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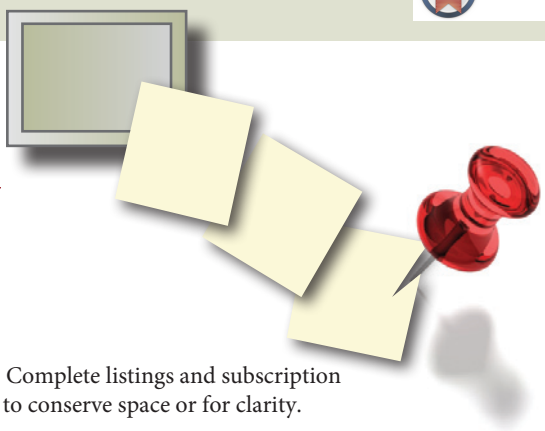

NetNotes

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Selected postings from the Microscopy Listserver from January 1, 2016 to February 29, 2016. 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:
agar embedding cells

Some advice please on colorizing agar for embedding cells. In the past, my osmicated pellets in agar blocks were easily trackable, but when the cells are in a thin layer the cells don't give much contrast, especially if not osmicated. I am collecting naked eukaryotic cells in suspension culture, but have problems bring down the smaller cells by centrifugation. Cultures containing cells are fixed in aldehyde or aldehyde/osmium cocktail, collected onto Millipore or Nucleopore filters, then held in place by adding warm agar over them on the filter. When cooled, I peel off the filter, leaving me with a thin layer of cells embedded in the agar. I then cut the agar layer into small blocks to enhance fluid exchange during subsequent treatments. My problem is, the cells are light colored to faintly black, and the thin layer of cells does not stand out well, particularly when in the various stages of Epon infiltration/polymerization. I tried adding some azure II blue dye I had on hand to the agar so I could see the agar blocks more easily, but by the time I got up to epon/acetone, the color was gone. Any suggestions for a dye that will withstand both the water based and acetone solutions, or an alternative way to mark the little agar blocks so I can more easily monitor them throughout the process? ***Steve Barlow sbarlow@mail.sdsu.edu Fri Jan 29***

I have had luck with fast green added at the 100% ethanol step. I make a saturated Fast Green solution in ethanol and add a drop or two at the 100% ethanol step. It kept the agar blocks rather colored through subsequent infiltrations and polymerization. Sorry I don't have handy what "saturated" means in terms of mg/mL but if you are interested I could probably dig that up somewhere. ***Tobias Baskin baskin@bio.umass.edu Mon Feb 1***

Not directly answering your question but making a remark: You are working with a cell monolayer, meaning approx. 50µm thin layer. This is so thin that you don't have to care about fluid exchange; I don't think that cutting your agar in small blocks will add anything. I would keep the whole agar block standing at the bottom of a jar, so you always know that the cells are on the upper side. ***Stephane Nizets nizets2@yahoo.com Tue Feb 2***

Specimen Preparation:
speckled TEM samples

I am having a terrible time getting clean TEM sections. I filter the primary fix mixture with a 0.1µm vacuum filtration unit; I've tried changing the water and am currently using RICCA ultra-pure H_2O; I have stopped en bloc staining altogether; I use Spurr's Low Viscosity embedding resin. Most recently I have been ethanol-washing all razor blades used during specimen collection; I use a 0.04% FSG quench after fixation (didn't seem to make a difference). The fine speckling is most noticeable in muscle tissue, although I see it in other tissues as well. I just looked at some freshly cut, unstained mouse foot pad muscle yesterday and there were the black speckles all over the place! The specks are tiny, much smaller than 10 nm. It almost looks like immuno-gold, except that I haven't done immuno-gold staining in years, and the specks are not rounded as you might expect with gold particles. They don't seem to be clustered at any specific organelle, and they are definitely in the tissue, not on the surface. Someone please help, this is driving me nuts! ***Debra M. Townley debrat@bcm.edu Thu Feb 4***

This could be a couple of things. First, did you use phosphate buffer? If your concentration was a 100 mM or over, the glutaraldehyde, osmium and phosphate can precipitate in the tissue. Check unstained sections to confirm. Also make sure all the glutaraldehyde is rinsed out of tissue before you get to osmium. Are the tissue pieces small? You can also microwave fixative to get better penetration. Be careful with using reduced osmium and then staining muscle. Lipid granules are affected with the post-section staining with uranyl acetate and lead. Apparently the lipid material gets lost after staining and rinsing, I have seen this phenomena and sometimes it will contaminate parts of the section. If you are sure it isn't your staining protocol, then it must be the fixation. ***Michael Delannoy mdelann1@jhmi.edu Thu Feb 4***

It would be interesting to see an image of your speckles. Wolfgang Muss wij.muss@aon.at Fri Feb 5

Image of speckled mouse muscle: http://s450.photobucket.com/user/wassupdoc1/library/Science ***Debra M. Townley debrat@bcm.edu Fri Feb 5***

Thank you for showing us the image with "speckles". I think that the problem might be in lead staining procedure. Please, look at Arvid B. Maunsbach & Björn A. Afzelius "Biomedical Electron Microscopy (Illustrated Methods and Interpretations)" 1999, Pages 207–228 <doi:10.1016/B978-012480610-8/50011-1> for explanation. We had also similar problems with lead carbonate precipitations in the past. Since our technician started to wear a face shield during sections staining with lead citrate the precipitates (contamination) in them almost vanished. There is an old paper on "How to remove such precipitates from ultrathin sections": J Kuo, "A simple method for removing stain precipitates from biological sections for transmission electron microscopy," *Journal of Microscopy* 120 (1980) 221–24. <doi:10.1111/j.1365-2818.1980.tb04140.x>. We tried this approach several times, but the results were not ideal in our hands. ***Oldrich Benada benada@biomed.cas.cz Fri Feb 5***

Difficult to say when we don't know your primary buffer / fixation protocol, but I would agree that it looks like it may be due to either precipitate from a phosphate buffer, possibly due to insufficient washing between glutaraldehyde/OsO_4 steps, or osmium 'peppering', usually produced with buffer reactions in the osmium steps, or prolonged fixation in osmium. I used to have similar problems, and resolved them by changing my buffer to HEPES or cacodylate, extra wash steps between the glutaraldehyde and osmium fixes, and secondary fixing in aqueous osmium as opposed to buffered osmium. Good luck resolving it - let us know how you get on! ***Natalie Allcock nsa2@leicester.ac.uk Fri Feb 5***

Specimen Processing:

allergy to histological solvents

After decades of using standard organic solvents for paraffin-section histology, I find that I've become highly allergic to the fumes. These include commercial preps like Citrosolv and Hemo-De, as well as xylene and toluene, which cause dizziness and pounding headaches. This has made staining and cover slipping very difficult, even with hood. Can anyone recommend alternative solutions for (a) dewaxing and (b) cover slipping with Permount? ***Daniel Blackburn dblackburn2000@yahoo.com Sat Feb 20***

I assume you're doing these steps in a fume hood. One thing we've found helpful for people who are sensitive to solvent fumes are charcoal-lined dust masks. Search amazon.com for "charcoal filter masks". We use the 3M type (but not for the prices here - we got ours through Fisher Scientific). So far, they've worked fine. Looks like you've already tried the various xylene replacements. ***Phil Oshel oshel1pe@cmich.edu Sun Feb 21***

A serious problem. By listing, I have come with suggestions. I have long used a Gum Damar formulation that I cannot offer, because I have prepared it in xylene. Note: for those who might be interested—or users of Damar—I have a liter that I will never use more than a few ml's over my reasonably extended age - now 76. Further, I don't do paraffin any more. The suggestions: If you can arrive at a solvent for Damar that is miscible with xylene/toluene, then it would be possible that you could prepare your own Damar. Note: Damar applied in the 1960s still has no sign of drying/cracking/crystalizing. Source: http://www.eco-house.com/shop/950-damar-resin-crystals-graded/. You might test a hydrophilic mountant. Source: https://www.emsdiasum.com/microscopy/products/histology/mounting_media.aspx. If your allergy is broad-band, then you may have to resort to the latter. If it is more specific - just for those you mentioned - then your options might include Canada Balsam that is very natural, but also often found with benzene or xylene or toluene. I have some that will never be used as it requires some solvent that might not raise a sneeze. ***Frederick C. Monson fmonson@wcupa.edu Sun Feb 21***

Image Processing:

batch convert emi to other format

We have an FEI TEM microscope. The STEM images Acquired in TIA software are saved in emi format. I can open them in TIA software, and can only export them one by one. That is quite annoying. Is there anybody who has any idea how to batch convert the images? ***Hongbing Yu 12hy1@queensu.ca Thu Feb 18***

TIA folder export: - Open menu Tools\Components. - Click "Install". - Type "folderexport.wsc". - "ok". Now that this component is installed, there will be an additional menu in the processing tasks. Check settings and hit Export. ***Wim Hagen wim.hagen@me.com Thu Feb 18***

Microtomy:

cryo-ultramicrotomy of rubber sections

Can anyone recommend a technique for collecting rubber sections during cryo-ultramicrotomy that enables the sections to lie flat (i.e. as wrinkle free as possible) on a TEM support grid? I currently collect my sections off the back of my cryoknife using a sucrose droplet, but as the sections warm coming out of the cryochamber on the droplet they contract and wrinkle before being deposited on a TEM grid (with or without carbon coating). Chamber temperature is -120°C. Any ideas are appreciated. ***Anand Badami badamianand@bfusa.com Mon Feb 8***

If the wrinkling happens during warming of the droplet, could it be because of the section surface characteristics? Adding salt to the sucrose should help if the surface is charged; multivalent ion salts would be more suited than monovalent. However if the surface is not charged but hydrophobic adding salt would probably increase the wrinkling, and in that case adding a hydrophobic component might help, such as serum albumin. Some detergents can do both. ***Jan Leunissen leunissen@aurion.nl Mon Feb 8***

LM:

iPhone microscope

Someone has asked me about microscope attachments for iPhone. I've seen lots of ads, but I haven't looked closely at any of the products. Do any of you have opinions about products that turn your iPhone or Android into decent microscopes? I'm not sure if she wants to go with a stand and slides, or just take nicely magnified images on the go. I think the former. ***Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Wed Jan 13***

Here at PNNL we've actually developed a low-cost (~$1) cellphone camera that magnifies up to 1000×, depending on the specification. Our lab has freely released the blueprints for 3D printing or you can order pre-manufactured lenses that clip onto pretty much any smartphone. We use them frequently in our demos to high school and middle school children and they work quite well once you get the hang of them. Here's a link to more details: http://availabletechnologies.pnnl.gov/technology.asp?id=393. ***Steven R. Spurgeon steven.spurgeon@pnnl.gov Wed Jan 13***

Though not as cheap as the PNNL design, some colleagues of mine have used this inexpensive, hardware-store-materials-based home-built design for outreach and liked it: http://www.instructables.com/id/10-Smartphone-to-digital-microscope-conversion/ ***Tyler Harvey trh@uoregon.edu Wed Jan 13***

I got 17 replies to my query about turning an iPhone into a microscope, and no two were the same. Most of them were along the lines of "I saw this one on the Internet...". If anyone is particularly interested, I can send a list of links or forward some of the responses. Here is the one that the person who requested feedback is looking at (and is not one of the ones any of you mentioned): http://thegadgetflow.com/portfolio/uhandy-microscope/ . It holds a slide and I think has a light source. ***Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Fri Jan 15***

TEM:

bias and gain correction on camera

We have a SIS Megaview III camera (SIS, then Olympus and now I see it is EMSIS "again") for over 10 years, a real workhorse, always performing well without complaining. Because it works so well I tend to forget about it and now I am just wondering if I need to update the gain and bias corrections (the correction pictures)? I can't remember the last time I did it but it is a long time ago. I know that it is not a lot of work to do it, I just wondered if there is any reason to do this regularly and if yes, what are the time intervals. ***Stephane Nizets nizets2@yahoo.com Thu Feb 18***

Since the blacklevel ("noise") and gain images are a necessary step for getting an artefact-free and evenly background, I think you should do this short procedure (depends what version of Analysis or ITEM software you are using) often. When I am installing or updating cameras at the customer´s site I ask for the most used magnification / spot size / apertures setting they use and do the correction images with this setting. But: I also know a customer who is doing these image sets prior to each high-res image he uses for publication (makes sense). ***Stefan Diller stefan.diller@t-online.de Thu Feb 18***

We have SIS MegaView II 16 years old, even older then yours Megaview III. Well, we used to record the correction images quite frequently, approximately once per month or two. As the CCD chip and scintillator were getting older, the interval for "shading correction" adjustment had to be shorter. After 15 years, we had to record every week a new set of gain and bias images. You can quite easily check whether you need to perform new adjustment of "shading correction" or not. 1. Take-off the sample holder out of the column 2. Adjust the illumination on the screen 3. Insert camera 4. Adjust beam intensity to ~50% 5. Take an image. Now measure the histogram of "Gray value distribution" in the recorded image (Main menu: Measure -> Histogram). Look at the course of the histogram line. If it is smooth and the distribution is narrow (~100 for MegaView II is OK) then you do not need to adjust "shading correction". Otherwise you should take a new set of gain and bias images. ***Oldrich Benada benada@biomed.cas.cz Thu Feb 18***

ESEM:

live image to external computer

There is a video out BNC on the back of the FEI Quanta 200 ESEM. In Low Vac mode, I can see the signal when connecting to an external view monitor. The signal is horizontal lines that change in size and number when I change image resolution or scan speed. Also B/C seems to adjust normally. What can I do to make this into a usable image signal to use for internet teaching outreach project? Is there a better way to get the SEM signal to the internet? ***Wallace Ambrose wambrose@unc.edu Wed Feb 10***

You can use Team Viewer or VNC to directly share desktop of your SEM with remote computer(s). If you want to grab video from BNC then decent video grabber card and some coding/configuring may be needed. I've used Epiphan PCIe in the past, though not with the FEI E-SEM that you have: http://www.epiphan.com/products/dvi2pcie-duo/ ***Valery Ray vray@partbeamsystech.com Thu Feb 11***

EDX:

strange performance at very low count rates

One or two of my users have observed a strange behavior on our FEI Talos F200X (FEI SuperX detector, Bruker pulse processor, Bruker software). I'm not going to call this an "error" or a "fault," just something strange. Hopefully the list's hive minds can reality check me. Let's say that on a very thin, low-Z sample, 400 pA might give 400 counts / sec on each of the four detectors (as seen in the TIA SuperX tab). 300 pA might give 300 cps. 200 pA might give zero. (Numbers are approximate.) I interpret this to mean that there is a low-end discriminator on the pulse processor or software somewhere, and below a certain count rate no counts are registering. Is this possible? Is there a setting I can drill down to in order to let my users push to lower beam currents on their beam-sensitive samples? This isn't a common problem—most of my Talos users are running ~5 nA, ~200 kcps beautifully—but I want to help all my users, not just those who can use the STEM as an electron sledgehammer. ***Chad Parish parishcm@ornl.gov Thu Jan 28***

We have a JEOL X-ray detector with a Thermo Pulse-processor and software and we can go way down in beam current to still collect X-rays. On a recent trip our engineer showed me a software switch to remove the "noise" peak counts from the spectrum and the reported count rate. But if you are literally not seeing any peaks with 200pA then there's a fundamental problem - call Bruker at once. ***Rob Keyse rok210@lehigh.edu Thu Jan 28***

MT

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