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Selected postings from the MSA Microscopy Listserver (listserver@msa.microscopy.com) from 4/10/04 to 6/10/04. Postings may have been edited to conserve space or for clarity.

LM – repairing old slides

Question: I would like to repair some old slides of lamprey cross sections. The glue has turned white all around the specimens. Where can I find information about renewing old slides that cannot be replaced? John H. Wahlert <John_Wahlert@baruch.cuny.edu> 12 Apr 2004

The woman who shares my lab space is the caretaker of the histology slide collection for the medical students here. She has about 250 sets of slides in her care. At the end of each course, when the boxes are turned in, she goes through them all and selects out those whose mounting medium has dried out, clouding the specimen as you described. She has been very successful in refurbishing these slides simply by soaking them (sometimes for days) in a staining jar filled with xylene. At some point, the old coverglass drops off. If the staining has faded, she re-stains them, and then remounts them with fresh media and a new coverglass. Most of these slides were originally mounted with either Canada Balsam or Permount, and she stills prefers Permount to remount them. Try it with one or two slides. Leona Cohen-Gould <lcgould@med.cornell.edu> 12 Apr 2004

FIXATION – osmium storage

Although we have always kept OsO_4 wrapped up to protect it from light because someone somewhere said that it was light sensitive, now I'm wondering if that is indeed true or not. I personally cannot really remember if it is, or if this is just a superstition. How do you people store your OsO_4, and is it really light sensitive or not? Garry Burgess <GBurgess@exchange.hsc.mb.ca> 20 Apr 2004

After many years of living with osmicated refrigerators, I tried an experiment by keeping the osmium tetroxide at the back of the chemical hood at room temperature. We only keep about 50ml at a time and the chemical hood is always on, so there didn't seem to be any vapor hazard (unlike the smelly refrigerator). After 5 years of doing this, I can say that there seems to be no harm in keeping 2% osmium tetroxide at room temperature and in full light every day. Now we keep the osmium tetroxide in full light and at room temperature and we have the added bonus of a clean fridge. *Paul Webster <PWebster@hei.org> 21 Apr 2004*

This is a quote from Advanced Inorganic Chemistry, 2nd Ed. by Cotton and Wilkinson on page 1005 under tetroxides: "Above ~180°, RuO_4 can explode, giving RuO_2 and O_2, and it is decomposed slowly by light; OsO_4 is more stable in both respects." The later statement suggests or implies that OsO_4 can also be decomposed by light because it does NOT say "OsO_4 is very stable and does not decompose or explode." So I always wrapped OsO_4 in Al foil as a minimum and kept all of it in a glass container tray is case of a broken ampoule. OsO_4 ampoules are not something you want out in the open on a bench anyway. For potential RuO_4 spills, use a crystallizing dish lined and padded on the bottom with paper towels. Unsaturated corn oil is recommended for OsO_4 but it's a mess to clean up. The article in the Sept-Oct 2002 issue of Microscopy Today on page 20 would indicate that the reaction rates are indeed quite different. Paul Beauregard <beaurega@westol.com> 21 Apr 2004

TISSUE PROCESSING – marking tissue

One of faculty here will be processing lengths of rat femoral artery for light microscopy and needs to keep track of the distal vs. proximal ends. She tried marking one end with India ink, but it was washed out sometime during the dehydration/xylene steps. Any ideas? Leona Cohen-Gould <lcgould@med.cornell.edu> 06 May 2004

When you use India ink it needs to be fixed to the tissue's surface you are labeling with a picric acid solution such as Bouin's fixative. Make the ink mark with the opposite end of a swab and then switch to the cotton tipped end of the swab. Dip into the Bouin's and dab the area marked with the India ink. Blot slightly dry with a Kimwipe and put back into formalin. It will be permanently fixed in place and can be processed and embedded in paraffin. Karen Bentley <Karen_Jensen@URMC.Rochester.edu> 6 May 2004

TISSUE PROCESSING – missing membranes

I have a colleague who has been trying to use TEM to look at isolated chloroplasts from a marine alga (Bryopsis). She has also tried looking at intact algae. The problem is that following a standard protocol (glutaraldehyde fix using isolation media, osmium, uranyl acetate en bloc, dehydrated in ethanol and propylene oxide, 'Epon' embedment; and lead citrate and uranyl acetate on-grid), the membranes along the perimeter of both the isolates as well as the supposedly intact algae appear fragmented, or missing. Mostly missing. The cytoplasmic matrix and internal membrane-bound structures such as thykaloids appear essentially normal in terms of electron density and membrane integrity, so on first inspection it would appear the membranes are disappearing following initial fixation, or, they are stubbornly invisible. We thought the missing isolated chloroplast membrane was attributable to the isolation procedure, until the intact algae also came up with no plasma membrane. Any comments on ways to keep the plasma membrane intact? Ann Lehman <Ann.Lehman@trincoll.edu> 03 Jun 2004

It does seem that osmium (and possibly the other stains later applied before dehydration) are not able to pass through the outermost plasma membrane of your marine alga plastids. The later on-grid staining might not be expected to be of any help if this is so. If the outer plastid membrane is not visible there is also not much chance that the thylakoid membranes will be. Could there perhaps be some algal glycoprotein "gunk" immediately outside the plastids that acts as a barrier to the fixative during normal fixation times. An enveloping carbohydrate goo is not always visible in thin sections although such envelopes are not uncommon in native prokaryote cultures. This thought is perhaps substantiated by my quick Google search: Kim et al 2001 report: "When the multi-nucleate giant cells of the green alga Bryopsis plumosa (Huds.) Ag. are injured, the protoplasm is extruded from the cells and can generate spontaneously numerous new cells. The cell organelles aggregate rapidly in seawater and become covered with a gelatinous envelope within 15 minutes". You seem to be dealing with quite a remarkable coenocytic plant here! Perhaps longer times and/or higher fixation temperature could improve matters. Although not directly relevant in your case, some help might come from the discovery recently by Walther & Ziegler (2002 Journal of Microscopy 208, 3-10) that cell membranes, which are notoriously poorly contrasted after freeze substitution, are rendered easily visible if 1 - 5% water is maintained in the substitution fluids. Perhaps freeze-substitution should be tried. Snap-freezing plus freeze-fracture is another technique good for viewing membranes and it has the added advantage of being unaffected by chemical permeability problems (viz. yeast cells). James Chalcroft <jchalcro@neuro.mpg.de> 03 Jun 2004 and 04 Jun 2004

Not all membranes are equally reactive with osmium. Protein storage vacuoles in plant cells have a region known as the "globoid" which contains phytic acid crystals. It was thought to be a non-membrane

bound region within vacuole's matrix. Conventional aldehyde and osmium fixation fails to show any sign of a membrane. When we used KMnO4 as a fixative, however, a unit membrane was present surrounding this compartment (Jiang et al., 2001 J. Cell Biol 155(6):991-1002). This type of phenomenon has been reported before. Plastid envelopes were observed to become progressively less distinct using conventional fixation in the fern Paesia but they could still be seen using $KMnO_4$. Mitochondria membranes in the same cells were always stained so it seemed to be due to changes in plastid membrane lipids and not some trivial problem related to the osmication procedure as a whole (Bell 1983 Eur J Cell Biol 30:279-282). So maybe your membranes aren't missing - just hiding! Tom Phillips <phillipst@missouri.edu> 03 Jun 2004

TISSUE PROCESSING - shrinkage

I am doing research paper on quantification of shrinkage due to fixation. There are some questions that I like answers to: (1) What are some of the methods used to measure shrinkage? (2) How can the effects of fixation be differentiated from other possible sources of shrinkage? (3) What are the practical and clinical significance of quantifying shrinkage due to fixation? Linda <hunny_pot@optusnet.com.au 10 May 2004

A great deal of research was done on these questions in the late 1940's-1960's and you won't find it on the internet. For starters, get a hold of "Principles of Biological Microtechnique" by John R. Baker, published in 1958. He discusses various fixatives, their penetration rates, how they do or do not promote shrinkage (in the fix or in subsequent processing), etc. Geoff McAuliffe <mcauliff@umdnj.edu> 10 May 2004

You may be interested in a paper I published a few years ago in which I measured the size distribution of cells in suspension after different fixation conditions. Develop. Biol 23:36-61 (1970). Joel Sheffield <jbs@temple.edu> 10 May 2004

TISSUE PROCESSING – embedding problem

I have a client that is having difficulty keeping their fly heads in their thin sections. The chitin tends to separate from the surrounding plastic. The head is taken off the fly, proboscis removed and then processed more or less using a typical procedure into epoxy. Robert Kayton <kayton@ohsu.edu> 12 Apr 2004

I have had this same problem with pupae and adults, and have tried many things but nothing worked well. The best results were obtained by doing all the trimming with a glass knife, taking only thin sections off the block at a time, less pressure at the Epon/cuticle interface. The cuticle at the bottom of the block should be sectioned off so that the knife hits tissue. In that the head is "lumpy", the first sections will still tend to pop out of the plastic. If there is a need to look at these, it is helpful to collect the thick sections on water, either in a boat or a drop of water at the edge of the glass knife and pick up the material with a loupe like EMS sells. I keep the water at the top of the knife by drawing a line of finger nail polish (old and cheap is fine) across the knife about 5 mm from the top edge. The polish sticks much better than wax. Time must be allowed to let the polish dry, so I do several at a time, usually after I break the knives so they will be ready when I need them. As the material gets large enough to pick up with a hair, I just transfer that to the slide and forget the excess plastic. Pat Connelly <psconnel@sas.upenn.edupsconnel@sas.upenn.edu> 13 Apr 2004

I had a similar problem with fruit fly legs. Two possible solutions (which I could not try in the study I was involved with) are: (1) Treating with a chitinase or (2) Taking tissue immediately after molting so the chitin is still relatively soft. Also check out papers in Nature 184:1584, 1959 (Beckel) and Trans. Am. Micros. Soc. 74:197-201, 1955 (DeGiusti and Ezman). Geoff McAuliffe <mcauliff@umdnj.edu> 13 Apr 2004

Many people have studied Drosophila tissues by TEM despite the embedding difficulties. I have done many thin sections of the eye and the epoxy usually separates from the eye surface boundary. I just collected the section parts that contain the tissue onto a grid with or without a film support. If you need to examine areas near the surface boundary a film support is usually necessary. Larry Ackerman <ackerman@msg.ucsf.edu> 14 Apr 2004

IMMUNOCYTOCHEMISTRY – gold labeling distance

When labeling e.g. Lowicryl sections using indirect immunogold (primary antibody - secondary antibody conjugated to gold), is there a consensus for the typical (or maximal, that may be easier) distance between the antigen and the particle? Michal Jarnik <M_Jarnik@fccc.edu> 09 Jun 2004

I have a an excellent book entitled "Electron Microscopic Immunocytochemistry" edited by Julia Polak and John Priestley, Oxford University Press, 1991. They state on page 74 of Chapter 3 entitled "Post-embedding Electron Microscopic Immunocytochemistry": "The minimum theoretical lateral resolution (i.e. distance between the centre of the gold particle and the epitope recognized by the primary antibody) that can be achieved with the protein-A gold technique is about 16 nm, using a colloidal gold particle of 3 nm. Using the immunoglobulin-gold technique with a gold particle of 10 nm the lateral resolution is approximately 21 nm. It is clear that the bigger the gold particle, the less satisfactory is the lateral resolution of the technique." Karen Bentley <Karen_Jensen@URMC.Rochester.edu> 10 Jun 2004

The statement in the Polak & Priestley book makes an assumption that when the specimen is dried, the antibodies and gold probe fall to one side (presumably in a random direction). As there is no evidence to support the statement, other than it was published in a book, may I propose the possibility that the gold particles have an equally good chance (or better) of drying on top of the antibodies. This would mean that the lateral resolution in practice is much better than predicted by what is expected theoretically. The resolution in three dimensions may not be so good in that the gold will end up above the antigen, but this should not concern us when we examine the sections in the TEM. The theoretical resolution predicted in the Polak and Priestley book, assumes the gold particles will be bound to the far end of the antibody molecule, when in fact the particles are more likely to bind closer to the hinge region (Baschong & Wrigley 1989 small colloidal gold conjugated to FAB fragments or to immunoglobulin G as high resolution labels for electron microscopy. A technical overview. J. Electron Microsc. Tech. 14:313-323), thus reducing the theoretical resolution predicted by Polak & Priestley. Anyone who performs immuno-EM on a routine basis will tell you that gold particles are very often much closer to the antigen site than could be expected if the gold was "falling off the pile of protein". Quite often the gold is located in precisely the location predicted.

A good demonstration of how precise immunolabeling can be is seen in the study by Sodeik et al (1997 Microtubule-mediated transport of incoming Herpes simplex virus 1 capsids to the nucleus. J. Cell Biol 136:1007-1021). An antibody to dynein (which attaches the capsid to microtubules) labels exactly 40 - 50 nm away from the virus, where the antigen is predicted to be. If the immunolabeling were to be as poor resolution as predicted by theoretical considerations, the technique could not be used for such high resolution studies as it has been applied. Obviously, we need more experimentation, and thus more data, to continue this discussion and to provide Michael with the answer he wants. Paul Webster <PWebster@hei.org> 10 Jun 2004

ESEM – merits of Tungsten vs. FEG

We are in the process of justifying, to the University, a proposal to purchase an ESEM. In preparing the business case, I am including the ten year financials for both FEG and tungsten filament machines. Given that we have to break even in that time, including covering our depreciation, the tungsten instrument is easier to justify in a purely financial sense. From a scientific and operational viewpoint, though, I was hoping some of you could provide opinions on the relative merits of tungsten or FEG. The range of samples we will be required to put in our ESEM will be anything you could possibly imagine from the Faculties of Engineering, Science and Medicine. Samples will include food materials (big dairy industry in New Zealand), polymers, conducting polymers, mammalian tissue, bacteria, fruit and vegetables, fish and chips, superconductors, ceramic coatings and bees knees. We will definitely need a hot-stage. Bryony James <b.james@auckland.ac.nz> 17 May 2004

We look mostly at hydrated biological samples. We have a tungsten gun XL30 ESEM TMP. For looking at bacteria and cells, a FEG-ESEM would be superior in terms of reduced damage and improved resolution. I find it hard to get medium/high resolution with wet biological samples. I feel FEG-ESEM would make this a lot easier. David Patton <David.Patton@uwe.ac.uk> 17 May 2004

In my experience, medium/high magnifications for wet specimens are limited mostly by specimens themselves and not by a type of emitter. While I can get a nice picture of gold on carbon at x100k, for most of my "real life" specimens magnification is limited by x20k (or less). Vladimir Dusevich <DusevichV@umkc.edu> 18 May 2004

The combined FE and ESED instruments are very pricey, expensive to maintain and, since you already have a FE, perhaps a bit redundant. We have a two-year-old tungsten variable-pressure and it is a popular workhorse that is in constant use. It can do high resolution or ESED but not both at the same time. If you need both at the same time, then you need FE-ESED. The thing we would most like to have is a cold stage, to look at hydrated samples without having to go to higher pressures. Mary Mager <mager@interchange.ubc.ca> 17 May 2004

I agree that resolution is more dependent on sample than microscope. What a microscope is capable of doing with an "ideal" sample often does not relate to the real world of biological samples which are anything but ideal! To get really good resolution you need to use higher kV values. These same values will result in the primary electrons penetrating too far into the low density biological sample resulting in substantial loss of signal in general and se1 & se2's in particular. Resulting image will be low resolution and often of much lesser quality than the same sample taken at a lower magnification. The problem of kV can be partially overcome by using a more coherent beam with greater beam density, i.e. Field Emission. FE allows one to work at lower kV values resulting in a se yield that is still adequate while minimizing sample damage. Resulting images are likely to be higher resolution (sharper with more detail) than comparable images at similar kVs from a tungsten instrument. However, there is a point where you go from useful magnification to "empty" magnification regardless of the gun type. This is sample dependent and must be determined accordingly. So it is unlikely that you will get any additional information from a bacteria sample at 100k than at say 50K. However, you will most likely get a much sharper image at 20k with FE than you could with a W filament. Debby Sherman <dsherman@purdue.edu> 18 May 2004

For ESEM in wet mode, a low voltage beam is not suitable. Even at 5 kV the noise level is too high. Vladimir Dusevich <DusevichV@umkc.edu> 19 May 2004