## Biomedical Applications: Pitfalls in the Practice

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At present most medical microprobe analysis is conducted on insoluble particulates such as asbestos fibers in lung tissue.[1,2,3] Cryotechniques are not necessary for this type of specimen. Insoluble particulates can be processed conventionally. Nevertheless, it is important to emphasize that conventional processing is unacceptable for specimens in which electrolyte distributions in tissues are sought. It is necessary to flash-freeze in order to preserve the integrity of electrolyte distributions at the subcellular and cellular level. Ideally, biopsies should be flash-frozen in the operating room rather than being frozen several minutes later in a histology laboratory. Electrolytes will move during such a long delay. While flammable cryogens such as propane obviously cannot be used in an operating room, liquid nitrogen-cooled slam-freezing devices or guns may be permitted, and are the best way to achieve an artifact-free, accurate tissue sample which truly reflects the *in vivo* state. Unfortunately, the importance of cryofixation is often not understood. Investigators bring tissue samples fixed in glutaraldehyde to a microprobe laboratory with a request for microprobe analysis for electrolytes. Having fixed the tissue in glutaraldehyde, the investigator believes that it is optimally preserved for microprobe analysis. While the sample is optimally preserved for traditional transmission electron microscopy, electrolyte distributions have been destroyed.

Not only is loss of electrolytes and other soluble minerals a problem, the addition of chemicals used with traditional chemical fixation is also a major problem. Lead, osmium and uranium are used routinely in electron microscopy laboratories. Arsenic is a component of buffers such as cacodylate. In histology laboratories, phosphorus from phosphate buffer and bromine from dyes such as eosin are problems. In electron microscopy and especially in histology laboratories, small particles of dust such as aluminum silicates are common contaminants. Glassware not properly washed or rinsed can contribute elements such as calcium or lead to tissues. We encountered this problem with a biopsy processed at another hospital. We avoided making an erroneous diagnosis by using the controls noted below. Scrupulous attention to cleanliness and to tracking all reagents and their associated buffers used in the preparation of the sample is necessary in order to conduct a knowledgeable microprobe study.

Once the sample has been placed in the instrument there are many additional potential artifacts. The most common problem is the lack of a peak from an expected element. The most common reason for this is either loss of the element due to poor specimen preparation as described above, or insufficient sensitivity on the part of the equipment. Conventional microprobe analysis with an energy dispersive x-ray detector on a typical scanning electron microscope is only sensitive in the 10 to 100 parts per million range. Thus the distribution of an element such as aluminum in tissues such as bone or neurons may be impossible to study with this technique, especially if some loss has occurred during specimen preparation. It may be necessary to use more sensitive techniques such as a laser or ion microprobe. However, the geometry of the sample stage and detector may be the problem, if aligned incorrectly, our sample holder can block the detector, resulting in no peaks detected. Another common problem is that the feature of interest may be covered by tissue. A particle should be free of tissue with its surface exposed to the beam in order to generate a good signal. It is surprising how little tissue is required to obscure large particles and to block x-ray detection. Histologic sections should be lightly coated with carbon and not sputter-coated with a metal. While it is true that the elements in sputter-coated asbestos fibers can still be detected, this technique only works because of the large mass of the particle. It is not recommended for ideal microprobe analysis. The sputter coat does prevent one other important problem, charging. A specimen which is charging may contribute no x-ray signal and thus be a "false negative". Usually this problem is obvious during secondary electron imaging. False positive peaks may arise from metals in the microscope (iron, chromium and nickel) as well as silicon from some

vacuum pump oils.

Once peaks are obtained, care must be taken to identify all peaks in the full spectrum. For example, it is easy to misidentify the M-alpha line for lead since it overlaps the K-alpha peak for sulfur. However, by identifying other lines for lead this mistake will be avoided. Two different types of spurious peaks may be present when there is a large mass of material contributing a strong signal - escape peaks and arithmetic doublets. The latter are peaks obtained at precisely twice the energy of the primary peak. Escape peaks are caused by charge buildup effects on the detector, and are either present at the excitation energy of silicon, which is the detector material, or at the peak energy minus 1.74 keV.

As a final check on all of the above problems, it is important during a microprobe study to utilize several different controls. An ideal study should include the full spectrum from the feature of interest, from tissue close to but lacking that feature, and from the blank stub or grid. Only in this manner can peaks contributed by, for example, the metal in the column, be understood and eliminated. Other useful controls are cases processed through the same histology or electron microcopy laboratory on the same day, but not including the feature of interest. These controls help to exclude the possibility of a spurious contaminant present in that particular run of embedding medium or fixative. This control is particularly important when a microprobe lab studies tissues from another laboratory where rigorous microprobe practices may not be the rule. ■


1. P. Ingram et al., *Microprobe Analysis in Medicine*, Washington, DC: Hemisphere Publishing Corporation (1989).
2. V.L. Roggli, in G.W. Bailey, Ed., *Proc. EMSA*, San Francisco: San Francisco Press (1988) 84.
3. V.L. Roggli and J.D. Shelburne, in D.H. Dail and S.P Hammar, Eds., *Pulmonary Pathology*, 2nd ed., New York: Springer-Verlag (1994) 867.



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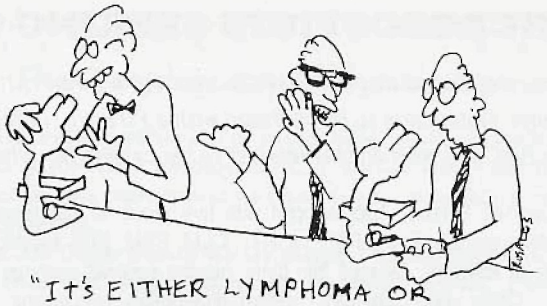


## Microscopy Society of America Undergraduate Scholarship Program

The Microscopy Society of America (MSA) Undergraduate Scholarship Program was initiated in 1988 to encourage students to pursue microscopy related careers. Since its inception the program has grown steadily and has provided support for over 35 undergraduate projects. It is encouraging that nearly all of the awardees have maintained an interest in microscopy oriented research and have gone on to graduate school, industry, or teaching positions.

*The program presently derives all its funding from the MSA and has* been able to support approximately 30 to 40% of applicants. This year, for the first time, the program is seeking matching external funds in order to maintain the favorable levels of support both in terms of the number of projects supported and the level of support for each.

The 1994 awardees are as follows:

**Sara L. Jung.** Junior. Univ. of Illinois. Supervisor: Richard Crang, Dept. of Plant Biology, Univ. of Illinois, Urbana, IL. Research: Adhesion inhibition and microscopic localization of adhesins from selected *Candida albicans* strains grown on human buccal cells.

**John J. Kostetsky.** Sophomore. Univ. of Massachusetts - Lowell. Supervisor: Changmo Sung, Ctr. for Advanced Materials, Univ. of Massachusetts - Lowell, MA. Research: Microcharacterization of SiC (Nicalon) fiber reinforced SiC ceramic-matrix composites by SEM and analytical TEM.

**Matt Robertson.** Junior. Wittenberg Univ. Supervisor: Nicholas Ziats, Inst. of Pathology, Case Western Reserve Univ., Cleveland, OH. Research: SEM analysis of proiein adsorption on biomaterials.

**Paul A. Smith.** Junior. Carnegie Mellon Univ. Supervisor: Michael Meltenny, Dept. of Material Science, Carnegie Mellon Univ., Pittsburgh, PA. Research: Microscopic investigation of aluminum manganese fine particle magnets.

**Sherry L. Spinelli.** Senior. SUNY Brockport. Supervisor: Craig Lending, SUNY Brockport. Research: 3-D *reconstruction of zeins in Zea maize L.* endosperm using immunocytochemical techniques.

The 1995 Undergraduate Scholarship Program is currently soliciting applications from students interested in conducting a research project which involves the use of **ANY** microscopy technique. Students should be sponsored by a member of MSA. The maximum award is $2,500. The application deadline has been extended to December 30, 1994.

Applications can be obtained from the MSA Business Office at Tel.: (800)538-3672 or FAX: (508)548-9053. If you have questions or require additional information regarding the program please contact either of the following:

Dr. R.M. Albrecht. Tel.: (608)263-3952/4162, FAX: (608)262-7420.
Dr. R.L. Ornberg. Tel.: (314)694-1184, FAX: (314)694-6727.

# MICROSCOPISTS SEEKING NEW EMPLOYMENT OPPORTUNITIES:

*As some individuals may not wish their current employers to know of their interest in looking for new employment, we have utilized a "box" reference system (not to be confused with a PO Box). In these cases, an employer can advise Microscopy Today of a potential interest and we, in turn, will pass on that interest to the candidate - who, then in turn, can contact the potential employer.*

☛ Certified Electron Microscopist with five years of experience in high-technology manufacturing R&D facility. OLM, SEM, EDS techniques used. Majority of analysis: layered thin films, plasma sprayed coatings and metal alloys. Other responsibilities: report distribution, purchasing, managing preparation lab. Very willing to relocate and acquire new skills! For resume and reference list phone Cynthia E. Schray at 707-447-5417.

☛ MATERIALS ANALYST - 10+ years experience in materials analysis. MS/BS Materials Engineering. Extensive expertise in optical microscopy, microanalysis, failure investigation and materials testing. Currently managing analytical consulting laboratory. Seeking technical or managerial position in materials analysis, QC, or product development. Salary negotiable. Location Open. Contact *Microscopy Today* Box 100.

☛ Skilled microscopist, 20 years experience, seeking employment in biological/biomedical research facility as optical and electron microscopist. Experienced in use of TEM, SEM, EDS, ultramicrotomy, cryothin sectioning, freeze fracture, freeze substitution and optical microscopy. Will relocate. Salary range $40-45K. Call (813)977-1135 and leave name and number. Thank you.

☛ Seeking senior level industrial or academic position to develop, promote, and utilize digital techniques in microscopy. Experience includes 15 years of image analysis software development applied to SEM, TEM, optical and confocal; instrument control and automation; engineering project management; technical publications; advertising; journal articles; operator's manuals; teaching; training; demonstrations and technical support. Contact *Microscopy Today* Box 101.

☛ Chemist/Microscopist experienced in textiles, polymers, food systems seeks R&D position. Will relocate. Experienced in SEM, EDX, polarized light microscopy, hot stage microscopy, FTIR-microscopy, image analysis (Quantimet, Image Pro-Plus, Optimas), technical photography, DSC, TGA, DMA, Haake, Instron, crytical point dryer, cryo-microtome, WordPerfect, Lotus, Paradox, Excel and Semicaps software. Contact *Microscop;y Today* Box 102.

☛ Over 20 years of experience involved primarily with biological EM: SEM, TEM, STEM, EELS, Cryo-SEM, Environmental SEM, Electron Probe Microanalysis, Cryofixation, Image Analysis ... seeks a challenging position in a dynamic environment. Contact *Microscopy Today* Box 103.

☛ Experience includes 37 years in materials technology/quality assurance with 28 years of failure analysis utilizing techniques including, but not limited to, SEM, TEM, XRF, XRD & Optical Metallography. nine years experience in NDE, including instructing at a junior college. Relocation possible to most of U.S. Contact Louis W. Hulse at (713)332-4493.

☛ As director of a NIH funded SEM Core facility for 12 years at world's largest comprehensive cancer center my experiences include: development of novel techniques, data interpretation, fiscal management, TEM, histotechnology, many other cell biology research applications. Looking for new challenges in innovative academic or industrial institution. Contact Kim Dulski at (713)792-8969.

☛ Postdoc in Materials Science and Engineering seeks position in Industrial/Academic R&D in Processing Development and Characterization of Materials: Ph.D in solid state physics with diversified experiences in materials science. Experiences: conventional and high-resolution TEM including JEOL, Philips and Topcon EM, high spatial resolution spectroscopies (EELS and EDX), CBED, SEM and x-ray diffraction. Contact *Microscopy Today* Box 104.

☛ Objective: TEM/Analyst, Semi-Conductor, Metals and Phosphors. Experience: SEM/Light Microscopist, Macintosh/IBM compatibles proficient, First to develop/routinely employ cross-sectional thinning of samples, Developed novel techniques to increase sample thruput by 80%, High vacuum/metallurgical background, 44 publications/4 patent disclosures, BA-Chemistry. Contact Chuck Buiocchi at (215)736-1424.

☛ M.S. Materials Engineering w/ Electronic devices option, 10 years experience. SEM (field emission, $LaB_6$, W), EDX/WDS, X-ray (single/double crystal, Laue), Auger, SIMS, RBS, IR/UV spectrophotometer, microhardness, interferometry, metallography, thermal expansion, titration. S-parameter, noise figure, I/V curve tracer, I probe, semiconductor parameter analyzer, infrared thermal analysis, life testing. Molecular beam epitaxy, ion beam enhanced deposition. Contact *Microscopy Today* Box 105.

☛ More than 10 years experience in TEM, SEM, immuno-EM, rapid freeze etch with rotary shadowing and routine histological technique, variety of special stains, immunofluorescence, immunoperoxidase, cell culture, animal care, perfusion, surgery and wide knowledge of histology and biomedical area. Available now. Call Mr. Hou at (904)373-5573 or (904)392-6244.

☛ Seeking new opportunities in biomedical sales/research to utilize my demonstrated abilities as Biological Microscopist (EMSA, Certified Technologist). Broad experience (including 10 years auditory research); tissue preparation for and use of LM, TEM, SEM; small animal surgery; microdissection procedures; microcomputer systems; darkroom/photographic procedures; interpretation of results; co-authorship. Contact *Microscopy Today* Box 106.

☛ SEM operator/lab manager position wanted. B.S. Geology, M.S. Geology/Geochemistry, 7 1/2 years combined experience in materials science, failure analysis, mineralogical examination and forensic science, including extensive familiarity with XRD. Seeking work in private industry or government (hard money position) in midwest or western U.S. M. Jensen: (702)322-8535 PST.

☛ A biologist with demonstrable experience in laboratory management, independent research (especially multidisciplinary), and publishing research results. Extensive experience in microscopic analyses, utilizing both photon (including polarizing, Nomarski, florescence) and electron (TEM and SEM) microscopes, specimen preparation, cytochemistry, freeze-fracture, and photomicrography. Contact *Microscopy Today* Box 106.

☛ SEM/TEM tech seeking the perfect position. I have 10 years of experience including the use of TEM's, SEM's, STEM's, EDS and related equipment in both medical and material environments. The right position will be in a technologically sophisticated lab offering both challenges and opportunities for advancement. All locations will be considered. Contact *Microscopy Today* Box 107.

☛ B.S. & M.S. in Geology. Field experience (mapping, sampling) and lab experience (rocks, minerals, metals, ceramics). Experienced analyst (optical, SEM, and microprobe): quality, problem-solving and research. Experienced operator: purchasing new equipment and debugging it, maintaining old and improving it. Interested in relocating to a challenging new position. Contact *Microscopy Today* Box 108.

☛ Qualified to operate SEM/TEM and X-ray analysis, process tissue, thin and thick section and photography. Certified from VA Hospital in Albany, NY. Interested in clinical or research work. Call Leslie I. Hughes at (518)458-7871.

- B.S. & M.S. in Biology and Certified Electron Microscopy Technologist. One and half years experience on transmission, scanning, cryo-electron microscopy, EDX and image analysis techniques. Willing to relocate anywhere in U.S. Please call me. S. Krishna: (518)456-1604.

- VERSATILE - MS. Inorganic Chemistry; A.B. Chemistry & Physics. Experience: optical mineralogy, semiconductors, concrete, environmental samples, chemical microscopy, limited microbiology, PLM, SEM & ESEM / EDS / IA, X-ray powder diffraction, FTIR, HPLC. Contact *Microscopy Today* Box 109.

- B.S. Biology, 1982; some graduate work. Electron Microscopy: routine processing, embedding, sectioning, staining, observation and photography, freze-fracture and freeze-etch techniques, immunogold labeling. Light Microscopy: cryostat sectioning, immunofluorescence labeling, photography and video microscopy. Supervisory experience and laboratory organization. Contact *Microscopy Today* Box 110.

- Ph.D Electron Microscopist. Interests: SEM/TEM analysis; operator training; quality assurance and technical lab management. Qualifications: 18 years experience (SEM/TEM/microprobe/XRD); 38 published papers on analysis of minerals, ceramics, chemical and nuclear waste disposal; managed commercial, government EM laboratories; certified TEM laboratory inspector (NVLAP); taught college SEM/TEM courses. Contact *Microscopy Today* Box 111.

- Electron Microscopist/Semiconductor Device Physicist. Ph.D. Chemical Physics, 20 years experience, expertise and interest in semiconductor device characterization and failure analysis using SEM and other electron beam and optical techniques. Hands-on experimental, instruction, research, publications, exposed to a very broad range of technical activities, ISO 9000 familiarity. J.D. Schick: (914)223-7393.

- MATERIALS CHARACTERIZATION. Ph.D. candidate (11/94) - Materials Science, M.S. - Nuclear Science, B.S. - Physics. Experienced in TEM, SEM, EDX, mass spectrometry, particle analysis, vacuum systems. Skilled at statistical analysis and quality control. Supervised laboratory services. Can write clearly. If your company needs an experienced, thorough innovator ot solve diverse problems, call (610)865-9031.

- Seeking academic position in Biology, emphasis in evolutionary/organismic biology. Ph.D. Entomology & M.A. Botany (UCBerkeley), M.S. Biology (Univ. Puerto Rico). Good grantmanship/publication record. About 4 yr postdoctoral experience (last 1.5 year in a molecular biology lab). Circa 8 year experience in SEM. Extensive teaching / some administrative experience in university/college. Contact *Microscopy Today* Box 112.



## The Employment Challenge

*In creating this list of summaries of microscopists looking for new employment, I could not help but reflect on my own previous challenges in seeking new employment.*

*With these thoughts in mind I have decided to keep this "list" updated on an on-going basis. I will, from time to time, publish the updated list in this newsletter.*

*Readers seeking new employment are encouraged to submit their "summaries" - or improve on those previously submitted. I will not charge for this friendly service but would not turn down article/material contributions to the newsletter.*

*And I will provide a copy of the current list to any employer wishing a copy - by fax or mail. Should readers not be directly involved in the hiring process at their establishment, they may care to draw this offer to the attention to those so involved. Again, I do not intend to charge for this service.*

*- - - Don Grimes, Editor*

## Polaroid Announces Winners of International Instant Photomicrography Competition

With his photograph featured on the cover of a recent issue of this publication, Dr. Gerald T. Baker, professor of entomology and director of the Electron Microscope Center at Mississippi State University, won the $2,500 Grand Prize in the Polaroid Corporation International Instant Photomicrography Competition. His Polaroid Type 55 P/N photograph showing the intricate surface structure of a giant silk moth egg, reveals a compex and geometrically precise design, as well as important clues about the condition of insect species.

Polaroid awarded $1,000 first prizes, as follows, in the competition's other categories, including one reflecting new techniques in confocal laser scanning microscopy, scanning tunneling microscopy and computer processed micrography.

**B&W Light Micrography Category:** Floyd E. Alberts, a research technologist in the Analytical Science Department at Ford Motor Company with a photograph showing thermal faceting in gamma-iron at 100x magnification.

**Microscopy Techniques Category:** John Georgiou, a researcher in the Department of Physiology at the University of Toronto, with his 800x magnification photomicrograph of a glial cell within a neuromuscular junction of a frog.

**Materials Science Category:** Vito Giannini, with the central Chemical Laboaratory at Italcementi SpA, Bereamo, Italyh, with his composition of calcite crystals produced by multiple exposing the Polaroid Type 55 P/N scanning electron micrograph at magnifications of 1,000x and 3,000x.

**Student Category:** Jim Wetzel, a Ph.D. candidate in zoology at Clemson University and a teaching instructor at Presbyterian College, Clinton, SC, with his Polaroid Type 55 P/N scanning electron micrograph showing the embryo of a dwarf seahorse at 11x magnification.

**Color Light Micrography Category:** Wutian WU, M.D., Ph.D., assistant professsor in the Department of Neurosurgery at Eastern Virginia Medical School, Norfolk, VA, with his photograph of sucrose crystals at 50x magnification.