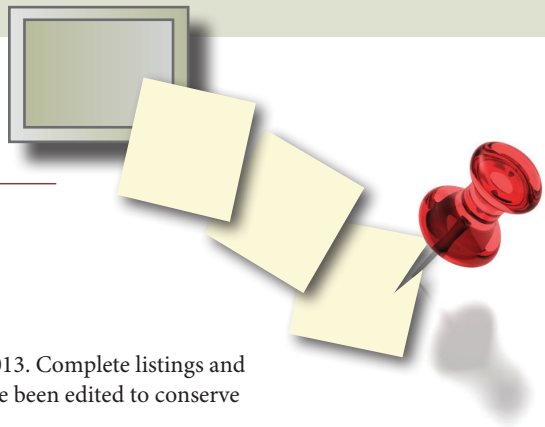
NetNotes



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Selected postings from the Microscopy Listserver from October 30, 2013 to December 31, 2013. 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:
LR White not polymerizing

We have encountered a problem with LR White polymerization using UV light. We have an immuno protocol that requires –20°C polymerization with UV after a progressive lowering of temperature protocol. The resin does not polymerize at –20°C, nor 4°C, nor room temp (well maybe a little at RT) using UV. It polymerizes fine at 50°C in an oven. Thoughts/suggestions? ***Christopher J. Gilpin gilpin@purdue.edu Tue Nov 5***

The times when LR White polymerization has given me conniptions generally involve 1) exposure to oxygen during polymerization, 2) outdated resin, and 3) residual amounts of osmium in the sample (this latter happens when LR White is used for other reasons than immuno work, such as wanting some specific cutting properties). 1) When using flat-embedding molds, make sure that the blocks you value are in the middle of a row of "dummies", all filled to slightly overflowing, then covered with suitably sized cover slip (Thermonox or glass). The outside blanks tend to act as buffers for the inside specimen blocks. 2) I am suspicious of LRW much over a year old. I tend to use older bottles for infiltration up until the last couple changes, but use newer resin for the last exchanges. 3) I have had LRW turn to the consistency of cottage cheese when there were trace amounts of OsO_4 left in the tissue. Had me puzzled big time until Dr. Tom Phillips said "Aha!!" and set me straight. If I think of anything else, I will shout "Aha!!" and pass it along. Randy Tindall tindallr@missouri.edu Tue Nov 5

Specimen Preparation:
algal cells for SEM

I am a materials engineer and I am assisting an external researcher in preparing algal samples (~10 µm) for SEM imaging. We have a recipe for fixation, fluid replacement, critical point drying (CPD) and coating, but my concern is this. If we go through the procedure and get to the critical point drying, how do we mount the cells on a stub prior to drying, so that they are not washed out of the alcohol solution when the CO_2 evaporates? Assistance greatly appreciated. ***James Weston james.weston@gmail.com Thu Nov 7***

First of all, you must have a suspension of cells. You can filter your cells through a Millipore filter. Your cells will remain on the filter (supposing that the pore size is smaller than the cell size). Then you can proceed with dehydration easily by dropping alcohol onto the filter. The cells will go nowhere. To proceed with critical point drying, you can put another empty filter on the filter that contains the cells. You can put some glue to the edges of the one filter and let the other filter adhere to the glue on it. Then you can then put the "sandwich" in the critical point dryer. Berillis Panagiotis pveril@apae.uth.gr Fri Nov 8

I'm not sure what you mean by "...washed out of the alcohol solution when the CO_2 evaporates?" There should not be any alcohol in the CPD when the actual drying run is made—otherwise you'll be drying from alcohol, and the cells will collapse. The best methods we've found are: 1) Collect the algal cells onto a membrane filter—the type with holes, not a torturous-path filter—mounted in a syringe filter holder or a filter column. Do your fixation and dehydration series in this apparatus as well. After the final alcohol wash, transfer the membranes with cells into the CPD device (use a basket or something to contain the filter membranes), do the flush/soak cycles to remove all the alcohol from within the cells and replace with liquid CO_2. Then make the run. 2) Instead of CPD, use HMDS (hexamethyldisilizane) **in a fume hood!**. This will take a bit of testing to find the right conditions for your cells, but still using the syringe or filter column, replace the alcohol with HMDS, using a transition series (2:1 alcohol:HMDS, 1:2 or add a 1:1 step), then 3 washes with HMDS. In or after the 3rd wash, place the filter in watch glass or similar with just enough HMDS to cover the filters, and cover the dish with a cocked lid or lint-free paper, enough to prevent dust etc. from falling on the samples, but leave space for evaporation. Algal cells will usually dry best at 60°C, 2–4 hours (the oven must be in a fume hood). Room temperature can also work—this is one of the things to test. There is a potential third method being worked on by Tobias Baskin that may well work for algae—I haven't tried it yet, myself. Philip Oshel oshel1pe@cmich.edu Fri Nov 8

We quite often work with cells in suspension. We have a protocol that works splendidly! We use the 12 mm round coverslips that fit nicely on our stubs. The coverslips are flooded with 0.1% poly-l-lysine and left in a dust free environment for an hour. After wicking off the poly-l-lysine, the prepared coverslip is flooded with the sample and left in a dust free environment for an hour. Remember to keep the solution on the coverslip and don't let it spill over the sides. After an hour, the excess solution is wicked away. The coverslips are put into a special holder made specifically to hold them for critical point drying. We purchased it from Tousimis (I've also used a small spring to hold the coverslips which is a bit more difficult). Once the coverslips are in the holder, they are dehydrated and critical point dried easily. The finished samples are mounted onto our stubs with double sticky carbon tabs and then sputter coated. If you need our protocol, please let me know off line and I can send it to you. Pat Kysar pekysar@ucdavis.edu Fri Nov 8

Specimen Preparation:
microwave

I am interested in accelerating preparation processes for light microscopy and transmission electron microscopy and maybe immunolabeling. Therefore, I am thinking about buying a lab microwave. I found three on the market, the PELCO Bio Wave, the EMS-820 and the EMS-9000 lab microwave oven. Who has any experience with these or maybe other lab microwaves? As they are quite expensive, I wonder if they are worth the money. ***Anne Heller anne.heller@uni-hohenheim.de Thu Nov 21***

We have been using a PELCO Bio Wave for about 12 years. We have been through 3 generations of the unit and loved every one of

them. The model we use now is programmable so we can set our times, wattage, vacuum, etc., store them then select the protocol we need. I have no experience with the EMS units but I'm sure they are comparable. Our clinical sample turnaround time is 2–4 days-fixation to images on the TEM! Processing the samples takes us only a total of 4 hours. Infiltration takes a total of 12 minutes microwave time. In regards to immunolabeling, I process the samples using the microwave (again, processed in 4–6 hours depending on the type of sample). However, we haven't used it for the labeling process. We embed the immuno tissue into LR White and polymerize in the microwave under water. I must say that cutting these blocks is 100% better when polymerized in the microwave. LR White can be brittle and the protocol we use gives us blocks that are much easier to cut! Pat Kysar pekysar@ucdavis.edu Thu Nov 21

Specimen Preparation:
yellowed glutaraldehyde

Every now and then, we receive TEM samples from outside sources, in yellow glutaraldehyde. When we call to investigate why they are using yellow glutaraldehyde (and not requesting fresh fix), the answer is always "the glutaraldehyde was colorless when we collected and shipped the sample". Can anyone out there explain how or why the glut would change color in transport? Is it detrimental to the tissue? Will our results be compromised? ***Rita Kenner kenner.rita@marshfieldclinic.org Mon Dec 16***

The yellow color is caused by reaction of glutaraldehyde with buffer components (typically Tris or free amino acids; generally with compounds possessing free amino groups). The best way is to avoid using Tris based buffers for fixation. If the buffer composition cannot be changed, you can use short prefixation (~15 min, half strength of fixative) followed by short wash with aldehyde compatible buffer and then use standard fixation procedure in compatible buffer with full strength of fixative (glutaraldehyde). Please look at following books for details: Principles and Techniques of Electron Microscopy: Biological Applications. M. A. Hayat, Cambridge University Press, 2000 pp. 543 and/or Fixation for Electron Microscopy (eBook Google) M Hayat, Elsevier, 2. 12. 2012, pp. 521. Oldrich Benada benada@biomed.cas.cz Tue Dec 17

I agree with Oldrich. There must be some amino acids or protein around but thing is that it doesn't matter. It actually tells you that your client has put some glutaraldehyde. Yorgos Nikas eikonika@otenet.gr Tue Dec 17

Having run a renal Pathology E.M. lab in prior years, I noticed that your e-mail address is listed as a clinic. By any chance, are you receiving clinical samples, and does your lab also receive samples in Michele's fix (Zeus transport medium)? If you receive samples in Michele's fix as well as samples in glutaraldehyde, the labs that are submitting samples to you in yellow glutaraldehyde are contaminating their glutaraldehyde with some Michele's fix when placing biopsies in their sample bottles. I was able to determine this years ago by careful tracking of the renal biopsies submitted to me from various hospitals. When I communicated the problem I was having to submitting renal Pathologists, and they stopped introducing a drop of Michele's fix into my glutaraldehyde bottles from forceps as they were obtaining biopsies, the problem was solved. The two solutions react to produce the yellowing and denaturing of the glutaraldehyde. In severely contaminated bottles of glutaraldehyde, the glutaraldehyde would actually turn milky yellow and would foam like soap if the bottle was shaken. The glutaraldehyde is cross-linking. Michele's fix is actually a transport medium without fixative properties, containing ammonium sulphate and N-ethylmaleimide. If cross-contamination of glutaraldehyde with Michele's fix is not the case, storing glutaraldehyde for prolonged periods of time under warm conditions can also cause the fixative to begin to polymerize and discolor, which is something else to investigate. Are submitting labs refrigerating their fixative, and using fresh fix? Ed Haller ehaller@health.usf.edu Tue Dec 17

Microtomy:
belt failure

The motor drive belt on our Ultracut E just failed after many long years of faithful service. I've managed to find a replacement belt, (it's actually just an O-ring) but I am a bit stumped on how to remove the hand wheel to be able to slip the new belt on the drive pulley. I can get the plastic cover off, but it looks like I might need a special tool to get further into the assembly. Has anyone out there done this themselves, or is there a repair manual available? ***Bradford Ross bradford.ross@botany.ubc.ca Mon Nov 4***

With some more concerted (and colleague consulted) fiddling, I managed to figure it out! Paul Webster hit the nail on the head saying that it has to do with the position of the hand wheel. The red dot on the cutting window adjustment part of the wheel needs to be lined up with the motor switch lever, and then you just turn the drive pulley until the wheel slides off. Then you just loop the belt around the drive shaft and shove it through the small notch in the microtome case and slip the belt onto the drive pulleys. I took pictures of the whole process in case anyone is interested. Bradford Ross bradford.ross@botany.ubc.ca Mon Nov 4

I made a pdf of the process as I went through it. If anyone wants to download it, I placed it on my Google Drive for anyone with this link to access it: (I may not leave it there for eternity, so get it while it's hot!) https://drive.google.com/file/d/0B3suda2jASIMbFBTdnhkN3dYRjQ/edit?usp=sharing Thanks to Wolfgang Muss for sending me a copy of the Ultracut service manual. The way the manufacturer tells you to do this is slightly different from my method, but they don't document the process visually very well, so I think my document may still be of some use to people. Bradford Ross bradford.ross@botany.ubc.ca Tue Nov 5

Specimen Preparation:
embedding resin

One of our students made some polymer sheets (polysulphone). She wants to microtome them for light microscopy. The sheets are thin and flexible, would it be easier to handle them by resin embedding prior to microtoming. The agar low viscosity resin we have requires curing at 60°C. She does not like anything over 40°C. In addition, the material is water sensitive. Any other resins to suggest? ***Zhaoxia Zhou z.zhou@lboro.ac.uk Wed Nov 27***

I have used a mixture of methyl and butyl metacrylates to embed samples for light microscopy. This requires UV polymerization, and a little bit of trial and error (with the light source geometry) to get bubble free embedment. But the stuff sections beautifully. If your sample is non-aqueous it should infiltrate directly. If this sounds promising, let me know and I can send you a protocol off-line. Tobias I. Baskin baskin@bio.umass.edu Wed Nov 27

TEM:
water temperature

What is the proper water temperature to cool the CM10 TEM? ***Josh Schorp jcsmtf@mail.missouri.edu Mon Nov 18***

From the FEI/Philips manual: Water temperature IN: minimum 6°C, maximum 20°C. Water temperature OUT: 20°C ± 2°C water flow maximum: 2.4 L/min, nominal 2.1 L/min. Rise in water temperature through microscope: 6°C at maximum load and maximum flow of 2.4 L/min. Phil Oshel oshel1pe@cmich.edu Mon Nov 25

I would rather use higher end of recommended temperatures—too cold water can result in condensation and corrosion. Vladimir M. Dusevich dusevichv@umkc.edu Mon Nov 25

The aim of water-cooling of electron microscope lenses is that the temperature inside the column be the same as that outside the column. Therefore, the chiller should be set to "room temperature." Roger Ristau roger.ristau@uconn.edu Mon Nov 25

You are being given good advice but perhaps an explanation is required. The water temperature specified for an instrument in most cases is overkill! Consider the manufacturer's situation, are we cooling 80 kV and 20,000×, or 120 kV and 500,000×, two totally different situations. The harder the instrument is run the more heat is generated within the lenses, the result of higher currents passing through the lens coils. But what happens when people switch off the electronics, no heat, so excessive cooling, and inevitably condensation; disaster! Instruments working at 200 or 300 kV will certainly be left on 24/7 so condensation is not a problem. But in laboratories that use the instruments irregularly, over cooling may be a route to big problems. If the instrument is to be left for long periods, with the electronics switched off, or in a stand-by mode, the water temperature should be adjusted to be no lower than a couple of degrees below room temperature. However, there should be a note on the instrument, so that a user would be aware of the adjustment and be able to return to "normal" values. Over cooling of a power transistor board, or the lenses themselves, may lead to numerous problems—the most catastrophic problem being objective lens astigmatism that is beyond correction, due to corrosion of the walls of the lens block. This does not happen in the short term, but with 15 years plus of over-cooling and I am sad to say I once had to condemn an instrument. The lens was beyond repair. Check your instrument out, place your hand on the objective lens and it should feel very slightly warm after a full day's work. If it feels cold then in my mind the water cooling is too much! Good luck, build a true "feel" for your instrument, none of us like being too cold! Steve Chapman protrain@emcourses.com Mon Nov 25

TEM:
weight of instrument

Does anyone have information on the weight of the CM10 and the high voltage unit? I cannot find any information on net weights in the manuals. ***Josh Schorp jcsmtf@mail.missouri.edu Wed Dec 4***

The values I find in the installation specs for weights in kilograms: Console + column 990 kg Power supply with HT generator 430 kg HT generator 100 kg Weight distribution 675 kg/m^2. This is from the "Space and Floor Loading requirements" page. Let me know if you need pdfs of the installation specs—I can send you those. Philip Oshel oshel1pe@cmich.edu Thu Dec

TEM:
Philips CM10 TEM specimen translation control stuck

The Y-translation control on our CM10 TEM stuck last week during "normal" operation by an "experienced" user. The symptoms include 1) stiff Y-control / the light on the control was on; 2) goniometer (specimen holder entry part) stuck at the left side and immobile (see photos at file:///W:/TEM%20specimen%20Y-translation%20control.html). I removed the cover of tilt mechanism and tried to find any things wrong mechanically. I also restarted the machine but no success. This happened once a few years ago and a service call was made. Due to the lack of service contract right now, we are wondering if there is a "simple" way to fix it by the guidance of experts out in the forum. Any tips/suggestions would be greatly appreciated. ***George Liu gul417@mail.usask.ca Tue Nov 12***

We have a CM-10 and frequently get a jammed right-side translation rod. (Y-drive, although the X/Y is confusing to folks, since the image rarely moves in the X or Y direction.) Question: Motor drive (foot pedals) or strictly hand-cranked? Can you remove and insert the specimen rod normally? A sticky Y-drive is common. Have you tried grabbing the translation rod up next to the airlock (above all the joints) and turning it? That usually works for us. Stuck all the way left should mean turning the top of the rod away from the column. Is the rod jammed with the goniometer motor both engaged and disengaged? And can you manually tilt the stage with the motor disengaged? If yes, then it is strictly a translation rod problem. Is the Z-drive centered, or at one or another end of its range of motion? If not centered, try that. Can you push on the end of the specimen rod, and move the rod laterally that way? Mimic the action of the translation rod by pushing sideways on the rod-end cap. If the rod moves freely, the problem again is most likely the translation rod, or where it engages the drive gearing to move the rod. If the rod doesn't move, it's jammed in the airlock, and there may be nothing wrong with the translation rod. If it's the latter, or the drive gearing in the airlock is jammed, or you cannot manually tilt the stage, you'll most likely have to pull the airlock off the column. First, though, hope for the best: the rod moves freely sideways, so the rod drive is jammed. But the rod can be turned at the uppermost end, where it joins the airlock. Push the rod to the middle and turn the rod carefully. This is a common jam, and gets frozen hard when a user first hits the jam and then keeps trying to move the specimen, tightening the jam. Careful work can still free it: if rod end is all the way left, turn the top of the rod away from the column; if all the way right, turn the top of the rod toward the column. If jammed hard enough, you may need a pair of pliers with padded jaws to get it started. BUT be careful! Done wrong, that will make things worse and potentially expensive! Is the rod stiff down at the handle? Reads like it, but loose set screws on the rods (not the U-joints) will also cause loss of stage movement. The rod spins freely then, however. Philip Oshel oshel1pe@cmich.edu Wed Nov 13

TEM:
cellulose nanocrystals

We recently got some samples consisting of certain percentages of cellulose nanocrystals embedded in some sort of epoxy resin. I sectioned them at 70nm, and tried a couple of methods for "staining" the samples with OsO_4. Vapor fixation and aqueous solutions were used for 1–2 hours, but I cannot seem to get any definitive results from the samples. The contrast in the images is very poor, and I cannot see anything that looks like what they are expecting to see. (Yes, I have tried different objective apertures and the like on the imaging side of things.) The most recent publication I could find on the subject was something from the 80's, and the methods section was not very clear on the TEM sample prep. Does anyone out there have experience with imaging this type of sample? ***Bradford Ross bradford.ross@botany.ubc.ca Mon Dec 2***

I also tried to image cellulose in a hydrocarbon matrix with no luck. We finally asked the customer for a sol gel of the cellulose and were able to cut that on our Quanta 3D FIB and only then got some very nice tilt series and reconstructions. We got some help from FEI on the reconstructions as they are only 1 hour from us. I am happy to share images with you and FIB tricks. It is very tricky to cut and lift them out in the FIB. But succeeded with a low KV ion beam and a PMMA recipe and judicious use of FIB protect layers. Pete Eschbach peter.eschbach@comcast.net Tue Dec 3

OsO_4 does some staining of cellulose in cell walls but the real staining comes from following up with lead citrate staining. The osmium serves as a mordent for the lead. Another suggestion I've come across is 2% OsO_4 after treating with sorbyl chloride. I have not tried this one. I have used barium permanganate as a post-section stain for cell walls. It's messy but does a wonderful job at defining wall substructures. I do not know the specific method of action but it may provide some contrast to finding the cellulose particles. It was originally published for fungi but I have used it for both fungi and

plants. Hoch, H. C. (1977) "Use of permanganate of increase the electron opacity of fungal walls," *Mycologia* 69: 1209–13. Richard E. Edelmann edelmare@miamioh.edu Wed Dec 11

TEM:

spherical aberration corrector ray diagram

Can anyone point me in the direction of a clear ray diagram of how a TEM spherical aberration corrector (C_s) works? I have had a good look around on the web and cannot find what I want. Some of the ray diagrams are just plain wrong, in my opinion. Some are complex but do not actually show what they claim to show. Many simply avoid the issue by having a rectangular box with no detail. I appreciate that the actual electron paths are complex multiple helices but personally, I find ray diagrams quite informative in understanding the basics of what is going on inside a TEM. A simple ray diagram can, for example, clearly illustrate how spherical aberration arises so it should be possible to illustrate the correction of C_s. Now, it could be that I am trying to over complicate the question and it really is very simple—a C_s corrector, in ray diagram terms is acting very much as a concave glass lens does for light? On the other hand, I have seen diagrams showing that C_s correctors are not rotationally symmetric about the optic axis—which is why they come in pairs so it isn't quite as straight forward as a concave glass lens. ***Larry Stoter larry.stoter@gmail.com Thu Oct 31***

We wrote a chapter on aberration correction some 5 years ago. It being an early text, it makes sure not to skip anything that more recent texts may take for granted. In other words, I believe that it explains the principles of aberration correction pretty well. It focuses primarily on STEM, but reversing the flow of the electrons in the diagrams (reciprocity does work!) so that they apply to CTEM applications should not be too much of a hardship. Here's the full reference: O. L. Krivanek, N. Dellby and M. F. Murfitt, "Aberration correction in electron microscopy," in: J. Orloff, ed., *Handbook of Charged-Particle Optics*, CRC Press, Boca Raton, 2009, p. 601–40. Ondrej Krivanek krivanek@nion.com Fri Nov 1

STEM:

measuring scanning probe size

What are the procedures to measure the STEM scanning probe size together with the probe current on a FEG(S)TEM (e.g., a Tecnai F20)? I measured the probe size at nanoprobe mode before I pressed into STEM mode, because in STEM mode the CCD sees a diffraction pattern scanning not the static probe. I recorded some lines on CCD when using the beam shift wobbles the nanoprobe. The FWHM was taken as the probe size at 400K× magnification. I took the screen read as the probe current. I end up with a ~1 nm probe with over 3 nA current at condenser aperture 150, spot size 1, 89 μA emission. Is that real? Any comments about my procedures? ***Zhaoxia Zhou Z.Zhou@lboro.ac.uk Fri Dec 6***

Your numbers sound fair or close, based on my FEI FEG experience (CM200ST), although I obviously don't know if they are exactly right. One thing that FEI does well and "some" vendors do poorly is that, while in STEM mode, you can release the diffraction button and see the direct probe on the florescent screen/TEM camera. Thus, you don't have to look at the nanoprobe spot size and then pop over to STEM, but instead, tune the probe in STEM mode, park the beam in the center of the field of view in TIA with the red circle/green crosshair tool, and release the diffraction button, which leaves the probe in STEM but changes the projectors to TEM. My major complaint with one particular non-FEI tool is that there's no way to see the sample plane instead of the diffraction plane during STEM. Ronchiograms are nice, but so is direct probe imaging. Chad M. Parish parishcm@ornl.gov Fri Dec 6

ESEM:

drying biological samples

I am a metallurgist with lots of high vac. SEM experience but very limited ESEM. I have biological researchers bringing in samples like rat hearts for ESEM. They bring the samples soaking in alcohol or water. The samples are hydrated and plump. We do ESEM on the samples (temperature and humidity control). I start the water vapor pressure high enough that water condenses on the samples in the ESEM and then turn it down just until the liquid on the surface goes away. When the samples come out—they look like damp leather. Does it sound like I am doing this right? Should the samples come out of the ESEM looking as plump and hydrated as when they went in? ***Robin Foley rfoley@uab.edu Wed Oct 30***

It is a really tough task—to observe soft tissue with ESEM. You can try re-wetting specimens every few minutes (change pressure so that you will see droplets of water on the specimen). You can try fixation prior to observation. Even better—perform all needed steps for conventional biological specimen preparation (fixation, dehydration, coating) and work in high vacuum mode. Best of all—show pictures to your customers, and if they are happy, be happy. Vladimir M. Dusevich dusevichv@umkc.edu Wed Nov 6

Image Analysis:

protein crystals

Is it possible that a very thin (membrane) protein crystal, consisting of maybe two or three layers of proteins in their respective lipid membranes, could exhibit, for example, a p3 symmetry in zero degree projection when the crystal as a 3-dimensional object doesn't have this symmetry? In other words, only the central slice of the Fourier transform parallel to the crystal plane would have 3-fold symmetry, but not a non-central slice. ***Philip Koeck philip.koeck@ki.se Fri Dec 20***

In general a 2D crystal with any higher order symmetry such as P3 symmetry will give you a (P3) symmetric projection map when imaged without tilt, but the projection map of such a crystal from a slightly tilted direction will not have any symmetry. This already applies for single-layered 2D crystals, and also applies to multi-layered 2D crystals or 3D crystals. For true 3D crystals, there might be other (e.g., orthogonal) views where other symmetries can be found in projection. But this doesn't apply to 2D crystals. If a structure has a screw axis, such as P312 or P321, then the structure is also P3 symmetric, and the same as above applies. Philip, I think you are right: If you have a non-P3 symmetry crystal, but place three layers of such a crystal on top of each other, rotated each by 120° with respect to each other, but centered onto the same point of origin, then the non-tilted projection map can well show P3 symmetry, even though none of the layers itself had that symmetry. In this case, processing that object under P3 symmetry would be wrong. Besides this, there are possible other sources of a fake P3 symmetry appearance: Hexagonally closest packing is a common way for proteins to squeeze together in a membrane. At low resolution, one sometimes gets the impression to have P3 or even P6 symmetry in such a case. But the low resolution might not allow conclusions anyway. Another way to get apparent symmetries is when the crystal is fragmented, so that different areas in the 2D crystal have differently oriented lattice vectors. If, for example, you are dealing with a P2 symmetry crystal that has as lattice vectors $\mathbf{a} = 100$Å, $\mathbf{b} = 100$Å, and an included angle of $\gamma = 120°$, and this crystal has cracks after which the lattice orientation changes so that the new **b** becomes the old **a** vector, then the overall appearance of such a crystal can be P3, even though the local areas aren't P3 symmetric. Henning Stahlberg henning.stahlberg@unibas.ch Tue Dec 24

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