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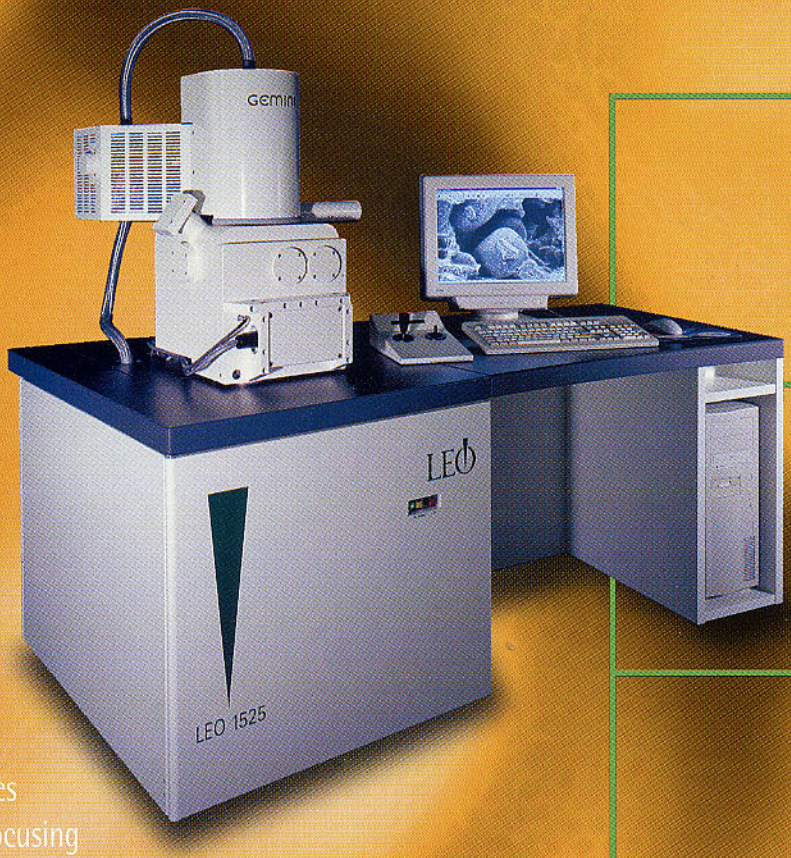

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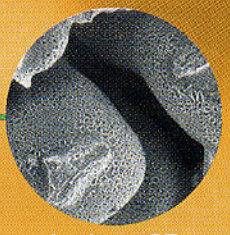

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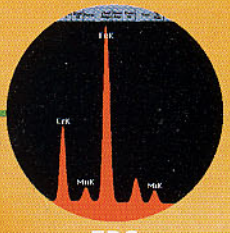

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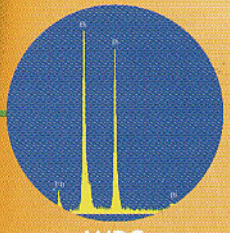

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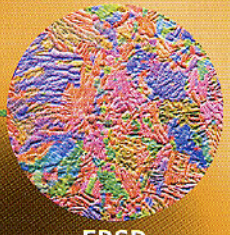

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WATCHING RAFTS MOVE WITHIN CELLS: A FLUORESCENCE MICROSCOPE-BASED TRANSPORT ASSAY

Stephen W. Carmichael and Jeffery L. Salisbury[1]
Mayo Clinic

Imagine a raft in a canal between point A and point B. On that raft is a visible (fluorescent) cargo. Also, attached to that raft is a motor that will propel the raft only from A to B (anterograde transport). When the raft gets to point B, another motor is attached that can propel the raft, and its cargo, and the anterograde motor, back to point A (retrograde transport). Within a cell, the canals are microtubules, and a lot is known about anterograde and retrograde transport in some systems, but these phenomena have not been directly observed in a living, intact animal. Until now, that is. In a pair of very interesting papers, the laboratory of Jonathan Scholey has shown us convincing micrographs of anterograde[2] and retrograde[3] transport in an important animal model.

Using the nematode Caenorhabditis elegans, Scholey's group introduced chimeric genes encoding a fusion of Green Fluorescent Protein (GFP) and several specific target proteins. Using a fluorescence microscope, they could visualize fluorescing dots that moved within the animal and measured the speed of the movement. Specifically, they looked at intraflagellar transport (IFT) in cilia of chemosensory cells that function as the "nose" of this worm. One of the proteins they studied, referred to as OSM-6, is a component of a macromolecular complex that is actually called an "IFT raft." Using the fluorescent GFP as a marker for the location of OSM-6, they could visualize and quantitate the movement of the IFT raft from the base to the tip of a cilium. In addition, they linked GFP to a subunit of a member of the kinesin family, molecules known to drive anterograde transport. Interestingly, they found that the raft (OSM-6), and its motor (the kinesin subunit), traveled at the identical speed of 0.65 µm per second. To bring these movements into macroscopic virtual perspective by scaling up from a microtubule with a diameter of 24 nm to a canal 1 meter wide, the virtual raft is moving along at about 94 km/hour (about 63 miles per hour). That's a fast moving raft with a powerful motor!

Concerned that the recording technique could give the same speed of travel for all proteins measured, Scholey's group observed the movement a ciliary transmembrane receptor (referred to as ODR-10) with GFP and measured its speed at 1.59 µm per second (blazing along at 153 mph in our scaled-up scenario). This established that the identical speeds recorded for the raft and the motor were not an artifact of the recording technique, but rather they were traveling together.

In the second paper, Signor *et al.* measured retrograde (from the tip of the cilium back to the base) transport of the molecules thought to be components of the IFT raft (called OSM-1 and OSM-6) and for the kinesin molecule (the anterograde motor that would accumulate at the tip of the cilium if not transported back). The speed of this return trip was about 1.1 µm per second (over 100 mph!). But it was known that a different molecular motor drives retrograde transport. To dissect out this part of the mechanism, Scholey's group worked with a mutant of *C. elegans* that lacked a specific molecule of the dynein family (referred to as the class DHC1b cytoplasmic dynein CHE-3). In the animals lacking this particular molecule, anterograde transport of the IFT raft was normal, but retrograde transport was inhibited, implicating this molecule as the specific molecular motor for retrograde transport of the raft, along with its cargo, including the anterograde motor.

These papers present a novel biologic assay for intracellular transport in an intact animal. In addition, they demonstrate the usefulness of the C. *elegans* model for studying transport. Genetic studies have identified 25 genes that are essential for ciliary function in this animal. Scholey's group have shown that specific gene products can be tagged (in this case, with GFP), or deleted, and the role of the gene product on ciliary function demonstrated. Since cilia (and the structurally similar flagella) are important biologic structures, this paves the way for even more interesting studies. ■

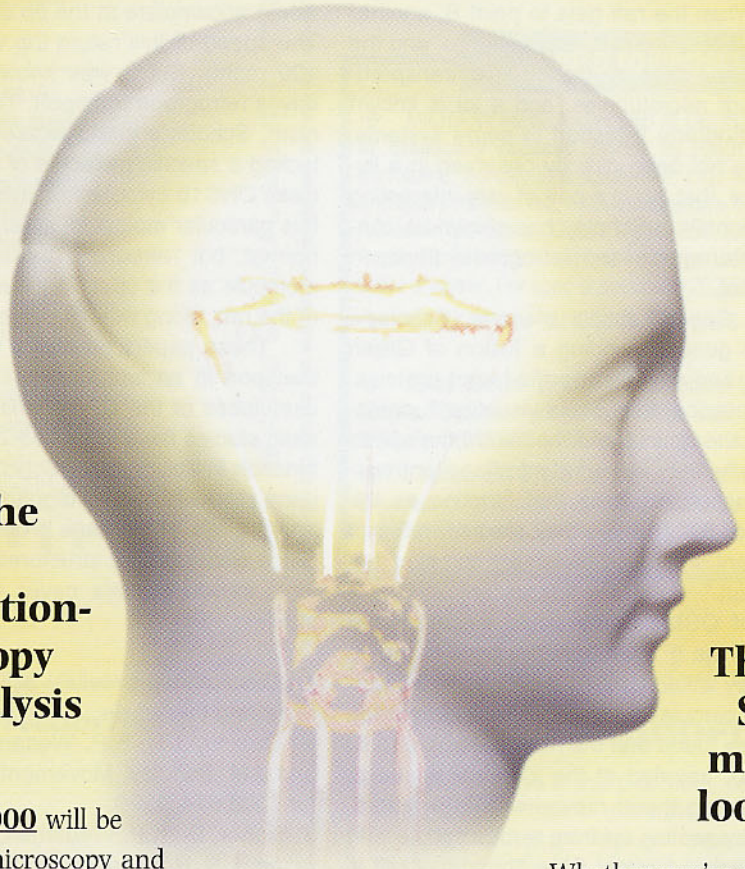
1 The authors gratefully acknowledge Dr. Jonathan Scholey for reviewing this article.

2 Orozco, J.T., K.P. Wedaman, D. Signor, H. Brown, L. Rose, and J.M. Scholey, Movement of motor and cargo along cilia, Nature 398:674, 1999.

3 Signor, D., K.P. Wedaman, J.T. Orozco, N.D. Dwyer, C.I. Bargmann, L.S. Rose, and J.M. Scholey, Role of class DHC1b dynein in retrograde transport of IFT motors and IFT raft particles along cilia, but not dendrites, in chemosensory neurons of living *Caenorhabditis elegans*, J. Cell Biol. 147:519-530, 1999. Video images from this study can be accessed at http://www.mcb.ucdavis.edu/faculty-labs/scholey/

INDEX OF ARTICLES

Smart



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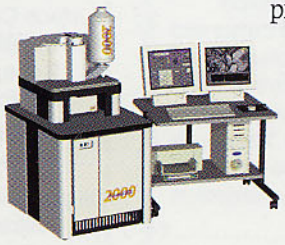

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NEW AND/OR INTERESTING IN MICROSCOPY

➲ NORAN Instruments, a world leader in microscopy and analytical instrumentation, annouces that Dr. Jon McCarthy has been named President. Jon McCarty has been with NORAN Instruments for 24 years, starting in 1975. His most recent asssignment with NORAN has been as Vice President, R&D and Technology. He has been very active in the microscopy community, including serving as President of the Microbeam Analysis Society.

➲ Marine Biological Laboratory Special Topics Courses have been announced:
-Analytical & Quantitative Light Microscopy: May 4/12, 2000.
-Microinjection Techniques in Cell Biology: May 16/23, 2000.
-Optical Microscopy & Imaging in the Biomedical Sciences: Oct.11/19, 2000.

For further information, contact Carol Hamel at MBL, tel.: (508)289-7401, eMail: admissions@mbl.edu

➲ Electron Microscopy & Microanalysis is a one-day course that will be held March 12th at PITTCON 2000 in New Orleans. The course is designed as an introduction to scanning and transmission electron microscopy and x-ray microanalysis for industrial analytical chemists. Numerous examples of the use of electron microscopy in materials analysis are presented.

For further information call (800)825-3221, visit the PITTCON 2000 web site (www.pittcon.org) or contact Dr. Mark Germani, MicroMaterials Research, Phone: (630)325-8170, eMail: mmrinc@wwa.com

A Request To Our Readers

Just as we are fortunate to be able to increase the size of this publication, the problem of finding interesting articles and materials has also increased.

I submit that a considerable number of our readers could help—either by directly providing material or by suggesting that others do so.

The only criteria is that the topic must be of interest to a reasonable number of working microscopists. Any length would be considered and we welcome images, in color or otherwise.

Should you find the publication of value, we would greatly appreciate your help!

- - - Don Grimes, Editor

FRONT COVER IMAGE

The image is an AFM micrograph of plasmid DNA deposited on a mica surface. The image was collected in air with a Digital Instruments MultiMode AFM. The specimen was unfixed and the preparation time was about five minutes. The image shows both supercoiled and relaxed forms of the plasmid DNA. This method can be used for extremely rapid high resolution analysis of a wide variety of nucleic acid and other biological samples.

Image provided by M. Lynch and E. Henderson, BioForce Laboratory, Inc. Ames IA, www.bioforcelab.com

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COMING EVENTS

February 3/4 '00: **Basic TEM Operation and Analytical Procedures**
February 15/17 '00: **Fundamentals of Asbestos Analysis by Transmission Electron Microscopy**
May 23/25 '00: **Fundamentals of Asbestos Analysis by Transmission Electron Microscopy**
October 17/19 '00: **Fundamentals of Asbestos Analysis by Transmission Electron Microscopy**
(MVA, Inc.) Norcross GA: (770)662-8509

✓ February 6/11 '00: **16th Australian Conference on Electron Microscopy** Canberra, Australia, peter.miller@cmst.csiro.au, www.anu.edu.au/EMJ/acem

✓ March 12/16 '00: **High Resolution Electron Microscopy in Materials Science Symposium** (TMS Physical Metallurgy Committee) Nashville, TN, Diane Albert, Los Almos Natl Lab: (505)665-2266, Fax: (505)667-5268

✓ March 12/17 '00: **Pittsburgh Conference** New Orleans, LA, www.pittcon.org

✓ March 15/17 '00: **TEM Specimen Preparation Course.** (South Bay Technology & FEI Company) Univ. of Central Florida, Orlando FL. Lucille Giannuzzi: lag@mail.ucf.edu

✓ April 3/4 '00: **Microscopy of Composite Materials V** (RMS & Oxford Centre for Advanced Materials and Composites) St. John's College, Oxford, U.K. +44-1865-248768, Fax: +44-1865-791237

✓ April 9/14 '00: **Light Microscopy For The Biomedical Sciences (LMBS).** (University of North Carolina) Chapel Hill, NC. Dr. Wayne Litaker: (919)966-1730

✓ April 11/14: **Analytica 2000** Munich Germany, Kallman and Associates (201) 652-3938

✓ April 9/13 '00: **FOCUS ON MICROSCOPY 2000** (12th Annual Meeting of International Conference on Confocal Microscopy). Shjirahama, Japan. http://lasie.ap.eng.osaka-u.ac.jp/fom

✓ April 11/13 '00: **MICRO 2000** (Royal Microscopical Society) London www.rms.org.uk

✓ April 30/May 4 '00: **2000 Annual Workshop on SIMS** Lake Tahoe, NE www.simsworkshop.org

✓ May 4/12 '00: **Analytical & Quantitative Light Microscopy** (Marine Biological Laboratory) Woods Hole, MA. Carol Hamel: (508)289-7401, admissions@mbl.edu

✓ May 9/12 '00: **SCANNING 2000** San Antonio, TX., Mary K. Sullivan: (201) 818-1010, Fax: (201)818-0086, scanning@fams.org

✓ May 11/13 & 15/17 '00: **Quantitative Image Analysis** (NC State University) Raleigh, NC. (919)515-2261, www2.ncsu.edu/cpe/

✓ May 16/23 '00: **Microinjection Techniques in Cell Biology** (Marine Biological Laboratory) Woods Hole, MA. Carol Hamel: (508)289-7401

✓ May 22/June 2 '00: **PASEM 2000** (Univ. of Maryland) College Park, Md., Tim Maugel: (301)405-6898, tm11@umail.umd.edu

✓ June 10/17 '00: **Optical Microscopy in the Biological Sciences.** (Univ. of Texas Health Science Ctr) San Antonio, TX. www.uthscsa.edu/gsbs/csbhome.html

LEHIGH MICROSCOPY SCHOOLS
✓ June 12/16 '00: SEM and X-ray Microanalysis
✓ June 11 '00: Introduction to SEM and EDS
✓ June 19/23 '00: Advanced Scanning Electron Microscopy
✓ June 19/23 '00: Quantitative X-ray Microanalysis
✓ June 19/22 '00: Analytical Electron Microscopy
✓ June 20/23 '00: Atomic Force Microscopy
✓ June 22/24 '00: Thin Specimen Preparation
✓ June 19/23 '00: Microdiffraction
✓ June 21/23 '00: Cryo SEM
For further information, contact Ms. Sharon Coe at Tel.: (610)758-5133 or by eMail at sharon.coe@lehigh.edu

✓ **3D MICROSCOPY OF LIVING CELLS**
June 19/29 '00: 3D Microscopy of Living Cells
July 1/3,'00: 3D Image Processing
Univ. of British Columbia, Vancouver, BC,
www.cs,ubc.ca/spider/ladic/course/bulletin.html

✓ June 22 '00: **16th Annaul Short Course on Molecular Microspectroscopy** Miami University) Oxford, OH. http://www.muohio.edu/~sommeraj

✓ June 23 '00: **2nd International Conference on Scanning Probe Microscopy in Biomaterials Science.** Bristol, U.K., Dr. Klaus Jandt: K.jandt@bris.ac.uk

✓ June 26/30 '00: **7th Asia-Pacific Conference on Electron Microscopy** Singapore. eMail: micngml@nus.edu.sg or medlab2@nus.edu.sg http://www.med.nus.edu.sg/micsoc/7apem

✓ July 9/14 '00: **2nd Meeting of the International Union of Microbeam Analysis Societies.** Kailua-Kona, Hawaii. www.microanalysis.org/iumas2000

✓ July 9/14 '00. **12th European Congress on Electron Microscopy.** Bruno, Czech Republic. http://www.eurem2000.isibrno.cz/regform.html

✓ July 27/29 '00: **International Kunming Symposium on Microscopy** (Chinese Electron Microscopy Society) Kunming, P.R. China. IKSM Office: IKSM@aphy.iphy.ac.cn

✓ August 13/17 '00: **Microscopy & Microanalysis '00:** (MSA) Philadelphia, PA. Annamarie Dowling / Mary Beth Rebedeau: (708)361-6045, rebgroup@earthlink.net

✓ August 22/26 '00: **Scanning Probe Microscopy of Polymers.** (American Chemical Society) Washington, D.C. Vladimir V. Tsukruk: ((515)294-6904

✓ September 3/8 '00: **11th International Congress of Histochemistry** York, U.K., www.med.ic.ac.uk/external/ichc_2000

✓ October 11/19 '00: **Optical Microscopy & Imaging in the Biomedical Sciences.** (Marine Biological Laboratory), Woods Hole, MA. Carol Hamel: (508)289-7401, admissions@mbl.edu

✓ November 19/23 '00: **First International Conference on Advanced Materials Processing** Rotorua, New Zealand. Prof. Nigel Sammes: n.sammes@walkato.ac.nz

Formaldehyde, Formalin, Paraformaldehyde And Glutaraldehyde: What They Are And What They Do

John A. Kiernan,
Dept. of Anatomy & Cell Biology, Univ. of Western Ontario,
jkiernan@julian.uwo.ca

Aldehydes are the most commonly used fixatives. They serve to stabilize the fine structural details of cells and tissues prior to examination by light or electron microscopy. Research workers, technicians, pathologists and others who regularly use aldehyde fixatives frequently do not appreciate the nature and properties of these compounds or the reasons for choosing to fix a specimen in formaldehyde, glutaraldehyde or a mixture of the two. Misconceptions are widespread also about formalin and paraformaldehyde, the commercial products from which formaldehyde-containing solutions are made.

Properties of formaldehyde and its polymers

Formaldehyde is a gas. Its small molecules (HCHO, of which the -CHO is the aldehyde group) dissolve rapidly in water, with which they combine chemically to form methylene hydrate, HO-CH_2-OH. This is the form in which formaldehyde exists in aqueous solutions; its chemical reactivity is the same as that of formaldehyde. Methylene hydrate molecules react with one another, combining to form polymers (Figure 1). The liquid known as formalin contains 37-40% of formaldehyde and 60-63% of water (by weight), with most of the formaldehyde existing as low polymers (n = 2 to 8 in the formula given in Figure 1). Higher polymers (n up to 100), which are insoluble, are sold as a white powder, paraformaldehyde.

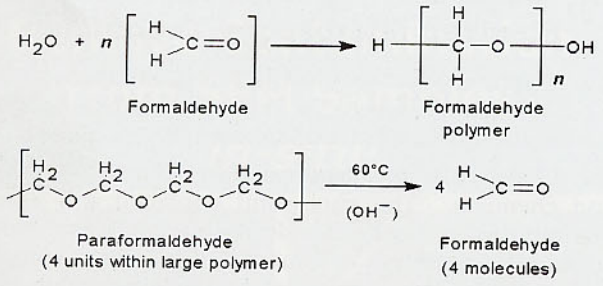


Figure 1: Formation of formaldehyde polymers (above), and depolymerizatiion of paraformaldehyde (below).

To be useful as a fixative, a solution must contain monomeric formaldehyde (or methylene hydrate, to be pedantic) as its major solute. Dilution with water breaks up the small polymers in formalin. This process is said to take a couple of days if plain water is used, but to be almost instantaneous when formalin is diluted with a buffer solution at physiological pH (Pearse, 1980). Hydrolysis of the polymers is catalyzed by the hydroxide ions present in the slightly alkaline solution (Figure 1). The big polymer molecules in paraformaldehyde need more energetic treatment. Heating is necessary, as is an added source of hydroxide ions. In one of the earliest paraformaldehyde-derived fixatives (Richardson, 1960) this was sodium sulfite, but the regular practice for at least 35 years has been simply to heat the paraformaldehyde to 60° C in water containing the salts used to buffer the solution to pH 7.2 to 7.6.

Formalin contains about 10% methanol, added by the manufacturer because it slows down the polymerization that leads eventually to precipitation of paraformaldehyde. A 4% formaldehyde solution made from formalin therefore contains about 1% methanol. It also contains a small amount of formate ions. These are derived from the Cannizzaro reaction, in which two formaldehyde molecules react together, one being reduced to methanol and the other oxidized to formic acid. Because of this slow reaction, the concentrations of methanol and formate in any formaldehyde solution increase slowly with prolonged storage (Walker, 1964). A solution of formaldehyde prepared from paraformaldehyde, which does not initially contain any methanol, is commonly used in fixatives for electron microscopy and in research applications. Satisfactory ultrastructural preservation is, however, also seen in tissues fixed in buffered formaldehyde generated from formalin (Carson, *et al.*, 1973).

Reaction of formaldehyde with proteins

The aldehyde group can combine with nitrogen and some other atoms of proteins, or with two such atoms if they are very close together, forming a cross-link -CH_2- called a methylene bridge. Studies of the chemistry of tanning indicate that the most frequent type of cross-link formed by formaldehyde in collagen is between the nitrogen atom at the end of the side-chain of lysine and the nitrogen atom of a peptide linkage (Figure 2), and the number of such cross-links increases with time (Gustavson, 1956). The tanning of collagen to make leather is comparable to the hardening of a tissue by a fixative (Hopwood, 1969).

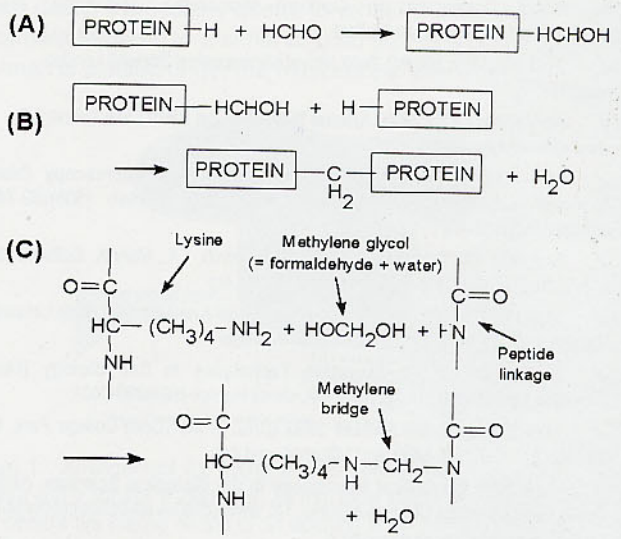


Figure 2:. Reactions involved in fixation by formaldehyde. **(A)** Addition of a formaldehyde molecule to a protein. **(B)** Reaction of bound formaldehyde with another protein molecule to form a methylene cross-link. **(C)** A more detailed depiction of the cross-linking of a lysine side-chain to a peptide nitrogen atom.

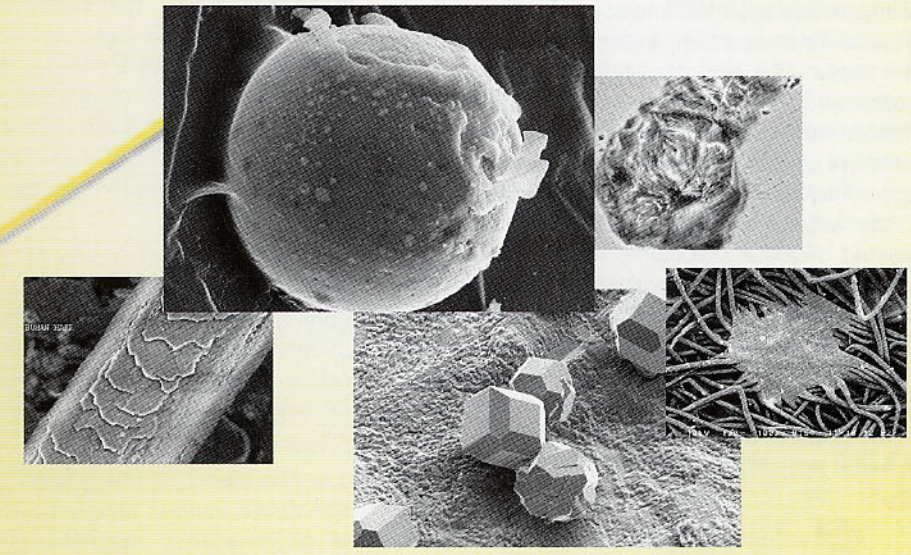
The fixative action of formaldehyde is probably due entirely to its reactions with proteins. Initial binding of formaldehyde to protein is largely completed in 24 hours (Helander, 1994) but the formation of methylene bridges proceeds much more slowly. Substances such as carbohydrates, lipids and nucleic acids are trapped in a matrix of insolubilized and cross-linked protein molecules but are not chemically changed by formaldehyde unless fixation is prolonged for several weeks.

Practical considerations relating to formaldehyde

This is the most important bit. Formaldehyde penetrates tis-

Continued on page 10

Hassle-Free



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Formaldehyde, Formalin, Paraformaldehyde And Glutaraldehyde: What They Are And What They Do

Continued from page 8

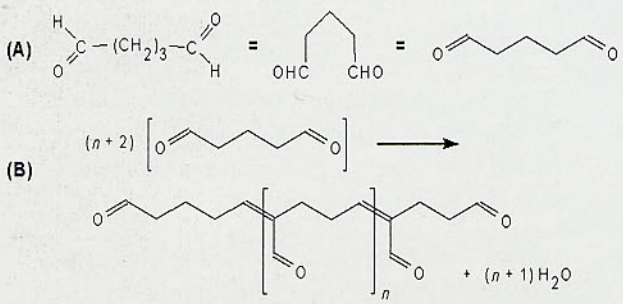
sues quickly (small molecules), but its reactions with protein, especially cross-linking, occur slowly. Adequate fixation takes days, especially if the specimen must withstand the osmotic and other stresses of dehydration and infiltration with paraffin. Brief fixation in formaldehyde (ideally delivered by perfusion) can stop or greatly reduce autolysis and confer slight hardening and some resistance (but not much) to liquids that are not iso-osmotic with the tissue. This can greatly improve the structural integrity of cryostat and other frozen sections, especially if followed by infiltration with a cryoprotectant such as sucrose (ideally 60% but more usually 15-30%).

When a specimen is dehydrated after only a few hours in formaldehyde, the largely unfixed cytoplasmic proteins are coarsely coagulated. Nuclear chromatin, which contains DNA and strongly basic proteins, is also coagulated by the solvent, forming a pattern of threads, lumps and granules. This is not unlike the appearance induced by fixatives that contain acetic acid, but it is less satisfactory for identifying cell-types on the basis of nuclear morphology. (After adequate formaldehyde fixation, chromatin displays a remarkably even texture, also of little diagnostic value but possibly closer to the structure of the living nucleus.)

Glutaraldehyde solutions

Before 1962 the only satisfactory fixative for electron microscopy was buffered osmium tetroxide. This preserves cellular structure by combining with lipids, especially in membranes, and by insolubilizing some proteins without coagulation, but it is expensive and toxic, penetrates tissues extremely slowly, and extracts much protein and RNA. With the introduction of glutaraldehyde (Sabatini *et al.*, 1962) electron microscopists had a more rapidly penetrating fixative that thoroughly insolubilized proteins and was cheap enough to deliver by vascular perfusion.

Glutaraldehyde has fairly small molecules, each with two aldehyde groups, separated by a flexible chain of 3 methylene bridges. It is HCO-$(CH_2)_3$-CHO. The potential for cross-linking is obviously much greater than with formaldehyde because it can occur through both the -CHO groups and over variable distances. In aqueous solutions, glutaraldehyde is present largely as polymers of variable size (Monsan *et al.*, 1975). There is a free aldehyde group sticking out of the side of each unit of the polymer molecule (Figure 3), as well as one at each end. All these -CHO groups will combine with any protein nitrogens with which they come into contact, so there is enormous potential for cross-linking, and that is just what happens (Figure 4). There are also many left-over aldehyde groups (not bound to anything) that cannot be washed out of the tissue.



Figuire 3: (A) Three representations of a molecule of monomeric glutaraldehyde. (B) Polymerization reaction of glutaraldehyde, showing an aldehyde side-chain on each unit of the polymer.

Practical aspects of glutaraldehyde fixation

Five important points must be remembered when using glutaraldehyde as a fixative for light or electron microscopy.

1. If it's to be any use as a fixative, especially for electron microscopy, the glutaraldehyde solution must contain the monomer and low polymers (oligomers) with molecules small enough to penetrate the tissue fairly quickly. This means you must buy an "EM grade" glutaraldehyde (25% or 50% solution), not a cheaper "technical" grade. The cheaper stuff, which is for tanning leather, consists largely of polymer molecules too large to fit between the macromolecules of cells and other tissue components.

2. The chemical reaction of glutaraldehyde with protein is fast (minutes to hours), but the larger molecules, especially the oligomers, penetrate tissue slowly. A rat's brain left overnight in a buffered glutaraldehyde solution and sliced the next day shows a colour change and harder consistency to a depth of 2 to 3 mm. Objects fixed for a few hours in glutaraldehyde are no longer osmotically responsive (Paljarvi *et al.*, 1979).

3. The free aldehyde groups introduced by glutaraldehyde fixation cause various problems. These include non-specific binding of proteinaceous reagents, notably antibodies, and a direct-positive reaction with Schiff's reagent. The free aldehydes must be removed or blocked by appropriate histochemical procedures, as described in textbooks (Culling *et al.*, 1985; Kiernan, 1999, Ruzin, 1999), before attempting immunohistochemistry, lectin histochemistry, or the Feulgen reaction or periodic acid-Schiff staining on glutaraldehyde-fixed material.

4. The thorough cross-linking of a glutaraldehyde-fixed specimen impedes the penetration of fairly large paraffin wax molecules. This makes for difficult cutting and peculiar differential shrinkage artifacts within the specimen. You can stain mitochondria nicely in cells surrounded by obviously abnormal spaces. This is an exaggeration of the inadequacy of formaldehyde and osmium tetroxide as fixatives to precede paraffin (Baker, 1958), and it also highlights the shortcomings of predominantly coagulant

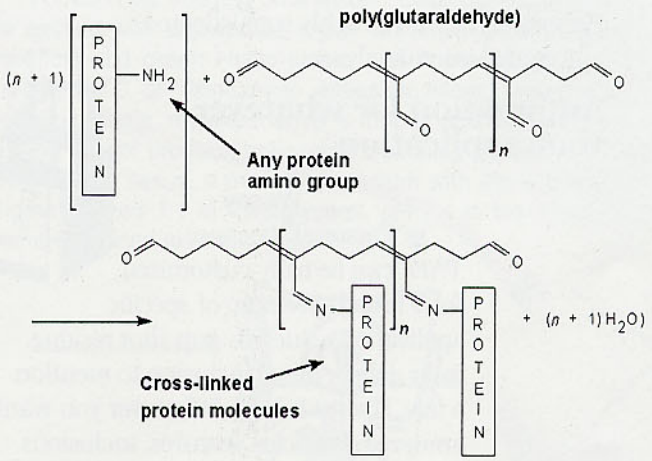


Figure 4: Reaction of poly(glutaraldehyde) with amino groups of proteins.

Continued on page 12

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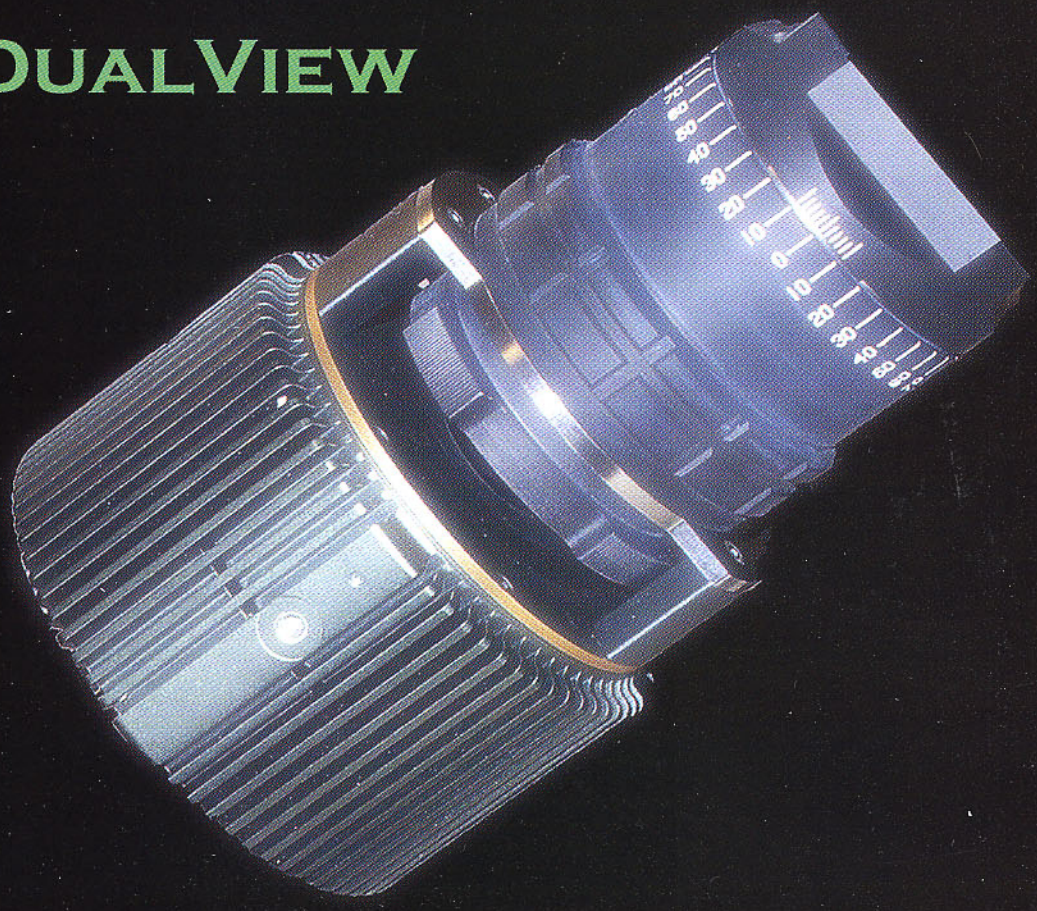

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Formaldehyde, Formalin, Paraformaldehyde And Glutaraldehyde: What They Are And What They Do

Continued from page 10

fixatives (AFA, Davidson's, Bouin, etc.), which preserve the micro-anatomy well but destroy or displace little things like organelles. Fortunately, plastic monomers penetrate glutaraldehyde-fixed tissue adequately. It has been shown that they do not enter every crevice (Horobin & Tomlinson, 1976), but there is enough support to allow the cutting of ultrathin sections for electron microscopy.

5. Immunohistochemistry, which requires as many intact amino acid side-chains as possible, is severely impaired by glutaraldehyde fixation. Nevertheless, clever people have generated antibodies to individual amino acids, that are glutaraldehyde-bound to protein. These allow the detection of soluble amino acid neurotransmitters such as glutamate, GABA and even glycine in presynaptic axon terminals in glutaraldehyde-perfused central nervous tissue (Hodgson *et al.*, 1985; Hepler *et al.*, 1988; Crooks & Kolb, 1992). Extensive cross-linking also results in the loss or severe reduction of most histochemically demonstrable enzymatic activities, though several are retained after brief fixation (Sabatini *et al.*, 1962).

Mixtures containing formaldehyde and glutaraldehyde

The combination of formaldehyde with glutaraldehyde as a fixative for electron microscopy takes advantage of the rapid penetration of small HCHO molecules, which initiate the structural stabilization of the tissue. Rapid and thorough cross-linking is brought about by the more slowly penetrating glutaraldehyde oligomers. This mixture is associated with the name of Morris J. Karnovsky of Boston. It is an example of a great innovation that was published only in an unrefereed abstract (Karnovsky, 1965). His original mixture contained 4% glutaraldehyde, which was a higher concentration than many people wanted to use (Hayat, 1981). Designations like "half-strength Karnovsky" became common parlance in the 1960s and 1970s. Fixatives of this kind allowed the definitive descriptions of EM-level histology that were accomplished in the 5 or 6 years that followed the introduction of Karnovsky's fixative, and they are still routinely used. ■

References

Baker, J.R. (1958). *Principles of Biological Microtechnique* (Reprinted 1970, with corrections). London: Methuen.

Carson, F.L., Martin, J.H. & Lynn, J.A. (1973). Formalin fixation for electron microscopy: a re-evaluation. *American Journal of Clinical Pathology* **59**: 365-373.

Crooks, J. & Kolb, H. (1992). Localization of GABA, glycine, glutamate and tyrosine hydroxylase in the human retina. *Journal of Comparative Neurology* **315**: 287-302.

Culling, C.F.A., Allison, R.T. & Barr, W.T. (1985). *Cellular Pathology Technique*, 4th ed. London: Butterworths.

Gustavson, K.H. (1956). *The Chemistry of Tanning Processes*. New York: Academic Press.

Hayat, M.A. (1981). *Principles and Techniques of Electron Microscopy. Biological Applications*, 2nd ed. Vol. 1. Baltimore: University Park Press.

Helander, K.G. (1994). Kinetic studies of formaldehyde binding in tissue. *Biotechnic & Histochemistry* **69**: 177-179.

Hepler, J.R., Toomim, C.S., McCarthy, C., Conti, F., Battaglia, G., Rustioni, A. & Petrusz, P. (1988). Characterization of antisera to glutamate and aspartate. *Journal of Histochemistry and Cytochemistry* **36**: 13-22.

Hodgson, A.J., Penke, B., Erdei, A., Chubb, I.W. & Somogyi, P. (1985). Antisera to gamma -aminobutyric acid. I. Production and characterization using a new model system. *Journal of Histochemistry and Cytochemistry* **33**: 229-239.

Hopwood, D. (1969). Fixatives and fixation: a review. *Histochemical Journal* **1**: 323-360.

Horobin, R.W. & Tomlinson, A. (1976). The influence of the embedding medium when staining sections for electron microscopy: the penetration of stains into plastic sections. *Journal of Microscopy* **108**: 69-78.

Karnovsky, M.J. (1965). A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology* **27**: 137A-138A.

Kiernan, J.A. (1999). *Histological and Histochemical Methods: Theory and Practice*, 3rd ed. Oxford: Butterworth-Heinemann.

Monsan, P., Puzo, G. & Marzarguil, H. (1975). Etude du mecanisme d'etablissement des liaisons glutaraldehyde-proteines. *Biochimie* **57**: 1281-1292.

Paljarvi, L., Garcia, J.H. & Kalimo, H. (1979). The efficiency of aldehyde fixation for electron microscopy: stabilization of rat brain tissue to withstand osmotic stress. *Histochemical Journal* **11**: 267-276.

Pearse, A.G.E. (1980). *Histochemistry, Theoretical and Applied*, 4th edn. Vol. 1. *Preparative and Optical Technology.* Edinburgh: Churchill-Livingstone.

Richardson, K.C. (1960). Studies on the structure of autonomic nerves in the small intestine, correlating the silver-impregnated image in light microscopy with the permanganate-fixed ultrastructure in electronmicroscopy. *Journal of Anatomy* **94**: 457-472.

Ruzin, S.E. (1999). *Plant Microtechnique and Microscopy*. New York: Oxford University Press.

Sabatini, D.D., Bensch, K. & Barrnett, R.J. (1963). Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *Journal of Cell Biology* **17**: 19-58.

Walker, J.F. (1964). *Formaldehyde*, 3rd ed. New York: Reinhold.

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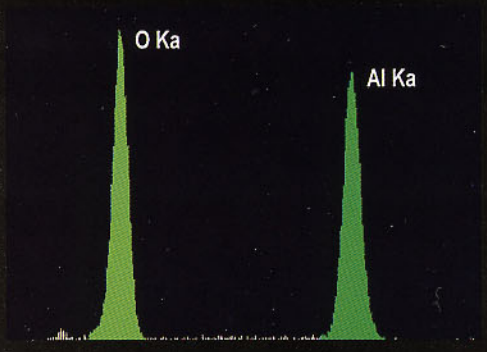

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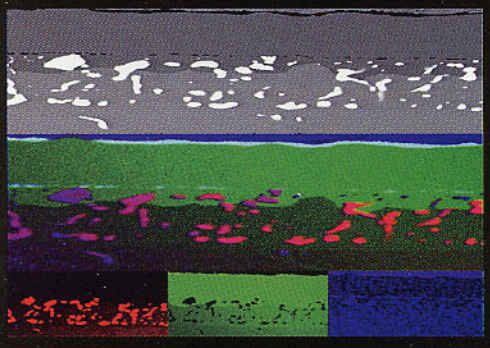

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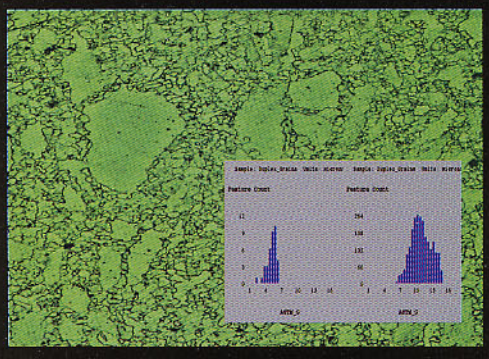

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Saga of the Chemical Microscope

Walter C. McCrone, McCrone Research Institute

The precursor of today's light microscope was invented probably in the Netherlands about 1600. In effect it previously involved a two lens system - objective and the eyepiece. As such it was termed a compound microscope. It differed from the simple one-lens microscope of Anthony Leeuwenhoek in the 1600's. The image was so bad, however, that most microscopists preferred to use the simple one-lens microscope. Leeuwenhoek, with his one-lens microscope, magnifying up to 280X, discovered foraminifera and many other "wee beasties", even bacteria and spermatozoa.

The first major improvement to the microscope came about 1830 when John Dolland produced achromatic lenses that made the compound light microscope far superior to even the 280X simple microscope of Leeuwenhoek. About the same time, William Nicol invented the calcite polarizing prism. This produced an instrument of great value to chemists as a microanalytical tool. During the balance of the 19th century, toxicologists and, especially, forensic investigators adopted the polarized light microscope as their principal investigative tool. Analytical chemists soon also recognized its value for identifying unknown substances and for studying chemical problems. Henry Sorby was one of the first such chemists, and by 1896 Emile Chamot was teaching a chemical microscopy course at Cornell University. Other universities soon joined the trend and began their own courses. Many Chemistry departments required such courses for their B.S. degrees. Most industrial laboratories incorporated microscopy and microscopists in their research laboratories.

Starting early in the 20th century we see the invention of other physical analytical methods that, for a variety of reasons, soon became popular and began to replace microscopy. X-ray diffraction about 1910; emission spectroscopy about 1920; transmission electron microscopy about 1930; infrared absorption about 1940; plus scanning electron microscopy, electron microprobes and x-ray fluorescence during the 50's and 60's soon convinced the scientific world that the light microscope had outlined its usefulness.

The universities gave up teaching chemical microscopy (even Cornell by 1980) and those of us who stuck to that venerable tool were subjected to criticism or ignored. I am not alone in believing, however, that dropping the use of polarized light microscopy (PLM) in the chemical laboratory is a great mistake. I have continued to use PLM to solve a variety of important problems like the identification of asbestos in all of its forms, studying the polymorphic forms of pharmaceuticals and explosives, and, less important, the fakery of the Vinland Map and the Turin Shroud. Partly because of my unpopular debunking of both the Map (VM) and the Turin Shroud, I have experienced considerable difficulty convincing today's scientists that the light microscope is still a valuable analytical tool. Dr. Harry Gove, a strong supporter of the more recent high-tech instruments, states in his recent book "Relic, Icon or Hoax?" "the trouble with McCrone is that his scientific techniques (PLM) are unsophisticated compared to AMS and PIXIE." Dr. Gove developed the Accelerator Mass Spectrometer (AMS) that was used to date the Shroud to the fourteenth century. Dr. Thomas Cahill of the University of Califlornia (Davis) used PIXIE (Photon-Induced X-ray Emitter) to "prove" the Vinland Map is most likely authentic. Dr. Gove admits the Shroud is Medieval but only because his AMS dated it to 1325 ± 65 years. I am certain that both the VM and the Shroud will eventually be accepted as fakes just as PLM proved both to be.[1,2]

The result of this attitude toward PLM and the absence of courses in chemical microscopy (another name for PLM) is the widespread conviction that chemists should use XRD, FTIR, TEM, SEM, SEM/EDX, NMR, GC/MS, and ETC to solve chemical problems. I use PLM instead and I solve problems quickly and confidently but I then include confirmation data from McCrone Associates who have, in addition to PLM, all of the above acronyms and more. The latter then convinces my client, or readers of my published paper, that I was correct.

So, let me detail the changes in PLM since 1980 and suggest who and what could reverse the situation. The records of the McCrone Research Institute (McRI) are an excellent source of the changes that have occurred since 1980. Worst of all, is the drop in enrollment at McRI: from 1589 (1989) to 485 (1999) a nearly 70% drop. The number of papers delivered at Inter/Micro (IM) meet-

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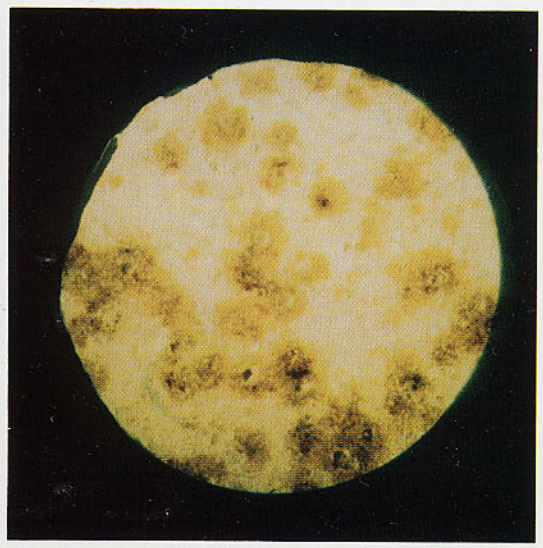

FIGURE 1
The spots on this drug tablet are brownish-red. This indicates a serious shelf-life problem: incompatibility of the components.

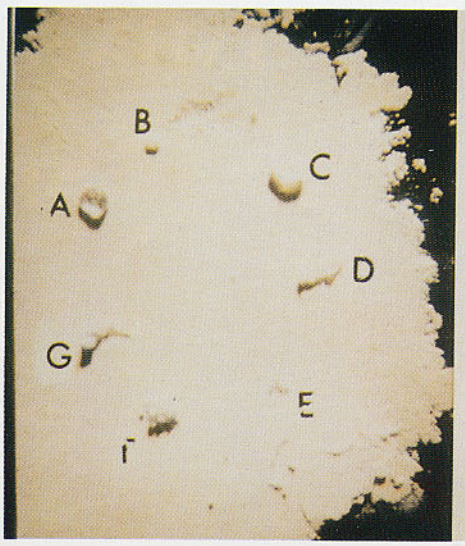

FIGURE 2A

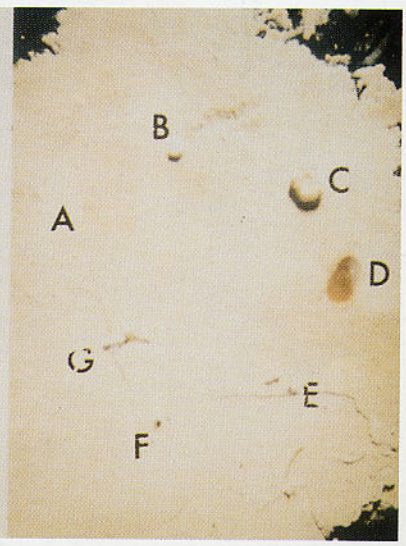

FIGURE 2B
2A shows a thin layer of powdered drug on which tiny known samples of the other components of the tablet have been placed. 2B shows, after heating 5 minutes at 80° C in a microscope hot stage, a brownish-red spot where D, the excipient (oxalic acid) was placed.

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Saga of the Chemical Microscope

Continued from page 14

ings decreased from 62 (1989) to 43 (1999), a 31% decline. Now, for the punch-line, of the 43 papers delivered at IM-89 only 13 have been published, 30 or 70% of those papers have not been published.

The net result of these disastrous decreases is the fact that few Lab Directors or Research Directors have ever used the polarized light microscope. They see only the age of the PLM and the availability of XRD, TEM, SAED, SEM, EDS, NMR, ES, MS, ESCA, Raman, IR, FTIR, DNA, etc. These highly sophisticated, automated, computer-controlled, and expensive instruments must be better than the venerable PLM. Yet, those of us who are lucky enough to be able to still use PLM know we can quickly and correctly solve forensic or, more generally, chemical product and process problems[1-3] that, if solvable by the hi-tech acronyms, would take far more time and money. I will give just one example of the direct problem-solving approach a microscopist takes to solve a vexing chemical problem. Figure 1 shows a drug tablet mottled with brown spots indicating incompatibility of some of the components that occurred during normal shelf-life storage. The microscopist took one look and thought that one of the excipients in these tablets had reacted with another component, very probably the drug itself. So, he pressed some of the powdered drug on a microscope slide and added on top of the powder layer (Figure 2a) single particles or droplets of each of the excipients (obtained from the drug company that submitted the problem). The slide was then placed in a microscope hot stage and heated to 80°C for 5 minutes; the result is shown in Figure 2b. Obviously, Component D, known to be oxalic acid, was the cause of the brown spots. The use of PLM is a direct approach to problem solving and its answers are "yes" or "no" with 100% certainty. Still, the use of PLM techniques like microcrystal tests and optical crystallography are being increasingly ignored in forensic and other laboratories [1].

This is a desperate situation. What can we do? The answer is that each of "us" must publish and lecture on examples of PLM success. We have been very lax in this respect. I admit my own sinful past and during the past year or so I've published half a dozen papers covering unpublished work done from 20 to 60 years ago[2-7]. I've also added a second week of American Chemical Society lecture tours to local sections scattered throughout the 50 states. It's up to all of "us" to concentrate on lectures and publications. After all, it's our job we're fighting for. I now understand what I've heard for years at universities: "Publish or Perish". ■

1. McCrone, Walter C. "The Vinland Map" Anal. Chem. (1988) 160, 1004-1018.
2. McCrone, Walter C. "The Shroud of Turin, Blood or Artists Pigments", (1990) 23, 77-83.
3. Evans, Hiram H. "Drug and Microcrystal Tests for Forensic Drug Identification, Microscope (1999) 47:3, p. 147
4. McCrone, Walter C., "Choosing Proper Chemical Problem-Solving Instrumentation" American Laboratory (October 1998) pp. 27-34.
5. McCrone, Walter C. "Attention Laboratory Directors", American Laboratory (April 1999) pp. 37-44.

6-8 McCrone, Walter C. "Identification of Organic High Explosives" I-III, Microscope (1993) 41:3, 161-182 (I); (1944) 42:2, 61-72 (II); and (1999) 47, 183-200 (III).

9. McCrone, Walter C. and Michael A. Bayard "Individualization of Hair" Microscope (1999) 47 3 pp. 129-134.

India Ink as a Tracer for Circulatory System Study in a Ganglion, With a Note on Using Ferritin, Lanthanum, and Horseradish Peroxidase for the Blood-Brain Barrier

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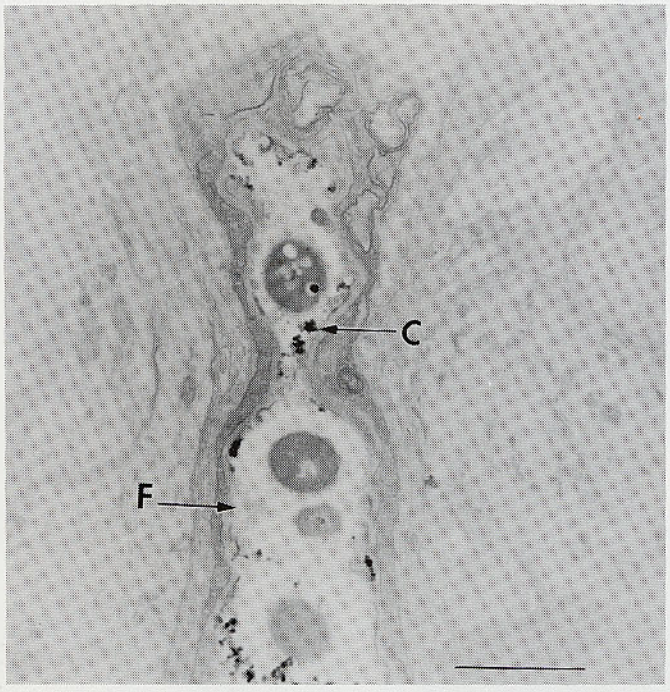
Several summers ago, while working at the Marine Biological Laboratory in Woods Hole, Massachusetts, George Augustine (Duke University) and I wished to look at the circulation in the giant synapse of the squid, *Loligo pealei*. At that time, Dr. Eugene Copeland suggested the use of India ink in order to see the circulatory system. The ink contains carbon particles that are visible under the EM. In addition, it is visible to the naked eye.

The India ink was first sonicated for 15 minutes and then 0.1 mL of it was added to the fixative solution (2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 with 0.8 M sucrose). The squid was perfused through the abdominal aorta initially for 30 minutes at room temperature. The giant synapse was then removed and submerged into fixative overnight at 4° C.

In later experiments, we included ferritin (Calbiochem) to the perfusate to see if it crossed the blood-brain barrier. First, the ferritin was spun down for 30 minutes at 30 thousand rpm to concentrate the ferritin. Finally, 5 mL of ferritin and 2 drops of India ink were added to 5 mL of 2X artificial sea water (ASW). The final concentration of ferritin was 2.5% in ASW (low Ca^{++}). After perfusing the squid for 30 minutes with this solution, the ganglia was removed and immersed into fixative overnight at 4° C.

Our main purpose was to be able to identify the blood vessels, and for this, the India ink worked very well. The carbon particles of the ink are larger and easier to see than ferritin. The ferritin did not cross the blood brain barrier, and demonstrated the tight junctions of the pericytes which form the blood vessels. We also tried horseradish peroxidase (HRP) and lanthanum. The lanthanum did pass through the blood vessels, while the horseradish peroxidase (HRP) did not. ■



Blood vessel of *Loligo pealei* filled with India ink carbon (C) and ferritin (F). Blood cells are visible within the vessel. Scale bar = 1 µm.

Reference:
Sanchez, M. E., C. M. Nuno, J. Buchanan, and G. J. Augustine. 1990. Contractions of the squid stellate ganglion. J. Exp. Biol. 152, 369-387. (We were able to identify the blood vessels based on our previous work with the tracers.)

Teeth and their Associated Tissues

Barry R. J. Rittman
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The preparation of histological sections of teeth can be both a rewarding and a frustrating experience. This is primarily due to the varying degrees of mineralization of the enamel, dentin, cementum and surrounding bone and the difficulty in retaining original relationships between the calcified and the soft tissues in the final stained section.

The tooth has a central pulp that is a gelatinous loose connective tissue. Surrounding this is dentin that forms the main bulk of the tooth, is composed chiefly of collagen fibers and is approximately 70% mineralized. The crown is covered with enamel, a brittle material that is approximately 96 - 97% mineral. Covering the root is a thin layer of cementum similar to bone in its mineralization (approximately 50%). The tooth *in situ* is held in a bony socket by a collagenous periodontal ligament, with the ends of its collagen bundles inserting into both the bone and the cementum of the root. The surrounding bone is a mixture of spongy bone surrounded by outer plates of compact bone.

One question frequently asked is why would anyone wish to carry out histology on a tooth that is obviously no longer of use to a patient. The answer lies in the wealth of knowledge that may be obtained by examining genetic imperfections, studying the process of remineralization, evaluating new methods for the regeneration of gingiva, and optimizing techniques for the retention of dental implants. Studies are carried out on the structure of enamel, dentin and cementum, patterns of growth during development, comparison of deciduous and permanent tooth development and factors which affect tooth eruption, occlusion, retention of teeth and tooth movement. Continuously erupting teeth such as rodent incisors are often used as models to study the development of the dental tissues as these teeth show cells in all stages of development.

Preparation of tooth sections is generally accomplished by one of two procedures. In the preparation of ground sections, the enamel is retained. In the other, processing involves removal of the mineral component so that tissue can be processed to paraffin wax and sections prepared. During demineralization, the enamel will fragment and is lost. These two general techniques complement each other in the information that they can provide.

Ground sections are prepared by slicing the tooth with an abrasive disc or diamond impregnated saw. During this process, thin slices in the order of 100-200 µm are cut but a considerable amount of the tissue between each section is lost. Sections may then be made thinner by grinding each surface with diamond powders or abrasive papers. With care, sections in the 5 to 10 µm range can be prepared, examined and stained. Ground sections are useful for examining defects in enamel and for general histology after appropriate staining but their preparation requires considerable technical expertise and soft tissues are lost. Thick ground sections can be used to examine carious lesions, orientation of dentinal tubules, retention of filling material, leakage around fillings, implanted materials in bony sockets etc.

A variety of techniques such as Nomarski Differential Interference Contrast can be used for transmitted light microscopy and confocal microscopy can be used to visualize structures at different optical planes in the sections. Using these techniques it is

possible to follow the course of dentinal tubules and growth markers in sections up to several hundred micrometers thick. Reflected light microscopy may also be useful. Staining the surface with toluidine blue, can provide a great deal of histologic detail as the stain generally only penetrates a few micrometers. The stained surface can also be reground to examine additional features that are deeper in the block.

Several methods exist to examine dentinal tubules. A common technique is to remove the pulp, place the tooth in India ink and subject to vacuum. Dentinal tubules are generally filled by this technique. Another technique is Colquhoun's method in which ground sections are soaked in mercuric chloride and then placed in ammonium sulfide. A fine deposit of mercuric chloride fills all spaces including the dentinal tubules. Thinner sections can then be prepared by grinding on abrasive papers.

A major problem with ground sections is the loss of soft tissues during the procedure. If soft tissues are to be retained then the tooth must be processed with a plastic such as methyl methacrylate. Sections may be cut with an abrasive disc and ground thinner using abrasive papers as before. Soft tissues are retained but the plastic embedding medium, unless removed, provides an impermeable barrier to the penetration of many dyes. Additionally, plastic sections tend to curl when they are ground down below a certain thickness. This curling can be minimized by grinding one side and then cementing this face to a plastic or glass slide before completing the grinding.

Fixation can be by perfusion but care must be taken that pressure is carefully controlled. Since the pulp is surrounded by a rigid chamber, excessive pressure is not easy to dissipate and may cause damage to the pulpal vessels. Penetration of agents into the pulp is slower than into bone and this may be improved by removing the apex of the tooth.

Developing teeth have a lower mineral content especially in forming enamel and can often be sectioned without subjecting them to demineralization. Attempts at a partial demineralization generally result in the enamel becoming fragmented and lost. Preferred methods of demineralization include disodium EDTA, sodium formate, and formic acid. EDTA with polyvinyl pyrrolidone appears to work well with many immunochemical techniques. With acid demineralization, tissues must be in a solution that is constantly circulating to prevent saturation of the solution with mineral and reprecipitation of mineral in the hard tissues. Round amorphous deposits of mineral appear to be deposited chiefly in dentin and once formed cannot be removed. Detecting the completeness of demineralization such methods as chemical end point, bending, slicing a portion off the tooth or probing with a needle are not advisable. X-rays are the preferred method.

While this decalcification process removes mineral, it usually does not remove dental filling materials. Amalgams are easy to detect and to pry loose from the tooth. Composite resin filling materials are usually not opaque on X-rays and often difficult to detect. Failure to detect and to remove them prior to processing to paraffin wax can result in considerable damage to the microtome knife.

Fixation, dehydration, and embedding will take longer, as tissues are denser and reagents penetrate at slower rates.

Sectioning is difficult, as the knife has to pass through a series of layers, each of which differs in hardness. Sectioning is gen-

Continued on following page

Teeth and their Associated Tissues

Continued from preceding page

erally best if slow and if the direction of cut is carefully selected to allow a gradual introduction to the most dense regions. Compression in one direction is a common artifact. Passage of the knife through layers that vary considerably in their density and hardness also often results in chatter.

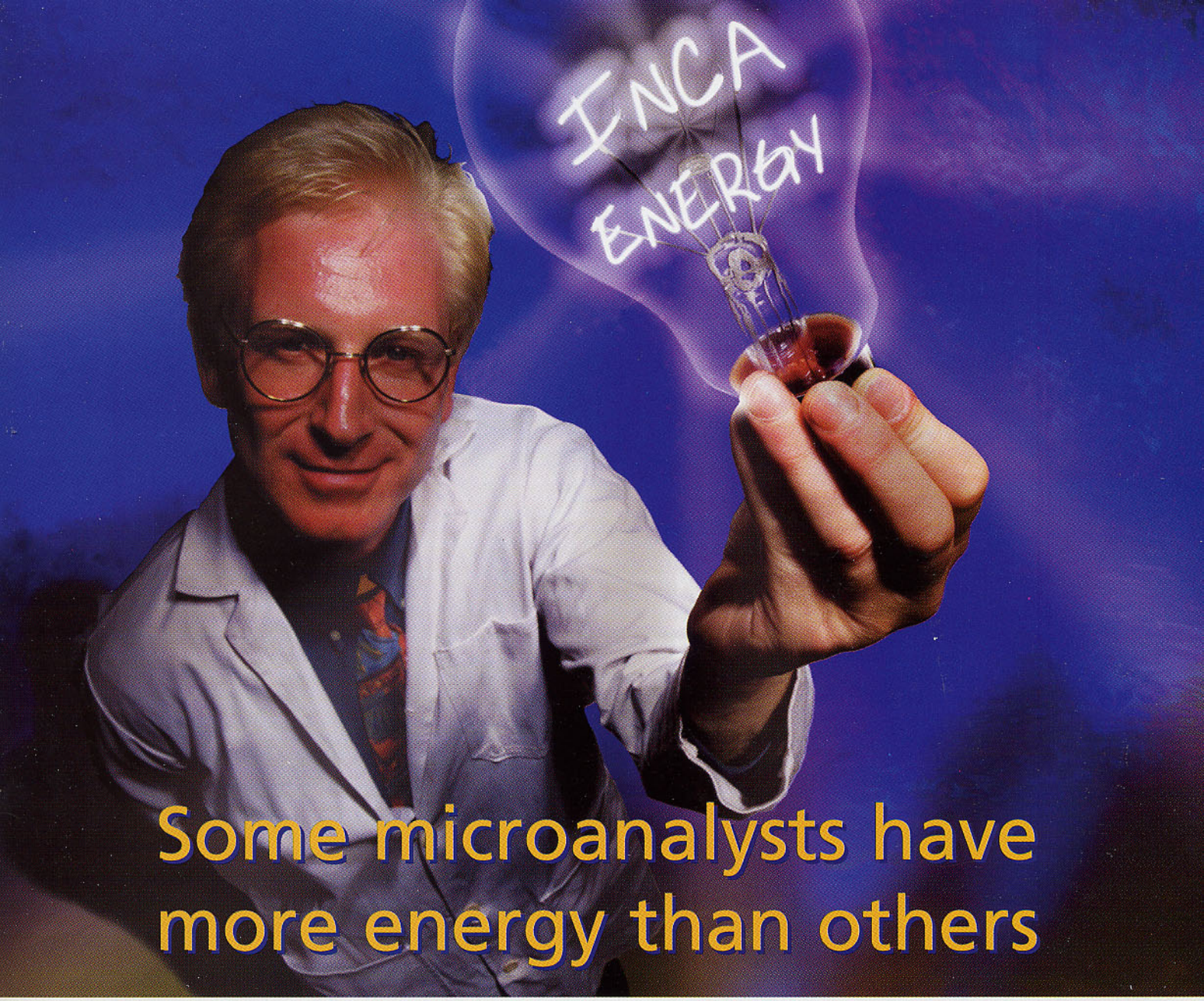
There is a tendency for the collagen fibers in the dentin to swell when sections are floated on a water bath, so when the section dries, folds form in the dentin and often in the surrounding bone. Staining of these areas then occurs from both sides of the section with a resulting darker stain in these areas and sometimes stain precipitation. These can be minimized or eliminated by carefully brushing the dentin with a fine paintbrush. The process is repeated until the hard tissue has dried, usually within a few minutes. This technique sounds crude but works.

Most of the standard staining techniques can be used on sections of bone and teeth but consideration needs to be given to the denser nature of the hard tissues and the slower rate of penetration of the reagents including the dyes. For general histological details, hematoxylin and eosin stain work well but best results are obtained when more dilute staining solutions than those for soft tissues are used. The use of very weak bluing and differentiating solutions is also recommended. Using these weaker staining solutions will accentuate growth and resting lines in the hard tissues. Even if using undiluted stain, more time will be required for staining hard tissues. A 5 minute van Gieson stain is necessary to uniformly stain the collagen fibers in dentin compared to 2 to 3 minutes for soft tissues. Sharpey's fibers embedded in the bone of the socket and the cementum of the root require an intense stain such as Heidenhain's Azan.

Adhesion of sections of hard tissue to the slide is a common problem especially with dentin, but can be improved by thinly coating the section of the slide with celloidin. Celloidin will, however, act as a barrier to enzymes and it will also permanently stain if certain dyes such as celestine blue are used.

Image analysis of teeth, implants and surrounding tissues is usually carried out using ground sections. With care, the amount of shrinkage and distortion with these is kept to a minimum, compared to the use of paraffin sections.

These comments regarding the preparation of hard tissue outline some of the major problems that can be encountered. Workers that have produced a stained section of a tooth with the surrounding tissues will, however, attest not only to the skill that was necessary to produce a section without wrinkles and with even staining, but also the beauty and organization of the tissues. ■



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OXFORD INSTRUMENTS

Surface Charging and Photoeffects on Semiconductor and Insulator Surfaces in AFM

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Having seen these effects often enough, and fielded questions on them from fellow AEM users, we'd like to share our speculations about surface charging effects on semiconductor and insulator surfaces. We do not claim the following to be authoritative, but just what we consider a reasonable explanation for some strange phenomena we have observed.

Q: Can charging have an appreciable effect on an Atomic Force Microscope (AFM) probe?

Yes! Of this we are absolutely sure. Marc Unger demonstrated this once by holding a wire connected to a Si sample while he dragged his foot across a carpet. His Si_3N_4 AFM probe (Au coated or not) would visibly leap toward or away from the sample surface, a deflection of tens of micrometers. (This is not recommend as a daily parlor trick as, if the cantilever comes too close to the sample, there will be an electrostatic discharge (spark) between the cantilever and the sample, which *does* damage the cantilever, and could damage the AFM laser! Applying a DC bias between the sample and tip also caused the cantilever to move. With the cantilever ~250 μm from the sample, a 50 VDC bias generated a 28 nN force on the cantilever. Charge-charge interactions are extremely long range, and they can often have an effect even before the probe is brought anywhere close to imaging a surface. This is all easily understood if the tip-sample interface is thought of as a capacitor, and a rough calculation is done of the number of charges expected for a given voltage.

Q: Can charging interfere with sample imaging?

When there is not a reasonably conductive electrical path from the sample surface to the tip of the AFM probe, and/or when something actively charges either of these two, large charge imbalances can certainly develop and we've seen them have major effects. Common symptoms include:

drifting tip deflection or oscillation magnitude at fixed height, even before engaging.

sudden changes in probe force, sometimes leading to disengagement during scanning, or premature engagement during approach.

an inability to engage the sample surface at all using "normal" engagement parameters.

excessive noise in the feedback-controlled deflection or oscillation signal.

With a Si_3N_4 probe close to a silicon sample, Marc Unger has observed "self-tapping" at between 0.1 and 10 Hz. The probe bends closer and closer to the sample, touches the sample, and springs back all at once. This is actually a pretty amazing demonstration of progressive charging. If the charge was not increasing, the cantilever position would be constant.

Q: What causes sample charging?

We suspect there are three common causes of sample charging, although we do not have experimental evidence for any of these. The first occurs on insulating samples, and mica is the classic example. When a sheet of mica is cleaved, the newly-exposed

surface often seems strongly charged. As mica is an insulator, this charge can not escape.

A second possible source of charge is bleed-off from the 400-some volts applied to the AFM's piezos and from other electrical components in the microscope. Although generally built of conductive metal and grounded through their umbilical cords, AFM's may sometimes be able to pump energy into the sample or probe faster than they can bleed it off. On home-built systems, or systems optimized for STM, the microscope may be only partly grounded, or not grounded at all.

The third effect is seen on semiconducting samples, and while it is only supposition, it makes sense given the evidence we have. Most semiconductor to metal junctions are diodes: that is, they allow current to flow in one direction but not in the other. Additionally, small-bandgap semiconductors (Si and Ge, for example) are readily photoexcited by the red laser light used in most AFM systems, and almost all semiconductors are excited by the trickle of UV light emitted by fluorescent lamps. When both the rectification and photexcitation conditions are present, the semiconductor can act as a solar cell, developing a sizable, light-intensity dependent potential difference between itself and the microscope on which it is mounted. Note that this can also occur for semiconducting probes mounted in metallic probe mounts, and thus both sample and probe can have the same charge. Silicon probes used on silicon samples are the most common example of this.

Q: What can I do to prevent this?

When there's no active charging going on, tools from outside the science arsenal often seem to do the trick. Anti-static devices based on ionized gas and/or ionizing radiation can effectively neutralize the charge on an insulating surface. We suggest either a "Staticmaster" Po-210 α particle-emitting strip (~$35), available from major photography supply houses, or a "Zerostat" piezo-electric ionizing gun (~$7O), available from Aldrich Chemical Co. (Milwaukee, WI). "Staticmaster" devices are often most effective if taped or otherwise held in place over the aperture housing the sample and probe during imaging. Note that these fixes are only effective when the troubling charge is residual on the sample, and not actively generated by any component of the sample or microscope.

When up against active charging of any sort, the sure-fire way to solve it seems to be providing a low-resistance electrical path from the sample to the probe tip. This can be difficult with insulators, but shorting the sample stage to the probe mount should eliminate any active charging taking place in such systems.

When you have a solar cell on your hands, the easiest part to defeat is the diode formed where the semiconductor meets the microscope. In our experience, electrically insulating a semiconductor from the microscope only makes matters worse. Instead, using a carefully selected metal that forms an "ohmic" contact to the semiconductor you are using should be most effective. For Si, indium acts as an effective ohmic contact. An easy-to-use liquid 'glue' can be made by alloying indium with gallium. Simply touching a blob of indium to a small bead of Ga and allowing the two to form a eutectic over a few hours provides a conductive liquid alloy that effectively discharges any Si sample and also holds the Si in place on a sample puck or stage. However, be forewarned: the toxicity of indium and gallium are thought to be low but have not been thoroughly studied, and the eutectic is extremely difficult to remove from both sample and substrate once applied. Marc Unger reports success with Si using carbon conductive tape, also, though we can not vouch for this being an "ohmic" contact.

Another sure-fire solution is to image the sample under a liquid (although we suspect a polarizable liquid like water or alcohol may be needed to eliminate a particularly nasty problem). Liquids reduce the effective range of electrostatic interactions to the point where they are generally negligible. ■

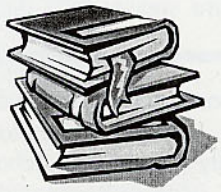
A postscript:

ImageSXM: A Modified Version of NIH Image for SPM Applications

Those who like using NIH-Image or a Macintosh for image processing should by all means take advantage of the hard work and generosity of Dr. Steve Barrett. His freeware "ImageSXM" program effectively integrates a slew of handy microscopy features into NIH-Image, and makes importing images from almost any scanning microscopy technique a snap. To find out more about ImageSXM, or to download a copy of it, visit one of the following sites:

Liverpool, U.K. (Univ. of Liverpool): http//reg.ssci.liv.ac.uk/

Pasadena, CA, U.S.A. (Caltech): http//reality.caltech.edu/imagesxrn.htrn [Note: NIH-Image is a freeware image processing program available at: http://rsb.info.nih.gov/nih-image/ and there is the address for the NIH-Image mailserver in the FAQ--MT.j



PHYSICS OF IMPREGNATION

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There appears to be some confusion as to how materials are impregnated or dried. Without getting into the exciting mathematical details best suited for persons who second language is Greek, I offer the following short explanation as to how materials are impregnated or dried.

If the object is porous, the porosity is described in terms of macropores (>50 nm), mesopores (50 - 2 nm), and micropores (2 - 0.8 nm). These three size classes of pores represent different flow mechanisms for the impregnation of porous materials. Solvent penetration of a non-porous particle by imbibing is similar mathematically to solvent penetration of micropores although the actual mechanism is quite different[1].

a) Flow through large pores or macropores is by bulk diffusion. In this case, the pores are large compared to the mean free path of the solvent molecule. Flow is greater when the pore size is larger or more pressure is applied. The kinetics are described by unsteady-state version of Flick's second law of diffusion[2] since rate varies with the degree of filling.

b) Flow through mesopores is by Knudsen diffusion[3]. In this case, the pores are smaller than the mean free path of the molecule. Flow is greater when the pore size is larger but independent of applied pressure. Over a limited change of temperature both bulk and Knudsen diffusion are essentially independent of temperature.

c) Flow through micropores or imbibing is by activated diffusion. In this case, the solvent molecules interact with the pore surface or molecular structure of the sample. The flow into the micropores or swelling of the material is exponentially dependent on temperature. At room temperatures equilibrium can take weeks or months.

On a more practical note, impregnation of a porous sample is faster in a vacuum. This can be done with a freezing trap with a separable ground glass collection tube. By adding valves to both the side outlet (to vacuum) and center inlet tubes (to solvent) and a liquid to be added to the sample under vacuum. For casting, we usually just made a deep aluminum foil pan, added the sample and then the resin. Place in vacuum oven, evacuate very slowly to degas, and then heat. ■

1. Patrick Meares, *Polymers, Structure and Bulk Properties,* 1967
2. S.P. Nandi, "The Unsteady State Diffusion of Gases from Coals," Ph.D. Thesis, The Pennsylvania State University, 1964
3. F.S. Karn, R.A. Friedal, and A.G. Sharkey Jr., "Mechanism of Gas Flow Through Coal," Fuel, 54, 1975

DiI and DiO Confocal Imaging of Chicken Embryos

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In my research, I am interested in understanding cellular morphogenetic events in early somitic myogenesis in the chicken embryo, and much of this work involves viewing fluorescently labeled cells in embryonic tissues. For this work, I am using 0.2% (weight/volume in 100% tetraglycol (Sigma)) concentrations of dil (1,1', di-octadecyl-3,3,3',3',-tetramethylindocarbocyanine perchlorate) and diO (3, 3'-dioctadecyloxacarbocyanine perchlorate) to focally label small areas (approximately 10 cells) of the epithelial somite known as the dermomyotome.

Dye delivery is by iontophoresis (3 second pulses at 100 µA). DiO is first solubilized in absolute ethanol, and then diluted in 100% tetraglycol. After dye injection and overnight embryo growth, embryos are removed from the egg, cleaned free of extra-embryonic tissues and fixed in 4% formaldehyde (in distilled water) for 4 hours at room temperature. The embryos are then washed in PBS and processed for microscopy or are stored at 4° C.

For confocal imaging, embryos are bisected down the length of the neural tube, and transverse cuts are made several somites cranial and caudal to the injected somite, isolating a small tissue piece.

For each specimen, a custom chamber is easily made by layering 2-5 strips of electrical tape on a clean glass slide (number of layers depends on size of tissue). A small chamber is cut-out with a razor blade and the chamber is filled with phosphate buffered saline (PBS). The tissue is then placed in the chamber and positioned with forceps. A coverslip is placed over the tissue and nail polish is used to seal the slide. The electrical tape chamber provides a fast and convenient way to view tissue material with minimal distortion for microscopy.

For confocal imaging on a Nikon PCM2000, diO and dil labels are excited with appropriate argon ion (lower power setting, 488 nm filter) and green HeNe lasers and fluorescence is captured through FITC or Rhodamine emission filters, respectively. For mounting media, my experience is that dil and diO are highly stable and do not produce noticeable bleaching. However, with antibody labeled tissue, a mounting medium with 2% n-propyl-gallate (Sigma), mixed 1:1 in PBS:glycerol, pH 7.4 in the electrical tape chamber is used to reduce bleaching. ■

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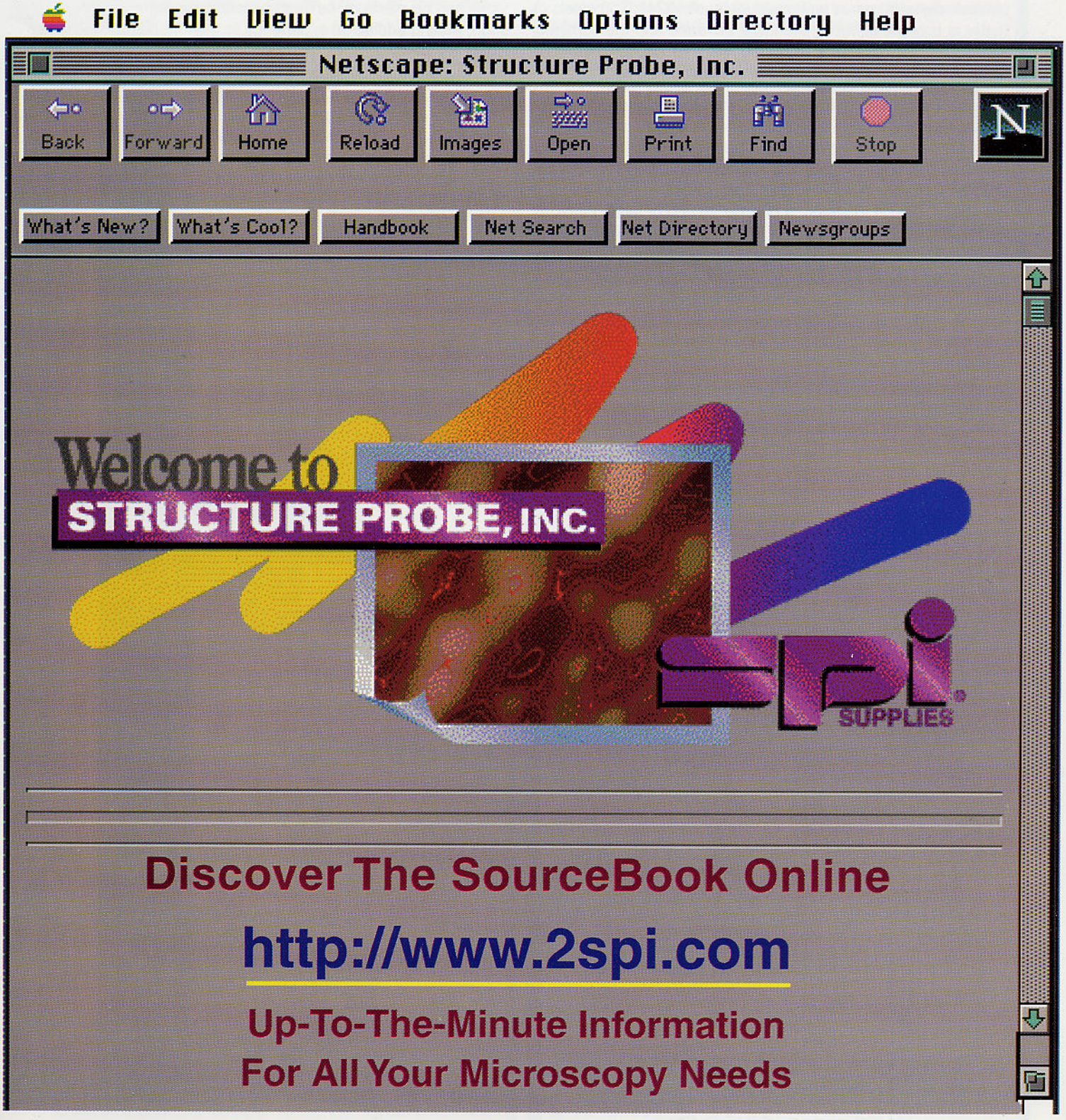

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An Affordable Digital Micrography To Interest Children (Of All Ages) In Science

Norman Lazaroff, Foundation for Microbiological Analysis

Inexpensive fixed-focus digital cameras can be used with homemade lens attachments to obtain good quality close-up images and photomicrographs. The current availability of inexpensive digital cameras with resolutions of 1024 x 768 pixels or better is brought about by technical advance and the continual introduction of competing new models and accessories. The digital camera which I use costs less than $150 now at a liquidation sale or internet auction, and the price continues to decrease. Although more expensive digital cameras are great to have, their advanced features are not necessary for introducing young students to the digital revolution in photography and for exploring the microscopic world.

It isn't difficult to attach a bracket to one of these cameras for holding a close-up lens or microscope eye-piece in front of the camera orifice. To minimize the necessity for critical alignment, good quality bi-convex lenses are used that are at least twice the diameter of the camera lens. In fact, with a surplus three inch diameter, 75 mm copying lens (costing $8.95) it is possible to get great macro-photos even without attaching the lens to the camera.

The lens brackets are made from 1/16" scrap aluminum, cut with drill and tin snips to match a card-board template corresponding to the camera. For macro-photography, the cut out piece of sheet metal is bent in a vise, after which a lens in a plastic retaining ring (made from a bottle-cap) is fastened in place with epoxy cement. The camera's electronic flash can be used for macro-photography if a neutral density filter (made from a darkened piece of negative film) is placed over the flash opening. Alternatively, with the flash turned off, the light from a small desk lamp is sufficient for either auto or timed exposures.

For photo-micrography, an ocular adapter (flanged aluminum ring from an old camera) is fastened to the bracket and fitted with a wide-field microscope ocular in a plastic sleeve. These devices can be made by seventh graders in a home or school workshop, adding additional experience to their introduction to science. Costs for hardware are minimal. Although good new lenses can be expensive ($22 or more) if obtained from optical catalogs, there are many possibilities for getting excellent used or surplus lenses. The costs for microscope oculars are part of the cost for microscopes, but even here obtaining discarded items from surplus equipment may be the most affordable way to go.

Required also are desktop computers with at least 32 Mbytes of RAM and sufficient fixed disk space to run the camera software on a Windows 95 or 98 operating system. Again, the marketplace has been very cooperative, providing great choices in computers, monitors and printers at low prices due to the competition of new models.

An advantage of the digital camera is that, along with its small size and portability, it allows images to be quickly screened and processed in many ways that are difficult and time-consuming with film images. Once downloaded from camera to computer, digital images can be stored, studied, edited, copied, printed or e-mailed without delay. Particularly useful for photo-micrography, is having the camera tethered to a serial or USB port of a computer while a specimen is studied under the microscope. Digital photos made in

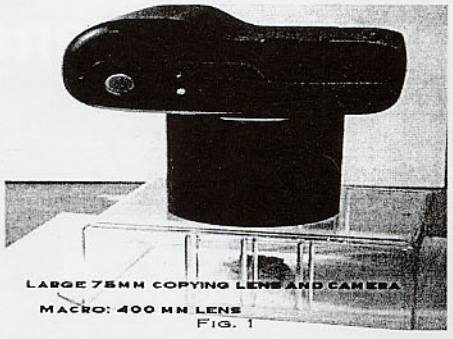

Figure 1. Arrangement for taking a macro-photo with a fixed focus digital camera and a surplus 3" diameter, 75 mm focal length copying lens. The lens and camera are placed on top of an opening in an inverted transparent polystyrene container. The specimen is approximately 2" below the lens and is illuminated from the side. This figure is photographed with the same kind of camera as the one shown but fitted with a 400 mm lens bracket and using the attenuated illumination of the camera's electronic flash.

Figure 2. Lens bracket holding a microscope eye-piece for photo-micrography. This figure was photographed with a fixed-focus digital camera fitted with a 400 mm lens bracket. Fluorescent illumination and auto-exposure.

this mode can be viewed in a minute or two while additional photos are being taken. This allows adjustment of the observational conditions until an appropriate view is achieved. Helpful is the ability to set the shutter speed with the computer's camera program and to make a timed exposure by clicking on the computer screen without having to touch the camera or microscope.

To enhance those options some additional equipment can be very useful although not essential:

DC Adapter. Although the camera runs well on AA batteries, a $25 DC adapter will save money and eliminate the need to change batteries when the camera is used indoors or tethered to a computer.

Storage Drive. A large number of images, (as from a class of students snapping digital pictures) can be managed more easily if a computer storage drive like a Zip Drive is available. This allows collections of the bulky photo files to be distributed on 100 (or 250) Meg disks for collaborative use on other computers.

Compact Flash Card Reader and extra Compact Flash Cards. Another useful option is to have a compact flash card reader attached to a USB or parallel port of a computer to facilitate rapid downloading of large numbers of images from camera memory cards. When the camera is not tethered to a computer and has collected many images, its filled flash card can be easily removed and replaced with an empty card. This allows the camera to continue to be used to collect more images even when it is not possible to download to a computer. Then while the camera continues "in circulation," the filled compact flash card can be delivered to a computer for downloading , viewing and using the images when it is practical to do so.

Shown here are digital photos of camera-lens arrangements and examples of the photos produced. Although not quite at the professional standard, I believe they would be of sufficient quality to enhance the interest and education of young students. ■

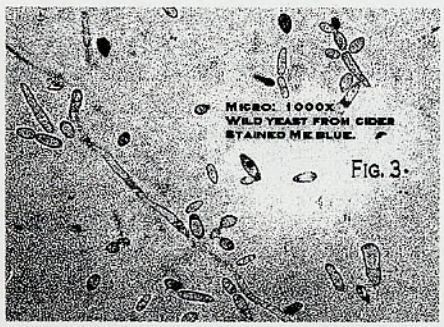

Figure 3. Photomicrograph, 1000x. Wild yeast in spoiled apple cider, stained with dilute methylene blue. Fixed focus digital camera equipped with eyepiece bracket attached to triple tubus microscope. Oil immersion objective.

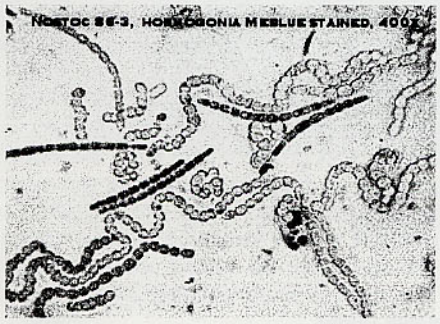

Figure 4. Photomicrograph, 400X. Nostoc sp. 86-3 hormogonia responsible for gliding motility, adhesion and aggregation in the nostocacean life cycle. Fixed focus digital camera equipped with eyepiece bracket attached to triple tubus microscope. The hormogonia are preferentially stained with dilute methylene blue.

MICROSCOPY 101

We appreciate the response to this publication feature - and welcome all contributions. Contributions may be sent to Phil Oshel, our Technical Editor at:

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PO Box 620068 *Fax: (608)836-1969*
Middleton WI 53562 *eMail: oshel@terracom.net*

Removing Stains and Paraffin from Clothes

To remove hematoxylin spots on clothing I have a small amount of acid-ethanol (70% EtOH and HCl) at home. When I see a spot on clothing before washing, I put a drop or two on the spot. The hematoxylin comes out with regular washing.

To remove paraffin from clothing I use a product called "Goo-Gone". It is available in most hardware and discount stores. If you see paraffin on your clothes before washing just squirt a little Goo Gone on the spot. If the clothes are already washed and a greasy area is spotted after drying (like a great greasy meal was enjoyed), squirt the spot with Goo-Gone and rewash and dry. This sounds like a pain, but I give a lot less clothing away to Goodwill. Also, Goo-Gone works wonders at taking off sticky residue like store stickers and tape residue.

Eosin stains usually wash out with normal washing. If not, try the acid-ethanol on them too.

Stain and paraffin removal can get into all kinds of chemistry and expensive products, but sometimes the best solution is in your lab or at your local store.

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What's Your Favorite Pet to Annoy?

I have noticed that there are many favorite materials used to make teasing hairs for EM thin sections. My favorite is cat whiskers, usually found near the cat's favorite lounging spot. Although they can be gotten by sneaking up on an unsuspecting pet and clipping the whiskers.

Pat Connelly, University of Pennsylvania
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And in response to a previous article on the topic of flexible needles by Tina Carvalho (University of Hawaii):

I was for many years an instrument maker and did most anything in that vein. She talks about fine needles for cleaning specimens—here is another, using a small diameter (1/16th or 1/8th inch) rod of nylon or delrin, heat middle slowly almost to melting point but not charred. Pull out straight out and hold until cool, then separate and choose the whisker you want. Nylon/delrin is/are very tough and yet flexible—as strong as glass. Or one could hold two ends together, heat until joined, and then pull out as above. Delrin is a bit tougher and black—easier to see.

Mario T. Sivilli, Micro-Tech, Tucson
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Constant Pressure Perfusion Fixation of Rodent Lungs

When examining the lung for experimental studies, especially those studies designed for morphological analysis (image analysis, stereology), it is important to have a standardized method for fixation of the lung tissues. By infusing the lung via the trachea with fixative under constant pressure, this goal can be achieved and allow standardization in the fixation of each lung sample. A constant pressure perfusion device can be constructed in many ways, but the simplest can be designed using a separator funnel. Tubing can be attached to the base of the separator funnel with a Luer-Lok adapter at the end. A cannula is inserted into the trachea of an excised lung, tied into place, and attached to the perfusion device, which is filled with the fixative of choice. (Specialized cannulas can be ordered or constructed by cutting off the sharp end of an 18 or 16 gauge needle.) It is important to purge out any air in the tubing before attaching the lung to avoid filling the tissue with air bubbles. The tissue is left to float in a beaker filled with the same fixative while attached to the perfusion device. The pressure is measured as the distance from the head of pressure (the top of the fluid level in the separator funnel) to the bottom of pressure (the fluid level in the beaker holding the lung tissue). This distance should remain constant throughout the entire procedure, topping-off as needed. For most rodent studies, 25 cm of pressure is desirable. Once lungs are satisfactory fixed, they can be removed, tied off to maintain the internal pressure, and stored for later trimming of the desired region of interest. One hour of constant pressure perfusion is satisfactory for formalin fixation, with an additional 12-24 hours submerged in a fixative filled container before trimming.

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Why Isn't There Arcing at the Metal Walls of a Microwave Processor?

When the magnetron is running, a pattern of "standing waves" is present in the microwave chamber that is characterized by an apparently stationary number of waves that span the inside dimensions. The chamber is sized so that some number of complete waves just fits inside, and the waves are at a null at the wall. A chamber like this is known as a "resonant cavity" and is characterized by the reflection, at the walls, of the microwaves so that their reflections coincide (more or less) with the incident waves. This same phenomena can sometimes be seen in a container of water that is being vibrated so that the waves on the surface form unmoving rings.

Anyway, the waves are, of course, electromagnetic waves in this case and the electric field component of the waves is a "zero" at the wall, so that no voltage potential is present to cause arcing. That's not to say that there is no current flowing in the walls, because there is. This current, in fact, produces the reflected wave, and since the walls are good conductors, there is little energy lost to heat in the walls.

It's interesting to note that it's the peaks of the standing waves that produce the "hot spots" in microwave ovens. One way to diminish the hot-spot problem is to force the standing wave pattern to change, *i.e.,* to force another resonant mode to alternate with the primary mode. In fact, there are probably several oscillation modes possible and by switching between these in rapid succession the hot-spots can be moved around to minimize their effect. This is the purpose of the rotating "stirrer" found in the top of many microwave ovens.

Michael M. Whittlesey,
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Sectioning Fat

I have worked for many years cutting frozen sections on skin specimens (for light microscopy) in a dermatopathology laboratory. In order to accomplish our goal of demonstrating a complete margin which includes the fat, my specimen must be frozen to a colder temperature than most tissues. My cryostat is set at -28 degrees C. However, this is not cold enough to demonstrate fat. Fat has to be chilled to around -50 degrees C. Remember, the knife blade and anti-roll plate must be at the right temperature as well. I use liquid nitrogen sprayed directly on to the block. I am able to do this because we keep the cryostat chamber free of debris and do not handle infectious cases. In a hospital setting, immerse a swab in liquid nitrogen, then place the swab onto the fat. When the fat turns white, section it. Sometimes, it also requires a faster rotation of the fly wheel to get a section.

There are many different techniques for sectioning fat, some as simple as giving the fat extra time to freeze. In most labs time is of the essence and techs are constantly seeking that one tip which will make their job easier while producing excellent sections. So even though the liquid nitrogen can be difficult to obtain it is well worth the trouble. Just recently, I saw first hand the need to demonstrate the fat with a complete intact section when a patient's slide showed scattered basal cells throughout the underlying fat in the margin. Give this technique a try. The doctors will be singing your praises when they see your fat sections.

Mequita Praet, Dermatology Associates
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Caveat on Time Differences Between Manual Staining and Machine Staining.

One of the biggest differences between hand and machine staining is how the surface tension of the reagent currently on the slide is broken and then replaced by the next reagent. When we stain by hand we exert much more and varied force than a machine does when plunging the slides into the reagent.

We also knock off more reagent when time is done so less reagent clings to the slide. A stainer (machine, not human) simply lowers the slides slowly, in a single plane, into the reagent. Even the agitation of machine staining is in a careful, single plane (up and down) movement. When we stain by hand we cause the reagent in the dish to bombard the slide from several angles and with greater force that breaks the surface tension in less time than a machine can accomplish. Therefore, a longer exposure time (of tissues to stain) may be required on a machine to yield the same results as hand staining.

When programming the machines I find it necessary to watch the hand staining carefully in order to make an accurate translation of a "dip" to a time value that the machine could reproduce. A "dip" in acid-ethanol in manual staining may not be reproducible by a machine. I may be able to use 1% acid-ethanol in hand-staining but have to use 0.5% acid-ethanol on the staining machine with a 2-second timing value to get the same results. Ten "dips" in a manual stain may require 30 seconds on a machine. Ten "dips" in a manual alcohol step may require 1 minute on a machine for the same results.

One of the things we need to remember is that the machine will move the slides exactly the same way for the programmed time. We humans (consciously or unconsciously) adjust our handling of the slides based on how the tissues or even the reagent looks.

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A Tip on Reducing Outgassing of Specimens in the SEM

The problem of dealing with the evolution of contaminating materials from plastics used to mount metallurgical, ceramic and mineralogical specimens is discussed on pages 75 & 76 of my book, 'Vacuum Methods in Electron Microscopy' (see http://www.2spi.com/catalog/books/book48.html for a description). Related topics also discussed are gas evolution from leaks, construction materials, specimen materials, and from cleaning reagents and procedures.

Contamination from mounting polymers can indeed be a very vexing problem, especially for SEMs that have field emission guns (FEG) and must operate with a relatively good vacuum in the specimen chamber.

Basically, what we found after a number of episodes of very serious contamination, was that it is necessary to be sure that the mounting polymers are mixed carefully and thoroughly, so that the correct relative amounts of polymer and hardener are used, and so that these components are thoroughly intermingled.

Then we found it to be necessary to be sure that after they are mounted the specimens are allowed to stand for a long enough period (at least 24 to 48 hours) to ensure that the mounting polymer is completely polymerized (moderate heating can sometimes be used to accelerate the polymerization reaction - even a 15 or 20 degree increase can have a significant effect).

Finally we ended up requiring that after curing all such samples had to be pumped overnight in a chamber of the type that is used to evacuate photographic film before it is placed into an electron microscope.

Such procedures did not totally eliminate the problem, but reduced it to a level where we could operate for a month or more before contamination built up to the point where cleaning of the chamber and apertures became necessary.

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Softening Tissue Blocks with Nair

Nair works well for softening paraffin embedded tissue blocks, especially on keratotic skin lesions. I just put enough Nair in half of a petri dish, or an empty plastic slide box. Set this on ice or a cold plate, put the block in the Nair face down, let it set for 3 to 5 minutes, then cut. With some tissues, plain old water works just as well with the same technique.

Many people who work only with paraffin swear by this for all kinds of tissue including (but not limited to, as the saying goes ...) uterus, bone that has been previously decalcified, but is still hard to cut, finger and toe nails and nail beds, and basically anything difficult to cut because it is too hard or fibrous.

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MICROSCOPY TODAY

The objective of this publication, perhaps unlike many others, is simply to provide material of interest and value to working microscopists!

It is produced ten times each year and mailed to just under 9,000 microscopists in the U.S. (each of which who have specifically requested subscriptions)—and to several hundred microscopists outside the U.S.

Due to the financial support of advertisers, the publication is sent at no charge to microscopists in the U.S. However, due to the relatively few international readers (and the resulting lack of advertising income) and the very high postage costs, we must charge for international subscriptions:

Canada, Mexico, Latin and South America: $40 for one year and $70 for two years.

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Individuals requesting subscriptions, or wishing to change/correct their current address, may use the form to the left. This completed form may be mailed or faxed, or information sent by email, or the information may be provided via our www site.

We greatly appreciate article/material contributions from our readers—"users" as well as manufacturers/suppliers. The only criteria is that the subject matter be of interest to a reasonable number of working microscopists.

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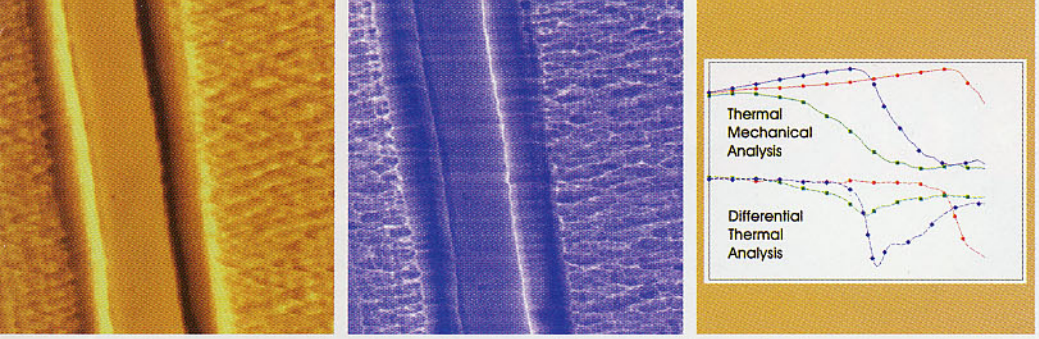
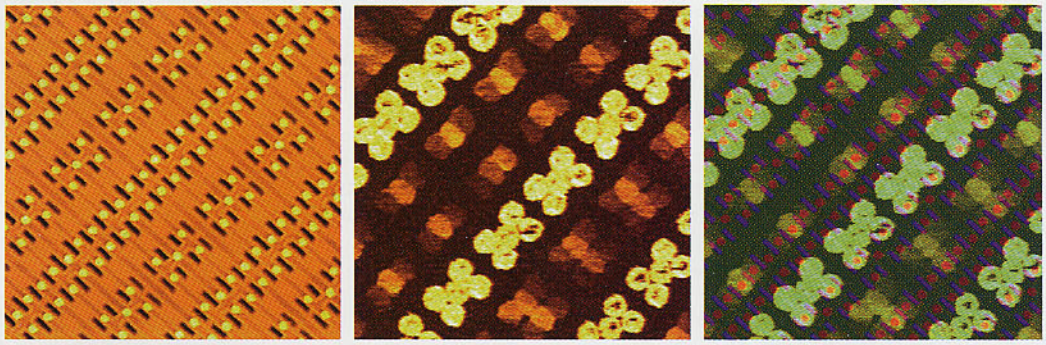


Using topography, thermal conductivity mapping and micro thermal analysis identifies layers in a polymer film. Courtesy of Duncan Price, Loughborough.



Topography and electrostatic force images combine to show misalignment of contacts and implants in SRAM.

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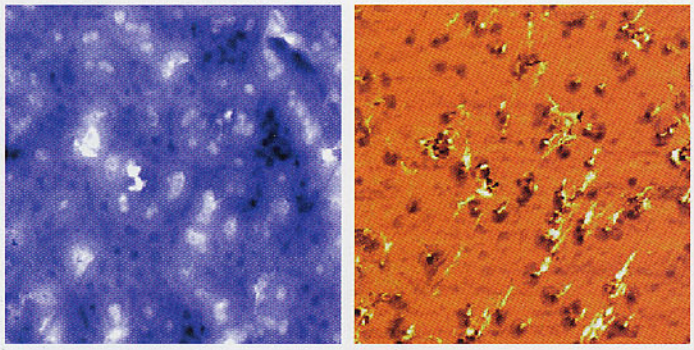

REGION GROUP	AREA µm²	AREA %
Total area	67.69	100
Silicone lubricant	3.101	4.6
Teflon particles	7.007	10.4

Topography and phase images show area and percent coverage of Teflon® and lubricant in support matrix. Courtesy of Steve Pratt, Kodak.

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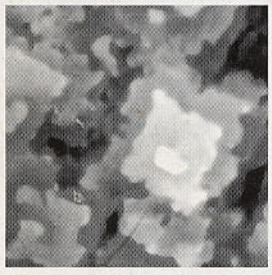

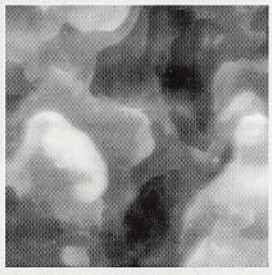

PEO at 60°C with evidence of melting clearly shown after 2 minutes.

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