage and transport of SEM samples but also whole TEM grid boxes: http://scienceservices.de/en/sample-stub-vacuum-desiccator.html They work really well, up to the point where they start questioning you at the airport security check where you'd be going with that "crystal ashtray". Guenter Resch lists@nexperion.net Tue Feb 13

Specimen Preparation
ancient grain

I have been tasked with imaging an ancient grain. It is 1000-year-old millet and I have one only! I have done SEM/TEM of grain before but not one so old. I am thinking SEM may be the way to go. The investigators would like to see the internal structure of the grain (if any) and I have no idea whether it will be 'normal', caramelized or powder inside! It must be fixed in order to be released from quarantine so my first question is should I use an aqueous fixative or alcohol? Any other advice would be gratefully accepted! ***Lisa O'Donovan lisa.odonovan@adelaide.edu.au Fri Feb 16***

It is a grain, so largely starch, meaning formaldehyde and glut will not fix much anyway. If alcohol is good enough to release from quarantine, use 70 or 80% ethanol, then go through to 100% ethanol. Either dry from ethanol or go to tert-butyl alcohol and vacuum sublimate at 20°C. After the 2nd or 3rd 100% ethanol, you could put the grain in liquid nitrogen and hit it with a razor blade, maybe gently crush it. You'll get a brittle fracture that will expose the starch grains. This part will be particularly fun if your grain is tiny. Phil Oshel oshel1pe@cmich.edu Fri Feb 16

Fixation in 95-100% methanol or ethanol may be better for your grain - there will be less tissue swelling. The dry grain will contain only about 8-10% water, so going into 100% solvent will be fine. A little water (i.e. 95% solvent) may help penetration. Methanol will penetrate a little better, but it will take some time for any solvent to get deep into a dry grain. Another option after drying would be high-resolution (± phase contrast) x-ray CT - it would quickly show you if the grain had any contents and give you an idea of what they are without breaking the seed. There are at least a couple of labs I know of in Australia that do this, one of them is here in Canberra at the ANU - https://ctlab.anu.edu.au/, and since your seed will be dead after going through solvent, you don't have to worry about the high kV x-rays killing the tissue. Millet seed is pretty small, so you'd get good resolution of the innards. Rosemary White rosemary.white@csiro.au Fri Feb 16

I like Rosemary's suggestion — mine about liquid nitrogen needs decent sized seeds. I've used it for corn and barley, but if you do have millet … oof. Phil Oshel oshel1pe@cmich.edu Mon Feb 19

Specimen Preparation
evaporating tin

I have been asked if our vacuum evaporator will put down tin to 1-micron thickness. We have a Cressington unit so it is not the principle, it is the practice. Does anyone have experience with tin or a thickness of 1 micron or both! ***Chris Christopher J. Gilpin gilpin@purdue.edu Mon Feb 19***

As the evaporated films get thicker, internal stress becomes significant. I am not sure how bad tin films are but I have had issues with the films peeling when they got much over 0.1 μm thickness. Henk Colijn colijn.1@osu.edu Mon Feb 19

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Considering that is more than 100 times the thickness you normally put down, that is an issue. I tried using evaporation to put down a thick layer of antimony on glass before. First, when I calculated the mass of Sb to put down a micron at a certain distance, I found I had to fill my tungsten basket to the full and maybe even run a couple of times. Then I think I found the thermal stresses Henk described. The layer curled up off the glass. Why do they want such a thickness? Warren Straszheim wesaia@iastate.edu Mon Feb 19

Specimen Preparation

critical point dryer

I am considering replacing an older critical point dryer for a multi-user microscopy lab that serves faculty and undergraduate student users for research projects, and trains undergraduates via courses. After training, users carry out their own specimen preps. Two models I have come across are the Leica EMCPD300 and the Tousimis Autosamdri-931. I would appreciate any comments from folks who have experience with either of these, as well as suggestions about other models I may have overlooked but should consider. Feel free to respond directly to me if you would like. ***Jan Factor jan.factor@purchase.edu Sun Feb 18***

Tousimis is good, I've used those a lot. Not the Autosamdri, but the previous model. Have you considered the Polaron "bomb"? The larger sample chamber (in the regular size) is very handy. SPI sells this as a rebranded product. I use it in a multi-user facility that teaches undergrads and grad students. Phil Oshel oshel1pe@cmich.edu Mon Feb 19

We have a CPD300 here that users have access to. One of our users is only trained on our bench top SEM and she uses it regularly, independently. Top tips for things that might catch out students: 1. As per the manual, the fillers should be on top of the holders. If you have them the other way around, the chamber doesn't fill up correctly 2. Do not overfill the chamber. Should be OK as long as the level is below the outlet hole at the top, otherwise you might have some liquid left at the end. It is easy to use with a simple user interface; it has been easy to train people on it. We use it quite a lot and it gives nice results. Matt Russell matt.russell@crick.ac.uk Mon Feb 19

EM

floor vibration & cutting the slab

I know this is a subject on which reasonable people can disagree, but I wanted to ask what has been your experience with cutting the slab to reduce low frequency vibrations? The floor appears to be moving in the horizontal plane at 1.6Hz (4.4 reduction factor needed) and in the vertical direction at 5HZ (3.1 reduction factor needed). ***Bryan Tracy btracy@eag.com Fri Feb 16***

3-4X attenuation of ambient vibrations is very difficult. Finding another location would be the preferred solution. A. John Mardinly john.mardinly@asu.edu Fri Feb 16

Do you know if the vibration is resulting from sources inside the building or from the general area outside? EAG is in between a number of freeways there in Sunnyvale and the 101 is not far away, so if it is the underlying ground below slab, you might make it worse cutting the slab. I am sure some of the experts on here have experience doing this and will advise. You might also check with Vibration Engineering Consultants - www.vibeng.com - in Pacifica, CA. This is their specialty. I would also be remiss if I did not suggest that you consider a Vibration Isolation Base Platform. The active type we offer suppresses vibration starting at 0.7 Hz and has 90% suppression by 2 Hz. I would avoid a passive type isolator as most of them have a resonant frequency right around 2 to 4 Hz, which would make things even worse for the vibrations you have. Likewise, if you decide to cut the slab, careful if you use an elastomeric sealant and check its resonant frequency. Have a look here at our line of Vibration Isolation Platforms for SEM/TEM/FIB: http://www.elementpi.com/active-vibration-isolation-platform-products/ Mike Toalson miketoalson@gmail.com Fri Feb 16

Those are very low frequencies. They should be in a range that an active compensation system should be able to handle it. Another thing to consider is the resonant frequency of the slab (even though it is damped by the ground). The more mass in the slab, the lower its resonant frequency. The install guides for our microscopes show that the microscope is much more sensitive at very low frequencies. The allowable vibration at 10 Hz is approximately 10× lower than at 20 Hz. Since too much mass can push the slab resonance down into the range where the microscope is more sensitive you may want to estimate the resonant frequency of your current slab and then consider whether to slice it or not. It would also be useful to measure the ground vibration away from the instrument to get an idea of the driving frequencies. Henk Colijn colijn.1@osu.edu Sun Feb 18

Cutting the slab is dangerous can may compromise the building. It is better to install active vibration isolation. I have a number of SEMs in Thousand Oaks, CA. One LaB_6 system is on the second floor. We use Herzan to reach vendor spec resolution with gold on carbon. Gianpiero Torraca gtorraca@amgen.com Mon Feb 19

One thing I do not think has been mentioned is that the soil under many slab floors has settled, and the slab is "floating" above without being supported. I have heard of holes being drilled and a foam material being injected under the slab to support and dampen vibrations. This is done sometimes, in addition to other vibration isolation measures. Darrell Miles milesd@us.ibm.com Mon Feb 19 20

TEM

beam always on

I'm using a Philips CM12 and I've been noticing recently I still have a beam and image with the filament supposedly desaturated. I have to turn the high voltage off to get the beam completely gone. I do not remember this scope doing this before. Therefore, I suspect something is not right but before I request a service call (we are a fugal company), I would like to confirm that the scope is acting abnormally. I am also fishing for ideas on what is wrong. ***Frank Karl frank_karl@ardl.com Wed Feb 7***

I have had a similar issue with our FEI Morgagni 267D (for most purposes similar to the CM12). I would bet the Wehnelt assembly is almost identical. For us it was almost certainly a filament break in such a way that it was shorting across the Wehnelt. I say "almost certainly" because in our case this happened at almost the same time that a bunch of electronics issues occurred and so we had mixed symptoms. However, replacing the filament (which we had only just replaced as part of our diagnosis of elimination), did the trick for us. At least it is a simple check. Duane Harland duane.harland@agresearch.co.nz Wed Feb 7

This is called dark current. It comes from the evaporation of filament material onto the insulator base creating a pathway for electrons to leak to ground, and therefore, creating a current in the filament that causes it to glow with only the kV on. It's not a problem but indicates that your filament is old. Kenneth JT Livi klivi@jhu.edu Wed Feb 7

SEM

tungsten filaments

We use tungsten filaments in our Hitachi S3400N VPSEM. Does anyone have any suggestions for extending filament life? We try to operate slightly under saturated except for EBSD or EDS mapping. I have heard of "seasoning" the filament but have never seen an actual protocol or talked with anybody who's actually done it. Any suggestions would be appreciated! ***Bil Schneider wschneider@wisc.edu Fri Feb 9***

With seasoning a tungsten filament, you perhaps (might) think of slow and carefully controlled "heating" of a new filament (as I have done that with every new filament after mounting in a TEM, a ZEISS109 with differential pumping system=rotary pump -for RV as well as an Ion Getter Pump for HV). IMHO 'slow heating' of the cathode filament

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before subjecting it to the "hard" working conditions might be (at least in my experience really 'is') of benefit regarding beam stability as well as maintaining your filament's lifetime. "Slow heating" means [after change of filament] at least running the new filament 1–2 hours "under-under"-saturated after achievement of an evacuation optimum of the chamber (not knowing whether you could see anyhow the filament's image in such a condition like in a TEM on the screen). You easily would / could see (if you e.g. have permanent vacuum-measurement monitoring ready) that on starting to "heat" (increase) the filament (current), the vacuum decreases significantly (at least slightly) because of "evaporation" of metal & impurities / contaminants. Then increase filament current a bit more.... and let "equilibrate" vacuum conditions. You might even find such decreasing vacuum after a third increasing of the filament heating near the "saturation" point. Another helpful requisite also would be to use - without using or even heating the filament first, an ACD (anti-contamination device - if available) with at least one if not two cycles of cooling first and pumping the chamber afterwards to get rid of most organic molecules you brought into the filament chamber by your cleaning and mounting procedures - let warm up the ACD and pump (RV and HV if possible) again. According to the specifications of the Hitachi S3400N VPSEM (I found on: https://www.ntnu.edu/documents/140082/1269041159/S-3400NSpecifications.pdf/6b94c26f-a9d4-4f36-82b3-7e9eb3603922) the possibility of doing so might be impossible due to the automatic settings of diverse functions but you should know about those possibilities from practical experience. Unfortunately, a modern VPSEM is not comparable to such an old piece of equipment like the TEM ZEISS 109 (vintage 1976) I worked with where maintaining and handling vacuum matters relatively easy... And, BTW, life time of a tungsten filament always depends on the "technical quality" and the handling - when mounting into the chamber - too.... Wolfgang Muss wij.muss@aon.at

First, what kind of filament life are you currently getting? If you are close to 100 hours, I would be satisfied. If you were only getting 30, then I would look for a problem. We got better life when we ran at a consistent voltage and did not have users doing the saturating. We went from average lifetimes of 25 hours to 80 hours. We also operated just under saturation conditions. We simply turned off the high voltage at the end of the session. We needed to check saturation and reduce current over time. As the filament thinned, it needed less current to reach the same temperature. Finally, we ran the filament a little further back from the front of the Wehnelt. That lessened brightness but increased life. Warren Straszheim wesaia@iastate.edu Fri Feb 9

Do you have a high vacuum gauge on the scope? With both our new Hitachi (SU3500) and our old JEOL (5600), the "ready" state comes on when the vacuum is in the high 10^{-4} Torr range (or even higher if there was a vacuum leak, outgassing sample, or other problem). Running a filament at such a poor vacuum will drastically reduce filament life and generally contaminate the column faster. I insist on having a gauge on any scope I run, and don't turn the beam on until the vacuum is at least mid-10^{-5} Torr. With vacuum gauges under $1000, they will quickly pay for themselves in terms of filaments and time lost changing them. I have ranted to both JEOL and Hitachi that they should make them standard on all scopes. By their own admission, half of their service calls are vacuum related, and with a gauge, the end user can often find a leak and save a service visit. In our case with the closest service in Ontario, it's a $2000 flight to get here. Should be a simple budgetary calculation, but Hitachi says I am the only customer in Canada who ever requested a vacuum gauge. I also let the filament cool 5 to 10 minutes whenever possible before venting. I do not have any hard evidence, but it just seems logical to me that exposing a hot tungsten filament to atmosphere is going to induce more oxidation (or whatever) compared to a cool one. In my experience, these precautions contribute more to filament life than slight undersaturation. Of course, you want to make sure you are not oversaturated, but I get 3-400 hours of the Hitachi filaments running in the "medium" gun setting. Jim Ehrman jehrman@mta.ca Sat Feb 10

Thanks to all who responded to my S3400N VPSEM tungsten filament questions. We do try to keep users from venting for 5 minutes after turning off the HV. We probably average 50 hours per filament lately, which is what aggravated me a bit, because the last box was more like 80 hours. One challenge is that some users change samples multiple times in a session. Then the next user is in variable pressure changing mag and kV, vacuum, etc... then someone will run an EBSD map overnight at 30 kV, 100 probe current. We ask a lot from the SEM, in general it is a workhorse. I did have several suggestions that seem promising: The Hitachi person called and suggested new filaments should not be turned on until Vacuum has worked for a couple of hours. He said they try to wait more like 15 minutes between HV off and venting. Several responders further emphasized "gently" saturating the filament over a few hours, keeping it slightly under saturated for general use, and using some gun bias to concentrate the emission area. One respondent made a great point about using a vacuum gauge on the gun and waiting until Vacuum is in the 10 to the minus 5 range. I honestly do not know what the vacuum is when the Hitachi lets you turn on the HV. Adding more spacers to push the grid cap further away from the filament tip was another suggestion. I was under the impression that however many spacers that each individual filament came with was the correct distance as measured at the factory to keep emission current appropriate? Otherwise I handle everything with gloves and care when changing filaments and occasionally use metal polish on the grid cap and threaded holder as well as the anode. More often, I will sonicate the grid cap and holder in methanol when the gun is open to change a filament. Thanks again for all the suggestions, I will repost this in hopes this collection of information might be useful to others with tungsten filament questions. Bil Schneider wfschneider@wisc.edu Sat Feb 10

In my experience, there are 3 very simple rules to extend the tungsten filament lifetime: 1. Vent the microscope by nitrogen and always exchange the sample and pump the chamber quickly. This will minimize the contamination of the vacuum chamber by water and speed up the pumping. 2. After getting HV ready signal (when the gun/chamber is pumped), wait several tens of seconds or longer before heating the filament - the filament lifetime depends on the pressure exponentially. Reaching lower 10^{-3} Pa in the gun is perfect to extend the filament lifetime. 3. Turn off filament heating manually and wait several minutes before venting the chamber. Like that, the filament will cool down properly. Venting the filament when it is hot decreases its lifetime significantly. By utilizing long waiting time, the filament lifetime around 1000 hours can be obtained easily if there is no vacuum leak in the gun. So sometimes it is a question of whether to wait longer and reach a very long lifetime or wait a shorter time and exchange filaments more frequently (changing tungsten filament is cheap and easy). For someone and on some machines, exchanging the filament might take several minutes only so it does not make sense to wait several minutes during each sample exchange procedure. For other users/machines, exchanging the filament might be a nightmare so it would be better to wait longer and extend the filament lifetime. Tomas Hrncir tomas.hrncir@tescan.com Mon Feb 12

I have just picked up this offering and with 50 years EM experience I fully agree with Tomas' posting. This procedure should be in your standard operating procedure for running a scanning electron microscope, any other way is just not good enough! Steve Chapman protrain@emcourses.com Tue Feb 13

MT