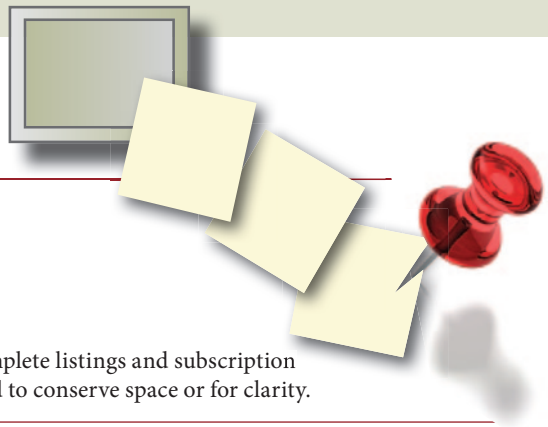

NetNotes

Edited by Thomas E. Phillips
University of Missouri

phillipst@missouri.edu

Selected postings from the Microscopy Listserver from July 1, 2012 to August 31, 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:
processing 2000 cells for TEM

We have a user of our EM Core who would like TEM images from a population of cells collected by flow sorting. The cell number is really tiny, just around 2000 cells. The best I can come up with is to pellet the cells, noting where the cells land (by example in another tube) on the inside of the tube (1.5 mL), then after removing the medium without disturbing the pellet of 2000 cells, add an aliquot of non-relevant cells, such as RBCs, to augment the pellet to the point of visibility, and pellet again being sure that the added cells will land on the same area as the 2000. I envision the precious 2000 cells being cupped by the cells from the augmenting aliquot. Does this sound reasonable? Has anyone done something like this? Is there any method that can be used with confidence? The cells are neurons from specific mouse ganglia and the objective is to look for herpes viruses inside. We will be happy to receive any comments or suggestions. ***Marcia Miller mamiller@coh.org Fri Aug 24***

Have you considered using agarose to gel the tiny pellet after fixation? Michael Delannoy delannoy@jhmi.edu Fri Aug 24

Vlad Speransky had a similar question in May this year and the possibility to use microdialysis tubing as is used in high pressure freezing applications emerged: Hohenberg, H., Mannweiler, K., and Mueller, M., "High-pressure freezing of cell suspensions in cellulose capillary tubes," *J Microsc* 175 (1994) 34–43. Cells might be collected in there, in fixative perhaps, and the tubing clamped shut. That way you would have a tiny packet that does not get lost as easily! Jan Leunissen leunissen@aurion.nl Fri Aug 24

There are many approaches for preparing small numbers of cells for TEM examination (flat embedding them on glass, centrifuging them to a specific location in a tube, pelleting them down in a sealed Eppendorf pipette tip, embedding them in agarose or gelatin). You could contact me off-line so we could discuss options and find a good approach to use. Your real problem will be in preserving the morphology of cells that have been through a cell sorter. It is a really disruptive process for the cells to go through and considering what they look like in the TEM, it is surprising that they are still viable. Is there a way for the researcher to let the cells recover before fixation? If not, then you will have to use the material given to you. Centrifugation is a problem because the cells will be very fragile, but there are ways around that too. Paul Webster pwebster@hei.org Sun Aug 26

My first thought when I read your message was the same as Paul: if the user wants to use TEM, it most probably means that he wants a good morphology. Of course the previous propositions are all valid techniques, but you may just be able to optimally prepare damage/unhealthy cells and then I wonder what sense that makes. If the cells are still viable after cell sorting, you may put them in a sterile petri dish with some medium and let them adhere to a suitable substrate for flat embedding. In this case 2000 cells are more than enough. Most cells adhere within several hours; this would leave them some time to recover too. May I advise you to consider taking appropriate measures when working with infected material (including disposal), especially when the material is infected with a human virus? Stephane Nizets nizets2@yahoo.com Mon Aug 27

Specimen Preparation:
LR White

I have in the distant past polymerized LR White in the freezer with UV light. My question is if this is possible/advisable at 4°C or even at room temperature? ***Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Fri Jul 6***

To my knowledge, LR White does polymerize with an exothermic reaction. There are mentioned at least 4 possible procedures: polymerized either chemically (by addition of a catalyst/accelerator) by elevated temperatures (classical polymerization oven, or also by means of microwave treatment) UV-irradiation (365 nm) in the cold (lowered temps 0°C–minus 20°C). Recommendations of most suppliers is to polymerize at least at 0°C down to –20°C, using UV light (30 W, for approx. 24 h). If you use the chemical accelerator (cold cure) this will end up with polymerization within at least 4–5 hours (at room temperature) which to me seems to be a rather short and "speedy" polymerization process. It also has been reported to have poor infiltration properties. To achieve slower, and perhaps more equal polymerization conditions, one can place embedding capsules into ice. Polymerization by heat treatment, as usually done with epoxide resins, is also possible. The oven is set to a temperature lower than 50°C for gelatin capsules (for BEEM capsules suppliers tell us: 60–65°C). All polymerization in gelatin or BEEM capsules with tight closing by firmly pressed Parafilm pieces over the cap to prevent oxygen and moisture interferences. Wolfgang Muss w.muss@salk.at Sat Jul 7

Thanks to all who replied; I got 10 emails and 34 out-of-office replies. I did not frame my question well enough, but it looks like everyone is just about as confused as I am about certain things, and the tech sheets about LR White from different vendors are inconsistent. LR White now comes either with a catalyst already mixed in, or with the catalyst (benzoyl peroxide) on the side that must be used for polymerization. The accelerator is an additional component that can be added for "cold cure," which means polymerization at room temperature, which is strongly exothermic so cold cure is a bit of a misnomer. LR White can be polymerized in an oven at >65°C if in polyethylene capsules, or at 50–60°C in gelatin capsules, and, according to some, as low at 45°C, but will not polymerize at 40°C. I knew this; I have used 50°C, but my current project is sensitive to even this heat. So LR White can be polymerized with UV. I have done this in the freezer (–20°C) in the past. My question was if it can be done at 4°C? My refrigerator is actually 6°C. Several people wrote that it has polymerized just fine at 4°C, but now I realize I have no idea how exothermic this reaction is, and if it is detrimental to antigen retention for immunolabeling. That was really my question, but I just didn't day so! The use of osmium tetroxide or even the presence of lots of metal (e.g., hemoglobin) can make the reaction so exothermic

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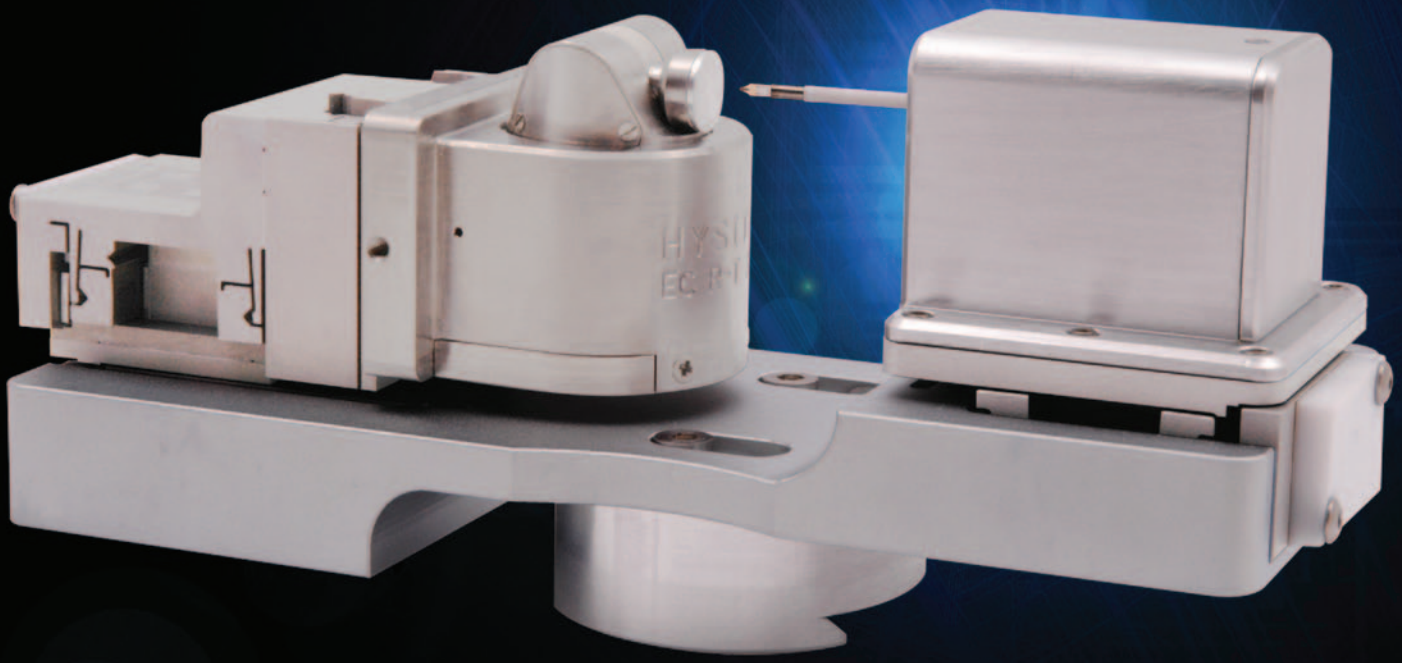

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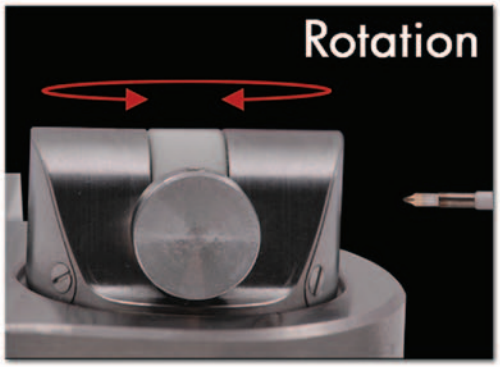

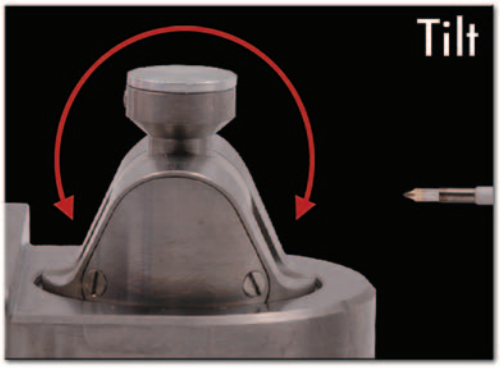

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that stuff really gets cooked. In addition, perhaps the optical density of osmicated tissue may prevent the polymerization by UV. Several people have the Pella UV cryo chamber and like it. So I guess now the question is: How exothermic is the polymerization at each of the recommended temperature/conditions? Could I have done it at 6°C and retained antigenicity? Then there's the whole microwave polymerization thing, which I have also done in the far past and didn't try here. I did polymerize in the freezer with UV, but it took longer than I expected (48 hours), and I have no idea how warm the tissue got. Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Mon Jul 9

Tina says, "How exothermic is the polymerization at each of the recommended temp/conditions? Could I have done it at 6°C and retained antigenicity?" My feeling is the reaction is pretty exothermic—I have seen polyethylene capsules deform from the heat of the reaction! I am 100% confident you can retain antigenicity at 6°C for antigen X but definitely not for antigen Y. Unfortunately, the only way to tell if you have antigen X or Y is to do the experiment. Armbruster et al., 1983, *J Histochem Cytochem* 31:1380–84 showed dehydration and embedding temperature influenced tubulin labeling at the EM level. See also my paper on a simple homemade device that uses a carbon dioxide gas cylinder to hold an aluminum block at between −20 and −45°C for processing and embedding tissues (Shoemaker et al., 2003 *Microscopy Research and Technique* 62:262–266). It is not as fancy as the Pella chamber but an inexpensive alternative. Tom Phillips phillipst@missouri.edu Mon Jul 9

It seems that your main concern is the maximum temperature during curing. That depends on many factors which seem to be getting jumbled together here. If the reaction was not exothermic, there would be no issue. There would be no generation of heat to cause a problem. The only question would be the temperature conditions necessary to get the reaction to proceed. However, given that the reaction is exothermic, the issue really becomes one of heat generation and heat transfer. A strongly exothermic reaction will definitely make the problem worse because more heat will be generated per unit of mass. A quick reaction will make the problem worse because that heat will be generated in a shorter time and will have less time to dissipate. More mass will make the matter worse because you have that many more units generating heat. Low heat conductivity will make the matter worse because once the heat is generated it will not be conducted away so well and will accumulate heating up the sample. You can't do much about the first factor. It will be a function of the resin and its reaction. You should be able to control the latter three to try to keep the temperature within your bounds. However, I don't think that I want to analyze the problem to determine what the heat rise will be for the specific conditions. I'd rather just try to push it in the right direction. You could use a case of an instantaneous reaction with no conduction to determine the worst case of temperature rise. Hopefully that is far worse than what you would see in practice. You might be able to control the speed of the reaction by the combination of reactants. If the resin will still polymerize with less accelerator, maybe you can just give it more time. You should also be able to control the speed of the reaction by the initial temperature of the reaction. I seem to recall the rule of thumb was a doubling of the rate for every 10°C increase in temperature. Of course, an exothermic reaction will generate heat which will warm things up and speed things up which will lead to an even faster release of heat which will. I have worked with small amounts of resin in my material applications. I could pot samples all day working with 1-inch molds. However, increasing the volume to 2-inch cubes led to situations where the resin heated to the point of smoking and nearly burning. We also don't think much of the exotherm involved with concrete curing. However, the mass of something like the Hoover Dam renders the exotherm a very real concern. I understand that was a major factor determining how fast the concrete could be placed—even with cooling lines. Temperature sinks in conjunction with high heat conductivity can do a lot to limit the maximum temperature. It could well be that the limiting factor will be the resin itself. Much can be gained by keeping the mold thin so that the heat does not have so far to flow. Like I said earlier, a full analysis would probably not be worthwhile. It brings back not-so-fun memories of partial differential equations from many years ago. Hopefully you can play with these factors and find some workable conditions. Warren Straszheim wesaia@iastate.edu Tue Jul 10

I had the person who wants to do the TEM immunogold labeling first try her antibody using fluorescence with some concentrations of glutaraldehyde in paraformaldehyde (it can tolerate about 1% glutaraldehyde) and with heat (it does not tolerate 50°C). From replies I've gotten it seems like any kind of polymerization of LR White may be too exothermic! Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Tue Jul 10

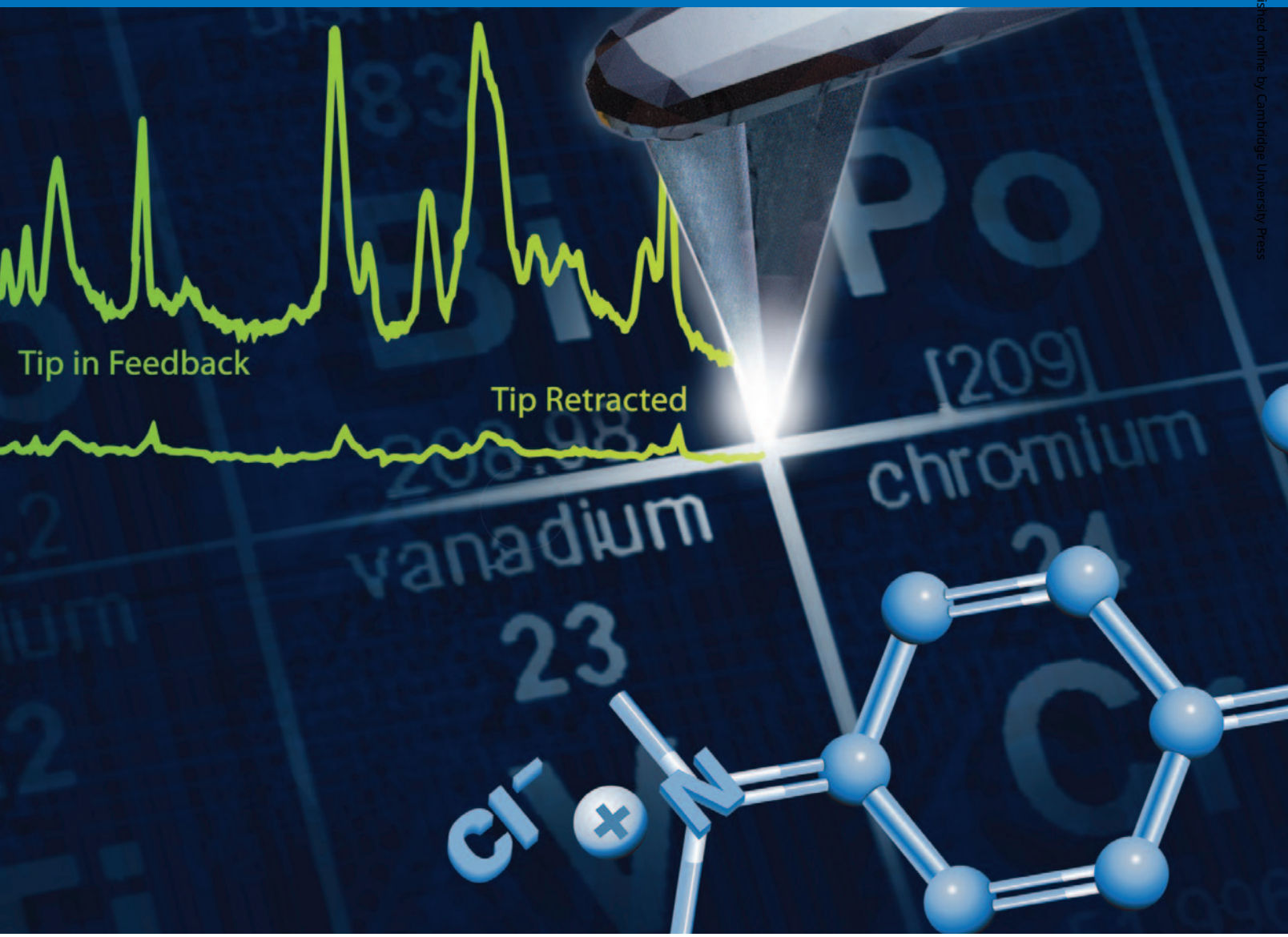
By now, the thread is a bit frayed, but let me add a couple of stiches. The issue is heat, more than temperature. Thus even though LR White polymerization raises the temperature a lot, you can minimize the amount of heat your sample gets by reducing the ambient temperature, increasing the efficiency of heat exchange around your sample and so on. It is definitely the case that antigens will suffer a lot more heat if you polymerize LR White in a 50°C box compared to a 4°C box. Whether that lower amount of heat is still enough to gork (scientific word meaning denature) your antigen of interest of course depends on that antigen. Besides lowering the temperature, it is also possible to protect antigens. I found that adding DTT to mixtures of butyl/methyl methacrylate did not block polymerization but did prevent the sample from turning brown during (also exothermic) polymerization and helped a great many antigens survive. I have no idea what DTT would do in LR White, but this is just an example of something that might protect your antigen during the polymerization reaction Tobias Baskin baskin@bio.umass.edu Sat Jul 14

Specimen Preparation:
lead citrate

I have traditionally used Reynolds' lead citrate made from lead nitrate but Venable & Coggeshall's (V&C) formula that uses solid lead citrate as a starting material always seemed to me a better idea. I decided to give the V&C protocol a shot but my first two attempts have failed. I used freshly prepared NaOH stocks made from boiled deionized water and carbonate-free concentrated 50% NaOH solutions. I used "carbonate free lead citrate" solid from a major EM supply house (albeit a bottle that was several years old). V&C suggest 0.01 to 0.04 g lead citrate in 10 mL of 0.01 M NaOH and I used 0.02 mg per 10 mL. In both my attempts, most but not all the solid goes into solution. This is naturally unnerving. I am about to revert to Reynolds' formulation unless someone has thoughts on what is going wrong. I am aware of other formulations like Sato, etc. but really want to restrict my efforts to V&C or Reynolds'. ***Tom Phillips phillipst@missouri.edu Mon Aug 13***

We use the same formula you mentioned with the solid LC. It looks like our final concentration of NaOH is different than yours. Ours ends up being 0.1 N NaOH. We use the same amount of lead citrate. Here is how we mix ours: Make fresh 1 N NaOH - 0.4 g NaOH in 10 mL ddH_2O Measure 9 mL ddH_2O and add 1 mL of 1N NaOH. Add 0.02–0.04 g lead citrate (we usually keep it at >2 < 0.3 g) Agitate until LC is dissolved. We put our lead citrate into a 10 mL syringe and use it from that. We do not filter. We also, oddly enough, do not have to boil water, etc. Until the lead citrate level gets to the end rubber of the syringe, our stains come out great. No problems! Patricia Kysar pekysar@ucdavis.edu Mon Aug 13

I used to make the V&C version but I have returned to the original Reynolds method with better results. I rarely had full solubility with the V&C version. Geoff McAuliffe mcauliff@umdnj.edu Mon Aug 13



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I have tried both methods. The solid lead citrate has never worked for us. Not sure why that is since like you we have made it with exact detail. No matter what we did, boil water, make fresh NaOH, sit inside a vacuum (HaHa) we always got a tremendous amount of precipitate. So we just stick to the plain old Reynolds' recipe and fortunately that works 95% of the time. Lita Duraine duraine@bcm.edu Mon Aug 13

Your water is well below 7 so additional NaOH will be needed. Check it out. Use water made slightly basic to wet grids before lead stain and first wash after. Pat Connelly psconnelly@gmail.com Mon Aug 13

I decided the uncertainty wasn't worth it and made a batch of Reynolds' lead citrate. It worked perfectly with the NaOH stocks I had. I appreciate all the comments I got both on list and off list. It appears I am not alone in having problems making the V&C method work. Now I need everyone to think good thoughts as I go over to the TEM and look at my grids later this week; I need all the positive vibes you can spare. Tom Phillips phillipst@missouri.edu Mon Aug 13

Thomas, I'm not sure if any of the replies you received about using solid lead citrate were related to Sato's, which uses solid lead citrate as one of its 3 lead salts, but a pre-condition in that formulation is for the lead citrate to be calcined. The calcined condition is obtained by heating a small batch of lead citrate salt at 250°C in a muffle furnace for 2–3 hours. It goes from a white color to a yellow-brown and becomes very powdery. We have used Sato's exclusively for the past several years and I have never had a precipitate form when making it; it's crystal clear but yellowish. I'm wondering if this is the difference in experiences. David Lowry dlowry@asu.edu Mon Aug 13

I've used the V&C lead for 30 + years. Here's how I do it: Bring deionized water to just shy of boiling to drive out any dissolved gases. Let cool slightly. Take 10 mL of that water and add 0.01 g of lead citrate powder or crystals, swirl gently. Add 1 drop of 10 N NaOH. Continue to swirl. There may be some undissolved lead citrate. Don't worry about it. Draw everything up into a 20 mL syringe. Store the syringe in foil. To dispense, replace the needle with a 0.2 micron HPLC filter. Be sure to use NaOH pellets in the dish when you are staining and use Reynolds Wash then water to wash the grids. Lee Cohen-Gould lcgould@med.cornell.edu Thu Aug 16

Specimen Preparation:

uranyl acetate disposal

Is there anyone who can provide advice/instructions/warnings, etc. with respect to proper (legal) disposal of uranyl acetate in the U.S? ***Bill Roberts william.h.roberts@usa.dupont.com Tue Aug 28***

Since no one else has tackled this, I'll give it a shot. My info is more than a decade old; however, at least this part is still valid: Different states have different rules, so the correct answer will depend on where you plan to dispose of the uranyl acetate. When I was last confronted with this issue, I was in NY, and the answer was that the radioactivity present in dilute uranyl acetate (1 or 2%) was not high enough to prohibit it being poured down the drain. Check with your safety office and anyone else who might have valuable input. Bill Tivol wtivol@sbcglobal.net Wed Aug 29

I am shocked! Uranyl is not only radioactive; it is a toxic and a heavy metal and as such accumulates in organisms. It shouldn't be poured down the drain! In my lab it is taken care of as a heavy metal contaminant with lead and osmium by a special company. Stephane Nizets nizets2@yahoo.com Thu Aug 30

As with Bill's reply, I'm sure this may differ from state to state, and may differ from institution to institution. There is enough radon radiation in the water supply in Florida from all of the phosphate in our soil that our Health and Safety officers told us to flush our uranyl acetate, even our 8% stain solution, down the drain. We use such a small amount (0.5 mL at a time, per stain run), that the dilution factor will render it harmless to the environment when compared to the amount of phosphate (and radon) present in river water. I'm sure this is correct. As an amateur paleontologist, I've put fossil ice-age mammal bone from my Florida adventures in my SEM before, turned on my EDS system with the E.M. beam off, and detected an X-ray signal, so I know the fossils are hot. I've been drinking Florida water for 32 years now.... No wonder I've been feeling kind of funny lately! Ed Haller ehaller@health.usf.edu Thu Aug 30

We have had a similar experience with uranyl acetate. Our radiation safety department came and checked the radiation output of a bottle of 2% uranyl acetate and found the signal was barely above the background. It was so low that they also said we did not have to collect the waste for disposal like we do with most of our other waste from sample preparation and staining. This may depend on where you live and the background presence of uranium. I would also think it depended on the amount of waste produced. We produce very little, maybe an mL a week of 2% uranyl acetate when we are doing a lot of negative staining. We usually use ~20 µL droplets when staining grids. Debby Sherman dsherman@purdue.edu Thu Aug 30

Immunocytochemistry:

storing slides

Today a student brought in a stained sample to image on the TIRF microscope. After she stained the sample on Friday her supervisor told her that because the sample would not be imaged until Monday, she should put it in fix, so she added the paraformaldehyde (PFA) to the sample. I asked whether she misunderstood and her supervisor meant just the buffer (essentially PBS), but he really did mean the 2% PFA. Does anyone else out there do this? Has anybody heard of this before? ***Michael Cammer Miohael.Cammer@med.nyu.edu Mon Jul 9***

If you store in PBS, you risk bacterial growth damaging the morphology. And both formaldehyde fixation and immunostaining are reversible so storage in fix seems like the appropriate thing to do. The only disadvantage is the need to do more rinsing before viewing. Tom Phillips phillipst@missouri.edu Mon Jul 9

From my experience with tissue cultures I can only say that too long fixation can induce quite some background fluorescence; not sure how much that applies to the sample in question. Also, PFA doesn't go along well with all fluorophores. What I can recommend is using PBS with 0.1% (w/v) NaN_3 to avoid bacterial or any other growth. I kept fixed tissue culture samples like that over a several weeks without any problem, should also work for cells. Just keep in mind that NaN_3 is quite toxic and deal with it accordingly. Christian Liebig christian.liebig@medizin.uni-tuebingen.de Tue Jul 10

TEM:

LaB_6 filament shelf life

I have a 6-year-old unused Denka LaB_6 filament. It has been stored in a N_2 cabinet the whole time. It was opened 3 years ago, but not used. I have heard some say that these filaments have a "shelf life" of only 3 years (which is why I did not use it 3 years ago). Is this shelf life real? Can I baby it into use with a slow outgassing process? ***T Van Fister van.fister@hp.com Mon Jul 2***

Check Denka's site and see what they have for information. I am more familiar with Kimball and they have a wealth of information about LaB_6. See: http://www.kimballphysics.com/cathodes-and-emitters/technology/technical-information. In particular: LaB_6-01 Technical Bulletin PDF - (1,653 KB) General Guidelines for Operating LaB_6 Cathodes. It states that, "A rough, white, powder-like deposit on the cathode itself indicates the cathode operating temperature is too low (not even the oxide can be evaporated), and the vacuum is excessively poor (too much oxide is being formed). A gun which

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is vented to air (dry nitrogen is preferred), without waiting for the gun structure to cool (a few minutes is appropriate), causes the cathode to become a dark blue to bluish silver color. Such a cathode can often be reactivated by over-temperature operation at 2000 to 2100 K for 10 to 15 minutes; the surface layer, presumably mostly LaB? evaporates away, exposing fresh LaB_6 underneath. As the emission reappears, it is important to reduce back to a normal 1750 to 1850 K operating temperature." The idea of a shelf life may be related to the oxide buildup. I don't know if Denka tips will survive the over-temp operation needed to clean the tip, but I've never had any issues with (or statements about) LaB_6 shelf life. If the crystal is purple, I'd go with it. If it's blue, you've really not got anything to lose in trying to recover it. Also see: LaB_6-07 Technical Bulletin PDF - (335 KB) Recovery of Emission from ES-423E LaB_6 Cathodes Following a Vacuum Dump. This gives more detailed information about the things that can happen to a tip and maybe still be recovered. Ken Converse kenconverse@qualityimages.biz Tue Jul 3

SEM:
viewing a diamond knife

I am having some problems with my diamond knife; my sections tend to have many scratch marks. Probably the knife has many scratches and the useful surface of the edge is quite reduced. I am curious to see what is causing the scratches, is it debris sticking to the edge or the edge itself is damaged. I would like to check the knife in a SEM, but I am not sure if the knife will be safe in the SEM conditions. Would the pressure or the beam heat affect the diamond or the cement that is holding the diamond in any way? Does anyone of you have any experience in seeing a diamond knife in SEM? ***Josif Mircheski jmircheski@us.es Wed Jul 18***

Thermal conductivity of diamond is huge and I can tell you from practice that small diamond crystals survive FIB processing and SEM observation just fine. I would not be too worried about damaging the diamond knife during regular SEM observation, at least not due to overheating by the electron beam. You have a different problem to worry about—diamond is dielectric and will charge under the electron beam even more then glass does. Coating the knife with metal or carbon to bleed off the charge would ruin it, so your best bet is to use environmental SEM and look at the knife in "high pressure" mode under H_2O atmosphere. You can try coating the knife with washable conductive polymer for observation in high-vacuum SEM, but the latter would hide tiny dents and scratches. Valery Ray vray@partbeamsystech.com Wed Jul 18

A long time ago I put my wife's engagement ring in a high vacuum SEM without coating. She was sure I was going to ruin it. I had no trouble at all using 5 kV and going to several thousand X to look at the edges of the facets. I've heard that diamond is non-conductive, so perhaps it was the accumulated dirt and grime on the stone that kept it from charging. Guess I should try this again, then clean it and see what difference I get. Ken Converse kenconverse@qualityimages.biz Wed Jul 18

Years ago I have observed an old diamond knife at 1 kV without coating. My main concern, rightfully or not, was heating under the beam and possible increase of adhesion of debris, so I used low magnifications—not higher than 1000×. I have found out a lot of sections stuck to the edge. After soaking overnight in Alconox and cleaning with a foam stick, the knife worked again. It was rather difficult to remove all traces of Alconox; I had to wash it (soak) for two days with several changes of water. As an additional benefit I got excellent wetting of the edge of the knife. Vladimir M. Dusevich dusevichv@umkc.edu Wed Jul 18

Thanks a lot for your suggestions on how to view a diamond knife in a SEM. Unfortunately, we didn't have an ESEM at hand at the time, so we tried to check it in low vacuum in a JEOL 6460LV with back-scattered electrons. The resolution was quite low and I didn't see what I expected to see. The knife does not show any visible sign of damage caused by the SEM, which was my biggest worry and the reason I consulted the list. I'll try the step Vlado suggested, wash the knife in a detergent and see if the scratches disappear. Josif Mircheskijmircheski@us.es Fri Jul 20

My son-in-law once made diamond knives; he now makes diamond scalpels for eye surgery. He first tried SEM for inspection, but he switched to a 200× "toy" QX-3 when they were first introduced. He routinely photographs edges before shipping & when returned for re-sharpening, which eliminates lots of arguments about who did what to the edge. One of the new handheld CCD scopes would provide somewhat higher resolution, but the old QX-3 still works for Bill. Caroline schooley@mcn.org Fri Jul 20

I did some research in the early 1970's in collaboration with the DuPont diamond knife group utilizing SEM and a special knife boat that allowed removal of the diamond/steel shank for ease of viewing. The Cambridge Stereoscan S4 did not have the capabilities of current instruments so I was looking at secondary electrons with accelerating voltage of 10–15 kV. The small radius at the edge tended to charge thus compromising the imaging so I coated the knives with gold:palladium in a vacuum evaporator. I observed small lumps or particles near the cutting edge of the diamond that were presumed to consist of epoxy resin deposited during sectioning. I did not find a method capable of removing the deposits without damaging the steel and epoxy mounting despite considerable effort including ultrasound, detergents and possibly weak acids (my memory is not clear about the details). These deposits clearly correlated with knife lines seen in ultrathin sections examined with a TEM. I was able to section with the coated knives and found that the coating generally sloughed off and did not affect sectioning. My conclusion was that these deposits were only removed during the vigorous mechanical polishing used when re-sharpening the knives so I did not publish these results. Larry Ackerman larry.ackerman@ucsf.edu Fri Jul 20

SEM:
back-scattered electron detector

I'm looking for some assistance in troubleshooting/operating a GW Electronics back-scattered electron detector system 47. It is connected to a Hitachi 4700 and has worked in the past, but there are not any current users that know how to operate the detector, and I cannot get much beyond noise out of it. The internet has been of little help so far, and it appears as though the company is fading out of existence. I am wondering if anyone has a manual or other reference material for this system, or is willing to help with some basic operation and troubleshooting tips. ***Edward Swanson ejs2163@columbia.edu Sat Aug 4***

Before you worry too much about the detector let's check that it is working, try these steps—1. Move to a 15 mm working distance as the ideal distance is said to be 12 mm from the detector. 2. Turn the gain to its highest level (by memory it has three levels) 3. Crank up the spot size/probe current as high as possible 4. Make sure all the segment switches are on positive 5. Slow the scan. If the above does not work, you have a real problem! Steve Chapman protrain@emcourses.com Sun Aug 5

SEM:
resolution

I am slowly losing resolution in my SEM images over the course of a few years. We have on occasion placed oils/greases in there for analysis as well as other materials that may outgas. Is this likely my

problem? What can I do now? ***Derek Hammill derek.hammill@carestream.com Mon Aug 13***

There is a device specifically designed to clean residual hydrocarbons from Electron Microscopes called a "downstream plasma cleaner." This puts oxygen radicals into the microscope, they react with the hydrocarbon contamination, volatilize it, and the volatiles are pumped away, restoring instrument performance. I don't know where you're based but you can find XEI Scientific Inc. for more details. Ian Holton ian@acutance.co.uk Tue Aug 14

That is likely the problem. A thorough cleaning of the column from top to bottom should make a significant difference. The downstream plasma cleaner might also help. One other thing to look at is the secondary detector. Are you changing the scintillator regularly? Does it have a brownish cast to it? Anything that lessens the contribution of the lowest energy secondary electrons (whether a dead layer from bombardment or a layer of contamination) first and foremost cuts into resolution, often without reducing overall signal by much. The finest details generally come from the lowest energy secondaries. Ken Converse kenconverse@qualityimages.biz Tue Aug 14

In a similar circumstance I have spent a happy hour or two with a hot air blower heating up the pumping manifold and the specimen chamber. Sounds a bit of a joke but I can assure you it vastly improved the situation. In truth the pumping line to the rotary pump should be changed if a really good job is the object. I automatically assume that in your situation the RP fluid is changed at least every 6 months. Steve Chapman protrain@emcourses.com Tue Aug 14

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