NETNOTES

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Selected postings from the Microscopy Listserver from February 16, 2009 to April 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.

SPECIMEN PREPARATION – waxy cuticles

I am working with a student who is investigating plant cuticles, a coating on the outermost layer of cells that consists of the polymer cutin and waxes. When examining this feature with TEM the waxes are often lost. From what she has read, this occurs mainly during the resin polymerization stage. The waxes typically melt at 52-56° C. Currently she is using Spurrs resin, which has been recommended in the literature for the type of plants (mosses) that she is examining. Possible approaches we are considering are: 1. Low temperature embedding using UV polymerization. We have equipment (Leica AFS) and experience with low temp embedding in Lowicryl HM-20 for immuno, but not for preserving waxes. 2. Microwave embedding in any resin. We have a Pella microwave with Coldspot, but haven't done much with plant tissue so far. 3. CryoSEM. We have a good FESEM, but do NOT have a cold stage, but would like to know whether anyone in this area (CT, MA, RI, NY) who does and would be able to help her with a one-time examination of her samples. If anyone has used these or other techniques for preserving waxy cuticles or a similar material, we would be very interested in hearing about your experience. Marie E. Cantino marie.cantino@uconn.edu Fri Mar 6

Try the paper below. I would use the SEM to look at an air dried sample first. My motto - always start with the easiest method! Sample preparation for scanning electron microscopy of plant surfaces - Horses for courses. A.K. Pathan, J. Bond, R.E. Gaskin (2008) Micron 39:1049-1061 david.patton@uwe.ac.uk Fri Mar 6

There are some additional possibilities: LR White can be UV polymerized; it does not even require the benzoyl peroxide to be mixed in (and that gives it years of shelf life at 4°C). A dual 4W "BLB" fluorescent unit a few inches above the samples is good; anything equivalent will work. You don't want it to polymerize too quickly so experiment. You can use aluminum weigh pans with a cover of Saran, Aclar, or Cellophane (good luck finding real cellophane today) film, or gelatin capsules - you need to exclude oxygen. Since you can embed from ethanol, the harsher acetone or propylene oxide can be avoided. Standard epoxy resins will also UV polymerize, similar conditions as above. Even with heated polymerization, it is accelerated by higher temps but even 50°C for longer times will work. Depending on the resolution required, the replication of the surfaces with dental impression materials has given excellent results from plant surfaces. See: A procedure for SEM of complex shoot structures applied to the inflorescence of snapdragon (Antirrhinum) P.B.Green and P.Linstead (1990) Protoplasma 158:33-38. Dale Callaham dac@research.umass.edu Fri Mar 6

I agree that UV polymerization can be used quite effectively with the acrylics and Vestopal, a polyester resin. I've not had much luck using UV to polymerize epoxy, however. Since UV does not penetrate very deeply into osmicated specimens (50-100 micrometer), specimens have to be really thin and you need to irradiate from as many sides as possible (or rotate the specimen). Even then... If you have a detailed protocol specifically for UV polymerization of epoxy resins, I would like to try it out. John J. Bozzola bozzola@siu.edu Fri Mar 6

We've only used UV polymerization in conjunction with epoxy for very thin samples - single cells, or thin sections being "re-embedded" after resin removal and immunolabeling or histochemistry – so osmium density was not a problem. Dale Callaham dac@research.umass.edu Fri Mar 6

I would freeze-dry or air-dry the leaves and look at them in SEM. If you can access to an environmental SEM you may look at fresh specimens also. Wax can cause separation of leaf tissue from adjacent resin. I believe that wax would not take up water soluble stain like uranyl acetate and lead citrate to be visible under TEM even if it is not dissolved during specimen preparation. Ann-Fook Yang ann-fook.yang@agr.gc.ca Fri Mar 6

SPECIMEN PREPARATION – TEM of fossil tooth

I have a researcher who will want to cut ultrathin sections of fossil tooth pieces (size ~ 3x5mm) for TEM observation. Since most of my TEM experience come from plant materials, I'm wondering if there are some special requirement/tricks for sample preparation (e.g. embedding), microtome (knife selection, cryo or RT) and post-staining etc. I Googled the internet but could not find much info/protocols to follow. Any suggestion and advice are greatly appreciated. Guosheng Liu gul417@mail.usask.ca Fri Feb 20

Use a FIB with lift-out. Especially if the fossils are valuable and you don't want to consume the tooth in making a TEM sample. John Mardinly a.mardinly@numonyx.com Fri Feb 20

SPECIMEN PREPARATION – retinas

I'm having problems with detached retina, not mine personally but samples I receive. When I embed 3 mm punch biopsies I have to bisect them. This causes the retina to detach from the back of the punch. I've tried simple agar sandwiches--agar in a plate, retina on top and then a drop of agar on top of the punch--they separate when bisected. They separate if you look at them wrong. If you can give suggestions as to how to keep my retina attached it would be great. I'd love to "see" how you keep them together. Paula Sicurello vapatpxs@yahoo.com Tue Mar 31

Are you sure they are not already detached by the biopsy procedure? I've dealt with many rat eyes, so I know what you are going through. They always split at the RPE/ORS interface. If you are fairly confident that they are intact when you get them, leave them whole through the processing....just drag it out terribly (30 minute dehydrations, many-stepped infiltration without accelerator, drawn out over 2-3 days before going into final resin) and then, when they are fully infiltrated, bisect them by cutting from the neural retina down through the sclera. You may still get detachments along the edge, but the middle should stay put. Lee Cohen-Gould lcgould@med.cornell.edu Tue Mar 31

SPECIMEN PREPARATION - SEM of fibrin clots

We have a student interested in looking at the morphologies (fiber arrangement and spacing) of fibrin clots treated with various stabilizing agents as a bulk sample, not as a thin layer. I have tried a few different techniques- CPD vs HMDS, ROTO, holding the clot between dialysis membranes in a cartridge arrangement (for the record this was a miserable failure),and processing the clot formed in an Eppendorf

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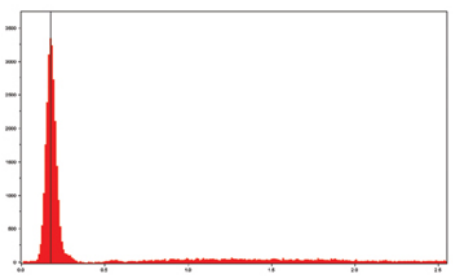

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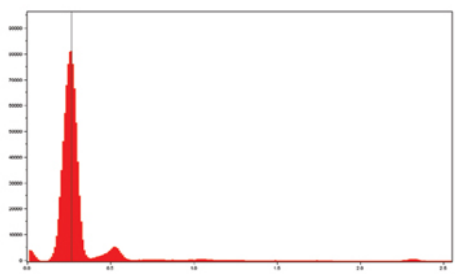

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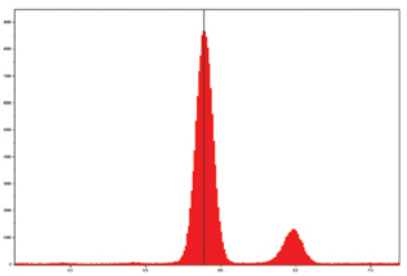

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tube- but continue to have issues with collapse of the clot. We've done a lit search but haven't been able to find a definitive technique for a bulk clot. It is important to note this is an SEM application, and that TEM micrographs are not what the student would like to present in his paper. I'm considering taking the clots to 100% ethanol and then freeze-drying, but my gut tells me the clots will still collapse. I am wondering if it is even possible to keep a bulk clot expanded. Should fibrin be thought of as a cable, without strength in compression? If so, how have people worked around this in the past? Derrick Horne dhorne@interchange.ubc.ca Tue Mar 24

During dehydration and drying the clot will collapse anyway. That is inevitable, unless you examine the specimen in wet conditions with ESEM, as people currently discuss in our listserver. In SEM, if the student wants to take measurements on the fibrin mesh he has to consider approximately 40% shrinkage of the specimen. To keep the mesh close to the original shape I think you have to attach the clot to a substrate that will also shrink -ideally some soft animal tissue- so it will not distort the mesh to any direction. Or, what about if you take out some red blood cells and leave more plasma to coagulate, dehydrate and CPD the bulk clot and then you look in SEM only at the periphery of the clot -maybe that will be fine. Yorgos Nikas eikonika@otenet.gr Wed Mar 25

SPECIMEN PREPARATION - liposomes

I need help in analyzing 200-300nm liposomes using TEM and SEM. What's the best way to prepare a suspension of liposomes for size analysis? Any suggestions/recommendations would be appreciated. David Osborn osborndc@umsl.edu Mon Mar 30

I have examined liposome suspensions by cryo-TEM in vitreous ice. You should keep in mind that in this, as other techniques, the liposomes are confined to a thin film. Flattening is very likely. This will most likely bias your size distribution measurements from projected area. In such cases we look at the apparent polydispersity and then use a complementary technique, such as dynamic light scattering to study the specimen. John Minter jrminter@rochester.rr.com Mon Mar 30

We have prepared for SEM liposomes and other nanoparticles by placing them on a filter paper. Please have a look at this publication: Scanning electron microscopy study on nanoemulsions and solid lipid nanoparticles containing high amounts of ceramides. Hatziantoniou S, Deli G, Nikas Y, Demetzos C, Papaioannou GT. Micron. 2007;38(8):819-23. Epub 2007 Jul 3. Yorgos Nikas eikonika@otenet.gr Tue Mar 31

TEM - Oval beam

I've got an oval beam as I started my TEM and it gets a line shape as I increase the intensity what seems to be the problem? I've tried to adjust the astigmatism, but didn't work out. Ahmad Ashkhaibi ahmad_ds@yahoo.com Tue Apr 7

You haven't given much detail so it could be several things. For instance have you just changed the filament recently or done any other work in the electron gun or condenser area? What happens if you adjust focus of the condenser lens from underfocus to overfocus - does the direction of the oval shape change? Have you tried checking the complete alignment of the condenser system eg gun tilt, gun shift, movable condenser alignment? Finally I apologize for asking but you say you have adjusted the astigmatism - I assume you mean the condenser astigmatism? If this has happened after a filament change then it could be a badly positioned filament or defective one. It could even be movement of the gun or condenser aperture. Other possibilities might include some form of wobbler or scan system inadvertently switched on. It might be useful to know what TEM you are using, as well. Malcolm Haswell malcolm.haswell@sunderland.ac.uk Tue Apr 7

What you are describing is known as Condenser Astigmatism. On your microscope there will be at least two devices for correcting the astigmatism, one for the condenser system, one for the objective system and possibly one for the intermediate system. I feel that when you say you have tried adjusting the astigmatism you have used the objective controls not the condenser. Try the following 1. With the beam on, adjust the second condenser (illumination or brightness on some instruments) to cross over, the smallest beam spot. 2. Increase the magnification to make the spot about 2 to 3cms across. 3. Decrease the filament heating until the beam breaks up into a spot and halo formation. 4. Adjust the illumination to focus this image as sharp as you can. 5. Adjust each condenser stigmator in turn until the spot and halo image is at its sharpest. 6. Repeat 4 and 5 until you have no improvement. 7. Heat the filament to the level you require for your tasks. Steve Chapman protrain@emcourses.com Tue Apr 7

SEM – oil shale samples

I was wondering if any of the mineralogist on this list have developed a sample handling and SEM examination protocol for oil shale rock samples. I've only just received an inquery regarding the possibility, but I have never handled such a sample. Our SEM is capable of environmental chamber pressures (FEI Quanta), but the work will require BEI imaging and EDX spectra. I imagine the vapor pressure from these types of samples can vary from nil to extremes - how would one determine before possibly contaminating the column if any particular sample was going to cause problems? Michael Shaffer michael@shaffer.net Mon Dec 1

I can't say I have a protocol per se, but I think you should be able to examine the shale without equipment worries. We purchased a VP-SEM years ago for use with concrete. We have used it with all manner of other materials including oily samples. I should probably point out that we do most of our work with BSE since an SE detector was not available for our Hitachi SEM. The situation might be different for the SE signal. We do probably 90% of our work in VP-mode since we routinely encounter insulating samples. We use 40-100 Pa of helium as our residual gas to bleed away charge. The helium scatters much less than air or nitrogen at the same pressure. We often sweep the pressure over a range to determine the minimum pressure required to eliminate charging. Since we are operating at a considerable pressure, we find that hydrocarbons are swept from the system. We have very little trouble with pump oil accumulating on the EDS window. By contrast, we need to clean the detector window on our other SEM (a conventional, high-vacuum scope) every 6 months or so as we see oil accumulating on the detector snout. My biggest concern would be with the vacuum "pulling" the oil to the surface of the sample. We see such an effect with embedded and polished samples where polishing oil finds its way between the sample and embedding medium. The vacuum pulls it to the surface and the oil runs over the neighboring material. I suppose

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that could happen with your samples. Maybe a higher pressure would minimize the problem. Maybe you could find areas less affected. Bottom line: I wouldn't hesitate to try it. Warren Straszheim wesaia@iastate.edu Mon Dec 1

SEM- magnetic materials

Electroplated nickel is ferromagnetic but, as far as I know, has no permanent magnetic field at room temperature. When examined in a high resolution SEM at 50-100 kX, is there any concern with this type of sample? Don Chernoff donc@asmicro.com Sat Mar 7

Put a magnet towards the sample. If north or south attracts the sample, it is magnetic. If so, using a magnetic immersion lens SEM is likely going to be an issue. Depending on the mag and magnetic characteristics of the sample and WD, either nothing will happen or the column could be warped...bent. Not a good scenario. For FEI/Philips SFEGs, use EDS mode. Poor resolution, poor S/N but the final lens magnetic field is off. For electroplated metals, it seems to me that a couple of KX ought to be enough. If not, use a LEO/Zeiss FESEM, in which case the sample characteristics are irrelevant. Gary Gaugler gary@gaugler.com Sat Mar 7

SEM – catholuminescence

What's the cheapest way of getting into catholuminescence (CL) of quartz? Can CL be put onto a benchtop SEM? cheers Ritchie Sims r.sims@auckland.ac.nz Wed Mar 18

Meant to get back to you sooner on this. The cheapest way to get to CL is to simply remove the Faraday Cage and scintillator from the secondary detector. That is your basic CL detector (monochrome). A dedicated CL detector generally has a larger diameter light pipe, but is still basically the back end of an E-T detector. A color CL detector is a whole other can of worms and I don't know of a "cheap" way there. I don't see why a CL detector couldn't be put on a desktop SEM, if there is a suitable port available and it can handle additional video inputs. Ken Converse kenconverse@qualityimages.biz Tue Mar 24

EDX – broken detector window

I understand that once a 'window' on an EDX detector is broken there is no way to repair it and the crystal is destroyed. Is this true and does this apply to all types of detectors? Margo Gill-Linscott analytic@rawbw.com Tue Feb 24

If the window is fractured, then you are out of luck. This means that you need to get the detector repaired. This is not an insignificant cost. But it makes the difference working and not working. Why did the window fail? This usually happens if the chamber is vented too quickly or for some other actions that are detrimental to the window. The "standard" windows are MoxD and are .3u thick. These are classified as SUTW (Super Ultra Thin Window) and are sourced from UT. I would strongly suggest that you look into why the window/film failed. You must not repeat this scenario. Gary Gaugler gary@gaugler.com Tue Feb 24

The EDS cannot operate with a broken light element window and the EDS will have to be sent back for repairs to the original vendor or a detector repair company. The latter is usually less expensive. This might run from $4K - 10K. Don Kloos dkloos@parallaxray.com Wed Feb 25

I believe that the "common knowledge" is not always correct. My understanding is that the SiLi crystal is usually destroyed by having the bias applied when warm. One of the ways to avoid that is to always turn the bias off before unplugging the detector. The bias supply will drain the voltage off, whereas unplugging the bias will leave a small, but very efficient, capacitor charged to the bias voltage for, perhaps, longer than it takes the detector to warm up. The broken window may actually be your only problem, although still expensive to repair, in part because the window is expensive and the Dewar must be re-pumped and leak-checked. Ken Converse kenconverse@qualityimages.biz Wed Feb 25

If a window on a detector blows, it depends upon how it blew will determine if the crystal is damaged or not. If the detector never sees atmosphere and is undamaged, it can run with the broken window without a problem. D Jones dljones@bestweb.net Wed Feb 25

I may have to stand correctable here but my experience says that the bias will automatically shut off if the FET and detector crystal are not cold enough. When the window breaks, that ought to cause the -750 V to shut off. The detector is a reverse biased drifted Silicon diode and the reverse bias creates a big depletion region. The output from the detector crystal is fed to a FET amplifier transistor which is also cooled to reduce noise. Its signal is then sent up stream to room temperature electronics and on to the pulse processor in the PC. Since the detector probe is under vacuum, venting the SEM chamber too fast or pulling open the door before full venting will likely crack the EDS window. AFIK, all makers use Moxtec windows, mostly the 0.3 μ thick SUTW. The detectors with no windows are a separate subject. Gary Gaugler gary@gaugler.com Wed Feb 25

I would suppose the new detectors might have a circuit to shut off HV, but I can certainly say that many old detectors did not. We have such a one still in service. We lost one crystal because it warmed up under improper conditions. I suppose it would be the pressure pulse that would rupture the window. We have not had one fail due to anyone slamming the SEM chamber closed or pulling it open - yet. I don't care to run the experiment on my nickel. Maybe the guys at Moxtek have run those experiments as part of their R&D. Warren Straszheim wesaia@iastate.edu Wed Feb 25

EDX- mothball liquid nitrogen chilled detector

Suppose someone wants to mothball a liquid nitrogen (LN2) chilled detector for a period of time. Is this feasible without significant damage? What precautions should be taken (bias off and voltage drained) prior to warming up, etc? What are the negative aspects of doing this (loss of resolution, presumably)? John J. Bozzola bozzola@siu.edu Wed Feb 25

I did this over a 1-month vacation period once, having been assured by the manufacturer that it was perfectly OK. The vacuum and resolution both deteriorated to the point of being unusable for quantitative work. After this had happened, and on further rather annoyed questioning, the manufacturer said that some deterioration in performance was to be expected. I chose to replace it, with one from a different maker! I will never, never do this again unless it is 100% unavoidable. Ritchie Sims r.sims@auckland.ac.nz Wed Feb 25

Hopefully others with more expertise in this area will chime in because my information is old. My understanding is that when Kevex brought out their windowless detector (too many years ago), what they found was that warming, in and of itself, was not a problem as long as the bias was gone. The big problem was condensable contaminants that would be captured by the zeolites in the Dewar,

released upon warming and then contaminating the SiLi crystal. They were perfectly willing to ship those detectors warm, but the appendage pump had a battery operated controller that shipped with it. Basically they were far more concerned about the quality if the vacuum than the temperature of the crystal. We've all heard lots of stories about how one person's detector warmed up once and was trashed, while others repeatedly warm their detectors and keep right on trucking. What I take from Kevex's experience is that it's kind of a crap shoot and depends upon what is in the vacuum part of the Dewar, whether from manufacturing residues or from leaking and what condensables might have entered through the leak(s). The other slight risk with warming is that if you've had a bad leak for a while, when the zeolites warm up, all the gasses they've captured are going to be released, possibly going to considerable positive pressure and blowing the window. It's probably not a concern unless your lN2 consumption is very high, indicating a poor vacuum in the Dewar, but thin windows make it more of a concern than Be windows. Is it PGT that made the LEAP detector? The Dewar looks nothing like your standard Dewar, doesn't hold much lN2, and functions for years going warm and cold, warm and cold. It may be that they got rid of the zeolites (hence the sorption pumping) to eliminate the possibility of contaminating the crystal upon warming. Ken Converse kenconverse@qualityimages.biz Wed Feb 25

Yes, that was what I concluded, the vacuum is everything, and in a windowed detector, warming up can allow gases to be desorbed from the zeolites (or whatever "getter" is used). If the vacuum deteriorates, the internals run a bit warmer because the thermal insulation is compromised. JEOL had for a while an integrated EDS detector which could be warmed and re-cooled repeatedly because it used the SEM vacuum to re-evacuate the detector, as I understood it. Seemed like a pretty good idea to me but I don't know if it was good in practice or if they still offer it. Is that what that LEAP detector was? Ritchie Sims r.sims@auckland.ac.nz Wed Feb 25

I have three EDS systems in my lab, and several times I did let them go to room temperature, each time unplugging them completely. No damage at all. Vladimir M. Dusevich dusevichv@umkc.edu Wed Feb 25

I second Vladimir's experience. I had used an EDAX Sapphire Dewar 204 detector and let it sit at room temperature for weeks. No problem. SafeFill was turned on when the detector was needed--it loads the LN2. Again, no problem. Nowadays, the EDAX Apollo 40 SDD makes a huge difference. I figure that all or most SDD maker's SDD systems will be vastly superior to legacy Si(Li) EDS detectors. There are argumentative issues with SDD specs but the fact remains, IMO, that SDD will kill Si(Li) and LN2 systems over time. The current generation of SDD is too phenomenal to dismiss. Money is always an issue. ROI and up-time are also factors. The SDD in and of itself was evolutionary and now it appears to me to be revolutionary with what I think is the third generation of SDD detector chips. The differences from Si(Li) are stunning! As

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a side note, the EDAX Apollo 40 has a small ion pump to maintain vacuum. This is very nice. I had suggested this some time ago for the Si(Li) flavors of detectors. I have no idea if they did this based on my suggestion. But they did do it. Thus, the getters issue is gone. This was an issue with non-LN2 detectors...old history. Gary Gaugler gary@gaugler.com Wed Feb 25

The detector Ken referred to is the Compact Detector Unit (CDU) made by EDAX. http://www.edax.com/products/sku.cfm?ProductCAtegory_Id=4247&Product_Id=1009&SKU_Id=1037 As with all EDAX's modern SiLi detectors, it was designed to automatically turn itself off when the LN2 was gone and the unit started warming up. The CDU can also cool down and stabilize very quickly, making it practical to leave at room temperature when not in use. Bottom line: things are going to vary according to detector design and manufacturer. Your best bet is going to be contacting the company which made the detector and ask them. Jeff Gschwend jgschwen@rcn.com Sat Feb 28

You are right on. The EDAX units automatically shut off if the detector tip temperature is not at spec. This prompts a call to their service folks. They are Johnny on the spot. From my experience, letting EDAX or legacy Rontec (UHV) detectors go dry for extended periods of time makes no difference. However, the Rontec UHV units have wimpy Dewars. So, that is the way that they are. But they work. They got bought up by some other company...nothing new about this. So you are right about contacting and getting credible responses (problematic) from the company. For new procurements, a list of specific requirements ought to be very helpful. Dr. Gary Gaugler gary@gaugler.com Sat Feb 28

Convergent-beam electron diffraction – thickness measurement

Recently I have started to work on convergent beam electron diffraction. So I have very basic questions. Please help me in gaining knowledge in the related field. My first goal is to determine the thickness of MAGICAL sample using convergent-beam electron diffraction (CBED). Here are my problems. 1. When I was taking a CBED pattern at lower symmetry, I was unable to find any K-M fringes on central spot <000>. I have varied camera length from 30 cm to 300 cm and also changed the exposure time from 0.1 sec to 30 sec. I was neither able to find any fringes on the central spot nor was able to find spot next to it. Basically to find thickness, we mainly require two spots next to each other. One is central spot and other one is <220> or <200> of CBED pattern. I was unable to get these two spots next to each other. Please tell me what are the variables need to be changed in JEOL 2011 TEM so that we get a pattern where spots are nearby to each other. 2. According to Williams and Carter text book, to get KM fringes, angle of convergence (2Î±) should be less than Bragg's angle (2theta). Can you specify how can we change angle of convergence and Bragg's angle in a JEOL 2011? If I'm correct, the angle of convergence is alpha selector and Bragg's angle is magnification toggle of JEOL 2011. If I'm wrong, please correct me. 3. Right now I'm using accelerating voltage of 200 kV. Does it have any impact on CBED patterns once I change it to 100 kV or 150 kV? Vishnu Mogili vishnu.mogili@gmail.com Wed Mar 18

1. If you are unable to see K-M fringes in your CBED disks, it may be that your specimen is too thin. The number of fringes increases with thickness and where your thickness is less than about half an extinction distance (for the reflection you are using Si(220) E=96 nm at 200 kV - so <50 nm and you may get nothing. Similarly if your specimen is exceedingly thick and you aren't using an energy filter, you might find the fringes are wiped out. Also if you have a large variation in thickness in the region probed, the fringes will blur into each other. Choose a very flat region of specimen. Check out J. Microsc 224 (2006) 187-196, where I describe some thickness by CBED experiments with silicon and P91 on a JEOL 2010. This work describes use of Vincent Hou's excellent DigitalMicrograph script for carrying out the thickness calculation. If you are capturing images using DigitalMicrograph, you can install this script - it makes the calculation a breeze - get it (Thickness by CBED) from the DigitalMicrograph Script Database (URL at the bottom of this message). Thickness determination is achieved by setting up two beam conditions - the (000) transmitted spot and another diffracted beam are intense - not two diffracted beams as your post suggests (to me). The choice of which beam to use isn't too important, but since the extinction distance varies with the reflection, then the minimum thickness you can measure is determined by your choice of diffracted beam (for the reasons mentioned in 1). It's best to use low index reflections - high index reflection have longer extinction distances. You only need to measure the fringe spacing within the diffracted beam for the thickness calculation. However, in order to convert this distance measurement into an angle, you need the transmitted beam present, since the distance from the edge of the transmitted beam to the edge of the diffracted beam corresponds to the angle 2theta (Bragg equation) which gives you the distance to angle calibration. To minimize measurement error capture the patterns at a camera length such that the spots span a large proportion of the screen. 2. Set up your CBED conditions so that the transmitted (000) and diffracted beams are large enough to almost touch. The larger they are the smaller the measurement error will be. However, if they overlap, you may find making the disk edge to disk edge measurement difficult. Experiment with both the alpha control on your JEOL and also the condenser aperture to understand how these affect your CBED pattern. 3. Changing the microscope voltage will change your wavelength, and since wavelength appears in the Bragg equation (nL=2dsin(theta)), your Bragg angle (half the disk edge to disk edge) distance will change (your patterns are bigger at lower voltage for a given camera length). Also, the extinction distance - which appears in the thickness equation will change. However, from a practical perspective the measurement you make will be correct at any voltage, provided you measure the fringe spacing and disk edge to disk edge distance correctly and you supply the correct extinction distance (ie don't use the 200kV value if you are working at lower voltage). Finally the CBED method is accurate but time consuming. If you have an energy filter, thickness mapping is much easier to do. However, you do need a good value for the mean free path in order to convert your map into true thickness (see the earlier reference on how to measure the mean free path). There is also a DigitalMicrograph script which will help you estimate the mean free path (Mean Free Path Estimator). It is described in the reference I gave you, and today I have posted a much improved version of it to DM Script Database (http://www.felmi-zfe.tugraz.at/dm_scripts/welcome.html) - it may take a week or so to appear. Dave Mitchell david.mitchell@emu.usyd.edu.au Wed Mar 18

3M Harry Heltzer Multidisciplinary Chair in Science and Technology

University of Minnesota, Minneapolis, MN USA

The Graduate School and the Institute of Technology at the University of Minnesota—Twin Cities invites applications and nominations for the position of 3M Harry Heltzer Multidisciplinary Chair in Science and Technology. This is a tenured and endowed position at the rank of associate or full professor (dependent upon qualifications and experience) in the area of physical and biological structures characterization using microscopy and imaging. Candidates must have outstanding academic and research records, with several years of successful research and teaching experience. A Ph.D. degree and dedication to teaching, graduate student advising, and regular and sustained interaction with industry are required. Candidates are sought whose research agenda will contribute to building cross-disciplinary and cross-college collaboration in one or more areas of strategic importance university-wide, including within the Institute of Technology and with other units at the University of Minnesota. This endowed chair is intended to foster industry-university research interaction and collaboration while advancing scientific and technological expertise in new frontiers of knowledge relevant to the Institute of Technology and 3M. Candidates with a background in any relevant areas of science or engineering are encouraged to apply. Department affiliation will depend on the candidate's area of expertise, with the possibility of a joint appointment with one or more units in the Institute of Technology or elsewhere in the University.

Applications should be submitted online at: https://employment.umn.edu, under Req. # 154636, and include a cover letter, curriculum vitae (including list of publications), research description/plan, statement of teaching interest, and contact information for three references. Review of applications will begin immediately and continue until the position is filled. For further information, contact Douglas Ernie at ernie@umn.edu.

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