Edited by Thomas E. Phillips, Ph.D.
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Selected postings from the Microscopy Listserver from December 15, 2008 to February 15, 2009. Complete listings and subscription information can be obtained at http://www.microscopy.com. Postings may have been edited to conserve space or for clarity.

CHEMICALS - acrolein storage

I have a client that has successfully used an acrolein fixative for difficult fungal specimens. His supply of sealed 10 ml ampoules of acrolein diluted in 0.1 M cacodylate has nearly run out. It seems our usual favorite EM chemicals suppliers no longer sell it. Sigma sells the pure stuff >99% in 10 ml ampoules. They cost about $50 each, and shipping is almost twice that. As we only need to use small volumes of fix at a time, we would like to dilute the 10 ml of pure acrolein to 3% in buffer, divide it into small aliquots, and store it somehow so it remain stable and viable for months to a few years. The question is how to store it. We think storage at -20°C, in glass vials with screw caps, stored inside cans with tight lids (like osmium solutions are shipped in) might be a good way to go. We would like to hear your ideas on storage of dilute, buffered acrolein, and how long we could expect it to remain viable as a fixative. We are quite aware of how nasty acrolein is. Any advice you can give us on safe storage for the long term will be appreciated. Gilbert Ahlstrand ahlst007@umn.edu Wed Dec 17

Can't you buy unsealed, empty ampoules that can be flamed to close? I would think this is essential for a volatile substance like acrolein. Tom Phillips phillipst@missouri.edu Wed Dec 17

Have you asked the EM Suppliers if they could make a special order for you? I would think that this would be the way to go. Over the years I remember both Electron Microscopy Sciences and SPI have advertised that they can get or make things on request and I suspect that others have also made this statement. You do not want to handle the pure chemical if possible. The last I had bought was from Kodak around 1973 and I know it works great mixed into a fixative for difficult samples. I remember it well for the 100 ml bottle tipped over when I was recapping it, spilling a bit onto my lab coat. Later, after not being able to breathe for what seemed to be a very long while (literally), I learned that diluted acrolein is what gives humans problems when exposed to tear gas. Patricia Stranen Connelly connellyps@nhlbi.nih.gov Wed Dec 17

CHEMICALS – uranyl acetate safety

A relatively new person in our EHS dept has informed us that uranyl acetate (UA) is a strong gamma emitter and should be stored and disposed of in a stainless steel containers and used in a stainless steel hood (or with other proper protection measures). This was a surprise to us as all former EHS staff has told us that though it does need to be disposed of with other radioactive waste, it can be used in a normal hood. We obviously want to be as safe as possible. Can you advise on any special handling procedures used for UA? Many thanks in advance! Danielle Crippen dcrippen@buckinstitute.org Wed Jan 7

As the sole worldwide manufacturer of uranyl acetate and other uranium compounds let me assure everyone that uranyl acetate is an alpha emitter and not a gamma emitter. When we manufacture these compounds we purchase the raw uranium in a depleted state from the government. There is no chance for error here. We do not use natural uranium. This means that the enrichable uranium U-235 has been removed, hence the name "depleted." Then U-238, which only emits alpha radiation is processed. If even by the slightest chance that U-235 were present then every alarm would go off in our facility because beta and gamma radiation is detected. I hope this answers everybody's concerns. Our products are sold exclusively through a distributor network and all of them have been instructed on this information. As for storage, good housekeeping rules apply. If anyone has any direct questions regarding this they can post or contact me directly. Alex Besenyo abesenyo@ibilabs.com Wed Jan 7

Did you have a chance to check the information from http://atom.kaeri.re.kr/ton/nuc7.html? Let me construct the decay tree. I'm sure everybody in this list can do it but, you keep insisting that "uranyl compounds are alpha emitters only" so, I will take the time to do the job and post in to the list. Let's start with U-238 which is the starting element in your compound. 1) U-238 decays into Th-234 by Alpha decay 2) Th-234 decays into Pa-234 by Beta decay 3) Pa-234 decays into U-234 by Beta decay 4) U-234 decays into Th-230 by Alpha decay 5) Th-230 decays into Ra-226 by Alpha decay 6) Ra-226 decays into Rn-222 by Alpha decay 7) Rn-222 decays into Po-218 by Alpha decay 8) Po-218 decays into Pb-214 by Alpha decay 9) Pb-214 decays into Bi-214 by Beta decay 10) Bi-214 decays into Po-214 by Beta decay 11) Po-214 decays into Pb-210 by Alpha decay 12) Pb-210 decays into Bi-210 by Beta decay 13) Bi-210 decays into Po-210 by Beta decay 14) Po-210 decays into Pb-206 by Alpha decay Pb-206 is stable so, it is the last element to be produced as a result of U-238 radioactive decay. I have constructed the above decay tree using the information from http://atom.kaeri.re.kr/ton/nuc7.html While constructing the above decay tree I have used the branch which has the highest branch ratio (above 99% in each case). Ayten Celik-Aktas celikaktas@gmail.com Fri Jan 9

Regarding the decay tree, I note from the provided link information that the half-life of U-238 is <<4.4689 years>>. It has been a while since my high school chemistry but I'm wondering how much Th-234 and associated beta emission danger we are really dealing with here; seems like there must be a very small amount of Th-234 produced with such a long half-life of the original U-238. Maybe you could comment on the danger of this. Dale Callaham dac@research.umass.edu Fri Jan 9

I work in a place that does not permit the use of uranyl salts for EM use because of the radiation dangers (and a healthy respect for ALARA), and am always trying to educate people about what these dangers are with facts. Thus I was very interested in this thread. I did have a question on the post by Ayten that I hope someone will answer: The website Ayten provided gives the branch ratio for decay of U-238 to Th-234 as 0.00005% (along with the half-life given in Dale's response). Would that not also make the amount of beta radiation small? It was interesting to read in Alex's post about how the presence of U-235 causes alarms to go off. Commercially available (United States, Electron Microscopy Sciences, technical data sheet for uranyl acetate, available at www.emsdiasum.com) is listed as 0.1% U235, so there is *some* U235 present, at least for this supplier (Ted Pella lists the composition as 0.3-0.4% U235). EMS also gives a reading for alpha and beta radiation for a 100 g sample, and gives a value of 0.51 μCi/g for the specific activity (they state

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a material with a value of >0.002 μCi/g is considered radioactive). Jessica Cervantes cervantes@bendres.com Fri Jan 9

I disagree with the assumption that depleted uranium (DU) is not radioactive and the implication by the word "depleted" that all the radioactivity has been removed. Here's why. I had 12 pounds of depleted UAc and a calibrated and certified "pancake" Geiger counter detector. It had no problem detecting background cosmic radiation and it had an up to date certification sticker on it. That amount of DUAc in those containers pegged my Geiger counter from three feet away. The one pound bottles were brown glass bottles, inside a plastic bag, inside a "tin" shipping can, and had labels that said, "(depleted uranium)" on them. So the counted radiation had to be gamma but U does not emit gamma radiation. It is the impurities from the decay of U that generate the gamma emitters. I have no doubt that some of the posters think their materials are not radioactive and their supplier's material may not be. So, we now have two schools of thought. It's not radioactive and there is radioactivity present. The only way to know for sure what you have and get a hint of the history of the manufacturing, is to take a reading on the purchased DUAc salt with a good quality Geiger counter and see what you get for a reading. My EH&S and safety people were totally shocked at the DUAc readings and said, "But this was made from depleted uranium. It's not radioactive." I relied, "Looks pretty radioactive to me." Like me, they believed the Geiger counter readings and not the MSDS sheets that didn't address the gamma emitting impurities, i.e. the amount of impurities from decay. In my opinion, the phrase 'depleted uranium' is misleading. There is a shipping exemption on this radioactive material, I was told. If the amount is one ounce or less, you don't have to label it or ship it as radioactive. My shipping clerk refused to ship any amount. So just because the bottle or packaging you received does not say "radioactive material," that does not mean small amounts are not radioactive. I think that's where the EM myth of not being radioactive might comes in. How do you know every last U-235 atom was removed and the uranium was zone refined and/or chemically purified? You don't. It doesn't say any of that on the bottle(s). Just measure the gamma radiation. Paul Beauregard beaurega@westol.com Fri Jan 9

Really, I have the feeling that we are making an elephant out of a mouse. How many times do you have to handle how much of depleted U? 2 times a year? 10 milligrams? How do you weigh your uranium salt? Do you pour all the content of the box on the bench, then take what you need? As someone said, I am more concerned about the chemical toxicity following ingestion (although even in this case it is probably fewer than few) than the radiations, except if you leave the box of U salt in one pocket of your blue jeans, which I wouldn't recommend (especially if you want children later). Stephane Nizets nizets2@yahoo.com Tue Jan 13

Indeed, from Pelco's measurements of their solution I estimate that if you held a 25 g bottle of their uranyl acetate against your skin for an entire year you would receive about 3.5 times the annual dose* you would get from background radiation sources [over the same year] - about 1,300 mRem. This would largely be from the gamma radiation [as you can assume the beta-rays and alpha particles are blocked by the glass jar, i.e. you kept the lid on, and your skin surface]. *Annual dose assumed to be 360 mRem [18% man made + 82% natural]. A radiation worker is allowed 5,000 mRem maximum occupational exposure. Keith J. Morris kjmorris@well.ox.ac.uk Tue Jan 13

CHEMICALS- cacodylate safety

I have a question about cacodylate buffer. I'm getting set to teach an introductory EM course for biologists to undergraduates. Having interviewed two of my three students during the previous semester, I know that I will be starting very much at zero. They had no to little knowledge of what "EM" is or can be used for before I spoke with them - they are exploring this new class. My plan is to take them through the preparation of plant, animal, and some sort of micro sample via traditional chemical fixation methods and keep it as simple as possible. I am inclined to steer clear of cacodylate buffer due to its toxicity and because they have enough to deal with already, and stick with phosphate buffer. However, I have noticed that most if not all of the animal tissue protocols I've been perusing use cac buffer. Is there any reason why I should keep it in the protocol? Kristen A. Lennon k.lennon@frostburg.edu Fri Jan 9

There are two ways of looking at this: first, phosphate and other buffers work as well as cacodylate, the difference between cacodylate and phosphate, e.g., is mostly preference and what one is used to seeing. So why use a toxin that can be avoided? Especially since some people (like me) are sensitive to the arsenic. The other way is: sometimes cacodylate is needed, and the students will have to be using other toxic materials in microscopy and chemistry and biotechnology and ... in other words, they need to learn how to properly handle such materials, and the sooner they learn the better, so use it. There, microambiguity. In our EM courses, I avoid cacodylate (because I'm sensitive), but we do make it available if needed, or if a student wants to use it for a project. Generally, though, when you're teaching about buffers, there are a lot more to discuss than just phosphate or cacodylate. If the class is doing aquatic critters (algae, protistans, tiny inverts, and suchlike), then the best buffer is 0.02 micron filtered water from where the critters were collected. Phil Oshel oshel1pe@cmich.edu Fri Jan 9

There is no compelling reason to use cacodylate buffers for an introductory class. If you need to add calcium to the fix you can use HEPES or PIPES, plenty of literature on these. Cacodylate was very convenient because it was a one salt buffer and Ca2+ ions did not precipitate. Hazardous waste disposal concerns have made its use difficult to justify. Geoff McAuliffe mcauliff@umdnj.edu Fri Jan 9

I have always found it strange that there is so much concern about the use of cacodylate in the EM laboratory. I concede that it is dangerous when handled incorrectly. However, I would think that phosphate buffer containing 2.5% glutaraldehyde is more dangerous, or even a 2% aqueous solution of osmium tetroxide. At what point should students start taking responsibility for handling chemicals safely and when do the trainers bite the bullet and make sure the training is adequate? During my training I remember very clearly how I was taught how to handle pathogenic bacteria and viruses. The instructions were very clear and very strict, and followed the same basic rules of how I was trained to handle radioactive material. The instructions were that these agents could make you sick and even kill you if you do not follow my instructions, so you have to handle them as follows.... When I moved into electron microscopy, the training I was given to prepare biological specimens consisted of being given access to a fridge full of chemicals

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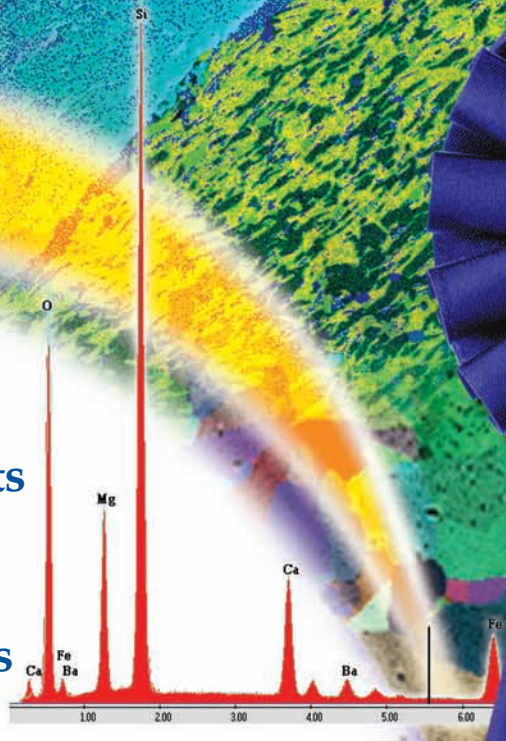

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and a written protocol. The brown spots on my hands appeared after a couple of hours but my corneas clouded over in about 30 min of my using the osmium tetroxide. I had been left completely unsupervised to handle these chemicals without any prior warning of their dangers. Interestingly, when my supervisor found out about my use of the osmium tetroxide, and what it had done to me, he blamed me! With proper training, the chemicals we use in the EM lab are basically very safe. We use small amounts of them and store them in closed cabinets so they should not affect our health in any way. Part of a good laboratory training course is teaching users how to handle toxic chemicals and how to pipette solutions without creating aerosols. Making sure that protective gloves are used correctly is another important aspect of this training. If we train correctly, then it should be sufficient to warn students that they are handling materials designed to chemically alter biological material. At this point, I usually remind them that they are made of biological material too. Paul Webster pwebster@hei.org Fri Jan 9

I naturally agree with Paul that with proper training that all the chemicals used in an EM are safe but that doesn't make it a good idea to use them indiscriminately. The comparison to bacteria and viruses is a red herring since those are typically the subject of interest as opposed to the selection of a buffer which is discretionary. Microscopists and other scientists inevitably generate hazardous waste but it is incumbent on us not to do so unless there is a scientific reason that it is the only way to do the experiment. It is true that glutaraldehyde in phosphate buffer is dangerous. But glutaraldehyde in cacodylate buffer is more dangerous. Accidental exposure would mean exposure to two dangerous chemicals. In addition, adding acid to glutaraldehyde in phosphate will change the pH but not release arsenic gas. Many studies have shown the good buffers to be suitable for LM and EM studies. I see no reason for any microscopist to cling to the use of cacodylate. Paul's own horror story of poor training and supervision is further evidence of why one should minimize all unnecessary risks. I doubt any trained scientist would use any chemical they don't fully understand outside of the hood and without gloves - that's a beginner's mistake. Unless one can guarantee being present at every step of a new student's processing, it would only be prudent to use the least toxic formulations. With experience, those students will be able to judge the risks they wish to take. Tom Phillips PhillipsT@missouri.edu Fri Jan 9

SPECIMEN PREPARATION - sand

I've cut some hard specimens over the years but never sand. We have a researcher who wishes to look at a section of a sand grain to study the distribution of nanotubes on the surface. Any suggestions on sectioning a grain of sand? Here's what I am planning: embedding in hard Spurr resin old, 50 degree diamond knife 2 mm/sec cutting speed John J. Bozzola bozzola@siu.edu Fri Dec 19

What kind of sand? I immediately think coralline sand, then I have to back up and remember you have different (Si) sand. When I have sectioned plant parts that have contained silica bodies (e.g., pineapple epidermal cells) (don't ever agree to do this!), the particles have torn out of the resin, but have often left behind some surface pieces and, importantly for you, anything sort of stuck to the surface of the particle. So I expect you will have holey resin sections floating on your water and a little pile of sand at the bottom of your boat, but you may have some nanotubes left in the resin. Pick up on coated grids. Cross fingers. Resort to high-resolution SEM. Come here; we are getting a new Hitachi S-4800 in two weeks with EDS and STEM, Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Sat Dec 20

For sectioning sand make sure you borrow a diamond knife from the director. Oops, you are the director! Seriously, ask the researcher to provide you with their diamond knife because sand/silt is hell on a knife. I've sectioned forams - they agglutinate sand/silt to form their shell (test). We embedded them with EMS' Araldite Embed 812. I can send you pdf's of the papers if you want to see micrographs. The 'sand' doesn't remain intact when it is sectioned - it fractures extensively. Have you considered ion beam milling? Maybe that would be the way to go. I've never done that but perhaps others could comment on ion milling. Beth Richardson beth@plantbio.uga.edu Mon Dec 22

I suspect that microtoming sand (esp. quartz grains) would be as impossible as microtoming Si because of hardness and cleaving concerns. Look through the ads in Microscopy Today for the vendors that sell instruments for ion prepping specimens. Any of them would be an excellent choice. Making friends with someone who owns a FIB would be another good idea for either SEM or TEM specimen prep of sand grains. Contact the instrument vendors and offer to co-author an article on electron microscopy of sand grains for Microscopy Today. I suspect it has never been done as there hasn't been any references to the literature so far. This might get you a foot in some manufacturer's application lab. Ron Anderson randerson20@tampabay.rr.com Mon Dec 22

They tried examination by SEM but needed better resolution than we could achieve with our conventional, older SEMs. I considered using HF to digest the sand grain but they nixed the idea since they wanted to show orientation of tubules relative to the surface. Oh, well. We did get sections using a diamond knife but the nanotubes appeared to be round globes rather than tubes. I'm uncertain if the embedding somehow messed them up or, more probably, nanotubes had not formed and we were looking at spheroidal materials instead. John J. Bozzola bozzola@siu.edu Tue Jan 13

SPECIMEN PREPARATION - etching resin

I have checked the listserver for Paragon staining of epoxy resin and found several very helpful comments. I tried using Martin's procedure without success. I came across another comment about etching the slides first. Does anyone have experience with this? I have since tried Richardson's stain and it just doesn't give enough differentiation. I am staining coral tissues that have been decalcified in 10% EDTA and fixed in 2% glutaraldehyde, post-fixed in 1% osmium and embedded in Spurr's. Sue Tyler sue.tyler@noaa.gov Wed Jan 28

I can't find my (antique) notes on etching at the moment, but I can sort of remember, and it might get you started while someone else comes up with something. I was etching epoxy resins with saturated ethanolic NaOH to do PAS. Make a solution of saturated ethanolic NaOH by dumping a lot of NaOH pellets in a bottle of absolute ethanol, like an inch and a half in a pint bottle. Put it aside for a couple of weeks until it looks like cognac. Soak slides in this solution in a Coplin jar for (and this is where I can't remember - Two hours? Two days?). Sections used to easily come off those old, plain slides, so I was careful not to agitate. I think they would stay on better with Superfrost Plus or treated slides. Go ahead and start making your cognac .. er.. etching solution and I'll look for

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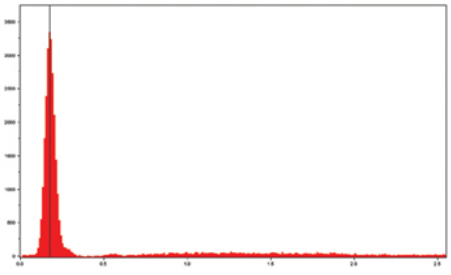

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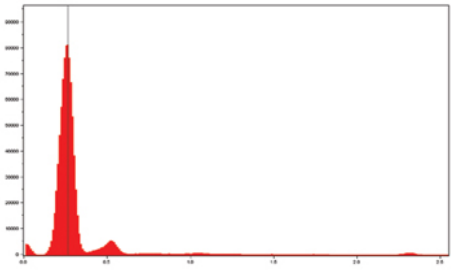

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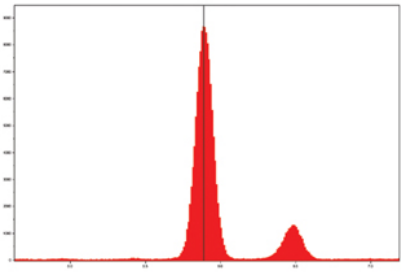

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my PAS protocol. Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Wed Jan 28

I prefer KOH in methanol. We use 5' etch, followed by 2 changes of absolute methanol. We published a protocol for Iron Hematoxylin/Eosin/Alcian blue (which gives pretty differentiated staining on marine inverts) a while back in Microskopie; I can send a .doc file to anyone interested. If you use PAS after etching, watch for staining artifact--epoxy embedding generates a bunch of them, at least with our invertebrate material. Julian P.S. Smith smithj@winthrop.edu Thu Jan 29

I found my old protocol, although I'll bet Julian's is more current, and may give you the differentiation you're looking for. Add 100-150 g anhydrous NaOH pellets to 250 ml freshly opened bottle of absolute ethanol. Allow to stand until cognac or deep rust-brown color, shaking occasionally, for about a week. Keeps 4-5 weeks. Store in plastic bottle, if possible. To remove resin from sections, immerse slides in solution for an hour or more; checking to see when resin is etched away (I remember this being about two hours for 0.5 micrometer thick sections). Drain well, but don't blot. Immerse slides in 4 changes of absolute ethanol, then proceed with staining, clearing, and mounting. This was originally for PAS (Periodic Acid Schiff) on gecko reproductive tissue. Now I want to try Julian's recipe on coral reproductive tissue... Tina (Weatherby) Carvalho tina@pbrc.hawaii.edu Thu Jan 29

SPECIMEN PREPARATION – plan-view TEM samples

Does anybody know how to protect plan-view TEM samples, which are thinned from one side only, from contamination during ion beam milling? Thanks very much in advance. Yisong Han y.han@sheffield.ac.uk Tue Jan 27

Evaporate salt (NaCl) on the side you want to protect. Dissolve the salt in water when you are finished ion milling and the contamination will flush away. Ron Anderson randerson20@tampabay.rr.com Tue Jan 27

Here are a couple possibilities: 1. Paint the side you want to protect with a layer of clear fingernail polish and dissolve in acetone after ion milling is finished. 2. I used to cut out a 3mm disk of a very thin sheet of mica and place that under the specimen. Any re-deposited material will stick to the mica disk. Craig L. Johnson cljohnson33@gmail.com Tue Jan 27

Just to add to Ron Anderson's and Craig Johnson's posts - if you use glycopthalate wax (which is often sold under a trade name of QuickStick or something like that), you can put a small amount into a few ml of acetone and paint it on the surface you want to protect. The wax is totally soluble in acetone and should leave no residue (okay, I guess a small amount of amorphous carbon on the atomic scale). I'm not sure that nail varnish would do the same. Richard Beanland contact@integrityscientific.com Wed Jan 28

SPECIMEN PREPARATION – stopping points

I'm wading through my first semester of teaching EM to undergrads and am in need of some advice regarding preparing animal tissue for TEM. Given the set-up of my class (that was not designed by me, mind you), we are forced sometimes to stop part-way through a protocol and store the tissue for a day or so. In my experience with plant tissue, I've done this at various steps; however, I need your advice on stopping points for animal tissue. Any advice on when tissue can be stored (and when it cannot), would be much appreciated. For reference, we are doing a "standard" primary fixation in glutaraldehyde/phosphate buffer, secondary in OsO4/phosphate buffer, dehydration in either ethanol or acetone and embedding in Spurr's. Kristen A. Lennon k.lennon@frostburg.edu Sun Feb 8

I have had best luck stopping at 70% ethanol. It is the only stopping point where I don't see problems. That said, my samples are not your samples. David Elliot elliott@arizona.edu Sun Feb 8

The point after glutaraldehyde fixation and 2-3 rinses is a good early point. After OsO4 and rinses is not bad too. Lower concentration alcohols is a big no. 70% is OK to leave overnight in the fridge and convenient to combine with uranyl acetate staining, making it 1.5% uranyl acetate in 70% ethanol. In general, any excessive time in alcohol or acetone causes extraction, so samples should not be left there for days. Same for diluted epoxy. Acetone has been reported to be less extractive during dehydration than ethanol, but it is harder to handle. There is one more point worth mentioning. I've never seen it described anywhere, but once had to fix an important sample on the eve of vacation. I reasoned that undiluted ethanol in the freezer (-20°C) should minimize any extraction and will also not protect from freezing damage. A week later, I embedded the samples, and all was perfect. Vlad Speransky vladislav_speransky@nih.gov Mon Feb 9

Buffer after glutaraldehyde or buffer after osmium are the best places to stop and store tissue. Storage in any concentration of alcohol (or acetone or propylene oxide) is not a good idea, cytoplasm will be extracted. This is well documented in the literature. If you can get to pure resin put the vials in the refrigerator overnight. Just remember to let things warm to room temperature before opening to avoid condensation. Geoff McAuliffe mcauliff@umdnj.edu Mon Feb 9

MICROTOMY - histo knife

If response to a query on histo knives:

We have a histo knife, however we don't cut thicker than 500nm and not on a routine basis. I have been said that like an ultraknife, its lifetime mainly depends on what you cut. Cut butter and it will survive you. Cut nanoparticles and quantum dots and it will probably not survive your grant. Cutting soft tissue in resin does probably not significantly affect it. Personally I couldn't imagine regularly semi-thin sectioning without a histo knife, it is so comfortable. Stephane Nizets nizets2@yahoo.com Thu Jan 22

I love our histo knife. I use an old diamond knife to face the block, then switch out to the histo knife. Sectioning is done at 0.33 µm. Life span varies with usage, type of sample (ie bone or cell culture phosphate crystals, etc are harder on the knife) I once had a histo knife that I had cut about , say 500-600 blocks / year, and I cut big blocks often, it lasted for 7 years! However, get glass, silicone, bone etc, and you could ruin a knife in a day. The time saved is really huge, the quality is very good, especially if you have to section some to get to "just the right depth". Well worth the money, and yes when I switched I did have a little bit of pride fall.... I can do glass well and it's an art... thing, that goes to the wayside quick after the pleasure of working with a diamond histo knife. So save old knives, use them to rough cut the already trimmed block, and you will get even longer life out of your knife. Lou Ann Miller lamiller@illinois.edu Thu Jan 22

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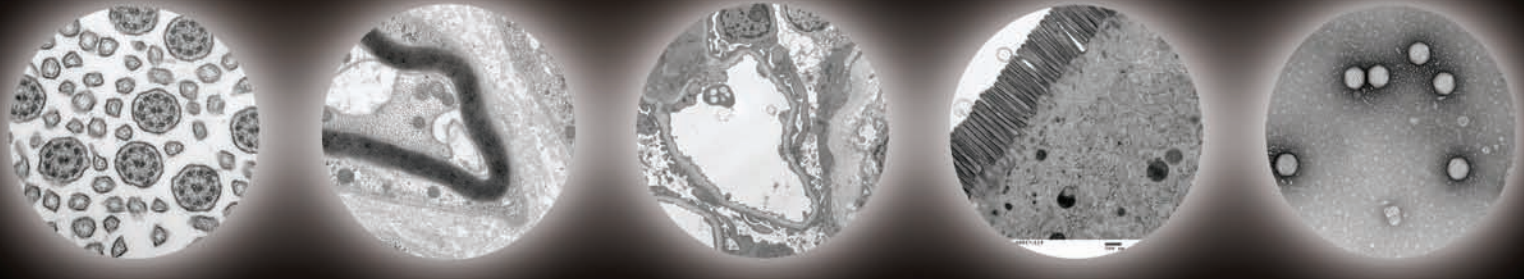

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Don't contemplate - spend the money, get the knife - make yourself happy. You will not regret it nor will you go back to using glass. Consider it a necessary luxury item - you will feel so spoiled every time you use it. Work productivity will increase tenfold. Everyone here uses them for 1um thick sections (plant material). Beth Richardson beth@plantbio.uga.edu Thu Jan 22

I agree with Beth. Do yourself a favor, get the knife! It will save you a lot of time. We use diamond knives for all of our thick sections. The only time we have to make glass knives is when we cut something that may have bone or hard material and then we also use glass to cut thins. If you take care of the knife as you probably do the ultra-knives, you will get a lot of sections off of it. Pat Kysar pekysar@ucdavis.edu Thu Jan 22

We purchased two for serial sectioning of fish embryos at 2 microns and are very pleased with them. For serial sections re-aligning for each fresh glass knife is out of the question. When one knife develops nicks (after many thousands of sections) and is being re-sharpened we use the second knife. Geoff McAuliffe mcauliff@umdnj.edu Thu Jan 22

I recommend the Histo diamond knife. At my previous job, we didn't have any complaints sectioning tissues and cell pellets at a half micron thick. By removing the chance of glass dust getting on the ultrathin diamond knife, I believe it lasted longer without nicks as well. When we used glass knives, we would face the block between glass and ultrathin diamond knife work (to remove any rare glass bits) using an old sapphire knife. I was glad to skip that step after switching to the diamond histo knife. For one project, I sectioned a 4-5 mm wide tissue section with the histo knife. I wouldn't have wanted to try that with glass! Gregg Sobocinski greggps@umich.edu Thu Jan 22

We have four large histo knives, usually one for trimming, two for everyday use, and one in perfect shape for when one of the others has to go away for re-sharpening. They are used to cut sections up to 2 microns thick, of quite large block faces - up to 3 mm across. They get re-sharpened at least once a year. Like Stephane, I'll never go back to routine glass knife use, the diamond knives save so much time. We only go back to glass for training and if the tissue might damage the diamond (chunks of rock in soil around roots, for example...). Rosemary White rosemary.white@csiro.au Thu Jan 22

In following this interesting chain, I should mention that we did quite a bit of experimenting with histos back in the '90's at our govt. lab in Ottawa. However, we were interested, not in thick sections of life science material, but in TEM sections of industrial materials like metal alloys. Courtesy of a free knife from a vendor (who was well aware of the intended material and still supplied it!), we had some interesting results: * Thin sections of an aluminum automotive alloy and some nanostructured Pd, were produced with no problem * Using EELS to measure the section thickness, it turned out that the histo sections, at least for Al, were closer to the set thickness than for regular diamond knives * The histo sections always came off in the boat as flat as pancakes, unlike the regular diamond ones which often had a tendency to curl like lathe shavings * However, the section microstructure was marred by what we termed 'continuous knife marks', ie closely-spaced grooves that suggested maybe a finely-serrated edge * Emboldened by these strange results, we tackled a material that had given us major headaches - thin TEM sections of embedded, amorphous, Fe-Nd-B magnetic alloy particles (~20-40 micron diameter and majorly hard). Other knives either suffered major edge damage (35 and 45 degree) with useless 'shards', or sections which were too thick and/or curled to be useful (55 degree). The histo? Semi-whole particle cross-sections that were relatively uncurled and close to the set thickness of 30nm, quite suitable for elemental mapping if we had wanted to do so. * Oh yes - it also cut flat, 1 micron thick sections of the Al alloy, and the edge remained undamaged, at least at the optical microscope level. Go figure. Dr. Tom Malis malis@nrcan.gc.ca Fri Jan 23

IMMUNOCYTOCHEMISTRY – colloidal gold

I have a researcher who will be wanting immunoEM on cell pellets. I'm going to embed them into LR White. What I want to know is what is the best type of gold to buy and from who? The researcher isn't sure what the source of the primary will be (rabbit, mouse, Sasquatch) so I was wondering if Protein A would be the best all around choice. I haven't done immunoEM in a while so I was wondering what's the latest and best in terms of optimizing the staining. Remember I have to go with a room temp embedding media, I have no acccess to low temp equipment. Paula Sicurello vapatpxs@yahoo.com Wed Dec 17

You really should know the source of your primary in order to decide which gold conjugate to use. While Protein A-gold is my, and many others', favorite - because it gives closer detection and only one gold (or less ;)) will bind to each primary - Protein A only binds well to IgGs from rabbit, pig, guinea pig (as well as some from human). So no, it is not an all-around choice. You could, though, use a bridging antibody - e.g., a rabbit-anti-mouse between that mouse monoclonal and Protein A. In this case, PAG will be indeed your universal probe, but you'll need to buy other Abs to bridge. Not meaning to hijack, but I would be really interested if somebody could comment here on which *primary* is be preferable in those cases when there is a choice? Or, should I say, *which polyclonal*, since we know polyclonal works better for immunoEM? Rabbit, guinea pig?.. Or, perhaps those IgYs from chicken - won't bind Protein A, but I remember hearing somewhere that chicken Abs tend to give "cleaner" labeling, while those from rabbit are among the "dirtiest"? As for source, I don't think you can go wrong with any. I wanted to blame it on gold a few times, but always turned out to be my fault in the end. Vlad Speransky vladislav_speransky@nih.gov Wed Dec 17

Sorry to diverge from the original subject, but you said "While protein A-gold is my, and many others', favorite - because it gives closer detection and only one gold (or less ;)) will bind to each primary" Please could you tell me why is it an advantage to have only one secondary bind a primary antibody? Multiple binding secondaries would increase the sensitivity no? If your fear is that secondaries would "displace" the localization of the primary, meaning less precision in space, why would several secondaries be less precise than one? Stephane Nizets nizets2@yahoo.com Thu Dec 18

Protein A is the smaller molecule compared to a complete antibody molecule in a secondary antibody gold conjugate. It also binds in a designated area on the primary antibody. This, at least in theory, is advantageous for the resolution of the labeling...but I would like to add that in experiments done over 25 years ago, using a three step labeling with protein A/gold as the final step there was little doubt as to whether the epitope was on the outside or the

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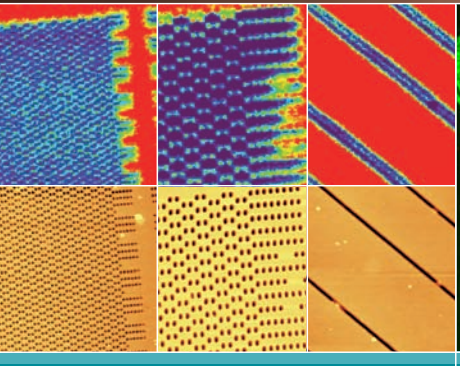
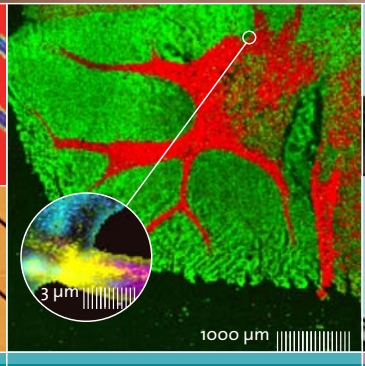


Raman stress and AFM topography images recorded automated at three different areas of a Si-device.



Raman large area scan of a hamster brain cross-section and high-resolution zoom-in Raman image at the marked area.

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inside of bacterial membranes, even though the detecting complex was all in all quite substantial and 'flexible'. Using a secondary antibody conjugate or antibody fragment (Fab or Fab2) might result in more gold particles per primary bound. But is this an increase in sensitivity? It certainly results in an increase in detectability, but if both protein A and a secondary antibody would recognize the same number of primary antibodies one still observes the same number of antigens. To get a more complete image it is also necessary to take the binding forces into account which is likely more favorable with secondary conjugates because of avidity rather than affinity binding. This helps keeping the label in place during washing steps for instance. All this is also related to the size of the gold particles. But that's a different story all together. Jan Leunissen leunissen@aurion.nl Thu Dec 18

Regarding your comment: "Multiple binding secondaries would increase the sensitivity no?". This is not exactly the case. By increasing the number of secondary antibodies binding to the initial bound antibody you only amplify the signal that is already there. To increase sensitivity, you have to bind more of the first antibody to the antigen. There are specific methods that aim to do this (e.g. antigen retrieval) but which occur before the antibodies (or affinity probes) are applied. I agree with Vlad that protein A-gold is probably the best way of visualizing bound antibodies at the moment. Not only does PAG bind 1:1 with rabbit polyclonal antibodies, but it can be used with unconjugated secondary antibodies (which are less expensive and easier to store than conjugated antibodies) as a bridge for when non-protein A -binding primary antibodies are used. Using one reagent for all visualization experiments needs makes it easier to monitor the reactivity of the reagent so that if one user finds the probe to be "not working", then the problem can be easily diagnosed, especially if the probe still works for all the other users (!!!). Using protein A gold also means that fewer probes are needed. I have only PAG 5nm and PAG 10 nm in storage which I used for all immunocytochemical needs. Our primaries are made in rabbit, mouse, goat, chicken and donkey. If I used secondary antibodies I would need a very large collection of antibodies conjugated to gold, which do not store very well over long periods (5nm and 10 nm gold coupled to anti-goat, mouse, rabbit, etc). Single gold particles (one gold per antigen), although thought to be insensitive when only a few are detected, can say much about the abundance of the antigen (fewer gold equals less antigen) and the location of the antigen. The small particles offer relatively high-resolution localization of antigens, which is a good thing. Paul Webster pwebster@hei.org Thu Dec 18

Regarding the sensitivity, I start with the hypothesis that the labeling will be used for quantification. It was my understanding (perhaps not right!?) that the amount of signal plays a role in the validation of quantification. Let's say that the amount of target protein is double in one cellular compartment in comparison with a second compartment. Compartment 1: 5 primaries and Compartment 2: 10 primaries. In Case 1 - If you have 1:1 secondary:primary this gives you: Compartment 1: 5 secondaries and Compartment 2: 10 secondaries. In Case 2 - If you have 10:1 secondaries:primary this gives you: Compartment 1: 50 secondaries and Compartment 2: 100 secondaries. Given that all values are accompanied with standard deviations (SD), chances are the SD in case 1 would make the difference not significant, while in case 2 the difference would be significant. Moreover, if you subtract the background from case 1 it has a huge impact on the final amount, while in case 2 it has few impact. I have not been trained for such things and I am the first to be sorry about it. My ideas come from a mixture of gut feeling and reasoning. I would be happy to be corrected if needed. Stephane Nizets nizets2@yahoo.com Thu Dec 18

Whether one gets an increase in signal/noise depends on a number of things. First, there may be a probability less than 1 for the primary to bind to the target even with a vast excess of primary in the reaction. This can be due to incomplete penetration into the region where the target is located, such as when the region is bounded with a membrane that is not too permeable to the primary. There may also be interactions of the target with other components of the region, such as formation of protein complexes that compete with binding of the primary. Second, there may be similar issues with the secondary. Third, incomplete washout of either unbound primary or secondary will result in noise, so in either case 1 or 2 the noise could be large and variable. Suppose, for example, that either the unbound primary or secondary is washed out to 90%, but secondary bound to primary is only washed out to 50% (since it is a larger complex). One can imagine conditions where 10:1 secondary:primary will result in a large noise component that cannot be determined from using a non-binding control, e.g., preimmune serum, that does react with the secondary. In your example above with 5 and 10 targets in compartments 1 and 2 respectively, case 1 would give 5 x (probability of primary binding in Compartment 1) primaries in Compartment 1 and 10 x (probability of primary binding in Compartment 2--not necessarily = that for Compartment 1) primaries in Compartment 2. Then using similar considerations for secondary binding and detection efficiencies, one would see signal (Compartment 1) and signal(Compartment 2) that are not necessarily 1:2 with either 1:1 or 10:1 secondaries:primary. In a somewhat related case--but not exactly the same--using a CCD to record TEM images one can have two phosphor screens one of which produces 5 photons per primary electron and the other of which produces 10 photons per primary electron. There is a SD for the number of photons per electron for each screen and an overall SD for photons in each pixel (which can be computed from the SDs for photons/ electron and for # of electrons). Although increasing photons/ electron will decrease the SD for that ratio, it will not decrease the SD for electrons, i.e. shot noise, so the increase in S/N will be limited by that shot noise. Increased photons/electron will make CCD readout noise negligible, so there is some gain to be had by increasing that ratio, but after a while that gain is not significant, which translates into the statement that the intensities for two compartments, as visualized by CCD signals, will not be distinguishable if the numbers of primary electrons are not distinguishable, regardless of the ratio of photons per primary electron. Bill Tivol tivol@caltech.edu Fri Dec 19

It is indeed a great point, the one about the advantage of having only one type of gold conjugate to keep track of. I've just been meaning to point out what that "more than one gold per each primary" binding really looks like in the microscope. It looks like loose clusters, some kind of rosettes. If the antigen is in a bordered compartment - granules, Golgi cisterns - you'll see your gold "spill over". Not nice, if you are interested in higher resolution localization.

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For my first immunogold labeling, back in 2000, I used 6 nm GAR. When I saw the clusters, I first thought these were some storage-related aggregates of either primary or secondary Ab - until I tried using PAG instead. Vlad Speransky vladislav_speransky@nih.gov Fri Dec 19

IMMUNOCYTOCHEMISTRY – choice of secondary antibodies

I've a question regarding secondary antibodies for immunolabeling. Are there specific ones that are best used for a sample embedded in LR-White and using a mouse monoclonal primary? I am planning to use a goat anti-mouse secondary for this, and wanted to see if anyone perhaps had some insight or preferences for specific types that work well. Or perhaps it is mostly the proper concentration that determines the outcome? David Parmiter parmiterd@mail.nih.gov Mon Dec 22

The choice for a particular secondary antibody is not much dependent on the type of specimen or embedding. It is different for the primary, however. Some seem to work well with one type of specimen and other primaries again with different types. You may want to choose a secondary that matches your primary (sub)class though for optimum results. I.e. an anti-IgG for a primary IgG. There are mixed anti IgG/IgM available too from several manufacturers, but in general "the tighter the fit" the better. The concentration.... that is a much neglected area. There are also many different ways the desired concentration is established, differing from user to user and not seldom even from experiment to experiment. A general guideline would be to use a concentration between 0.1 and 1 µg/ml which is in accordance with the equilibrium constant for antigen-antibody interactions. One would ideally incubate just long enough to reach equilibrium in binding and to get reproducible results from one experiment to the next. Higher concentrations may give faster results, higher intensity by labeling of low affinity epitopes for instance, but also an increased background risks. Lower concentrations resulting in the opposite pattern. Longer incubation times may result in higher background levels, or when specimens are vulnerable (pre-embedding, cryo ultramicrotomy sections) in loss of integrity of the specimen's ultrastructure. It is a matter of balance. The concentrations used are in general lower than the ones applied in fluorescence or "DAB" microscopy as even a low particle background is somehow experienced as more annoying than a similar low background with a fluorescent secondary and/or peroxidase-labeled antibodies.....The way we perceive things analogue signals seem to be more forgiving than yes/no particle based systems! Jan Leunissen leunissen@aurion.nl Mon Dec 22

As usual, Jan gives some excellent advice. I want to clarify one point he made since I routinely run into students and clients who fail to understand a bit of basic immunology. Most commercial secondary antibodies are "anti-IgG (H+L)". The H chain is class specific but the L chain isn't. An anti-IgG (H+L) antibody will also stain IgM and IgA. If you want a class specific antibody, you need to get one made against the Fc portion. I also agree with Jan's advice of keeping the concentration as low as possible. 70% of the time, I use 1 µg/ml. In about 20% of the time, I find using the primary at 10 µg/ml gives better results and sometimes even need to for the secondary but much less frequently. Tom Phillips phillipst@missouri.edu Tue Dec 23

SEM – current state of the art for biological specimens

At the risk of being labeled a heretic, I'd like to know the current state of biological SEM. Here's the deal, I am teaching a class on bio SEM and just sat down to prepare a lecture on modern biological SEM. I have lots of nice books with cool SEM pictures, but I noticed they are all about 20 years old. Heck, I even did some nice bio SEM, but that was, well, 20 years ago. I tried to cruise the web to see if I could get up to date on what's happening, but web access to journals from here is pretty lame. So is the offering of ultrastructural type journals at our library, like, there aren't any. I do get a few journals and I can access a few on the web, but most of the papers in the searchable index from J. Cell Biology, etc. that have SEM are, well, pretty old. Is there much modern research using SEM being done in biology these days? Mostly I see the SEM being applied to materials type research. Not that the biology work that has been done is not elegant, it just seems like a lot of it is finding its way on to journal covers or 'coffee table' picture books after being colorized in Photoshop. I am OK with that, and if the kind of SEM that I know, simple fix, dehydrate, and critical point dry is the state of the art, then I will feel better getting my students to do these things. But if there are sources for something new, I would like to be able to tell them about it. I mean, after all, how many fly eyes, bee's knees, and pollen grains can you look at? Jonathan Krupp jmkrupp@ucsc.edu Wed Feb 4

You ask a relevant question. As a biologist who has gotten deeply into SEM in the last decade, I am surprised that there is not more use on our side of the fence. I suspect that most of the recent advances in SEM tech have been driven my materials folks and that the biologists simply don't know about them. Let me give you my perspective on where I think the advances are. 1. Environmental SEM. It is now possible to examine a fully hydrated specimen in the SEM. The sample needs to be frozen or at least cold to minimize evaporation but otherwise not processed at all. I am aware of people who use ESEM to study root hairs and also floral meristems. I suspect animal scientists use also. The point is that fixation critical point dry and coating are all avoided and instead the sample is directly viewed. The drawback is that once the sample is out of the machine it cannot be viewed again later. And the resolution of an ESEM is no better than a conventional tungsten filament model. 2. Wet chambers. A rather recent (5 years or so?) development are thin membranes that the beam can pass but gas cannot. This allows fully hydrated samples to be viewed at ambient temp and pressure in chambers built with the top surface being that special membrane. Thus living cells can be grown on the membrane (inside the membrane) and viewed in the SEM while they are alive. It is of course a question how much radiation damage the beam will induce. Still the cells are certainly viable for a while. I have seen papers using this tech on animal tissue culture cells. There was quite a lot of excitement when these chambers hit the market but I don't know how successful they have proven in the "real" world. The above points are geared for eliminating fixation and looking at samples as close to living as possible. This is clearly difficult given the fundamental high-vacuum nature of the SEM beast. The other direction where advances lay is in the high vac, high resolution end of things. Here the advances in SEM design have been phenomenal. The field emission gun allows resolution to be obtained that is almost as good as the best TEMs and certainly far beyond what the good old fly eye SEM was capable of seeing. 3. High resolution. With the field emission gun, macromolecules can be resolved. I among others have taken advantage of this to study the ultrastructure of the plant cell wall. Cellulose microfibrils are on order of 10 nm and these can be readily imaged with FESEM. What is particularly useful for me

is that I can put my plant stem 1 mm by 10 mm in to instrument, get good low mag survey views and crank up the magnification to the ultrastructural level in any cell on the sample that is appropriate. The easy moving between low and high magnifications is extremely helpful where extensive sampling is needed or when rare features need to be found. Other biological systems that have been profitably studied with FESEM include the cytoskeleton, bacterial biofilms, biomineralization, and nuclear pore complexes. All of these cases involve complex three dimensional structures and the FESEM lets you image them at high resolution in the intact (or semi-intact) state. 4. Low voltage. Along with the sharper probe of the FESEM comes the ability to work at really low accelerating voltage. In the recent vintage FESEM's it is easy to image at 1 kV and possible to go down even as low as a tenth of that. This has several advantages. First, many samples are destroyed or damaged at 30 kV or even at 10 kV. Second, the lower the beam voltage the more exclusively the image comes from the surface. This increases the resolution in the depth direction. Both low voltage and the smaller probe size allow much thinner metal coats to be applied, and in some cases no coat at all. For the work I have been doing on the cell wall, I use ca. 1 to 2 nm of Pt. This is an order of magnitude less than typical gold coats for conventional tungsten SEM work. 5. Backscattered imaging. Of particular importance to biologists is being able to image backscattered electrons. This allows the localization of gold probes, as in gold conjugated secondary antibodies. With the latest instrumentation, one can collect electrons from selected energy (or angle) levels and mix signals from several detectors. Thus, the SE detector can show the topography of the sample while the BSE detector can pick up the signal from the gold and the two are overlain seamlessly. The sensitivity of the detectors is such that the gold can be imaged at high contrast even with a sample lightly covered with platinum. I took advantage of this (with slightly older not quite so performant technology) to detect gamma tubulin in the plant cell cortex. Others have localized antigens on the plasma membrane and cell wall, to name a few. There have been parallel developments in elemental analysis (EDS and related modes) and I expect biologists have taken advantage of these too but it is not my area, so I don't know. Perhaps a helpful netizen will chime in. Summarizing, you are right, recent use of SEM in biology is a little bit buried. However, these uses will be well worth your digging up. Tobias Baskin baskin@bio.umass.edu Thu Feb 5

A quick answer: "Biological Low-Voltage Scanning Electron Microscopy", H. Schatten and j. Pawley, eds. Springer, 2008. Lots of good information in there. The major advances in Biological SEM have been in cryo methods and high resolution (which pretty much means low voltage) methods. SEM using gold-conjugated antibodies and other ligands are being done. There are also environmental SEM and fluid-chamber SEM using backscattered detectors. Correlation studies of e.g. gold-conjugated primary antibodies followed by fluorescently labeled secondaries should (I hope) become more common. I suspect most of the advances are in the primary literature, but not necessarily in the microscopy journals. Rather, in journals like "Immunity", etc. Then again, one reason old texts are still good is that there isn't necessarily a need for new methods, "just" new studies. Philip Oshel oshel1pe@cmich.edu Thu Feb 5

Our lab deals with dental biomaterials and we have an SEM. The biological part comes into play for tissue engineering, e.g. see-

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ing if cells are in scaffolds, are they happy with the surface they are on, etc. We've also looked at bacteria in microleakage studies. Some of these problems are unsolved but improved upon in 20 years. From what I've seen of research, the more diverse the background you have, the more apt you are to come up with a better solution to a problem. Remember Edison, the inventor of the phonograph and practical light bulb? He had to come up with a complete system to make the light bulb work outside of the lab. He studied how the gas company distributed gas, an already tried and true system. And the woman who invented the circular saw blade did so because she was using a spinning wheel while the males of her family were pushing and pulling a saw through wood. It is connections of old and new. Ron L'Herault lherault@bu.edu Thu Feb 5

I agree. Biological SEM has changed and materials applications are more plentiful. However, in my opinion and experience, there is still room for the "old ways". Delta has a wonderful library of old SEM proceedings and there are many good papers in them. (Let's hope that "old" doesn't mean "obsolete" or we'd all be in trouble.). It does seem that environmental, low vacuum and high resolution SEM are most relevant nowadays, however, many biological labs don't have the luxury of owning them. As a technician who is responsible for SEM processing and training on a standard SEM, there is still a call for different biological preps. Your students would benefit from learning how to process cells (suspensions or grown on filters), OTO, cryo fracture, maceration, backscatter, replicas etc. Basic theory and hands on are crucial. When I was a student, we used the book "Preparation of Biological Specimens for Scanning Electron Microscopy" published by Scanning Electron Microscopy, Inc. and compiled by Judy Murphy and Godfried M. Roomans. It's from 1984 but it still has a lot of ideas for your class. I'm not sure if it's still in print but there used to be one there at Delta. The more protocols they learn, the better equipped they will be when they graduate. Pat Kysar pekysar@ucdavis.edu Thu Feb 5

The type of microscopy has to fit the question. SEM can be a very valuable biological tool if the question is appropriate. For instance, we do a lot of cryoSEM so that we do not have the artifacts (shrinkage, etc) that go with critical point drying. I have one investigator who is interested in chemical crystal formation in plants. She does cryo and fractures the plant tissue to reveal the crystals in their native state without any possibility of extraction during aqueous fixation, etc. Other uses revolve around food processing, biomedical products, and pharmaceutical applications with samples such as starch, collagen, and synthetic hydrogels. Documenting bacteria growth on food products before and after treatments to eliminate the bacteria would be meaningless if you had to process the sample as that would likely also remove bacteria. CryoSEM would preserve them on the sample. Some samples can be done with traditional critical point drying even though there may be some distortion. An example would be to monitor cell growth on various substrates. Cells would be expected to shrink but their overall distribution would stay the same. Many plant tissues do well with CPD but others (very young plants) require cryoSEM. And the list goes on for applications to biological (or hydrated samples in general...). Debby Sherman dsherman@purdue.edu Thu Feb 5

I have to agree that SEM isn't used much in biomedical research, but disagree about why. SEM is not used much in biological work, biomedical in particular, but I think it is used more than indicated here, and that it is under used. I worked on many SEM biomedical/bioengineering projects at UW-Madison involving cultured cells, biofllms in catheters, implanted medical devices, bone fractures, and others. And using gold-conjugated antibodies to study cell-surface receptors. SEM is a much better method for this than TEM or confocal, as the entire cell surface can be sampled at high resolution, instead of just very thin cross-sectional slices that could easily miss the receptors, or instead of at light microscope resolution. Studies like this can raise and answer questions like: are the receptors distributed at random on the cell surface or in patterns? Is there any relation between receptor distribution and cell surface structures like ruffles, etc.? And so on. There are many biomedical molecular/genetic questions that could be addressed with SEM, there just are few people thinking of them. The problem isn't "how useful is the technique", but how people are thinking and how much they know about what techniques are available. Most molecular/genetic people know little or nothing of microscopy, or EM, much less SEM, so they never consider them useful techniques. That just means they don't know much about the technique, not that it's not useful. Philip Oshel oshel1pe@cmich.edu Fri Feb 6

I agree with Phil on this, and it may be partially due to the EM community not reaching out to researchers with information about the capabilities of our techniques. It's easy, for us at least, to fall into the routine of just reacting to the researchers coming through the door with their projects and a preconceived idea of the best way to approach them. We often have clients come in with completely inappropriate protocols that they want us to follow, simply because they have seen them in a journal article and believe that they are universally applicable. Quite often they may be completely uninformed about other, more appropriate, techniques. Being a bit more proactive in getting information out in the form of workshops, brown bag lectures, newsletters, etc., might do wonders in renewing interest in things like use of SEM technologies in biological research. Randy Tindall tindallr@missouri.edu Fri Feb 6

SEM – cooling

I was just tossing around a few thoughts, and I was was wondering if anyone has experience cooling two instruments with one chiller. My thinking is that you should be able to daisy-chain the diffusion pumps together and achieve proper chilling, but the trade-off would be a decrease in the required temperature coming out of the chiller. Does the flow rate have to be augmented somehow as well? Perhaps the better solution is to put a Y connector in the chiller lines and run the two in parallel? Justin Kraft kraftpiano@gmail.com Tue Feb 10

I run a few instruments off the one cooler, they are in parallel, with individual flow-control valves and water-flowmeters. Remember not to have your cooled water below the dew-point! Ritchie Sims r.sims@auckland.ac.nz Tue Feb 10

Daisy-chaining is not a good idea. Most systems are also cooling some of the electronics and that needs to be done with the cooler water. Also, having very warm water at the top of the second DP (and its water baffle) would be very counter-productive by allowing a great deal more back-streaming. A "Y" is fine as long as you put 2 flowmeters on the outlets so that you can be sure each instrument is getting the proper flow. If one has a higher resistance to flow (due to mineral or corrosion build up, or just a different design), it could end up with insufficient flow. The biggest question is whether or

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The facility houses a JEOL JEM-3010 TEM with an Environmental Cell, a Noran Energy-dispersive X-ray Spectroscopy (EDXS), and a Gatan Image Filter Model GIF 200. In addition, there is a Hitachi H-600 TEM and a sample preparation facility. The facility details can be found at http://www7430.nrlssc.navy.mil/facilities/emf/index.htm.

The successful applicant is expected to initiate and maintain an active research program that utilizes the facility while managing the day-to-day operation and maintenance of the facility. The Announcement opens March 15 and closes April 15, 2009.

Applicants are encouraged to visit the websites at https://chart.donhr.navy.mil and click on search for jobs, type in NE9-1310-03NRL0152-DE for the level 03 position or NE9-1310-04NRL0152-DE for the level 04 position and press enter to obtain qualification information and instructions on how to apply. Point of contact for this position is Carolyn Gilroy, 228-688-4660 or carolyn.gilroy@nrlssc.navy.mil.

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not the chiller has the cooling capacity for 2 instruments. A pump can be fairly easy to upgrade, but the BTU capacity of the chiller is fixed (and sometimes less than advertised). If you're anywhere near the max for the chiller, in terms of BTUs, either get a second chiller or get a larger chiller. It's not that unusual to run 2 systems off one chiller, but they must run in parallel with separate controls and the chiller must have enough BTU capacity. Ken Converse kenconverse@qualityimages.biz Tue Feb 10

Running the 2 in parallel is better since the cooling water will be at the same temperature for both systems. The flow rate through a DP should be set so that the water coming out should be slightly warm to the touch. I think ours run ~1 gal per minute. Most chillers have pumps that can supply a fairly large water flow. They then have a pressure valve which shunts the excess flow back into the tank (kind of like the fuel pump in your car). An inline flow meter is a really useful device. It is an immediate indication if you have any kind of blockage restricting flow (e.g. algae, corrosion, etc). I also put a 1 - 5um cartridge water filter in the waterline just before it enters the instrument. Hendrik O. Colijn colijn.1@osu.edu Tue Feb 10

EDS – Beryllium and copper

In response to a query on detecting beryllium with EDS or taking advantage of its low backscatter coefficient: Yes, beryllium rich phases should exhibit lower atomic number contrast via BSe. If you have a beryllium-copper alloy that has been precipitation hardened, the CuBe phase should be dispersed evenly throughout the matrix as numerous spherical precipitates. They should also appear gray in optical brightfield and full wave polarized light if I recall. If you are seeing isolated inclusions, they may be oxides, which will appear ruby red in full wave polarized light. Joseph M. Oparowski joseph_oparowski@bose.com Fri Jan 23

Most assuredly it is possible to detect Be with EDS - which obviously must have a UTW detector. Although we were using biological materials that are mostly carbon, it is absolutely no problem with higher Z materials such as Al. I haven't tried Cu but as long as there are no other overlapping peaks around 0.11 Kev it should not be a problem. One caveat: you do have to be careful to tweak the "threshold" for the pulse processor to minimize the potential detector "noise" that can creep in! Here is a reference of ours from a few years ago: Butnor KJ, Sporn TA, Ingram P, Gunasegaram S, Pinto JF, Roggli VL. Beryllium detection in human lung tissue using electron probe X-ray microanalysis, Mod Pathol. 2003; 16(11):1171-7 Peter Ingram p.ingram@voice.cellbio.duke.edu Fri Jan 23

It would be a big surprise to me to see beryllium in a bar, as it is normally used to precipitation harden copper for springs. Also, the precipitates would be expected to be very small and well dispersed, not in inclusions. John Mardinly a.mardinly@numonyx.com Fri Jan 23

EDX – Sn and Pb ratios

We were analyzing some samples of tin solder with varying but small amounts of lead. The solder was electroplated as a film (~7-10 μm) on copper. We need to know % of lead in the samples with reasonable accuracy. The amounts of Pb varied from about 0.5 to 3% based on information from another quantifying technique. We collected a spectrum for 120 sec using 30 kV (3000-5000 cps) in an area and then repeated this on the same area two other times under identical conditions and immediately following the previous spectrum. Each time the ratio of Sn to Pb varied significantly. We are at a loss to explain why there was such a difference in the ratios from the identical area. Could this be due to something going on due to the repeated sampling of the same area? Any idea as to why we had such poor reproducibility? Debby Sherman dsherman@purdue.edu Wed Feb 4

I had many years experience analyzing electroplated SnPb solders on Cu using table-top micro-xrf instruments (Seiko, Fischer, CMI, Thermo, MXRF units). It's obviously not quite the same as SEM/EDS in terms of excitation, but here are some ideas that may help: 1) Sn Ka and Pb La intensities vary with solder thickness AND composition. If your sample is not infinite with respect to these energies, you will get varying results depending on where you measure (or what angle). In your case, the layer is almost all Sn, so the film infinite thickness is about 50 μm or more with respect to Sn Ka. 2) If you're using Sn La and Pb Ma (or even Pb La to a lesser extent) then the film infinite thickness is much lower. I'm not sure what lines you are using, but you are accelerating at 30KV, enough to excite Sn Ka. 3) Solder is notoriously non-uniform in plated and hot-flow processes. I found in studies that the composition varied with depth as well. In addition, an intermetallic Sn-Cu layer begins forming at the Sn-Cu layer interface which can affect results, especially for thinner layers. Are you measuring EXACTLY the same area and getting drastically different results? 4) If it is just reproducibility, what are your net count rates for Sn and Pb? Peak/background? 5) In SnPb/Cu on circuit boards, the presence of Br in the FR4 / G10 substrates interfered with Pb La considerably and somewhat with Pb Ma. Consider the interference from Br. 6) How are you quantifying the composition and thickness? (They're related unless you're measuring an 'infinitely thick' layer and/or using the lower energy lines.) Don Kloos dkloos@parallaxray.com Wed Feb 4

What was the morphology of the analyzed area? Spikes of Sn will change after being whacked by a 30 KV beam. Why did you use 30KV? For all Ma peaks, 5-6KV out to do the job. You could double check this at 20KV for La peaks. The geometry of the specimen will make a difference and so will the effects of being scanned. Did the scanned areas show polymerization rectangles? If so, that pushes up H, C and O. So all other elements degrade in %. Of course not due to EDS non-detected H. I would suggest trying 6KV, then 10KV and finally 15KV. See what you get. Did you detect any Cu? Is the sample surely grounded? Is the probe current near the same from one collection to another? If not, why not? That will make a big difference. If I did not say so here, I have to build a spectra for special specimens that start at 3KV and wind up at 20KV. This also assumes that your specimen is at the EDS analytical WD. Can you supply a sample for off-site comparison? I'm working on Pb and Pb-free components (mostly ICs). The RHOS thing is somewhat nebulous. In my opinion, definitely not precise. Gary Gaugler gary@gaugler.com Wed Feb 4

Was there any drift to the SEM image during the analysis? My assumption is that there was a slight drift in the signal over the 120 second collection time, and you moved to a slightly different chemistry in the sample. The solder probably does not have a very homogenous microstructure, so your image shifted to a different microstructural feature. Check to make sure your sample is adequately grounded and maybe select a larger area to collect the spectrum from, so there is more of an averaging of all of the microstructural constituents. Most likely there are small globules of lead, so you're drifting over these regions during the analysis. BSE should show the segregations. Gerald Shulke gas19@chrysler.com Thu Feb 5