Edited by Thomas E. Phillips, Ph.D.
University of Missouri
phillipst@missouri.edu

Selected postings from the MSA Microscopy Listserver (listserver@msa.microscopy.com) from 08/15/06 to 10/15/07. Postings may have been edited to conserve space or for clarity.

SAMPLE PREPARATION – staining lipids for TEM

I am looking to visualize lipid droplets in C. elegans and keep them looking black. Has anyone tried methods as described by Boshier, 1984, in Stain Technology using 1% p-phenylenediamine in 70% ethanol during dehydration in order to prevent extraction and keep them black? Or another method I am interested in trying is Tannic Acid-p-phenylenediamine as described by Guyton and Klemp in The Journal of Histochemistry and Cytochemistry, 1988? I would like to keep them black and be able to see the mono or double membrane. These articles are fairly old, so I am wondering about the relevancy today. We have currently tried high pressure freezing with freeze substitution in 2% osmium/0.1% uranyl acetate/acetone. The results are varying colors of lipid droplets, we would like to be able to see the membranes more clearly, we are looking for the text book black lipid droplets. I will be adding 5% water to the next batch of freeze substituted samples, but this will not keep the lipids from being extracted by subsequent processing. Suggestions? Rhonda Allen <rra@stowers-institute.org> 29 Aug 2007

This reference might be of interest to you. I have tried the technique discussed in the paper, it is quite straightforward, you equilibrate the sample in an imidazole buffer and add osmium to the solution, I have really good lipid staining with the technique. Here is the reference. "Imidazole-buffered osmium tetroxide: an excellent stain for visualization of lipids in transmission electron microscopy" S. Angermuller and D. Fahimi (1982 Histochem J. 14(5):823-35. Neeraj V. Gohad <neerajg@clemson.edu> 30 Aug 2007

SAMPLE PREPARATION - staining polysaccharides for TEM

I have been asked to look at some samples containing a polysaccharide base and, as I have never looked at this before I was wondering about the best stain to use. I will probably try it unstained with EFTEM but would also like to compare that to a stained sample. I wondered if the usual suspects could be used (uranyl acetate, lead citrate and osmium tetroxide) but thought that maybe someone had had more success with another stain. Colin Veitch <colin.veitch@csiro.au> 25 Sep 2007

Include tannic acid in the fix, and UA or OsO_4 in the post-fix. Stain sections using KMnO4 followed by Pb (especially Sato's Pb), if you embed in Araldite or in some Epon formulation that excludes NMA (=MNA), which reacts badly with MnO4. Look up my list contributions for the past year or two for details. It stains agarose, and in quick-frozen freeze-substituted material, even preserves and strongly stains the kinky wormlike molecules of 500,000 MW dextran. Dextran washes out and away in non-frozen tissue fixed with aqueous tannic acid. Mike Reedy <mike.reedy@cellbio.duke.edu> 25 Sep 2007

Staining will depend on the form of the polysaccharide. One staining technique is based on the light microscope PAS reaction and has been usefully used on plant polysaccharides. It involves oxidation using periodic acid of the vic-glycols of the polysaccharide chain to aldehydes, linking these to thiocarbohydrazide (TCH) or thiosemicarbazide (TSC) and visualizing with silver proteinate. It relies on the C1-C4 linkage found in many polysaccharides but can be affected by side chains. Also crystalline cellulose is said not to be stained. Pre-existing aldehydes can be blocked using saturated dimedone in 1% acetic acid at 60°C. A method I have successfully used is Dimedone block (saturated in 1% acetic acid) 2h. Wash in distilled water 15 min. Periodate oxidation 25 min. Wash in distilled water 35 min. TSC 1% in 10% acetic acid 3.5 h. Acetic Acid 10% 15 min. Acetic Acid 10% 15 min. Acetic Acid 5% 5 min. Acetic Acid 1% 5 min. Distilled water 5 min. Distilled water 5 min. Silver Proteinate (fresh in distilled water) 30 min (in dark). Wash distilled water. More details can be found in a number of general works e.g. Lewis PR, Knight DP (1992) Cytochemical staining methods for electron microcopy. Vol 14, Practical methods in electron microscopy. Ed. AM Glauert. Elsevier. Ian Hallett <ihallett@hortresearch.co.nz> 25 Sep 2007

Another thing that might work is ruthenium red. It is described in an old paper by Luft, and I gather it goes for the carbonyl groups. Carol Heckman <heckman@bgnet.bgsu.edu> 26 Sep 2007

SAMPLE PREPARATION – Thermanox coverslips

We are using TEM to look at cells that are grown on Thermanox coverslips - however, when we try to section them for TEM the Thermanox pulls away from the epoxy and we are left with our cells sitting along the edge of the section, which is largely unstable in the TEM. As such, we have to remove the Thermanox and re-embed them before sectioning, which rather defeats the purpose of using Thermanox. Does anyone have any tips for preparing cells on Thermonox for TEM? Martin Saunders for Peta Clode <peta.clode@uwa.edu.au> 05 Sep 2007

I would be interested in feedback on this too. Meantime we are using Millipore filters to circumvent this problem. You have to avoid certain solvents, but infiltration with ethanol diluted epoxy resin is OK as long as you have extra 'pure resin' steps to remove trace ethanol before embedding, and use a flat bottomed resin/solvent resistant LM embedding mold to avoid handling the floppy filter. After polymerization with a thin layer of resin, the filter can be trimmed to fit into a conventional EM mold for sectioning on edge. Alastair McKinnon <a.d.mckinnon@abdn.ac.uk> 05 Sep 2007

What has worked for us recently (with Aclar and sapphire discs) is to peel the Aclar off post polymerization and add a second layer of the same resin to the block face where the cells are. This seems to work much better than leaving those cells hanging out there. What resin are you using? A second treatment is to be sure you are curing the resin under vacuum. The resin tends to stay very smooth against the Aclar (should for Thermanox as well) but if not under vacuum there tends to be some roughness which prevent decent sectioning. Regardless of what resin we have used the first method works pretty well. Garnet Martens <gmartens@interchange.ubc.ca> 05 Sep 2007

One method of doing this is to use the "pop-off" technique. Note that this also removes the cover slip, so if you need a cross-sectional view of the cells including the attachment area between the cells and coverslip, this method will not work for you. However, if your main problem is that the cells end up on the edge of the section and are therefore unstable for viewing, the pop-off method works just fine. Grow your cells normally on the coverslips and process them through your final pure resin infiltration stages. Then fill your embedding molds with resin and leave a small meniscus at the top. Put your coverslips, cell side down, on top of the meniscus on the embedding capsules, and polymerize. When the blocks are still somewhat incompletely polymerized, remove them from the oven and bring them to room temperature. Put some liquid nitrogen in a small, insulated container and either submerge the coverslip end of the block into the liquid nitrogen or hold it in the vapor phase. You may hear some crackling sounds, which is usually good. After a few seconds of freezing, remove the block from the LN_2 and try to peel off the coverslip. If it snaps right off, great! If not, try again. It may take a few tries, but when the coverslip comes off, the cells will remain in the resin and may be sectioned as a monolayer. Look at the block under a dissecting scope to identify areas with plentiful cells, trim away the excess (you may want to save the trimmed chunks as backups), and carefully set up your approach on the microtome. There is only a monolayer of cells, so you will need to start collecting sections as soon as they are big enough to pick up on grids. You will be sectioning from the side of the cell, which attached to the coverslip, up to the top of the cell. A couple cautions-sometimes (rarely) a block will shatter in the liquid nitrogen. If so, it will usually hang together and you can find pieces with cells and remount them on another block. Also, trimming the block

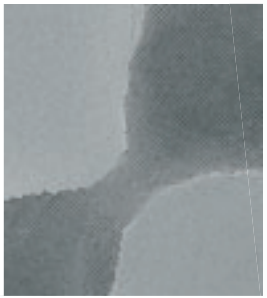
Conquering the sub Ångstrom era

Ultimate performance, stability, and flexibility—FEI's revolutionary Titan is the first and only platform designed to realize the full potential of corrected S/TEM imaging and analysis. With directly interpretable, sub Ångstrom image resolution you will, quite literally, see things you've never seen before. Investigate property/structure relationships with atomic resolution. Clearly visualize the interfaces, discontinuities and crystalline defects that are often the key to material properties. Analyze elemental composition, chemical bonding states, electronic band gaps, and more with unprecedented spatial resolution. If you know S/TEM, you know this truly is a revolution.

If your research requires ultimate performance, and your success depends on generating ground breaking new results, call us. We'll be happy to give you an unfair advantage.

See more at fei.com

Background image: High resolution STEM image of a grain boundary in gold at the atomic level. Courtesy of: C. Kisielowski, NCEM, UC Berkley. Image is coloured for artistic impression.



face and taking the initial sections need to be done carefully so you don't accidentally lose all your cells. Making extra blocks is good insurance, as always. Finally, Thermanox coverslips generally are much easier to use than glass, simply because glass likes to shatter when it is removed from the cold block face, leaving lots of little pieces that must be removed individually. That said, this is a very reliable and fairly easy technique. Randy Tindall <tindallr@missouri.edu> 05 Sep 2007

To support the edges of your sections, you can mount them on Formvar- coated grids. That will keep them from curling and melting under the electron beam. Dotty Sorenson <dsoren@umich.edu> 05 Sep 2007

Following on from Garnet's reply have at look at the paper: The axonal transmission of Herpes simplex virus to epidermal cells: a novel use of the freeze substitution technique applied to explant cultures retained on cover slips. Journal of Microscopy Vol 192, Pt 1 Oct 1998 (pages 69 -72) Although the method described is used with Thermanox and Lowicryl HM20 resin, the procedure works as well with epoxy resin and Lowicryl HM20 resin. I like the idea of using a light vacuum as suggested by Garnet, I haven't tried that. Allan Mitchell <allan.mitchell@stonebow.otago.ac.nz> 05 Sep 2007

This happens quite commonly when examining monolayers. Several things to try: 1. Use a supporting substrate (Formvar/carbon for example) to collect the sections. 2. Grow the cells on epoxy substrate (if they will attach). Then layer on the epoxy for the embedding and the cells should be sandwiched. John Bozzola <bozzola@siu.edu> 05 Sep 2007

SAMPLE PREPARATION - dewaxing leaf surface for SEM

I have a client who needs to remove wax from the surfaces of leaves so that she can count stomates in the FESEM. A first try at soaking the leaves in 2 changes of xylene, 10 minutes each, didn't do much at all. The leaves are highly papilose (is that a word? Have lots and lots of pappillae) and have lots of wax. Peels are not an option. Tina Carvalho <tina@pbrc.hawaii.edu> 05 Sep 2007

You could try sluicing the surface with chloroform, that sometimes works well. Sally Stowe <sally.stowe@anu.edu.au> 05 Sep 2007

When at a previous lab, I processed one batch of leaves for SEM using standard ethanol dehydration with CPD and got wonderful shots of waxy cuticle with stomata peeking through. A second batch was dehydrated in an acetone series, followed CPD in 100% ethanol and the surface was beautifully cleaned of wax. It worked for me. Randy Tindall <tindallr@missouri.edu> 05 Sep 2007

If the chloroform doesn't work, you might look into methods for making trichomes and papillae fall off. I think if you freeze the leaf in LN_2 you can shear them off. But this is just something I heard of for isolating the trichomes, not for looking at a trichome-free leaf. But the leaf stomata might be easier to see with the hairs gone? I am sorry I can't point you to a citation, but maybe the tentacles of Google can oblige? Tobias Baskin <baskin@bio.umass.edu> 05 Sep 2007 >

In addition to using acetone or chloroform to de-wax plant surfaces, as others have already mentioned, another solution to use is acidified DMP (2,2-dimethoxypropane). DMP is actually intended to be used as a dehydrating agent. Right after post-fixation water rinses, you can add DMP and it reacts with water to produce equal parts ethanol and acetone so it dehydrates by chemical conversion of water (endothermic) rather by physical diffusion of water out into a dehydration series. You can go direct into the critical point dryer (using CO) after 2nd change of acidified DMP (2x, 10-15' each). Usually add 1 drop (0.05 ml) of conc. HCl to 100 ml DMP. DMP has the side effect, or in this case the benefit, of de-waxing plant surfaces. The classic paper for DMP dehydration in EM is: Rapid Chemical Dehydration of Samples for Electron Microscopic Examinations. L.L. Muller and T.J. Jacks. The Journal of Histochemistry and Cytochemistry. Vol. 23, No. 2, pp.107-110, 1975. Other papers: 2,2-Dimethoxypropane, a rapid dehydrating agent for scanning electron microscopy. W.S. Johnson, G.R. Hooper, B.F. Holdaway, and H.P. Rasumssen. Micron, 1976, Vol. 7:305-306. Rapid Chemical Dehydration of Biologic Samples for Scanning Electron Microscopy using 2,2-Dimethoxypropane. Morton D. Maser and John J. Trimble, III. The Journal of Histochemistry and Cytochemistry. Vol. 25, No. 4, pp. 247-251. 1977. Optimization and Investigation of the Use of 2,2-Dimethoxypropane as a Dehydration Agent for Plant Tissues in Transmission Electron Microscopy. J.R. Thorpe and Diana M.R. Harvey. Journal of Ultrastructure Research, 68, 186-194 (1979). Hope this helps, or gives you another option to try for de-waxing plant surfaces or for dehydration in general. Gilbert Ahlstrand <ahlst007@umn.edu> 05 Sep 2007

SAMPLE PREPARATION - osmium tetroxide on Lowicryl sections

A colleague I know wants to know has anyone used osmium tetroxide on post-embedded immunogold labeled Lowicryl sections to make microtubules or other membranes stand out? Kenneth Dunner <kdunnerj@mdanderson.org> 23 Aug 2007

Our solution to the common poor section contrasting of fast frozen, freeze substituted, and low temperature HM20 Lowicryl embedded material was first presented on microtubule barrels of isolated centrosome: Histochemical Journal 27, 240-246 (1995). Treating Lowicryl HM20 sections like fresh tissue (= post sectioning fixation) can produce sections with contrast and morphological preservation equivalent to Epon sections using standard TEM stains. This technique was then applied to whole mammalian cells low temperature processed into HM20, resulting in "Epon-like' contrast of whole cells with well defined membranes and microtubules: Current Biology 5 (12): 1384-1393 (1995). The technique applied to isolated virus samples: Journal of Virology 73(3): 1931-1940 (1999). The technique has also been applied to low temperature processed yeast, bacteria, and plant cells with equally good results (no published images). "Post-Sectioning fixation" (after sections immunolabeled and in final water rinse) 1) Freshly prepared and filtered 2% tannic acid, pH 7.2 for 10 minutes. 2) Rinsed three times in 1.0% sodium sulphate, 5 seconds each. 3) Distilled water rinses, three times for 1 minute each. 4) 2% glutaraldehyde in water, 10 minutes. 5) Distilled water rinses, three times for 1 minute each. 6) 1.0% osmium tetroxide in water, 10 minutes. 7) Distilled water rinses, 4 times for 1 minute each. 8) 5% uranyl acetate in 50% ethanol for 10 minutes. 9) Distilled water rinses, three times for 1 minute each. 10) Repeat steps 1 - 3 = optional; only if you want to enhance microtubules to the maximum. 11) 5.0% lead citrate in water, 4 minutes. 12) Distilled water rinses, four times for 1 minute each. I have only used HM20 and so do not know if this will work with the polar Lowicryl K4M. Material lightly aldehyde fixed and processed using the Progressive Lowering Temperature method often benefits from the post-sectioning fixation for section contrast as well. Brent Gowen <bgowen@uvic.ca> 25 Aug 2007

SAMPLE PREPARATION – premature polymerization

I have two problems with using an automated tissue processor to throw out to the list for advice. For almost a year now we have been using an automated tissue processor for LM, SEM and TEM specimen processing and have been generally quite satisfied with the performance of the device. 1. However, we have had some problems getting consistent, good infiltration of epoxy resin into some tissues, both plant and animal. We use an epoxy resin, Embed-812, in a formulation which usually infiltrates samples completely when we do infiltration in vials on a rotator outside of the tissue processor or infiltrate in our microwave processor. In the tissue processor, samples are carried in plastic multi-well mesh baskets, stacked one atop another. Depending on well size, the mesh holes in the basket bottoms are 0.2-0.4 mm diameter, the rectangular slots in the sides average 0.5 x 4.6 mm. The viscosity of even this low viscosity epoxy resin seems to be high enough so that exchange from one resin change to the next through the pores is not good. We do agitate the stack of baskets in the resin at about 1 cycle per second, usually 1-4 hours per resin change. The resin (mixed in batches with accelerator added, stored in freezer) is at room temperature for about 20 hours during a run. When infiltration problems occur, they are sometimes severe enough so that no useable sections can be obtained. 2. The only time I used LR White acrylic resin

11 MEGAPIXELS

High Definition Digital TEM Cameras

AMT's HOT NEW LINE

TEM INTEGRATION

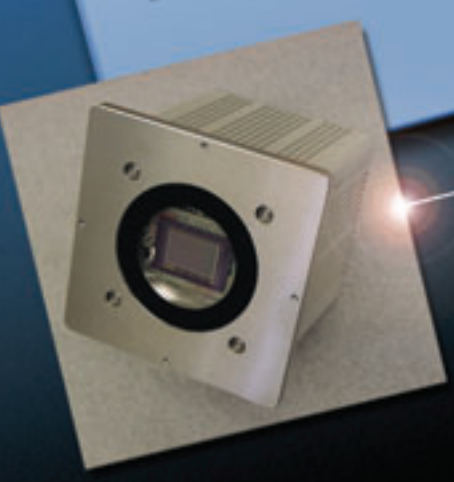
FASTEST DISPLAY

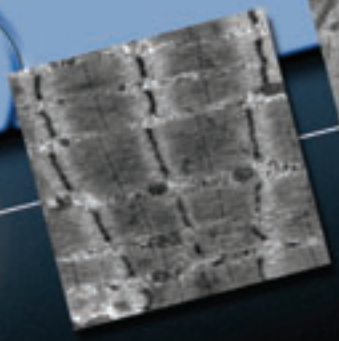
LARGEST FIELD OF VIEW

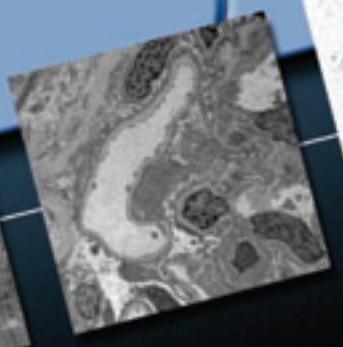
BUILT-IN RELIABILITY

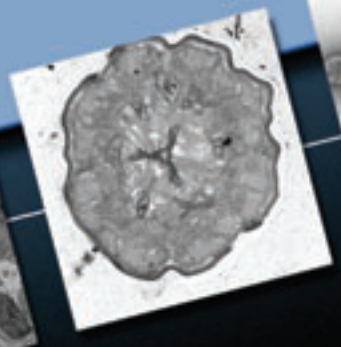
PROVEN SUPPORT

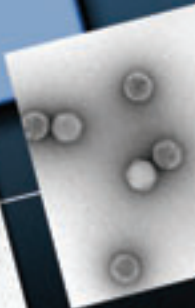
SIDE MOUNT & BOTTOM MOUNT











AMT

Advanced Microscopy Techniques Corporation
3 Electronics Avenue • Danvers, MA 01923
978-774-5550 • www.amtimaging.com

(very low viscosity) I believe the infiltration to have been good. The problem with that run was that in the 3rd and final change of LR White, the resin polymerized completely! The unit was operating at ambient temperature of about 24 C. The tissues (all pancreases) had been osmicated. Our mechanical shop sliced the baskets apart on a lathe, I dissected out the tissue blocks, and the run was saved. I duplicated the run just using a stack of empty specimen baskets and no polymerization occurred. Has anyone had these problems with tissue processors running resins and what solutions have you found to your satisfaction? We would appreciate getting some protocols that produce good infiltration of resins into plant and animal specimens. Gib Ahstrand <ahlst007@umn.edu> 25 Sep 2007

I can't address the automated processor problems, but I have had similar problems with LR White and osmicated tissue. In my case, the resin formed a cottage cheesy sort of texture, rather than a hard polymerization, but the run was ruined just the same. Perhaps if it had completely polymerized, we could have saved it, but the curdling happened during the infiltration stage with resin:ethanol mixes. Fortunately, we had more samples. My sense is that if the resin is near or past its expiration date the problem is worse. Other people have reported problems with osmium and LR White also. I did successfully embed osmicated tissue in LR White later, using very new resin. Coincidence? Randy Tindal <tindallr@missouri.edu> 25 Sep 2007

Sorry if I missed this part of the discussion already. We never try using LR white osmicated tissue because the osmium blocks UV light during polymerization resulting in a either a very brittle block, or an incomplete polymerization. If you have to use an acrylic resin, try using LR gold (I know, I know, a terrible EM resin) with the polymerizer that allows for rapid (minutes) polymerization at room temperature without UV light but polymerized on ice to even out the polymerization. You still have to work fast (about 30 minutes polymerization) but can do the infiltration without the accelerator added. This has worked for us in the past. Garnet Martens <gmartens@interchange.ubc.ca> 25 Sep 2007

This issue has cropped up in the past. Premature polymerization is not necessarily related to osmication of the tissue but is often related to older resin or resin that has had poor storage, a frequent problem was overheating during transport and also to some components in the tissue or extraneous media. Like many people we have for many years bought the uncatalysed resin and made up a bottle only when we need it. We have had no problem in the 12 or so years we have done this. Below is a quote from Roy Gillett of London Resin when we first raised the issue many years ago. "The reason this pre-polymerization occurs only with tissue must be something to do with a tissue constituent catalyzing polymerization. Older resin is much more susceptible to this than fresh monomer because of the significant polymer growth that will inevitably have occurred in the monomer. The most likely 'endogenous catalyst' from previous experience is likely to be an amine or peroxide moiety in the tissue." Ian Hallett <ihallett@hortresearch.co.nz. 25 Sep 2007

SAMPLE PREPARATION - Au on C and Al/W dendrites

I've spent quite a good deal of time Googling for some clues as to how one goes about making a Au on C (also sometimes called gold islands) resolution sample and also an Al/W dendrite sample (which I tried once and didn't seem to get what I was expecting). I couldn't come up with anything about the processes. Does one evaporate or sputter the Au? What type or grade of C is the best substrate? Does one cool the Al/W mixture quickly, slowly, or doesn't it make a difference? I'm going to play with these some, but sometimes it's nice to not have to reinvent the wheel. Ken Converse <kenconverse@qualityimages.biz> 20 Aug 2007

It has been a while since I made these, but I will try to remember. To make the best gold-on-carbon islands, you evaporate pure gold onto polished spectrographic-grade graphite, and then post-heat the sample to make the islands coalesce a bit. Takes a few tries to get both the amount of gold and the post heating right. Some of the ones I've seen also have a bit of evaporated tin on top of that; it makes little balls decorating the big balls and is better for a FESEM and high resolution testing. To make the Al-W phase, just put pure Al in a W basket and heat it up until the aluminum melts, then evaporates. Soon after that, the W basket will break at one of the arms, because the Al-W alloy formed is very brittle. The basket with the cooled Al in it will show the dendrites and a pretty Al-W phase. I'm not sure if cooling fast or slow matters, it cools pretty fast when the wire breaks. Mary Fletcher <maryflet@interchange.ubc.ca> 20 Aug 2007

SAMPLE PREPARATION – sputter coater glass chamber

I have a sputter coater that has a chip on the top edge of the glass chamber. I have heard of a procedure that can be used to epoxy the chip so it will not grow any larger and prevent losing vacuum. Does anyone know of the epoxy procedure? Or does anyone have any other ideas on how to fix the chip without replacing the entire glass chamber? Andrea Gusman <amgusman@ucdavis.edu> 27 Sep 2007

I have fixed several chips in my sputter coater glass chamber. I fill the chip with five-minute epoxy or other epoxy glue, making sure it is completely filled and a bit proud. When the epoxy is fully cured, I polish the whole top flat with our medium-to-fine (320 to 400 grit) SiC grinding wheel. Then it seals fine. I believe this was also written up in a previous Microscopy Today. Mary Fletcher <aryflet@interchange.ubc.ca>27 Sep 2007 Electron Microscopist

Any of the supply houses can sell you either the five minute epoxy or we also sell a bell jar repair epoxy. JD Arnott <d@laddresearch.com> 27 Sep 2007

Depending of the diameter of the bell jar, it's possible to grind it new again. A glassblower has done this a couple of time, once with a 4" and the other time with a 12" cylindrical bell jars. Jacques Faerber <jacques.faerber@ipcms.u-strasbg.fr> 28 Sep 2007

An alternative: If your sputter coater chamber volume is not too great, you might consider substituting a cylinder of Lexan polycarbonate for the glass one. This will be less likely to break if bumped or dropped, is inexpensive and easily replaced. Do not use Plexiglass, as its not as strong as Lexan. The Lexan one I have is 3" high, 4" inside diameter with 1/8" thick walls. At most colleges and universities, the scientific apparatus shops will likely have it, or you can purchase from vendors. Gilbert Ahlstrand <ahlst007@umn.edu> 28 Sep 2007

SAMPLE PREPARATION - sputter coater thickness

I am trying to determine how thick my sputter coater will coat at different time intervals so I am conducting an experiment. I made a small block out of Spurr's resin, sputter coated it, and then placed the block back in the mold and covered the gold layer with more Spurr's to sandwich it between resin. I then trimmed the block and cross sectioned it on a microtome to obtain thin sections. I placed the thin sections in the TEM and measured the gold layer. The time intervals I used were 30 seconds, 90 seconds, 1 min, and 2 min. Does anyone know how thick of a layer a sputter coater should coat with a gold target at those time intervals? Or what is a suitable thickness for SEM samples? Also does anyone have any other suggestions on ways to determine the thickness besides thin sectioning? Andrea Gusman <amgusman@ucdavis.edu> 03 Oct 2007

The way you are doing this is exactly what one should do for a check of the system in the conditions you use. The TEM measurement of the layer will be an accurate value to use. For given conditions this should be very repeatable. The thickness of the layer is dependent on many things including the design of the coater/electrodes. For a given unit the coating thickness will be directly proportional to the current and time; the current is affected by the kV applied, the gas (typically Argon), and the pressure of the gas. If you have a manual for the sputter coater it may give a formula that accounts for the design of the system and lets you plug in the parameters you can control (voltage and current, and time). Contact the manufacturer if you don't have a manual, or else get back to the list with the make/model of the machine and the parameters you are using and someone will probably be

https://doi.org/10.1017/S1551929500062015 Published online by Cambridge University Press

able to help. Note that sputtered layers much below 10 nm are likely to be discontinuous and in cross section it may appear as a discontinuous band. Dale A. Callaham<dac@research.umass.edu> 03 Oct 2007

Ballpark, the thickness is roughly 10 nm/minute in our Denton Desk II with gold or gold/palladium. But, this is strongly dependent on the gas pressure in the chamber, current, target (Au, Au/Pd, or something else) and target-sample distance, so that figure may not be relevant to your coater. The second question, how thick for samples, is entirely dependent on 1) what kind of samples (how conductive are they, what's the surface topography [smooth, setose, etc.], 2) what accelerating voltage do you need (low voltage, less coating maybe), 3) how are the samples mounted? better the electrical mounts, therefore path to ground, can allow for thinner coats 4) what resolution (what magnification) do you need? The higher the magnification/resolution the thinner the coat required (thick of looking at a lawn after a the first snowfall. If the snow is light, it coats all the grass blades, but you can still see each individual blade and its veins; if the snow is heavy, all you see is the snow. 5) Etc. So there's no simple answer to "how much?" It depends on "what questions?" Phil Oshel <oshel1pe@cmich.edu> 03 Oct 2007

Phil is exactly right. Sputtering is a very dynamic process that will coat your samples with varying amounts of conductive metal depending on the parameters described. For our purposes in SEM we overlook many of these variables and think we are "controlling" the process but we really are not. I only need to use the uneven wear of the sputter target as an example of the un-evenness inherent in the process. The only way to get something close to a "reproducible thickness" is by a FTM (Film Thickness Monitor) which ignores the dynamics of the process described by Phil but calculates the amount of metal deposited on a Quartz Crystal, sort of a "Micro-balance" method. This method is not without its own flaws but is as good as it gets for our purposes. It's much more complex than I will go into but leaping through some of the hoops you've been describing will only get you stressed and not provide any meaningful results in the end. If FTM is not an option, best to do a sequential coating of a specimen till it looks good (Contrast/Resolution with minimal charging) in your microscope, then use those parameters as the starting point for future work. Al Coritz <sampleprep@earthlink.net> 03 Oct 2007

A fast way to evaluate the layer thickness is to put the sample on an OM glass slide, and coat both together. After coating, one look at the color of the layer on the slide, by transparency in front of a sheet of paper (at daylight). Blue is in the 5-10 nm range, then green is up to 20 nm, green/yellowish is 20-30 nm, yellow more then 30 nm, and the transparency disappears (against the daylight) with more than 100 nm. At the same time, one can control the size and homogeneity of the deposit, versus current, distance to the target, and gas pressure. With the way you have used, combined with this one, you can calibrate the slides. The needed thickness is depending on many things: what kind of SEM you have (W source or FEG), what kind of sample you are looking at, what are the performances/quality of the coater (ultimate vacuum, quality of the magnetron head, is the power supply in current or voltage regulated, etc.). The "thickness" is a bit a misleading concept, in that matter. Most sputter coaters give on most samples gold grains, and not a layer (sugar powdered on the cake and not butter spread on the bread). What one needs is the smallest grains, separated by a distance short enough to allow the electron to tunnel from one to the next. And in most cases, one has big grains, separated by a too long distance. One must then let the grains grow in size (and thickness) until there is a contact between them. And then, the film is too thick, like a foot of snow on a ploughed field, instead of only a layer of frost. If you need to look at a tree in the field, it doesn't matter, but if you are searching the seeds between the groves, you must try something else. I would say: put the least gold necessary to have the picture you need, and try, in the same time, the lowest beam energy which gives a resolution adequate with the details you want to see. Jacques Faerber <jacques.faerber@ipcms.u-strasbg.fr> 03 Oct 2007

EM - Wi-Fi and RF interference

The Institution has decided to push wireless internet access into every corner of the campus. They also intend to boost the RF (cell phone) signal strength. I operate 3 TEMs, a LEO EFTEM, a cryo-Tecnai and an CM10. I worry that the TEMs will be affected by the signal frequency and strength from these new sources. I am being asked specific questions about which frequencies are harmful to our operation and am having trouble answering. If anyone has any experience or resources to help me communicate the scope of the problem it would be much appreciated. Bob Harris <bharris@uoguelph.ca> 05 Oct 2007

We did extensive testing on a lot of wireless devices, and the only interference we found was when a walkie-talkie was transmitting right next to the pre-amp of the EDX system, which smeared the EDX peaks badly. Aside from that, there was no effect on the electron beam (that was for a 2010F, but the SEM guys did similar testing and found no effect, so don't worry. John Mardinly <john.mardinly@intel.com> 06 Oct 2007

Your EM column and the electronics are encased in metal parts and cabinets that are grounded, I hope. A Faraday cage is a shield. Shielding is used to contain or keep out electric fields and RF. So your EM is a nearly perfect Faraday Shield, if properly grounded to the earth over a low resistance path to a driven earth ground. The comments from John and Alan state that they don't see the problem. I can see why. Here's a little paradox of sorts. How come a wireless RF transceiver right next to your PC is not interfering with your PC but it does with your grounded EM thirty feet away? The FCC and manufacturers are a few shielding steps ahead of us. Even the inside surface of a plastic PC case is metallized and grounded to reduce interference. Paul Beauregard <beauregа@westol.com> 08 Oct 2007

TEM – contamination

We have had problem in recent years with contamination on our TEM. As a consequence, we have to change objective aperture strip as frequent as every 4-5 months. We use liquid nitrogen daily. We clean film plates periodically. We minimize the time when we have to open the camera chamber to change films. But the problem persists. I suspect the reason we are getting more contamination than years ago when we only looked at resin embedded samples on scope is that we have had increasing number of users who look at nanoparticles. Unlike biological material, these nano particles may not inhere to support film as well therefore may "fly" away when beam hits them. This problem can be worse when particles are not in a single layer on support film. Also, users often do negative staining of these nanoparticles in order to see the coating. I assume too much PTA or UA dried onto the grids can contribute to the problem as well. We use our scope at 75 KV and usually use 10 or 20μm objective apertures for adequate contrast. This means there are only 1 or 2 apertures we can use in a standard objective aperture strip. Objective aperture strips are very expensive (about $1300/ea). Obviously we cannot use them as disposable items so often. My questions are: 1. Do you have many users looking at nanoparticles or negatively stained materials on your scope, If yes, how long do you think I should expect an objective aperture to last based on your experience? 2. Is there anything else we should do to minimize contamination and prolong the life of objective apertures? Hong Yi <hyi@emory.edu> 08 Oct 2007

We look at over 3000 negative stained samples for viral diagnostics every year. We have to scan at 20,000x, and we print at 200,000x. In my experience, both the moly and platinum apertures have very limited time of use between cleaning when you try to use them for any high magnification/high resolution work. This is especially true where the samples are negatively stained. With an average workload of 60 samples/week, we would be cleaning the apertures every 1-2 weeks. In short, I find them unsuitable. I would never work a microscope without a gold foil objective aperture. The high energy of the beam burns off contamination as it gathers on the aperture. Periodically (every 2-3 months) I focus the beam and run

https://doi.org/10.1017/S1551929500062015 Published online by Cambridge University Press

it around the edge of the aperture to clean it up. Don't know if that helps, but I do it any way. I keep both a 20 and 30 micron gold foil aperture in the scope, and usually use the 20. I replace the 20 micron aperture maybe once a year - it is usually more like every 18 months. I use the 30 when the 20 is getting a bit dirty and we haven't got any spare 20 apertures in stock. The major problem I have is keeping the specimen grid holder clean. We clean that about every 2 weeks. Every thing works well between annual cleanings with these protocols. Paul R. Hazelton <paul_hazelton@umanitoba.ca> 08 Oct 2007

I fully understand your problem and respect the ideas passed on by others, but have you tried running at one kV step higher. A higher kV will not "cook" your sample so quickly and will be far less sensitive to contamination on the apertures. I have many clients working in the biological field, particularly with virus work, who routinely use 100/120/125kV, give it a try? Two positive steps with just one change! Steve Chapman <protrain@emcourses.com> 09 Oct 2007

SEM - tilt correction

I have a periodic two-dimensional image that is tilted. I'm working with a Philips XL30 and tried to correct for this tilt through tilting the stage and using the tilt correction feature. Tilting the stage has no effect on the periodicity of the 2D image but using the tilt correction feature does. What exactly is the tilt correction feature doing here? Justin <jjb42@pitt.edu> 13 Sep 2007

The two methods do very different things. The tilt on the stage will physically tilt the stage so that you can make your sample exactly normal to the beam. I find that small tilts of the stage make very little difference to the image, because of the large depth of focus of the SEM. The "tilt correction" of the SEM electronics is a manipulation of the X and Y rasters to electronically compensate for a tilted stage. In the old days we often used the SEM with the stage tilted 45 degrees towards the SEM detector, to improve the signal. If measurements on a flat surface were important, the "tilt correction" could take a square grid and remove the foreshortening caused by the stage tilt. You can imagine what that would do to a sphere. My suggestion is to use the stage tilt to make the sample as normal to the beam as possible and avoid electronic tilt correction. Put a 2D calibration sample into the SEM with your sample to check the results. Mary Fletcher <maryflet@interchange.ubc.ca> 13 Sep 2007

"I find that small tilts of the stage make very little difference to the image, because of the large depth of focus of the SEM." This will depend on what is being imaged. "Tilt Correction" is ideally suited to planar/near planar specimens. Consider an integrated circuit composed of a series of perpendicular tracks. Image it untilted and measurements in X and Y should be the same scale - track widths and separation constant. Tilt the IC to a high angle, with tilt axis parallel to one set of tracks, and the apparent width of these tracks will decrease - whilst the others will remain constant regardless of angle of tilt. In effect the magnification differs in the 2 directions. "What exactly is the tilt correction feature doing here?" The "Tilt Correction" usually operates by cramming in more scanned lines along either X or Y - so that they are a constant distance apart on the specimen surface. Thus compensating for the change in magnification. Applying a tilt correction to an image of a flat specimen, tilted along an axis parallel to a scan direction, will produce a corrected image on which true measurements can be made despite the tilt and regardless of the orientation of the features being measured. "Tilting the stage has no effect on the periodicity of the 2D image but using the tilt correction feature does." However if its a more 3 dimensional object you are looking at things may be very different. To take another extreme case: spherical particles will appear round regardless of the angles at which they are viewed. They will still appear as spheres when tilted. Use the tilt correction on these and the image will be distorted rather than corrected. There are pictures of just this effect in some SEM text books - there must be images of this type on the web somewhere - anyone know where? So the effect and use of stage tilt and tilt correction both depend on what you are looking at - and what information you want. "In the old days we often used the SEM with the stage tilted 45 degrees towards the SEM detector, to improve the signal." So doesn't this help on newer SEM's ? A useful side effect of the distortion produced by specimen tilt is that the increased magnification in one direction allows surface irregularities to be seen more clearly in some types of specimens. <ab78@esc.cam.ac.uk>

SEM - wt% or atom%?

When I perform an EDX analysis in SEM (Oxford Instrument's INCA software), I obtain two values for each peak: weight % and atom%. I guess that weight % represents the integration of each peak area, whereas atom% represents weight%/atomic weight. Now the two values can significantly differ, and thus the element ratio of my samples can also be significantly different. And that is precisely what I want to know! Now, please allow me to ask some questions: - why is the integration of the peaks in the EDX spectrum called "weight %"? - Does the intensity if the peaks in EDX depend on the atomic weight? In other words, do heavier elements produce more x-rays than lighter elements? It is a hard for me to believe this, because electron shells are the same for light and heavy elements (a K shell is a K shell), however it is the only reason I can see to calculate an atom% value. - Which one of the two values to use, in which case and why? Stephane Nizets <nizets2@yahoo.com> 23 Aug 2007

The weight% and atom% are simply two ways of expressing compositions. The weight% is typically output because materials scientist will almost universally use weight% to express phase diagrams. The area under an X-ray peak is called the integrated peak intensity (after the background intensity is subtracted.) All of the X-ray microanalysis techniques for bulk samples have to account for atomic number, density, and fluorescence effects. These corrections correct for the production of X-rays as a function of depth (the phi-rho-z curve), absorption of X-rays as a function of depth, fluorescence as a function of depth. Now all of these corrections depend on the composition. The integrated peak intensities for the elements are used to iteratively calculate the compositions. These calculations in the various correction routines are easier to perform using the weight% values for concentrations. I suggest that you look at the Goldstein et al. book for SEM and Microanalysis. To calculate the atom% of Fe in FeO is easy. It is 1/(1+1) = 0.5 or 50 atom%. For Fe2O3 it is 2/(2+3)= 0.4 or 40%. To calculate the wt%, you need to use the atomic weights of the elements. For Fe in FeO, you have 1*55.85/(1*55.85+1*16.00) = 0.777 or 77.7 weight%. For Fe2O3 it is (2*55.85)/(2*55.85 + 3*16.00) = 0.699 or 69.9 weight%. Once the software finds the wt%, it uses a relatively simple algorithm to calculate the atom%. You should check any introductory materials science book having a chapter on phase diagrams to see how it is done. To answer your third question, they are equivalent. When you do an oxide, doing the oxide by ratio, it is easier to do it by atom%. Now the other question that you asked is again tied up in the stuff that I said above, but there is another thing that you should be aware of and that is the issue of fluorescence yield. There are two competing physical processes that can occur to relieve the excess energy in an atom when a core electron is ejected from that atom. The first is X-ray emission which you are familiar with. The second is Auger electron emission (After Pierre Auger). Auger electron emission is a two electron emission process. Let's take a K shell excitation example. Just as in the emission of a K-alpha X-ray, an electron from the L shell drops into the K shell, but instead of the excess energy coming off in the form of a an X-ray, the excess energy left is enough to ionize another L shell electron. This would be the emission of a KLL Auger electron. Auger electron spectroscopy if a surface analytical technique since the Auger electrons will loose an indeterminate amount of energy if it is emitted within the bulk of the sample and it is just added to the backscattered electron background. X-rays and Auger are competing processes. The X-ray fluorescence yield is the probability of an X-ray being generated when a core shell electron is ionized. The X-ray fluorescence yield is higher for heavier elements than lighter ones. The auger yield is more prevalent for light elements. This is just another natural physical thing that the analyst is fighting against

NETNOTES

when doing light element X-ray microanalysis. Scott D. Walck <walck@southbaytech.com> 25 Aug 2007

I'm in complete agreement with Scott. Also, you can learn a bit more from any textbooks of Modern Physics. The Answer to your question at the end is "Yes". The K shell of atom A is different of the K shell of atom B as far as their binding energy is concerned. Also given the fact of the existence of ONE vacancy in the K shell, the probability to generate X-ray photons is deferent from Z1 (element one) to Z2 (element two), so are the energy amount of any individual X-rays dictated by characteristic orbit energy differences. Please note, when you do quantification, you need to take into consideration of a factor called "ZAF", standing for "atomic number", "absorption", and "florescence". For example, a total integral of a lighter element A peak generally carries more weight than the same total integral of a corresponding heavier element B beak assuming the primary energy of the e-beam is sufficient. Wt% and at% are equivalent (please find that in any General Chemistry book.) Yes, reading some more books shall help! Chaoying Ni <cni@udel.edu> 25 Aug 2007

I meant given wt%'s of elements A, B, C., their at%'s can be calculated, or vice versa, of course, unless you would definitely prefer a specific stoichiometry for some elements among the A, B, C. Chaoying Ni <cni@udel.edu> 25 Aug 2007

It has been more than fifteen years since I taught this subject; however, if I remember correctly most programs for electron microbeam analysis report analysis results in weight fraction (i.e. grams unknown per gram total) rather than in either weight percent (gms unknown per 100 gms total) or atom percent (atoms of unknown per 100 atoms total). I believe the basic reason for this is that the fundamental equation for the number of atoms of the unknown element that are ionized by an incident electron, per centimeter the electron travels through the specimen, is given by an equation of the form: dn/dx = Q (N C d/A) Where dn/dx = the number of atoms of unknown atoms ionized per cm the electron travels in the specimen, Q is the ionization cross-section of the unknown atoms, N = Avogadro's number, C = the concentration of the unknown atoms U, d = the density of the sample, and A = the atomic umber of the unknown U. To make the dimensions come out right the quantity in parentheses has to have the dimensions of (atoms per cubic centimeter), and this requires the concentration of the unknown to be expressed in weight fraction, i.e.: {atoms U/At Wt U x gms U/gm total x gms total/ cc)/(gms U/ At Wt U) = atoms U/cc This requirement for the use of weight fraction in the fundamental equation for the ionization process then carries through to the calculation of intensities and through all the subsequent ZAF corrections (or other machinations) used to produce the final results. Some programs may contain subroutines to convert to other units of concentration, but I think fundamentally most yield results in weight fraction. You can find this matter discussed in more detail in various text books on the subject such as 'Scanning Electron Microscopy and X-Ray Microanalysis' by Goldstein, et. al. Wilbur C. Bigelow <bigelow@umich.edu> 28 Aug 2007

I think Michael's earlier explanation is a little confusing, especially the statement "EPMA... is more sensitive to mass% than atom%". In fact since the column density actually defines x-ray generation volume, Jim Bigelow is right, to calculate the number of ionizations, not only weight fraction, but atomic weight, density and number of atoms must all be included for a rigorous calculation of these electronic (atomic number) dependent interactions in solid materials. However, the fact remains that these atomic

https://doi.org/10.1017/S1551929500062015 Published online by Cambridge University Press

level interactions (fluorescence, absorption, energy loss, etc) at these keV energies are dominated by electronic (atomic number dependent) effects, not mass effects. X-ray excitation itself is essentially independent with respect to atomic mass (the EPMA technique is not sensitive to mass). Most atomic processes involving electron-solid interactions at energies below 100 keV are well correlated with atomic number (and electron energy) and only give the appearance (in compounds) of being more related to mass fraction than atomic fraction because A/Z is approximately a constant over the periodic table. And also involved is the historical fact that we have terms for mass (weight) fraction and atomic (number of atoms) fraction but no corresponding term related to "Z" (atomic number) fraction of compounds. If you do not agree, simply check any table of atomic properties and note (for example) that x-ray fluorescent yields increase monotonically with Z, even in the three places in the periodic table where atomic mass decreases and atomic number increases. EPMA calculations originally started with the mass based first approximation simply because it gave a more accurate first approximation than an atomic fraction first approximation (due to the aforementioned relative constancy of A/Z) and eventually also because most of the corrections (as Jim mentioned) to the intensity ratios are based on mass normalized (e.g., mass absorption coefficients) or mass unit (e.g., stopping power) calculations. In the case of electron backscatter loss, there is no theoretical physical basis for calculating average backscatter in compounds using mass fraction. Pure momentum exchange occurs in the case of a perfectly elastic interaction of an electron with a 180 degrees scattering angle. The largest effect is elastic scattering off a hydrogen nuclei (a 0.05% mass effect), and this effect decreases further with increasing atomic mass. And of course in most cases the scattering angle is not 180 degrees so the mass effect is even smaller. The funny thing is that early models using mass fraction for average backscatter in compounds not only did better than atomic fraction averaging (this isn't surprising for reasons already mentioned), but mass fraction models even did better than simple Z fraction averaging because the A/Z ratio is not quite constant across the periodic table (mass increases faster than Z of course). However, this entirely unrelated effect (due to stellar nucleosynthesis s and r-processes and nuclear stability properties) just so happens to push the averaging calculation in the proper direction to serendipitously account for effects of nuclear screening by inner orbital electrons in larger atomic number atoms (which is why the backscatter curve gets flatter at higher Z- because one measures fewer than expected backscattered electrons due to screening effects). To obtain accurate predictions of average backscatter using a Z based fraction, one must also include a term for nuclear screening by inner orbital electrons. More modern methods (e.g., Monte Carlo programs such as Penelope) using actual scattering cross sections implicitly take these considerations into account and are therefore even more accurate than any simple fractional based models. Gory details can be seen here: http://epmalab.uoregon.edu/UCB_EPMA/download/Compositional%20Averaging%20of%20Backscatter%20Intensities%20in%20Compounds%20(M&M,%202003(.pdf http://epmalab.uoregon.edu/UCB_EPMA/download/Compositional%20Averaging%20of%20Backscatter-%20Reed%20and%20Response%20to%20Reed%20(M&M,%202003).pdf. John Donovan <donovan@uoregon.edu> 28 Aug 2007

SEM - secondary electron detection

My Failure Analysis group is acquiring a new high resolution FE SEM and have investigated three well known leaders in SEM engineering and manufacture. I transported the same samples to each performance demonstration to assure accurate comparison and analysis of the equipment. One of the microscopes obtained a series of SEI micrographs with layers in oxide otherwise not present in the images from the other SEMs. Subsequently, the presence of these layers was confirmed in BEI mode. Why are the layers present in the data from one SEM and not the others? I know signal detection varies based on parameters and geometry of the microscope (solid/take-off angles, detector distance, working distance, etc.?), but does it vary enough in different instruments to this extreme? Unfortunately, I can't divulge any more information regarding the proprietary sample! Thank you, in advance, for your time! Regards, Marissa Libbee <mlibbee@gmail.com> 04 Oct 2007

In response to the questions asked regarding my inquiry: SEM: JEOL JSM-7500F. w-dist: 3 mm. Semi in-lens detector with r-filter. 1kV. Marissa Libbee <mlibbee@gmail.com> 04 Oct 2007 04 Oct 2007

In my experience it is possible to find an instrument that handles one particular sample different to other similar instruments from competitive manufacturers. kV, spot size, detector position(s), chamber accessories, but most importantly working distance all play their part. Some instruments are able in their upper detector (through the lens mode) to include a contribution obtained from converted backscatter which add BSE contrast to the image. This is usually achieved by adjusting the working distance allowing the BSE to strike components, generating the secondary's which are sucked up into the upper detector. In some instruments the difference between 3 mm WD and 6 mm WD can be quite staggering! Do not panic; it is probably real data you are picking up. This is one reason why I insist on clients taking critical specimens to all the appropriate manufacturers for demonstrations, you never know what you will find and it is found to be on some occasions instrument specific. Be happy you found this out. Steve Chapman <protrain@emcourses.com> 04 Oct 2007

EDX - SDD detectors

We are in the process of examining EDX systems for our newly acquired JEOL 2010F TEM. The microscope will be used mostly for materials/nano work where we anticipate high magnifications, small analysis spots, and correspondingly low count rates. Additionally, we expect some light element work on occasion. Up to now we have looked at "traditional" systems from the major manufacturers fitted with LN_2 cooled SiLi detectors. We are however, aware of the increasing presence of Peltier cooled Silicon Drift Detectors (SDD) supplied with EDX systems primarily from the SEM and XRF markets, although most of the comparable TEMs we have seen are equipped with SiLi detectors. It is our understanding that SDDs have recently improved in performance with advertised resolutions comparable to, or better than, SiLi detectors and have generally been known for having high throughputs. It would seem that having an LN_2 Dewar hanging on the microscope also introduces noise and vibration into the column. On the other hand, we have heard that SDDs have an efficiency fall off above 12kev and that they can be expensive to repair. What are the consequences, if any, of this fall off? Our naive assumption is that we would not see the K alpha peaks for the higher Z elements but the lower energy L and M peaks for these elements would still presumably show up. Further, should we have any concerns about possible radiation damage from high-energy back scattered electrons striking the detector in an SDD? In short, we would appreciate any comments or suggestions from the listserv in regards to these issues or any other issues we may have missed regarding the use of an SDD detector on a high resolution field emission TEM. Greg Strout <gstrout@ou.edu> 05 Sep 2007

SDD has certainly had major advances. There are 10^2mm, 20^2mm and 40^2mm detectors. There might be some 30 units. So far, my results are from SEM. But there is strong correlation with STEM. I am using EDAX 402 mm SDD...Apollo 40. The latest generation of SDD by EDAX is totally awesome. They replaced their Cryospec Clemenko cryocooler system with this new SDD. They have 102mm or 402 mm. I am using the Apollo 40. I can dump a huge amount of counts at it and still keep DT low. The trick is to keep DT and resolution in an area/zone where peaks are discernable. This means that if you want high cps, you will need shorter time constants. That said, resolution will degrade. If your Z list is wide, that is not a problem. If close Z values, one must suffer longer collection times and poor resolution between Z. I cannot imagine a SDD that has the same resolution at every time constant. You, as the operator, have to make a tradeoff between clock time and accuracy. SDD is the future, IMO. Si(LI) is history. Gary Gaugler <gary@gaugler.com> 05 Sep 2007

NETNOTES

I do not agree that "Si(Li) is history." TEM's are hampered by low count rates, especially if one desires to do more than simple spectroscopy and would like to do compositional line profiles and spectral imaging. There are at least two vendors I know of that offer 50 square mm detectors, and they offer tremendous advantages in count rates. Also, Si detectors are much thicker that drift detectors, which transmit (and do not detect) most X-ray's over 12 KeV. Elements with L series in the mid 10KeV range are difficult to deal with due to overlaps. Working with the K series in the 15-25KeV range offers significant advantage for those elements. The only barrier for some TEM's is these detectors may not fit, and for our 2010F, the anti-contamination device (inside the pole piece) had to be modified. The parts were expensive, but JEOL had the parts as a factory configuration and the service engineers were able to pull the gun, split the column, put everything back together, and start pumping in 7 hours, so don't be afraid of doing the modification. For us it was well worth it. John Mardinly <john.mardinly@intel.com> 05 Sep 2007

ELECTRON DIFFRACTION

When looking at a single crystal and obtaining a diffraction pattern (SAED) obviously the electron beam enters the crystal at some angles relative to the crystal's lattice. The d spacing changes as a result of tilting. From this one can determine the zone axis. Therefore one must tilt (single or double) to get a specific zone axis or even get a diffraction pattern that will yield d spacing for a zone axis. How then do people claim that the d spacing will always be the same regardless of the tilt of the sample or how the sample is positioned in the objective lens on the sample holder? Example, we were looking at a gunerite crystal and it was claimed that the d spacing was X and therefore this was enough evidence to conclude that the crystal was gunerite. I don't understand. Don't you have to find a zone axis by determine the d spacing and the interplaner angle then consult literature or a computer program to determine what zone axis you have and if the spacing matches then you might have that mineral? Jim Richards <jrichards@macslab.com> 25 Aug 2007

As you tilt a crystal, different lattice plane sets diffract. So, the d-spacings of lattice planes are 'fixed', it's just that at different crystal tilts, different d-spacings appear in the diffraction pattern. Having said that, unless you are very careful, identifying phases on the basis of simple selected area diffraction patterns is asking for trouble big time: 1. SAED can only reliably 'select' a known area down to regions of ~1 um. If you do SAED on smaller areas, aberrations in the imaging lens mean that higher order reflections come from regions different from the lower order reflections. So, SAED with multi-phase samples and small crystal will be asking for the wrong answer. 2. The calibration of any TEM camera length is only accurate to ~5%, unless you use an internal calibration with known d-spacings. 3. Taking into consideration the above, you should really do exactly as you say - find a zone axis, identify the axis and measure interplanar spacings/angles. Even so, you need to keep in mind that the accuracy of measurement is ~5%, so you then need to be able to exclude other possible phases with similar crystal structures and d-spacings. 4. EDS analysis is a big help - knowing the approximate composition allows you to exclude many possible phases quickly. 5. Personally, I would never use SAED - small probe diffraction methods are much better, allowing you to reliably do electron diffraction from small regions. If you use convergent beam electron diffraction, the pattern symmetries allow you to identify the crystallography very reliably. Larry Stoter <larry@cymru666.plus.com> 26 Aug 2007

https://doi.org/10.1017/S1551929500062015 Published online by Cambridge University Press

XRF in SEM-ED

Could you please share your experience/comments on usability of X-ray fluorescence as combined with SEM-EDS. The method seems promising and supplementary to EDS for analysis of heavy elements, but is not very popular, why? Leszek Kepinski <l.kepinski@int.pan.wroc.pl> 18 Sep 2007

I ran samples by EDS on TEMs and SEMs, and also separately by WD-XRF on a Rigaku spectrometer. If I was not sure of the EDS results on a trace element and I had enough sample, I would press it on boric acid with a WC polished insert and run it by WD-XRF to confirm the element was there. WD-XRF is more sensitive, IMO, but takes much more sample than a TEM. You might want to consider joining the XRF-L list and submitting your question to get a different point of view on WD-XRF versus EDS. I belong and it is free. It is fairly active but not as active as this list server. Here is the link. http://listserv.syr.edu/scripts/wa.exe?SUBED1=xrf-l&A=1 I guess I better defend EDS on this list. I cut thin sections of anti-reflective coatings on lenses. The limit of EDS detection is usually taken to be 1% on the volume of material being analyzed. I was able to detect a TEM invisible layer in an anti-reflective layer multistack system that was only 10 angstroms thick. I got a half inch high peak by EDS on this anti-reflective stack layer. This was the smallest feature I was ever able to detect by EDS. Paul Beauregard <beaurega@westol.com> 18 Sep 2007

There are some early instrument offerings of XRF in the SEM on the market, for elemental analysis. I have found these methods to be immature technology and a few years off from really being a useful tool. One would think XRF in the SEM would be a natural considering the success it has had as a stand-alone technique in a benchtop unit. But, the positioning of the detector relative to the sample is time-consuming, difficult to repeat with sample changes, and overall (currently) not conducive to the type of environment SEM's offer - multiple operators, general ease-of-use, and the quick, semi-quant chemical capability available from EDS. My vote is to stick with SEM/EDS for now - and wait out XRF in the SEM for a few years yet - there is a very good reason why only small companies are offering this technology and the big players, Oxford and EDAX, have not yet entered the game - the technology is not yet competitive and profitable. My solution, get a good benchtop XRF (Horiba, Rigaku, EDAX, or other) and compare the spectra via SLICE, which reports to compare XRF to EDS spectra fairly well. I don't have specific experience with comparing the two techniques with SLICE, but it's reportedly possible. Andy Fisher <andyfisher4@bellsouth.net> 19 Sep 2007

I first saw XRF on an SEM ~30 years ago. It was an interesting implementation, using a slide with something like 6 targets, selected by the user to optimize sensitivity for elements of interest in his particular application. It is one of those experimental techniques which seem to have a number of advantages yet is not attractive enough to make it commercially viable. A particular problem is that it combines well established techniques in a different way - this disrupts established 'political' territories. The XRF people do 'spectroscopy' and live in one lab, the SEM people do 'images' and work in another lab. When something comes along which crosses traditional boundaries, a lot of people get very uncomfortable and defensive, sometimes with justification but I do wonder whether lab politics is taking priority over scientific enquiry? Funding mechanisms also cause problems, with nobody being prepared to support techniques that they feel should be funded by somebody else. To get a 'new' instrumental method accepted, especially commercially, is both a huge investment and requires enormous luck. Benchtop SIMS and X-ray tomography seem to be struggling. Then there are He ion microscopes and nanoSIMs, neither of which have yet, in my opinion, made a major impact. On the other hand, STM and AFM exploded out of nowhere. The benchtop TEM/STEM appeared at an exhibition a few years ago... and disappeared. I've recently seen a proposal for a FE-SEM which would fit in your hand. Larry Stoter <larry@cymru666.plus.com> 19 Sep 2007

GENERAL TECHNIQUES - canned air

Have any of you run into the current bane of canned air that includes anti-inhaling ingredients? It is supposed to prevent "huffing." In so doing, the cans basically deny all use for what is normal for these. Like cleaning mirrors, specimen holders, cover slips, etc. The most recent is Falcon Dust Off. Useless. Are there normal, historical canned air products still available? Do these need a background check and a ten day waiting period to purchase? This is ridiculous. Gary Gaugler <gary@gaugler.com> 31 Aug 2007

Unfortunately, I can't help directly. Was not aware that the "dusters" are being corrupted. However, thanks for the heads-up. For the most part, I quit using them a number of years ago. I added a little plumbing in the lab and distribute compressed nitrogen to quick-disconnects scattered about the lab. Plug in the nozzle valve/hose and voila! I do regulate down the pressure a bit. Woody White <nwwhite@bwxt.com> 31 Aug 2007

The idea of nitrogen gas is good, but may run afoul of the safety people. About 20 years ago I convinced a new department chair that the cost and inconvenience of the air cans dictated a change. Safety people were very iffy about the use of nitrogen; they felt it presented a safety risk for oxygen deprivation from rooms it would be used in. They suggested using the building's compressed air system. All we did was put a filter in the line to remove oil and particulates - needed it for our Airfuge anyway. Now I have three plug-ins around the lab, a coiled line and a pistol with different nozzles that came from the local auto supply store. Works great, no fees for nitrogen, or tank rentals. Paul R. Hazelton <paul_hazelton@umanitoba.ca>31 Aug 2007

I know this isn't the perfect solution, but I have a 5-gal "portable air tank" (available from NAPA auto, and other sources for about $35) with added fittings for filling from a N_2 cylinder and the pistol-style fine tip blow-gun for dispensing. It has a built-in gage and valve. It should give good clean gas if compressed N_2 is all you ever put into it. Dale Callaham <dac@research.umass.edu> 04 Sep 2007

Along this same line of thought, does anyone know of any in-room sensor that can be purchased that emits an alarm if oxygen levels get low. I am thinking of something that works like either a smoke alarm or a carbon monoxide detector. We need to use ethane gas for our cryo procedures and the safety people are causing an uproar about that. These people tend to make a "one size fits all" type of regulation that often has little relevance to specific situations. If anyone has any specific product I would appreciate the info. Norm Olson <nholson@ucsd.edu> 04 Sep 2007

Back when we still had a darkroom with rotary doors, the safety people asked us to put in extra heavy duty hoses for the nitrogen guns that we used to blow off dust. We thought they were going overboard at first when they chose 12,000 psi rated brake hoses, but this was judged much cheaper than oxygen sensors, and when we thought about the scenario they were designed to prevent, we thought it was OK. What is the scenario? Imagine a hose fails in the middle of the night or weekend. The room fills with nitrogen, displacing all of the air. A person entering the darkroom through the rotary doors would be breathing 100% nitrogen, and would pass out after taking one breath. Nobody on the outside would see them. Anyone entering the darkroom would suffer the same fate. These would become fatalities. Basically, the consequences of a nitrogen leak were deemed so severe that 12,000 psi rated brake hose nitrogen lines seemed reasonable when used in darkroom with rotary doors. John Mardinly <john.mardinly@intel.com> 4 Sep 2007

We are a commercial testing laboratory and we use nitrogen lines (have a hook up coming from the plant supply) to Run some of our equipment and also to blow off samples in our metallography area. This works out a lot better for us due to the fact that the plant air lines sometimes get water in them. We are talking about getting quite a substantial stream of water out of an air line, instead of just air. Not good! The thing we have done for safety is to install oxygen sensors in the vicinity of these nitrogen supply lines. And these sensors are connected to very loud alarms and large red

https://doi.org/10.1017/S1551929500062015 Published online by Cambridge University Press

Microscopy AND Microanalysis

Table of Contents Preview

Volume 13, Number 6, December 2007

Special Issue: Atom Probe Tomography

Feature Articles

Book Review

Calendar of Meetings and Courses

Microscopy and Microanalysis website: http://www.journals.cambridge.org/jid_MAM

Indexed in Chemical Abstracts, Current Contents, BIOSIS, and MEDLINE (PubMed)

HUMOR

Dear Abbe

Dear Abbe,

I've heard a lot about the use of femtosecond pulsed X-rays for the imaging of molecular interactions, even getting down to subatomic resolution. What is your opinion of this? Will we ever have an X-ray microscope that can see atomic particles?

Wondering in Wichita

Dear Wonderbread,

Every time I think about using X-rays to image something I am reminded of that unfortunate 1957 incident involving Superman and the Piscataway All-Girls School in New Jersey, but let's not dredge up ugly memories and revive old lawsuits. X-rays were given their unique name because we really have no idea what they are. As my good friend Wilhelm Conrad Röntgen would have told you "Setzen Sie nicht die Hand Ihrer Frau innen dort!" X-rays are as unpredictable as a rabid ferret in your swim trunks. You use them at your own peril. In my opinion if you are really intent on looking closely at subatomic particles get yourself a good old fashioned super-collider and a Leitz Wetzler camera. Then sit quietly and wait for something interesting to happen.

Dear Abbe,

I am having trouble finding the right person to interface with. I've tried various commercial options and OTC aroma attractants. I'm looking for a similar model as myself—serious about science and Star Trek conventions. What can I do to increase my statistical probability of finding a compatible partner?

Lonely in Livermore

Dear Lonely,

Quit your whining! There are plenty of nerds out there with similar programming. You just need to use the right approach and say those sweet nothings that will get their Bessel functions modulating at high frequency. Fortunately for you, I was quite the lady's man and have compiled a variety of pick up lines from various sources.

-According to Heisenberg's Uncertainty Principle of QM, we may already be in love right now.
-Honey, you're sweeter than 3.14159
-I'm not being obtuse, but your acute guy.
-I wish I were a derivative so I could lay tangent to your curves.
-Your eyes have a perfect wavelength of 563.4 nm
-What's that great perfume? Vacuum grease?
-If I were an enzyme, I'd be DNA helicase so I could unzip your genes.
-You're more special than relativity.
-You must be a good benzene ring, because you are pleasantly aromatic.
-Your eyes are like limpid pools of primordial ooze — and I'm the amoeba who longs to swim in their depths.
-You're sweeter than glucose.
-You know, it's not the length of the vector that counts; it's how you apply the force.

It's a jungle out there. Herr Abbe has all the moves and can dispense advice like nobody's business. For answers to life, the universe, and everything, write to his personal assistant at jshields@cb.uga.edu

NETNOTES

flashing lights outside of the rooms where nitrogen supplies are present. The alarms are set to go off if it registers oxygen less than around 19% or 20%. The sensors are serviced on a regular basis. Kelly A. Ramos <kellyaramos@eaton.com> 04 Sep 2007

The MSDS can be found here: http://www.falconsafety.com/assets/falcon/msds/75-37-6.pdf The stuff is Ethane, 1,1-Difluoro so it is Difluoroethane rather than yours which is tetrafluoroethane. The label does not list the "bitterant" but warns about its presence. The product code of the propellant is 152a. The other propellant is 143a, which is probably what you have. Since the can does not state the propellant type, I suppose if it says "Contains a bitterant to help discourage inhalant abuse" it uses 152a. Without the warning, it is probably 143a. In any event, Falcon says that the bitterant model with 152a is for computers, electronics, optics, etc.exactly what we need. The alternate 143a is for flamability reduction applications. I'll get some cans from Office Depot and try both versions. I usually get the dusters from a camera store since they use the dusters to clean lenses and front coated mirrors. So that ought to be safe stuff to use for EM and LM. So, I guess that either type is not useless. Oh, Falcon makes a point of the cans NOT being canned air. Gary Gaugler <gary@gaugler.com> 04 Sep 2007

You can get small portable oxygen sensors which are useful also. However, they have a limited battery life, and they need to be recalibrated preferably annually at minimum, just like the permanently installed oxygen sensors, as outlined by Kelly. Rosemary Dr Rosemary White <rosemary.white@csiro.au> 04 Sep 2007