NetNotes

Edited by Thomas E. Phillips
University of Missouri

phillipst@missouri.edu

Selected postings to the Microscopy Listserver from May 1, 2011 to July 1, 2011. 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:
polyvinylpyrrolidone in fix

In doing a literature search I came across a paper recommending the use of polyvinylpyrrolidone (PVP) in glutaraldehyde fixatives to help minimize changes due to osmotic pressure. In our case we would be using it on mouse intestinal tissue. Bohman and Maunsbach (1970) Effects on Tissue Fine Structure of Variations in Colloid Osmotic Pressure of Glutaraldehyde Fixatives. J. Ultrastructure Research 195–208. We often do add sucrose to our fix for osmotic control but I have never used PVP. I would appreciate comments from those who might have experience using it. ***Debby Sherman dsherman@purdue.edu Wed May 4***

I used PVP many, many years ago when I was doing TEM on the renal medulla, very hypertonic down there. How much the PVP helped versus how much adding lots of NaCl to the fix to make it hypertonic helped is open to question. I got the recipe from Bohman's work on the kidney which had very nice EMs. J. Ultrastruc. Res. 47:329–360, 1974. I actually met him some years later, nice guy and a good scientist. I have also done TEM of mouse duodenum and never had a problem getting good fixation with 2.5% glutaraldehyde in buffer. I think I used 0.08 M cacodylate in those days but probably phosphate as well. I did not perfuse but just flushed the lumen with fix from a small syringe with a 25 g needle. Final answer, it is probably not necessary but there is enough small intestine in a mouse to try adjacent pieces of tissue in fix with and without PVP. Geoff McAuliffe mcauliff@umdnj.edu Thu May 5

Specimen Preparation:
shelf life of resins

What are your ideas about the shelf life of embedding resins? We have a bunch, some very old (a few back to 1983), and I don't know what to do with them. Are they good to use or should they just go? I know I could mix up test sets, but I'm not sure I can spare the time. In addition, some of the things on the shelf I have never used. Some are old & open, some old and never opened. All kinds of things, DMAE, DMP-30, BDMA, Spurr's components, Epon 812 substitutes, some Araldites, DBP, NMA, DDSA, Quetol, Maraglas, and Cardolite, LR White, the list goes on. All have been stored at room temperature in a glass door cabinet in the lab; most are in brown glass bottles. I'm ready to purge the lab of old, unused things, but don't want to discard anything that would be useful for the future. Happy to share a complete list and give away what we don't need. ***Jon Krupp jkrupp@deltacollege.edu Thu May 5***

In a slightly peripheral but related question, I was wondering about the shelf life of Permount. We have a very old bottle in our lab, and it's been opened some time in the past. We rarely do alcohol/xylene series embedding, in fact, never have during the time I've been there. But if we were to, would an old bottle of Permount still be usable, or would the xylene base evaporated off too greatly to be usable? Peter Werner germpore@sonic.net Thu May 5

Having attempted to use an old bottle of Permount, I'd suggest that you dispose of it now and get a new bottle when the time comes to coverslip again. If you can get the cap off you'd be lucky and the fluid is most likely at least partially dehydrated and thick to the point that it will not flow properly under the coverslip. Patricia Stranen Connelly connellyps@nhlbi.nih.gov Thu May 5

The DMP-30 has a very short shelf life and the LR White should have been in the refrigerator. Any old open bottles of NMA and DDSA and anything else that is supposed to be anhydrous need to be discarded. Unopened bottles of Epon 812 substitutes should be fine for years. My best suggestion would be to make a list with the lot numbers and send it to the suppliers and ask their opinions. Saving all the things in the world is not worth a failed embedding of an important experiment. Patricia Stranen Connelly connellyps@nhlbi.nih.gov Thu May 5

Specimen Preparation:
polystyrene beads for TEM

A researcher wants to study the uptake of 200 nm polystyrene (PS) beads using TEM of sectioned materials. I believe the beads will be dissolved in any solvent we use (including ethanol). I suggested other nanoparticles, but the researcher believes PS would be best for the work. I was hoping to use ethanol with the epoxy resin (to avoid more powerful organic solvents like acetone and polypropylene) but the manufacturer of the beads said any organic solvent would dissolve the beads. Anyone have experience using PS beads and embedding? ***John J. Bozzola bozzola@siu.edu Wed Jun 29***

You might want to remember that some of the Epon substitutes will also dissolve PS. I have used LX-112 from Ladd and it works well with PS. I assume that there are others that do also but EMbed-812 will not work with it for it melted my small polystyrene Petri dishes. Patricia Stranen Connelly connellyps@nhlbi.nih.gov Wed Jun 29

You could try Tokuyasu cryosectioning, I guess? Michal Jarnik jarnikm@mail.nih.gov Wed Jun 29

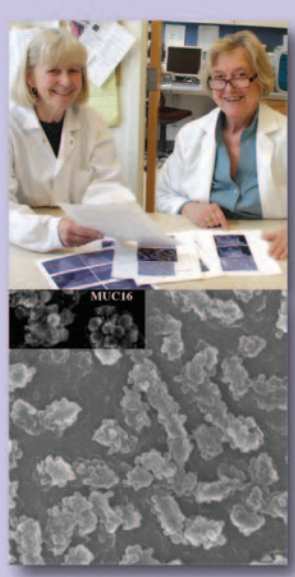
While there is no doubt that acetone dissolves plastics, I would be surprised if ethanol would do any harm to them. I would recommend bypassing the acetone step and directly embedding in ethanol/resin mixture after dehydration. Just be sure to leave no trace of ethanol in the resin. In any case it is quite straightforward to test the resistance of the beads to ethanol or anything else: leave them in ethanol overnight and check by SEM. Stephane Nizets nizets2@yahoo.com Thu Jun 30

If you wish to test what solvents dissolve polystyrene try a low cost styrofoam cup. It dissolves is seconds if you try to fill it with acetone. I tried it in chem lab decades ago, the lesson sticks. Since many alcoholic beverages are served in #6 PS cups, alcohol should be fine. Roseann Csencsits rcsencsits@lbl.gov Thu Jun 30

Specimen Preparation:
HMDS

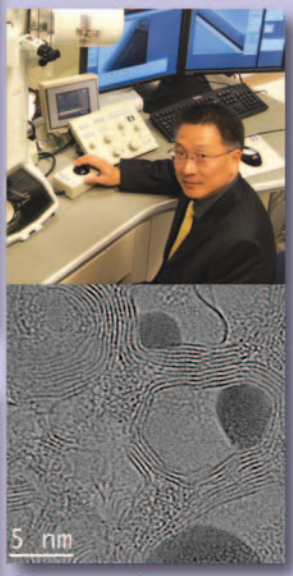
I wanted to test HMDS (hexamethyldisilazane) as an alternative to critical point drying for SEM of plant material and insects. 100%

Microscopy in good company.



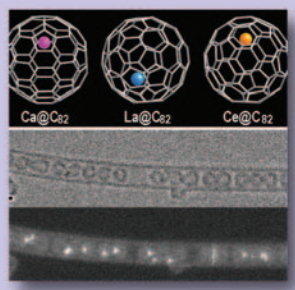
Ann Tisdale, Dr. Ilene Gipson
Schepens Eye Research Institute

Corneal surface



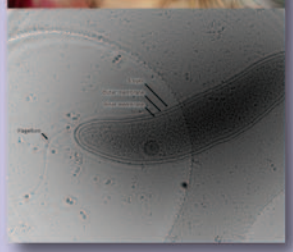
Dr. Moon Kim
University of Texas - Dallas

Pd nanocrystals

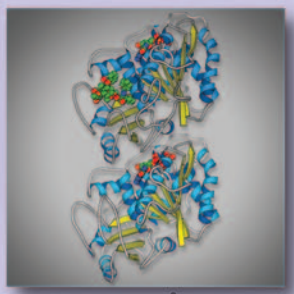


Ca metallofullerene peapod
K. Suenega, AIST

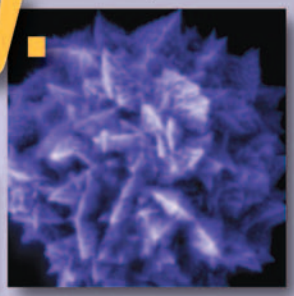
Dr. Elizabeth Wright
Emory University



Caulobacter Cb13b1a cells

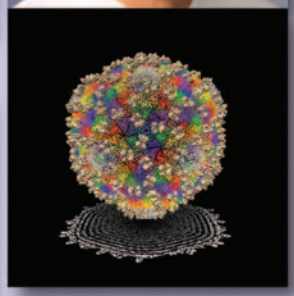


Tubulin structure 3.5Å – Berkeley Lab
Lowe, Li, Downing, and Nogales

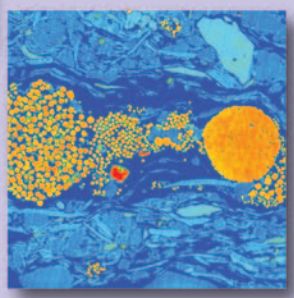


Catalyst - C. Cabrera
University of Puerto Rico

Dr. Wah Chiu
Baylor College of Medicine



Bacteriophage Epsilon 15



Oil shale cross section

The McCrone Group - Tadarida brasiliensis hair

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HMDS was not available and we only had 98% (greater or equal). Nonetheless, I prepared a sample and everything seemed to be fine. Drying was ok and there was no problem with the vacuum. However, I observed a slight discoloration of the gold target during sputter coating—the non-sputtered gold surface turned grayish. Have you experienced a similar phenomenon and can you explain it to me? Is it a consequence of bad drying and/or the wrong quality of HMDS? ***Edith Stabentheiner edith.stabentheiner@uni-graz.at Wed May 11***

We use 98% pure HMDS and have never noticed discoloration of our gold target. I would like to open out the discussion. Should I look for greater than HMDS of greater than 98% purity for dehydration of cultured human cell lines? Dave Patton david.patton@uwe.ac.uk Wed May 11

We use 98–99% HMDS and have not noticed any discoloration of our sputter coater targets. It may be that you did not completely remove the ethanol, or did not completely dry the sample from HMDS. How did the pump-down go in the sputter coater? Did you have trouble getting to vacuum at first? Did you do a few purges with argon before coating in order to completely flush out all the air and any adsorbed water vapor, etc., from the chamber? Phil Oshel oshel1pe@cmich.edu Wed May 11

I believe the discoloration was the result of incomplete drying of your sample, but could also be due to sample outgassing. You may need to extend your dehydration steps. What I recommend, also, is to place samples in a 45°C vacuum oven after HMDS and pull a vacuum on the sample overnight to insure the sample is completely dry if you are working with a thick or difficult sample. A graduate student in my lab is working with shark skin, which contains a lot of connective tissue. She was unable to coat 6 samples at a time in my sputter coater. There was too much sample outgassing with her samples even with the overnight baking in the oven. With the 6 samples in the chamber, our gold-palladium target discolored, also, and her samples never coated properly, although a plasma formed in the chamber. She asked me to try another run with the coater, and I had the same results as she did with another set of samples. I noticed that I needed almost no argon gas to produce a plasma. At first, I thought I was having a problem with my gas feed and disassembled my needle valve and cleaned it, but that was not the case. The same phenomenon occurred with the next set of samples. These were samples that had been processed, oven dried and been in a bell jar over desiccant for weeks. This led me to conclude that the samples were outgassing. Extended baking at 45°C did not cure the problem. When we reduced the sample number to 3 in the chamber, all three samples coat fine. Try placing fewer samples in the chamber with each coating run as well. Ed Haller ehaller@health.usf.edu Wed May 11

Specimen Preparation:
perfect loop

I had the chance to acquire a Perfect Loop recently, thinking that I can increase the quality of the collected sections. Unfortunately, it was not that much helpful (at least in my hands). I lose plenty of good section when picking them up with the "perfect" loop. The grids that I routinely use are slot grids coated with Formvar, and it is very difficult to transfer from the loop to the grid. I bought tabbed grids in order to handle them better, but then I had to cut the tab, so I had to buy cutter tweezers. All in all, a tool that didn't deserve the investment. However, before I leave it in some box and forget it for years, I'd like to hear if there is anybody out there that has used it or still uses it and if there are any special tricks that I can apply. In the meantime, I will still call it the "Imperfect Loop." ***Josif Mircheski jmircheski@us.es Fri Jun 10***

Here is a how I use it: after cutting the ultra sections, I separate them (by ones, twos, threes etc.) and then lower the loop on top of them, making sure that the rim of the loop does not touch the sections. Then, I raise the loop, trying to minimize the water droplet taken by the loop by raising the loop near the edge of the boat. Then, while holding the grid with tweezers (grid is single slot, Formvar coated), I touch the loop on the grid surface, trying to match the orientation of the section with the orientation of the slot so that it sticks to the film only, but not on the metal. The tricky part is there—the presence of the tweezers' tips prevents the loop and the grid from sticking perfectly and easily transfers the section. This is the point where I lose most of the sections. I used the tabbed slot grids in order to remove the tip away from the contact area, but then I still have problems while picking up the sections and transferring them with the loop at the grid. Also, the tabs need to be removed, so I have to put three more steps in the handling of the grid—I lose grids there, too. So, I guess this explanation can help somebody to tell me what I do wrong, or where I can improve. And, I have been trying various modes, for months now. Josif Mircheski jmircheski@us.es Fri Jun 10

I also use the perfect-loop for naked grids and it works perfectly. It is most useful when ribbons are not achieved and sections are all individual. I tried it with coated grids for a short while and had trouble like you described. I did not hold the grid but had it sitting on a piece of filter paper. I got the best results by first filling the open loop with as many sections as would fit. That way, when they moved with the water being wicked out, there were still sections covering the slot of the grid. Getting the final bit of water out between the grid and the loop was where I had the most trouble. I finally gave up as it was easier to take the time to trim better and make ribbons of sections. I pick them up the old fashioned way of dipping the grid into the water of the boat, hooking the top of the ribbon over the edge of the grid and lifting the grid up at a fairly steep angle so that the rest of the ribbon lays nicely across the slot of the grid. Newly coated grids or ones that have been glow discharged are needed to do this for older ones tend to be hydrophobic and the sections will "run away." Patricia Stranen Connelly connellyps@nhlbi.nih.gov Fri Jun 10

The best technique I've tried in getting sections onto a coated slotted grid with consistent results is to use homemade support films (e.g., Formvar or Butvar) and a domino rack (EMS# 70620). The domino rack is a great investment. I use it every time I transfer serial sections on coated slotted grids. After casting the film on the water surface, the domino rack is carefully used to pick up the film. The film on the rack is allowed to dry. Sections are picked up with the slotted grid. (The grid serves now as your loop.) The slotted grid containing the sections suspended in water is carefully placed on the film on the rack and allowed to dry. This technique is carefully described in Biological Specimen Preparation for Correlative Light and Electron Microscopy by David Moran and Carter Rowley. After reading your post, I wanted to start looking for my commercial perfect loop. It's been missing for a while but I don't think I need it for now. Claire Haueter chaueter@bcm.edu Fri Jun 10

Immunocytochemistry:
immunogold for SEM

Does anyone know of literature references or vendor technical notes that mentions labeling of surface antigens on cells in tissue explants? I am looking for large probes (~100 nm) for SEM studies. ***Vickie Kimler vakimler@med.wayne.edu Wed May 25***

I would recommend using nanogold labeled probes followed by gold enhancement. The Nanoprobes kit worked great for us—see: Meagher, C.K., H. Liu, C.P. Moore, and T.E. Phillips. 2005. Conjunctival M cells selectively bind and translocate Maackia amurensis leukoagglutinin. Experimental Eye Research 80(4):545–553. Thomas E. Phillips phillipst@missouri.edu Wed May 25

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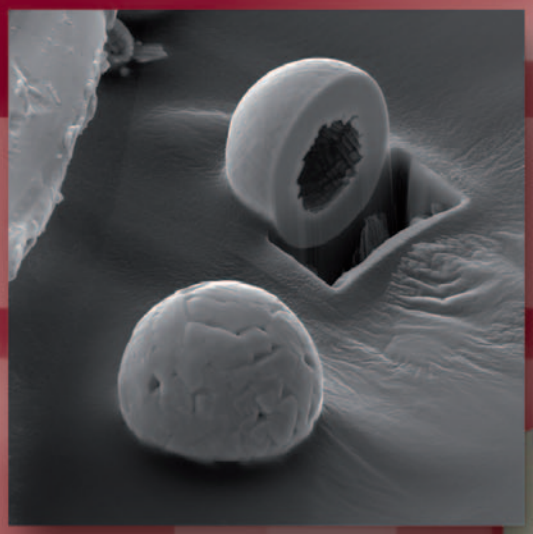

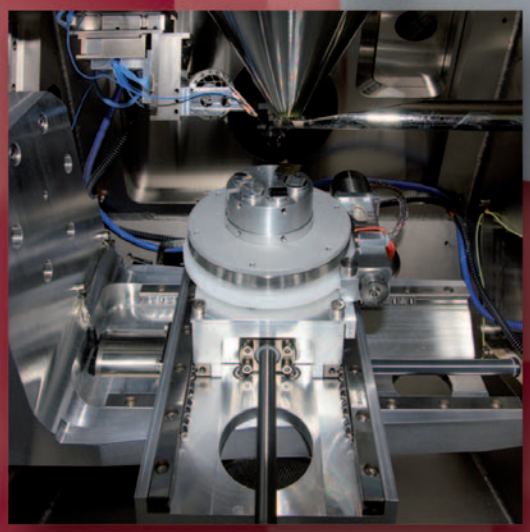

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One reference paper on this topic is here: Schroeder-Reiter E, Houben A, Wanner G (2003) Chromosome Research 11: 585-596. FE-SEM of chromosomes with nanogold . . . have a close look. Reinhard Rachel reinhard.rachel@biologie.uni-regensburg.de Thu May 26

EM:
service contracts

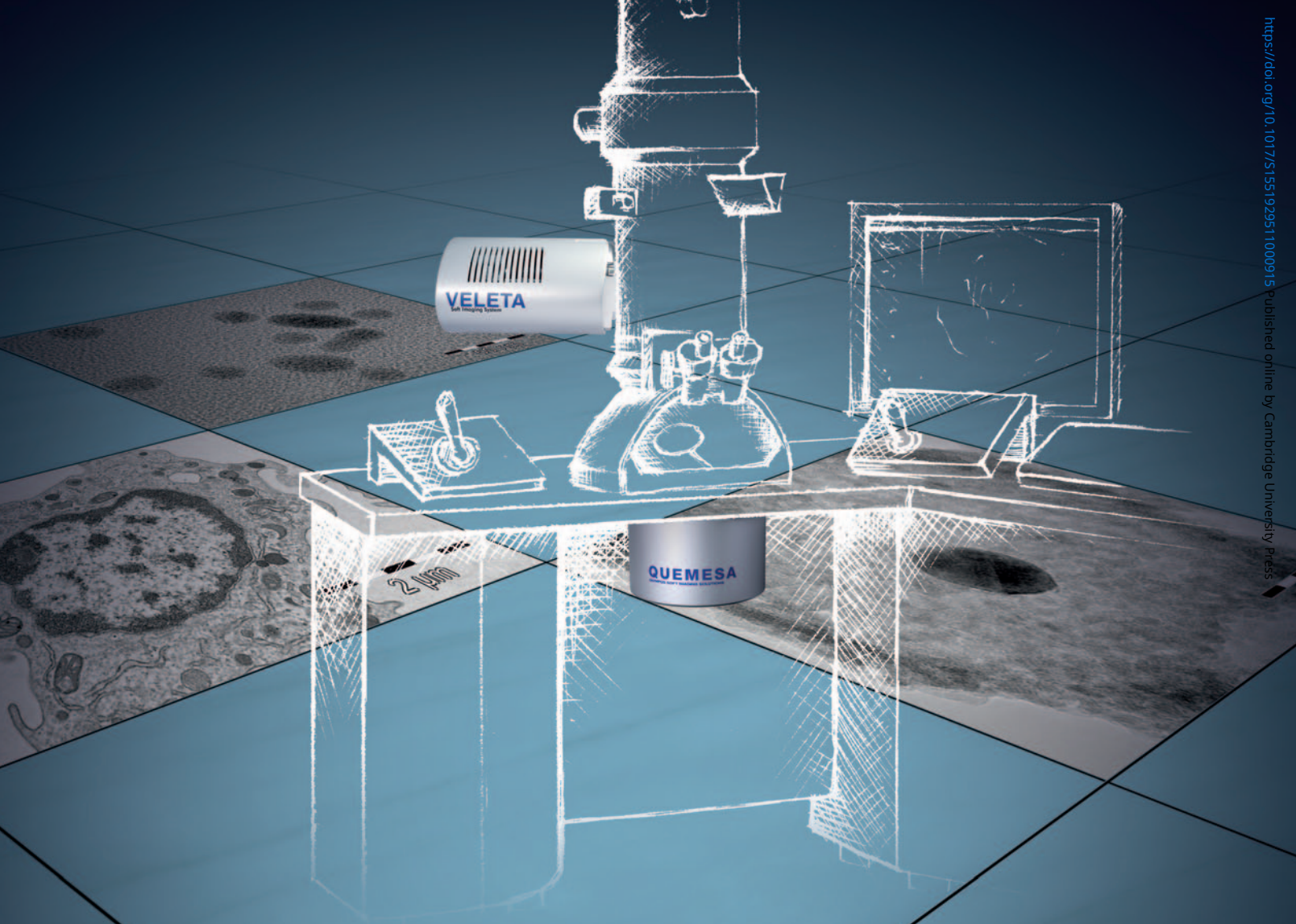
We are caught on the horns of a dilemma. For nearly forty years, Delta College has trained EM technicians for positions in academia and industry. For most of those forty years our instruments, now numbering 3 TEMs, 4, SEMs, an AFM and an FIB, have been covered by service contracts. Our instruments are used all day, every day for student training. As you may have heard, funding for California schools is getting so tight that the bean counters are turning to drastic measures to make ends meet. Among pressures put on us is a requirement that we enroll at least 20 students/class, nearly impossible in our advanced, hand-on classes. In addition, the administration is now reluctant to authorize service contracts for our instruments. Many or our microscopes are older and require more repairs due to their constant use as training instruments. We have received good service from our scope vendor, but it is expensive, mostly because we have so many instruments. The administrative people only look at the bottom line and when they see the big number, they go ballistic. We are afraid that if we drop our current service contracts that the manufacturer will not pick them up if we want to come back after a year off their service. We are afraid that if we go to pay as you go with the manufacturer, we will be low in their priority list, something not good with so many students depending on microscope time to finish projects. We are afraid that if we can find a cheaper, independent service provider, parts will be hard to get and the price of the parts would be in addition to the cost of the service agreement, whereas parts are included in our current contracts. This is getting long. Bottom line is we are asking for any advice or suggestions about how to approach this. I have heard all the horror stories about 3rd party insurance plans, etc. Are those stories true? Is it true that vendors move you to the bottom of the list without a contract? What is your experience if ever faced by this kind of situation? What kind of strategy has worked, or not? ***Jon Krupp jkrupp@deltacollege.edu Thu May 12***

The service contract for a new microscope is worth trying to include in the price paid for at least about 3 years. During this time keeping a record of each and every little thing that goes wrong will be useful to whoever eventually takes over the maintenance and repair duties. A service contract costs on average about 10% of the purchase price, so a three year contract is a substantial saving that will please the finance people. The service you get is very dependent on the people who work for the service departments of the individual companies; often cutting edge instruments are unknown quantities to the field service engineers. You don't want to waste money while the instrument is down for the engineer to be educated on-site; so try to get the first few years with a service contract (beyond the warranty period). Don't sign-off the instrument until you are happy that everything is working, the installation needs to settle the instrument, so try everything yourself before being satisfied. Eventually though there is a time when you are going to be better off without paying the huge bills to the service manager. It has to happen and will depend on you and your staff, to take the brunt of the work, using instructions of friendly service engineers. I don't believe the stories about low priority, in fact I'd argue they want your money; and so long as you have the quotation matched to the purchase order, you are on the schedule, same as everyone else. If you have many instruments from one company and it makes financial sense to hire an ex-service engineer (it is down to respect they feel from you) it's a good route, if you get the right one. Parts are often sold from a separate office to the service manager, so again if you have the numbers and they have the stock you are going to be fine. For instance we wanted some resistors and didn't want to wait three months for delivery, so alternatives were found that we believe are as good. Rob Keyse rok210@lehigh.edu Thu May 12

I always advise institutional users to stay with OEM service contract if they can, or to purchase a service contract from the reputable third-party service provider as a second choice. In the beginning of this year I've heard from two industrial users of FIB equipment that they are not allowed to have a service contract with anybody and forced into "pay as you go" mode. Well, guess what—one of them already has her FIB "down" for over a month, and there is nothing I or anyone else can do about it until the institution shells over US $20K in parts and commits to pay for labor of changing them. Think twice. Valery Ray vray@partbeamsystech.com Thu May 12

We went off contract last year and had to wait over 4 months for a service. The engineer said that if it had been a breakdown the delay would have been the same. Dave Patton david.patton@uwe.ac.uk Fri May 13

Dave's message compelled me to explain a difference between service contract and one-off service call from the vendor's perspective. For most of FIB/SEM equipment service providers, both small and large, equipment service is a business activity; it must be profitable or could not continue. Delivering service and making it possible also costs money: most of users do not realize how enormous are the aggregated expenses of labor, travel, sourcing parts, re-designing or substituting obsolete components, warehousing, bean counting, etc. When you buy a service contract from the certain provider, it not just gives you reliable support with predictable response time, but also gives that service organization reliable stream of revenue and predictable work load. Stability allows to plan and structure business, retain and maintain necessary resources, source and stock parts, etc. If customer goes off service contract then situation becomes completely unpredictable: he/she may call in for service, but also may do self-service, hire internal service person, or do whatever else. In such situation managers of the service organization have no other choice but to prepare for the worse and assume that there will be no service calls from this customer. To insure survival of the organization, cost-saving measures follow immediately: engineers are laid off, facilities are closed or downsized, parts are not procured and not stored, and so forth. These measures are inevitable—otherwise service organization simply could not continue to exist. Reduced resources will usually be matched to the needs of



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contract-paying customers, with little to no spare capacity. When odd service call comes from the customer without the service contract, it is treated as such—one-off, single-case business, which may never return. Yes, it is nice to get some extra revenue, but (already reduced) resources of engineering time and parts will be given to this call only if and when all the needs of service-contract customers are taken care of, and not a split second earlier. The bottom line is that service organization simply cannot provide more service then what it paid for, and overall level of service users receive will always degrade in one way or another, as the service revenue decreases. Valery Ray vray@partbeamsystech.com Fri May 13

Very well stated. The only thing I might add is that the customer with a service contract is guaranteed certain things, such as preventive maintenance, operation to specification, perhaps even guaranteed maximum down-time. The one-off customer has none of this. The service organization is contractually bound to the contract customer. Period. Yes, the one off cash is nice, but the regular customers are far more valuable in the long run. They often are happier customer, also. To start with some of the more obvious places: If the system is down because service is not readily available then A) your students and researchers are not able to do what is required/needed in a timely manner, B) you need to send your work out to another lab and pay by the hour to use their systems or, C) your customers take their work elsewhere (to a competitor) to be done on a timely basis. Even the bean counters can see some of the costs involved here, although they are likely to "overlook" at least some of them. There are some less obvious costs, also: A) You bought a system with 3.5 nm resolution but through many little problems (death through a thousand tiny cuts) that have accumulated over time, it will only produce 20 nm resolution. First, you may not be able to do the work that needs to be done, resulting in similar expenses to above. Second, if your response is, "I don't need better than 20 nm resolution for my work" then a considerable amount of money was wasted in the original purchase. B) Productivity goes down because the people using the system (whether "tool operator" or "microscopist") know it doesn't operate properly and become discouraged. The people with the service contract are happier because they don't have to constantly worry about their system. They are confident that it will remain operating up to spec. The people without the service contract may actually be spending as much money, if not more, but the amounts aren't always obvious. In addition, they have to constantly worry whether or not their system is going to be able to do the work that needs to be done. That's why they are less likely to be happy. The benefits of service contracts are not entirely obvious and this shows very clearly in scientific grants. It's not all that difficult to get someone to give a lot of money for a building that will have their name on it. It may be even easier to get someone to give money for a fancy state of the art piece of equipment that will have a bronze plaque on it. There are no bronze plaques adorning service contracts, hence grant money for such unseen things is more difficult to come by. Ken Converse kenconverse@qualityimages.biz Mon May 16

There is a new option that is floating around the University of Texas system. An independent company has signed a deal with UT system to save everyone 25% on their service contracts (not just EMs—a whole range of equipment, as long as it is a parts and labor contract). They get you to move to an "on demand" arrangement with your existing service provider. They pay all the bills and take the chance that you will spend less than a full price contract. Of course there is the issue with how service providers prioritize contract versus on-demand customers. This company will freeze the cost for 4 years and as part of the arrangement with UT system (with qualifying contracts and equipment) will not decline coverage. I am tempted but don't like the idea of having less priority when I make a service call. Christopher J Gilpin christopher.gilpin@utsouthwestern.edu Mon May 16

Don't do it. I dealt with a system like this back in my previous position. Worked fine as long as we only did routine maintenance and a maybe a small changing-out-a-part repair. But the first time we did something unusual to them but perfectly normal for EM—tracing a stray EM field—the company demanded we pay the full bill. Because they don't pay for room tests. Didn't matter that the EMF test is routine for diagnosing imaging problems, didn't matter that the test was a minor part of the bill, didn't matter that . . . etc. etc. Also didn't matter that I specifically asked them a series of "what if" questions about what they covered—like the above—and their answer was always "you're covered." We got back on an instrument vendor service contract and were much happier. Now what I do is keep track of time-and-materials charges for any repairs or maintenance and anytime some admin type thinks of dropping the service contract, I show them the comparison: service contract vs. time-and-materials (T&M). The contract has always been cheaper for any year other than ones in which the instruments never needed more than routine PMs. In more than one case, as someone mentioned earlier, a single repair visit if charged as T&M would have cost >2× the annual price of a full-option service contract. Mind, CMU generally is a believer in service contracts, unlike some places I've been, so that helps, but it still is very useful to have the contract cost vs. T&M cost comparison handy. Philip Oshel oshel1pe@cmich.edu Mon May 16

TEM:
large UPS systems—stray fields?

I am considering installing an inverter type UPS to keep my FEG TEM running during power outages/blips and to provide it with clean power. My options for locating the UPS and battery stack are limited. I obviously want it located as far from the microscope as possible and plan on putting it in the plant room for the microscope, adjacent to the lens/ion pump power supplies. Does anyone have any experience with, or advice on, potential interference issues between UPSs and TEM systems? ***Dave Mitchell drg.mitchell@sydney.edu.au Fri May 13***

The Phase One UPS I had on my 2010F at Intel had a requirement from JEOL that it be at least 20 feet away from the microscope. I measured 35 milligauss coming out of that thing. We kept it 50 feet from the microscope. The new lab at ASU has the UPS located in the attic at the far end of the building, and there is extensive shielding between the UPS and the microscopes. You definitely want the UPS as far from your column as possible. John Mardinly john.mardinly@asu.edu Fri May 13

The UPS attached to the FEI Polara FEG TEM at Caltech was located in an adjacent room, ~10 m from the scope. The Haskris coolers, air compressors, and some of the electronics were also in this room. We saw no interference from the UPS. Bill Tivol william.f.tivol@aero.org Fri May 13

TEM:
dislocation density measurement

A student studying Ni-based super-alloys asked how to measure dislocation densities using a 2000FX TEM. I understand that grains can be tilted to certain orientations to make dislocations visible/invisible, eventually the burgers vector of the dislocations can be worked out. Does that mean at no orientation all the dislocations are visible? How do we then quantify the density of dislocations? Any advice, references,

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books, manuals are highly appreciated. ***Zhaoxia Zhou z.zhou@lboro.ac.uk Fri May 20***

Contrast from dislocations can only arise if the crystal is oriented in such a way that the strain field scatters the Bloch waves set up by diffraction. Although dislocation contrast in aluminum was discovered by Peter Hirsch and co-workers back in 1956, Archie Howie and Michael Whelan explained the dynamical diffraction contrast of imperfect crystals with phenomenal results. Much of this is contained within what is considered to the 'The Bible' of electron microscopy: *Electron Microscopy of Thin Crystals* by Hirsch, Howie, Whelan, Pashley & Nicholson. Several different editions of the book exist and it is still highly recommended as a text book. Mike Loretto's book *Defect Analysis in Electron Microscopy* is a shorter, and in some ways more accessible book to consult, with helpful diffraction patterns and Kikuchi line maps in the Appendices that assist navigation to the required diffraction conditions. More recent books include Barry Carter & David Williams, *Transmission Electron Microscopy,* which is aided by a selection of some the most beautiful pictures of defects to have appeared in the past 55 years. Some of the best images seared into my mind date back from the 1970s! All of these books (and many more) will tell you that successful dislocation analysis requires setting up well known diffraction conditions for each crystal grain. Therefore, no single orientation can give you all the information you want. Identifying dislocation Burgers vectors requires multiple images of the same set of dislocations under diffraction conditions to see which ones disappear, i.e., dislocations are identified by a process of elimination. However, it is reasonably straightforward to find a set of reflections in which all dislocations will eventually appear. Therefore, the dislocation density can established only if all crystal grains have each diffracted strongly with several different reflections. Jon Barnard jsb43@hermes.cam.ac.uk Mon May 23

TEM:

LaB_6 upgrade

We have a JEOL 1200 EXII TEM and we were wondering if it is good enough in which to use a LaB_6. The engineers seem to have mix opinions, as does the great Google. I would like comments from anyone who has or has had a 1200 fitted with a LaB_6. If you have one, which one? The microscope is behaving itself very well, no leaks everything is as good as it gets given its age. With one fitted do you notice any significant difference when working at the top end? ***John Mitchels john.mitchels@gmail.com Thu Jun 16***

LaB_6 filaments have to work in a good vacuum. There are some instruments like your TEM working with LaB_6 in a gun pumped by DP or turbo, but most of the LaB_6 TEM work with high vacuum in the gun provided by SIP. If your vacuum system is very good, you may use LaB_6 but you must understand that you will work on an edge. Anyway, you may try without danger for your instrument and you will see if LaB_6 stay safe during one year (good) or one month. Please note that you may have to use another Wehnelt cap for this kind of filament and you have to change distance between filament tip and Wehnelt cap. First heating of this filament must be done very slowly (about one hour) after almost one night of pumping. Working with LaB_6 is very comfortable because the brightness is very high and it may increase significantly top end results. Nicolas Stephant nicolas.stephant@univ-nantes.fr Thu Jun 16

I would not recommend to work with a LaB_6 in a (T)EM pumped by a DP or turbo only. For me, and from our 20 year experience, an IGP or ion getter pump (is SIP the same?) plus a nitrogen-trap is a must. Other people may comment on this or have other experience, though. The lifetime of a LaB_6 in our TEM (heavily used by bio-people; though, no cryo) , pumped by ODP, plus IGP plus nitrogen trap (all are always on, during day time) is 3 years, up to 3.5 years, in the last 20 years of use, now. I was told that the lifetime in a turbo-pumped TEM (bio, too) is about 0.5 to 1 year only, at best. It is not my own experience. Everybody can decide what this means, and what to do. Reinhard Rachel reinhard.rachel@biologie.uni-regensburg.de Thu Jun 16

IGP (ion getter pump) and SIP (sputter ion pump) are different names for same device. Roger Ristau raristau@ims.uconn.edu Thu Jun 16

We have the same model JEOL 1200 EXII as you do and it has worked flawlessly for 25 years with LaB_6 filaments. It has diffusion pumps not turbo. We get anywhere from 900 to 1500 hrs of filament life—and that is running at fairly high emission currents of ~20 μA. One caveat: it helps to keep a clean column (vacuum). We have a custom-built LN_2 cold trap that lasts for up to 48 hrs. Peter Ingram p.ingram@cellbio.duke.edu Thu Jun 16

Use of LaB_6 cathode in a vacuum system without an IGP and differential pumping is possible but impractical. Even if all following rules will be enforced: have LN trap cold before heating filament; turn off filament heating before specimen exchange; never heat up filament before gun/column vacuum fully recovers after specimen (or film) exchange, LaB_6 will not likely last for even 1000 hours. Much less at higher emission setting. Vacuum for LaB_6 must be better then 10^{-6} Torr. Very difficult to maintain without IGP and differential pumping system. I consider LaB_6 use justified when it lasts at least 2000 hours at low/medium emission. My average service time experience for LaB_6 is above 3500 hours in a UHV system, with the record approx. 7000 hours and still going. This particular Denka cathode was installed on Philips CM-12 in 1997 and still works 14 years later. Vitaly Feingold vitalylazar@att.net Fri Jun 17

Like Nicolas said here below, it's all a matter of vacuum. Because of the well pumped gun (usually turbo or ion pumped), LaB_6 SEMs usually have a filament life of way above 1000 hours. If you take the same SEM and exchange the LaB_6 filament for a Tungsten one, you'll be able to get way above 1000 hours with the tungsten filament. So, in theory if your SEM gun has a very good vacuum, you should be getting a very high lifetime from each tungsten filament. If not, you could expect to get good result with the LaB_6 filament, but keep the lifetime of your current tungsten one. Keep in mind that the LaB_6 filament is exponentially more expensive than tungsten. It'd be best to consult with JEOL in any case before you decide to do this. Roy Golombick roy@picotech.co.il Wed Jun 22

I beg to differ. I have not seen any significant increase in tungsten filament life in an ion-pumped gun versus a normal gun in good condition in an SEM. Poor vacuum will definitely shorten a W filament's life, but a better vacuum makes very little difference due to the fact that W filaments run hotter than LaB_6 and evaporate faster. The reason poor vacuum accelerates their demise is due to oxidation on top of evaporation. No W filament I've ever seen has lasted more than about 300 hours at operating temperature in an SEM, 10^{-7} T vacuum notwithstanding. My observations may not hold for a TEM because TEMs run at lower temperatures/emission currents and therefore evaporation rates should be significantly lower. Ken Converse kenconverse@qualityimages.biz Wed Jun 22

I usually agree with your comments but this time I have to throw in a curved ball. I often work with automated analysis SEMs which have a tungsten filament life of upwards from 2500 hours; admittedly we run at 11 micro amps emission with the filament well

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back. The instrument are not used by electron microscopists so the idea behind this long life being that the service technician is able to fit a new filament on their six monthly service visits. We worked hard to obtain this life and one of the features that we found that was very important in our development was the vacuum level. Thus we run with an ion pump on the gun and the difference in filament life is considerable. This is no criticism of the basic instrument, which exhibits a gun vacuum level in its standard form the same if not better than its competitors. Another area that the gun vacuum level influenced was the high voltage stability. The instruments need to run for up to 24 hours unattended and it is most important for the high voltage to remain absolutely stable over that entire period of time. Double plots of high voltage level and gun vacuum show that once the gun vacuum settles so does the high voltage. Thus the operators wait for this stability period prior to starting a sample run; proven to be the best practice. As a second point I know LaB_6 users who have been forced to use tungsten whilst waiting for a new source and they have also commented on the increased filament life. Steve Chapman protrain@emcourses.com Wed Jun 22

The 11 μA emission current leads me to think that the lower temperature enhances the effects of good vacuum. As for a more normal system, I've found Amrays follow the manual, 40 hrs, ion pump or not, unless the vacuum is especially poor. JEOLs seem about the same except their typical life is 100–200 hours. I did have one dry pumped ETEC that would run for weeks and months 24-7 in the 10^{-8} T range, but it was run slightly undersaturated because life was more important than stability for the particular experiment. Maybe you can shed some light on this thought. I've always wondered how much effect the physical gun design has on the temperature required for a particular emission current. Your example seems to indicate the same thing that TEMs seem to indicate and that is that the lower emission currents, and consequent lower temperatures result in oxidation being a larger factor than evaporation on filament life, so better vacuum has a greater effect on lower temperature filaments. Any thoughts? Ken Converse kenconverse@qualityimages.biz Wed Jun 22

I seem to have spent most of my life playing with electron guns. How often I have moved filaments forward or backwards, sharpened tungsten filaments to obtain LaB_6 type performance, lifted up anodes and checked out different cathode shapes! To most people in laboratories a gun is what the manufacturer gives you, but with the good old tungsten hairpin that may be considered to be just a starting point. Total cathode geometry and the changes that you are able to make regarding filament life in one direction and performance in the other, are outside of the norm. Add to this raising the anode to improve performance and few appreciate how you may transform an instrument. On tungsten filament temperature I have a great deal to say but as usual from me it is probably controversial? I rarely fully saturate the gun! I am a great believer in under saturating. I see no point in throwing away filament life and with modern stabilities there is usually no point in doing so. An amazing amount of work may be accomplished and for most SEM analysis there is no point, it hardly can be said to improve X-ray resolution! I only saturate when working at the absolute limit at a particular kV and I challenge anyone to see a visual degradation in any images I record through using this technique; but only up to 100,000×. The big bonus for most labs is this technique really does improve tungsten or even LaB_6 filament life. Thus I agree with you with low filament currents it is oxidation that is the final tungsten filament killer not evaporation! Too hot a filament and it just evaporates but be more thoughtful and life is extended. We improve the vacuum and the life is extended even further; but probably at the cost ($) of an ion pump! Steve Chapman protrain@emcourses.com Wed Jun 22

If you are working above 10 kV and at a maximum of 5,000×, I see no point of moving to LaB_6. LaB_6 is brighter than tungsten and running at a lower temperature has less chromatic aberration. The problem with LaB_6 is that you must have a very good vacuum or the possibility of a discharge could be catastrophic! A discharge may seed a crystal in what is meant to be a single crystal tip. Unfortunately over time this seed crystal may grow and eventually you have a double emitting source. Nice for fancy images but not for science! Thus great caution is required when running up the LaB_6 filament and changing kV in order to prevent discharge occurring. If you were always working below 5 kV and looking at images I would suggest LaB_6 as due to the higher brightness and lower aberrations this source will always better tungsten at these levels. Steve Chapman protrain@emcourses.com Fri Jun 24

TEM:
acquiring Holz lines

I am trying to acquire HOLZ lines in [000] transmitted spot of CBED patterns for point group determination purpose, but have been experiencing difficulties in acquiring/recording images of HOLZ lines. The TEM I am using is Philips EM-20 with Gatan camera. On the phosphor screen, I was able to see HOLZ lines for higher index zone axis patterns and they seem to be clearer under 80 kV than 200 kV. However, when I inserted the Gatan camera to take pictures, I noticed that the quality of the diffraction images was too bad—the HOLZ lines were blurry, and some areas were too bright, making the nearby HOLZ lines invisible. Does anyone on this list have similar problem before? Any suggestions on how to record sharp/clear HOLZ images would be much appreciated! ***Chuan Zhang zhangchuan827@gmail.com Thu Jun 16***

There are four points to consider when taking convergent beam electron diffraction patterns (CBED) on a digital camera: (1) Correct focus (diffraction lens). If your camera is far from the viewing screen, then the CBED pattern can appear to be out of focus and the HOLZ deficiency lines are quickly lost. I have used a Gatan Imaging Filter to acquire (energy filtered) CBED patterns and always have to fine-tune the diffraction focus to get a nice sharp (000) CBED disc—the condenser aperture should appear sharp when the diffraction focus is correct. (2) Sampling rate. If your binning is too high or the camera length is too small the modulation transfer function (MTF) of the camera may be blurring the HOLZ deficiency lines too much. Try using single binning and/or a higher camera length. You may have to try several different areas because HOLZ deficiency lines tend to narrow with thicker areas. (3) Dynamic range. Because of the highly inhomogeneous distribution of intensity in a diffraction pattern, it is easy to saturate the camera. I have found using multiple exposures with a small acquisition time to help tremendously. For example, I have taken diffraction patterns with >100,000 counts per pixel on a 16-bit camera (max. 16396) by using the multiple frames option (DM > Camera > Acquisition Parameters > Acquire > Processing > Frame Sum (No. of Frames). Ten or twenty frames can make a significant difference to the visibility of weak features. (4) Spot size (used in conjunction with point (3). I've found that using a smaller probe with less current leads to better CBED patterns (and using a larger exposure time). Most samples have a thickness gradient so the CBED pattern you see are an incoherent mixture of CBED patterns from each point on the sample that is irradiated, i.e., smaller probes "average out" the CBED pattern over a smaller area. However, if your

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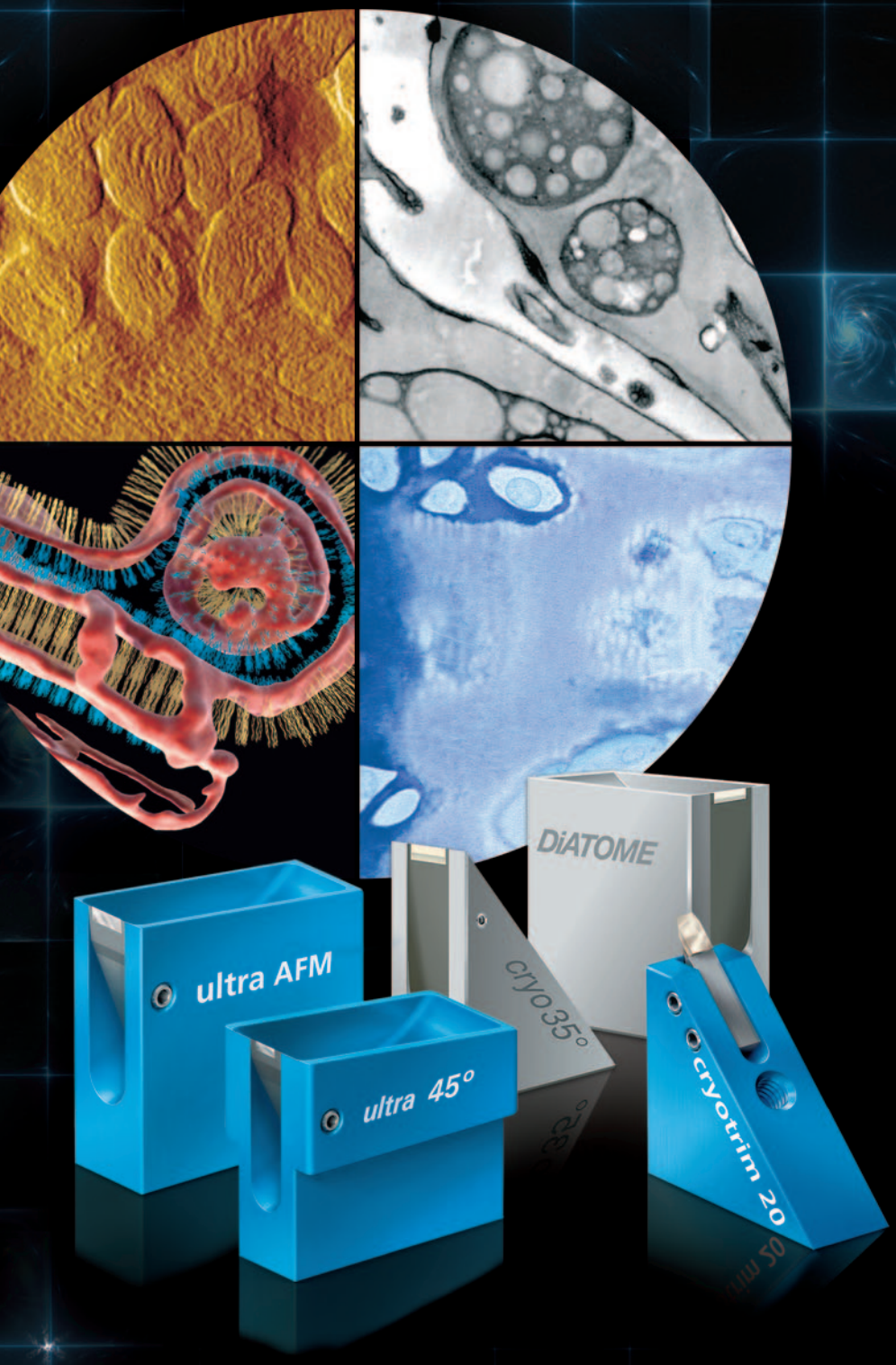

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samples are flat and clean, this shouldn't make too much difference. Jon Barnard jsb43@hermes.cam.ac.uk Fri Jun 17

TEM:

EDX and magnetic parts

We have an SDD EDX system on our TEM and routinely get Co, Cu, and Fe in the spectra. I understood that these elements are from the polepiece and other components in the sample area. When a student submitted a manuscript with these elements in the spectrum, along with the other elements of interest (In, Sb), a reviewer said that the TEM instruments are strictly restricted from using the magnetic impurities/parts, so there must be other sources for iron. Comments? How would you respond to this reviewer? ***Debby Sherman dsherman@purdue.edu Wed Jun 22***

While the Fe, Co, etc. are undoubtedly from the pole-piece, I wonder why you see such a significant contribution to the spectrum. As long as the objective aperture is out, even the scattered electrons should go down the bore of the pole-piece. Bremsstrahlung from the condenser apertures may have some effect, but it should be small. Analytical scopes have improved dramatically over the years and I thought stray signal was pretty much a thing of the past. Pretty much every microscope I've used in the past 25 years has almost no X-rays from the p-p. Do you have an unusual scope? You can reply off-line if you don't wish to identify the scope publicly. In my experience, the EDX collimators on TEM detectors don't shield very much, so the improvements in stray signal have been due to changes in the column itself. Henk Colijn colijn.1@osu.edu Thu Jun 23

I agree with Henk that you should not see the elements from the p-p if the detector geometry is optimized. You need to request for service to figure out where is the problem. Normally the possible spurious source is the grids if you use, as well as the specimen holder when it is translated far way from its zero position. Zhiping Luo zhiping_luo@hotmail.com Sat Jun 25

SEM:

stigmation

We have a Hitachi S4700. Last year it started to have stigmation issues. The left stigmation would peg out and we were not able to focus past 5000×. This was on every sample, in fact magnetic samples tend to do a bit better with stigmation. The problem gets worse the longer we use the scope. To date the service persons have rebuilt the column, installed a new source, replace the objective lens (or the bottom unit if you don't want to consider it an objective lens). We are waiting on the bottom cover of the polepiece, this might take some time considering it will be coming from Japan. I was wondering if anybody else has had this issue and if so was it every corrected. ***Clarissa Wisner cvierret@mst.edu Thu May 5***

You didn't mention whether or not the engineers have looked at the signal going to the stigmator coils. If they haven't, have them do that. In my experience on other brands if there is noise on the signal at under 500 kHz with an amplitude over about 10 mV, you can have uncorrectable astigmatism. Ken Converse kenconverse@qualityimages.biz Thu May 5

I've never experienced this problem on my SEM and don't recall ever seeing any posts associating it with Hitachi SEM's. I've used an S-4700 for the past 9 years so I pay pretty close attention to posts associated with Hitachi. I recently had a stigmation issue with mine but it was related to sample material migrating to the BSE detector. A quick cleaning and we were back on line. John A. Robson john.robson@boehringer-ingelheim.com Fri May 6

MT

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