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Selected postings from the MSA Microscopy Listserver (listserver@msa.microscopy.com) from 04/15/08 to 06/15/08. Postings may have been edited to conserve space or for clarity.

SPECIMEN PREPARATION – acetonitrile as a dehydration agent

I remember well that acetonitrile (AN) has been advised as a substitute for propylene oxide (PO) between ethanol dehydration and Epon embedding. I have always used ethanol and then PO until now but I am eager to use AN instead. Now I wonder if I could not use AN for dehydration too, so it could be used all along! For dehydration factors like penetration time, lipid extraction and hardening of the tissue are important to take into account but having no experience with AN, I have no idea. Do some of you use AN for dehydration and Epon embedding? Are there differences between ethanol and AN dehydration (time, hardness,...) to take into account? Also, I have to split the whole procedure into 2 days, usually I stopped in ethanol 70%. Where should I stop until next day with AN? Stephane Nizets nizets2@yahoo.com Apr 17

I have only ever-substituted acetonitrile in place of the PO, I've never tried to use it to dehydrate, only for the infiltration steps. It works great for that purpose. Jo Dee Fish jfish@gladstone.ucsf.edu Tue Apr 29

I tried dehydration and Epon embedding with only acetonitrile as agent. I used it instead of ethanol dehydration then PO (propylene oxide) then Epon in my classical protocol. Well...it didn't work. The sections contain holes or cracks that really look like bad polymerization. Especially artifacts (holes or cracks) are visible along the nuclear envelope and with lipidic organelles like the mucus-filled vacuoles of goblet cells (I tried with pieces of gut). Nothing is visible in LM on semi-thin sections, only under the TEM. Apart from the artifacts, the morphology is excellent and the contrast too. I can only guess that acetonitrile dehydration requires longer or more frequent steps than ethanol. My dehydration protocol for small pieces of organ in ethanol is 2x10' per step (50%-70%-95%-100%) and I did the same with acetonitrile. I could of course wash 3x or extend the incubation time but I wish not to experiment further with acetonitrile. Stephane Nizets nizets2@yahoo.com Apr 29

SPECIMEN PREPARATION - wicking or blotting grids

The following replies were made in response to a query about the best material with which to blot grids.

Whenever I had something like that being necessary, for instance for removing surplus methylcellulose over ultrathin cryosections, I used wet filter paper, or wet tissue. That will remove the solution to be blotted off very slowly. Jan Leunissen leunissen@aurion.nl May 19

If speed is an issue, you could try using 'paper points'. These are thin enough (there are different sizes so that you can pick the correct wicking speed) that you can have lots of control over the process. David Elliot elliott@arizona.edu May 19

The only materials I have experience with of that wet slowly and are brittle are nitrocellulose (and similar) membranes, used for protein/RNA/DNA blots or in micropore filters. The wet filter paper idea seems the simplest and cheapest, though! rosemary.white@csiro.au May 19

Living multiple lives as both microscopist and molecular virologist I think I've probably used most options for doing negative staining, and have done all sorts of transblotting. The first thought would have been points or filter paper cut so that you had no loose fibers for wicking as with Dave Elliot's point. However, Rosemary's observation is probably best. Your description of the brittleness and flow rate for what the post-doc used fits something like Zetaprobe right down the line. Go over and borrow a little from your local contact in the molecular biology group and try it. It should do the job. Paul R. Hazelton paul_hazelton@umanitoba.ca May 19

I don't know what your original material might be but I have tried many different filter papers for the purpose of slow and even wicking of grids and have found that a #40 Whatman has a nice rate. Bob Underwood underwoo@u.washington.edu May 20

As already suggested, wet filter paper (Whatman Qualitative grade "1") is ideal. If you hold the piece of wet filter paper at an angle so that it contacts the edge of the underside of the grid that also helps to pull the suspension down rather than straight to the edge. Ted Dunne drteddunne@yahoo.com May 28

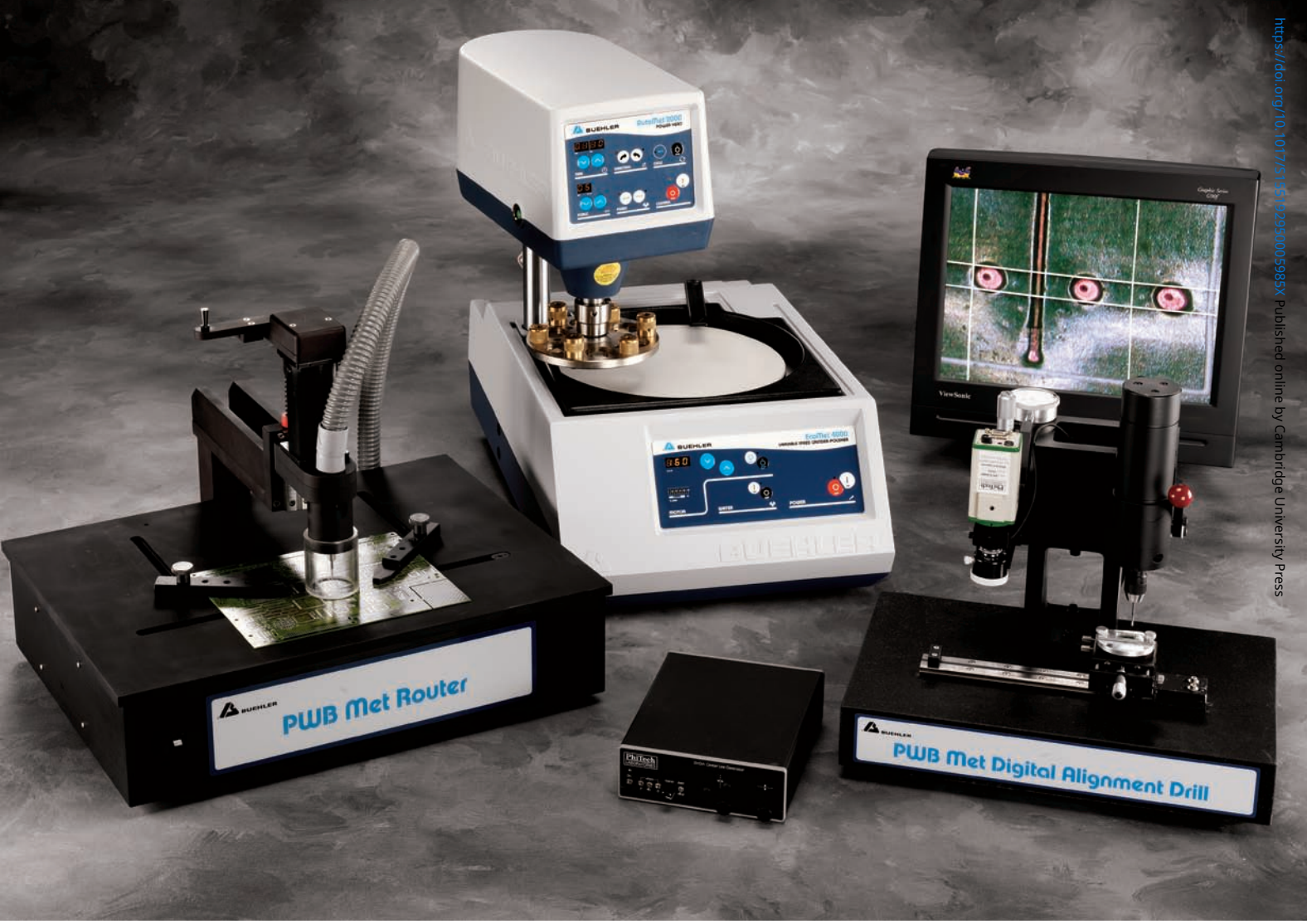
SPECIMEN PREPARATION - sample mounting

What is a good method of attaching a silicon chip to an aluminum SEM stub, so that: a) the electrical contact is good, allowing high-resolution imaging (50 kx or higher); and b) the mechanical stability is good, so that the specimen can survive shipping? I am aware of the following materials: colloidal carbon in isopropyl alcohol conductive carbon adhesive tabs and I am curious about the experience of other microscopists. Don Chernoff donc@asmicro.com May 9

Normal way is to use carbon double sticky tabs. If the die is SOI, then coat the outer edges with colloidal Ag. This will prevent it from charging. For shipping, single stub storage tubes work well. Another mounting method is to use colloidal Ag to mount the die. Care is needed to avoid wicking over the edges of the die. Gary Gaugler gary@gaugler.com May 9

We have had problems in the past with high-resolution imaging (>100,000x) of samples secured with double-sided carbon adhesive discs, although I use them all the time for routine, low-magnification work. At high magnifications, the sample crept under the beam. I use cyanoacrylate ("super glue") to hold the sample securely, then a bit of colloidal carbon in one corner to ground them. Unfortunately, you cannot then recover them. I have also used a hot-glue gun to secure samples, again grounding with colloidal graphite. Soaking in acetone later will remove the colloidal carbon and loosen the glue, so the sample can be removed. Mary Fletcher maryflet@interchange.ubc.ca May 9

I will second Mary's problems with double-sided carbon tabs. I have the same trouble with double-sided carbon tape. I use colloidal carbon paint to mount my samples for high magnification work, making sure the paint is fully dry before I put the sample in the SEM. I've also used Mary's SuperGlue suggestion. Use minimal glue and ground with carbon paint. CrystalBond thermal adhesive works well and has the added ability to be removed with acetone. Becky Holdford r-holdford@ti.com May 9



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I too have experienced stability issues with carbon tape. In my line of work, the Z axis is also critical and carbon tape contracts for many hours after the SEM is evacuated. I have had very good results with putting the silicon chip directly on an aluminum sample holder and using single sided SEM compatible copper tape with nickel colloid adhesive to tape it down. I use my tweezers to burnish the tape onto the chip top, edges, and aluminum holder. The conductive adhesive on the edges of the chip gives a good electrical connection. One nice feature of this technique is that you do not have any solvents that have to dry before imaging. The negative side of this technique is that some part of the chips top will have tape covering it and adhesive residue is left if you peel off the tape. Acetone will remove the residue. I have successfully shipped samples all over the world that have been prepared this way. Richard Stallcup rstallcup@zyvex.com May 11

Single sided copper tape works fine. I have also successfully used double sided copper tape on the aluminum stub instead of the double sided carbon tape. Smitha Dronavilli vvi@cypress.com May 12

SPECIMEN PREPARATION – drying carbon paint for SEM

I use a hot glue gun to stick my samples to the stubs, since I have found in the past that carbon paint is not always a reliable glue. Just paint over the glue with a bit of carbon paint, after the glue is set. Mary Fletcher maryflet@interchange.ubc.ca May 28

Depending on the sample type I use either of three methods: A cheap hair dryer, an old vacuum chamber, or a heat lamp. Coming soon method #4: New lab poster! "For urgent work, give me one week. For impossible demands, give me one day". Evelyn York eyork@ucsd.edu May 28

The use of carbon paint to mount specimen-carrying stubs in a scanning electron microscope is a potential source for a serious vacuum problem, as described on page 76 of my book, "Vacuum Methods in Electron Microscopy". Often the user smears the entire under surface of the specimen stub with a thick coating of carbon paint, places it on the microscope stage, waits a few minutes until the visible ring of paint around the bottom edge of the stub appears to be dry (assumes all of it is dry) and proceeds to pump the instrument out. What usually happens then is that this user comes around complaining that the SEM's vacuum system is not functioning properly because it is taking an abnormally long time to pump down. What happens in a situation like this is that whereas the visible ring of carbon paint around the bottom edge of the stub appears to be dry, the large quantity of paint underneath the stub remains un-dry, and the solvent that it contains is slowly diffusing out through the "dry ring" causing the rate of evacuation to slow down markedly. A better approach is to set the stub on the stage dry, and then to place a few small isolated dabs of carbon paint around the bottom edge of it. Three or four such small dabs will dry quickly, and if allowed to become truly dry, will usually hold most stubs in firmly place. There will then be no trapped paint to bleed solvent into the vacuum system, and operation should proceed smoothly in a normal manner. -- Wilbur C. Bigelow bigelow@umich.edu Thu May 29

SPECIMEN PREPARATION – dispersing particle for SEM

I am interested in liquid suspension/dispersing techniques that will allow me to disperse small particles (10 to 0.1 microns in size) onto an SEM substrate so the individual particles are well separated (i.e. no clumping/flocculating etc.). While I suspect this is a well-known problem in SEM sample prep, maybe with some known techniques, I have been trying things without any outside help so far. I have had some success using ethanol with a few drops of a high quality commercial surfactant, but after the ethanol evaporates the oily surfactant is left as a coating on the particles, which makes SEM impractical. The material I am working with are small inorganic (mineral) particles. However, the material aside, I'm wondering in particular if the life science side of the EM house has some suggestions that might help. Roy Christoffersen roy.christoffersen-1@nasa.gov May 21

The closest I come to a problem like yours is putting 10 nm Au particles on cryoTEM grids. They have a tendency to aggregate, and I want them well-dispersed, so I try to evaporate the water they're suspended in rapidly. I have found that putting the grids in a 50-70 degree oven does the job. You can also try to tailor the properties of your SEM substrate. If the particles bind to the substrate, then they should have less tendency to clump. Another thought is to use an atomizer to deposit small drops of your suspension onto the substrate. If the substrate can be kept warm, the drops will evaporate quickly, so the particles should be in fixed positions, more or less, and this should give you a good distribution. Bill Tivol tivol@caltech.edu May 21

Chuck Garber described a technique for separating particles in an email to the listserver some time ago which I've attached below. I don't know if it will help in your situation, but it is probably worth looking at. Cheers, Henk Colijn -- [From: Garber, Charles A. * EMC.Ver #3.1] -- Oldrich Benada wrote: I need some advice. I was asked to do some analysis of silica particles (size distribution) for chemist in our institute. Particle size should be in the range of 3 to 6 um. I do not have any experiences with such sample. Could someone give me a tip how to prepare sample for TEM (or SEM)? Charles Garber reply: The problem is that those pesky silica particles don't know that they are supposed to separate and stay away from each other when dispersed in a liquid followed by a droplet of this liquid suspension being placed on a solid surface. They tend to agglomerate very quickly leading to a difficult- to-analyze situation, especially using automated means of analysis. You are correct in that the size range expected could be on the order of 3-6 nm. This is the ideal application for the camphor/naphthalene method which I described several years ago. Credit for the technique, or at least the one who taught it to me was an innovative microscopist then working at the DuPont Experimental Station in Wilmington, DE by the name of Robert P. Schatz, in about 1968, now deceased. Take a 60% camphor/40% naphthalene mixture and heat it to twenty or so degrees above room temperature on a hot plate in a small beaker or flask, the two organics are miscible in each other and this is the eutectic composition. Once a clear liquid, add a small amount of the silica (not more than 0.1%), which disperses quite readily. Then, using a pipette, take out some liquid and put a drop onto a carbon coated glass slide, at which time the drop is instantly frozen solid (it is at room temperature). Put the slide into your vacuum evaporator to pump out all night, and the "magic" is that the solid eutectic sublimes at room temperature at a rate that by morning, it is completely gone, leaving the silica particles uniformly dispersed on the carbon film! The rest is obvious. You can pick this up on

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a grid, as is, or in order to bring out more contrast, Pt/C shadow, probably using an angle not more than 30 degrees. You can float the "replica" off of the slide directly onto a grid and viola! you have particles completely dispersed, virtually no doublets or triplets, and a field quite amenable for automated image analysis (as a bonus). One important further suggestion: Sometimes these silica particles tend to fuse together as little "chains". If you suspect this is happening, be sure to take the micrographs as stereo pairs because you can in fact capture this three dimensional spatial information. Disclaimer: We do not sell either the camphor or naphthalene so have no vested interest in whether people use this method or not. It is just a really neat method for the preparation of fine particle samples in this size range. We are obviously set up to use this method as a service for others, however. Charles Garber - reposted by Henk Colijn colijn.1@osu.edu May 21

FYI - The published version of the camphor-naphthalene article is: Thaulow, N. and E. W White: General method for dispersing and disaggregating particulate samples for quantitative SEM and optical microscopic studies, Powder Tech, 5, 6, 377-379, 1972. Andrew Anthony Havics ph2@sprynet.com May 21

I would make sure that your substrate is very flat: a glass cover slip or vitreous carbon. Suspend your particles in pure alcohol and sonicate for a minute, don't use the surfactant for the reasons you mention. Put one drop of your suspension onto the substrate and it should spread out quite well. The alcohol should evaporate quite quickly, but particles at that size range should not agglomerate too badly. You should be able to find good areas on your sample to examine. Mary Fletcher maryflet@interchange.ubc.ca May 21

Another way to disperse particles dry is by simply pouring slowly into the top of a long tube or pipe held vertically over your substrate. The pipe should be something like 3-4 feet long and 2-3 inches in diameter. Just hold it vertically over an adhesive substrate. The fall through the still air in the tube breaks up the particles and reduces agglomeration. John Mardinly a.mardinly@numonyx.com May 21

There are numerous strategies that might be worth a try for your samples. It will require significant trial and error to determine what works best for your samples and analytical needs. All will have advantages and disadvantages and prep artifacts so you will need to experiment and decide what produces the best dispersion (particle loading, particle separation, and substrate) for your samples. In general, if you are starting with a dry powder there are a few paths you might try (in no particular order). 1. Dry "puffing" of the sample in a box with your sample substrate. A home-made "puffer" box is pretty cheap and easy to make. You basically use compressed air to puff the dry sample into the air around a sample substrate and allow it to settle. Works well for narrow size distributions of larger particles (~500nm or greater). 2. Resuspension and filtration. Suspend your sample in water (or other solvent) with a surfactant, ultrasonic briefly, and filter onto polycarbonate membrane filter. Remove a section of the filter, place it on a substrate and carbon coat. This is a good general purpose method, but dispersion of particles can be difficult with some minerals (clays in particular). Your sample will also end up on a carbon-rich substrate, but methods are available to produce carbon films supporting particulate on TEM grids (See any of the TEM asbestos analysis methods) 3. Spray mounting. This is basically making a heavy suspension of your sample in a solvent and airbrushing it onto a suitable substrate. One of the microscopy supply vendors makes a spray mounting device. Makes good dispersions of fine particulate, but introduces the possibility of size fractionation during prep. 4. Drop mounting. Sounds like you have already tried this, but if you try different solvents and try heating your substrate slightly to allow for rapid evaporation it might work. 5. Film casting. Suspend sample in collodion solution (~2% or less) and cast a thin film on H20 surface. Pick the film off the water with a polished substrate and heat to sublimate the collodion. This takes a lot of trial and error to get the technique down and to figure out the best particle loading. Your particles also need to be able to withstand higher temperatures and need to be insoluble in H2O. Those are the few that come to mind. There is opportunity for significant amounts of creativity so have fun playing with this. Just beware that some will introduce significant prep artifacts depending on the particle size. Lehigh used to offer a course called "Particle Characterization and Preparation, Microscopy and Analysis" during their summer Microscopy School. It was taught by John Small and Cynthia Zeissler et al. from NIST. All of these general methods can be refined to suit what you have and what you want to do, but all will likely introduce artifacts on the particle size distribution. Bryan Bandli bbandli@d.umn.edu May 21

SPECIMEN PREPARATION - sputter coating

A question regarding applying conductive coatings. We're in the process of procuring 2 new FESEMs and I need to update our sample prep equipment. We have an Edwards Auto 306 and a vintage (workhorse) Polaron E5000. I am aware that we need to update to a new sputter coater with e.g. Pt/Pd targets; however it has been suggested to me that we use the Edwards Evaporator for all our coatings needs. I don't think that this will suffice - but apart from the evaporator being slower, can anyone help me build a list of reasons why we need a new sputter coater? Emer emer.ryan@dit.ie Apr 18

I have been operating an EMS575X Turbo Sputter coater for nearly two years. Besides the fact that it is fast as you already knew, the samples rotate so all sides get an even coat and the machine is built to be compatible with a "Film Thickness Monitor" (FTM) which we chose as an option. This has proven to be a great help and money saving component in that the sputter coater now stops sputtering automatically when the desired coating thickness is obtained - no guessing or timing! Less electricity is used per run and a pre-determined amount of target is used for each run (thickness easily changed for different types of samples) eliminating wasted expensive metal. The negative was the additional cost of the FTM. Patricia Stranen Connelly connellyps@nhlbi.nih.gov Apr 18

I am going to put a disclaimer up front. We manufacture and sell the IBS/e ion sputter coating system. Now, having said that, consider a sphere sitting on the tilting and rotating holder of your sputter system. Now consider the amount of time each surface element of the sphere is pointed at the sputter target. When your stage is tilted at 90°, if it doesn't stay there longer to account for the amount of time to cover each surface element with the same amount of material as when the stage is at 0°, then the coating over the surface will not be uniform. In other words, the stage should spend less time going through 0° than when it is turning around at 90°. Further, because you want to coat the sample uniformly, it

is not beneficial to coat the sample fast. You want to grow the film slowly so that you have better control over both the film thickness growth and the uniformity of the film by allowing your stage to go over all possible angular ranges over the duration of growth. In our system, I will actually purposely grow some films slower to enhance the uniformity of highly topographic samples. Magnetron sputter systems are fast, but they have a couple of disadvantages. Magnetron systems can "spit" material sometimes. These are larger particles that can be incorporated into the film. Also, the distribution of the sputtered material changes over time as a function of the wear pattern in the target. This can affect the accuracy of a quartz crystal thin film monitor system. Don't forget that you also have to run the unit a little before coating to get rid of any surface oxide on the target. Different target materials oxidize differently, so the amount of time that you have to pre-sputter the target is dependent on the material. In practice, what this means is that you have to have a shutter for your sample so that it is not coating the sample while you are conditioning the target. On the other hand, an ion beam system has a fairly stable deposition pattern over time. The deposition rate has a cosine distribution that doesn't matter too much about how the target is eroded over time. It is slower, which I think is a good thing for high resolution SEM coatings. And it does not "spit". One other thing that separates the systems: You can't deposit magnetic materials easily with magnetron sputtering. Why is this important? Several investigators are using Ni and Fe thin films to use as catalyst layers for the growth of carbon nanotubes. Another thing is that you can readily change targets with an ion beam. In our system, we can have up to four targets that can be switched while under vacuum. The Gatan system has two targets. What this means is that you can use these systems to put down multi-layer thin film coatings that can be used for calibration samples and standard samples for TEM. I've actually performed the calculations for using a bi-layer thin film with known thicknesses to act as a thin film binary standard for AEM. I just haven't done the experiments yet. Scott D. Walck walck@southbaytech.com Apr 18

We had an older dual beam VCR Group microsputter coater mounted on an Edwards 306A. Both units integrated fine. However, it was a bear to change the Edwards back and forth between microsputtering and other thermal evaporations. 1. The Edwards is a fine unit but it is oil pumped. That was not a big problem with our Cr coatings but others wanted a turbopump on it. 2. We had to cover all the feed throughs with a steel bell jar that was more like a collar. The collar was very heavy and had a large diameter. The microsputter coater fit on top of this collar. It took two of us to change back and forth from the microsputter coater mode to regular Edwards' modes. 3. You need lots of bench space for the bell jar, the heavy collar, and the sputter unit. A desktop sputter unit is MUCH smaller, lighter, and takes less bench top space. 4. Cycle times are longer with a large free standing large bell jar evaporator. They have their place and uses. However, then there is the LN_2 trap issue and waiting for cooling and pump down with LN_2. Then you might need a rotator system. Do you really want to go there? I took a 30+ year old vacuum evaporator from another group that was headed to the dumpster and rebuilt it. It was not fun. Some nut opened all the valves and pumped the thing overnight with the diffusion pump heater on and the vent valve open. Don't hire a plumber as a vacuum technician. The diffusion pump chimney had carbon foam in it the next day. That's just one of the 15+ things that were wrong with the evaporator before I got it. It took three months of my spare time to finish the rebuild. Once I got it working, then I had to convert my new used evaporator from a dual thermal boat unit. I installed a Ladd carbon rod and tungsten basket unit in the evaporator. So I got the new smaller evaporator unit working and we seldom had to change the Edwards over again. What's the moral of the story? That's how much I/we hated converting the Edwards. If you are a weight lifter, you will love converting back and forth. I eventually passed on converting. The dedicated carbon rod evaporator setup I built put a stop to the repeated conversions between carbon to chrome to carbon to Pt, to carbon, etc. Paul Beauregard beaurega@westol.com Apr 18

There is a new bench-top high vacuum platform for research & development from Oxford Vacuum Science. It uses thermal evaporation, providing a real, low-cost alternative to electron beam technology: www.oxford-vacuum.com. Dietrich von Diemar didi@specs.com Apr 18

SPECIMEN PREPARATION – coating for SEM

Does someone in the community have some experience/opinion about the osmium coating for FE-SEM as a replacement for conventional gold coating? Any particular comment about Os vs Au or Au/Pd or Pd/Pt for biological specimens (collagen, cells, etc) Alejandra acamacho@rd.us.loreal.com Alejandra Jun 2

Consider Pd and Ir. If EDS is not an issue, these will work well. The caveat is to coat at high vacuum. This is typically about 15-20mT. With rotation, you can get a very nice coating. At high magnification, Au/Pd and Au are seen. Pt works well too at high vacuum. Gary Gaugler gary@gaugler.com Jun 2

SPECIMEN PREPARATION – evaporating thick Al layers

We have several researchers who need to coat some wafers with 1-2 micrometers of aluminum. We tried using our conventional technique (10 cm of 10 mil Al wire evaporated from a tri-wire tungsten filament) but can only obtain extremely thin layers. What should we be doing to obtain the thick layers? John J. Bozzola bozzola@siu.edu May 16

I had success producing thick Al layers by evaporating Al foil in a basket of W, which can be obtained from a number of EM supply houses. The basket has two arms and is conical with the wire wound to form the part that a ball of crumpled foil will fit into. I think that one could use as much foil as necessary, since Al has a relatively low boiling point. (The same is not true for Ti, which can melt and short enough W wire that the temperature never achieves the >3000 K necessary to evaporate it.) I cannot say how flat or uniform the layer is except that Al evaporated onto plate glass makes a good mirror. You could also evaporate as usual, then repeat until a 1-2 um layer is built up, but this may not be practical unless you can get ~100 nm or more per individual layer. Bill Tivol tivol@caltech.edu May 16

Thermal evaporation of aluminum from W baskets is problematic. The molten Al reacts with the W to form a eutectic (Al-W dendrite sample anyone!) and the W wire often breaks before you get a very thick film. Also, when making thick (>100nm) metal films, internal stress in the film becomes a problem. I've had films peel

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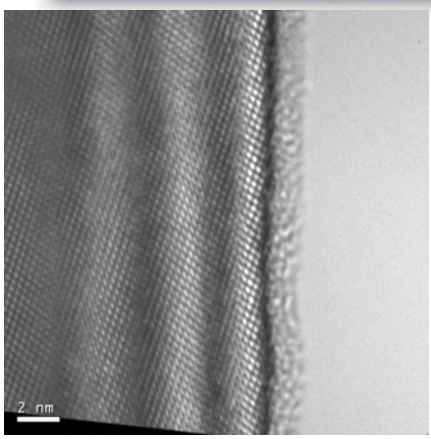

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from the substrate due to the stress. I'm not sure if this is more of a problem with thermally evaporated films compared to sputtered films or not. Perhaps someone more familiar with the deposition processes can further clarify the issue. Since thick films are grown, there must be ways to do it. Would it be better to grow a thick film by electro-deposition similar to the way they grow TEM grids? Hendrik O. Colijn colijn.1@osu.edu May 16

IMMUNOCYTOCHEMISTRY – brain tissue for EM

Can you all lend some expert advice as how to optimize a protocol for post-embed labeling on brains? I have a user who has been doing post-embed labeling on brain tissue which has been embedded in Araldite with the traditional fixation and embedding protocols (Karnovsky's, OsO4, ethanol, propylene oxide and Araldite). She has to etch her sections and other damaging things which limit ultrastructural beauty and labeling prettiness. I'm suggesting LR White but I want to know what the experts are doing. Paula Sicurello vapatpxs@yahoo.com Jun 11

The specimen as you describe it is probably not the best situation to start with, although there have been reports of successful immune-labeling on tissue that was OsO_4 fixed and epoxy embedded. So if that is what you get, you might have to give it a go. LR White embedding means you have to go through another run of specimen preparation. While you are at that...is there no way you could consider a pre-embedding approach? Jan Leunissen leunissen@aurion.nl Jun 11

Using Epon may not be far off the mark when it comes to brain immunocytochemistry. There is a group in Norway who have been successfully using it for a while (O.P Ottersen). More recently they switched to Durcupan (Gundersen et al J Cerebral Blood Flow & Metabolism (2001) 21, 41–51; http://www.nature.com/jcbfm/journal/v21/n1/abs/9591036a.html;jsessionid=178E36E3B37B167B1C682EA6ED331497). Although other resins may be more favorable, they will bring other problems. For example, LR White is a very good solvent that may take away brain lipids. You may also encounter polymerization problems too if you are not using this resin routinely. There will also be a different contrast that your collaborator may not appreciate (low contrast myelin) Of course, the final answer to your question is to try one or two different options and see which works best for your antigen and antibody combination. Have you considered cryosections of cryoprotected material? This would be an excellent approach for antigens that are not washed away during aldehyde fixation. The only disadvantage could be that the myelin will not have the black appearance usual for tissue treated with osmium. Interestingly, someone just told me that adding uranyl acetate to the pick-up solution will create the black (artificial) color if you really want it. Paul Webster pwebster@hei.org Jun 11

There are many approaches for immunogold labeling in brain tissue. Post-embedding labeling on conventionally fixed and Epoxy resin embedded material is one of them, however this method is only suitable for a small category of antigens, among which is a group of amino acids used by the brain as neurotransmitters. The antisera against these small amino acids usually are raised by immunizing rabbits with the respective amino acids that are conjugated to a carrier protein (e.g. BSA) using glutaraldehyde. Therefore, the preservation of the epitopes of these amino acids in brain tissue is not incompatible with glutaraldehyde fixation, and this is one of the reasons conventional embedding is fine for the labeling of these amino acids. The labeling described in the paper Paul listed in his reply is an example of this type of labeling. However, the majority of immunogold labeling in brain tissue is for antigens that are far more "vulnerable". Although using milder aldehyde fixative, omitting OsO_4, and switching to LR White resin may minimize the "damage" to the antigens, I do not recommend this approach for brain tissue. In brain tissue, the identification of neuronal structure relies heavily on the integrity of membrane ultrastructure. In addition, many labelings are for membrane bound proteins such as receptor proteins, channel proteins, or transporter proteins. In its protocol, LR White embedding is usually accompanied by the omission of OsO_4 fixation which means lipid is not stabilized so more likely to be extracted or "melted" during dehydration and thermo polymerization. For this reason, LR White embedded brain tissue will not give the adequate membrane ultrastructure required. If your user has a particular reason to insist on post-embedding labeling, I would recommend high-pressure freezing, cryo-substitution, and embedding in Lowicryl. There have been many publications using this approach for brain tissue showing good membrane integrity. In general, the far more common approach, or I should say the approach people usually start with for immuno labeling in brain tissue is pre-embedding labeling. One aspect of immunolabeling in brain tissue is to survey the distribution of the protein in a large and structurally heterogeneous area before dissecting out the right area for EM analysis. For this reason, pre-embedding labeling offers a major advantage because the labeling can be seen with LM in large Vibratome sections that cross any region of the brain. Pre- embedding immuno labeling has other benefits as well which we can discuss later. As Paul mentioned your user might want to try one of two different approaches, however, he/she should not try randomly. Again, find out first what is being labeled and what questions need to be answered with this labeling. Back to Araldite embedded material, if the etching is done right, he/she should be able to get beautiful ultrastructure. Hong Yi hyi@emory.edu Jun 12

It all depends on the antigen you are looking for. That said, it is unlikely that traditional EM processing will allow you to find what you are looking for. Before proceeding, I suggest a literature search to see how others have localized this antigen at the EM level. This could save someone a lot of time and trouble. Geoff McAuliffe mcauliff@umdnj.edu Jun 12

DIGITAL IMAGING – resolution

I have a dilemma: I have acquired images for a client on our TEM, which is equipped with a Gatan Erlangshen ES500W camera. Using Digital Micrograph, I acquired images in the DM3 format. I then batch convert to Tiff or BMP, and gave the client both the original DM3 files as well as the converted ones. I instructed them on opening the DM3 in Image J, which was not a problem. The problem is that once in Image J, they need to be converted into tiff, or other common file type for use in other programs, i.e., for preparing a manuscript. My client has a problem with a "resolution loss" once the file type is changed. The image is about half the size (~1.3MB), but their main concern is that the dpi is only 72 (when opened in Corel), and that they won't be accepted if they have that low of a dpi. I haven't been able to find any information on how important the dpi number is, and I'm a bit skeptical as the image looks to be the same quality as the

original. My client thinks I should change the settings on the camera so the original image is larger than 3MB, but I don't even know how/ if this can be done! Sarah Aubut saubut@nrcan.gc.ca May 28

Your image size is not determined by the dpi - that is the display size and is a function of the software and/or output device (i.e., monitor or printer). Displaying the image at 72 dpi or 300 dpi makes the image look bigger or smaller on screen but the resolution isn't changed. The resolution is determined by the 1200 x 800 pixels (or whatever the image size is in pixels). Thomas E. Phillips phillipst@missouri.edu May 28

Digital imaging has so many things controlled by various things from the acquisition to the final output. Like Tom said, the image resolution is set by the pixel resolution at acquisition time. But ugly things can subsequently happen. A new document in Corel is typically 72 dpi by default unless changed; the assumption is that you are developing images for computer viewing and computer monitors are approx 72 dpi. Now if you drop an image onto a page at that default resolution you have to grab the "handles" and drag it to the desired size on the page. Here is the problem: when you click "Apply" (in Corel9....) it re-samples the image to the 72 dpi page resolution and the original resolution is gone. If you had started with a page at 300 dpi (near photographic) then an 1800 pixel wide image is 6" wide on the 300 dpi page and if printed on a good 300 dpi printer (true 300 dpi like a dye sublimation printer, or equivalent). I think that if an image has no scale data in the tag fields (TIFF) Corel will treat it as a 72 dpi image by default so the hypothetical 1800 pixel-wide image is (1800/72) inches in hypothetical size. There is wealth of information about digital imaging on the Molecular Expressions website: http://micro.magnet.fsu.edu/primer/digitalimaging/index.html Dale Callaham dac@research.umass.edu May 28

Another thing that might be related to the reduced file size is the bit depth. Image acquired from the CCD camera is likely being 16 or 32 bit. But it has to be converted to 8 bit TIFF file in order to be displayed properly in normal software such as Windows Image Viewer or MS-Word. This process will cause some information loss and reduce the file size. Fan Li lixxx326@umn.edu May 28

EM - algae in chiller

We have a conventional SEM, a FEG-SEM (the most recent acquisition) and a TEM, all with water circulation provided by the same chiller. Lately we have noticed some algae forming inside the hose of the FEG-SEM, which is a bit more transparent than the rest. We have changed the water in the chiller, but with no results. My questions are: 1) Is it possible to use some product to remove these algae without damaging any part of the microscopes? If not, what can we do? 2) Do you use distilled water in such chillers? Isabel Dias Nogueira isabel.nogueira@ist.utl.pt Apr 16

Preventing the growth of algae in cooling water systems is discussed in detail in my book "Vacuum Methods in Electron Microscopy". Two chemicals commonly used to prevent algal growth in such systems are Chloramine T and dichlorophene. Both of these chemicals can be obtained from various specialty chemical companies. You can also probably obtain algaecides from companies (and merchants) that sell water beds, and swimming pool equipment. I do not recommend the use of ethylene glycol for several reasons that are discussed in my book. Also, remember that algae require light in order to grow, and so you can substantially inhibit

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their growth by fully excluding light from the system (i.e. cover the reservoir with a light-tight cover, and use fully opaque tubing). Changing from ordinary tap water to distilled water will probably not give you much of an advantage, except for possibly minimizing the formation of a bit of scale in the heated parts of a diffusion pump. However, the amount of scale formation should be quite limited since it is a closed system containing a limited amount of water. Wilbur C. Bigelow bigelow@umich.edu Apr 16

When we got a new water re-circulator a few years ago, they told me that, if it got some algae in it, empty all the water out, fill it with tap water, run it around for a while, then empty the tap water out and replace with distilled water. The tap water in most cities now has enough chlorine in it to kill the algae and flush it out, but they want the re-circulator run routinely with distilled. The other thing I have heard is that just one or two drops of bleach in the tank will kill any organisms without harming any components. Mary Fletcher maryflet@interchange.ubc.ca Apr 16

I would keep all Cl out of the system. If any remains, it will corrode the cheap brass most systems use. This brass is high in Zn and is much more susceptible to corrosion than Imperial brass (but it cost $1 more). For my Haskris chiller, I run 10% ethylene glycol with distilled water. I try to find the purest distilled water I can. I've taken selected drops from different distilled water jugs and put on a clean microscope slide. Let dry and see what remains. Most will have residue. The one that has the least or none is the one to use. Zeiss chose clear hoses for my SEM but I see no problem since using ethylene glycol. Be sure to change chiller filter(s) about twice a year. Gary Gaugler gary@gaugler.com Wed Apr 16

To protect our chillers from algal growth and freezing (chiller lives outside) we run them with the heat transfer fluid Hexid A4 from applied thermal control. http://www.app-therm.com/product_hexid.asp. It's pricey but we have no problems with algal growth. I have no commercial interest, just a satisfied customer. A. Christine Richardson a.c.richardson@durham.ac.uk Apr 17

To restrict the growth of algae in the fluid reservoir, I recommend the reservoir cover be kept in place and that all recirculation lines be opaque. This will eliminate the entrance of light, which is required for the growth of most algae. The use of Chloramine-T, 1 gram per 3.5 liters is recommended. Other algaecides can be harmful to the unit's internal components. We use a Thermo recirculating chiller for our Hitachi TEM. Thermo recommends distilled/deionized water with 0.05 to 0.1 megohm-cm reading. Highly distilled/deionized water, above the 3 megohm-cm region, may become aggressive and is not recommended for use with units with wetted parts other than stainless steel. Distilled/deionized water in the 15 megohm-cm region is definitely aggressive and should not be used. Do not use a deionization (DI) filter with inhibited EG. A DI filter will remove inhibitors from the solution rendering the fluid ineffective against corrosion prevention. Also inhibitors increase fluid conductivity. Proper reservoir cleaning is necessary. Make a monthly visual inspection of the reservoir after initial installation. After several months, the frequency of cleaning will be established. Use a soft cloth for cleaning; do not use steel wool or other abrasive materials. They can scratch the steel surface and initiate rusting. IPS Gill ipgill@gmail.com Apr 17

As suggested by one of you, I ran some tests to make sure we had algae in our system. To my surprise, the composition of the deposit I removed from the hose was mainly Fe, Zn and Cu... I suppose they come from the water pipes, and that our filters have not been changed as often as they should have been. Apart from changing the filters, cleaning the water deposit of the chiller, is there anything else I can do to remove this deposit from inside the microscopes? Isabel Nogueira isabel.nogueira@ist.utl.pt Apr 22

I am not sure I am the only one that sent you a suggestion. I sent you the procedure and suggestion to determine if you were dealing with either green algae or green copper carbonate residues. Either case is possible. So that is absolutely the first task. I am glad that someone found out what I did. Green does not mean that algae are the only cause of green deposits. See the Microscopy.com archives on Jan 21, 2008 for algae and carbonate reactions involving CO_2. As shown in the attachment article sent to Microscopy Today and you, the chemical reaction of CO_2 will attack copper sources including brass. I suspect that your zinc is from alloys like brass and not galvanized pipes. Impellers on pumps can also be bronze and slowly eroded away over the years. So tin can also be found. The iron could be from a lot of sources but stainless steel fittings or tubing makes a lot of sense. Chlorides will pit corrode stainless steel. You can never eliminate all sources of copper, its alloys, and iron in instruments or chiller loops. Water filters can catch precipitates in suspension such as calcium and magnesium carbonates (city water contamination) and/or copper carbonate (erosion of copper and its alloys). A water filter will not stop the scaling by itself but is required at the inlet of every instrument, in my opinion. The scaling problem is Ksp based and that involves dissolved ion concentrations. Removing the residual or plugging deposits that you can't see is tricky. Servicemen use Lime Away or CLR to remove the scale. Both these products contain a mild acid. They are citric acid and phosphoric acid based. Some phosphates can be insoluble. Dilute hydrochloric acid works to remove carbonates but it has some risk. You run a water circulation loop into an open bucket using a small circulating pump. Start out with a low concentration of HCl. All sorts of CO_2 bubbles and black rubber particles can be seen in a white bucket exiting the return line. In the bottom of the bucket, put a couple of old dark copper US pennies or other equivalent copper coins. They must be oxidized. Once the initial bubbles are formed and any blockage is opened up a bit, you can then slowly add HCl while still circulating. Do not dump HCl additions onto the copper coins. Remove them temporarily while adding HCl. When you see the copper penny coins turn bright and the copper oxide is removed by the return line solution, then that is the highest HCl concentration you should use. You have started to attack the copper tubing oxides in your microscope and the pennies are your indicator for that attack. Immediately switch to pure DI water from another bucket and flush the system. Do not recirculate any water at this point. This flushing can take a while and requires frequent water dumping and refilling of the buckets. Monitor the output of the circulation from the microscope with silver nitrate to test for a white silver chloride precipitate. Once you think you have not detected any more chlorides from any residual HCl, flush a few more volumes of pure DI water and switch to freshly flushed chiller lines. Circulate fresh DI water for a few days. Test for chlo-

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rides. If you see chlorides, flush the system again. When you find no chlorides, add your algaecide, hopefully a low PPM level of a quaternary ammonium salt surfactant and not any chlorine based "bleach". Try to seal the open reservoir airtight. It is the source of both CO_2 reactions, whether algae or copper carbonate based. As CO_2 is dissolved in the copper erosion case, the solubility product (Ksp) of copper carbonate will be exceeded before calcium carbonate. Once you exceed a Ksp, you will start to form scale. The ion concentrations will build up from CO_2 mixing in the open reservoir, even in a HVAC heat exchanger based system. Monitor any transparent lines for a green color build up in the future. If you see the green build up, then you did not exchange the old water with new DI water often enough (3-6 months). You must replace the DI water periodically to prevent exceeding the Ksp of any carbonates that are sure to form. It helps to have valves installed so that one can selectively flush the various branch lines to a diffusion pump or lenses. Deposits do not form uniformly. The output from DPs will scale more than the inlets. It would seem that hot water would keep the ions in solution. That is not what I/we found. So the green monitor tubing line should be after a DP, if possible. Check all the flow rates after cleaning out any lines. Check them against the flow specifications of the manufacturer. You said that you were surprised at the composition of the green deposits. I was not. I spent two months killing algae that didn't exist by following my supervisor's algae idea. The more Chloramine-T we added, the darker green the water turned. I took our water and added HCl and then ammonium hydroxide. The water turned green in acid and blue in NH_4OH base. After a few blue and green color cycles back and forth, I looked at him and said, "I don't think we have a blue-green alga problem in our chiller. This is how dissolved copper ions perform chemically." Like you, I got the message and eventually did some relevant inorganic testing. I would recommend having a serviceman flush the scale out with acid. If you have a good idea of how the lines run in the scope, then you can try descaling yourself. Paul Beauregard beauregа@westol.com Apr 23

EM - maximum length of vacuum lines

The oil mechanical vacuum pumps (a few each of belt drive and direct drive) for our 4 EM's are currently located in a service spline directly behind the instruments. Currently, the pump to scope distance is about 4-5 feet using rubber hose. Our safety folks are asking that we relocate the pumps. This will involve 1) moving them into the scope lab itself or into another room at the end of the hallway. I'm worried about noise and vibration if we relocate into the lab. If we put all the pumps into a single room it will involve long vacuum line runs. Safety insists we will use hard tubing. The vacuum line runs could be up to 20 or more feet between the pumps and EM's. Also, the tubing run will involved 2 right angle bends. How far can I locate the pumps from scopes? Does it matter if the bends are mitered? Should I use larger diameter tubing than the current rubber hose? Owen Mills opmills@mtu.edu May 7

Very simply the pumping speed is such that it follows the inverse square law. The further away the pump, the slower the pump down. Bends in a pumping line slow the procedure even further. When we design an instrument we expect the rotary pumps to be within 5 feet of the instrument and the facilities provided with the machine are designed to reduce vibration. The only area that you may not like is the noise from the pump, however to use an acoustic cover is a far better solution that to have the pumps at the distance that you indicate. Steve Chapman protrain@emcourses.com May 7

I would like to know the justification for moving the pumps. Is it that the exhaust is not properly ventilated? Could it be that lab equipment is not allowed in utility spaces? Fire code? http://www.lesker.com/newweb/technical_info/conductance_calc.cfm Jim Quinn jquinn@www.matscieng.sunysb.edu May 8

I have to agree with Gary. You need to know why they want them moved. Is it a fire hazard, a fume issue, etc. You might point out to them that it is far more dangerous to have the pumps in the same room than to have them isolated. This involves inhaling the fumes, etc. If they persist, you must insist that they put in writing that they will pay to fix any problems (e.g., poor vacuum to the instrument) that result from this move. They must realize the consequences of this forced move. John J. Bozzola bozzola@siu.edu May 8

Conductance of tubing is inversely proportional to tubing length, not inverse square. However, it is proportional to 4th power of diameter of tubing. So, slight increase in diameter will compensate for the increased length. Doubling diameter would enable one to increase length 16 times for same conductance. Kristian Ukkonen kristian.ukkonen@iki.fi May 8

I have been involved with several instances of moving rough pumps, and being a former student of Will Bigelow, dutifully calculated the conductance using the class notes that are now in his text book on vacuum technology. OK, the conductance and pumping speeds match, but what will surprise you is that the rough pump line suddenly has a volume that has increased by quite a lot, and when you hit that button that says "Pump", the "WHOOOMPF" you will hear the first time that roughing valve opens will really be a shock. Consider carefully whether your system can withstand the internal turbulence that this will cause. EDX vendors believe that pumping turbulence throws particulate material around with sufficient force to put pinholes in EDX detector's thin windows. There is also potential for physically moving thin apertures, lifting pole pieces, and who knows what else. The best thing to do here (aside from keeping pumping lines short) is install a throttle valve where the roughing line connects to the microscope. One way to do this is insert a butterfly valve in the large diameter roughing line in which you have drilled a small hole, maybe on the order of 0.25-0.5 inch. Close the butterfly valve before pumping, and then open it after the initial surge has subsided. John Mardinly a.mardinly@numonyx.com May 9

The "WHOOOMPF" sound and much longer pumping time which you are describing will indeed take place if entire roughing line is vented and if roughing pump is stopped during the vent. One way to deal with it will be to install a throttle valve, as you described, but in this case one still has to deal with increased pumping time due to the need to pump air from the volume of roughing line during each circle. Another way of running instrument with long roughing lines is to install into the roughing line electric (or pneumatic) shutoff valve as close as practical to the place where roughing line connect to SEM, and to control this valve from the relay which intended to stop roughing pump during vent and start it during pumping, in such a way that the valve is closed during vent and opened during pumping. Roughing pump should be connected

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directly to power and run all the time, even when instrument is vented, thus keeping roughing lines constantly evacuated, so this volume does not need to be pumped each circle. The operation, as far as SEM is concerned, will be the same as if roughing pump, with pumping speed limited by the conductance of the long roughing line, was installed where the shutoff valve now is. This will save pumping time with, but have downside of increased hours on the roughing pump (bill pump rebuilding costs to Safety Department. Valery Ray vray@partbeamsystech.com May 9

The question of the acceptable length of a rough pumping line has been pretty well covered by others who have already commented on this matter. However, if you want to see an actual calculation then you can find one on pages 44-45 of my book, "Vacuum Methods in Electron Microscopy" If you do install a pumping line of considerably greater volume than the existing one, and if the pump-out valve is located at the end of this line nearest to the instrument, then you should give serious consideration to the phenomenon mentioned by Dr. Mardinly. That is, assume that the vacuum chamber is at atmospheric pressure, the pump-out valve is closed, and the pumping line is evacuated. Then if the pump-out valve is suddenly opened the sudden rush of air from the chamber into the rather large volume of the vacuum line could indeed cause a serious turbulence in the instrument chamber. Wilbur C. Bigelow bigelow@umich.edu May 12

EM - magnetic interference from UPS

Do uninterruptible power supplies (UPS) cause magnetic interference to EM? If so, what solutions for UPS are in use in EM labs. The question came to us as to whether a UPS in rooms adjacent to EMs might interfere with them. Aryeh Weiss aryeh@cc.huji.ac.il May 15

We did some measurements on UPS last fall, before the installation of a new TEM, as we took the opportunity to add UPSs on the SEM and the existing TEM. The question was where localize them, on one hand to answer the same question as you and, on the other hand, to avoid long cables between them and the scopes. The specifications of the manufacturer give only some indications in the MHz/GHz range, to assure that there are no interferences with phones, WiFi etc. For EM, it's more the 0-10 kHz band which is important. We did the measure with a 100 kHz spectrum analyzer and a coil, in a room far from all power lines, at distances from 3 and 6m. The results 6 m away show that compared to the local background (UPS out), if it is powered on without load, frequency between 400 and 1500 Hz increase a bit (from 0.2 to 0.4 mG). With a resistive load of 6 kW, frequency between 1500 and 3000 Hz increase from the same order. The UPS itself has a frequency at 8 kHz of less than 0.05 mG, with its harmonics at 16, 32, etc kHz. The conclusion was that if we put each UPS 3-5 m away from its scope (in the technical room, next to the scope) we will be quite sure. The noise from the power line supplying the scope and its power supply itself will produce much more interferences. And it's the case. For the SEM, there is a level of less than 0.2 mG near the column with the UPS without load, and it goes up to 0.4 mG when I switch the SEM on! Jacques Faerber jacques.faerber@ipcms.u-strasbg.fr May 15

It depends on the particular make and model of the UPS. For the Phase One (Now Danaher) UPS for our 2010F, JEOL recommends over 30 feet distance from the UPS to the column, and that is for a 200KV microscope! John Mardinly a.mardinly@numonyx.com May 15

EM - filament life

We have problem with filament life on our Tecnai12, with diffusion pump and IGP. The scope is 5 years old. We are using W filaments supplied by FEI. We are getting between 20 to 30 hrs, rarely up to 50 hrs of life from a filament. Usually we operate at low emission settings, less than 10 μA. The filament current is stable and does not fluctuate more than 10% of the selected value. Scope performance has not changed. We have had several attempts by FEI engineers to find out the cause which they have failed to do so far. This problem is not operator related since we have experimented with saturating carefully the filament and then leaving the beam on at 120kV until the filament burned out without anyone using the scope during that period. The filament lasted 27 hrs. This problem has started about a year and a half ago. Before that we had unbelievably long filament lifetimes. The record is 1097 hrs! Usually we used to get lifetimes consistently over 300 hrs, with typical time between 500 to 700 hrs. Since the problem developed we have replaced the IGP, the ceramic insulator in the gun, gun chamber O-rings, specimen stage air-lock valve, we have tested different Wehnelt caps, boxes of filaments old and new, the outputs of the power supply have been measured and they are stable and within the specs and nothing seems to make a difference. The Wehnelt cap aperture size and distance is consistently the same as at the time we were getting hundreds of hours of lifetime. Typically the scope is operated at vacuum in the range of 10 to 20 log readings which corresponds to 1x10-7 to 5x10-7 Torr. At the same vacuum levels we used to get very long lifetimes. Any clues or suggestions what could have gone wrong? Krassimir N. Bozhilov bozhilov@ucr.edu May 8

If the electronics are stable (especially the filament drive ... I don't consider 10% anything close to stable, 1-2%/hr is satisfactory), then I would be looking for a vacuum leak in the immediate gun area. You haven't said exactly where you're measuring the vacuum. That can make a big difference, depending upon the vacuum design. One can very easily get pressure differences of a decade or more between sections of the vacuum system even without a significant leak. Have you put a leak detector or RGA on it? Ken Converse kenconverse@qualityimages.biz May 8

Take a good look at the glass/porcelain base of the burnt filaments that lasted less than 30 hrs. Can you positively see deposited (tungsten) material on the base surface around the filament? Additionally - do you have at least an impression, if not positive observation, that emission current somewhat increases on its own at any time during filament life? Irrelevant to arcing. Does not have to go up steadily all the time from installation to burn-out. But at least a few hours of slow steady increase, however small. If any of the above is a 'yes', then I might have an idea. Vitaly Feingold vitalylazar@att.net May 10

The answer to both questions is yes. There is definite deposit of W on the base even for filaments that have lasted about 20 or so hours. I have observed once that the emission current increased quite significantly, from about 8 μM to about 40 μM. After switching off the HT and starting again the emission was normal. I cannot tell if this occurs frequently, since I am not using that TEM very often by myself. I need to talk to users and find out. K. N. Bozhilov <bozhilov@ucr.edu> May 10

NETNOTES

All right, then I suspect the deposit creates an electrical conductance pass between Wehnelt and filament. If you look at the schematic diagram of HT generator, you will see this pass being electrically parallel to gun bias resistors. These resistors are switched back and forth for gun bias control in automatic bias circuit; that is common in modern EM guns. The less resistance - the higher the bias setting- the more current forced from the filament. This deposit may effectively increase the gun bias, with obvious consequences for filament life expectancy. You may prevent this deposit from connecting the circuit, in the following way. Find a piece of copper/brass/bronze thin sheet or thick foil, some 10 mil to 20 mil will do. Actually can be any metal that will not corrode and is easy to solder. Cut a washer from this material. This washer must be soldered on top of filament holding nut, just 2 or 3 points soldering, no durability required. The external diameter of this washer must be little less than the external diameter of the filament holding nut, and the internal diameter must be much smaller, just enough to accommodate filament pins. Make sure of the following: a) distance between filament pins and inner diameter of washer must be not less than 2.5mm, (3mm is good) - do not make it too large, otherwise it will not work; b) washer must not touch filament base - suppose it will bend and come very close to the base, that is OK, but must not touch the base; c) washer does not have to be perfectly even and round, yet (!!!) all must be flat and smooth, with no bumps and sharp points - solder, edges of the washer, etc. Deposit will still form. But the washer will 'cast the shadow' on the outer part of filament base, so the deposit will not close the circuit. The actual reasons for excessive tungsten evaporation/erosion may vary. Perhaps you have bad batch of filaments, which is less likely. Or perhaps your HT generator circuit is outside the specifications which is more likely. Additionally, if my guess was correct, then a filament base with very rough surface may prolong filament life. Bottom line, this method worked for me, on Philips CM series TEMs and it is much preferred to any other remedy as long as it works. Actual configuration of this additional element may vary depending on Wehnelt design for a particular TEM model. Vitaly Feingold vitalylazar@att.net May 10

P.S. A possible alternative - again presuming my guess was correct- a Kimball Physics LaB6 filament might be a solution. It has carbon heater and carbon does not evaporate much at these temperatures. In fact I installed this filament on a CM-10 with same problem a couple of months ago and it works so far without the modification that I described in the previous posting. Too short a time to be certain whether it is a fix or not. Vitaly Feingold vitalylazar@att.net May 10

Vitaly mentioned the Kimball-Physics LaB6: on our heavily used CM12 - the predecessor to the Tecnai 12 - we used and are using Kimball-Physics LaB6 filaments - only three in the last 10 years. The vacuum of the TEM was (very) good, always, and still

is .... I keep my fingers crossed. This means: I am a very satisfied customer ... and I do recommend this type. Reinhard Rachel reinhard.rachel@biologie.uni-regensburg.de May 12

Some Kimball Physics LaB_6 cathodes may be mechanically instable within first 50 or so hours of use, and gun tilt might need repetitive adjustment before the cathode becomes stable. Worst case - cathode may have to be re-centered. However, these cathodes take lots of punishment and still work for years. Leaky/dirty specimen airlock, fast heating/cooling of LaB_6, vacuum accidents, arcing, etc. In other words multi-user environment with little supervision. Vitaly Feingold vitalylazar@att.net May 13

TEM - FFTs in focusing and removing objective astigmatism

I was just wondering, how is a TEM focused using fast Fourier transforms? Also, how do FFTs compare to Fresnel fringes for focusing and removing objective astigmatism at high and low magnifications? Sven Ernst sven.ernst@student.curtin.edu.au Apr 25

FFT from a thin amorphous film contains information of the objective phase contrast transfer function (PCTF). By fitting the 2D intensity distribution in a single FFT image, one can measure the amount of mean defocus and the focus variation as a function of the azimuth angle, i.e. astigmatism. This is how focus and astigmatism are measured using the FFT image. Compared with the Fresnel fringe method, the FFT method is far more accurate at high magnification and high resolution conditions, with a typical accuracy of 1-2nm in both focus and astigmatism measurement. At low magnifications, the Fresnel method is preferred since it is easy to do and the accuracy requirement is not high. Also the FFT image can be used to do coma-free alignment, a must for obtaining HREM images under the right focus condition that can be correctly interpreted using the structural model. At low magnifications, rotation alignment is adequate. Ming Pan mpan@gatan.com Apr 25

FFT from any image contains frequency information (usually the phase information is not used). As such, an angular change in the Fourier domain (ex. 2-D intensity image) can represent astigmatism; and broadening in the Fourier domain represents spread of data. In general, the eye perceives a change in small frequencies as focus changes. Thus minimizing angular features minimizes astigmatism, and minimizing spread mathematically (stochastically) or visually by looking at a 2-D representation of the Fourier domain will improve focus. Andrew Anthony Havics ph2@sprynet.com Apr 26

SEM - low keV imaging

We have a FEI Quanta FEG SEM and would like to optimize it for low keV imaging. I really don't care if my microscope can perform above 2keV. The normal alignment procedure is to start at 30keV and align the top part of the column and then go to 1keV and align the lower part of the column. I am thinking that if we were to align the top part at 2keV and align the bottom part of the column at 1keV that we would be more optimized for 2keV and below. Does anyone think that this is a good idea or are there better ways to get optimized low keV images. R Richard Stallcup rstallcup@zyvex.com May 10

Yes, you are on the right lines for most instruments, but I do not know the FEI FEG too well. The lower the kV that you use to align the instrument, the better it should be at the lower levels. This is standard practice for most W SEM but in that case we often raise the anode to keep the gun fields optimized for best performance. Steve Chapman protrain@emcourses.com May 11

SEM - interference

We have a JEOL 5600LV and we are having an ongoing problem that JEOL say is an 'environmental issue'. We get interference on the images that manifest as bars down the image on the higher scan settings (this is a single solid bar on the right hand side of the image on scan 3, 3 bars on scan 4 across the whole image) These bars do not always seem to vary in their position with changes in KV and WD adjustments. Although the pronounced nature of the bars can be partially reduced by increasing the KV. The effect on the image is was intermittent and now it seems to be permanently present. The lines represent as solid bars running vertically down the screen. It's not caused by vibration. Essentially we want to find out if it is a field, we have had JEOL in to try and track down the field but with no success but they are adamant that there must be one despite being unable to locate it themselves. We don't really want to spend money on an expensive cancellation system if that is not the problem so has anyone out there had experience of: (a) Shielding microscopes cheaply in order to ascertain if it is a field. It doesn't matter if it's really crude it's just for initial diagnostic purposes. (b) Similar problems with ageing microscopes indicating another possible fault? (c) Any situation/equipment that may have produced similar effects. John Mitchels john.mitchels@gmail.com May 13

External magnetic field interference is always worse with lower KV and longer working distance. It usually manifests itself as line to line displacement of the image. There should also be a way to synch or unsynch the raster to 60 cycle to see it get worse. If none of these behaviors are evident, it is probably not an external field. As far as chasing down the source goes, the simple Extech 480823 that you can now get at Amazon.com for $99 http://www.amazon.com/gp/product/B00023RXDC will enable you to find the field and measure it easily. Another thing to look at is very dirty power. I'm not sure how to measure that, but I'm sure your local electrician can. In that case, a UPS or isolation transformer can solve the problem. Of course, the microscopy power supply should have some giant capacitors to do a significant amount of isolation, and maybe JEOL needs to look at that. If your SEM is old, capacitors could easily be not performing properly. John Mardinly a.mardinly@numonyx.com May 14

You say it is an ongoing problem, but give me the impression they were not always there. You also indicate they were intermittent when they first started appearing, and are always present, now. How long ago did they first start to appear? Have you had the system for a while, where it performed nicely, before the current symptom started to appear? You do say "aging microscope" near the bottom. Has there been any new construction, or equipment installations, in the vicinity of your system? Was there a repair, parts replaced, on your system? If so, is there a relationship, time wise? First, to answer your question about shielding, there is no effective way to easily and cheaply block outside fields. Even expensive Active Isolation systems have their limitations. Are these bars directly vertical, or do they angle down across the screen? If the bars look basically the same at 15 kV and 4 mm working distance, as they do at 1 kV and 15 mm working distance, then it is probably from within the

system. If JEOL cannot "find", and show you the "environmental" influence, then it is probably not there. You should be able to pick up, and see, a field that is strong enough to influence the beam that much. If the bars are vertical, then I find it difficult to believe an outside field would be that synchronous with the scan of the SEM. I had a JEOL 6400F SEM that had a problem from the start. JEOL had pre-checked the room for fields and vibrations, and had given the room their blessing. After the system arrived, and was set up, there was an intermittent quirk in the image. JEOL could not figure it out, or stop it, so they decided it was from fields outside the system. They could not detect them, or show them to me, but they must be there! After I had relocated the system to a different building, several miles away from the first, to a pretested and approved room, I still had the exact same symptoms. (Just to be clear, my symptoms were different than yours) They thoroughly checked the system again, and then brought in an active field and vibration blocking system, thinking they were going to prove it was the environment, and not the system. They were wrong. If they can't detect, and see the environmental fields, I don't believe they are affecting your system. Darrell Miles milesd@us.ibm.com May 14

You have clearly carried out some tests for fields but here are more. 1. Long working distance (25-30mm) does the problem become greater? 2. Short working distance (5-7mm) does the problem become less? Or 3. Low kV (5) does the problem become greater? 4. High kV (30) does the problem become less? Each of these series proves the field problem if you have a change. Third test I have rotated a column 45 degrees, the question being is the problem the same or does it change? A change in physical orientation providing a change in the problem indicates it is external as internal (microscope) faults will stay exactly the same. Steve Chapman protrain@emcourses.com May 14

SEM – digital camera

I work with an Hitachi S-570 SEM that is currently setup with a Polaroid film holder, and was previously setup with a 35mm film camera. I am wondering how easily a digital camera could be substituted for the 35mm film camera. If possible, it would be much more affordable then refitting the microscope with a digital system. Sarah Aubut saubut@nrcan.gc.ca Apr 16

The Polaroid back or the 35mm option are still film. They capture line scans as the image is displayed in slow scan on the capture back/port. The problem is that digital cameras are one shot devices--they take a pix of what is there. As such, they do not integrate an image. The final image is not on the record CRT. I can't think of any way to do this digitally unless using a passive or active digital capture system. A digital camera is not going to work, in my opinion. However, if you find a workable solution, please let us know. What is missing is a frame buffer that would store all line scans and present them as a final (total) image as TV which could be frame grabbed as 640x480. But a good record CRT is good for about 4000x3000 lines/inch. Gary Gaugler gary@gaugler.com Apr 16

It is possible to fit a digital camera onto an older SEM. I used a Nikon D70S, focused onto the recording CRT of our Hitachi S-2460N and I took some nice images with the digital camera in the "bulb" mode. That's the key: you must have a digital camera that will "open the shutter" for the entire exposure and close it afterwards. Not many digital cameras have this feature. In operation, I find the image of interest, open the digital camera shutter with a wireless remote trigger, start the SEM slow scan exposure, wait until the SEM slow scan is over, close the digital camera shutter using the wireless remote. These images are as good (noise-wise) as one would capture with conventional film. The resolution is not as good a film, of course. Yes, there are a lot of tradeoffs. It is an inconvenience to operate in this mode--unless you are used to using film. Then, it's nearly the same as shooting conventional film. Once the microscopy is complete, remove the memory card from the camera and download them to your computer. One advantage of this method is that the magnification bar generated by the SEM will be recorded on the digital image (unlike digital capture systems that insert their own). The cost of this system is basically the cost of the digital camera and modification of the existing film camera holder to accept the digital cam ($1,200 total, in our case). Having said this, I must confess that on our other microscope, also an S570, we retrofitted a 4pi digital capture system that we are quite pleased with. On the 2460, that I described above, we only rarely need digital images greater than 1 MB. So, we use the X-ray analysis system to capture images. When we need higher resolution digitals, then we could use the Nikon camera. By the way, an article describing the use of a Canon digital camera to capture images was published in Microscopy Today several years ago. You might check the archives or perhaps the author could give us the citation. John J. Bozzola bozzola@siu.edu Apr 16

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There are two major considerations when using a digital camera to take slow scan images. I have a background in astronomy, so I'm very familiar with CCD devices and the inherent difficulties with them. I also tried to set up a digital SLR on a Topcon/ISI ABT-SX40. First, I'm going to make a couple of assumptions. I am going to assume that the digital camera is a digital SLR, like the Nikon D70. I am also going to assume that it has a 'bulb' feature, and that it is going to be able to be mounted directly, sans lens, to the photographic system on the SEM. The two major concerns are the alignment of the scan and the size of the chip. The chip is made up of 'buckets' (pixels) which basically count how many photons hit the bucket, and register that as an intensity value on the final photo. These buckets are arranged in a nice x-y pattern. If the scan of the microscope is such that the scan line overlaps two rows, or is at an angle to the x-y orientation of the CCD pixels, you can get a bleed-over effect that causes some distortion in your final image. The Nikon D70 has a pixel size of 8x8 microns, and a resolution of 3000x2000 pixels, so it might not be as obvious an artifact at first, but once you start zooming in, you will see it. This can be corrected, though, given some careful alignment when setting it up. The second major concern is the size of the chip. A 35 mm film negative will record an image in a 36 x 24 mm area. The CCD chip is physically smaller, recording an image in a space of 24 x 16 mm (For a Nikon D70- you can get this measurement off of the specs on your camera). This being said, when you view your images, you have to take into account that you are either not going to get the full image- if you are photographing without any compensating lenses for size, for instance, you might lose the micron bar and information lines at the bottom of the image, for example, or you are going to be off on your measurement calibration if you do correct the optics to capture the full image. Of minor concern is pixel saturation. When the shutter is open for a longer period of time, and a line dwells in a spot for longer than a microsecond, you have the potential of maxing out the intensity resolution of the pixel. This was a problem that we ran into with using CCD cameras on telescopes- a nearby star would be bright enough to "Fill the buckets" of the CCD pixel, which would then overflow into neighboring pixels. CCDs for the most part are more sensitive to light than film, so make sure that the intensity of the photo CRT is set at the lowest possible value to register a satisfactory image, and you won't max out the intensity on the CCD. I hope this helps. I know I seem to go off on the D70, but it's the camera I have, and therefore have the booklet with the specs sitting in front of me. Justin A. Kraft kraftpiano@gmail.com Apr 16

These are important concerns. Yes, I used a digital SLR with a Nikon Micro Nikkor lens that was focused on the recording monitor. When recording the image, I adjusted gamma so that the image was rather flat in appearance (and then did a final adjustment in Photoshop). I included as much of the photo-CRT as possible (including the magnification scale) and only had to minimally crop the final captured image to exclude areas outside of the photo-CRT. I am very pleased with the results, but realize that it is somewhat inconvenient (maybe not even practical) if you have multiple or infrequent users who may not remember all the steps. John J. Bozzola bozzola@siu.edu Apr 17

EDX problems

Today I have the impression that our EDX detector coupled to our Tecnai G20 TEM microscope is just going crazy. The Dead Time (DT) is constantly on 100% when I want to analyze a particle (objective aperture out). I use classical copper grids and I took care not to be near a grid bar while reading. Here is what I did to extend the diagnostic: 1) When I position the beam on a grid bar, the CPS (counts per second) go up to 300 000 and DT 100%, as expected 2) When I target the center of a grid hole with the objective aperture out, I get a count of 30 and DT 100% 3) When I take the specimen holder out and insert the objective aperture, I have a CPS of 5 and a DT of 100% 4) When I take the specimen holder out and take the objective aperture out, I have a CPS of 0 and a DT of 0%, but sometimes it goes suddenly to 100% then come back to 0% (frequency, about every 2 sec) 5) I can retract the detector, in this case I get the same result of point 4) I am a biologist, so I cannot understand much of what is happening, but for me it look like the processor is down. What I wonder is why the counts look correct while the DT is crazy. How can I have a CPS of 5 and a DT of 100%? I never let the Dewar warm up (but there is a protection in this case) and the only thing I did recently is calibrate the instrument (last week). The instrument is from EDAX. I contacted them 2 weeks ago to ask for instructions on how to calibrate it, but still got no answer. So I don't expect much help from their side. Stephane Nizets nizets2@yahoo.com Apr 18

You didn't mention if you tried this test: Set up your normal operating conditions, e.g., objective aperture out, etc. then, turn off the e-beam. If you still get 100% deadtime and various count rates, it could mean that your detector FET, attached to the SI(Li) crystal, is blown. If deadtime and counts go to zero, check the condenser aperture, could someone have pulled out the condenser aperture? That would result in very high beam currents down the column perhaps resulting in enough counts to paralyze your system in the test conditions you presented. As for EDAX, don't give up so soon, contact Steve Mann there, by phone. He's been quite responsive to issues I've had on my EDS systems in the past. Or, give it a rest overnight. Then try again. Possible software glitch? Gilbert Ahlstrand ahlst007@umn.edu Fri Apr 18

A possibility, considering you mentioned a re-calibration. Be certain the low energy end of your spectrum is properly inhibited. This is usually a "threshold" adjustment. Uninhibited noise at the low end (typically below Be/B) will be counted in the DT value. Another possibility, as mentioned is a bad FET pre-amp. Another is poor Dewar vacuum / blown thin window. One thing that "got" me was when I forgot to be certain my IR camera illumination was off (it defaults to ON when the viewing program is executed). The detector can see the IR and goes berserk! Woody White nwwhite@babcock.com Apr 21

High deadtime is a common problem related to the EDS maintenance. Several possibilities for this: 1. Thick ice layer buildup on the window. Consult the manufacture how to remove it. For Oxford INCA, simply run the detector conditioning program (refer to http://www.oxinst.com/wps/wcm/resources/file/ebd6db4f1d7f723/EDS-Hardware-Explained.pdf). This should be done in weeks/months. 2. Detector needs correct re-calibration from time to time (in months/years). If still not working, unlikely your service can be skipped. Zhiping Luo luo@mic.tamu.edu Apr 22