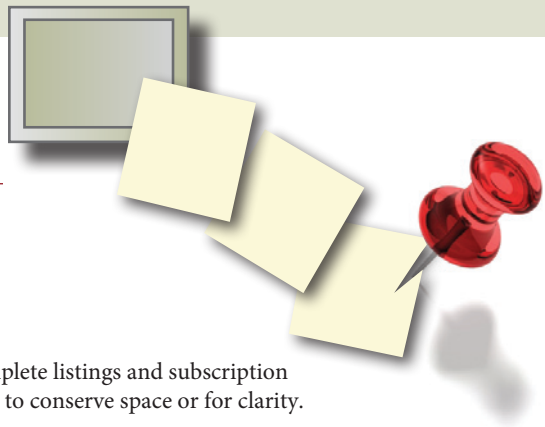
NetNotes



Edited by Thomas E. Phillips
University of Missouri

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Selected postings from the Microscopy Listserver from July 1, 2015 to August 31, 2015. 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:
cells on coverslips

I have one user who wants to do a TEM study of cultured neurons on a coverslip. She wants to preserve elongated nerve processes. We cannot go with suspension culture and make its pellets. Can anybody help me here? ***Ravi Thakkar ravi.thakkar369@gmail.com Thu Jul 16***

I have had success looking at drosophila testes that have been adhered to a coverslip and then processed into resin for TEM. I followed a method by J P Chang where you process the samples normally (I used a glass petri dish to contain the solutions with something on the bottom to hold up the coverslip and make it easier to pick up), infiltrate on a shaker plate and then embed on a mold that I made from silicon. To separate the coverslip from the resin I dipped the sample in liquid nitrogen briefly and didn't notice any changes in the tissue. The paper is: Chang J.P. (1971) "A new technique for separation of coverglass substrate from epoxy-embedded specimens for electron microscopy," 37, 370–377. You can also process as normal, infiltrate in the glass petri dish on a shaker plate and then use BEEM capsules to embed. You put some resin (don't fill it right up) in the BEEM capsule and then carefully invert it onto the area of interest and carefully place in the oven. The capsules can be quite easy to tip over. They can be separated using liquid nitrogen also. Jordan Taylor j.w.taylor@massey.ac.nz Thu Jul 16

No problem, you can flat-embed the cultured cells on the coverslip. Process the coverslips + cells as you would if you had pellets. You can probably use just glutaraldehyde, no formalin, since these are spread cells. I'd also add 1% monomeric tannic acid to the glut and/or OsO_4 to help preserve the membranes. Do the resin infiltration with a nutator or the like, but try to limit the degrees of tilt (and slow speed), so you don't get the fluid everywhere. Go through to 100% resin as usual, then cut the bottom & cap off of a BEEM capsule, invert over the cells, Carefully fill with 100% resin – don't get air bubbles! - and polymerize. After polymerization, drop the coverslip + BEEM capsule in LN_2, and the capsule with pop off. Trim and section. Phil Oshel oshel1pe@cmich.edu Fri Jul 17

Specimen Preparation:
wear pattern of used sputter target

We have Denton Desk II Sputter coater. Attached is the image of the glowing sputter target. Should it be replaced? https://www.flickr.com/photos/97321550@N08/19760866061/in/dateposted-public/

Ravi Thakkar ravi.thakkar369@gmail.com Thu Jul 16

This is the normal glow pattern of a Denton Desk II in use. We'd need to see a photo of the target itself when not it use. Top open, looking directly at the target to get a good image. If there are any holes in the target, especially in the area of the plasma annulus, the target is toasted and needs to be replaced. Phil Oshel oshel1pe@cmich.edu Fri Jul 17

With a dubious target of 57 mm, I, with fresh gloves, remove it and lay it on a vertically oriented dissecting scope lamp. In a darkened room, one can easily detect pinhole defects in the anulus. Fred Monson fmonson@wcupa.edu Fri Jul 17

The other replies have been very good—light will help show a pinhole in the target when viewed from the other side in a dark room. I wanted to add a few points that might be helpful for other deposition systems also. For thicker targets, a profile gauge often used by woodworkers to duplicate a profile can be used to help determine the depth of the wear-groove. This gauge has a row of pins that slide when pushed and match the profile of the item being pushed against. This is helpful in harder-to-reach targets if the gauge can fit. Often the mounting plate for the target is made of another material (stainless steel or copper) and this will show up in EDS or other analyses when the deposited film is analyzed if the wear track has broken through the target sufficiently. Finally, a good metals reclaimer (and sometimes the target supplier) will be willing to pay for the remaining high purity metal target "scrap." This may help in purchasing the replacement target. Allen J. Hall ajhall@prairienanotech.com Fri Jul 17

Specimen Preparation:
protozoa

What is the current thinking regarding conventional SEM of protozoa? I have some Trichomonas tenax fixed in 2.5% glutaraldehyde that need processing for SEM (and TEM) and I was hoping to use my standard procedure through ethanol/HMDS. Will this be adequate, or will critical point drying yield superior results? Also, what is the best method of transition? Does the sample need filtering, and how do I process the filter? Would they adhere to a subbed coverslip, or will they all just wash off? If using HMDS, can I process them in a centrifuge tube, and just sprinkle the dried sample onto a sticky tab? We have some microporous specimen capsules that I use for critical point drying, but fear the protozoa are too small, and there is not enough sample, to effectively retrieve them after processing. If anyone does regular processing for TEM and has a reliable protocol for resin embedding protozoa, that would also be very useful! Any suggestions would be welcome. ***Natalie Allcock nsa2@leicester.ac.uk Fri Jul 17***

I filter protozoa onto a membrane filter (pore size determined by the critters, but anywhere from 0.45 µm to 8 µm). First, sputter coat the filters on both sides, so you filter the beasts onto a conductive surface. Then add fix (1–1.25% glutaraldehyde); obviously in your case, just filter the fixed critters. ethanol:HMDS series 2:1, 1:1, 1:2 then 3 X 100% HMDS. I find most protistans dry best at 60°C. You can CPD the filters with critters, but some (sometimes most) of them may come off in the CPD and be lost. Other times, this works fine. Just be sure you know which side of the filter your critters are on - the simplest way is to look at the pattern on the filter support, as it will be embossed on the filter. You might also try drying from tert-butyl alcohol. There's an article about this in the May 2014 issue of Microscopy Today. I never use microporous capsules. I find they shed particles and clog CPD valves. TEM: try processing with whatever your usual protocol is.

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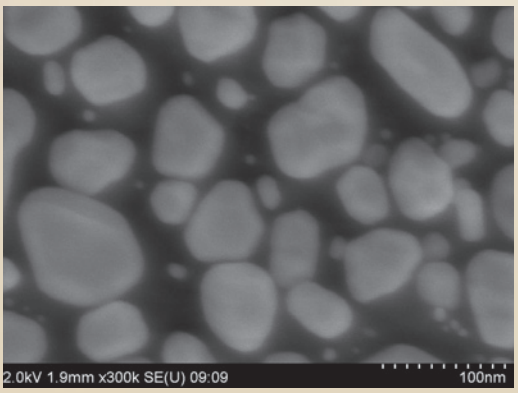
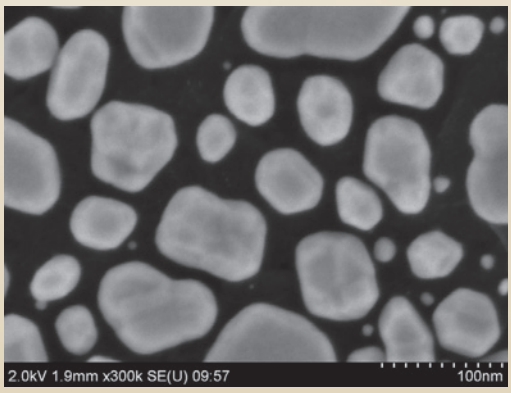


Mesoporous TiO2 Before UV Cleaning



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Protistans are so variable, you really have to experiment, or get a known good protocol from someone who does your particular organism - *Trichomonas tenax*, here. From processing euglenoids, we find even congeneric taxa can vary greatly in their processing requirements, for both SEM & TEM. Phil Oshel oshel1pe@cmich.edu Fri Jul 17

Protists are notoriously variable things; different but closely related strains can respond very differently to the same fixation protocol. So you might get good results the first time you try, or you might have to experiment. To answer your questions in order: I usually use a CPD for SEM prep, and I've gotten good results with that. However, there are a few different types of CPD, some of which require careful operation. Thus, the results may depend both on the type of CPD and the operator. I've also used tert-butanol in a freeze-dryer with adequate results, although I've gotten better results in some cases with the CPD. I've never used HMDS but I've heard very good things about it. For mounting, I've used both poly-L-lysine-coated coverslips (the round 12-mm types, which unfortunately tend to be expensive) and Isopore filters. Give the cells about an hour on the coverslip and they're stuck. Isopore filters don't require any treatment at all: I fix my cells in a Petri dish and just plunge them through the filter and that's enough. Of course you want to be careful not to apply back-pressure when removing the cartridge, handle anything with cells adhering to it gently, and keep it wet! A few seconds at most out of ethanol is fine, though. As for TEM, I 'trap' my cells in 2% agarose after fixation. I do this by pipetting a shallow puddle of molten agarose onto a fresh slide (use a transfer pipette or a cut-off Gilson: narrow pipettes will clog!) and then pipetting 1–10 µl of concentrated fixed culture directly into the puddle, trying to suspend the cells directly in the middle of the water (agarose?) column. After the agarose sets, I cut out a ~1-mm cube around the cells, and do my dehydration and embedding with the cube. I'll typically get 4–6 cubes from a single fixation. One nice aspect of this protocol is that, for the resin-containing stages, the sample will float if it's not ready for the next change! I also have colleagues who instead spin down their cells and dehydrate and embed them 'free' in an Eppendorf tube, but my cultures are usually too sparse to deal with the amount of material one loses in that procedure. Aaron A. Heiss aheiss@amnh.org Sat Jul 18

Specimen Preparation:
paraffin sections for TEM

I have one user, who wants to carry out TEM analysis of his Histology samples. He wants only histology slide stained with H&E. Has anyone done this kind of work before? Kindly give the best suggestion from your experience. ***Ravi Thakkar ravi.thakkar369@gmail.com Fri Aug 28***

If you mean preparing an H&E section for TEM, I suspect you are in for a disappointing result. I haven't done it for an H&E section but have done it for an unstained paraffin section. I osmicated the section, infiltrated with resin and polymerized resin on the surface of the slide. I then popped it off the slide using liquid nitrogen and mounted en face on an epoxy block so I could section it. The tissue was recognizable in the TEM but the quality of the tissue preservation was terrible. It looked vacuolated. I am always amazed how "acceptable" tissue fixation looks like at the LM level but how bad it looks like once you go to TEM. TEM is tedious and demanding enough with optimally fixed tissues so it is best to handicapping your chances before you even start. Unless the tissue is something incredibly rare, it is worth repeating with proper fixation and embedding for TEM. Tom Phillips phillipst@missouri.edu Sat Aug 29

It is not often that someone, via the MSA Listserver, requests such measures/procedures. This is probably due to the decrease of med.-diagnostic EM-ists (unfortunately many medico-diagnostic EM-Labs have been cut and more will become extinct) out in the wild. He or she must be a young pathologist or clinician, I guess. Seconding 100% Prof. Phillips's opinion (and making no difference between an H&E stained=deparaffinized or unstained paraffin section): "Garbage in, Garbage out". If the tissue is initially fixed poorly then poor / bad preservation of ultrastructural detail will be the "natural" consequence. But sometimes careful and sophisticated reprocessing of - selected areas of - either tissue from whole paraffin blocks or also a H&E-section only can yield some interesting and unforeseen result in terms of diagnosis (viral, bacteria, etc. etc., see also below). So in the end: It always will depend on the circumstance and task of the study whether such a re-embedding/reprocessing (which might be sumptuous and/or a bit complex to accomplish) will yield something of value (e. g. especially something diagnostic) - worth to be documented. In my 35 years EM-career I have done about 100–150 re-embeddings (from paraffin blocks as well as from deparaffinized H&E and/or pre-embedding IHC-sections) and the scientifically useable yield was estimated at about 65–70% (esp. for evaluating the preembedding immunolocaliztion of markers in DAB-treated immunohistochemistry sections). Some examples for your convenience: Estrada, J.C. et al., "TEM of Paraffin-Embedded H&E Stained Sections for viral Diagnosis (an Unusual Papovavirus Case)," *Microscopy Today*, Sept. 2005, pp. 22–24. See also, for example, J. Burns, "Preparation of thin epoxy resin sections from thick sections of paraffin-embedded material," *J Clin Path* 23(7) (1970) pp. 643–45; or Van den Bergh Weerman M.A., Dingemans, K.P. Rapid, "Deparaffinization for electron microscopy," *Ultrastructural Pathology* 7 (1984) pp. 55–57; or S. Widéhn, and L.-G. Kindblom, "A Rapid and Simple Method for Electron Microscopy of Paraffin-Embedded Tissue Ultrastructural Pathology," 12 (1988) pp. 131–36; or Lighezan, R. et al., "The value of the reprocessing method of paraffin-embedded biopsies for transmission electron microscopy," *Romanian Journal of Morphology and Embryology* 50(4) (2009) pp. 613–17. Wolfgang Muss w.muss@salk.at Mon Aug 31

I know you just asked if we've already done this kind of work, not what we think about it. However I must second both Thomas and Wolfgang. Technically it is not such a big challenge but we like to know if what we do makes sense somehow. You rarely give yourself the hassle of TEM preparation because it is cool. Usually one has a precise purpose in mind. If your colleague/client wants fine morphological information, he will be very disappointed. Stephane Nizets nizets2@yahoo.com Tue Sep 1

Specimen Preparation:
preparing frozen insects for TEM

We're interested in processing moths for TEM, but the moths have been stored in a 0°F (-18°C) freezer for a few weeks or months. Will it still be possible to immerse these moths in glutaraldehyde and process them for conventional TEM? I understand the ultrastructure may not be optimal, but has anyone had success processing frozen insects for TEM? ***Joe Mowery joseph.mowery@ars.usda.gov Thu Aug 20***

Summary (you don't need the details to take a decision): "Not optimal" is mildly put. Leaving biological material unfixed at –18°C (it is probably the worst method to preserver biological material) and expecting to see anything meaningful in TEM is not realistic. If you want to use TEM, you are interested in fine morphology right? Well you won't get fine morphological information this way. Details (if you want to know why): 1) Upon freezing and especially slowly freezing like it happens when you put a specimen at –18°C, the water in the tissue and cells crystallizes. Nice spikey crystals. The crystals don't care about the biological structures (like membranes); they grow

through them, perforating them. Upon thawing, the membranes are literally cut in pieces, leaving you with a biological soup with very few original morphological properties. 2) The cellular enzymes have had plenty of time to do whatever job they have to do. 3) Big osmotic issues. Slowly freezing produces extreme osmotic forces because while some water freezes, the remaining water, which is in liquid state, sees its salt concentration dramatically increase. Hope you can convince your colleagues to invest time and money in something more useful (like reading a book about the usefulness of fixatives). Stephane Nizets nizets2@yahoo.com Thu Aug 27

Microtomy:

DMSO substitute

Material: sulfonated polystyrene-co-divinylbenzene spheres (0.5 mm diameter). I'm able to obtain microtome sections of the non-sulfonated PS-co-DVB beads using a diamond knife with a boat filled with DMSO at −55°C. I can section the beads stand-alone by gluing them onto a metal stub, or by embedding them in Epon. The problem is that the sulfonated beads like to swell once they contact the DMSO and the sections break apart/dissolve. Dry sectioning attempts have failed since the beads are relatively soft and completely crumple on the knife when there is not a liquid to relieve the compression. I'm looking for a substitute for DMSO that will not dissolve the sulfonated sections... something non-polar? ***Nathan Velez nrvelez@lbl.gov Thu Jul 9***

What is the glass transition temperature (Tg) of the non-sulfonated PS-co-DVB copolymer? I expect it would be appreciably higher than room temperature. Regardless, the crumbling of the sections on the dry knife indicates too low a cutting temperature. **RESPONDER RESPONDER EMAIL DATE OF RESPONSE**

The first pieces of information a microtomist needs to know about the polymer are (1) its composition (polarity, solvent susceptibility, etc.) and (2) the Tg of the polymer. Best microtomy results for virtually any polymer will be obtained by cryosectioning at or slightly below the Tg or at room temperature for high Tg polymers. Finally, my decades of experience in cryoultramicrotomy of polyolefin plastics/elastomers and block copolymers led me to believe that DRY sectioning is the best way to section these materials. I know that others have success in this area but I found that liquid cryoultramicrotomy just wasn't worth the trouble caused by wetting of the back of the knife and the sample, swelling of the sample, residue of DMSO on the sections, etc. Gary Brown microscopy.gmb@gmail.com Thu Jul 9

Image Analysis:

calculating wall thickness

Has anyone written a plugin for ImageJ/Fiji that will calculate the perpendicular distance between an inner and outer line at various positions? We need to measure the wall thickness on isolated plant cells, some of which look like cross-sections of a cylinder. Now, if they were all nearly circular cylinders with even wall thickness, that'd be easy. But these cells are far from perfectly cylindrical in shape and the wall thickness is uneven. In the past, we just made 4 measurements of the wall at fixed N-S-E-W positions and calculated the average thickness. But with the imaging tools available today, it should be possible to write a plugin that will do something like this—run a ball along the wall, which shrinks and swells according to the wall thickness and record the ball diameter at every position, for example. That would not only give us the average thickness but some useful stats about variability (per cell and per sample) as well. ***Rosemary White rosemary.white@csiro.au Wed Aug 12***

The 2D filament plug-in might do the job, although more will have to be added. With this plug-in, you can make "snakes" that follow edges, so you could find the inside and outside edges of cross-sections of the cells. Higher contrast works better, so using this with cryo data can be problematical. After you have traced the edges, you are still left with the task of determining the distance between them, but maybe someone else on either list can help. Bill Tivol wtivol@sbcglobal.net Wed Aug 12

EM:

radon 222 and 220 in ultra-high vacuum system

I am curious to know if anyone has encountered Radon 222 and Radon 220 in your ultra-high vacuum systems. All of my valves are metal bellows, VCR fittings with Ni gaskets, large flanges are conflat with copper gaskets. From the fore output of the turbo, to the inlet of the mechanical pump there are QF flanges with Viton gaskets. Approximate size of manifold is ~15 liter. At 60°C, my ion pressure is ~1.4×10^{-8} Torr. With the residual gas analyzer, my Rn 222 peak averages between 3.4×10^{-13} down to about 6.2×10^{-14} in ion current. The 220 peak closely follows. The water vapor peak averages about 5.3×10^{-13} ion current. Helium 4 leak testing reveals no leaks at ~1.0×10^{-9} sccm-atm. Where is the radon coming from? ***J. Allen Williams, Jr. oddioeng@aol.com Fri Aug 21***

The source of the radon could be the soil, depending where you are. For example, there is a formation called the Reading Prong in PA and NY that is very high in Rn, so much so that installing a heat pump system, which requires that the building is well sealed, leads to dangerous levels. Since EM's are typically in sub-basements, one would expect the highest Rn concentrations there. In fact, unless you have a few grams of radium lying around, the soil is certainly the source. Bill Tivol wtivol@sbcglobal.net Fri Aug 21

TEM:

high-tension shut down

We have an FEI Tecnai Osiris TEM in our lab. The high tension of the microscope has turned off suddenly without any warning or error information several times during our operation even though the vacuum and the supplies were all good. And we are not allowed to turn on high tension after that. Does anyone has similar experience or know what the reason is and how to avoid it? ***Hongbing Yu 12hy1@queensu.ca Mon Jul 27***

Could you elaborate on what you mean by saying "we are not allowed to turn on high tension after that?" Do you mean that functionality is disabled, or do you mean that somebody forbid you to do so? Valery Ray vray@partbeamsystech.com Mon Jul 27

I would first check the SF_6 pressure in the gun to make sure it is 6 bars. You can also look at the HTI board located in the Power Cabinet to check for indicator LEDs. Let me know if any of the LEDs are on and I can tell you possible issues. Did you hear any sounds just before the high tension shut off? John Schreiber js51@princeton.edu Mon Jul 27

TEM:

beam sporadically forms a dot

Our Philips CM10 TEM has a beam issue—it looks like it is in a dark field mode, concentrated as a tiny point and could not be spread out. But suddenly it might be normal for a few seconds to less than a minute without touching anywhere, then go back to point beam again. The oil diffusion pump and ion getter pump vacuum readings are not ideal but seemed to be working before. Could anything else be the cause of this problem? Where should I check first? ***Guosheng Liu gul417@mail.usask.ca Thu Aug 27***

It might be the board regulating current for C2 condenser. You can check the current passing through C2 in this way: Go to Parameters page on the information CRT screen and press Display Currents.

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Then you can monitor second condenser (C2) current. Go to Single mode, select C2 and look what happens when you turn the Intensity button back and forth. If there is no change in current level for C2 then the trouble might be in the current regulating board. Oldrich Benada benada@biomed.cas.cz Fri Aug 28

Definitely sounds like one or more of the lenses is cutting out (check them all on Parameters page, as Oldrich suggests). We are having the same issue with our CM10 caused by insufficient water flow through the heatsinks (MRU and LMH) in the lower right of the microscope (if you feel the two hoses at almost ground level to the left of the rotary pump, one of them will be the return with very hot water). The water flows around the column (top and bottom circuits of the column in parallel), then through the MRU and LMH heatsinks, then out of the microscope (the diff. pump water circuit is in parallel with this). If the microscope is on for an extended period, as well as the MRU/LMH water causing the lenses to cut out, we also have the lower part of the column heating up (feel by hand on the surface). We are going to solve it by looping out the MRU+LMH part of the circuit, and instead feeding it a separate water supply from our chiller circuit. There is a blow-off option that can help clear this circuit. The connector for this is on the right when you have the back of the microscope off. When you pull out the connector, a switch will turn off the microscope automatically. This quick-coupler is then connected to the inlet to push out water + debris from the lenses, MRU, LMH part of the microscope's cooling circuit. If you do this, make sure you know where the air will go when it is forced out of the return line of the microscope-we have had engineers make a fountain out the top of our chiller. We have a drain we can open. Ben Micklem ben.micklem@pharm.ox.ac.uk Fri Aug 28

If the beam is a pinpoint of light, the most likely cause is water flow. You will see on the Lens Current page that all the main lens values will be close to 0. You should have water flow gauges that are located near the mechanical pump. These have magnetic floats in them and if the flow is low, they will trip the sensor located near the bottom of the gauge which will shut all the lenses off. These gauges only read the flow going to the upper and lower column. I would install two more gauges that read the water flow to the ODP and the Electronics. The proper water flow through each leg is 0.7 liters/ minute. John Schreiber js51@princeton.edu Fri Aug 28

Also wanted to mention that there are regulators that you can you use to adjust the water flow to the different legs of the water circuit. If you have those max out, then you will need to determine what is blocking each circuit. John Schreiber js51@princeton.edu Fri Aug 28

I don't know if the float meters were a later modification on CM10s, but ours doesn't have them. It just uses a temperature sensor in the heatsinks in the electronics part of the circuit. Our CM100 has all the float meters as you describe. Ben Micklem ben.micklem@pharm.ox.ac.uk Fri Aug 28

Yes, you are correct. The early CM10's did not have a site glass for the water flow. In which the water had only two splits in the circuit. From the water input, half went through the ODP and half goes thru the Lens and electronics. The Power Booster safety circuit shuts off the lens if the heatsink get too hot. I would still recommend adding the flow gauges to adjust the proper water flow. John Schreiber js51@princeton.edu Fri Aug 28

First of all I want to thank you for the prompt replies regarding the pinpoint beam issue on our CM10. In summary, the majority of comments considered that the bad water flow led to lens current interruptions intermittently due to the lens power heat sink's overheating. This is most likely what is happening in our CM10. Our CM10 has been with cooling issues for years. We have replaced a circulating water chiller for the system, and also monitor the temperature for the column with temperature-sensor-type stickers. We have used CLR to clean the cooling system regularly (last time was half year ago)—despite this, the temperature of the bottom part of the gun column is still high, around 82–96°F (28–35°C) during operation (e.g. 80 kV). The temp setting for the chiller is at 14°C now. Definitely there is still some clog in the cooling system. The top part of the column temperature used to be OK (always around 20°C) but today it is 28°C. Anyhow I'll first do the CLR flush (at least overnight) to try to boost water flow. When I checked the lens current, it indicated that Projector (1&2) lens were down. Here are the readings (twice) when beam was normal and pinpoint:

C1	424/294	424/424 mA
Obj	110/115	120/117
Diff	787/549	787/795
Interm	3/3	3/124
Proj1	2190/1522	5/4
Proj2	1789/1248	–1/–1

My question is: is there any way to check the cooling hose from outside of the column which links to the projector lens part? Except for the bad cooling water for the lenses, it also could be the problem is related to the failure of a C2 condenser board, projector lens fuse, or dirty beam

path or even HT board as others pointed out in the comments. I'll check these later as needed. Guosheng Liu gul417@mail.usask.ca Fri Aug 28

SEM:
preparing microbeam standards

Does anyone have any tips or a good method for mounting standards in a standard block for SEM/Probe analysis? We got the Smithsonian microbeam standards recently and are trying to figure out the most effective way to create our own in-house standards block. Any help or advice would be much appreciated. ***Erin Summerlin es.smrln@gmail.com Mon Jul 13***

We mount all of our standards in acrylics pucks with 35 pre-drilled holes. The advantage is that when the epoxy shrinks as it cures, the whole mount shrinks without introducing any cracks that catch abrasives, oil, etc. Details are here: http://probesoftware.com/smf/index.php?topic=172.msg1436#msg1436

John Donovan donovan@uoregon.edu Mon Jul 13

SEM:
imaging of starch grains

A food scientist here is interested in gluten and gluten-free baked goods and dough, and is looking at starch grains from things like breadfruit flour. These are all "gushy" preps. What we've been doing is freezing pieces in liquid nitrogen (decided it wasn't worth trying to use a better cryogen for this), then throwing them down on the benchtop to "cryofracture" them, then freeze-drying them, since food scientists tend to have good freeze-dryers. Then mount on stubs, coat, and they've been pretty good. Of course, in this case, trying to decide what's a starch granule vs. a fat glob has been fun, but these guys think they know. (I reserve judgment.) If the material would not fracture by dropping it on the bench, we used the razor-blade-hammer-pop it open technique. ***Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Sat Aug 15***

Starch grains are fun. I did some from barley in the past - the USDA people in the barley lab (because of the brewing industry in Wisconsin). What are you trying to image? Specimen prep: If dry, like corn kernels, just break open the kernel. Cryofracture is fun, but not needed. Poke out the starchy endosperm and spread it on the stub. Sputter coat with Au/Pd as per usual. If wet - dissected from fresh, moist grains, then: Either dissect and allow to air dry - you won't affect the structure of the starch grains themselves and treat as above or fix with a normal formaldehyde/glutaraldehyde fix, use an extended—like an hour or more—dehydration series and critical point dry. Dissect some more and spread on a stub. If they're looking at how the grain is digested once the seed germinates—as the barley people were, then you must fix and dehydrate. But! It's also worth going the simple "do as little as possible" route. Starch grains are very tough and are very hard to break open—hitting with a hammer just produces individual grains. I've even tried cryofracture and not broken open a grain. But, the seeds do use enzymes and "open" the starch grains, producing pits. The walls of the pits have really neat light-dark layering. Starch grains left behind by baking, I don't know. I've looked at bread dough and not seen starch grains, nor did I see any in the (fully baked) pretzels I did. Donuts, though... there's a hole in my studies, there. Phil Oshel oshel1pe@cmich.edu Sat Aug 15

Is there a reason they haven't tried the old fashioned way and looked at these samples with crossed polars in a light microscope? Starch grains give a distinctive Maltese cross. Barbara Foster bfoster@the-mip.com Sat Aug 15

I wondered this myself! But for imaging starch breakdown - seeing the holes develop, and also for getting a quicker idea of the relative size, shape and abundance of small and large granules, SEM is quick, and you can keep the samples and look at them again if necessary. Here, people from the starch lab extract the starch, wash to remove protein and other contaminants, dry it, spread on a sticky carbon tab on a stub, image with BSE at 10 Pa (no coating necessary). For higher magnification or resolution, gold-coat then image at 20 kV under HV. Generally no fixation or other processing because in the various rinse steps you tend to lose the smaller B-granules which people here are very interested in. It's also easy to do and this way once trained, the starch folk can do this without needing my input. Just have to make sure not to dwell on the grains too much when focusing or they tend to crack, especially if uncoated. Rosemary White rosemary.white@csiro.au Sat Aug 15

SEM and EDS:
elemental Hg analysis

Anyone have experience looking for trace amount of elemental mercury in samples? ***Fern Stones stones.fern@dol.gov Tue Jul 7***

I examined some river sediment with EDS, and while I wasn't specifically looking for Hg, I did find many elements. I also had enough overvoltage to see even the *K*- lines. There are two problems that you face: The first is that EDS is not sensitive to amounts much smaller than 1%, and the second is that Hg is volatile, so the amount under the beam will be continually decreasing. If you have access to WDS, it will be easier to find small amounts of Hg before it goes away. Bill Tivol wtivol@sbcglobal.net Sat Jul 11

My acquaintance with mercury in the SEM is with a tooth filling, a mercury amalgam! Acquired when a student broke a tooth, the "specimen" proved to be very interesting as an EDX investigation. It was made up of mercury, silver, tin and copper. We picked up mercury if we jumped to a new area but, after dwelling too long on an area, the mercury may not show. For those who would like a nice EDX test specimen, ask a dentist, they often have a pot full of potential specimens. Without any doubt, specimens like this make great teaching material. Steve Chapman protrain@emcourses.com Sun Jul 12

SEM/EDS sensitivity can be much better. I've gotten better than 500 PPM detection limits, using an SDD at high currents (5 nA or so, but still less than a probe) for about 1 minute acquisition times (~15–20 million counts in the spectrum) with very careful sum-peak stripping. Can't speak to volatility, but I suppose a cold stage plus area scanning might work if a yes/no answer is all that's needed and that meets your definition of "trace". The Hg $L\alpha$ line is in a nice place WRT potential overlaps, with the exception of Ge *K*, which is still more than 100 eV away. Depending on the sample and the spatial resolution requirement, XRF might be a better tool for this if the sample is something like Bill's river sediment and you need one or two more orders of magnitude in sensitivity. No beam heating issues. Rick Mott rmott@pulsetor.com Sun Jul 12

I would suggest inductively coupled plasma- optical emission spectroscopy (ICP-OES) or inductively coupled plasma mass spectrometry (ICP-MS) if you have access to them. ICP-MS is capable of ppq in the right conditions, and frequently in the ppt. Samples do not need to be liquid, they are digested or ashed, then digested, for introduction into the instruments. While SEM-EDS or XRF might be capable of detecting the element, the conversion from excitation volume, ZAF correction, and peak area to a ppm or similar number is non-trivial. Allen J. Hall ajhall@prairienanotech.com Sun Jul 12

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