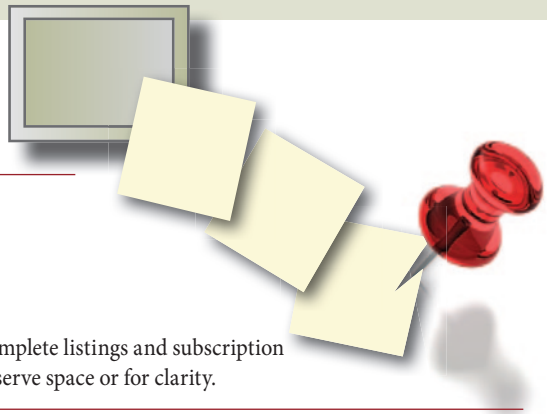
NetNotes



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Selected postings from the Microscopy Listserver from October 11, 2009 to November 30, 2009. 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:
fluorescent dye for lignin

A student here wants to find a fluorescent dye for lignin so that she can see it on her (mostly red) autofluorescing grass. The grass will be subjected to various harsh treatments to rid it of the lignin, but so far it seems that all the treatments result in lignin being redeposited on the material in droplets. We can see the droplets with SEM, but would like to find a good way to quantify the amount of remaining cells walls (cellulose) that is covered by lignin droplets. I think the ultimate goal is to use image analysis to get some numbers. Any ideas? ***Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Wed Nov 25***

Calcofluor White 2MR stains cell walls & fluoresces white, but I'm not sure if it is specific to lignin. Acidic phloroglucinol is a standard lignin stain, and it looks like it should be fluorescent, but I'm not sure (3 hydroxy groups bound to alternate carbons of a benzene ring). http://www.botany.hawaii.edu/faculty/Webb/BOT410/410Labs/LabsHTML-99/Xylem/Labxyphlo99.html Should be easy enough to get some and find out. But, have you checked for autofluorescence? That's a common problem with plant cell walls, and if I remember right, lignin is a contributor to this. Philip Oshel oshel1pe@cmich.edu Wed Nov 25

I agree with Phil. Lignin autofluoresces. Calcofluor is typically used for cellulose identification (under UV light) and Phloroglucinol-HCL for lignin (at the light level, no fluorescence needed). Here is Zheng-Hua Ye's protocol for lignin staining: Add a drop of 1% phloroglucinol in 100% ethanol onto a section and then add a drop of conc. HCl. Lignin will be stained red in a few minutes. And, check out Ruiqin Zhong's paper on Ectopic Deposition of Lignin in the Pith of Stems of Two Arabidopsis Mutants. http://www.plantphysiol.org/cgi/content/full/123/1/59 or you email me for the pdf. Beth Richardson beth@plantbio.uga.edu Mon Nov 3

Specimen Preparation:
coating for polymers

I have a question regarding sample preparation for TEM and SEM. For SEM of polymers, since they are often non-conductive, we are depositing a metallic coating to take care of the accumulated charges during analysis. However, for TEM, we are microtoming thin polymer sections (70–100 nm thickness) and mounting them on a copper grid, and directly observing without using any coating. The accelerating voltage for SEM is of the order of 10–30 kV whereas that for TEM is of the order of 200–300 kV. Why is a coating generally not required for TEM of polymer samples even if we are using a higher accelerating voltage? Is it due to the thinness of the specimen so that electrons can pass through it? Or is there any other reason? ***Rajeev Rajvihar rajeevrajvihar@gmail.com Tue Nov 3***

Excellent question. Ultrathin (TEM) specimens are less prone to charging than bulk samples for several reasons: 1. Less mass (as you already observed) to accommodate the static charge, 2. Proximity to a metal (usually copper) grid that dissipates charges to ground. Nonetheless, TEM specimens do build up static charges, resulting in specimen drift or even ejection of smaller specimen parts, usually onto your objective aperture. If the specimen is in close proximity to a grid bar (or lying over a bar), usually there is little problem with static charging. However, with non-conducting, particulate specimens that are far away from a grid bar (and isolated), you will see major drifting, jumping and disappearance of the specimen due to charging. In that case, a light coating of carbon (by thermal evaporation) will usually solve the problem. Carbon substrates are less likely to show charging but they are fragile. Most people use Formvar/carbon substrates, primarily for stability. Just make sure the specimen is on the carbonized side and that the carbon overlays the grid. I got a charge out of your question. John Bozzola bozzola@siu.edu Wed Nov 4

There is also, in my opinion, a charge balancing effect. My understanding is that the upper surface of the sample is charged by incident electrons. With an objective aperture inserted just below the sample, backscattered electrons from the aperture charge the lower surface. The charge on the two surfaces tends to roughly balance, stabilizing the sample. The demonstration that this is happening, it to load a polymer or similar sample with no objective aperture inserted, or remove the aperture after loading the sample. The sample tends to explode ... especially with fresh samples. After having been exposed to the electron beam for some time, samples seem to "harden" and become resistant to exploding. Larry Stoter larry@cymru666.plus.com Fri Nov 6

Fluorescence Imaging:
live cells with viruses

I am getting a room ready for BSL-2 level virus imaging. I can't get any information regarding how safe the cells are once the virus has been transfected into them. I have a BSL-2 level bio-hood, I have a room with a door and I have a brand, spanking new deconvolution with FRET and an incubator for live cell imaging. I need to know if the people in the room are safe from the virus once it's inside the cells. I can't get the air pressure changed in the room so I need to know what other precautions we might need to take. Thanks to all who can give me any pertinent information. ***Paula Sicurello vapatpxs@yahoo.com Tue Nov 3***

Unfortunately, you do not say what the virus is. However, the information you give suggests you need to work at level 2. You also talk about the virus being transfected, as opposed to infection. As a result, it is not really clear what you are working with. The rules are different if you are talking about plasmids, viral vectors which are not replication competent, viral vectors which will replicate, virus, etc. However, as a general rule of thumb, it is best to presume that anything infectious remains so until it is inactivated. Being inside the cell is no guarantee of protection. Once the cells are infected, and replication in the cell has proceeded you may have production of infectious progeny, even if you didn't start with infectious material to start with. Therefore, the material should be kept in your level 2 area until it is inactivated. Inactivation means fixation with glutaraldehyde. Waste material should be treated with either glutaraldehyde or the material autoclaved. However, if you do that, do not chemically inactivate the

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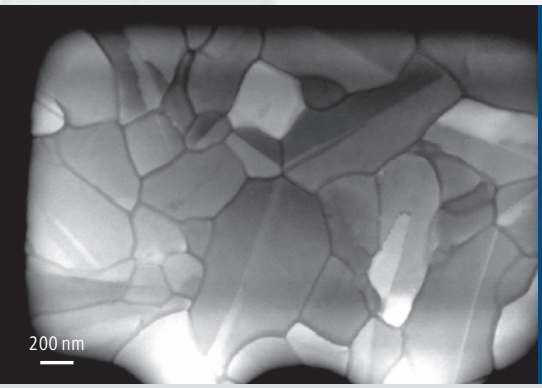

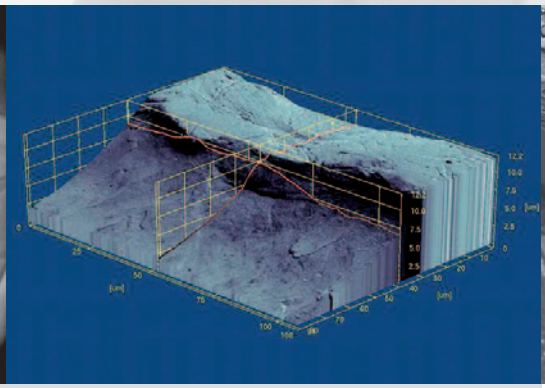

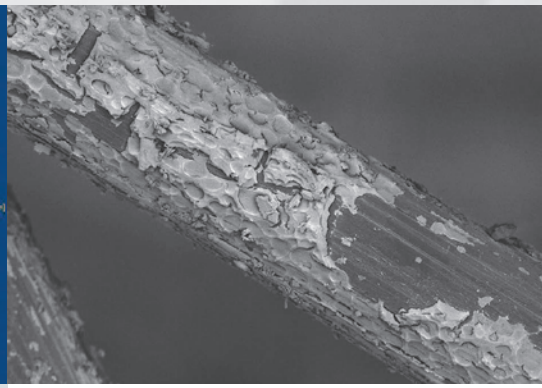

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waste or cells. It is not good for the people who are around, or, for that matter, the autoclave. I will send you a copy of my lab safety manual for your information. It will be in Word form so you can adapt it for your use. While it will have the different things I make the staff sign off on, it does not have the MSDS sheets for the individual viruses we have in the lab, or some of the things which I simply reference to elsewhere for saving time and paper. I will also send a .pdf of the final form so you can see how it is put together—things sometimes go awry when we open in different computers with different printers. Also, it guarantees you can open it if you can't open word. Note, the manual is for my level 2 gastroenteric virus lab, recently modified for a lab partner my Chair gave me who is doing Borna Disease Virus work. It is not for my EM lab. The rules are relevant to what you need. Also, you really should have the room at negative pressure, or it is not a proper BSL-2 room. Paul R. Hazelton paul_hazelton@umanitoba.ca Tue Nov 3

As Paul said if you work with a BSL-2 virus, consider it infectious at all times. One cannot be sure that the cells do not release virus particles in the medium. On the good side, all cells in culture are protected in sterile dishes/plates, so a direct contamination is not possible, but you must always consider possible accidents. As long as I know BSL-2 containment does not require depressurized rooms (it starts with BSL-3). Working with security–wanting virus requires specific rooms and behavior; this is not subject to personal opinions. I guess that your concerns come from the sharing of the material (microscope and accessories) for BSL-2 and non-BSL-2 applications. Here is my opinion: Only trained persons should work in that project (makes sense but you never know). Do not mix persons involved with the BSL-2 project and those who are not in the same lab. Optimally, the microscope and accessories should stay in the same room as the one used to manipulate the cells (at least for the time needed to finish this project). If this is not possible (but this is really advisable), the cells should be transported in a closed container. Take en-route accidents seriously! The pieces in contact with potentially infected material should be systematically disinfected after each manipulation, as well as the pieces in contact with the gloves (did I say you need to wear gloves?). Any accident/spilling, even the slightest, should be taken seriously and managed accordingly (and the personal made aware of that). The key here is to use aware and trained personal and take the routine disinfection seriously. In brief: Be careful not paranoid. Stephane Nizets nizets2@yahoo.com Wed Nov 4

Instrumentation:
air table gas consumption

We've just had a Kinetic air table delivered for our new Zeiss Apotome scope and I'm casting about for a source of air for the table. Anybody out there have an idea how much gas is consumed by such a table? How often (roughly) would we need to change a standard nitrogen cylinder? Alternatively, if we provide air with a compressor, how often could we expect the compressor to cycle in the course of a day? We're mainly concerned with noise, and the compressor provided by the vendor seems a little expensive. Can we get away with a less expensive solution? ***James M. Ehrman jehrman@mta.ca Thu Nov 19***

We are fortunate to have gas/vac/air available in all of our hoods so we just tapped into the line for air and added a pressure regulator on that line for the anti-vibration table. Any chance of doing that in your building? Beth Richardson beth@plantbio.uga.edu Thu Nov 19

Air consumption is going to be rather variable depending upon whether you have a large floor mounted table or a benchtop table, how much you actually touch the scope while using, and how much of the time it is being used. I've seen SEMs on large floor mounted Micro-G tables that would go for many months, sometimes years, on a single 220 cu. Ft. nitrogen tank. Other times, they would only last a couple of months. If they are trying to sell you a Jun-Aire compressor, I can tell you that they make all the racket of a modern refrigerator. If noise is an issue, it is a good solution. The only noise you will generally hear is the unloader valve at the end of a pump cycle. These compressors use the same basic compressor that a refrigeration unit uses, rather than a piston or diaphragm pump. They must be heard to be believed. No interest other than I like customer sites that have them. No noise, no high pressure tanks. Ken Converse kenconverse@qualityimages.biz Thu Nov 19

Jun-Aire's are definitely great, just very expensive. We used a $290 compressor from Home Depot for quite some time on our 2010F when I was at Intel and located it in an equipment chase. The only caveat with the cheap compressor is that if you are actually consuming air through leaks or air table actuation you must be diligent about draining the water from the reservoir on a regular basis, or it will fill up and pump water into the air table pistons, which really creates a mess. Also persistent water retention in the reservoir can lead to corrosion of the reservoir which can lead to the reservoir bursting. John Mardinly john.mardinly@wdc.com Thu Nov 19

Instrumentation:
Freon

I work with a JEOL JEM 200CX and the Freon used as dielectric in the gun has been consumed, I would like to exchange this gas to another, because Freon is forbidden to be commercialized (It is responsible for the destruction of the ozone layer). My question is, what gas can I use instead the Freon? In high-tension installations sulfur hexafluoride (SF_6) is used, but I don't know if I can use it inside the microscope column. ***Telmo Nunes telmonunes@hotmail.com Wed Oct 21***

Some years ago we changed the Freon gas in our JEOL TEMs, including a 200CX, from Freon to SF_6. Apart from changing the gas inlet fittings to allow us to use a standard SF_6 gas cylinder instead of the JEOL cans we made no other changes. We pumped out the Freon from both the tank and the gun, filled them with air and then pumped out again. We then filled them with SF_6 to the same pressure as the Freon (tank 0.15 kg/cm, gun 3.0 kg/cm^2). The 200CX ran happily until we scrapped it about 7 years later, the other instruments are still running at 200 or 400 kV. Mr. Ron Doole ron.doole@materials.ox.ac.uk Thu Oct 22

My recollection is that JEOL HT tanks cannot be directly converted to SF_6 because they cannot withstand the required pressure. You will probably need to purchase a new unit from JEOL. John Mardinly john.mardinly@wdc.com Thu Oct 22

Basically, Freon materials are liquids at room temperature and enter gas phase when hot. This gas is then cooled and the liquid phase reappears. SF_6 is a gas and you would need pressure to ensure enough insulation. FEI used to use SF_6 in the HV connection at the top of the column. This SF_6 came in a small gas cylinder. When the tank uses a liquid for insulation, there is no pressure. Is this the case for your tank? If you can find out what Freon is in the tank, there are replacements for some of them: http://www.hvacmechanic.com/refrigerants/refrigerants.htm. This lists the refrigerants (not used for this in HV tank applications). Check to see which available material has the highest dielectric constant. Also see if it not on the phase out list. Air is also on the list and let's hope that it is not phased out. Gary Gaugler gary@gaugler.com Thu Oct 22

Instrumentation:
vacuum systems

We are having some discussions regarding the most appropriate vacuum system for a 120kV LaB_6 TEM dedicated to biology

samples—ultrathin epoxy embedded sections at magnifications less than 100k and a digital camera (no photographic film). The choice is between a cascade oil diffusion pumped (DP) system achieving 4 × 10^{-5} Pa and a turbo molecular pumped (TMP) system. Two points in favor of the DP system: 1) It recovers rapidly after specimen insertion (15 second turnaround) and is more forgiving with beginning users. We have graduate student and post-doctoral users. 2) The TMP significantly increases the price of the service contract. Do you have any recommendations based on your experience? ***Larry Ackerman Larry.Ackerman@ucsf.edu Thu Oct 29***

The service contract price tells a lot. When there is a major problem with a DP, you just clean it, add new oil and away you go (except for the occasional heater). Even using exotic oils, you're probably well under $1k for materials and you can do it yourself. There is essentially no routine maintenance required. A TMP absolutely requires regular maintenance, and when (not if) anything goes wrong, you're in for at least $2.5k and you can't fix it yourself, in most cases. These are not forgiving pumps. Power consumption is low, though, and water cooling may not be necessary. As to which is cleaner, it all depends... There are lots of variables and either one can come out as cleaner or dirtier. I've seen mighty clean DP pumped systems and pretty filthy TMP pumped systems. I've also seen the reverse. Ken Converse kenconverse@qualityimages.biz Thu Oct 29

No question about it: for your system, a DP is the way to go. Having said that, if you have in mind to do cryo work at some future time, then possibly consider TMP. But, as was pointed out, it is an extremely expensive proposition throughout. Remember, too, if one ever fails out of contract, you're looking at $25–30K in repairs. Our newest biological TEM is DP, even though we could have had a TMP. Service contracts are often 30–50% less, as well. John Bozzola bozzola@siu.edu Thu Oct 29

I agree with others that an oil diffusion pump would probably be the best choice for Larry Ackerman's TEM. While a turbo-molecular pump will give a cleaner vacuum, specimen contamination does not sound as though it will be a major problem for the type of specimens and the magnification range he says he will be using. However, oil diffusion pumps are subject to many problems that can cause very serious consequences for an electron microscope. In one incident that I very well remember the failure of a diffusion pump system resulted in such a high concentration of oil vapors entering the column that the window on the viewing chamber was covered with a thick coating of oil that we couldn't see through it. Cleaning the column after this event was a tremendously difficult task, and it is unlikely that we really got all the oil out. Anyone not thoroughly familiar with the characteristics and operational requirements of this type of pump would be well advised to review the discussion of them in Chapter 5 and Section 9.2 of "Vacuum Methods in Electron Microscopy" (ISBN 1 85578052) Wilbur C. Bigelow bigelow@umich.edu Thu Oct 29

Good point Dr. Bigelow makes about DP vacuum disasters. In the modern instrument, with automatic valving, this "should" not happen. If it does, that's when a service contract more than pays for itself, believe me. John J. Bozzola bozzola@siu.edu Thu Oct 29

Having experience of almost 20 years with one particular LaB_6 TEM of one manufacturer, which has been used almost exclusively for "biological" TEM, here is a brief comment / summary: this TEM has worked absolutely reliably for almost all of the time. No DP disaster; I am keeping my fingers crossed. The TEM was and is on about 355 to 360 days a year, and it has: a rotary pump; a DP (which is great, cheap, reliable, and appropriate); an ion getter pump (IGP), which is an absolute must, in my opinion, if you want to operate the LaB_6 (usually, one LaB_6 is at least 3 years in—heavy—operation; with, on average, always more than or at least 10 to 15 different operators; including teaching; users: undergrads, PhD students, postdocs; average 40 (30–55) hrs per week, with sometimes frequent, sometimes rare specimen changes, depending on the user). —We always have the LN_2 trap filled—always. And, an additional "cryo-finger" in the objective lens part, which was nice and useful for occasional cryo-work. My secret? None. Regular supervision, regular and tight teaching and supervision. Only regular maintenance, once or twice a year and a second IGP after about 15 years. Work: sections, freeze-etched samples, negative staining, as usual for biologists. 2 years included cryo–no vacuum problems. Concerning the question from Larry: For sure, if I had to decide, I would choose an identical or at least similar machine, under similar conditions. —This means, not only the DP matters, but also the logic of the vacuum control, the valves, the IGP (for the LaB_6), the LN_2 trap, and if you can afford it, the cryo-finger. Reinhard Rachel reinhard.rachel@biologie.uni-regensburg.de Thu Oct 29

TEM:
cleaning column

I would like to know if it is necessary to clean a column in TEM after the collapse of a turbo molecular pump? We had to change the fluorescent screen because of a debris fall out so I am wondering if the entire column might be contaminated. ***Dorota Wadowska wadowska@upei.ca Thu Nov 19***

Short answer—yes, based on my experience. The coating from the screen probably is scattered up and down the whole column. Randy Tindall tindallr@missouri.edu Thu Nov 19

Yes, I have seen this as well. And be careful as you clean—I've also seen fluorescent hand- and footprints all over a lab from this exercise! The stuff gets everywhere. And if you have any other debris in the column, you really must get this out as well, especially if magnetic. Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Thu Nov 19

Thanks for your input about column and turbo molecular pump. My next question is: how to clean a column? Would you do it yourself or leave it to a service person? Dorota Wadowska wadowska@upei.ca Fri Nov 20

I don't know about others, but there is absolutely no way I would tackle cleaning a TEM column myself. I have done SEM columns in the past, but that's a different critter altogether, seems to me. Are there labs out there that do this in-house without trained service engineers? I'm curious. Randy Tindall tindallr@missouri.edu Fri Nov 20

We do not clean the TEM column ourselves. No way. Far too complicated, in my opinion, for our group / our department / faculty. Done by service people from the manufacturer. Reinhard Rachel reinhard.rachel@biologie.uni-regensburg.de Fri Nov 20

For many years we had one of those amazing EM technicians (largely self-taught) who was unafraid to tackle any problem with a microscope. About 10 or 11 years ago, our EM420 had a leak in the lens cooling circuit, such that water was dripping inside the column and onto the phosphor viewing screen! Larry courageously disassembled the entire column, repaired, cleaned and reassembled it so that it performed very well for 8 or 9 more years. Larry retired before I came on board so I, to my deep regret, did not have the opportunity to sit at the feet of this Jedi Master. Roger A. Ristau raristau@ims.uconn.edu Fri Nov 20

Gosh, you all are wimps! I have taken apart and cleaned the columns of a Philips 201, Philips 300, Hitachi H-600, Zeiss 10/A and a little of a Zeiss 912. Yes, except for the last, they are all really old scopes, and quite amenable to being disassembled. The person asking the question did not state what kind of TEM, so it really depends. My Zeiss 10 manual is very detailed about cleaning the column, and easy

to follow. The others were more seat-of-the-pants. But a TEM is easy; it's a bunch of coils stacked up on each other with a cleanup tube that comes apart in sections for cleaning. Some fixed apertures that may be interesting to try to remove and clean. If it's an older, not overly-digital-sensorized instrument and they have some intelligent help, it's doable. But if they have the money, hire a professional! With new O-rings. On the other hand, I would never take apart my SEM! OK, wait, I've done that, too, but not to put back together! I used to rebuild VW engines. On the ground, not through the tailpipe. When you live in the middle of the Pacific ocean, you learn to become self-sufficient. Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Fri Nov 20

Go Tina! BTW, SEM columns are generally simpler than TEM columns (and even VW engines) so have at it! Ken Converse kenconverse@qualityimages.biz Fri Nov 20

Ah, but I have a Hitachi S-4800 cold cathode field emitter with all kinds of detectors that I'm a little afraid of. Although I did help install it plus another one the very next day, when Hitachi sent out their female engineer (we girls scared the boys), so I'm pretty comfortable with it. Here's what I did to our old S-800 http://www.flickr.com/photos/koolau_arts/3246764752/in/set-72157613221785575/. I keep parts around to show people as I train them. Alas, whoever will eventually replace me will probably not ever have had to take apart and repair this kind of equipment. Maybe they shouldn't have to. It's like the new cars with "black boxes." Although there is hope: I talked a kid through rebuilding a broken Penning gauge a couple of weeks ago, and the SEM got pumped down and he's forging ahead on getting it going. I took my car in for service yesterday—I can't even tell you what's under the hood. (OK, that's not true—it's a kind of a performance vehicle and I pretty much understand the specs, but I would never try to even stick my hand in there.) Are you "old guys" training up the youngsters? Oh, shoot, I remember being the youngest person in the place. Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Fri Nov 20

Maybe this is an urban legend, there's the story of the faculty member who assigned the cleaning of an ISI tabletop SEM to his graduate student. He was leaving for Europe and wouldn't be able to do the cleaning herself. He simply told the student that the solvents, wipes and Q-tips were in a locker near the instrument. He wrongly assumed that the student had seen or assisted others in the process. The student carefully removed the gun from the top of the column, placed a glass beaker in the specimen chamber and gently poured acetone down the column! John Bozzola bozzola@siu.edu Fri Nov 20

Probably not urban legend. Here's a version I can absolutely confirm (although I didn't do it, but arrived only minutes after it happened). Technician cleaned the TEM column parts with acetone and re-assembeled, not completely dry. Noticed a drop of acetone had fallen on the fluorescent screen. Removed the window and blew on it with canned air. The entire room fluoresced for a couple of years. Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Fri Nov 20

Here's one that did happen to me. I was at a faculty meeting (in Philadelphia), when my graduate student burst into the room shouting "the electron microscope is on fire!" I ran over to the room and, sure enough, the serviceman was stamping his feet on the floor to put out a fire. Here's the story: his method of cleaning Pt apertures was to flame them and drop them (while hot) into a beaker of acetone. Normally, this was not a problem as this would not ignite the acetone. This time, however, his Bunsen burner was too close to the 250 ml beaker and he reported that the flame appeared to jump from the Bunsen over to the beaker, where it went up quite vigorously. In his panic to cover the beaker, he tipped it over, sending flaming acetone along the countertop (all over my Nikon electron flash unit) and onto the floor. He attempted to stamp out the fire, igniting his pant legs. The fire was extinguished with CO_2 but he had burned his hands and shin severely enough that he went on medical leave. He never came back to work. He left permanent footprints on the tile floor and the EM company never reimbursed us for the damage. I won't flame the company, by revealing their identity. John Bozzola bozzola@siu.edu Fri Nov 20

We used to clean our JEOL 100CX column, gave it a thorough clean just after I arrived here because oil would condense on the viewing window and had to be wiped off every month or so. This was considered normal. Also got the workshop to take the diffusion pumps apart and clean out the tarry residue—what diffusion pump oil turns into after 20+ years without being changed. The gun was surprisingly clean—only a faint brown residue in the chamber. Changed all the vacuum hoses as well to try to stop oil coming out and into the column. All this improved things a bit–only had to wipe the oil off the window every 6 months. After a couple of years, it developed an incurable vacuum sequencing problem (don't get me started), it was just worn out and oil-soaked, so we retired it (to my great relief, I have to admit). Not sure I would do this with a new instrument, but we didn't have anything to lose with the old one. Rosemary White rosemary.white@csiro.au Fri Nov 20

SEM:
astigmatism

As biologists we usually deal with poorly contrasted/bad conductive material in REM and they are not optimal for astigmatism correction. What would you have advise, a commercial or a make-it-yourself sample, to correct astigmatism up to 50,000× in REM? ***Stephane Nizets nizets2@yahoo.com Mon Oct 12***

Having a specific sample for correcting astigmatism is not going to help, beyond finding out what the intrinsic astigmatism is in the column. Any sample that charges is going to have varying astigmatism depending upon the exact area scanned (for those who are not familiar with REM, it is raster electron microscope, otherwise known as SEM), the beam current, the length of time scanned, and the scan rate (especially the horizontal scan rate). Generally speaking, reducing the accelerating voltage, reducing the beam current, and increasing the horizontal scan speed will reduce charging and the associated astigmatism. One thing that can help is to focus and stigmate carefully on an area very close to where you want to collect your image, then shift the field of view, quickly make any minor corrections and take the picture. The bottom line is this: If you haven't stigmated, you haven't focused. Stigmating the beam is an integral part of focusing and cannot just be set once and forgotten. It must be done every time you want a good image, especially at high magnifications. Ken Converse kenconverse@qualityimages.biz Mon Oct 12

I am often asked to examine some fairly smooth surfaces for their fine structure. Often, the sample seems so featureless that I cannot easily focus. In those cases, I take one of two approaches. In one scenario, I start on the edge of the sample and do as much focusing and astigmatism correction as possible. Corners are usually better than edges since it is hard to correct for astigmatism with a single straight edge. The other scenario is a judicious level of uncleanliness. A little bit of dust can be a good thing as it provides something to focus on. Certainly a lot of dirt or debris could obscure the important features or foul an otherwise good image. However, a small dust particle in the neighborhood of a feature of interest is preferable to an edge hundreds of microns away. Warren Straszheim wesaia@iastate.edu Mon Oct 12

Get a gold on carbon "standard" from Pella, et al. or Agar. Choose the gold particle size to be compatible with your magnification and resolution. 50,000× is not bad. The trick is to get some large chunks

of gold along with small ones in between. This is not going to solve your stigmation situation since each beam condition and specimen need custom stigmation. But what the gold on carbon will tell you is whether your column needs alignment or your apertures are dirty. For biological samples, you have to find a discerning feature and stigmate on that. Not always easy. Gary Gaugler gary@gaugler.com Mon Oct 12

EDS in TEM:
SiLi and SDD detectors

We're writing a grant for a new TEM which will include EDS to be used for life sciences and materials sciences. I'm more used to SiLi detectors on SEMs, so I would like some input from the materials folks on what to look for in EDS detectors on TEMs, and the pros and cons of SiLi vs. SDD. I've got all the usual references, so I'm looking for user experiences. Vendor responses off-line welcome. Note: TEM location may well limit us to 120kV, 200kV would be better, yes, but likely not do-able. ***Philip Oshel oshel1pe@cmich.edu Wed Nov 18***

Referring to the article by Dale Newbury, NIST, referenced below, there is only one major parameter where a SiLi detector outperformsanSDDdetector:thecaptureefficiencyforphotonsabove10 KeV. http://spectroscopyonline.findanalytichem.com/spectroscopy/Featured+Flash+Component/The-Revolution-in-Energy-Dispersive-X-Ray-Spectrom/ArticleStandard/Article/detail/609461 Rick Ross richard.ross@allisontransmission.com Wed Nov 18

This concerns SEM not TEM, which was the point of Philip's message. As I understood it, the real advantage of SiLi detectors are in high count rates, which specifically never happen in TEM. Actually sensitivity is a major point for EDS analysis in TEM. I would tend to think that SiLi detectors offer no real advantage in these conditions, but I would be grateful if everyone would care to share their comments on this question with the community. Stephane Nizets nizets2@yahoo.com Thu Nov 19

Microprobe:
Crystalbond

Does anyone know if Crystalbond embedding samples can affect or contaminate the microprobe? I would appreciate any suggestion. ***Marcello Serracino marcello.serracino@igag.cnr.it Wed Oct 14***

Our Quickstick 135 is a similar composition to the Crystalbond. When we did the work on the plasma cleaner, we used it as an intentional contaminant. See the following paper for the details: Surface Science Aspects of Contamination in TEM Sample Preparation, J. T. Grant, S. D. Walck, F. J. Scheltens, A. A. Voevodin, Proceedings of the Materials Research Society, Workshop on Specimen Preparation for Transmission Electron Microscopy of Materials IV, eds. Ron M. Anderson and Scott D. Walck, Pittsburgh, Vol 480 (1997). When we tested the samples in the TEM, you could have the balls of the materials just next to the beam and you would not get any excessive increase in contamination on the sample. It wasn't until you intentionally put the beam on the wax did it boil off an evaporate into the vacuum. I have used it to bond samples in the SEM without problems. As long as the sample does not get hot or the beam hits the material directly, it doesn't seem to be an issue using it. I prefer not to use it, but if I want to polish the sample after examining it first, I'll use it. Scott D. Walck swalck@southbaytech.com Thu Oct 15

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