NetNotes

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Selected postings to the Microscopy Listserver from January 1, 2012 to February 29, 2012. 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:
uranyl acetate

I have been having a problem with getting uranyl acetate to go into solution. I have been using the same bottle of uranyl acetate (UA) for over 22 years. I have not had any problems when I was using nanopure water, but now that I am using distilled water, I cannot seem to make a 3% solution of UAaq. Is there anything I could do to help it go into solution? I am near the end of the bottle. Maybe I should just buy another bottle. Or try to get the lab to get the nanopure water working again (I think they need a special filter). Or would adding a little methanol help? I am open to suggestions! ***Barbara Plowman bplowman@pacific.edu Thu Jan 19***

I had the same problem making a 2% solution about 20 years ago—using old UA. As soon as I started using a new supply the UA went into solution as I thought it should. If your UA comes in a can, it is not a bad idea to store the bottle in the can between uses. I have found that this extends the useable life of the crystals until I empty the bottle. Patricia Stranen Connelly connellyps@nhlbi.nih.gov Fri Jan 20

I have no experience with aging uranyl acetate. But am curious how time could cause a crystal to become insoluble. Many chemicals on the lab shelf absorb water over time (they often form cakes). This makes them difficult to weigh out, but I cannot think why it would hinder solubility. Alternatively, could an oxide be forming on the surface of the grains, an oxide might be insoluble (think rust). If the UA powder is not super fine, I guess you could try to grind it with a mortar and pestle to expose fresh surface, although from a safety point of view that seems dubious (UA aerosol—yum!). Anyway, I'd be interested to hear if anyone actually knows how time leads to insolubility. Tobias Baskin baskin@bio.umass.edu Fri Jan 20

I think this problem has to do with the pH of the water that is used. Probably the pH of the nanopure water was higher than the distilled water. Hans Janssen j.janssen@nki.nl Fri Jan 20

I know that uranyl acetate is generally considered as only slowly/poorly soluble in water. I would suspect that acetate salts would decompose more readily than some other salts and so I had always just assumed that old uranyl acetate just slowly oxidized producing a less soluble mixture. Certainly I had heard that 10+ year old uranyl acetate was less soluble than a fresh supply so I had always refused kind gifts of uranyl acetate if it was old. Malcolm Haswell malcolm.haswell@sunderland.ac.uk Fri Jan 20

I had used the same water source with both samples of UA, old and new. I know that the pH stayed the same most of the year so I do not think that the pH changed from morning to afternoon of the same day. If I remember correctly our pH usually ran in the 6.5 range. I know it was well below a pH of 7.0 any time I tested it. Reply to Malcolm: I had trouble getting the old UA to go into a 2% solution over a period of time. It just kept getting worse to the point that I had it mixing for up to two days (under a metal can to exclude the room lights) which had me questioning what was wrong when I knew that others made 4% UA with no difficulty. At that time I "borrowed" a gram from another lab, which I knew was going into solution fine according to my EM Tech friend. I then used my water, glass bottle, etc. the same manner that I had done before with my old UA. It went into solution within a short period of time so I ordered a new supply of UA from the same supplier of my old bottle and the borrowed UA and have not had a problem since. I suggest that those who offer me gifts of old UA send the bottles out the next time they have a pick up of radioactive waste. I have tried several old supplies over the years and found none to go into solution readily. Reply to Tobias. When I started in EM back in 1971 my supply of UA was in the form of coarse crystals, which I was instructed to grind fine. I had a dedicated mortar and pestle for this purpose. I was very glad that when I ordered my first new bottle of UA some years later that it came as almost powder-like crystals. This is how my UA has come since that time. Since it is now 2012, and I am still alive, I guess I did a good job of not inhaling the dust! According to a very old edition of the Merck Index, UA was used as a snuff. Hard to believe with all the cautions on current MSDS sheets. connellyps@nhlbi.nih.gov Fri Jan 20

Well, this has been an interesting discussion about what happens with aging uranyl acetate (UAc) salts. Although I am certainly not a chemist, I suspect several events (some already mentioned by other contributors) are taking place to cause the salts to become less soluble: 1. Degradation of acetate. Acetates are notoriously unstable and break down with time. If you detect a strong smell of acetic acid (carefully, by wafting the air above the container), then decomposition is taking place. 2. Photolytic decomposition. Most people keep the UAc away from the light; however, this is not always the case. I've seen solutions sitting on top of counters for long periods of time in clear, glass containers. You should cover the container with aluminum foil and keep it refrigerated (to cut down on light even more). 3. Radiolytic decomposition. UAc (even depleted, U238 Ac) is weakly radioactive, giving off alpha, but also beta particles and gamma rays as part of the decay process. Over time, these will degrade most chemical compounds. In fact, ever wonder what was coating the inside of your UAc staining vessel? That insoluble material is uranyl oxide (Teply & Tulik 1963. Formation of Peroxidic Precipitate in the Radiolysis of Uranyl Nitrate Ketone Solutions. *Nature* 200:671–672). Now, for some "popular" trivia. Anyone ever hear of a Revigator? That's a water vessel lined with uranium that releases radon into the water! Back in the 1920s they were sold to fitness folks to "restore water's lost element" and invigorate the body . . . Frightening! You can occasionally find them on EBay and I am surprised they are even allowed to be sold. John J. Bozzola bozzola@siu.edu Fri Jan 20

Specimen Preparation:
carbon dioxide tank stability

I happen to have a cylinder of CO_2 for critical point drying that has been here for 3 years or so, untouched. I need to start up critical

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point drying again, and I am just wondering whether there is any reason to be suspicious of this "old" CO_2? Seems like it is just a simple gas in a tank, so what could happen? Contamination from the tank liner? CO_2 is a pretty decent solvent. I would be delighted for people to say, "You are crazy! Nothing wrong with it!" I have someone else's samples coming in and I'd hate to ruin them. So I am being careful. ***Tobias Baskin baskin@bio.umass.edu Thu Feb 23***

Chances are good that it would be OK; however, if the specimen is irreplaceable, then I would order another tank. Around here such a tank is about $14. If the procedure gives strange results, and you use the old tank, you will always wonder if that was the cause. John Bozzola bozzola@siu.edu Thu Feb 23

I am using the same large CO_2 bottle since nearly ten years without any negative happening. Stefan Diller stefan.diller@t-online.de Fri Feb 24

I have a really big bottle of CO_2 since 12 years, no problems. However, if you have really "critical" specimens, like cultured cells or sensitive tissue, use CO_2 of highest purity ("water-free"), though it is extremely expensive; the standard CO_2 contains a lot of water and is useless in CP-drying of biological specimens. I can imagine that the water content in standard CO_2 could cause corrosion within the bottle over the years. Over here in Germany bottles have to be "mirrored" in the inside by removing the valve and thorough inspection. Peter Heimann peter.heimann@uni-bielefeld.de Fri Feb 24

I haven't gone as long as some of the other folks, but I routinely have a tank for 2 years or more without problem. I agree about the problems of water in CO_2 tanks, but there is a cheaper-in-the-long-run solution: put a no-go water filter or desiccator in the line. This way you don't need to buy the really expensive CO_2—you do need a siphon tank—but I do recommend getting a "food-grade" siphon CO_2. These might have water problems (nonissue with a filter), but they won't have any of the oils that can contaminate non-food-grade CO_2. Mind, I'd also use an oil filter and a particle filter. Phil Oshel oshel1pe@cmich.edu Fri Feb 24

I would like to add my experience on the CO_2 as a possible cause of sample damage during preparation for SEM. Until recently I had never detected any problems with the industrial rate CO_2 I use for CPD. Maybe water contamination as Peter points out, is a problem in countries where the air is humid, but not in Athens (well, at least the nice climate in Greece remains unspoiled). The last couple of years I found a shop that refills the cylinder on the spot. They have a big tank and they use a pump to refill the cylinder. Since that time I detected some little spots occurring sporadically on my samples and they look like something oily. Because they are very small <30 microns they were puzzling me, until I came with the idea that they are oil droplets coming from the refilling pump. Has anybody else noticed such a problem? Please have a look at http://www.eikonika.net/v2/photo_list_nikas.php (it's an animal epithelium). Yorgos Nikas eikonika@otenet.gr Fri Feb 24

TEM:
current center vs. voltage center

Looking for info on what to tell my students regarding current center vs. voltage center. I have often settled on current center because, for me, I would rather have the image stay in the center when focusing. I have been told that the current center and voltage center seldom are exactly the same and the operator should pick one or the other for alignment. What is the general practice in labs? Try for both, pick one, or punt? ***Jon Krupp jkrupp@deltacollege.edu Wed Feb 29***

I'd have to go to one of the TEM books to define current and voltage centers, but it depends on your scope (and sometimes preference) what to align. On the primitive end, the HVEM current center was aligned by moving the objective lens upper pole piece, and the voltage center was not aligned, but both the Polara and the Titan from FEI had electronic alignments for both centers. I am no longer employed by FEI, but I was a few years ago. Bill Tivol wtivol@sbcglobal.net Wed Feb 29

Both are to align the beam to the objective lens. Generally for materials science lattice imaging, generally near Scherzer focus, the voltage center is done. The high voltage is varied and the beam is tilted to minimize image movement with high voltage change. For biological imaging and especially with biological tomograms where defocus values vary over many microns, the objective current center must be done. The beam tilt is aligned to minimize image movement with changes in the objective lens. The two alignments are slightly different and vary from scope to scope. If specified in the original order they can be pretty close to spot on but that is seldom written into the specs. Roseann Csencsits rcsencsits@lbl.gov Wed Feb 29

With the stability of the high voltage being the most sensitive area of an instrument it is important to have the centre of the high voltage on the axis of the instrument; voltage alignment. The historic comment on current and voltage alignment are that the former is for convenience the latter for resolution. The two should not be far apart but when pushing an instrument to its limit it is voltage alignment that results in the higher quality image. Check it out? Caution, when dealing with biological specimens that may require a wide range of objective current settings when working over a wide range of magnifications, then current alignment is the best route as it keeps the image on the centre of the field of view. Steve Chapman protrain@emcourses.com Wed Feb 29

To follow up on Steve's comments. One of our TEMs is a BIOTWIN meaning that the objective lens configuration is designed to increase contrast for biological samples. In this case the manufacturer's recommendation is to center using current alignment although voltage alignment could also be used if desired. When you purchase a TEM, you often have a choice of objective lens configuration and the choice is made based on projected use for that instrument. Thus an instrument designed for low atomic number, poor contrast samples such as typical biological ones will be different than that chosen for nano materials or other materials where resolution is more critical. Debby Sherman dsherman@purdue.edu Wed Feb 29

We had a PhD student who looked into this years ago (Rudiger Meyer). I remember reading in his PhD thesis that the voltage center (where the beam energy is wobbled) is mathematically equivalent to finding the coma-free axis, whereas the rotation center is not. The two always differ by a few milliradians. For low-resolution work we do the "Rotation center," i.e., current center, but insist on using the coma-free axis/voltage center for any sort of chemical mapping using EFTEM or high resolution imaging (this can be found in the "Autofilter Tools" panel on FEI instruments, e.g., Tecnai or Titan). For EFTEM the correct voltage center is important otherwise large image shifts occur as the HT is adjusted. Otherwise 3-window maps are prone to losing the edges of the map. I hope this helps. Jon Banard jsb43@hermes.cam.ac.uk Wed Feb 29

It might be useful to read: The importance of beam alignment and crystal tilt in high resolution electron microscopy <http://orproxy.lib.utk.edu:2053/science/article/pii/0304399183900062> *Ultramicroscopy*, Volume 11, Issue 4, 1983, Pages 263–281 David J. Smith, W.O. Saxton, M.A. O'Keefe, G.J. Wood, W.M. Stobbs. Especially section 4. We would always start with a good voltage centering on our cold FE Hitachi HF-2000, to prep for a following coma-free

alignment for high-resolution imaging. After carefully doing a coma-free tilt adjustment, we always found one tilt axis was very close to the value found by voltage center, whereas the second tilt axis was noticeably off by a small amount (don't recall the numbers . . .). Larry Allard allardlfjr@ornl.gov Wed Feb 29

On FEI/Philips microscopes there are several different alignment options: (a) Current center aka rotation center: with the beam spread, the objective lens current is wobbled; any image shift is minimized by adjusting the beam tilt coils. Good enough for low resolution work, e.g., tomography. (b) Coma-free alignment: the beam tilt is wobbled some milli-radians around the center value. This causes beam tilt induced astigmatism and defocus (coma) due to the Cs of the objective lens. The difference in focus and astigmatism between the plus and minus beam tilt has to be made symmetric by adjusting the beam tilt coils. This has to be done for both the x and y direction of the image. For any high resolution work, coma free alignment is the preferred method. It's a quick-and-dirty alternative for a Zemlin tableau, which is superior and used e.g. in the tuning of image Cs correctors. Many people iterate rotation center and coma free alignment a few times, this is not necessary: rotation center is the rough alignment to bring things close, coma free then is the final fine-tuning. (c) Voltage center (only available for energy filters): the accelerating voltage is wobbled, image shift is minimized by adjusting the beam tilt coils. This alignment however affects the entire beam path in the condenser system, the objective lens and the projector system as all lenses are kept constant but the accelerating voltage is changing. There are options for compensation of beam intensity (= beam size) and beam shift. This is only needed for convenience when using an energy filter for certain applications where the acceleration voltage is changed. Wim Hagen wim.hagen@me.com Wed Feb 29

TEM:
X-ray radiation

We have taken almost all the shielding from our Tecnai G20 down in order to make some repairs and now that I am ready to mount them again, I ask myself: Should I ask FEI to come and control the X-radiation? We work mostly at 100–120 kV but sometimes we use 200 kV so X-rays should be considered, but is there a risk of leak after unmounting/remounting the shielding? ***Stephane Nizets nizets2@yahoo.com Wed Feb 8***

I would suggest that it would be very important to get either the manufacturer's engineers or someone who is trained to that standard to do the re-assembly and X-ray check afterwards using the appropriate type of meter rather than any old Geiger counter. Most of the training I had in the early days emphasized the need for scrupulous checks after a major re-assembly or modification and it was even more important for 100 kV+. In the olden days we had a Siemens IA that spectacularly failed radiation checks when operated at 100 kV but was fine at the lower voltages. Malcolm Haswell malcolm.haswell@sunderland.ac.uk Thu Feb 9

Many thanks for taking the time to share your thoughts with me. I appreciated it very much! The majority (5 versus 2) thinks that, although the risk is minimal, it would be well advised to control the X-ray leakage of the system after remounting. One person mentioned the opening the column which I think is a good point (actually she talked about breaking the column but I don't think I am strong enough). I didn't say it but we didn't open the column, in which case I think it is very important to check the X-ray leakage after remounting. Stephane Nizets nizets2@yahoo.com Thu Feb 9

TEM:
shift parameters

We have a JEOL JEM 2000 EX II behaving oddly. Each time I change magnification the whole set of shift parameters (condenser and projector) are recalled with a set that is totally wrong. I can correct it each time, but as soon as I change magnification, the microscope is recalling the wrong set of parameters. Is there any instruction for storing a set of shift parameters for all magnifications? Same for diffraction! Thanks in advance for any help. ***Marco Arienti marienti@tiscali.it Wed Feb 22***

We have a JEOL JEM 1200EX II so I'm assuming it has the same computer system. There are four magnification ranges (M1 to M4) and the one between 10K and 250K (M3) is the reference range. I suspect that the actual numbers may vary between microscopes with different objective lenses, etc. I should think that if the alignments change at every magnification, then you have to call service. There are adjustments between ranges for beam and image alignment and a procedure that involves "teaching" the microscope various settings, like astigmatism and making it remember the settings. Before you go delving into the procedure however you must write down the values from the computer (including perhaps the Hex values). Type PRTEST (space) M2 for a listing. Make one change at a time and save the result in case you need to go back. For example, use image shifts between ranges and PL align within a range. To save changes you need to enter "learn" mode by first entering DADJ (space) 1—then make a change —finish with DADJ (space) 0. This is a form of programming and you need to start slowly and save often. For different voltages we use the user free control (UFC) that is explained well in the manual. But if you have wrong values, call service. Rob Keyse rok210@lehigh.edu Wed Feb 22

It seems that you need to change the battery of the memory card of your TEM. This card is located behind the right console and removable easily after switching off the TEM; then you will be able to measure voltage of the battery. Of course, all alignment values need to be adjusted again when RAM's content is lost. Nicolas Stephant nicolas.stephant@univ-nantes.fr Wed Feb 22

EDS:
silicon drift detectors

We have a new silicon drift detector (SDD) Oxford detector on our S-4700. I was wondering if most owners of these systems keep them in Operate mode, or only cool them down when users are scheduled. Are there any issues that may be encountered? ***Patricia Scallion pscallio@dal.ca Thu Feb 2***

I posed the same question to my Oxford service engineer and he told me that they recommend leaving the detector in operate mode. I haven't been able to detect any adverse effects in detector performance. Bryan R. Bandli bbandli@d.umn.edu Fri Feb 3

We also have an Oxford SDD and the technologist who installed it recommended that we keep it in standby mode when not being used in the near future. Translation: if it would be used within 12–24 hr, keep it chilled. Any longer periods, go to standby. My personal opinion is keeping it chilled all the time is putting an unnecessary "strain" on the Peltier electronics. John J. Bozzola bozzola@siu.edu Fri Feb 3

I have spoken with Oxford's applications people and they suggest leaving it in Standby except when needed. It takes no more than 5 minutes to cool down to the operating temperature. Alan W Nicholls nicholls@uic.edu Fri Feb 3

MT