NetNotes

Edited by Thomas E. Phillips
University of Missouri

phillipst@missouri.edu

Selected postings from the Microscopy Listserver from July 1, 2010 to August 31, 2010. 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:
section adhesive

I seem to remember that one can dissolve "Scotch tape" in acetone to make a glue for helping to adhere sections to grids. Does anyone remember how much tape per unit of solvent? ***Geoff McAuliffe mcauliff@umdnj.edu Thu Aug 19***

Re: Palade, D.C. Histologic Techniques for Electron Microscopy, 2d Ed., 1964, Academic Press, NY, NY., p. 208. "Unfortunately, carbon films do not adhere well to copper grids. However, it is possible to pre-coat the grid wires with an adhesive that helps. This author has, from time to time, used an extremely dilute solution of rubber cement in carbon tetrachloride. Or, the adhesive on a short length (about 4 in.) of cellophane tape, can be dissolved in an ounce of chloroform. The grids should be dipped in this and quickly dried on filter paper. The adhesive must be so dilute that there is no tendency for it to form films or strands across the grid openings." I have seen this recipe using xylene/toluene on double-stick tape by immersing the tape and removing it after some time—but I cannot remember the source. Fred Monson fmonson@wcupa.edu Thu Aug 19

Here is the prescription from "Electron Microscopy in Molecular Biology": Dissolve the glue from 10 cm of adhesive tape in 50 ml of chloroform. Store the solution in a dark bottle with a tight-fitting stopper. ... citation: Sommerville, J.; Scheer, U., eds. *Electron Microscopy in Molecular Biology: A Practical Approach*. Oxford: IRL Press, 1987, pp. 147–148. Some comments: We use Scotch Magic Tape and dissolve the glue from the tape for one or two minutes. The rest of the tape is then removed with tweezers. Finally, the "sticky solution" is filtered through dry filter paper. We are using quite a recent brand of Scotch tape: Green box, Scotch Magic No. 810 (invisible); 19 mm × 33 m. It seems to work. Oldrich Benada benada@biomed.cas.cz Fri Aug 20

This is a home-made recipe. 1. Take 15 cm tape as it is used by decorators and cut it in 1 cm parts. (Don't stick them together). 2. Fill a beaker with 15 ml chloroform and place the 1cm-pieces inside under stirring. Stir for 1 minute. 3. Cover the beaker with aluminum foil and keep it in the hood for 90 minutes. 4. Decant the solution and centrifuge in a resistant tube (Falcon) at 1300 rpm at a bench centrifuge to get cellulose cut off. 5. Take the supernatant with a glass pipette and transfer it in a glass for storage. 6. Dip the grid briefly in the solution and let it dry while you hold it in a horizontal position. Dilute the solution if it clogs the grid space. Stephane Nizets nizets2@yahoo.com Fri Aug 20

About 10 cm in chloroform works for me. Just shake for a few seconds and you can remove the tape. Julian R. Thorpe bafg3@sussex.ac.uk Fri Aug 20

Specimen Preparation:
bacteria for ESEM

We recently purchased an FEI Quanta 250 SEM that is capable of ESEM. We are trying to image bacterial cultures (E. coli and B. cereus) and can't seem to see anything. We've looked at many papers to get starting parameters and tips, and still no luck. We've tried the following: 1) using the cold stage and ESEM mode, we've tried the following parameters: temp 4–5C, 300–600 Pa, RH 50–90%, 5–20 KV, spot size 4–5. We've tried a) smearing the culture on a coverslip and attaching it to the cold stage, b) adding the culture to filter paper (0.4 and 0.2 um), and c) directly dropping culture into the cold stage stub. 2) using low vac, smeared the culture onto a coverslip. Parameters were 10 KV, 130 Pa, and spot size of 4. We also tried adjusting all these parameters up and down and still no bacteria. 3) using high vac, smeared culture onto a coverslip and coated with Au. We also coated filter paper with sample on it. Parameters were 12.5 KV and spot size of 4. We tried adjusting these parameters up and down and still no bacteria. Does anyone have any suggestions? Any input is greatly appreciated!! Ideally, we don't want to have to stain, fix, or wash the bacteria. ***shafermr@whitman.edu Thu Jul 8***

Before going with ESEM, you need to get reliable results in high vacuum. It looks like your problem is your culture medium. When you smear culture on a substrate, after drying out you will have bacteria embedded in dried salts, sugars, proteins. Since SEM is able to see only the surface of a specimen, bacteria will be blocked by a layer of dried media components. The same is true for ESEM: we can see only the surface. If your specimen is submerged in a water solution, you will see only the surface of the solution. You need to dry out the solution, so that bacteria will be above it, but then again, it will be covered with dry components of the media. If your culture can survive for a while in distilled water, then you have a good chances to see it in the ESEM mode. Put a drop of distilled water with bacteria on the specimen stage (keep the stage far from the objective lens and EDS detector during initial pumping, otherwise droplets from the degassing water may damage or contaminate them). Changing pressure or temperature let the drop evaporate slowly, and you can observe your specimen. For best results you will need to fix it. If the culture cannot tolerate pure water, then you have to fix it. Good luck, Vladimir Vladimir M. Dusevich dusevichv@umkc.edu Thu Jul 8

If the bacteria cannot tolerate distilled water due to ionic strength and/or pH, you could try NH_4HCO_3 or NH_4Ac, which are volatile buffers that evaporate along with the water, so there is no residue. Bill Tivol wtivol@verizon.net Thu Jul 8

Specimen Preparation:
carbon-coating with sputter coater

Has anyone successfully carbon-coated Formvar films using a sputter-coater with a carbon filament? I hear the carbon can be uneven and leave a chunky mess. If you have refined this technique I'd like to hear from you. The vacuum evaporators on campus are down at the moment so I am seeking an alternative method. ***Beth Richardson beth@plantbio.uga.edu Tue Aug 24***

Just a quick note about carbon film quality. Many years ago I worked with Edwards high vacuum on a joint project where we tested all the different vacuum methods for making a carbon film. One point stood out above all others, the better the vacuum the better the film! Run your sputter coater to try to obtain the highest vacuum possible prior to coating. Steve Chapman protrain@emcourses.com Tue Aug 24

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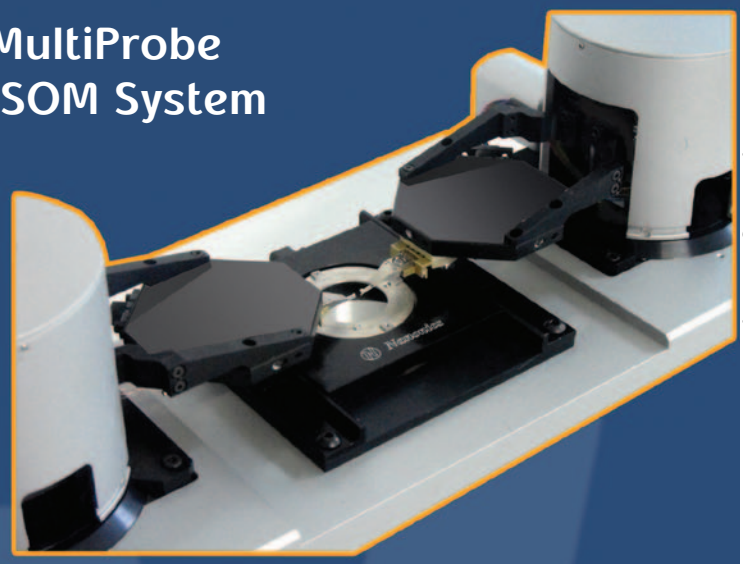

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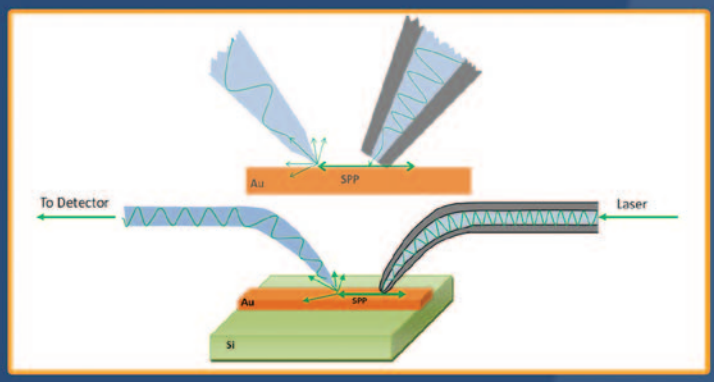

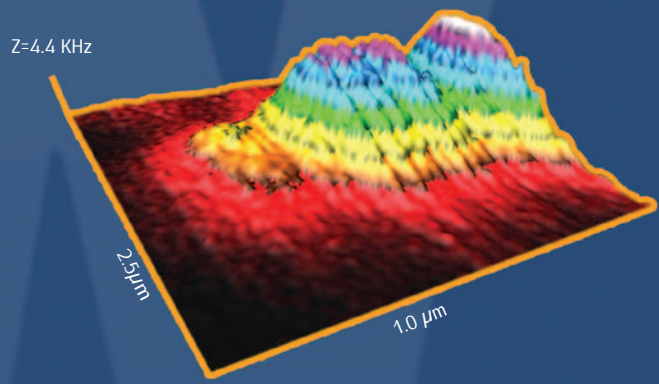

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Been thinking a little more on your behalf. Use the type of carbon string that is like an old fashioned boot lace, it's almost a round tube. They normally burn out in about 4 seconds so run for just 3; in the last second the string throws carbon rocks at the specimen! Steve Chapman protrain@emcourses.com Wed Aug 25

Specimen Preparation:
cutting Silica glass using FIB

Any suggestion for cutting a 30 µm thick Silica glass that has been coated on a Si wafer using the FIB? ***Alex Chou chou_msa@yahoo.com Wed Aug 25***

The thickness of the film will provide a challenge with the milling time. Using enhancing etch chemistry can speed up the process. Depending on requirements for precision of your cut, and the size of the work-piece there might be several solutions: 1) Use IEEE etch to speed up removal of the trench in the SiO_2 if the desired FIB cut is not much wider than the thickness (i.e., 30-50 µm). 2) If you need to produce a cross-section at a precise site, perhaps micro-cleaving the wafer piece to the site or nearby can lead to a suitable result. 3) There are fast speed ion milling tools that use hard-mask approach to produce cross-sections. 4) A wafering saw could be used with micron-scale precision to cut the film near your feature. Jerzy Gazda jerzy.gazda@ceriumlabs.com Wed Aug 25

1. You may have to a do thick coating if it does not affect your application. 2. For some platforms/setups, you can fire the electron beam simultaneously to neutralize the i-beam charging. The e-beam current has to be 3–5 times of the i-beam. Some details: D J Stokes et al 2007 J. Phys. D: Appl. Phys. 40 874 3. Some platforms have a gas needle based neutralizer. Jing Fu jing.fu@monash.edu Thu Aug 26

Specimen Preparation:
re-using osmium

I recall reading somewhere or hearing from someone that the OsO_4 (used for fixing TEM samples) can be reused by adding some hydrogen peroxide. Could someone tell me if this is true and if anyone has experience in reusing the OsO_4? It is quite valuable, so if this is true I'd like to save as much as possible. ***Josif Mircheski jmircheski@us.es Thu Jul 29***

It is possible to re-oxidize OsO_2 or osmium chloride back up to OsO_4. Years ago, the late Dr. Chuck Garber told me it was "impossible." I read an item on the Internet from the Asian subcontinent that said essentially that an unopened bottle of OsO_2 powder had a black Os stained PE bottle cap liner. So it appeared to me that he was wrong and the cap was slowly stained with OsO_4 over a few years. In my research, I found that the reaction will proceed but it is extremely slow with even strong oxidizers. There is some rate determining step that is slowing the reaction down, in my opinion. I had no proof back in 2001 other than a very slow reaction rate. Is this practical? Not really and I surely tried all sorts of common oxidizers. As for using hydrogen peroxide with Os, I wouldn't! For me, osmium dioxide catalyzed the decomposition of 30% H_2O_2 as a very violet foaming reaction back in 2001. That's just a little heads up safety tip. Mixing Os salts and the oxidizer H_2O_2 is a not a good idea. Paul Beauregard beauregа@westol.com Fri Jul 30

I agree with Paul: because of the redox potential for the OsO_4<>OsO_2 reaction a very strong oxidizing agent would have to be used to regenerate OsO_4 from reduced solutions (used fixative). Even if this were to work, this would require serious safety precautions. Secondly, the mix would have to be purified after the regeneration. However, there is a procedure to recover "unused" OsO_4 from used fixative by distillation. I have done this on a regular basis in the grey past, would have to search for the detailed procedure. Let me know if this would be of interest, I could have a go at it. Before embarking on such an adventure, please be aware that such distillations need to be well-controlled as well because of the chemical hazard posed by OsO_4, especially in the vapor phase. Jan Leunissen leunissen@aurion.nl Fri Jul 30

A detailed description how to re-distillate used OsO_4 is given in: *Swiss Chem* 8 (12):43–44, 46 (1986). However, in my opinion, the apparent and practical efforts are in no proportion to the price you have to pay for a new supply. I recall that in the 70's we got money for our Os-waste from Degussa (a German company specialized in rare metals) but nowadays in my experience no company even takes the waste back for free. Peter Heimann peter.heimann@uni-bielefeld.de Fri Jul 30

Specimen Preparation:
polymer for TEM

I'm trying to get a TEM image of quantum dots embedded in polyacrylate polymer. I wanted to use a microtome to get thin sections, but the manager I contacted thought the resin might dissolve or in some way alter the acrylate. What would be the best way to go about treating the sample so that I can analyze it? ***John Katahara john_katahara@brown.edu Wed Jul 21***

You do not specify the form of the polyacrylate, but if it is a block, I assume you would just have tried to section it. If the polyacrylate is in small beads, which would need to be further embedded in order to be sectioned, and if the shape of the polyacrylate needs to be preserved (hence the manager's concern), one possible solution is to embed the specimen in amorphous ice using a high-pressure freezer and cryo-sectioning it. I think that there is such equipment within a few hours drive of Brown, and it may be possible to arrange to have your specimen prepared. Bill Tivol wtivol@verizon.net Thu Jul 22

Specimen Preparation:
electro-polishing Ni-based superalloy

Can I ask for some advice on TEM sample prep using jet electro-polishing? Sampe is Ni based super alloy with a content of gamma prime. We were electro polishing in 5% perchloric acid in methanol, using a Struers TenuPol, –40°C, flow rate of 10 or 8 and a voltage of 30 or 35 V. We are struggling to get a large enough transparent area for any useful TEM. We are either getting many small holes or 1 massive hole . . . very little transparent area. Any advice is highly appreciated. ***Zhou z.zhou@sheffield.ac.uk Tue Jul 13***

The recipe you are using should give you good results. I have used it on pure nickel and austenitic stainless steels of many different kinds with considerable success. However, a couple of things you can play with are listed below: You mention using a voltage of 30 to 35 V. Many electropolishing recipes suggest a particular voltage, when in fact they should specify a current. It is current that will control the rate of electrochemical dissolution of your specimen rather than voltage. In fact, the voltage will vary with the age of your solution. As you polish more specimens, the metal ion concentration will increase and the resistance of the solution will change (generally fall). Therefore, if you are keeping voltage constant during your preparation, you will be using higher currents as you continue polishing. The current you to use should be around 120–180 mA for double sided thinning. Generally I start at around 120 mA and then for subsequent specimens I increase the current in steps of 20 mA until I hit a sweet spot. I then stick at that current and during electropolishing endeavor to keep the current constant by varying the voltage. If you use too little current, you will effectively anodize your specimen and it will be covered in oxide and will be generally poor. If you use too much current it will perforate very rapidly—you won't be able to control it, and you'll

Microscopy Society of America Awards

Nominations are now open for the Microscopy Society of America Annual Awards. The awards process is one way in which the Microscopy Society of America recognizes the significant and diverse contributions that individuals make to our field. Deserving nominations for consideration should be submitted online no later than December 15th, 2010 to:

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The Main Society Awards Are

Distinguished Scientist Awards

These Awards recognize preeminent senior scientists from both the Biological and Physical disciplines who have a long-standing record of achievement during their career in the field of microscopy or microanalysis.

Burton Medal

The Burton Medal was initiated to honor the distinguished contributions to the field of microscopy and microanalysis of a scientist who is less than 40 years of age on January 1st of the award year. (Please note the change in the selection criterion regarding age.)

Outstanding Technologist Awards

These Awards honor technologists from both the Biological (Hildegard H. Crowley Award) and Physical Sciences (Chuck Fiori Award) who have made significant contributions such as the development of new techniques which have contributed to the advancement of microscopy and microanalysis.

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This Award was initiated to recognize outstanding volunteer service to the Society as exemplified by Mort Maser, who served the Society for many years with great dedication. This award is made to honor an MSA member who has provided significant volunteer service to the Society over a period of years.

Further details of the nomination process can be found on the society webpage at:
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end up with a specimen which looks like an aperture—large hole, no thin areas. Nickel is ferromagnetic, and your superalloy may be too. If so, you'll want to make your blank foils as thin as possible. I generally aim for around 100 µm for non-ferromagnetics but grind to 60–80 µm for ferromagnetics. This helps minimize aberrations in the microscope. You do need good metallographic practice to make sure you do not inject a lot of mechanical damage into such thin specimens and obviously handle with care to avoid bending. These foils should be perforating after perhaps 20–40 s of electropolishing. If this is occurring in much shorter times than this, then you may be using too much current. In terms of flow and stop sensitivity. I always leave the termination sensitivity on maximum. Flow is of second order importance. On a Tenupol 3 I set the flow rate to about 1/3rd full scale. A couple of other points—as soon as the termination alarm sounds, remove the specimen and plunge it into a beaker of methanol and then wash in fresh methanol as quickly as possible. Thin areas of foil will corrode very quickly. Finally, single-phase materials in the annealed condition generally electropolish beautifully. Introduce second phases or cold work and you can get local perforations around the second phases or pitting along dislocations. Your foil can end up looking like an Australian road sign in the outback (peppered with bullet holes). You may have to settle for what you can get. Finally, even when you get a good recipe, what works today, may not work so well tomorrow. Electropolishing is one of the more perplexing mystic arts. Dave Mitchell drg.mitchell@sydney.edu.au Tue 7/13/2010

Specimen Preparation:

cleaving sample

I am trying to view the thickness of a Si/SiC superlattice that I have been laying down on some Silicon wafers. The images from the SEM show a messy surface (debris) near the superlattice as a result of the method used to break the silicon along its cross-section. I use a diamond scribe to scratch the backside of the substrate and then a corresponding force to break the silicon piece along the scribed line. What is a good method for breaking this silicon substrate to view the cross-section and get adequately clear images of the coating thickness without having debris from the break? ***Andrew Kustas abkustas@rams.colostate.edu Tue Jul 27***

A method that has worked on occasion for me is to scribe (diamond scribe) a short line (4–5 mm) on the front side oriented, as best as I can eye-ball, to a ⟨100⟩. Then cleave with firm but constantly increasing pressure (surprise break style) on a pair of glazier's pliers (e.g., Fletcher-Terry Co 6" Glass Cutter Pliers No. 06-111). For smaller pieces, just a nick on the edge with thumb pressure at the ends and a piece of W wire as a fulcrum works well. This approach works well with a number of thin (and not so thin) films on Si wafers. John Heckman jwheckman@earthlink.net Tue Jul 27

Immunocytochemistry:

etching

In the ongoing quest to improve our immuno-results, and learn more about working with Lowicryl HM20, we have come across a number of papers recently where people have pretreated their Lowicryl HM20 sections before immuno-labeling with saturated NaOH in absolute ethanol, for 2 to 3 seconds. This is followed by a wash then preincubation in 0.1% sodium borohydride. This does seem like quite harsh treatment, and I am curious as to the purpose of the saturated NaOH step, and its origins. Have any of you come across this before? Has anybody got the original reference? ***Allan Mitchell allan.mitchell@stonebow.otago.ac.nz Thu Jul 29***

Sodium borohydride is usually used to reduce autofluorescence. I have had modest success with it in the distant past. I have etched lots of Epon sections with sodium ethoxide (NaOH saturated ethanol). I usually did this to increase staining of osmicated tissue with hematoxylin and PAS or safranin O. I have tried it with Epon for colloidal gold labeling and it may have increased the signal but it increased the noise much more. I have never tried it with the Lowicryls. I believe HM20 was originally conceived as a more hydrophobic version of K4M so theoretically shouldn't be as good for immuno-staining but I also found it as good as K4M and much easier to section. However, if you are doing LM work with 0.5 µm sections, I strongly recommend butyl-methylmethacrylate since you can extract all the resin with acetone. I haven't done LM immuno on sections with Lowicryl or LR White or LR Gold resins since I discovered BMMA. It may work at the EM level but my very limited tries weren't especially promising. For EM, I usually use LR Gold but often try them all. Tom Phillips phillipst@missouri.edu Thu Jul 29

I am probably not the best IHC specialist of this list but here is what I understand: Ethanolic solutions of NaOH are used for etching, which is revealing the antigenic sites embedded in the resin. This is indeed a harsh treatment because it is not easy to etch the resin! For this reason, the treatment must be very brief and must be followed by thorough washing. All treatments have a downside and clearly etching may destroy some antigenic sites too, so for some it works and some others not. As for borohydride, I am not sure but this is a reducing agent and it may be used to reduce the free aldehyde groups present from the fixation with glutaraldehyde. Apart from that it may also simply open certain bonds that can hamper the immunological reaction. Stephane Nizets nizets2@yahoo.com Fri Jul 30

Immunocytochemistry:

background issue and immersion vs. floating

I'm trying to label some root nodules and have noted in 4 separate samples that I'm getting apparently specific labeling of what appears to be starch granules, with manageable background in other parts of the tissue. That part is fine. However in all samples when I get off the sections (LR White, UV polymerized in an icebox), I am finding very even and heavy immunogold binding to the carbon support film. No Formvar, just carbon. Nickel grids. The controls incubated in buffer instead of primary antibody are clean. Any idea what's going on here? I'd be interested in people's thoughts on the deep, philosophical and practical ramifications of floating grids on drops vs. full immersion of grids in drops during the labeling process. I come from a long line of floaters, but some of my best friends are immersers. Is there a true way or is it all good? ***Randy Tindall tindallr@missouri.edu Fri Jul 30***

When I was doing immunocytochemistry on LRG thin sections, I found a significant advantage of immersing over floating. I do not know if it was 2× as much labeling but maybe. Have you tried acetylated BSA or cold water fish gelatin in your blocking buffer? I also like to add 1% normal serum from whatever animal the secondary was made in. I am a big fan of long incubations for primary and secondary staining and often use 4 hrs to block before starting overnight incubation in the primary. Unrelated to the problem you asked about but often with polyclonal primaries on plant material, it pays to pre-absorb the primary on a control tissue extract if the antigen is a transgenic expressed protein or some bacterium not present on normal nodules. Thomas E. Phillips phillipst@missouri.edu Fri Jul 30

Image Processing & Analysis:

electron diffraction patterns

I would like to know if there is software that can calibrate the distortion of electron diffraction patterns that were recorded by Digital Micrograph. I want to calibrate the distortion in all the directions from the central spot. I will use a ring pattern (Au) for a standard. ***Hiromi Konishi hkonishi@wisc.edu Thu Jul 1***

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Capitani et al. wrote an interesting paper on the topic of diffraction pattern distortion (*Ultramicroscopy* 106 (2006) 64–74). They measured the distortion using an Excel spreadsheet. You might contact the authors and see if they will share their spreadsheet with you. I am unaware of a DigitalMicrograph-based solution for this measurement. That said, it might make an interesting addition to DiffTools (www.dmscripting.com/difftools). I'll have a think about developing one. For simply estimating whether visible distortion is present you might experiment with the 'Locate SADP Centre' tool which overlays a series of rings on the pattern. The CHT Diffraction Analysis tool (available from the above web site) can also be used to find the centre of a polycrystalline pattern and if elliptical distortion is present in the diffraction rings, then this will be very evident in the Hough transform images. Unfortunately, it does not quantify the distortion. Dave Mitchell drg.mitchell@sydney.edu.au Thu Jul 1

I found a script on the Digital MicroGraph Script Database hosted by the Graz University of Technology that does what you are asking. The link to the script to calculate distortions in the diffraction pattern introduced by the projector lens stack is here: http://felmpc14.tu-graz.ac.at/dm_scripts/freeware/programs/Diffraction-Rings-Distortion-Analysis.htm A link to the database is here: http://www.felmi-zfe.tugraz.at/dm_scripts/dm_scripts/ The summary of the script's function is as follows: "This is a script package to characterize diffraction ring distortion in TEM. Due to the distortion introduced by the TEM projection lens, diffraction rings obtained from a fine crystalline sample are of elliptical shape instead of round." I have no connection to this script nor have I tried it so I can't comment on its function, but it seems to do what you want. There are many other scripts in the database should this one not be what you're looking for. Christopher Winkler microwink@gmail.com Thu Jul 1

Teaching:

sand

I am trying to get a lesson together for some local high schools. I am using the LHS GEMS Microscopic Explorations for inspiration. One of the exercises uses sand to tie in microscopy, geography and observation. I have two samples of sand, one from Hawaii's North Shore and maybe one or two from around the Santa Cruz, CA area. Anybody have some from a collection to spare or willing to get some and send it to me? ***Krupp jkrupp@deltacollege.edu Thu Aug 19***

MSA's Project MICRO has a sand pile, ably managed by Heidi Ullberg of McCrone. You'll find it at http://www.microscopy.org/education/projectMICRO/classroom.cfm Caroline Schooley schooley@mcn.org Thu Aug 19

Indeed, I have been humbled. Take a look at the list available, it will blow you away. Jon Krupp jkrupp@deltacollege.edu Thu Aug 19

EM:

floor stability

We will be installing a new 200-KV LaB_6 TEM within the next few months, and our university has allocated a nice lab in the basement of our older (ca. 1972) building for the instrument. However, the environmental survey showed an on-going, 18-Hz, 25-μm vertical oscillation in that room due to the building's HVAC. Also, the floor lacks resiliency in some areas, maybe due to shifting over time of the soil below, so occasional, nearby foot traffic could affect image stability. The survey company offers an active vibration-cancellation system to remove the low-frequency noise. Though I am not opposed to this, my first priority is to address any issues with the building foundation before installation. Therefore, I have spoken with several contractors about the possibility of forming an isolated region of the floor to support the TEM. My understanding is that the vibration propagates readily through the rigid cement floor, which lacks sufficient mass to damp the oscillation. It would seem that the ideal floor has 1) large mass and 2) weak coupling to the driving force. One practical possibility I investigated is to install a detached, separate section of floor on concrete piers, set deep (12–20 ft) into the ground, upon which the TEM column would rest. Now, I am not a civil/mechanical engineer, but my naïve impression is that if the piers extend deep enough, the earth would essentially act as a massive load. The underlying soil is relatively soft, so the vibrations from the building would not substantially propagate through the earth from the building to the TEM. In addition, this should decouple the column from the higher-frequency traffic in the building. The employee of the microscope manufacturer with whom I spoke was adamant that this was a ridiculous idea, and would actually make the vibration worse. He believes it would require an impractically massive foundation to impede these oscillations, that the earth would not damp the vibration, and that the active cancellation system is our only option. Of course, we will consult with engineering experts before cracking into the building foundation, but I am sure a number of people out there have dealt with these issues, and have opinions on how to handle such things. Sure, I would like a new, custom-designed building—heck, throw in an aberration-corrected FE-TEM while you're at it—but that isn't reality. All I am looking for is one small patch (~1 m^2) of vibration-free floor. ***Phil Ahrenkiel phil.ahrenkiel@sdsmt.edu Thu Jul 29***

We've had a lot of discussion with Michael Gendreau of Colin Gordon & Associates about the merits (& demerits) of isolation slabs, i.e., small patches of floor that are mechanically 'decoupled' by use of some medium with a very different mechanical impedance such as elastomer, air, etc. However, I know our circumstances are different (new building vs. already existing building), but there are a few simple principles that Michael's analysis demonstrated beautifully (using data taken from our already existing isolation slabs). An isolated slab is, in itself, a resonator with resonant frequencies determined by the density and elasticity of the material as well as the spatial extent (modes of oscillation). Generally, the bigger and heavier the mass, the lower the resonant frequency: a large single mass is better than lots of little isolated slabs if you need the resonant frequency shifted downwards. Isolated slabs are ineffectual below their resonant frequencies (the wavelength of the vibrations are much longer than the slab dimensions and the slab moves easily), they amplify the vibrations in the vicinity of the resonant frequency* before giving attenuation with approximately a $1/f$ dependence for high frequencies. Given the 18 Hz oscillation you have, you would probably need a very large and deep mass to get a resonant frequency low enough to get some attenuation, probably something much larger than the 1 by 1 m^2 you're suggesting. If the noise is a relatively sharp tone (single frequency), the active noise cancelling system would probably work. If the noise is spread over a wide band of frequencies, it is going to be much harder to remove. You said that source appears to be HVAC. Could the air-ducts be retro-fitted with noise-damping baffles? Or the duct-work re-configured to remove most of the flow away from your workspace? In our case we had amplification factors of between 3 and 10 times for different microscope/slab systems. Jon Barnard jsb43@hermes.cam.ac.uk Tue Aug 3

TEM:

beam drift after filament change

I installed a new filament in our Hitachi H7600 yesterday and all seemed well after gun alignment etc. However, today it appears that the beam just wants to wander its merry way around the screen as if we were merely suggesting it stayed centered, rather than it being steadfastly forced into place by the strict electrostatic and magnetic forces the microscope imposes on it. The wandering isn't a great distance mind you, just a few cm this way or that, tending to fluctuate in a roughly diagonal path across the screen. The drift is mostly a very

smooth and slow motion, but occasionally the beam will jump 5 mm or so, and then continue on its lazy drift. Also, it doesn't seem to drift back and forth from one point to another, just away from center when you place it there with the beam shifts. It then goes off in one direction and stays put when it moves as far as it wants/needs to. This causes a bit of a problem when imaging at higher magnifications as you might imagine. We suspect that we may have a box of questionable filaments, since we are only getting between 60 and 140 hours out of them without any obvious change in microscope operating conditions. Gun vacuum seems good at around 4 × 10^{-7} mbar.) Are there any other obvious things I can check that might be causing this? Help me pin down my wayward beam! ***Bradford Ross bnross@interchange.ubc.ca Tue Aug 10***

I strongly suspect that you have a bit of "crud" (technical term) in your column that is charging. It may be as simple as piece of dust or lint that entered when you were changing the filament or it may be something that was in a harmless place but got moved when the gun was vented and/or re-pumped. There are two hints in your description. The first is that the beam drifts, you re-center it and it drifts again (in the same direction?). The second is the occasional jump in position. Sounds like a discharge from some nonconductive "crud." Clean the column. Ken Converse kenconverse@quality-images.biz Tue Aug 10

This looks like a classic charge situation. There must be a piece of dirt charging in your column which will act as a deflection coil as the charge grows and as it discharges the beam will fall back to its original position. The more erratic the movement is the poorer the contact between the dirt and the column; a distinction between a hair and a lump of material. A column clean would be my suggestion. Steve Chapman protrain@emcourses.com Wed Aug 11

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