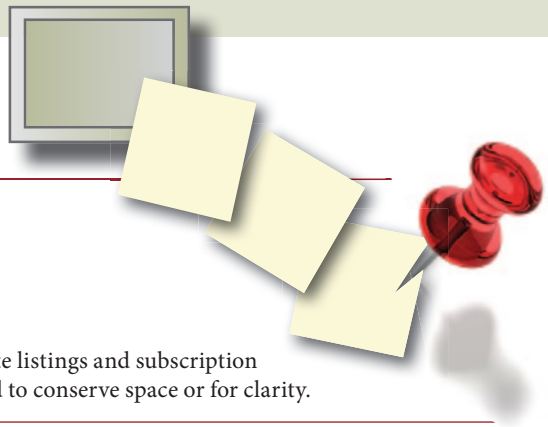

NetNotes

Edited by Thomas E. Phillips
University of Missouri

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Specimen Preparation:
microwave processing

Does anyone have experience in use of a microwave oven in biological sample preparation for the TEM? Any comment about microwave based sample preparation. We are dealing with ultrastructural medical diagnostic specimens using TEM. ***Ravindra Thakkar ravi.thakkar369@gmail.com Fri Jun 22***

We have used a microwave for processing clinical samples for many years. I would not process any other way. It has cut our turnaround time from a week down to 1–2 days! Native kidney biopsies are completed (including scoping) in 1 day. We have had no problems or issues with the processing. The major advantage I see is the fact that infiltration of the resin takes a total of 12 min! 3 min in 1:1, 3 min in 3:1 and 3 min twice in 100% resin. We do not polymerize epoxy in the microwave—just not practical for our purposes. However, we process LR White samples for immuno (we also do research for campus PI's) in the microwave and I find that polymerization of the LR White in the microwave makes trimming and cutting sections from the block so much better. Pat Kysar pekysar@ucdavis.edu Fri Jun 22

I can almost copy the e-mail from Pat Kysar (UC Davis), with our experience on microwave processing, with some minor modifications: we process kidney samples regularly, since >2 years now, and I very much prefer these samples against conventional RT processing (i.e. processing for many long hours with too many and long steps of dehydration and infiltration/ embedding). Turnaround times: from 4 days (including polymerization), down to 4 hours (again incl. infiltration and polymerization of Epon! works great!), resulting in a specimen that can be sectioned instantly. Here, we continue on the next day, with semi-thin + LM, trimming and sectioning for TEM. Yes, you can in fact do this on the very same day, if you are focused on a very special aspect, only. If you aim to do a more detailed visualization of details of kidney ultrastructure, I would argue that you may need one to even a few days on the TEM, alone. Note: here, we have time slots of 3 hours only, on the TEM, which need to be booked in advance, due to a tight scheduling scheme of TEM time. Nice to read that LR White can be processed in the microwave, too—which conditions are used for infiltration + polymerization of LR White? BTW: we used kidney samples—processed in the microwave with little OsO4, 0.1%—and Epon embedding also for immuno-labeling—and it is worth trying: it works! (Depending on the initial processing / perfusion fixation of the kidney, and the amount and kind of antigen present in kidney.) We have to restrict our experience to microwave processing in the AMW (and we think this is important to note)—and another note added: we are just a satisfied customer (Leica), no financial interest. Reinhard Rachel reinhard.rachel@biologie.uni-regensburg.de Sat Jun 23

Specimen Preparation:
using Formvar coated slotted grids

I have having huge problems getting my ultrathin sections to go on Formvar Coated grids without a big fight with the sections and the water in the boat of the diamond knife. Because of the surface tension of the water, I am having a big problem getting the section onto the grid without the making a lot of wrinkles in the Formvar coating, or the section or both. Is there a trick to this that I don't know about? ***Garry burgess gburgess@dsmanitoba.ca Thu Jun 14***

I have not worked with slotted grids, only regular (square) and hexagonal mesh, but the problem you describe is not uncommon. If by "fighting" with the sections you mean that they tend to want to run away from the grid when you are trying to pick them up, or because you are having trouble getting your grid into the water, that is because grids tend to be hydrophobic. Two things that should help: (1) Use a static-free polypropylene specimen cup to condition your double-distilled water prior to filling the boat. Ted Pella has them (Static-Free Plastic Cups, 30 ml, Prod. #12901). I'm pretty sure they ship to Canada. Fill the cup, wait a couple of minutes, then use a needle syringe to fill your boat with water from the cup. (2) Glow-discharge (plasma clean) your grids just prior to picking up sections. This will make your grids hydrophilic. The discharge is good for about an hour; I usually plasma clean my Formvar + carbon grids while the ultramicrotome is churning out thin sections. With conditioned water and plasma cleaned grids, there is very little resistance at the water's surface when you slide your grid in, so there will be no wrinkles on the film due to the grid bending. In my opinion, using static-free water is better than adding Photo-Flo or solvents like acetone to your boat to reduce surface tension, which most knife manufacturers advise against anyway. One other method that was recommended to me is to store Formvar coated grids in the refrigerator until you are ready to use them; this supposedly keeps them hydrophilic. I found that did not work with our Formvar + carbon grids. If you use only plain Formvar film, storing your grids at 4°C might help. To avoid wrinkles in the sections, I relax them with chloroform vapor before picking them up (just a couple of quick waves because you don't want to over-stretch them either). I use locking tweezers and release the grids onto filter paper in a Petri dish. I then place the Petri dish in a 60°C oven for 10 to 20 minutes to ensure good adhesion and fewer sections washed off during post-staining. Gigi Kemalyan singinggardenersx2@live.com Fri Jun 15

I see lots of interesting ideas and methods for picking up serial sections onto Formvar-coated slotted grids. As all involve immersing the coated grids in water and picking up the sections from below, I think they will require lots of skill and patience. A simpler method is to use uncoated slotted grids as pick-up loops. Align the batches of serial sections on the side of the knife boat and cover each row with an uncoated grid similar to the coated ones. Make sure each grid is completely wet on the bottom surface and then pick it up while keeping it horizontal. The water drop will stay in the slotted hole and the sections will remain on the surface of the drop of water. You will be able to transfer the grid, water drop and sections onto

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a Formvar-coated slotted grid held in a clean pair of forceps. Align the slot in the pick-up grid with the slot on the Formvar coated grid and leave to dry slowly. The sections will remain flat and dry down onto the Formvar surface. I think there is a similar version where the grid with the drop of water and sections are placed on Formvar sheets created over the holes of a sheet of Perspex. Once dry, the grid, with Formvar and sections are removed. Have fun trying this—I promise you it will be easier than picking the sections up from below. I got first the idea from an old (1970's) paper which I do not remember the reference for (or the authors—maybe it was Galey and Nielssen). Paul Webster pwebster@hei.org Fri Jun 15

A somewhat similar method was used by Marinozzi to pick up sections and isolate them in a plastic loop. It was quite useful for cytochemical procedures that were aggressive for metals such as for the detection of glycol-conjugates using the Thièry method. Way back, I used the perforations of 35 mm film to transfer sections; that might work for slotted grids as well. Marinozzi, V. "Cytochimie ultrastructurale du nucleole. RNA et proteines intranucleolaires," *J. Ultrastruct. Res.* 10, 433 (1964). Jan Leunissen leunissen@aurion.nl Sat Jun 16

Reading the other posts reminded me of one more method, using a "Perfect Loop for ultramicrotomy." EMS sells them and also has the how-to illustrations on their product page. Not cheap but it works well, at least for regular grids. Might be worth a try with your slotted grids. http://www.emsdiasum.com/microscopy/products/preparation/ultramicrotomy.aspx#70944 Gigi Kemalyan singinggardenersx2@live.com Sat Jun 16

Many people wrote to me off-line to point out that EMS has a "Perfect Loop" that will do the same thing I described for picking up sections. I agree that the Perfect Loop is useful for picking up sections to place onto a regular grid where orientation is not essential. However, the original question was for advice on how to pick up sections onto a slotted grid. This is a single slot grid which is longer than it is wide. Orienting a ribbon of sections onto the Formvar-coated slot will be very difficult is using the Perfect Loop, which has no orientation. Using a clean slot grid makes it possible to orientate the sections before they dry down. Maybe there is a market niche for an oval-shaped Perfect Loop! Paul Webster pwebster@hei.org Sun Jun 17

I used to use the slot grid as the loop, with regular success. Briefly: A domino rack was coated with 2.5% Formvar in dry chloroform, and the grids were "coated" (to make them sticky) by dipping in 0.5% Formvar and air drying immediately on filter paper. Put the coated domino rack onto a 60° C hotplate. Line your sections up in the boat, and using the slot grid, dull side down, as your loop, pick up sections from above the water. The sections will always be in the hole with this method. Hold the loaded slot grid above a hotplate for a few seconds to flatten the sections, and then gently place onto Formvar film over a hole in the domino rack, record position of grid on rack. Allow to dry for at least an hour, allow to cool, store in desiccator overnight, and then gently "punch" the grid out of the rack. The hex key tool in the Leica cryo kit is perfect for this. Stain the Shiny side! Domino racks are a copolymer coated metal rack with 5 mm diameter holes in them. (Available from EMS # 70620. No commercial interest.) There is a reference with the product listing on the EMS website. Rosey van Driel rosey.vandriel@deakin.edu.au Mon Jun 18

Our lab doesn't have a glow discharge unit so we mainly use the Formvar-coated bridge method as described by J. Carter Rowley and David T. Moran (1975). "A Simple Procedure for Mounting Wrinkle-Free Sections on Formvar-Coated Slot Grids," *Ultramicroscopy* 1, 151–55. Where we differ from their method—we submerge the slot grids at an angle keeping the slot filled with water and then scoot the sections into the slot with an eyelash tool rather than lower the slot down onto the sections. The sections will float in a meniscus of water and after you place the grid down onto the Formvar-coated bridge the sections will dry down onto the Formvar. We usually let them dry down for 2 h but that may be overkill. And, we don't try to coat the slots with carbon after picking up the sections. The Formvar alone seems to give enough support. Anti-capillary forceps are a huge help when using slot grids and I'm a big fan of Synaptek 2×1 mm slot grids with dots. I pick up the sections with the dot side up so I know to stain the grids dot side down. Roesy van Driel gave good tips in her reply (dipping the grids prior to use to make them "sticky"), etc. though we haven't tried using a hotplate—maybe the heat and humidity here in Georgia allows us to skip that step. Beth Richardson beth@plantbio.uga.edu Mon Jun 18

Specimen Preparation:
gold on carbon test specimen for SEM

Is there an easy way to make a gold on carbon test specimen for SEM? ***Stefan Diller stefan.diller@t-online.de Thu May 3***

If you just want gold islands on a carbon surface, you can evaporate carbon onto a formvar-covered grid, then briefly evaporate a small amount of gold onto that, but if you want both islands of gold and other islands of graphite, you will need to deposit the carbon in such a way that the graphite islands form, and I do not know the procedure for that. It may be more cost- and time-effective to buy a combined test specimen from one of the supply dealers, which should be about $50 or somewhat less. Bill Tivol wtivol@sbcglobal.net Thu May 3

Specimen Preparation:
insects for SEM

Can anybody give me some advice with SEM preparation of insects (I am working with honey-bees now and need to go through dehydration with ethanol for final critical-point-drying), especially these topics: How to kill insects without getting these cramped legs? Use of chloroform? Any tips how to bend and glue the legs of critical-point-dried specimen to the stub? ***Stefan Diller stefan.diller@t-online.de Fri May 25***

The legs become flexible after critical point drying. You may arrange them anyway you want before gold coating. Ann-Fook Yang ann-fook.yang@agr.gc.ca Wed Jun 6

Specimen Preparation:
separating the smallest of particles

I have a size fraction of <75 µm rock particles that I need to further separate into >45 µm, >15 µm and <15 µm. I've tried wet sieving with precision fabric, but because the 15 µm sieve has only 10% open space it took much longer than anticipated, and still did not pass more than 40–50% of that size. I'm looking into jet sieving, but thought I'd ask this group about their experience with wet sieving, jet sieving, and other possible methods. ***Michael Shaffer mshaffer@mun.ca Tue Jun 12***

I don't know jet sieving but you are clearly in a delicate zone (between 100 and 10 µm). Particles bigger than 10 µm usually sediment fast in water (of course it depends on the density), so to select particles under this size you just suspend the powder in water and leave the suspension for 5 min on the bench and then pour the supernatant. Repeat the process 3× and the results should be quite good. For even smaller particles (sub-micro) centrifugation works well. I suppose it may be possible to use higher density liquids (than water) in order to select bigger particles but I have no experience with that. Stephane Nizets nizets2@yahoo.com Wed Jun 13

Thanks Stephane. Others had suggested sediment rates as well. However, there exists a relatively large density differences between the minerals in these powdered samples, the most important of which are quartz and hematite/magnetite, and carbonates between. Subsequent analysis of the modes would depend on there being absolutely no partitioning of minerals relative to size. Michael Shaffer mshaffer@mun.ca Thu Jun 14

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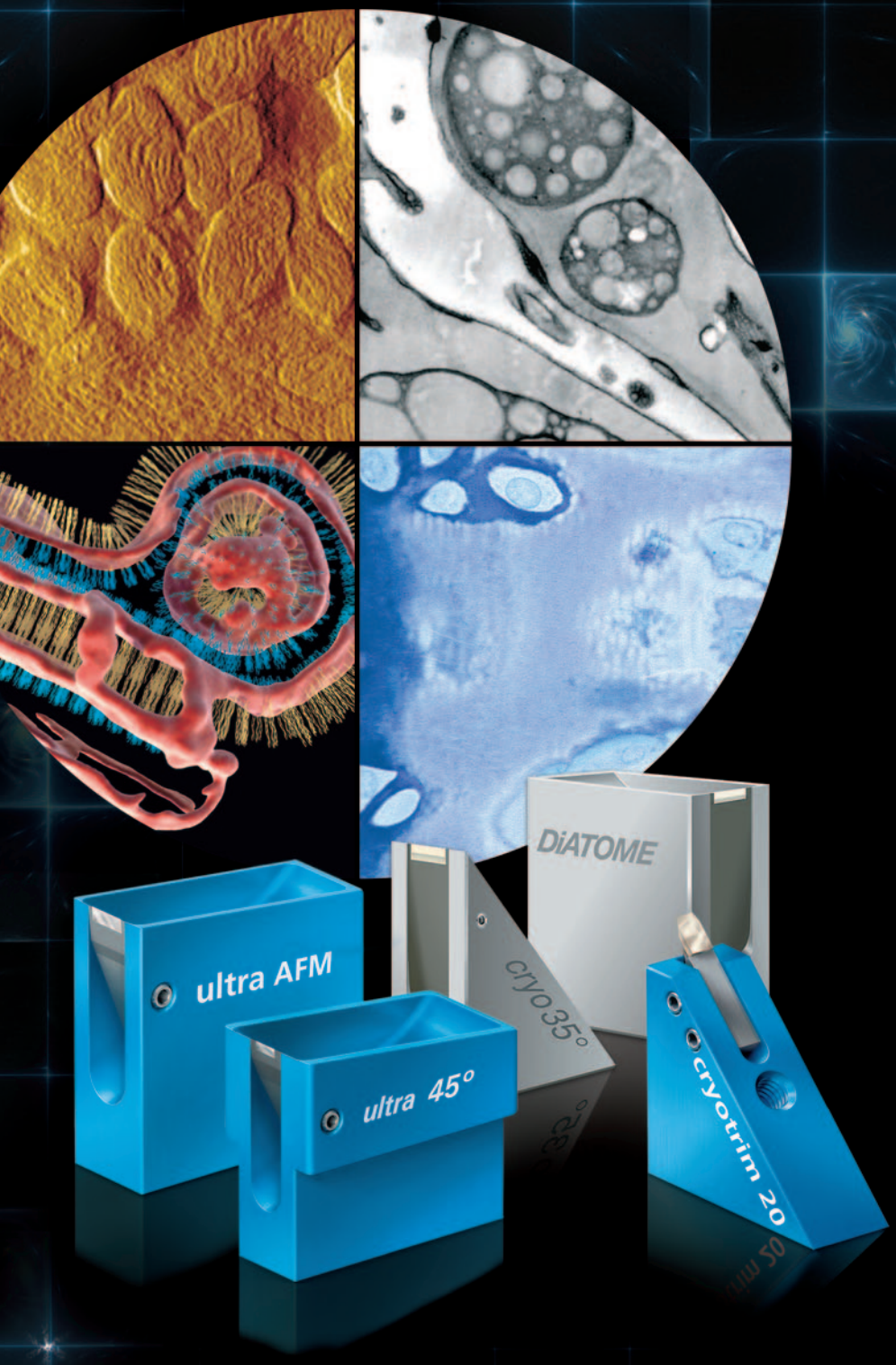

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It occurred to me that sieving might go faster if you suspend your particles in a less viscous solvent, such as ethanol (other organics or Freons may also work, but they would definitely require you to work in a hood, and they might evaporate too quickly). Agitating the sieving apparatus while separating could prevent small particles from hanging up on the cloth could improve your yield. Do each of the minerals yield the same fraction during the process, or does one have a higher yield than another? Bill Tivol wtivol@sbcglobal.net Fri Jun 22

Thanks Bill for the interest. I did try "wet" sieving with ethanol, which certainly worked better than agitated dry sieving. However, the smallest of particles for both the 48 µm sieve and 15 µm sieve still remained not passed, which is the problem when I want to quantify the minerals in each size and with the quantification being via area in 2d section (SEM). The study is also about any fractionation for different minerals with respect to size—e.g., as if lower density minerals might be smaller than more dense minerals, so I expect size fractions to differ for different minerals. I guess I'm a bit surprised I received no responses from users who may have experience with "jet" sieves. These use agitated air flow together with suction to deaggregate and agitate particles while trying to suck them through the sieve. I don't know how well these might work for particles this size. After all, the smallest of sieves (e.g., 20 µm) do not have a lot of open area. Michael Shaffer mshaffer@mun.ca Fri Jun 22

Image Processing:

ethics

I'm looking for official or semi-official imaging ethics rules. Does anyone know a source? ***Robin Foley rfoley@uab.edu May 18***

The Microscopy Society of America's Policy on Digital Imaging can be found here http://microscopy.org/resources/digital_imaging.cfm. I have reproduced it below. The MSA position on digital image processing has been approved as follows: "Ethical digital imaging requires that the original uncompressed image file be stored on archival media (e.g., CD-R) without any image manipulation or processing operation. All parameters of the production and acquisition of this file, as well as any subsequent processing steps, must be documented and reported to ensure reproducibility. Generally, acceptable (non-reportable) imaging operations include gamma correction, histogram stretching, and brightness and contrast adjustments. All other operations (such as unsharp-masking, Gaussian blur, etc.) must be directly identified by the author as part of the experimental methodology. However, for diffraction data or any other image data that is used for subsequent quantification, all imaging operations must be reported." This policy was formulated by the Digital Image Processing & Ethics Group of the MSA Education Committee and was adopted as MSA policy at the Summer Council meeting August 2–3, 2003. Nestor J. Zaluzec zaluzec@aaem.amc.anl.gov Fri May 18

For imaging in biosciences, some years ago, an Editorial and a Feature on this topic were published in *JCB* (*The Journal of Cell Biology*): Rossner, M. and Yamada, K. M. (2004) "What's in a picture? The temptation of image manipulation," *J Cell Biol* 166, 11–15. doi: 10.1083/jcb.200406019. Rossner, M. (2008) "A false sense of security," *J Cell Biol*, 183, 573–74. doi: 10.1083/jcb.200810172 Oldrich Benada benada@biomed.cas.cz Fri May 18

Instrumentation:

cleaning a vacuum pump inlet filter

I've recently become the proud new administrator of a Hitachi SU-1500 SEM system. I come from a background mainly in optical microscopy, and while imaging on an SEM has been straightforward enough, it is the first time I've maintained an instrument with any kind of vacuum system. I was told by the Hitachi techs to change the oil in the rotary vane vacuum pumps that power the evacuation system periodically. Consulting the vacuum pump manual for the procedure on this, though, I also notice that it also recommends cleaning the vacuum inlet filter about twice a year. That raises some questions. Is this a point of maintenance that actually needs to be carried out when the pump is fully attached to a vacuum system, as opposed to one in which the intake valve is continually taking in air from the surrounding environment (including any dust) rather than drawing out air from inside the EM system? And if so, what are the conditions under which it would be OK to disconnect the vacuum line from the EM to the pump? The default state is for the EM chamber and column to be evacuated, including when the system (including the pumps), are turned off. Would disconnecting the line backfill the EM with air with potentially bad consequences? Alternately, if I have the EM in Air (nonevacuated) mode, then the pumps are by default running. I can unplug the power cords one pump at a time to work on them, that would seem to be OK, but they normally both run even in Air mode, so I'm not sure if I should be taking them offline in that mode either. Dilemmas! Unfortunately, the SEM manual's coverage of the vacuum system is not particularly good, so it leaves a lot of open questions. I'll run these questions by the Hitachi tech next time I talk with her, but was wondering how other users treated this particular point of maintenance, or whether you bothered with it at all. ***Peter G. Werner germpore@sonic.net Wed Jun 27***

I have to say I am very puzzled because I have never heard of an inlet filter on an electron microscope! Are you sure they are not talking about the exhaust filter, which I agree should be maintained or replaced every 6 months? If the exhaust simply ends in a mushroom shaped unit I would very strongly suggest that you replace it with a good quality filter as offered in EM accessory manufacturers catalogues. The pump should be disconnected electrically, switched off, to change the fluid, but you do not have to switch it off to change the exhaust filter. But I suggest you do switch off to change filter so that you will not be exposed to the nasty exhaust fumes. Steve Chapman protrain@emcourses.com Wed Jun 27

I've never seen an inlet filter on a pump; rather I've encountered such filters on the microscope air inlets. Even older TEMs (Hitachi HU-11 and H500) had an air inlet desiccator filter. Basically, it was Drierite or silica gel granules with a large piece of cotton wadding to prevent loose powder from entering the microscope. We never had one on our SEMs (Hitachi S570 and 2460), though we did hook up the air inlet on the 570 to a tank of dry nitrogen gas. Our most recent SEM, an FEI 450FE, came with a HEPA-like, micro-filter on the air inlet to prevent the intake of particulates into the EM. They are basically replaced once or twice a year and the old ones discarded. No filter on the pump inlet, though. John J. Bozzola bozzola@siu.edu Wed Jun 27

Actually, I have seen them used extensively on Leybold roughing pumps, only on the exhaust side. It's a stainless steel trap integrated into an O ring assembly for a KF25 flange. The trap sits inside the pump and filters out any large chunks of debris that may fall out of the exhaust filter. Gary Easton garyeaston@scannerscorp.com Wed Jun 27

I believe Peter is referring to the various forms of molecular sieves (might be zeolites or steel or copper wool) that are usually placed near the inlet of the roughing pump to limit backstreaming of pump oil. Some of the units are sealed and considered throw-aways, although one can flush them with acetone and then thoroughly dry them, preferably under vacuum and heat, before putting them back in the vacuum line. Others (notably Edwards and M. E. Taylor) can be opened and the contents replaced. I don't know if his system has a turbo pump or an oil diffusion pump, but either way, my suggestion would be to shut down completely before opening any roughing lines. With isolation valves, the high vac pumps can be left for short periods

without backing (if the vacuum system is tight) but since Peter is new to vacuum systems, Murphy is watching, and "everything takes longer than it takes," I would strongly suggest a complete shutdown. Ken Converse kenconverse@qualityimages.biz Wed Jun 27

Instrumentation:
molecular drag pump

Recently I had Company X rebuild a 5010 Alcatel molecular drag pump (MDP) that was not pulling the vacuum I needed. This MDP serves as a backing pump to a larger turbo pump. Upon receiving the rebuilt MDP I placed it back into the vacuum setup but was not able to get the MDP to accelerate to full capacity, the rebuilt MDP pulls a vacuum to ~1000 Pa. The backing pressure (from a diaphragm pump) is roughly 6750 Pa, a backing pressure that has always been sufficient for "my" 5010 Alcatel MDPs. Since confirming proper backing pressure I have contacted Company X stating my issue and they retested the rebuilt MDP, and still state that the pump pulls a vacuum of 6×10^{-6} mbar (6×10^{-4} Pa). I have a second MDP, another 5010 Alcatel MDP, which accelerates properly (better than 1 Pa) under five minutes when used in the exact same vacuum setup where the rebuilt MDP will not fully accelerate. To me, using this second MDP allows the backing pressure and potential leaks within tubes/etc. to be ruled out as a possibility for the rebuilt MDP not to operate at full capacity, agreed? Are there other options for me to test whether the rebuilt MDP is operating correctly? Am I able to lean harder on Company X when I know a virtually identical MDP performs properly within in my vacuum setup and when I replace the functioning MDP for the rebuilt MDP and the result is basically failure? Any suggestions or thoughts on this matter will be greatly appreciated. ***Joseph Heintz josephheintz@gmail.com Tue May 8***

When you said that you used the same vacuum setup when testing the second pump, did that mean that you used the same controller as the first pump? If they are testing the pump and it gets up to speed at their test facility, then the pump is probably OK. You have only a couple of potential problems. (1) You have a problem with the controller. It also could be a problem with connectors and cabling. Since the pump worked during testing at company X, the connector on the pump is probably OK. Check your cables and your cable connectors. (2) You have a vacuum leak. Anytime you make or break a vacuum connection, you have the potential for a leak. Look at your O-rings and your sealing surfaces for scratches on that first pump. (3) You have a problem with the backing pump. Since you tested that with the second pump, this shouldn't be a problem. Scott D. Walck s.walck@cox.net Tue May 8

I assume you just put pump 2 (good one) in the same position/tubes, etc. as pump 1 (bad one). Did you also switch controllers? There are 2 controller boxes, although one may be stuffed away somewhere. (I think it failed in the past.) If the bad pump is still bad when hooked to the same controller as the good pump, then you've got a case. Also, how's the turbo running when hooked up to the bad pump? Speed OK, sounds good? How about the gauge you're using? You tried it on both pumps, yes? If only on the bad pump, see how the gauge reads on the good pump. Philip Oshel oshel1pe@cmich.edu Wed May 9

TEM:
LaB_6 filament

My lab owns a JEOL 2010 TEM. Recently, the JEOL engineers came to do a preventive maintenance for our system. They changed a new LaB_6 filament and did a thorough check on any possible leakage and the power stability because before the maintenance, our filament was creating issues. The filament current (we set at 104 μA to 106 μA) drops noticeably faster to the background during usage and finally burns off in about 4 months). While I hoped that the maintenance would clean up the column and stop any possible leakage (they did detect a leak at V2, which is around the camera chamber), the new filament which we are using this time doesn't look promising either. First, at instrument daily start-up, we observed that the filament was unstable twice. Once when the current suddenly dropped from 101 μA to zero when not in use. The second time is when the JEOL engineer was removing the sample holder. For the second case, the engineer told me that it is not because of any discharge from the filament by observing the change in the SIP. On the other hand, I try to observe the shape of the LaB_6 filament in its under saturated condition. I did see 4 lines from different directions connecting to a dot (supposedly it should be the tip, please correct me if I am wrong), but they seems to be tilted at an angle as the dot is not at the center of the beam observed under the viewing screen. I try to use the gun tilt in attempt to bring the filament back to the center of the beam but it did not seem to be working. What I observed is that the lines and the dots are fading fast into the beam (which also darkens) when I do the gun tilt. I would like to ask (1) If anyone has experience the fast dropping beam current during usage, if so what could be the possible reason? (2) If I cannot bring the tip back to the center when using the gun tilt, what could have happen or what other alignment I could have use? ***Yee Yan Tay one_twinklestar@yahoo.com.sg Tue Apr 24***

I am mainly familiar with Kimball LaB_6 cathodes in SEMs, but a couple of things jumped out at me in your message. A Kimball tip with the 15 μm flat should never be run above about 60 μA or it will fail prematurely. Check with the manufacturer of your tip for more details. Second, although I've had very little to do with TEMs over the years, most typically run at much lower emission currents than SEMs, in the range of 10–15 μA, I believe. What does your user's manual recommend? The emission current could be the whole answer to your short filament life. Ken Converse kenconverse@qualityimages.biz Tue May 1

We use Kimball LaB_6 cathodes in our FEI CM20 TEM. Let me add a couple of points to those made by Ken. (1) Filament current—we typically run ours below 40 microamps. It has been quite a while since I used a JEOL TEM, but if I recall correctly, there is typically a dark current, so Yee Yan's 106 microamp current may include the dark current—please let us know the value of the emission current—dark current. (2) LaB_6 cathodes need to be saturated slowly. I wrote a DigitalMicrograph script to saturate my cathode over about 10 minutes and do the required sample tracking chores while it runs. I should also note that one should check saturation again after 10–15 minutes because it can creep up. It is too easy to inadvertently over-saturate the cathode. The cathode should be turned down slowly to minimize thermal shock. I really wish our CM20 had a manual gun isolation valve so I could close the valve with the cathode saturated during sample exchange . . . (3) The cathode lifetime is also a function of vacuum. How good is your gun vacuum? John Minter jrminter@gmail.com Tue May 1

Our background current is 101 micro Amp. I have checked with JEOL, 106 micro Amp is their recommended beam current to be used in our machine. I should check with them the cathode. But I think it's the brand from Denka. I will let you all know the details of the of the LaB_6 filament. Yee Yan Tay twinklestar@yahoo.com.sg Wed May 2

TEM:
magnetic samples

We work on a JEOL JEM-2100f. I was just browsing the JEOL website and they mentioned a JEM-2100 LaB_6 has an objective mini lens hence Lorentz microscopy is a default feature or we can do magnetic samples in it. 3rd line in 4th paragraph: http://www.jeol.com/products/electronoptics/transmissionelectronmicroscopestem/200kv/jem2100lab6/tabid/123/default.aspx but same is not said about 2100f. As both have same lens configuration (other than one extra condenser lens in LaB_6 system) shall I presume it is true for 2100f also? Can

anyone tell what is the gap between objective pole piece in HR objective pole piece in JEM-2100F? Can we image magnetic nano particles normally in it (i.e., without switching off the objective lens)? ***Amit amit.welcomes.u@gmail.com***

I am very interested to know what you find out. We have a JEOL 2100 and I have just recently been looking for an explanation of the reference to Lorentz microscopy mentioned at the link you provided. On the features/capabilities document it says "Field Free Imaging—Lorentz Microscopy—Foucault/Lorentz—Standard." Also, "Lorentz mode standard: Foucault imaging from 100× to 40k× (inexpensive option)." In another place: "Low Mag optics/Lorentz Microscopy—Standard—simple upgrade to full Foucault imaging up to 40k×." So, they may be referring to a change of pole piece, which I don't really want to pursue, if it means lower resolution. Otherwise, I'm guessing they just mean to work in low-magnification mode (with the OL off), but that doesn't seem adequate for analyzing nanostructures. But if there is some software that would make it easier to work with magnetic samples, I would be very interested. I'm hoping to get some feedback from JEOL soon. Phil Ahrenkiel phil.ahrenkiel@sdsmt.edu Wed Jun 13

TEM:

Wehnelt bore diameter

We changed the LaB_6 filament of our JEOL 3010 TEM last week and we noticed a problem with our spare Wehnelt, during the cleaning process. The Wehnelt cap's bore diameter was larger than I remember. We compared its diameter with the one from another TEM (JEOL 2100), and it was really big. The diameter was ~2.1 mm compared to ~1.5 mm. We confirmed that the Wehnelt cap has the same part number for both microscope models. We do change filaments once a year, and the microscope is 13–14 years old. After installing this Wehnelt, we could not adjust properly the gun tilt/shift. So, we had to clean the Wehnelt that was originally installed in the TEM and order a replacement cap. After talking to the people here, I suspect that the person that was keeping the TEM before me was using a Dremel tool to clean and polish the Wehnelt. More, I suspect he also used diamond-polishing paste instead of the POL polishing compound to do the cleaning. Sure this will remove LaB_6 deposit much faster, but can someone over polish that much? ***Carlos Kazuo Inoki carlos.inoki@lnls.br Mon May 21***

Yes, I have seen a similar "problem," but in that case, I investigated and found that they had chronic illumination astigmatism due to a very miss-shaped cathode aperture. Please understand that the cathode with a larger aperture may have been appropriate for a tungsten hairpin system. A smaller cathode aperture may have been used in order to attain the higher bias levels that LaB_6 require. Steve Chapman protrain@emcourses.com Mon May 21

I think Steve is right. All of the LaB_6 instruments we had came with two grid caps, one for W, one for LaB_6. Sometimes it was hard to tell the difference. Jonathan Krupp jkrupp@deltacollege.edu Mon May 21

As one who has been using 1 µm diamond paste and a Dremel tool for most of the past 30 years, I will say that, yes, it could be that the previous user was over-enthusiastic, but I'm more inclined to think that your "spare" Wehnelt was for a tungsten cathode rather than a well worn Wehnelt for LaB_6, especially if it is still symmetric. Ken Converse kenconverse@qualityimages.biz Mon May 21

Steve, that is a good point. At first I also thought it was a Wehnelt for tungsten filament. But people told me that we never had a Wehnelt for tungsten in our lab. I also talked to our JEOL service engineer and he told me that the JEOL 3010 used LaB_6 filaments only. Carlos Kazuo Inoki carlos.inoki@lnls.br Tue May 22

I visit many laboratories in a year and you would be surprised at what we find when searching through the "spares." I am sure the information that the service technician gives you is correct according to the JEOL specification but as others have mentioned to cause such a remarkable change in the cathode aperture has not been seen before. My conclusion is that at some time a tungsten cathode has been used in the laboratory, maybe an older instrument or delivered as a spare in error? Steve Chapman protrain@emcourses.com Tue May 22

SEM:

high voltage instability

What effects may be occur if we have high voltage instability in an SEM? What are the diagnostic indications? ***Arazeshi arazeshi@gmail.com Tue May 15***

Just to keep it simple, high voltage instability in an SEM would be best displayed on a slow scan, the image moving in and out of focus. It is extremely rare for this type of instability to appear due to the final lens being unstable. I have only seen the final lens unstable once in 40 years and that was a prototype! Tell us more if your instrument does not follow the above. Steve Chapman protrain@emcourses.com Wed May 16

EDX:

magnetic samples

I'm working with magnetic materials for STEM and EDX mapping. However, sample drift makes it terribly hard to get any useful info from my EDX maps. Are there adjustments that you can recommend for better mapping conditions? On the same note, how can I tell if my column/lens has been contaminated with any magnetic material? ***Serene Ng serene_ng@dsi.a-star.edu.sg Wed Jun 20***

Ferromagnetic samples can escape from some specimen holders; it is best if some kind of a screw-type mechanism holds these samples securely. If the column is contaminated with magnetic samples you might find problems correcting image astigmatism or see distortions in diffraction patterns. IF you have a complete record of the settings of the alignments (e.g., beam tilt for objective optical axis) from the installation and perhaps the strength of the objective stigmator, you can compare these values with those you currently have using a carbon film test specimen (e.g., combined test specimen with gold islands and graphitized carbon). If for example the beam tilt is far away from its "normal" value you might suspect contamination. Sample drift is usually associated with thermal equilibrium (or lack of it rather), since the sample holder is often contracting for some time after insertion. If the magnetic force is great enough to overcome the spring return force, that often holds a tilted sample cup against its drive mechanism, you might find the whole sample cup is flipped towards the vertical and any chance of microscopy is lost. If you can reduce the amount of magnetic material present (e.g., by preparing a FIB specimen) or dilute the number of particles you should be able to reduce magnetic distortions and if your sample is loose it might be moving to escape the substrate. Finally try to work at the highest voltage possible, assuming your samples are stable and securely mounted. Robert Keyse rok210@lehigh.edu Wed Jun 20

EDS:

calibration drift

I calibrated my EDS (Energy Dispersive X-ray Spectroscopy) but, two weeks later, the X-ray spectrum is out of calibration. What causes this problem? ***Zabihi mansoreh152@gmail.com Sat May 12***

The last time this happened to me, it was a fried power converter chip in the power supply—about $250 total to fix. Time before that, it was a DC/DC power supply chip on the acquisition control board in the computer. $50 fix. The other suggestion was a bad chip in the

detector pre-amp (but it wasn't this). Philip Oshel oshel1pe@cmich.edu Mon May 14

FIB:
active voltage contrast

I am looking for a contract services lab that can perform Active Voltage Contrast. We have some scribe-line via chain structures, fabricated in tungsten, with 2 micron by 2 micron terminal pads. The over-all size of the test structure is 40 microns by 80 microns. This far, this structure has resisted our advances using passive voltage contrast. ***Bryan Tracy bryan.tracy@spansion.com Fri Jun 22***

There are two tricks that I have for stubborn passive voltage contrast samples. One is for overall floating structures where whole chain gets dark in a FIB—use black "Micron" 005 pen from Sakura brand to ground one end of the chain—these pens produce fairly conductive traces with a width of about 200 μm and are great for drawing long grounding lines under a microscope—final connection to the chain is by FIB metal. Another trick is for chains with a "soft" disconnect—use "huge" beam currents, up to nA range, to get enough voltage drop to see location of the defect. If you have a FIB with vented chamber (a-la FIB200 and all the later variants) then with a small hardware hack and a bit of sample prep effort you can start doing active voltage contrast in about an hour. Disconnect the stage current cable from the sample holder (it is fairly useless for the originally-intended purpose anyway) and hard-ground the sample holder to the stage. Use the disconnected cable and its feed-through to bring in voltage from regulated DC power supply (±10 V range)—your FIB is ready. For the sample preparation—attach a short piece of a bonding wire to the piece of wire-wrap wire, attach the wire-wrap wire to the sample by a drop of epoxy, bend bonding wire close to your chain in question, trim the end of the bonding wire with an ophthalmic scalpel, and fix it with drop of Ted Pella's colloidal silver—all this takes about 30 min under inspection microscope. Almost done: load the sample into your FIB, attached wire-wrap wire to the and of the freed stage-current cable, pump the chamber, make final connection of the chain to the silver dot at the end of the bonding wire, and apply voltage from the power supply. If for any reason none of the above works—any lab that has an in-situ manipulator on the FIB should be able to do active voltage contrast, for example … EAG in Sunnyvale, or maybe even one of Universities next door? Valery Ray vray@partbeamsystech.com Fri Jun 22

EDS:
device to re-pump an EDS detector

It seems that we'll have to re-pump our EDS detector. I must now ask our workshop to make the T tube with the tool to open the plug on the detector and the pumping output. I can imagine how it might look, but if someone would have a sketch to send me, it would be nice and save me some time, in particular as the critical dimensions are probably in inches and not metric. ***Jacques Faerber jacques.faerber@ipcms.u-strasbg.fr Wed Jun 13***

When we had this issue, we replaced the back panel of the detector with one that had a standard vacuum valve attached, and then we could just attach a line to pump out the detector. I do not know whether the housing on your detector is capable of this modification, but it solved our problem completely. Bill Tivol wtivol@sbcglobal.net Wed Jun 13

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