NetNotes

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Selected postings from the Microscopy Listserver from January 1, 2011 to March 2, 2011. 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:
hexamethyldisilazane (HMDS)

I'm looking for a reference to explain just how/why HMDS works to dry specimens for SEM observation. There are plenty of references showing that it does, in fact, work and produces good results in many cases, but I have yet to find an explanation as to the "why." Is it that HMDS has the right physical properties (surface tension) to evaporate without causing drying artifacts? Alternatively, is there something about the silanization of the sample that helps make it more robust, or both, or am I completely lost (quite likely)? ***Bryan Bandli bbandli@d.umn.edu Wed Feb 2***

I believe it has to do with very low surface tension. It also does not work well across the board. I understand that it works well with insects, but one of my sons did a science fair project comparing dried, freeze-dried, and HMDS treated green pepper. In his case the HMDS didn't appear to be any better than drying. His best results were from plunging small pieces into acetone cooled with a Peltier cooler, and then transferring to another Peltier cooler in a vacuum evaporator until dehydrated. He was not able to compare it to critical point dry prep as we didn't have the requisite CO_2. Obviously, the plunge freezing was not done at typical temperatures, so there was probably ice damage in those samples, but they looked the best of the bunch. Ken Converse kenconverse@qualityimages.biz Wed Feb 2

Drawing from my cleanroom experience. . . . HMDS is used as a photoresist adhesion agent for Si wafers. It binds to hydroxyl groups. It makes sense to think of as having mordant properties, strengthening the specimen with Si. The low surface tension also plays an important role. http://www.transene.com/hexa.html. Edward Basgall ejb1176@gmail.com Thu Feb 3

Specimen Preparation:
SEM sample storage

One of our EM lab users asked me a question: what is the best way to store SEM samples? I know you'll all say that the best is to process and coat first. He wants to store them in 2% glutaraldehyde and process later since he does not have time now. ***Dorota Wadowska wadowska@upei.ca Wed Feb 2***

To my experience, specimens can stay in glutaraldehyde for an indefinite time. I have processed samples after >3 years stored in glutaraldehyde inside the fridge, and they looked the same as others being processed within a few days. In rare cases, I've seen mushrooms developing in samples that stayed only a few weeks in glutaraldehyde. There, I suspect that the glutaraldehyde was old, samples were dirty from the beginning, or some other unidentified factor was present. If you process and coat the specimens, I think it is better to view them soon, otherwise they can affected by humidity and the morphology deteriorates. In conclusion, I think it is not a bad idea to have your samples staying in glutaraldehyde until you have the time to view them. Yorgos Nikas eikonika@otenet.gr Wed Feb 2

Glutaraldehyde can deteriorate over time and produce a white precipitate that could coat the specimens. For long term storage it is not a good idea. Ralph Common common@msu.edu Wed Feb 2

Store for how long? What kind of specimens? I have stored soft tissue samples for a couple of days in glutaraldehyde with no problems, and crustaceans with well sclerotized cuticles for several days, but lightly sclerotized crusties and body parts don't like it. Note! Watch the pH! The fix can cause dissolution of Ca^{2+} in the crustacean exoskeleton. When I had to process, store, then transport samples from Antarctica to Chicago, I ran them up into 100% Pel-Dri (back when it was allowed) or 100% HMDS. This worked. The samples fish lateral-lines and lateral-line end organs, and they compared well to freshly fixed, processed, and dried end-organs collected in Lake Michigan. The sample vials where completely full of fluid, with a 10:1 or 20:1 fluid:specimen volume ratio. But the best way is to fix, process, dry, mount, and stick in a desiccator. Don't coat. The expansion and shrinkage of the samples when moving them from the desiccator to the SEM and back can cause fine cracks in a sputter-coated metal layer. Coat when ready to examine. Philip Oshel oshel1pe@cmich.edu Thu Feb 3

Immunocytochemistry:
post-embedding FluoroNanogold labeling for TEM

Some of our antigens are difficult to label in mammalian fat cells and muscles, such as particular lipid droplet proteins. I have tried several methods of antigen unmasking and probe maintenance in post-sectioning fluoronanogold labeling, including absence of uranyl acetate in the LR-White embedding medium, reduction of osmium post-fixation concentration to reduce etching propensity of silver-enhanced gold probes, antigen unmasking with oxidizing (sodium metaperiodate) and reducing (sodium borohydride) agents and Tris base pH 10. I have reduced my glutaraldehyde concentration to 0.10% and maintained paraformaldehyde at 2%. Pre-embedding fluoronanogold labeling appears to work with the absence of glutaraldehyde in the fixative, however the ultrastructure is quite compromised, as one would expect by TEM. I have been thinking now of using LR-Gold, until I hear from some experts on any other suggestions. ***Vickie Kimler vakimler@med.wayne.edu Mon Jan 24***

I had a similar situation and had successfully used pre-embed labeling with FluoroNanogold. In the post-section fluoronanogold labeling, there might not be enough cross linking of your proteins to keep them stabilized especially during the dehydration and embedding steps. Antigen unmasking might not be the issue there but rather the proteins have been extracted. From what I recall lipids rely on osmium tetroxide and uranyl acetate fixation. Assuming you have abundant amount of these proteins, if you have already tried the gradual low temperature approach during dehydration and resin infiltration steps and there is still no labeling, I would try another approach. You mentioned that pre-embed fluoronanogold labeling worked but the ultrastructure quality was quite compromised. That

https://doi.org/10.1017/S1551929511000319 Published online by Cambridge University Press

sounds promising when you get similar labeling pattern as in previous light microscopy data. In this case, you might be able to fine-tune the fixation step and permeabilization if the latter was used. In my experience, this approach worked better for the Flower, a synaptic vesicle protein in the neuromuscular junction (NMJ) which we were working on. We tried several fixation combinations but found that overnight 4% paraformaldehyde fixation achieved sufficient labeling. Although ultrastructure quality was not the same as routine TEM, the organelles were still recognizable. We were a bit lucky because the NMJs lie close to the muscle surface and go only as deep as 5–8 microns into the tissue. Also, we worked on fillet-dissected third instar Drosophila larvae and did not need 50 micron thick Vibratome sections. Even so, we still needed permeabilization steps but not as drastic as used in regular immunofluorescence. After trying different detergents and concentrations, we added 0.01% Tween 20 in the blocking step. The nice thing about using FluoroNanogold is we can examine the fluorescence labeling before proceeding to next steps. In addition, you can post fix with 3% Glut and use lower concentration of osmium tetroxide prior to dehydration and embedding so the sample quality should be more enhanced. I am not an expert in Immunocytochemistry like Drs Paul Webster and Jan Leunissen and others out there but one thing I learned as I was getting into this is that there is no single labeling approach. Pre-embed method is not suitable for all samples but would likely work on cell monolayers/suspensions, Vibratome sections and samples where antigen is easily accessible. We try to fit the best immuno-EM method to your samples which we all know usually takes time. I hope you have plenty of time to optimize the method for your samples. I will send a PDF of the Flower paper so that you can check the micrographs. A detailed pre-embed method with Fluoronanogold is on the supplemental material. Claire M. Haueter chaueter@bcm.edu Tue Jan 25

Light Microscope:

stuck objective

What tricks/tools do people suggest for unscrewing objectives from the turret when you cannot loosen them by hand? ***David Knecht david.knecht@uconn.edu Wed Feb 16***

Buy a strap wrench. If the rubber slips on the objective barrel, use channel pliers to grab the rubber strap and turn. Don't squeeze excessively. Focus on turning with the pliers. You may be able to use leather gloves with the pliers, but the teeth of the wrench could penetrate and cause gouges in the outer case. A small sheet of thick rubber may also work, but strap wrenches are cheap and store easily. Gregg Sobocinski greggps@umich.edu Wed Feb 16

I want to thank you all for your suggestions and report back. I put WD-40 on the threads with a plastic Q-tip, waited a while, then used a small strap wrench (Harbor Freight Tools) and I was able to get them all loose. David Knecht david.knecht@uconn.edu Sat Feb 19

TIRF:

laser alignment question

We have the Nikon TIRF system and have three laser lines going into the TIRF arm via a single fiber. When we project through the 100× objective through the sample onto the wall, we see that the lines go through the sample at different angles. (You can see a picture of the projection at approx 45 degrees at http://www.flickr.com/photos/mcammer/5359189090/.) It is also noticeable in the TIRF images that the field depth is different for each wavelength. Is this unavoidable due to the different wavelengths or is it possible to align the optics better so these spots would be more coincident? ***Michael Cammer michael.cammer@med.nyu.edu Sun Jan 16***

That is a beautiful photograph of the unavoidable relationship between the angle of incidence and the wavelength of light used to illuminate the sample. It stems from the fact that the relative index of refraction of the glass-water interface is slightly different for each wavelength. To ensure that the penetration depth is nearly equal for all laser lines, you would have to offset each laser beam to a different specific radial position on the back focal plane of the objective—and these relative spacings would change as well if you wanted a different penetration depth. You can work out what these spacings need to be by examining the basic equation for the TIRF penetration depth (you have to assume you know the wavelength dependent index of refraction of the sample which ranges from 1.33 to 1.38) and you'll find that they are on the order of a few tens of micrometers depending on the specific penetration depth you desire (see http://micro.magnet.fsu.edu/primer/techniques/fluorescence/tirf/tirfintro.html). There's a nice little ImageJ plug-in that can be used: http://rsbweb.nih.gov/ij/plugins/tirf/index.html. John Oreopoulos john.oreopoulos@utoronto.ca Sun Jan 16

Thanks for the reply. Reading it and the referenced websites jogged my memory. A few years ago we were having problems with the first commercial Olympus TIRF system because we could not get consistent evanescent waves with the one angle adjustment with the laser lines we had from 405 to 568 nm that were delivered via a single fiber (it was worse when we later added a 633 or 638 nm laser). I suggested we pump each laser in through a separate path that could be angled independently. We did not build it, but I think Olympus now sells a TIRF system that does this. Another issue is that when I first heard about TIRF maybe 15 years ago, it was introduced as a ring illumination at the outer edge of the back aperture, not as a single point or crescent at the periphery on only one side. A ring, or at least a series of points around the periphery, seems like a better way to provide a uniform field due to aberrations from coherent light in the imperfect optics. Any thought on this? Michael Cammer michael.cammer@med.nyu.edu Sun Jan 16

Yes, you are right. See e.g. this paper about this very topic: Fiolka, R., Belyaev, Y., Ewers, H., & Stemmer, A. (2008). Even illumination in total internal reflection fluorescence microscopy using laser light. *Microscopy Research and Technique*, 71(1), 45–50. doi:10.1002/jemt.20527. Janne Hyoetylae janne.hyoetylae@stud.unibas.ch Tue Jan 18

EM:

radiation risk

I am thinking about the EM radiation for an expectant mother. Are there any policies regarding the use of SEM and TEM by pregnant employees? Thank you very much! ***Xiaolan Wu xiaolan.wu@dartmouth.edu Tue Feb 15***

In our Facility (located in Austria) we had the pleasure to deal with this situation a year ago. What we found out was that with the maximum X-ray emission of our 100 and 300 kV TEMs as measured in the acceptance test (approx. 200 nSv/h), it takes an expecting mother under intensive, but realistic working conditions more than 2 years to reach the maximum dose allowed here by law. To confirm this, we had both microscopes re-measured by an external, certified expert. It turned out that close to our 100 kV machine, the radiation level is even below environmental radiation, as the column shields more than it emits. Subsequently, we received an official safety clearance from the local work inspectorate for both scopes. We also had our pregnant colleagues carry live dosimeters with them, which we checked daily, to make sure the conditions at the microscopes did not change. Guenter Resch guenter.resch@imba.oeaw.ac.at Wed Feb 16

We have found, after extensive testing (over the last 15 years) that if you have an instrument that was bought after 1987 (when the oldest of ours, that we tested, was purchased) and it has only been modified by either the manufacturer or had ancillary equipment added to it by one of the XEDS companies or Gatan, or the like, that there is absolutely MINIMAL leakage of radiation from the instruments. For the past 15 years we have had radiation badges located permanently next to the microscopes right where the operator sits. These badges are developed every three months and replaced. The three oldest sets of badges, one on our JEOL 2010F (was on the JEOL 2000FX when we had it), one on our FEI SEM and one in my office show the following exposures:

Last 3 Months	Year to Date	Lifetime (July to June)	(Since 1995)
JEOL 2010F	<10 mrem	<10 mrem	78 mrem
FEI XL30FEG	<10 mrem	<10 mrem	133 mrem
Office Monitor	<10 mrem	<10 mrem	21 mrem

These numbers are from Landauer (www.landauerinc.com) and are listed as "Dose Equivalent." Office monitor is line of site to one of the few windows in the basement, assume it is cosmic ray readings that make it higher, but that is just a guess. Annual exposure limits, according to Landauer, are 5,000 mrem for the body and organs and 50,000 for extremities and skin. General Public should expect to see 100 mrem/year. As far as I can see, our sensors have indicated that we are getting about the level that Jo and Joe Average see in the streets. We still encourage anyone who is worried, or thinks they might be pregnant, or wanting to become pregnant, to obtain and wear a dosimeter when ever operating the instruments. We do not mandate dosimeters, neither does the University or the State of Michigan. John Mansfield jfmjfm@umich.edu Thu Feb 17

Facility Operation:
problem clients

Yesterday, on my very old SEM, a student user accidentally engaged the lock on the tilt. When the stage would not tilt, he decided to force it and snapped off the 5 mm diameter steel rod that you move to tilt the stage. He REALLY had to force it to cause this to happen. I'm sort of wondering if he beat it with a hammer—but I don't see any signs of that sort of damage! What response do all of you take when someone screws up like this? How do you prevent it? Also, what kind of training do you give your students prior to letting them loose on an SEM? We're about to replace our SEM with a new one with lots of features. I'm trying to get a feel for what normal training practices are and what works and doesn't work in your facility. I've had two incidents similar to this in a year (steel valve bent at 45 degrees on sputter coater), and it seems like too many. I might need some improvements in my training methods! ***Robin Foley rfoley@uab.edu Sat Feb 26***

When I was running a facility, I always tried to be patient with the perpetrators of such events. Even the best of us did dumb things when learning and this is part of their education—to become people who will not mess up in the future. If you control them too tightly, they will never learn. After all, science comes from people willing to do things that have not been done before. In our lab one extreme case involved a student drilling a hole into the chamber of an SEM—while it was running. The hard part of the whole deal is convincing those responsible for funding that this is OK. And convincing yourself that this is OK even when the instrument damaged is a new and expensive one. Alwyn Eades jae5@lehigh.edu Sat Feb 26

I agree with Alwyn that "doing dumb things" is part of the education. As facility directors/managers, however, I believe it is our job to teach/train our users to do as few "dumb things" as possible, especially when these "dumb things" have a big consequence. It is costly to repair, and prevents other people doing research (the other important mission of the facilities). We have a written policy that if a student damages an instrument due to improper operation, the PI has to agree to pay for the repair (if any). Even education is not free and we all learned that part. With that said, we always try to teach students beforehand, to avoid any unnecessary damage to the instrument. One thing we always tell the students is the "two fingers rule"—Nothing on the instrument (TEM, SEM, confocal) requires forces greater than what your two fingers can handle. If you cannot open/close anything with your two fingers, STOP. That works pretty well, in most of the circumstances. Zhaojie Zhang zzhang@uwyo.edu Sat Feb 26

I have been teaching microscopy and managing/directing a good sized service/multi-user EM facility for over 20 years (additional time as a user or service provider in smaller facilities). I am very happy to say that in that period we have had extremely few user accidents. I attribute that to three major reasons: 1) Good training for all users before they are given their flying license. 2) Support by facility staff to assist, answer questions, and continue to train/educate on a routine basis. 3) A certain amount of fear that users can lose access privileges if they misuse instruments/break rules/do not respect other users. (1) requires offering (and requiring) courses where students learn basic theory and hands-on use of major instrumentation . . . during which they are instructed in both what to do and what not to do. If you are not willing to put in the time required to train/educate users, then you and the instruments will run into problems at some point. It all starts from learning correctly in the beginning with the emphasis on good habits, sufficient understanding to make good choices, and thus get good results. Users training users is problematic in my experience, as bad habits get passed on and magnified over time. 2) A good relationship between facility staff and users results in users who are not hesitant to ask for help. This means knowing your users, showing an interest in them and their projects and offering assistance when asked. You will quickly learn who needs some additional help along the way and who can work truly independently. 3) I don't apologize for this one. Facility rules are there for a reason. Down instruments due to user error affect many and that is unfair. The primary rule I have always enforced is that users to do not attempt to fix anything at any time. What seems simple often is a sign of much more significant problems that can be averted if the staff knows about them and responds accordingly. Failure to follow this rule results in much closer scrutiny and potential loss of privileges (rarely necessary). All users may not perform at the same level but that does not mean that all cannot learn to use instruments with respect for the instrument and their fellow users. Part of our jobs as facility staff is to teach, train, and support to insure that this happens. We may not be 100% successful, but the number and severity of incidents will decrease significantly as a result. Debby Sherman dsherman@purdue.edu Sat Feb 26

The Australian Centre for Microscopy and Microanalysis (ACMM) at the University of Sydney, is the largest facility of its kind in Australia. We have 7 TEMs, 6 SEMs, 2 atom probes and lots of sophisticated light and optical and x-ray equipment. This brings in over 400 registered users annually, approximately 1/3rd of whom are new users. Effective training is essential to ensure users get up to speed quickly, and also to ensure that costly mistakes don't happen. The training regime we use is as follows: 1. New users attend a meeting with relevant academic/technical staff to determine what

they are trying to do, how best to do it, and on which instrument. 2. Training (for TEM) is usually 3 × 3 hr sessions either with a technical staff member or with one of our competent grad students. Users are taught from a standardized manual, which shows step by step procedures with lots of pictures. Users are encouraged to annotate their own copy of the manual. 3. After training users are given 1–3 supervised solo sessions, where tech staff get them started and drop in frequently to assist with any queries. 4. Once the user is confident running the machine solo they are assessed. This is two part process. The first part is a 40 question multiple choice quiz, covering basic theory, instrument operation, safety etc which is covered during training. Nobody fails the quiz—we use it as a safety net to fill in gaps in users' knowledge. The second part is an assessment, where the user goes through setting up, operating, and shutting down the instrument—it's open book so they can refer to the manual. This is supervised by technical staff to ensure quality control. Users need to be able to do all the basics without undue hesitation and without harming (or nearly harming) the microscope. 5. Users who fail the test are given more training. In some cases lots more, until they get it right. 6. Users on instruments can always get immediate help from one of our technical staff by calling our Duty Microscopist number. This avoids having users wander corridors in search of staff, and avoids them getting bored and trying to fix things themselves—which is usually where things go bad. Despite all the above, trained users stuff things up occasionally, usually through carelessness. Such users are retrained in whatever they stuffed up and watched carefully thereafter. We avoid tearing strips off users who do bad things as it discourages them from asking for help or owning up to mistakes. We strongly encourage "if in doubt—ask." Dave Mitchell drg.mitchell@sydney.edu.au Tue Mar 1

History:
early EM and ultramicrotomy

I recently read this article about Ernst Ruska and thought of sharing it with the list, many of you may have already read this but it's truly a fascinating read. http://www.mpg.de/english/illustrationsDocumentation/multimedia/mpResearch/2006/heft03/Electron_Microscopy_Ernst_Ruska.pdf. ***Neeraj V. Gohad neerajg@clemson.edu Fri Jan 7***

It is very interesting. It doesn't mention why Ruska could not have received the Nobel Prize way back in the 50s when the microscope was new. The concept was patented by somebody else, a Mr. Rudenberg, from the Siemens Company, based on a visit to Ruska's laboratory. It has all been written about by Ruska in his book. Carol Heckman heckman@bgsu.edu Sun Jan 09

As the article describes, the early version of the instrument could only reach modest resolutions and we have to remember that the thermal advancement ultramicrotome by Porter and Bloom was reported in 1953. We know now what the combination of TEM and ultramicrotome did for biology and other fields, and gradually many crucial discoveries were made using the TEM which eventually could attain atomic resolutions. This is somewhat similar to discovery of laser, many of the key people who worked on the laser during its conception were awarded the Nobel prize later. Luckily the TEM didn't go through the lawsuits and patent battles that the laser did. This may be true of our times too, we have yet to fully grasp the potential of recent advances in super-resolution optical imaging. Neeraj V. Gohad neerajg@clemson.edu Wed Jan 12

The Porter-Blum microtome was mechanical advance. Simple, reliable, unbreakable. Caroline Schooley schooley@mcn.org Wed Jan 12

My mistake, I meant mechanical, Porter-Blum MTI was indeed mechanical, but you get the point. neerajg@clemson.edu Wed Jan 12

I know at least 2 old microscopists who made glass knifes by breaking window glass—one of them here at CMU. He went to construction sites to get the broken windows. I'm running down the reference and (I hope) an image, but one of the early tries at ultramicrotomy was sticking razor blades on a centrifuge rotor, then mount the sections on the tub, close the lid and turn on the centrifuge. The sections where then picked up from inside the tub, having been flung willy-nilly around the inside. The image is used in the microtomy lecture to convince the students thin sectioning could be a lot worse than they think it is. Phil Oshel oshel1pe@cmich.edu Thu Jan 20

Indeed. We are in a building that was often broken into. Years ago, I made it my business to collect the old door panes—they were a tinted glass, about 1/4″ thick, and had just the right temper to make excellent knives. Joel B. Sheffield joelsheffield@gmail.com Thu Jan 20

I just remembered seeing a print ad from some science journal published the 40s or 50s (before my time!) for an early ultramicrotome. It was a high speed motor spinning some type of blade. The concept was that the block was advanced into this buzzsaw and you were supposed to catch the sections flying off. At the time I think the view was that ultrathins could only be cut at high speed. The real kicker was the ad mentioned the motor was also suitable for use in centrifuges. Crazy. Tom Phillips phillipst@missouri.edu Thu Jan 20

Sorry for the delay in response to this topic, but due to an error on my on my part my previous communication went astray. For those who may be interested in the history of ultramicrotomy I do have a copy of an article in PDF form which traces the development of the ultramicrotome from "wedge" sections in the late thirties where the thin end of the wedge was (hopefully) transparent to electrons, through the high speed era (actually up to 57000 rpm), overcoming embedding limitations and finally discussing the first generation of commercial instruments. Please contact me on terry.cooper@btinternet.com and I can attach the missive, Terry Cooper terry.cooper@btinternet.com Wed Feb 2

Fascinating article. Some of my recollections (possibly with some inaccuracies but the best I can recollect) involve Fernandez-Moran who is credited with developing the first diamond knives for use with ultra-microtomes as well as a cryo ultra-microtome and a number of other interesting developments. I worked for Fernandez-Moran at the U. of Chicago for a few years starting in 1963. This was after he was forced to leave Venezuela and then did a short stay at MIT before being recruited by U. of C. He had a microtome, presumably of his design, that only he used on rare occasion. Almost all the imaging done in the lab was with negatively stained samples. He, of course, had his diamond knives. The technique to make them was perfected at the lab for neurological research that he built in Venezuela. (He was forced to leave Venezuela when the government was taken over by a military coup and he was on the wrong side, but that's another story.) He had a workshop there with his diamond cutters, etc. After developing the knives, he sent them out to the leading investigators of the day to get them to try them so that they would then buy them. Dupont picked up on the idea and also began making them for sale. Moran had patented the process so was able to sue Dupont and did win. I believe he later agreed to give them rights to make and market the knives. Moran's lab at Chicago was quite a place. It was a semi-clean room lab in the basement of the Research Institute. The floors were raised so that all the water and vacuum lines for the microscopes were underneath with mechanical pumps and water recirculators a long ways away from the microscopes. He had 3 Seimens 1 and 1A TEMS on vibration mounts with the raised floor cut around the microscope bases so that moving a chair would not affect the TEM stability. A motor generator located in

the attic of the building provided stable power. I started out as a technician and we all wore white nylon lab coats, white rubber shoes that we washed weekly, and little white hats. Visitors suited up in lab coats with plastic bags over their shoes. Pre-pumps to evacuate the film cameras were located two floors up. We would put on our red goggles to retain our dark adaptation, put plastic bags over our plastic shoes, and shuffle up to get new film cameras . . . looked like Martians! It was an interesting time. There were also a couple of Japanese scientists working on an early Hitachi microscope trying to set resolution records. They would literally disassemble the microscope after just a few tries to clean it and get ready for the next attempts. They were the only ones with the patience to deal with that microscope. Later Perkin Elmer Company came along and helped with design changes to make it more user-friendly and thus more marketable. Pointed filaments were also made in the lab. E.F. Fullam came one time to see how they were made and then was able to start selling them commercially. Moran built a helium-cooled microscope to improve resolution using superconducting lenses. I do not know if it was originally his idea or if he "borrowed" someone else's idea. However, they built a liquid helium recirculator system, again with most of it in the attic 4 stories up. The helium flowed through a special jacket on the microscope that encased the entire upper part of the column through the objective lens. They managed to get a few images but then Moran lost interest. A series of health problems shortly thereafter led to closing down the lab and end to a very interesting few years. Louie Ouwerkerk was a Dutch engineer who, along with the great U. of Chicago Instrument shop, designed and built a lot of Moran's ideas. Debby Sherman dsherman@purdue.edu Wed Feb 2

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https://doi.org/10.1017/S1551929511000319 Published online by Cambridge University Press