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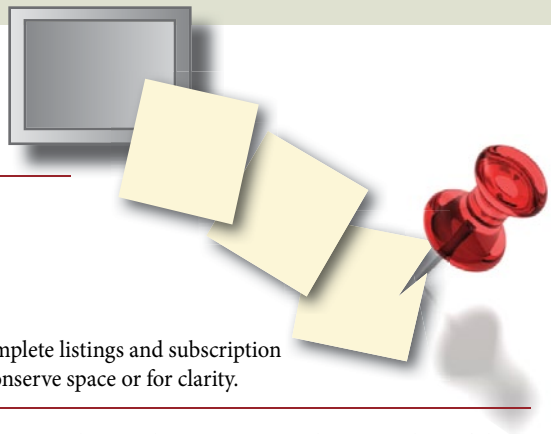

Edited by Thomas E. Phillips
Bond Life Science Center, University of Missouri, Columbia, MO 65211

phillipst@missouri.edu

Selected postings from the Microscopy Listserver from August 16, 2009 to October 10, 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:
stain for myelin in LM

Does anyone have a favorite stain for myelin in plastic thick sections? We have been given one protocol using P-phenylene diamine in absolute ethanol, and we will give it a try, but if there are any other pet techniques that you would be willing to share, we would love to try them, too. ***Randy Tindall tindallr@missouri.edu Tue Sep 1***

It's been 25 years since I worked for a neuropathologist, but here's what I remember (and could dig up): We did a lot of morphometry (in black & white film), so the paraphenylenediamine stain (lipids stain dark brown over a light brown background) worked well. We used a 1% aqueous solution (in distilled H_2O, vigorous stirring to dissolve everything). As I recall, staining occurred on the slide, on a 80°C hot plate for 15-30 sec and then rinse with distilled H_2O. This was a very dependable, if not very exciting looking stain. We also used 1% Toluidine Blue (in 1% Na borate), but not often. The pathologist's favorite (because it looked like H&E) was the Paragon stain. 0.73 g toluidine blue, 0.135 g basic fuchsin in 100 ml of 30% ethanol. The staining protocol was a bit different for Epon-like plastics, as opposed to Spurr's. For Epon plastics, we stained on the hot plate for about 15-30 secs, rinsed gently with distilled H_2O and then we checked the stain on a scope while it was still wet. Sometimes it needed longer, so it was back to the hotplate. Spurr's was exposed to the stain for only 10-15 sec, with a light sprinkle of Na borate powder on top of the stain and then rinsed with distilled water. I seem to recall this was a temperamental stain and difficult to get "just right". Douglas W. Cromey dcromey@email.arizona.edu Tue Sep 1

Specimen Preparation:
carbon coating

I would like to ask your expertise with regard to the problem I have encountered with my carbon coating procedure. I tried to coat 7 stubs of rock fragments all at the same time. However, when I viewed them under the SEM, 3 out of the 4 stubs exhibited coating peeling off that looks like shavings. Would this problem be sample related? I used our SPI carbon coater with 1.3 mm diameter carbon string. ***Melina Miralles From mmiralles@pi.ac.ae Wed Sep 9***

I have seen peeling coatings in other circumstances. It usually had to do with trying to put down a thick layer for other than microscopic purposes. The thick layer built up internal stresses that led to curling. Did you try to apply the same thickness of coating as normal? You should have. Adding additional samples does not require extra time or coating material. The material is there to do the coating whether you have 10 samples, 1, or none. The material will go to coat your chamber if there are not samples there to receive the coating. Did the vacuum reach the same level as normal? Our sputter coater has a good vacuum pump, but we run into cases where samples outgas significantly and extend the time it takes for the coater to reach a good vacuum. Using 7 samples instead of 1 could extend the time. I do not know the SPI coater and whether it works based on vacuum level or time. Perhaps a poorer vacuum leads to peeling. You might try coating fewer samples at once or letting the system pump longer and see if the problem disappears. We have had some samples whose nature seemed to prevent good coating. We coated many samples at once, but only a few had the problem. They seemed to be holding a liquid so that our gold coating did not stay in a layer but tended to bunch up into large islands. I found that if I pre-pumped the samples under a higher vacuum - I let them sit in my SEM chamber for a couple hours - that the samples took a coating better. Apparently, there was some phase that slowly volatized and that extended time under higher vacuum removed it. It is only rarely a problem, but I have considered using our high vacuum evaporator to pre-pump the samples if the problem recurs. Warren Straszheim wesaia@iastate.edu Wed Sep 9

Adhesion problems for coatings are typically a surface preparation problem. I would double check your cleaning procedures for your samples prior to coating. Some coatings are not compatible with some surfaces. Are the good samples and poor samples the same material? Often, you can change the surface properties with a plasma preparation. For example, polymer surfaces are often treated with an Argon plasma to modify the wetting characteristics of the film going down on the surface. Scott D. Walck swalck@southbaytech.com Wed Sep 9

I ran into this problem after coating the inside of my bell jar with a releasing agent. Too much carbon buildup and it would peel right off during flashover falling on the samples below. About every third coating now I hit the bell jar with a can of air to blow off any peeling film and have not had problems since. Scott Whittaker whittaks@si.edu Wed Sep 9

I have applied the same amount of carbon as I usually use on other samples and there was no significant increase in the time it took to reach a good vacuum. Anyhow, I will try washing off and drying the samples as most of you suggested and play around with the amount of sample per batch of coating cycle. I would also look and see any trend with the sample composition of the good ones versus the bad ones. I cannot try the suggestions that use other equipment aside from the ones I mentioned, as these two are our only workhorses in our SEM lab. Melina Miralles mmiralles@pi.ac.ae Thu Sep 10

Specimen Preparation:
growing cells on grids

Some researchers are interested in growing cells on grids. Can you provide me with some help? ***Lennell Reynolds l-reyjr@northwestern.edu Thu Sep 17***

I did this about 25 years ago as a very young student! I used both mesh and 1x2 mm slot grids, gold, and Formvar coated. I can't remember if I carbon coated. It's possible I coated with poly-L-lysine to give the cells a start. I was working on crustacean neurons, and I was a bit frustrated that they grew along the grid bars, which is when I switched to slot grids. My main problem was anchoring the grids down in the medium so they wouldn't float. Can't remember the solution (I'm not being much help, here), but it's possible I grew some upside down, floating on drops. You may have to play around a bit to

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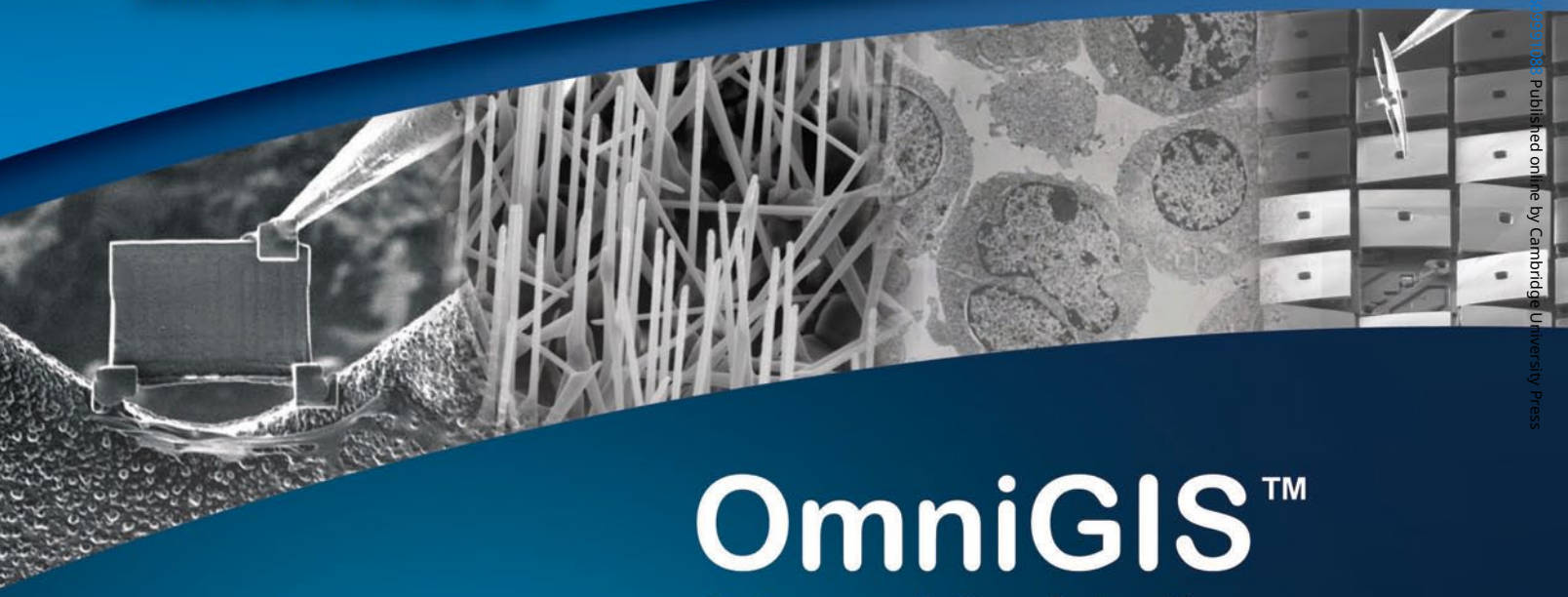

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see what makes the cells happy. The good news is that I was then able to fix, dehydrate, and critical point dry the neurons on the grids, and then do 400kV TEM and then SEM on the cells on the grids. I still have them. They were remarkably robust. Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Thu Sep 17

Are nickel grids reactive with the culture medium or toxic? If not, one could use a magnet below the dish to hold Ni grids down. Dale Callaham dac@research.umass.edu Thu Sep 17

Did you leave the grids stuck to the cover-slip when picking up the Formvar coating? Removed individual grids after processing? That might help keep the grids sunk in the medium. Pat Connelly connellyps@nhlbi.nih.gov Thu Sep 17

I think this is what I did - I must have picked up the Formvar-coated gold grids on a slide and then kept the entire slide/grid/Formvar sandwich submerged in medium. Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Thu Sep 17

Guess I'll chime in on this one. This goes back 30+ years but I still know the details. Use either gold or Ti grids, 200 mesh. I never found nickel to be good in the long run. Formvar films floated on water were used, and then grids placed on the film, picked up with 22 mm square or 18mm round cover slips. After air drying, the Formvar was coated with carbon and the coverslip/grid complex was irradiated with UV for about 24 hrs for sterilization. The grids were then ready to be used. Coverslips were placed in Petri dishes, seeded with cells and incubated as usual for the cell types. Cells on the coverslips were washed with serum depleted medium, fixed with glut and processed through osmium and dehydrated and critical point dried. Grid free areas of the coverslips were processed for TEM, embedded, and the plastic (generally) easily separated from the coverslip (due to the Formvar). Fixation times were reduced to 30 min per step and dehydration schedules were similarly shortened due to the thin nature of the specimen. I never published anything but the procedure was used by the Porter group. I know there are publications but I no longer have any available due to having retired a couple of years ago. Roger Moretz rcmoretz@gmail.com Thu Sep 17

Why all the old protocols? - growing cells on grids took on a new life with structural cryo-EM. Here is an example: Koning RI, Zovko S, Bárcena M, Oostergetel GT, Koerten HK, Galjart N, Koster AJ, Mieke Mommaas A. 2008 J Struct Biol. 161(3):459-68. Cryo electron tomography of vitrified fibroblasts: microtubule plus ends in situ. Paul Webster PWebster@hei.org Thu Sep 1

We published a technique to grow cells (bacteria, in our case) on a variety of surfaces, including sterilized TEM grids. It worked beautifully and should work in your situation. You would only need to substitute gold-coated grids. These are not solid gold grids, but copper grids that have been electrolytically coated with gold. The coated grids are less fragile than solid gold. I believe you can obtain them from Electron Microscopy Sciences (and possibly other vendors).Here is the reference: Bozzola JJ, Johnson MC, Shechmeister IL. 1973. In situ multiple sampling of attached bacteria for scanning and transmission electron microscopy. Stain Technol. Nov 48(6):317-25. I have a copy that I can email to anyone who might be interested. John J. Bozzola bozzola@siu.edu Fri Sep 18

Specimen Preparation:
HMDS

I have heard that one can use hexamethyldisilazane (HMDS) to dry samples for SEM - instead of using a critical point drier. Does anyone have experience/protocols they'd be willing to share, for using this chemical? ***Karen D. Moulton kmoulton@usm.maine.edu Mon Sep 21***

There are several methods in the literature. The protocols mostly depend on what you're doing -- what do you want to do? The general comment is "Use in a fume hood!" Philip Oshel oshel1pe@cmich.edu Mon Sep 21

Lots of experience- Processes as usual for EM and for final drying transfer out of 100% alcohol step to 100% HMDS, 2-3 changes for the same time as your alcohol steps and then let air dry (work in hood-not pleasant stuff). Slower drying seems better on samples that are more delicate so most of the time I wet a filter paper with HMDS in a Petri, dump on the wet sample, and partially cover to allow slower evaporation. Most animal, cellular, and insect/invertebrate tissues do well, hit or miss with plant and fungal tissues with no apparent predictability as to what will/won't work. I have had problems in the past with samples sticking in plastic vials/tubes so do this in glass or dump into Petri with filter paper. Like all EM smaller is better. I do not have an actual reference handy, just been using it for a long time. You may get protocols that specify alcohol/HMDS dilution series, but I have not found it necessary. Scott Whittaker whittaks@si.edu Mon Sep 21

I am using HMDS for cell cultures (and some other specimens) with good results and simple protocols (everything done in a fume hood): After removing the last change of ethanol add HMDS. Keep in open vessel. After 10 minutes pipette out most of HMDS (leave just enough to cover specimen) and let it air dry. If EDS should be performed, treatment with HMDS is not the best choice: it infuses specimens with silicon. Vladimir M. Dusevich DusevichV@umkc.edu Tue Sep 22

Specimen Preparation:
oily film for cryo-SEM

Anyone use a Gatan Altos to freeze and image wet/oily samples for cryo-SEM? I am having trouble finding the right holder for my samples that have the consistency of thin oil. The sample tends to roll off hats, rivets, and not give me a good drop of material for freezing. ***Gordon Vrdoljak doc.vrdoljak@gmail.com Fri Sep 25***

Try using a thin walled drinking straw mounted within a cryo holder and syringe the fluid into it. I have used similar technique for other difficult cryo specimens. Steve Chapman protrain@emcourses.com Fri Sep 25

Specimen Preparation:
LR white

I've recently returned to immunolabeling after a 10 year break and have run into a problem with LR White that I don't remember. I am embedding virus-infected tobacco leaves but I suddenly can't see the tissue in the block to section it. Since samples are not treated with osmium, it was never easy, but I do not recall it being the hit or miss matter it is now. When I finally do get sections, the cell walls appear to be bleached - they are a clear white, much lighter than the cell contents and even lighter than the surrounding plastic. This is with uranyl acetate and lead citrate staining that in the past gave me very lightly stained cell walls. To make matters worse, the quality of preservation is totally unacceptable and labeling tests have been uniformly unsuccessful, even positive controls. My fixation/embedding protocol is one I used many times in the past without problem. I fix in 4% formaldehyde and 0.3% glutaraldehyde in phosphate buffer, and dehydrate samples in a graded series of alcohol.. I've tested both the formaldehyde and glutaraldehyde in conventional osmium treated LX112 embeddings and preservation is okay so it seems to be the LR White. I'm wondering: has anyone else had this problem, and if so, what did you do about it? I checked the date on the LR White and it is still fresh. Another question: has anyone had success immunolabeling tissue fixed in osmium and/or embedded in LX112? ***Margaret Dienelt margaret.dienelt@ars.usda.gov Fri Sep 25***

This won't help your preservation problems but as far as visibility

goes, I have found in my work with methacrylate resins that adding a microlitre or two of ethanolic fast green at the 100% ethanol stage stains plant material well and does not interfere with immunowork. I think I make a 1 or 2 % fast green solution. Tobias I. Baskin baskin@bio.umass.edu Fri Sep 25

Did you use an accelerator with LR White? The accelerator is benzoyl peroxide and that could explain a lot of your symptoms. If no accelerator was used, then we have to consider other possibilities: inadequate fixation by the aldehydes, extraction by various organic solvents used for dehydration, length of time in various liquids (alcohols, resins, etc.). John Bozzola bozzola@siu.edu Fri Sep 25

Specimen Preparation:
fine grain coating

I would like to get some suggestions for choosing a target for the finest grained coating that can be sputtered using a Denton Desk II sputter coater. ***John Mardinly john.mardinly@wdc.com Mon Sep 28***

We use Baltec coaters that might have different sputtering currents, but at low currents, Cr gives the best coverage and smallest grain. The disadvantages are: quick oxidation (< 2 weeks usefulness of specimens) and with some soft polymeric materials (resist) we can get implantation of Cr under the surface and distortions. Ir was also reported to work better that Au/Pt/Pd. Jerzy Gazda Jerzy.Gazda@spansion.com Mon Sep 28

I use Pd and Ir (obviously not at the same time) in Denton Desk IV TSC. The Desk II does not have a turbo (MDP) so you are probably limited to 30mT. In this case, I would probably opt for Ir if its EDS signature does not impede your specimen's signatures. With turbo, I coat at 15mT and cannot see the coating at 600k×. Settings are 60% power, 120 seconds, 20% tilt/rotate, 15mT. No way to tell if this is the "finest" grain film. EBSD on Pd film on field oxide on Si wafer does not work. I figure that this is because the film is less than 50nm and the beam just pushes through the film. EDS confirms huge Si peak with very low Ir or Pd peak(s). Gary Gaugler gary@gaugler.com Mon Sep 28

Specimen Preparation:
dark backgrounds in SEM

I am looking at insects mounted on wires in the SEM. Is there an easy way to reduce secondary electron emission from the stub below so that the background appears dark behind the insects? My wires are glued to an aluminum stub with silver paint. ***Marie E. Cantino marie.cantino@uconn.edu Tue Sep 29***

It sounds like the stub is mostly bare Al. You would need low Z covering material to reduce secondary electrons. Try putting down some colloidal Carbon paint. It will likely meniscus over to the bug wires. But, since these are not the legs or feet, it should not matter. Gary Gaugler gary@gaugler.com Tue Sep 29

We like totally black backgrounds, whenever possible. To achieve this you need a very flat substratum composed of a low atomic weight. Even polished aluminum stubs are not totally flat (you will see scratches) and Al still gives enough signal to make the background lighter than wanted. We have good luck mounting specimens on glass coverglasses or pieces of microscope slide. Even when specimens are coated with Pd/Au, the background is quite dark, often black. If you cannot do this with the present specimens, then follow Gary Gaugler's advice and put carbon paint behind your specimens. This will also give a dark background. John Bozzola bozzola@siu.edu Tue Sep 29

You have had some good advice so far. One thing I have not seen yet is to mount the pin in such a manner as to tilt the sample and look at the structure over space. We examine thousands of pin mounted insects here and that is the preferred method. The background structure of the stage can still be visible however. To mitigate, my predecessor variously painted the stage with carbon paint. Effective, but to obtain a thick enough coat to stop beam the paint flakes off over time. Not really recommended. He also used carbon tape over everything. More effective, but sticks dust/dirt/debris which is then visible. Also difficult to remove. He also carbon coated glass and stuck back there. Very effective but difficult to make work. I ripped all that out and used Teflon foil. Worked beautifully but very hard to stick down. That is when I found graphite foil from Alfa Aesar. Easy to work with, cut and stick, washable and featureless, no off gassing or flaking etc... We also use it as backgrounds for stubs and stage and since we use pin type stubs we raise them a bit and place folded sheets under stubs that swing around as the sample is tilted and rotated- always being in the background. Stuff is not all that inexpensive, but I am on my second sheet in 10 years so no pain there. Scott Whittaker whittaks@si.edu Wed Sep 30

Immunocytochemistry:
nickel grids

We have some users who wish to examine biological sections mounted on nickel grids in our JEOL 2100 cryo TEM (200kV). These will be examined with either a JEOL standard quick exchange holder or a JEOL non-analytical double tilt holder. In both cases, the specimens are held in place by a retainer. This is screwed down on one side only of the specimen. Retaining force is then applied to the specimen via the residual springiness of the retainer. Question 1: Is there any realistic possibility that a nickel grid may get pulled out of the holder by the field and get stuck on the polepiece with this type arrangement? Question 2: The biological folk are using nickel grids for immunogold work. I'm not a biologist, but it seems that using a ferromagnetic material for a support grid, is best avoided wherever possible. Are there other types of grid (non-ferromagnetic - Al, Mo, nylon etc) which are compatible with immuno-gold labeling? ***Dave Mitchell david.mitchell@emu.usyd.edu.au Wed Sep 30***

I have never heard of any problems with grids being pulled out of the holder by the field in the TEM. The likeliest problems are grids clinging to forceps and occasional weirdness with image (distortions, etc.) with severely magnetized grids. If you just do not want to use nickel, gold grids will work well for immuno work, but they are more expensive. Just do not try to label with ultra-small gold probes and then use gold-enhancement to increase their visibility after labeling. Obviously, the grid will be "enhanced" right along with the gold nanos. Finally, I have seen people use plain, old copper grids for labeling with success. The idea that copper grids are not to be used for immunolabeling is embedded in the EM psyche, but I have wondered many times how important that really is. Never hurts to try alternatives, just for fun if nothing else. Randy Tindall tindallr@missouri.edu Thu Oct 1

I can address the "Why we don't use Cu grids for immuno-electron microsocpy" question, having experienced the problem. It only applies if you use Cu grids and Tris buffers for the labeling - the Tris etches the Cu and you wind up with lovely blue drops of Cu-laden buffer & severe debris on the sections! Cu grids with phosphate-based buffers are fine for IEM. I've never tried anything other than Tris and phosphate for immuno-electron microscopy so I don't know what other buffer salts will do. I've never had problems (other than the expense & tendency to fold up into tacos) with Au grids. Au-clad Cu grids have been iffy; they are cheaper than plain Au & definitely sturdier, but the cladding on the batches I've tried has not always been complete - I lost part of an experiment to the Tris-Cu curse - so only use those with phosphate buffers. Tamara Howard thoward@unm.edu Thu Oct 1

From my experience, copper grids are perfectly fine as long as

they have support film covering the copper and your incubations are not too long. If PBS is allowed extended contact with copper (like overnight), yes, I saw the drops turn bluish - and then the grids were very dirty under the microscope. But if you invest a little time in making your Formvar film solid, should be no problem, and definitely worth the handling convenience. "Copper dirt" is something you either have or do not have, and you will know if you have it, so I would not worry about that simply as a possibility. A good alternative to Ni that I have found is gold-gilded copper grids. I remember, a few years ago, someone on this list was warning me how perilous could this be should the Au coat get scratched enough for the Cu underneath to get exposed - all kinds of electrochemical forces will be unleashed - but this has never happened to me. Gilded copper grids are easy to handle and, unlike Ni, they take Formvar coating well. Finally, the only case when you will really need Ni grids is if you use Ultrasmall or Nanogold label and grow it by silver or gold enhancement. But if you were doing that, you would not be asking the question?. Vlad Speransky vladislav_speransky@nih.gov Thu Oct 1

As others have said, I have tried, by mistake, copper grids for EM immunocytochemistry and had precipitation problems that I don't think were linked to Tris based buffers. I use mostly HEPES or PBS and not sure which type caused the problem but I use nickel exclusively for immuno-work. Tom Phillips PhillipsT@missouri.edu Thu Oct 1

EM:
clogged plumbing

A question regarding a vintage Philips CM12 TEM, which has shown gradually diminishing cooling water flow. We have backwashed it, swapped out filters etc, which helped, but it is still not getting enough cooling, and instability at 120kV may be related to this issue. I believe the system may simply be clogged with scale/oxide crud. I am wondering if we run some cleaning agent through it, whether this will help. My first thought would be either dilute acetic acid (white vinegar), or one of those commercial solutions you see advertised for descaling shower heads, cleaning rust stains from baths etc. Has anyone done anything like this with any success, and if so, what did you use? ***David Mitchell david.mitchell@emu.usyd.edu.au Tue Oct 6***

CLR works fine. Just add to chiller reservior and let it circulate best it can overnight. Might take a couple of days to completely clear plumbing. We have a CM12 that had the same problem. If you have any questions contact Russ Buyham at FEI. Don Kierstead donk@ardl.com Tue Oct 6

We've had good luck with a commercial product known as "CLR" (Calcium, Lime, Rust) sold by Jelmar. In the US, we can find this stuff in our local hardware stores. It contains lactic and gluconic acids and surfactants. I'm sure you have something similar down under. We dump it into the reservoir of our water chiller and let it run for several hours (up to overnight) and then flush the system several times. We refill with distilled water and add a bit of Chloramine T and some sodium bicarbonate to adjust the pH (very slightly basic). In addition, I maintain a 1 um cartridge filter immediately before the water enters the microscope to catch any algae floating through the system. Most of the filter housings here are clear so that you can see the filter. I would enclose the filter in a black trash bag to minimize light exposure. Keeping the entire water system dark also helps reduce the growth of algae. Hendrik O. Colijn colijn.1@osu.edu Tue Oct 6

TEM:
z-height and focusing

We have a 200 kV TEM. For a radiation sensitive sample I have to operate this instrument at 80 or 100 kV. In this case I cannot bring the sample to focus by changing z-height. Flipping the sample does not help either. It is away from focus about 100 microns. I then use the focus knob to bring the sample to focus. As a result, what kind of artifacts, distortions etc. should I expect? ***Ayten Celik-Aktas celikaktas@gmail.com Fri Sep 4***

Let us consider the problem a little deeper. You are unable to adjust the specimen to the normal operating position - the eucentric point I assume? Changing the basic focal length of the objective lens changes the magnification, the resolution and the contrast - if you require a higher lens strength the focal length is shorter (higher magnification, higher resolution but less contrast). All that will change in your case is you seem to have a longer focal length than normal, thus ,when you bring the sample in to focus using the lens strength control, you are obtaining true focus , but at a longer focal length and at a slightly lower magnification. Under the above circumstances you will not have any imaging artifacts, just more contrast and less resolution. Steve Chapman protrain@emcourses.com Sat Sep 5

Thanks for your insight. If I will have a change in magnification, then I should be repeating my magnification calibration, right? Is there any formula to estimate how much of a change I will have when I use focus knob excessively? Ayten Celik-Aktas celikaktas@gmail.com Mon Sep 7

Yes, you should under normal conditions set up at the eucentric point. This action will have set the physical focal length to that required by the instrument design. This, as I have mentioned, determines, resolution, contrast, and magnification level. For many years, I have used TEM outside these parameters. If I wanted a higher resolution than the manufacturer offered I would lower the stage (focus clockwise) increasing the objective lens strength, the magnification is increased and the resolution. Conversely, with low contrast levels from a biological sample I have raised the stage as increasing the specimen to objective aperture distance results in an increase in contrast. You will need a magnification calibration sample in order to calibrate the instrument over the magnification range that you require. You will almost certainly find that there will be a simple % change related to the lower level of objective current than normal; but I am unable to offer a formula for you. Steve Chapman protrain@emcourses.com Sat Sep 12

TEM:
adding a digital camera

Does anybody have an experience with adding a digital camera to a TEM? My impression from watching this list is that it may not be an easy task because older microscopes have been offered for free "to good homes". Am I right? ***Halina Witkiewicz halina@ucsd.edu Fri Aug 21***

Last year I made a rig to hold a "35mm" standard digital camera with macro lens precisely aligned and angled to image the fluorescent screen on my TEM. The $700 camera produces 11 megapixel images. The results were slightly distorted since the screen is in the 45 degree viewing position but otherwise no one could distinguish the resulting image from one taken with standard film and scanned at 1200 dpi. Not all TEMs are equally adaptable but it is possible to get good results for about $1000! Larry Ackerman Larry.Ackerman@ucsf.edu Fri Aug 2

It should be possible to add a digital camera to most older TEMs. Normally, you would remove the film camera and install the digital camera system in its place. Or, with TEMs that have ports above the viewing chamber (for accommodating a 35 mm film camera) you could install a slide-in digital camera system at that location. Digital cameras for TEMs vary in price from $25K up to $150K or more. You may be able to buy a used one, even. If you do the installation yourself, as we did recently, you can get a reasonable system for $25-30K. It definitely will not be a fancy system, but it will produce publication quality images. I believe most people give away older TEMs since parts are no longer available, they cannot afford to keep them running,

or they no longer use TEM enough to justify keeping one online. Realistically, the digital camera (even the cheapest one) is worth more than many older TEMs (even if the TEM is still operational). John J. Bozzola bozzola@siu.edu Fri Aug 21

I was wondering if anyone had done what Larry Ackerman describes. I have not gotten around to making the rigid frame, but got good pictures just by holding the camera against the glass. The distortion Larry mentions can be easily corrected in Photoshop by multiplying the distorted dimension of the photo by the cosine of the angle of your screen, for example .707 if your screen is at 45 degrees. You have to uncheck the "constrain dimensions" box in Photoshop Image -- Image Size. Ralph Common rcommon@msu.edu Fri Aug 21

I'm sure purists would give many reasons why this is not a good approach (low resolution of viewing screen, dirt on screen, etc.). But one cannot argue with the price of this system. Furthermore, it may be perfectly adequate for capturing study images, and even (gasp, gasp) publishable ones. Obviously, markings on the screen (cross hairs, bull's eyes, etc.) would nix some uses. Go for it! After all, if it doesn't work out, you still have a digital camera for lab use. John J. Bozzola bozzola@siu.edu Fri Aug 21

We just added a digital camera to our very old JEM 100 CX-II. The only hang-up was that the manufacturer didn't have a flange fitting for our column when we ordered the camera, since they hadn't anticipated putting a camera on such an old 'scope. The sales representative and I found 3 other labs with the same model EM that were interested, so the company made the fitting. It took a while for me, since I was their first, but now they have the specifications and any subsequent orders should go quickly. Now if I can just find the time to learn how to use the darned software! Lee Cohen-Gould lcgould@med.cornell.edu Mon Aug 24

SEM:
cleaning column pole pieces

How should I clean the condenser pole pieces? For other components like Wehnelt cap, anode, etc., I use abrasive paste for metals and acetone to wash it, but I'm not sure if this is treatment might be too strong for pole pieces. ***Davide Cristofori dcristofori@unive.it Wed Aug 26***

Several years ago someone told me they cleaned their Wehnelt and its aperture by sonicating in Sparkleen (glassware detergent from Fisher) or its equivalent. I actually thought this was stupid until I tried it. It worked very well. I've always left the pole pieces to the field engineers, but if I were to touch one, that would be the only way I would try to clean it. Paul R. Hazelton paul_hazelton@umanitoba.ca Wed Aug 26

A service technician from Zeiss told me never to sonicate pure iron pole pieces, so I never did this. From another technician I learned this: 1- depending how dirty the surface is: cotton swaps and diamond polish with 0.25 micron grain size; no polish like Wenol or normal household metal polish. 2- brake cleaner. 3- soap and water. 4- acetone or ethanol several times. 5- drying at 60° C. It works well with surfaces in my LaB_6 SEM. In my older Cambridge days, when I had to clean pure iron pole pieces a lot, I used this also. Stefan Diller stefan.diller@t-online.de Wed Aug 26

Stefan makes a good point that I had not thought about, that is the composition of the part to be cleaned. Depending on surface finish, sonication may be very bad, and that could well be for pole pieces, whose finish and integrity is so important. As said, I have always left that to the service people. Paul R. Hazelton paul_hazelton@umanitoba.ca Wed Aug 26

The main consideration when cleaning pole pieces is "Will I remove any metal?" Any technique that removes metal, no matter how careful you are, will eventually spoil the pole piece by changing its shape. I feel pole pieces that have individual poles could be washed in a solvent in an ultra sonic cleaner. I fail to understand how vibration will alter the pole piece shape if cleaned individually? However pole pieces where the two poles are fixed together (often soldered) should never be placed in an ultrasonic cleaner; the vibration may crack the solder interface! I had client who used a dental drill with a polishing head to clean the mouth of the cathode (cathode aperture) for 15 years. They manufacturers service technician noticed that he could not correct for condenser astigmatism, even after twice cleaning the condenser system. I became involved when they asked for my advice and I suggested the technician take a good look at the cathode? Sure enough the aperture was an ellipse! I was also brought in on an objective lens astigmatism problem where once again the manufacturer's technician had tried everything he could think of. The lens was water cooled with water that was too cold and condensation had attacked the body of the lens producing permanent astigmatism, too great to compensate. A warning to all that the water flow is for the situation when the lens is on (100kV typically 5 amps of current being passed), but what happens when the electronics are switched off overnight, should you switch off? I still marvel at the wondrous ways people use to clean cathodes. It is a very simple task that should take about 15 minutes, the time consuming element is often the filament alignment. Ammonia solution (NH_4OH) at a concentration in excess of 25% will clean a cathode in an ultrasonic cleaner in about 10 minutes in most cases; ammonia being a solvent for tungsten. Wash away the solution and rinse for 30 seconds in alcohol, dry as rapidly as possible and check with a hand lens. Steve Chapman protrain@emcourses.com Wed Aug 26

SEM:
scanning speed in EDX

Theoretically the scanning speed in SEM should have no effect on EDX analysis, am I right? What about reality? Does the scanning speed influence the EDX analysis in any way? I am trying to determine if a redox reaction occurred at the surface of a material. If these reactions occurred, I should have a thin metallic film at the surface of the material. If I analyze the surface from an angle normal to the surface ("above"), what would be the optimal HT? The film is expected to be thin, so on one hand it may be advisable to use high HT to increase the signal. However on the other hand a high HT would penetrate deeper, making the part of the signal due to surface material less significant (basically, I would analyze the bulk material, not its surface). What would you advise? A HT as low as possible? I am aware that this method is probably not optimal, but I am not sure if I can do something else. I have no machine to make nice clean cross sections, I fear that the film would detach if I simply break the material with a hammer. The ionic species which are expected to be deposited are Ag and Cu. ***Stephane Nizets nizets2@yahoo.com Wed Aug 19***

Since the EDX detector is gathering all the x-rays from the sample, it doesn't know or care whether the beam is scanning or still or what area the beam is scanning. You should just be aware that the SEM's scanning pattern includes a "pause" at the upper left-hand corner of the scanned rectangle and at the left-hand side of every line, so the x-rays will not be evenly acquired from the whole area scanned. Some SEMs have a special "Analysis Mode" that moves the beam evenly on a rectangle to give an even x-ray distribution over the area.

If you want to analyze a thin metal film on top of a substrate, you would be advised to lower the kV and look for the x-ray *L* lines of the metal. Increase the beam current to increase the signal. Use an accelerating voltage no more than twice the energy of the metal's *L* line. If you use a high kV and try to get the *K* lines of your metal, you will get too much x-ray flux from the substrate and will not be able to detect the x-rays

from the thin metal film on top. Mary Fletcher maryflet@interchange.ubc.ca Wed Aug 19

SEM:
elemental sulfur

As many of you are probably aware, elemental sulfur will evaporate within the SEM chamber. However, we would still like to try using SEM-platformed image analysis to measure sulfur in metallurgical hydromet processing by-products. Does anyone have a good idea of how fast sulfur will evaporate? That is, during the 1st hour, it's difficult to see it going anywhere, but give it a day in the vacuum and it may be difficult to find. We would like to think that less that 10% would disappear in the 1st hour - does anyone have more experience with it? We would also like to think that our ESEM would be designed such that evaporated sulphur would not pose problems for the electron column ... true? ***Michael Shaffer michael@shaffer.net Tue Oct 6***

I think that your best bet would be to find an SEM with a cold stage. You need to look at the vapor pressure versus temperature value for sulfur. Then you want to be well below the temperature that corresponds to the vapor pressure equal to the pressure in the SEM. Scott D. Walck swalck@southbaytech.com Tue Oct 6

I agree that a cold stage would be advisable. Vapor pressure depends on pressure, so the cold will definitely help. However, I do not know how much an E-SEM will help. The issue is one of the partial pressure of S vapor, not just the pressure within the chamber. You could have 10 torr of pressure in the chamber, but the sulfur partial pressure is probably coming entirely from the elemental sulfur in the sample. Unless you can feed sulfur vapor as the environmental gas, the sulfur in the sample will sublime to bring up the sulfur pressure in the chamber. It will just be a question of time. It may be worse if the subliming gas is being quickly pumped away keeping the partial pressure of S close to zero. I suppose some sacrificial chunks of elemental sulfur off in a corner would help if they could be the source of sulfur vapor rather than the sulfur in your sample. I was thinking of saying that the E-SEM or VP-SEM would be good in that the sulfur vapor is being swept away by the atmospheric gas of the sample chamber. However, if it is being effectively swept away, then that means your sample would continue to sublime and you still have your original problem. That takes you back to Scott's suggestion of a cold stage. It will serve to lower the equilibrium vapor pressure which will be better for your scope. Since the question is one of rate, maybe you can observe crystals of pure elemental sulfur over time. You should be able to document the rate at which the size changes. Warren Straszheim wesaia@iastate.edu Wed Oct 7

You could freeze your sample by setting a low temperature on your ESEM Peltier stage. This is not the same as a cold stage obviously and I have no idea if it would be helpful - can anyone comment? Dave Patton david.patton@uwe.ac.uk Wed Oct 7

My tables show the vapor pressure of sulfur as:
1e-8 torr - 13°C
1e-6 torr - 19°
1e-4 torr - 57C°
I think that if you can keep the temperature down (i.e. below 10°C), you should be OK with looking at sulfur containing materials. A Peltier stage should be adequate. A rough rule-of-thumb for deposition is that you get 1 monolayer per second at a partial pressure of 1e-6 torr. This can easily be derived using the ideal gas law and the kinetic energy of a gas assuming a sticking coefficient of one. While the loss of mass from the sample is indeed a concern, I would be more concerned with the redeposition on other surfaces within the chamber, especially the polepiece of the SEM. The e-beam may ionize the sulfur vapor molecules causing them to stick more tenaciously to the nearby surfaces. In this sense, I think that trying to increase the sulfur partial pressure by setting blocks of sulfur in the chamber will, in the long run, be very undesirable. The less sulfur in the chamber the less contamination you will have. Hendrik O. Colijn colijn.1@osu.edu Wed Oct 7

Instrumentation:
repairing small wires

We have a Amray 1645 with 4 BSE diodes that have broken leads (the really small gold wires, not the larger ones). These are way too small to solder without special equipment. The preamp and amp are working and I would like to move the system to our Amray 1600. Ideally we would just buy a new detector, but as you all know, budgets are tight. Does anyone know of some off the shelf diodes that could be used for a homemade BSE detector. There are lots of small electronic parts dealers, I'm assuming that some of the diodes they would work and not be too expensive. Any ideas? ***David Waugh dwaugh@kent.edu Sat Oct 3***

I have had success in the past using automotive rear window heater repair paint, it has no strength so you have to use epoxy to hold the wire firmly in place then the paint to make the electrical connection. Ritchie Sims r.sims@auckland.ac.nz Mon Oct 5

I just got my Think Geek catalog and it has "wire glue" http://www.thinkgeek.com/gadgets/tools/b70c/ that makes me think I would use either my silver conductive paint or carbon paint for SEM mounting. Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Mon Oct 5

Digital Camera:
resolution

I just wanted to discuss some reflections I made while discovering the new "5MP color digital camera" from Olympus. Let us make a bit of calculation: 1) The best resolution of this camera is 2560 pixels length × 1920 pixels width. 1) Zeiss reports a best resolution for its 100× objective at 0.26 µm (green light) 2) The field of view of a 100× objective is around 0.20 mm (this information was hard to find so I am unsure of it). Now to simplify the calculations, I will ignore that the camera takes only a part of the field of view. I will consider that the diameter of the field of view takes the whole 2500 pixels of the camera. Let's calculate the pixel size at best camera resolution with a 100× objective: 0.20 mm or 200 µm divided by 2500=0.1 µm. One pixel is thus 0.1 µm in size. This is more than 2× smaller that the best resolution of this objective! This means that the optics has a lower resolution that the camera. I conclude that even a camera with half this resolution would be more than enough. Would you agree with me? ***Stéphane Nizets nizets2@yahoo.com Tue Oct 6***

That looks like a pretty fair assessment to me. We have an old Pixera camera on our light microscopes. The recorded field of view is between 850 and 1000 µm at 100× depending on the microscope. That's using the 10× objective on our reflected light scope along with a 10× photo-eyepiece. The field of view would be 100 um or less with a 100× objective. That converts to 0.04 µm per pixel at full 5-MP resolution. Indeed, that is overkill. I do not mind oversampling some. However, I would not want to pay much premium to get a 5 MP camera if a cheaper one would capture all the information. Those extra pixels might be helpful at lower magnifications depending on the resolution available with those lenses. I will let you do the calculations for your particulars. I would look at the issue of sensitivity. More pixels mean less light per pixel. The available light is spread around more bins. Of course, you should have the option for binning multiple pixels together to increase the signal and reduce the noise. However, there might be some loss in sensitivity since you have more boundaries and their attendance dead area between pixels. I hope that the dead areas got thinner as the pixels got smaller. If so, that may not be an issue. Warren Straszheim wesaia@iastate.edu Tue Oct 6

From a purely mathematical standpoint you are correct at the 100× magnification. You have just enough resolution at 2×. Remember information encoded in the image is encoded in contrast separation, thus you need a difference in contrast between 2 pixels to carry any information. And then there is actual use. I am assuming this is an interpolated camera where the chip is laid out in a color mosaic, each pixel assigned one color only R,G,or B. Color information is interpolated from adjacent pixels around the one being "read". If you look at crisp edges, a higher resolution interpolated camera is going to have less edge artifact thus images will look a little crisper. Also, redo your calculations with the 10× or 4× objectives and see what you come up with. Bet you are not capturing everything there. Scott Whittaker whittaks@si.edu Tue Oct 6

Thank you for your helpful comment and for correcting me with the fact that one needs 2 pixels to get contrast! The given resolution for the optics at 0.26µm is optimal. I think that in practice most of us probably do not reach it. Actually I was fair in my calculations because (1) the camera does not span the entire field of view (2) the optics is not always optimized - this is the difference between theory and practice, if you already noticed it (3) the "real" resolution (of a labeling for example) is probably lower because of technical constraints (the methodology introduces errors). Now some of you say that the resolution of the camera makes sense at lower objective powers: 10× or 4×. I did the calculations: field of view: 2.5 mm or 2500 µm at best. That means 1 pixel is 1 µm. This is approximately the resolution of the optics. Now you have to agree that one does not image at 10× in order to push up the resolution. At this magnification power, one does not really care about the resolution, it is more to have a large field of view. If you want to resolve objects approximately 1 µm big, you don't do it at 10×, you do it at 63× or 100×! It may be relevant if you plan to project a 10× image on a 2 m × 2 m white wall, which is not very common. Otherwise, I doubt that the eyes would see a difference, or that scientific information would be gained. This means that the gain at 10× is not relevant for me neither. As for the explanation of Scott with regard to RGB pixels, I did not quite get it. Stephane Nizets nizets2@yahoo.com Tue Oct 6

I think your reasoning is good but there are some considerations missing. A definition of resolving power states that resolution is the minimum distance between two points that can be seen as two separate ones. So, you should need three pixels, two for each point and one for the separation. Let's say two dark pixels separated by a clear one. If each pixel is 0.1 µm, the resolution would be 0.3 µm or compatible with the optical resolution stated. Francisco José Kiss kiss@demet.ufrgs.br Tue Oct 6

There's a nice little JAVA app at Nikon's microscopy website that may help in the discussion: http://www.microscopyu.com/tutorials/java/digitalimaging/pixelcalculator If you push the magnification to the highest levels, the best resolution needed is 5 megapixels. Mind you, that's for a monocolor CCD 1" in diameter, where every pixel on the CCD contributes to the image. The Bayer Filter used in nearly every color-sensitive digital camera spaces out the r, g, and b pixels. Thomas C. Trusk truskt@musc.edu Tue Oct 6

The thing I keep forgetting, possibly because it's so counterintuitive, is that the lower the magnification of the system, the higher the pixel count required to capture all the detail. This was first brought to my attention by a little handbook (I think) put out by Zeiss, but a quick Google found a similar explanation (without any calculations). From the Leica website, the page appropriately titled "Beware of Pixel Mania": http://www.leica-microsystems.com/press-media/our-customer-magazines/resolution-pathology-diagnostics/patgology-diagnostics-resolution-may-2009/beware-of-pixel-mania/ Here's the interesting bit: "At low magnification, the microscope is usually able to deliver more details to the camera than it can capture. At high magnification however, it is the optical system that limits the amount of detail that a camera can capture. At 1× magnification the instrument delivers about 14.3 megapixels of information to the camera, while at 16× this figure drops to 2.6 megapixels." As I recall (and all this is from memory), with 100× oil the number of pixels required to capture all the information delivered by the optical system is ridiculously low. So people doing low magnification histology, etc. need the highest pixel count cameras. Jim Ehrman jehrman@mta.ca Tue Oct 6

Since it is numerical aperture (NA) that determines resolution, you don't necessarily need less pixels at higher magnification. For example, if the move is from 40×/0.6 to 100×/1.4, the pixel requirement is essentially the same. Esteban Fernandez fernandezg@missouri.edu Tue Oct 6

The Bayer filter cuts down on resolution in order to achieve color capture. Furthermore, typical pixel sizes are 4-6 µm. Gary Gaugler gary@gaugler.com Tue Oct 6

I believe that should be 2 pixels. That is, if you formulate the resolution question differently ... ie, "How many pixels are required to discriminate 100 particles in close proximity?", you would need only 201 pixels, which allows for your criteria that a pixel be between, but converges on a 2:1 ratio. The OP's query is legitimate, but there is nothing wrong with using more pixels than necessary. I also notice that some of the newer cameras can bin neighboring pixels for reducing noise .. but that may be normal for modern cameras designed for microscopy. Michael Shaffer michael@shaffer.net Tue Oct 6

Core Faculty User Rates

As a manager of a university based TEM facility operated as a service center, I have been asked to look into the matter of adopting a fee structure that would consist of two user rates for the same instrument: a standard hourly rate for infrequent users and a discounted rate for frequent "superí users." Information has it that such, or similar user rate structures, currently exist at other universities. My immediate thought is this will not pass muster with the feds re: you cannot charge different users different rates if they are paying with federal monies. The argument presented to me in defense of the super user rate is we would be offering two products, e.g., single quantity vs. discounted large quantity. My question for the listserv: is anyone aware of any similar university base TEM facilities that have adopted this "two-product" fee structure for the same instrument and would you happen to know if they've (using this scheme) been audited by the feds? Another question for the listserv: what of a proposal for a user rate system that incorporates a maximum cap on the total dollar amount per month for instrument usage? In other wors, all users initially pay the same user rate, but for the frequent users the total cost for the month is limited to a set amount. This still smacks of different rates for different users. I have not been able to locate clear and direct statements in the OMB circular A-21 for a rebuttal to the aforementioned user rate proposals. ***Tom Rawdanowicz tarawdan@ncsu.edu Thu Sep 17***

You can check out the fee structures the University of Sydney charges at the following link. http://atomic.emu.usyd.edu.au/emu/index.jsp As you'll see, the more hours that clients buy, the cheaper it gets. David Mitchell david.mitchell@emu.usyd.edu.au Thu Sep 17

If you are charging back to federal grants, you cannot use a 2-tier structure. The government always gets the "best" rate. If you charge one federal project at one rate and another at a different rate, the feds are not getting the "best" rate somewhere along the way. One possibility is to have all users pay a fixed rate up to a certain quantity,

then a lower rate above that amount. It would be more bookkeeping to keep track of hours below and above the trip point, but I think it would be legal since all the projects have the same rate structure. You may need to talk to your sponsored program lawyers to get a more informed opinion (and to cover yourself). I think Tina Carvalo (tina@pbrc.hawaii.edu) ran into similar issues with rates a number of years ago at U of Hawaii. She may be able to clarify the situation. Henk Colijn colijn.1@osu.edu Thu Sep 17

Over the past three years, we have used an annual subscription approach, where subscribing users who pay over $1000 during the subscription "drive" get an additional 25% match from the lab toward use of the equipment. The matching portion expires after one year. I have also offered subscribers some free sample prep, or unlimited use of our heating stage, which was purchased with state funds. Subscribers are also allowed to sponsor limited use of the equipment to support new proposals at no cost. The subscriber approach helps to draw in a large pool to refresh the account for purchasing the service contract, which cannot typically be met by whatever balance remains in the account. (To give myself some credit, the balance was negative when I got here, so things have improved.) However, we ran into a problem this year with our accounting office, which does not permit pre-payment for services. The result is that our program has had to pay for the service contract, so nobody gets a discount, but we cannot continue this indefinitely. I think the subscriber system makes sense, in principle, because it encourages greater use of the equipment. Unless the university commits to a fund for buying service contracts, the obvious source is grants, and a discounted rate or match give the users some incentive to commit their funds in advance. However, I would not charge different rates based solely on frequency of use. Phil Ahrenkiel phil.ahrenkiel@sdsmt.edu Fri Sep 18 MT

https://doi.org/10.1017/S1551929509991088 Published online by Cambridge University Press