NETNOTES

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Selected postings from the MSA Microscopy Listserver (listserver@msa.microscopy.com) from 6/11/03 to 8/10/03. Postings may have been edited to conserve space or for clarity.

IMAGE PROCESSING – RGB to CMYK

Is there a way to convert an RGB image to a CMYK image without having the colors go "haywire"? Tobias I. Baskin <baskin@marlin.bio.umass.edu>

Sure, I do it all the time for Microscopy Today. With Photoshop 7 there is a very efficient automatic conversion: while viewing the RGB image, click "Image"/"Mode"/"Convert to Profile." Select "Working CMYK – US Web Coated (SWOP) v2". in the "Destination Space" drop down box, click OK, and you are done. There may be similar procedures in earlier versions of PS. Ron Anderson <microtod@optonline.net>

SEM - native silica aerogel

I am trying to look at a native silica aerogel in a Hitachi S4700 FE-SEM. I want to look at a fractured specimen in a 100 to 200 nanometer range. The problem seems to be optimal sample preparation. I can view the sample with a gold or gold/palladium sputter coat, but in order to view at the magnification I'm looking for the coating is substantial enough that it fills in some of the pores or open pathways and totally coats over the primary silica particles. I have a comparison of gold sputtered onto a glass slide with a Balzer Coater, 40 mA and 1.5 inches to the top of the jar at 10, 20 and 30 sec. The ten second sputter coating viewed by SEM at x250k and 200 nm somewhat mimics photographically the channels and porosity of the native silica. At the higher sputter times of 20 and 30 sec the pores and channels begin to close or fill in. I have also tried a carbon deposition coating. It's lighter in density not filling in pores and channels and not obliterating primary particles, but doesn't seem to be conductive enough to dissipate charging in a manner that lends itself to anything but low magnification photos. I've tried mounting on carbon tape, and/or carbon paint but the isopropanol dissolves the sample. I've tried varying degrees of the carbon and gold coat. I've tried silver epoxy; I've even tried, after the coating running a thin line of the carbon paint up the side of the sample onto the area to be viewed. Of course with the native silica being so easily degraded the carbon paint was actually eating away the silica aerogel and breaking the contact of the existing coating. I've tried running a thin copper wire or copper tape from the face of the sample to the sample holder. The problem being there is no good method of adhesion to the sample. I have simply run out of ideas. Does anyone please have a solution? Linda S. McCorkle <linda.s.mccorkle@grc.nasa.gov>

For high magnifications I usually coat with Au/Pd at 10 mA for 20-60 sec., depending on the roughness of the specimens surface. Usually, when coating is thin enough, I do not observe it for magnifications up to 100-150k. Sticky tape and carbon paint are not stable enough, so I prefer to glue specimens with Quick Gel superglue and let it dry overnight.

Vladimir M. Dusevich <dusevichv@umkc.edu>

In my evaluations of FE-SEM's, that included the Hitachi 4700, we examined uncoated silica aerogel samples. I mounted them using carbon paint on a stub. We used a voltage of about 1 kV and a working distance of about 2 mm if I recall correctly. There were some issues with drift, but we used a continuous average mode, soaked the sample at a lower magnification than what we wanted to take the micrograph for a few minutes, focused out of the area that we took the picture and brought it back in and took the picture when the image stabilized. We were able to look at all of the samples in the 50kX -150kX range on all of the systems without too much trouble. Scott D. Walck <walck@ppg.com>

You might try varying the accelerating voltage to see if you can find a voltage that results in a non-charging (or very minimally-charging) sample. You might try anywhere from 0.5 kV to 2 kV. Another alternative is to find a colleague with a variable-pressure SEM with a secondary electron detector that functions in the VP mode (as opposed to the BSD). Becky Holdford <r-holdford@ti.com>

Do you have access to an ion-beam coater? We routinely use one with a platinum target for high resolution SEM of subcellular structures, gels, etc., and I don't see coating effects until 250,000X for 4 nm coatings, or 300 - 400,000X for thinner coats. Normally we use a 1 - 2 nm thick coating. Philip Oshel <peoshel@wisc.edu>

One of the other responders to your post mentioned the technique of moving around on the specimen to minimize localized charging on the specimen surface. This technique takes a little practice, but is the best way to manage a specimen like silica aerogel. At Lawrence Berkeley Laboratory, we sometimes wrapped the specimen in aluminum foil, with a small hole approximately 2-3 mm in diameter through which we would view the specimen. This worked well for Auger analysis too. Ken Gaugler <ken@gaugler.com>

SEM – charging of biological specimens

We are looking at biological specimens (tissue explants) coated either with platinum or carbon (details below) and having problems with charging. The charging is much worse on the carbon coated specimens compared to the platinum coated ones. Our protocol is to fix the tissue (2x2x2 mm) in paraformaldehyde/glutaraldehyde (or 2% PF) for several hours and then osmicate for 2 hours, dehydrate with an ethanol series, critical point dry and mount on a stub with conductive tape. The edges of the specimen are painted with silver and then allowed to dry. The specimen is then either sputter coated with platinum at 10 mA for 70 seconds or high purity carbon for 3 seconds. Specimens are stored in a desiccation chamber until viewing in a Hitachi FE-SEM at 5 kV and 20 μA at magnifications ranging from 1-20k and sometimes up to 50k. The carbon coated specimens are colloidal gold labeled and viewed with both BSE and SE detectors. I don't have a feel for how much of this problem is "normal" and one has to live with it like so much else in EM! But if anyone out there has some ideas or tricks to reduce charging, we are willing to try it. I would also be interested in anyone's nomination for a great reference book on SEM techniques. Tom Phillips <phillipst@missouri.edu>

I usually do 20 mA for 60 sec on a Denton Desk II coater at 75 mT. These three variables determine the film thickness. If after coating you get charging, the coating is too thin or not grounded. Consider that some "conductive" tabs are not. Silver the top of the tab to the stub at some point and re-try. A small exposed area of the stub created by cutting off a piece of the tab will allow the coating to connect the tab surface to the stub...ensuring a good ground. The other option is to increase coating time. You should not see charging when using BSE. Gary Gaugler <gary@gaugler.com>

With silver paint being used, the sample should be well earthed to the support. Presumably, the support is well earthed/clamped to the sample stage in the microscope? If so, then the sample must be inherently the problem - Is the sample undercut or spongy? We have sometimes re-coated from three or four directions by laying the sample on its side or tilted at 45° or thereabouts. This can help. Keith Ryan <k.p.ryan@btinternet.com>

The problem can be minimized, but it helps to understand its origins. For introductory reading, the textbook by Postek et al. can't be beat. It is what I use for classes in SEM. It will explain a number of different ways you can get charging. The answer to all of the problems is the same - maximize conductivity in the specimen and between the specimen and mount, such as the stub. Carol Heckman <heckman@bgnet.bgsu.edu>

1) Always look near the closest area to electrical ground on the sample. If you need to look further away, then you should place a conductive path closer. 2) Height on the sample (distance to the target) is very important in a coater. 3) Rotating the sample in a coater is important. 4) Try various HV/EHT in the SEM. 5) Try different scan averaging and times. Pixel averaging is usually the worst for combating charging. Line averaging is in the middle. Frame averaging is the best. Anonymous posting.

Have you tried the OTOTO technique? It should work for secondary imaging, but not for the immuno work since osmium is only 3 atomic numbers from gold. On the other hand, 3 seconds seems to a very short time for carbon coating, depending on what the deposition rate is for your sputter coater. Sputtering for a longer period seems to be a possibility and/or tilting the sample and sputtering from different angles should help. As you say, some samples are just difficult to make conductive. Bill Chissoe <wchiss@ou.edu>

Gold is the standard that most people use for sputter coating, so you must be aware that platinum takes twice as long to put down. You do not say what type of sputter coater you use (important as there are about five different styles of coater needing different techniques) but if it is a coater graduated in kV, I would say that 70 seconds at 10 mA was much too short and with too low a current. I would expect 20 mA for 1.5 to 3 minutes absolute minimum with 5 cm target to specimen distance. Secondly, if your materials have complex or rough structures you will need to coat at least two different angles. Coat for 2 min with a 45° tilt in one direction and then 2 min with a 4° tilt in the other. Carbon coating is much less efficient than sputtering, so unless you spin or tilt the specimen during coating, one would expect the results to be far worse. You are using a very low kV and an extremely low emission current (unless you have a FEG which I guess you do?) which should be ideal, so I would also ask which Hitachi machine you are using? If you have a double detector system use the lower detector as this relies less on SE, reducing the level of visible charge. Upper detectors are almost pure SE detectors so are prone to showing charge. What type of conducting tape do you use? The only good tapes are double sided carbon, any other style and in my experience you are asking for trouble. Steve Chapman <protrain@emcourses.com>

Since you're stopping at 50kX, there are several things you can do, depending on heat sensitivity of your samples which I assume is minimal. Coat longer, as you've already discovered. Use a higher current or higher pressure. This depends on the brand of your coater, and how close the sample is to the target. 100 mT and 10 mA is what we use for one coater and 120 to 140 mT and 30 mA for the other. Raising the pressure is better – you get a shorter mean free path (MFP) for the sputtered metal atoms, therefore, they come at the sample from more angles and you get a more uniform coating on samples with lots of topography (at the microscopic level), like most biological samples. There is not much absolute difference in MFP between these two pressures, but there is about 20% relative difference. What is the pressure you're using? 100 mT? Try 120 mT unless you've got one of the coaters that control the current by controlling the gas pressure. If so, then drop the sample stage 1 to 2 cm and raise the pressure to 120 mT or so. "Conductive tape" is less conductive these days, it seems. Don't just ring the samples with Ag paint, but run a line of paint from the sample to the metal stub – "wire" the sample to the metal, so the electrons have a direct path to ground. I did this with setose mouthparts from crustacea, and it worked fine at 20kV. Carbon: sputtering or evaporating? I'm skeptical of sputtered carbon, although I could easily be wrong. I prefer to use carbon thread in a vacuum evaporator, and place the sample on a spinning + tilting holder to get evenness of coat. But, we do our colloidal gold imaging with a Pt coat of 1 to 4 nm. The gold beads show up fine in the SE, and even better in the BSE, if you've got the right BSE detector, a high resolution one. A "normal" BSE detector needs higher kVs and big spot sizes, which will kill your resolution. For books, I'd recommend Michael Postek et al. "Scanning Electron Microscopy: A Student's Handbook". Paperback and the best text available. M.A. Hayat has "Biological SEM" that's good to have, but if you want one book, get Postek's. Philip Oshel <peoshel@wisc.edu>

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Microscopy CD-ROMs For Children: A Bibliography

Caroline Schooley, Project MICRO Coordinator, MSA

Project MICRO (Microscopy In Curriculum - Research Outreach) is the Microscopy Society of America's precollege educational outreach program. Its goal isn't to recruit young microscopists; rather, MICRO uses microscopes and magnifiers to introduce scientific observation and inquiry as a way to see the world. The program is designed for middle (and upper elementary) schools, because at that level it's possible to reach all students before they must choose elective subjects in high school, and because middle school teachers often aren't prepared to teach science well, and want help.

MICRO reaches its student audience in several ways. The centerpiece of the program is a highly successful teacher's manual, *Microscopic Explorations*, written by professional science educators at the Lawrence Hall of Science (LHS) of the University of California at Berkeley. *Microscopic Explorations* is used in organized programs sponsored by MSA local societies and at least one university. *Microscopic Explorations* is part of the LHS GEMS (Great Explorations in Math and Science) series, and its classroom use is taught by LHS-trained "GEMS Associates" in workshops throughout the U.S. and around the world (this summer (2003), in many cities, at MSA's San Antonio meeting and even in the Philippines!). *Microscopic Explorations* is sold nationally in bookstores, educational catalogs and on the Internet, which makes it available almost anywhere.

MICRO supports its program with a website, *www.msa.microscopy.com/ProjectMicro/* that provides a lot of information and advice that can help anyone who is interested in microscopy education. This CD listing is part of a much more extensive bibliography of books, videos, CD-ROMs, and website links available on the website. They aren't all for children in middle school; many are "adult" and are listed to help teachers, parents, and volunteers. Anyone compiling a list like this can use help. If you are aware of other relevant CD-ROMs, please contact the Project MICRO Coordinator, Caroline Schooley, at schooley@mcn.org.

Biodisc, Inc. **Enclycopedia of Biological Microslides** With a 100 page, 8 1/2x11" textbook and a complete image index, $95.00 from Clearvue/eav, 6465 N. Avondale Ave., Chicago, IL 60631-1996, (800)253-2788. Order 384CD 2555 for Mac (7.1 or later) or #84CD 2456 for Windows. Also available directly from Biodisc, 800-453-3003. Over 4700 plant and animal color photomicrographs make this a bargain in comparison to the cost of an equally comprehensive slide collection. Images may be moved and magnification changed, which somewhat mimics actual microscope use. So many images on one CD-ROM means limited resolution, however; pixels are obvious on full-screen images. Grades 7-12.

BioScope Initiative 2000 **Cell Structure and Function** $44.95 plus shipping for a single user, $295.00 site license (25 users), with a 63 page users manual. Order from www.ylearn.com. or (800)320-6715. Autostarts on both Mac and Windows; requires Flash software (included). The BioScope Initiative of Purdue University has just released this CD; it will be joined soon by similar CDs on **Genetics** and **Microbiology**. The title of this one is a bit deceptive; about half of it is "pure" microscopy, rather than cell biology. It uses a web browser format, so Internet-aware students will find it very easy to use. It's interactive and well designed, with good graphics. An active Internet connection is desirable, since there are frequent links with the BioScope website (www.bioscope.org) for supplemental information, current hotlinks, quiz answers, etc. The historical introduction to microscopy is outstanding and the how-to-do-it instructions for basic compound microscope use are clear and grade-appropriate. There is a unique, informative section that describes and compares many modern microscopies. The user can select a sample from a short list and view an actual image of it as seen with each of the microscopes. The cell biology is good, too, including a clever "build a cell" sequence. The medical topics must have been chosen by teenagers or someone who knows them well; the microbiology of pimples is included! Good quizzes can be used for review or test. Grades 9-14. ***RECOMMENDED***

Carlson, S., editor 2000 **The Complete Collection of the Amateur Scientist on CD-ROM** About $35 from software retailers. Published by the Tinker's Guild, Menlo Park, CA 94025,www.tinkersguild.com. For Mac or Windows. Requires a Web browser; Netscape 4.0 is recommended. **The Amateur Scientist** was a popular monthly column in the old-format **Scientific American** magazine. This CD-ROM version, edited by one of the last contributors to the old column, is better than a print collection of the old articles, since on the CD they're indexed and rated for safety and current relevance. There are over 1000 projects, making it a gold mine for science fair participants. There are both chronological and topical indexes. Although there is a "microscopy" category, it contains less than a dozen adult-level articles. Adult.

Carolina Biological 2000 **Neo-Slide Collection** $59.95 each or $379.95 for all seven. Purchase from Carolina Biological, 800-334-5551 or www.carolina.com; requires Windows 95, Mac 7.5, 16 MB RAM, or better. The teacher who is doing "wet" microscopy still needs prepared slides for many topics, and they're expensive. These CDs provide a usable substitute at moderate cost, although one of the CD-ROM atlases might be a better investment for a comprehensive survey of animal tissues; the CD in this series selected for review had just 50 images. The text accompanying each image is well written and informative, and a brief multiple-choice quiz is available with each. Magnification change and measurement are possible. Titles are: BA-40-1308A **Viruses and Bacteria**, BA-40-8792A, **Protozan Diversity**, BA-40-8766A **Algal Diversity**, BA-40-1342A **Fungi Diversity**, BA-40-1350 **Plant Diversity**, BA-40-1060A **Animal Cells and Tissues**, and BA-39-1130 **Human Disease**. Grades 6-14.

Clearvue/eav 1998 **How to Use the Compound Microscope** $75.00; from the publisher at 800-253-2788; 6465 N. Avondale Ave., Chicago, Il 60631-1996. Requires Mac(68040) or Windows 3.1 or 95. The CD adds the teaching amenities that CDs do so well: editing and report writing, multiple choice tests, and individual student records. Instructions on the use of a compound microscope are clear and well illustrated. The advice on the use of a substage condenser may be a bit too simple (move it to adjust image brightness), but most middle school microscopes don't have condensers. Poor advice is given on the use of oil immersion objectives. A major error in the original video is repeated; benzene or ether are suggested to clean lenses. Ether is very flammable, and benzene is a biohazard; neither has any place in a middle school science lab. Half of the CD content is a survey of microorganisms, and there is good advice on preparation of hay infusions and wet mount slides. High school.

Clearvue/eav **Microscopic Anatomy** $110.00 from Clearvue/eav; order #84CD 2589; for either Mac (7.0) or Windows. This is a less comprehensive collection than Biodisc/Clearvue's **Encyclopedia of Biological Microslides**; there are 1200 mammalian images. It does include electron micrographs, plus nearly 2000 test questions. If this CD is too expensive, a good atlas is available on the Web at http://www.udel.edu/Biology/Wags/histopage/histopage.htm Grades 10-12 and adult.

Corel Corporation **Corel Professional Photos** About $25.00 per CD-ROM; contact Corel, 1600 Carling Ave. Ottowa, Ontario K1Z 8R7, Canada, phone (613)728-8200 or FAX (613)761-9176 for the names of retail dealers. For both Mac and Windows. Copyable professional stock photos are an excellent source of high quality images for classroom use. Corel has hundreds of titles; two relevant ones (each with 100 images) are #645000, **Microscopic Images** (colorized SEMs of biological subjects) and #610000, **Sand Grains of the World** (stunning images of shell, gemstone, and other mineral sands). . Adult.

Corel CD Home 1997 **Beyond the Naked Eye** *$32.95 from the Edutain*ment Catalog as item #353049, call 800-338-3844; or other retail sources. For DOS, Win 3.1, and Mac; installs easily. Corel's three-part *Life Science Mystery* series begins on this CD with the microscopic world of bacteria and viruses. Two situations are presented to the student-scientist: diagnosis of a bacterial disease and creation of an AIDS-awareness program. There's a lab in the research center, with an incubator (for the bacteria), light and scanning electron microscopes, and a harassed technician. The microscopes are used effectively, but the emphasis is on microbiology; scientific method is presented well. The small-world adventure continues in the second CD, **The Green Files** (not reviewed) and concludes with **Crisis at the Anamalia Research Center**. The reviewed CD is similar to **Science Sleuths** (see below), but for somewhat older students; grades 8-12.

Cubic Science, Inc. 1997 **Virtual Physics** $34.95; from Cubic at 800-383-6363 or #712001 from The Edutainment Catalog, P.O. Box 21210, Boulder, CO 80308; 800-338-3844 . Minimum system: Windows95 or 3.1 (Pentium 75) or Mac (68040) with 12MB. Waves, light, mirrors, and lenses are among the experimental topics covered with animated tutorials, movies (very low resolution), and review problems. There is a simple "space aliens"

NETNOTES

TEM - carbon coating replicas

I need some advice in order to optimize the carbon coating process of my replicas for TEM. Alessandro Mattozzi <alessandro@polymer.kth.se>

We can share our optimized conditions for carbon coating. The carbon rod which we use is about 1/4" in diameter. We sharpen one end (about 1/4" length) of rod to 1/8" in diameter. In our experience, the vacuum in the evaporating chamber is the most important parameter for making the high quality carbon films. A higher vacuum is better. Normally, we use 1.5 X 10^{-6} torr. The best current value is really dependent on the vacuum which you use. Lower vacuums need a lower current to get sufficient speed of carbon film growth; higher vacuums need a higher current value. However, the carbon film made under low vacuum is easily oxidized and the film is very soft and prone to breaking. Another tip is that the speed for growing the carbon film is related to the current value. However, the maximum current for growing a high quality carbon film is limited by the value that will cause you to see a spark coming from the filament. The spark is a large group of carbon molecules which will mess up your carbon film. Wendy Zhang <wendy@grid-tech.com>

I agree with Wendy Zhang: A better vacuum results in a better carbon film quality. But, using Electron Gun evaporation in combination with good vacuum delivers an even better result. The carbon must be well dispersed to produce a fine, uniform and stable film. Thermal evaporation is not an effective method to produce mono atomic vapors. It's more like clouds of 10-15+ atom clusters. Electron beam evaporation utilizes bombardment of carbon by accelerated electrons. This method is more effective in forming nearly mono atomic carbon "clouds" and therefore better carbon film homogeneity/uniformity. This process also may be better controlled than thermal evaporation. Finally, you may produce very thin, uniform and stable films. Personally, I am using 1.2-1.5 nm thick films for routine work in shadowing and negative staining. Sergey Ryazantsev <sryazant@ucla.edu>