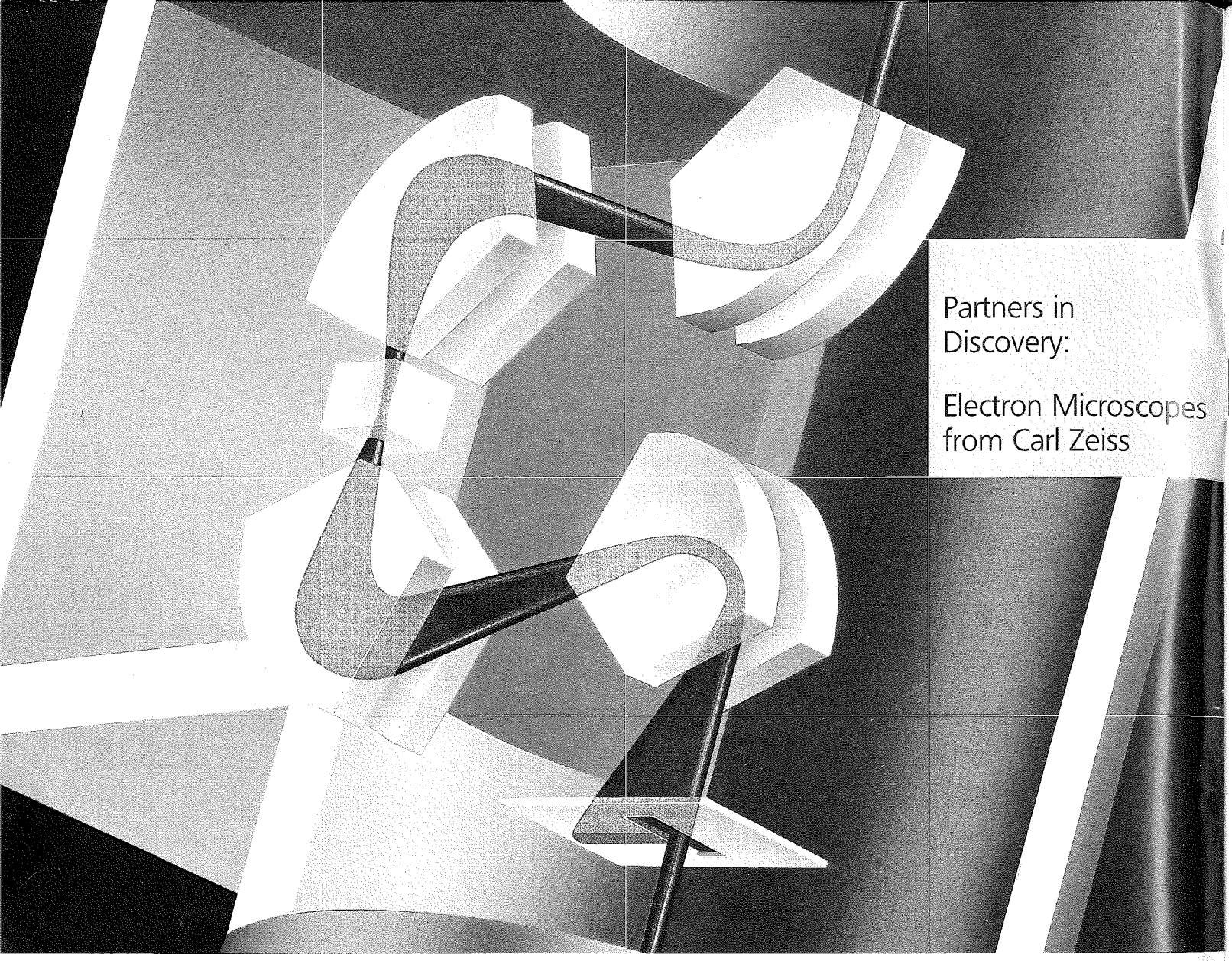
JOURNAL
VOLUME 24, NUMBER 2, 1993
ISSN 0196-5662



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TEXAS SOCIETY FOR ELECTRON MICROSCOPY JOURNAL
VOLUME 24, NUMBER 2, 1993
ISSN 0196-5662

Louis H. Bragg, Editor
Department of Biology, The University of Texas at Arlington, Arlington, TX 76019

Texas Society for Electron Microscopy
"For the purpose of dissemination of research with the electron microscope."

ON THE COVER
Scanning EM view of the trachea of a sheep 30 minutes after exposure of an isolated segment to cotton smoke at 37°C. The characteristic initial response of the epithelium to toxic agents in smoke is sloughing of intact, beating ciliated cells. Note the conical shape of the cells, which extend like cornucopias from a narrow end attached to the basal region to a disk covered by cilia. Approximate magnification X 7000. See Abdi S. et al, Am. Rev. Resp. Dis. 142:1436, 1990, and Barrow RE et al, Lung 170:331, 1992. Photo — Robert A. Cox, Shriners Burns Institute, Galveston, TX 77550.

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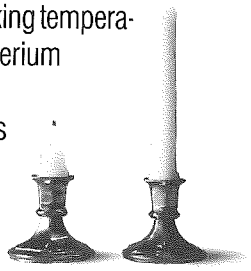

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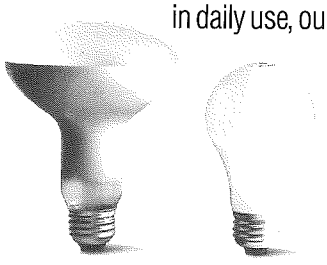

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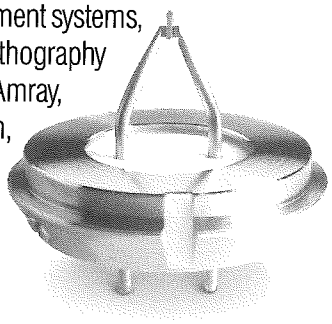

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President's Message

1993 and 1994 promise to be a period of continuing growth as we have strong programs underway and excellent meetings planned for Galveston and Denton. The meeting in Corpus Christi was a winner both in terms of enjoyment and in terms of gaining scientific knowledge; the in situ workshop was especially excellent. The meeting in Denton will feature demonstration of high resolution TEM studies of problems in the physical sciences. This is a momentous year for yours truly, since I am in the process of recovering from a move of 50 miles from Baylor College of Medicine, where I held forth for almost ten years, to the University of Texas Medical Branch in Galveston, where I may have taken on too many jobs as pediatric pathologist on the autopsy and surgical services and director of diagnostic EM and of the research EM lab at the new Shriners Burns Institute. I look forward to welcoming you to Galveston Island for the Fall meeting which corresponds with publication of this issue. It is a charming "city with a past" as well as the home of an excellent medical complex. We hope to welcome even more guests than usual for this meeting, including the contingent in the Houston metropolitan region and many members scattered throughout Texas and neighboring states. We especially welcome Joe Mascorro, who will be joining us to invite our members to participate in the annual meeting of the Microscopy Society of America in New Orleans next summer. The program for this meeting includes some new features. We are offering two workshops, scheduled so that members can attend both. Laszlo Kömúsz and Jan Minshew will be demonstating methods of cryosectioning, and Gwen Childs, one of the leading scientists at UTMB, will be offering a workshop on *in situ* hybridization. We also have an outstanding invited speaker, Stanley Erlandsen, from the University of Minnesota, who will he escaping the onset of winter long enough to educate us about the extraordinary power and scope of application of high resolution low voltage scanning electron microscopy.

Our parent organization, the Microscopy Society of America, is also continuing its growth and is at the leading edge of technology in all areas of imaging of small things, including various atomic force imaging systems as well as confocal and video light microscopy, computer assistance and all the variants of electron microscopy. The Local Affiliate Society office which oversees our activities has a new head, Peter Ingram, who is a fascinating character with broad interests in jazz and the arts as well as an outstanding electron microscopist with a long record of leadership in local societies. We look forward to working with him. The Texas Society for Electron Microscopy continues to offer our student members the opportunity to compete for an award of subsidized travel to present their work at the national meeting.

The Texas Society for Electron Microscopy Journal will also be changing leadership with the next issue, and all of us join in our gratitude to Louis Bragg for his excellent leadership and hard work in producing the *Journal* regularly. We wish David Garrett the best of luck as he takes the reins, and I want to urge all of you to support his efforts by submitting interesting work for publication. This is a way to publish methods, ideas, speculations and overviews which may be of great value but may not fit the usual guidelines of research journals. Publication in the *TSEM Journal* does not preclude later publication in a national journal. Many of us have valuable experiences to share, and the *Journal* is an excellent forum for sharing them. Thanks to Lynn Gray and Ann Rushing, some of our collected experience is published in this issue in the form of the proceedings of the "favorite methods and inventions" symposium at the Corpus Christi meeting. As always, your contributions to meetings are greatly appreciated, whether in the form of posters, 10-minute talks, comments, or suggestions for speakers or workshops. Do you want to participate in minisymposia or round table discussions? Your continuing contribution of time and ideas to your organization ensures its continuing vitality. We are on the way to becoming "the best ... microscopy society in the country."

Sincerely,

Hal K. Hawkins
President, 1993-1994

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Treasurer's Report

TEXAS SOCIETY FOR ELECTRON MICROSCOPY
TREASURER'S REPORT
For Period Ending September 20, 1993

ASSETS ON JANUARY 1, 1993:		
Certificate of Deposit No. 113515	$3,651.24	
Certificate of Deposit No. 2414483036	1,688.30	
Certificate of Deposit No. 9005997	4,109,97	
Checking Account No. 44059412	1,619.57	
TOTAL		**$11,069.08**
CHECKING ACCOUNT RECEIPTS:		
Dues	$2,396.00	
Spring 1993 Meeting Registration	965.00	
Workshop	45.00	
Exhibitors	850.00	
Donations and Grants	173.75	
Guest	120.00	
Journal Advertisements 23:2	260.00	
24.1	2,625.00	
Miscellaneous	298.97	
Checking Account Interest (Account No. 70072962)	40.29	
Subtotal		$7,774.01
Certificate of Deposit No. 9005997 to Checking Account No. 70072962		4,138.35
Close Checking Account No. 44059412		1,077.28
TOTAL		**$12,989.64**
EXPENSES:		
Journal, 24:1	$2,240.31	
Office Expenses	152.69	
Mailouts	585.55	
Spring 1993 Meeting	2,340.08	
Workshop Refund	45.00	
Student Competition/Travel	330.00	
Student Representative	55.00	
Plaque	74.40	
Invited Speaker	285.83	
Legal Fees	1,832.82	
Checking Account Service Charge (Account No. 44059412)	23.10	
TOTAL		**$7,964.78**
ASSETS AS OF SEPTEMBER 20, 1993:		
Certificate of Deposit No. 113515	$3,928.63	
Certificate of Deposit No. 2414483036	1,688.30	
Checking Account No. 70072962	5,024.86	
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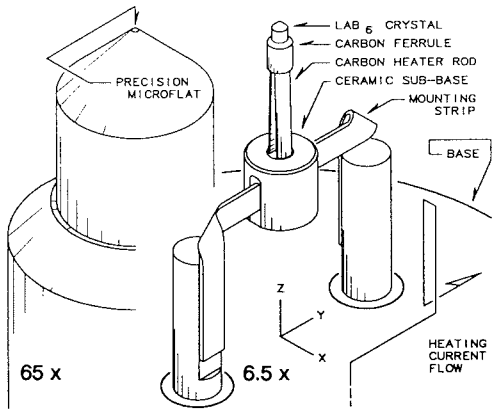

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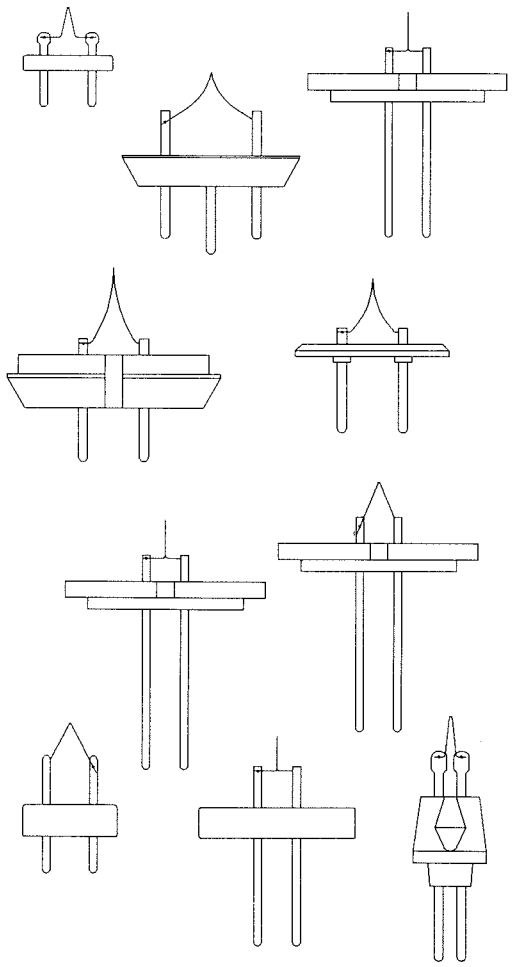
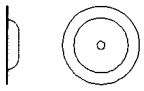


WEHNELT APERTURES



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Signature of advisor *(for student applicants)*:_____

Signature of applicant:_____ Date:_____

EMSA Local Affiliate Societies *(Choose one)*

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☐ Appalachian Regional Electron Microscope Society
☐ Arizona Society for Electron Microscopy and Microbeam Analysis
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☐ Michigan Electron Microscopy Forum
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☐ Minnesota Electron Microscopy Society
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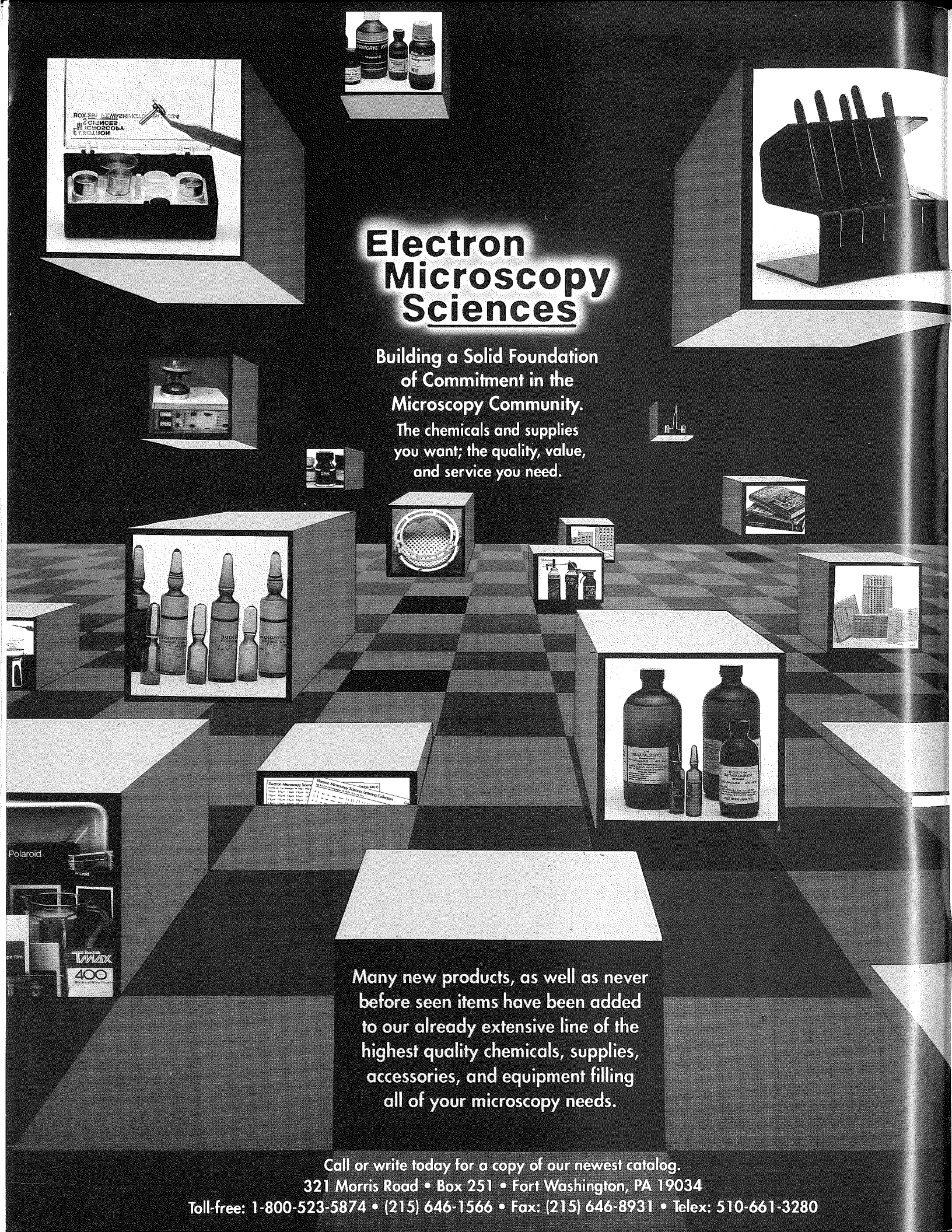
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ELECTRON MICROSCOPY TECHNOLOGIST

—(BIOLOGICAL SCIENCES)—

GENERAL ELGIBILITY REQUIREMENTS:

1. Membership in EMSA.

2. ONE of the following conditions must be met:

   — 2 years (60 credits) college or equivalent, including science and TEM (1 year laboratory) courses; science courses to include one each of chemistry, physics and biology; math through trigonometry

   — 1 year (30 credits) college or equivalent, including one course each of chemistry and physics, *and* 1 year of recent full-time work experience (within the past 5 years) in a TEM laboratory

   — high school diploma and 2 years of recent full-time work experience in a TEM laboratory

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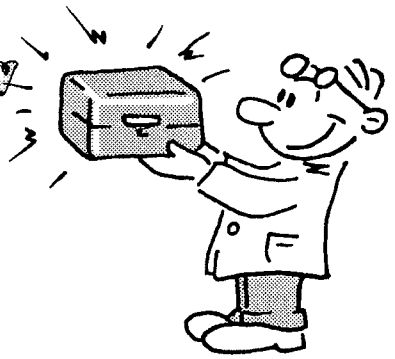

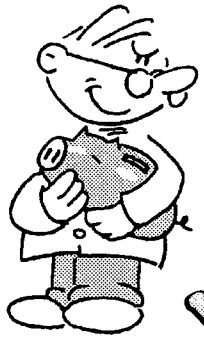

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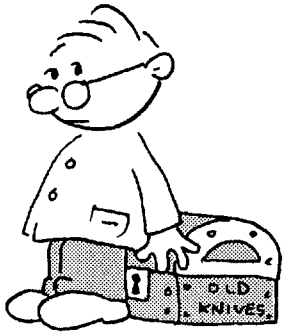

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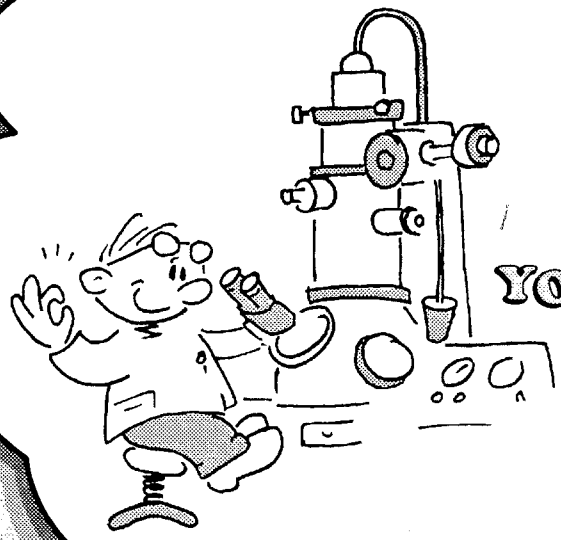

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CALENDAR OF MEETINGS

SPRING MEETING OF TSEM

March 24-26, 1994

Denton, Texas

See Details Elsewhere In This Journal

PRACTICAL ASPECTS OF SCANNING ELECTRON MICROSCOPY

March 14-18 and 21-25, 1994

University of Maryland Short Courses

College Park, MD

Contact: Tim Maugel (301) 405-6898

FOOD STRUCTURE ANNUAL MEETING

May 7-12, 1994

Toronto, Canada

Contact: Dr. Om Johari (708) 529-6677

SCANNING '94 (FAMS & SEEMS)

May 17-20, 1994

Charleston, South Carolina

Contact: Mary K. Sullivan (201) 818-1010

13th INTERNATIONAL CONGRESS ON ELECTRON MICROSCOPY

July 17-22, 1994

Paris, France

Contact: Secretariat ICEM, 13,67 rue Maurice Gunsbourgh, 94205 Ivry sur Siene cedex, France

TECHNICAL TIPS FOR THE E.M. LAB: PART I

A COLLECTION OF SELECTED TECHNIQUES AND INVENTIONS PRESENTED AT THE SPRING, 1993 TSEM WORKSHOP

Dr. Ann E. Rushing[1] (TSEM Program Chairman, 1992-93)
and
Dr. Lynn D. Gray[2] (TSEM President, 1992-93)
co-editors

INTRODUCTION:

Scientists using electron microscopy must periodically deal with situations where the tool or item we need is either too expensive, not available on the commercial market, or not quite right for the job. We then have to become inventors and engineers to solve the problem or to get the particular job done in an acceptable manner. Many of the techniques we use in these situations are native to our own laboratories or have been handed down from academic generation to generation. Others are "revived" from earlier publications or inventions. However we came by them, they make our laboratory lives easier. Most don't seem to find their way into the scientific literature yet these laboratory pearls of wisdom could and would benefit other colleagues if they simply knew about them (or remembered them). Part I represents a collection of items which were presented at the Spring 1993 TSEM Workshop in Corpus Christi. The contributions vary from inventions to modifications of other inventions to techniques. We plan to publish Part II in the next *Journal* issue — that is, depending upon the cooperation of our inventive colleagues in TSEM! We hope our readers find these items helpful in their own laboratories. We also hope that our readers will contribute their own inventions and techniques in Part II.

We begin with a review of the operation of the MT-1 manual microtome (as presented at the Workshop) by Randy Scott followed by descriptions of various inventions and devices by several TSEM members including Hal Hawkins, Carolyn Corn, Pam Neill and Lynn Gray. There should be something here for everyone. Enjoy!

[1]Dr. Ann E. Rushing, Department of Biology, Baylor University, P.O. Box 97388, Waco, TX 76798-7388.
[2]Dr. Lynn Gray, Dept. of Cell Biology & Env. Sci., The U.T. Health Center at Tyler, P.O. Box 2003, Tyler, TX 75710.

USE OF THE MT-1 ULTRAMICROTOME

Randy Scott
The Electron Microscopy Center
Texas A&M University
College Station, TX 77843-2257

INTRODUCTION:

Keith Porter and Joseph Blum developed the ultramicrotome which was produced as the Sorvall MT-1. The microtome is a keystone in our field, as is the electron microscope, because the instrument was perhaps the first truly practical device for cutting thin sections. It has been said that the MT-1 helped open the door to modern cell biology. The instrument is small and at the same time rugged enough to section many difficult samples. A great advantage offered by this machine is that the operator gets direct feedback because it is totally a hands on operation. This is a foundation machine for modern microtomy and the fundamental knowledge of its use should prove to be beneficial to the accomplished microscopist. It was with these thoughts in mind that we proposed to demonstrate the MT-1 in the Workshop meeting.

MATERIALS AND METHODS:

The equipment used for this demonstration include the MT-1 ultramicrotome, a compound, binocular microscope, glass knives with tape troughs pre-mounted and sealed, well-fixed and embedded tissue, a hot plate, and a microtomy kit consisting of fine forceps, a single hair brush, a tuberculin syringe, a vial of xylene, distilled water, microscopy slides, stain and a selection of grids. The equipment was arranged in a comfortable fashion on a 30 inch high table with the microscope and the hot plate to the side of the microtome.

PROCEDURE:

The block of tissue was trimmed to a regular trapezoid face approximately 3mm high. The bottom of the trapezoid measured approximately 3mm across and the top measured about 1.5mm across. The relatively small block face makes sectioning easier on the MT-1.

The microtome advance was reset and the arm was placed in mid-down-stroke position to facilitate mounting the knife at the proper level. It was demonstrated that the knife should be positioned at the bottom edge of the block face with a slight forward tilt of the front face of the knife. The knife was secured in this position. It was pointed out that this tilt is called the rake angle.

The trough on the knife is then filled with distilled water delivered from the syringe. It is best to over-fill the trough and then draw the water down until a slight negative meniscus is seen in the trough. The light source, binoculars and knife carrier are adjusted to allow the operator to see the reflection of the knife on the block face. Here it was noted that we were demonstrating what is probably the most trying aspect of microtomy because it is at this point where the most unpleasant things can happen. The microtomist can damage the carefully trimmed block or ruin the knife. The edge of the knife can be seen and a dark space on the block face will narrow as the knife is moved to cutting position. Note that with the 15X viewing microscope, with practice, the knife can be advanced to a point where the sections will be coming off after a few

strokes of the operator wheel. Slow and steady rotation of the crank is continued as sections appear in the trough. The thickness of the sections can be estimated relative to their usefulness in the microscope by observing the interference colors generated by them. Note that irregular thickness will show as irregular color patterns in the sections. It was pointed out that gray and silver are seen on a good thin section.

One technique used was that of spreading sections with xylene vapors prior to placing the support grids on top of the sections as well as immediately after. The grids were then lifted from the trough and stained, rinsed, blotted dry and then stored.

THICK SECTIONS:

Perhaps one of the best present day applications of the MT-1 is the use of the instrument in obtaining semi-thin sections for optical microscopic screening of material for proper fixation or for location of desired structures. The operator can cut the entire cross-section of the idealized one millimeter cube of tissue. It was demonstrated that a square block face can be nicked on one corner to aid in the orientation for further trimming if a block is selected for closer study with the electron microscope. The set up is as described previously for thin sectioning but the thickness control is regulated to full advance which gives an advance of 0.5 micrometers per cut. If thicker sections are needed, then the microtome can be set to bypass the knife the right number of times to allow for the right thickness.

The sections can be transferred from the trough to a precleaned microscope slide by various methods, however, the simplest method by far is to use the point of fine jewelers' tweezers. A drop of water is placed on the slide and the sections are transferred one at a time by placing the point of the tweezers under a corner of a section and lifting straight up, then slowly lowering the section placing the bottom corner of the section on the water drop. The section will float free on the water drop. After drying, the section may be stained for optical microscopy.

DEVICE FOR TAKING MULTIPLE, UNIFORM TISSUE SECTIONS

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INTRODUCTION:

Electron microscopy laboratories which process biological materials may have need for a disposable tissue sectioning device which is both inexpensive and easy to construct. One can be made using double-edge razor blades and glass microscope slides. It can be adapted to the specific needs of the laboratory, such as varying the thickness of the sections, taking multiple sections from one site, and taking reproducible sections from multiple sites. After the slices of tissue are embedded, the thinner ones can be reviewed under a dissecting microscope with substage lighting if necessary. The instrument works best with fixed or lightly fixed tissues.

MATERIALS AND DISCUSSION:

The materials needed to construct the knife are as follows: (1) double-edge stainless razor blades (we use Gillette brand), (2) glass microscope slides, size 3x1 inch, 1 mm thick, and (3) Super Glue®. The basic model consists of one glass slide used as a spacer and a double-edge razor blade glued to each side. This basic model can be modified to cut thicker slices by gluing 2 or more glass slides together, and likewise, thinner slices can be obtained by using 2 or more glass cover slips (instead of slides) together.

If more than 1 slice is needed from an area, 3 razor blades and 2 slides can be used. This modification requires gluing an additional slide and an additional razor blade to the basic model. Deeper sections may be taken if extra blades are glued to the sides of the two cutting blades in the basic model and the spaced slide is glued much higher on the cutting blades. This increases the depth of the cut and gives the blades more stability. A third blade or scissors may be used to cut the bottom of the parallel section or sections to remove them from the bulk tissue. Soft unfixed tissues do not cut as well, but sections can be obtained if they are thicker. The Super Glue holds better if a little skin oil is applied to the upper surface of the blade before it is glued. The Super Glue will eventually give way in an aqueous environment, but many sections can be obtained if the blades are not left standing in these solutions. The modification for cutting deeper sections is not as sturdy and fewer sections can be cut.

AN INEXPENSIVE FILTER HOLDER FOR USE ON THE COPY STAND

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INTRODUCTION:

Sometimes its necessary to use filtration for color correction or to produce a desired background color when using the copy stand to make color transparencies or internegatives. An example that immediately comes to mind is making white letter/blue background slides using Kodak Vericolor Slide Film. Some of the most versatile and inexpensive filters to use are the square gelatin or acetate filters. These are sold either separately (for color correction, i.e., Kodak Wratten gelatin filters) or in color printing kits (Cibachrome printing filter sets with several grades each of yellow, magenta and cyan filters). Metal holders for these

filters are commercially available, however, they are expensive and hold only one filter of a specific size. If you need to stack filters for the desired effect, these holders are impractical. The device described herein will house several stacked filters and was made of readily available materials for minimal cost. This holder has been successfully used in our laboratory for several years and can be used on 35 mm or medium format cameras without modification. If properly altered, it could be used on large format or enlarging lenses.

MATERIALS AND METHODS:

Materials and equipment required for filters up to about 3½" square include the following: 2, 4"x4" squares of heavy matteboard, preferably flat black; a strip of Velcro **loop**-half, approximately 12"x¾"; a strip of Velcro **hook**-half, approximately 2"x¾"; about 2' of stiff wire (I used 12AWG insulated electrical wire), cut into 2, 1' long pieces; black electrical or gaffer's tape; scissors; wire cutters; stapler capable of stapling through matteboard and a scalpel or X-acto knife.

Cut out a window in the center of both pieces of matteboard (about 2½" - 2¾" square) so that the openings and edges are flush when the pieces are taped together. Hinge the two matteboard peices along one outer edge with electrical tape so that the holder opens like a book. The black sides of the matteboard should be on the outside. Staple small pieces of the Velcro hook and loop opposite one another on the inside, front edges of the matteboard "book". These will lock the filter holder closed once the filters are placed inside. Staples seem to hold the Velcro better than glue. Glue an inch or two length of Velcro loop-half on the top, inside half of the matteboard on each side of the open window. These strips will act as soft, pressure pads to keep the filters in place.

The rest of the device consists of side support arms to suspend the holder from the camera lens and a strap to attach the arms to the lens. Each support arm is made from about 6" of the stiff wire. For each side, the length of wire is bent into an even "U" shape. The bottom of the "U" is bent at 90° to fit under the bottom of the matteboard holder where it is securely taped in place. The top ends of the "U"s are bent down to form "belt loops" for the strap. The remaining length of Velcro loop-half is passed through the belt loops to secure the holder to the camera lens. A small piece of Velcro hook-half is stapled to one end of the strap so that the strap is adjustable to any lens size. The side support arms can be bent as needed to accomodate various diameter lenses.

DISCUSSION:

Keep the filters in their proper container to prevent scratches and dust accumulation. When you are ready to use them, select the desired filters and stack them evenly; open the holder and slide the filter stack in. Close the holder (the Velcro should keep it closed and the pads should keep the filters from sliding around on one another) and strap the device to the camera lens tightly. Keep the filters close to the lens (aberrations and dust will not be in focus if you have the filters close). Don't expose filters to hot copy stand lights any longer than necessary or they may warp. The head may also loosen the tape on the holder so check it fairly often. Modifications can be made to the length and bend of the arms so that the device can be used on virtually any lens.

A SIMPLE METHOD FOR FLAT EMBEDDING IN HYDROPHILIC RESINS

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Dept. of Pathology, Univ. of Texas Medical Branch
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INTRODUCTION:

Hydrophilic resins such as L.R. White and Lowicryl K4M are often used for electron microscopic immunostaining since they are less denaturing to antigens. They are sensitive to the presence of small quantities of oxygen, however, and require a truly anaerobic environment to harden properly. L.R. White is normally embedded in gelatin capsules with all air bubbles carefully excluded. This presents a problem when tissue samples must be oriented prior to sectioning (skin or epithelia for example). Previously, specimens had to be embedded, then cut out and trimmed and fastened to other blocks to produce oriented sections. Flat embedding can be accomplished in sealed jars made anaerobic by including Gas-Packs which generate a hydrogen atmosphere, but this method is cumbersome and a little expensive. At the last meeting, Laszlo Kömúsz described a method for flat embedding in L.R. White using cryomolds. I have confirmed that this is a very useful method, and added a minor modification which allows us to easily prepare L.R. White blocks identical in shape to those we use routinely for diagnostic electron microscopy.

MATERIALS AND METHODS:

Tissue-Tek Cryomolds are a trademark product of Miles Lab-Tek division, Napierville, IL, distributed by Baxter Healthcare Scientific Products Division; their cat. #4557, 25x20x5 mm size is necessary for this method (smaller sizes don't work). L.R. White resin is manufactured by the London Resin Co., London, UK and distributed by multiple suppliers; we obtain ours from SPI Supplies. Rubber bands and embedding ovens are readily available. Ted Pella's #106 blue silicone rubber embedding molds have 10 cavities per block which measure 11x6.4x4 mm. Using a sharp single-edge blade such as a Weck blade, a cut can be made into the Pella rubber mold just above the bottoms of the cavities to provide a flat 2.5 mm-thick rubber mold open on both sides. It is easy to trim this perforated rubber sheet so that two cavities just fit into a cryomold. Then L.R. White resin is added to the top of the cavity and tissue specimens are carefully placed in the point of the blue rubber cavities with labeling slips. A second cryomold is carefully pushed into the first so that all air bubbles are brought to one side and extruded as they fit tightly together. They are then secured together with a rubber band passing over all four sides. This

is then placed flat in an embedding oven at 55-60°C and left overnight. A good hard block which easily fits into the standard microtome chuck is the result.

DISCUSSION:

Laszlo Komusz's method works quite well if sufficient care is taken to exclude the last stray air bubbles. Of course, Chien molds or other special shapes of flat molds could also be used to produce special shapes for special purposes. We have found it useful to stack multiple layers of very thin specimens such as amnion on top of one another in tilted flat molds after dipping in resin to provide more surface to examine in the final thin section. This could also be done with L.R. White or Lowicryl. Pella has recently come out with a line of Teflon flat embedding molds which can be used for L.R. White embedding if they are covered with Aclar film. Although we have not yet evaluated this method, I expect it will also work well.

When using our method, we normally discard the cryomolds (which come in boxes of 100) but re-use the trimmed Pella rubber molds multiple times. Rick Giberson of Pella tells me that the blue silicone rubber molds will lose their normal consistency after prolonged use in L.R. White embedding; apparently, the devilish resin eventually diffuses into silicone rubber. Of course, trimming a few more blocks is easy to do. The recent publications of Laszlo and Julian Heath at Baylor's Children's Nutrition Research Center in the *Journal of Histochemistry and Cytochemistry* amply illustrate the kind of beautiful results which can be obtained when studying well-oriented epithelial samples by colloidal gold immunostaining.

A SIMPLE STORAGE METHOD FOR COLLOIDAL GRAPHITE

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INTRODUCTION:

Carbon based adhesives such as conductive carbon paint or colloidal graphite are frequently used in the laboratory for a general specimen adhesive as well as an SEM mounting medium. These adhesives are designed to air-dry rapidly at room temperature. This property also makes colloidal graphite difficult to store. The more the bottle is opened, the thicker the paint becomes as the solvent evaporates. Thinners are available, but the thinned paint doesn't seem to be as good as the original. Eventually, one may end up with several bottles of dried colloidal graphite which cannot be remixed. Aliquoting the conductive paint into smaller containers solves this problem.

MATERIALS AND DISCUSSION:

Aliquots (1ml) of stock colloidal graphite are transferred into 1.5 ml microcentrifuge tubes with Safe-Lock™ tops (Eppendorf #2236326-3). The stock bottle is then resealed with polyethylene sealing tape (Cole-Parmer #N-08279-10). Three to four working containers should be made at a time. The tubes with "lock-tops" do not need to be sealed and they can be opened several times before they dry out. When they do dry out, throw away the microcentrifuge tube and only a ml is lost; thus, all of those bottles of thick and dry colloidal graphite are a thing of the past.

A CABLE RELEASE FOR USE ON CAMERA AUTOWINDERS AND MOTOR DRIVES

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INTRODUCTION:

Most professional 35 mm cameras can either be equipped with motor drives/autowinders or come with built-in automatic film winding. Often, this is a desirable feature to have when remote camera operation is necessary (ex: when the photographer is hidden when photographing wildlife subjects), when you don't want to touch the camera for manual film advance, or simply for speed and convenience sake when shooting on the copy stand. Common cable releases will trip the shutters but will not activate motor drives (some of the newer cameras with built-in auto-film-advance are exceptions), so the film must be advanced manually. In order to trip the shutter and activate film-winding, a cable release must be plugged directly into the supplied socket in the motor drive itself. These specialized cable releases are commercially available from the major camera manufacturers and some generic photographic suppliers, however, they may not be stocked routinely in the local camera store and they are likely to be quite a bit more expensive than a common cable release. When one of us (LDG) needed such a cable release and it wasn't stocked in the local camera store (Mr. Enloe's), Mr. Enloe described the following device (based on information from a former customer) which served the purpose well at a fraction of the price of a commercial unit. From Mr. Enloe's description, I (LDG) was able to build the unit without any special tools

or equipment, using readily available materials.

MATERIALS AND METHODS:

The device is constructed of a plastic film cannister (the type commercial 35 mm film comes in) with a contact switch attached to the outside of the cap via an opening drilled or cut in the center of the cap. The contact switch housing is fitted in the opening and secured by its accompanying nut on the inside of the film cap. Speaker wire (cut to desired length) is run inside the cannister from the switch terminals (soldered to them) through the length of the cannister and finally through another opening drilled in the cannister base. It is a good idea to run the wire through a rubber grommet fitted to this opening to prevent abrasion of the wire as it is pulled through. A phono plug (2 mm dia.) is soldered to the terminal end of the speaker wire, outside the cannister. The wire can be virtually any length desired and can be pulled up inside of the film cannister for easy storage. It is simply pulled out through the hole in the cannister base when the cable release is to be used. The cannister easily fits in the palm of the operators's hand and the thumb is used to press the contact switch. Plug the phono plug into the camera's motor drive socket and you're ready to go.

The equipment needed for the project is as follows: soldering iron with fine tip; small diameter, resin-core solder suitable for electronics; wire strippers or sharp knife; drill and drill bits of appropriate size for switch housing hole and wire hole. The electronic materials were obtained at Radio Shack: 2.5 mm (3/32″), two-conductor phone plug; momentary contact switch (we used an Archer Momentary Pushbutton Soft-Feel Switch, SPSY contacts rated at 3A at 125V AC, cat. #275-1566); speaker wire, 2-conductor, 24 AWG stranded (we used Archer Speaker Wire, 50 ft. roll, cat. #278-1301). The film cannister was a standard Kodak 35 mm plastic cannister and the rubber grommets can be obtained at various electronics stores.

DISCUSSION:

There are many types of momentary contact switches available and any kind will work, so the device can be modified to suit the user. The length of speaker wire can be whatever the user desires, as well, depending on the intended application — i.e. remote shutter tripping from a wildlife blind vs. use on the copy stand. Likewise, the film cannister could be replaced by a plastic certifuge tube or other holder for the switch. The 2.5 mm phono plug will fit a variety of autowinders and motor drives including the following: Canon Motor Drive MF, Winder F (both for the manual F-1 camera), Motor Drive MA and Winder A (for Canon A series cameras) and autowinders from Olympus OM series cameras. You could check the socket size for your own camera's motor drive and use a suitable size phono plug if it isn't 2.5 mm. The entire cost of the device was under $5.00 at this writing.

A PETRI DISH STORAGE CONTAINER

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INTRODUCTION:

Commercial storage containers for Petri dishes are available, but they are expensive and the storage containers themselves can be difficult to file. When Petri dishes are used to store and archive samples, a large number may be needed. Coffee cans with plastic lids may be used for this purpose and they are free.

MATERIALS AND DISCUSSION:

Coffee cans (size 12 to 13 oz.) fitted with their plastic lids are the correct size for storing stacked Petri dishes. One coffee can will hold 13 dishes, size 100x10 mm. Retrieval of the dishes from the coffee can is achieved by using an easily contructed carrier which lifts the dishes out of the can. The carrier is made by using a strip of paper approximately 1x20 inches long. This strip is inserted into the coffee can and the end-flaps are left out. The dishes are placed in the coffee can on top of the carrier strip and the strip ends are folded across the top of the dishes for easy storage before the lid is snapped on. Coffee cans can be labeled and a storage log maintained for quick access to samples. The cans stack very well. It should be noted that coffee cans will rust; therefore, they should be washed and oven dried.

REMOVAL OF LEAD CITRATE AND URANYL ACETATE PRECIPITATES ON L.R. WHITE SECTIONS

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The Woodlands, TX

INTRODUCTION:

L.R. White is a hydrophilic embedding agent which allows aqueous solutions to easily pass through thin sections. Conventional methods for eliminating uranyl acetate precipitates (Hayat, 1986) suggest treating sections with 0.5% oxalic acid for 12 sec., 10% acetic acid for 1 min., or 2% aqueous (or ethanolic) solution of uranyl acetate for 1 min. Lead precipitates are eliminated by treatment with 2% aqueous uranyl acetate 2-8 min. at room temperature or 10% aqueous acetic acid for 1-5 min. Unfortunately, these methods may destroy the continuity of L.R. White sections. Our lab has modified the conventional methods of Hayat to

avoid or eliminate stain precipitates on L.R. White sections before and after they have been under the electron beam, while keeping the section intact.

MATERIALS AND DISCUSSION:

Our first objective is to prevent stain precipitates from forming during the staining process. Warm distilled water is used for the final rinse of the grid to avoid uranyl acetate precipitate. A 10 ml beaker of distilled water is set into the curing oven (50°C) during the staining procedure and taken out for use prior to the rinse step. To avoid lead precipitate during the staining process, add one sodium hydroxide pellet in 10 ml distilled water. Stain with lead, dip once in the solution, then wash with distilled water.

We have successfully removed uranyl acetate and lead salts from L.R. White embedded sections by dipping the grid in 0.25% filtered oxalic acid. Retain the section 3-4 times longer than the original stain time. This method has worked in our laboratory on sections that have not been under the electron beam for prolonged exposure (30 min. or longer).

REFERENCE:

Hayat, M.A. 1986. Basic Techniques for Transmission Electron Microscopy. pg. 225.

AN AIR-TIGHT CONTAINER FOR SMALL AMOUNTS OF OsO_4

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INTRODUCTION:

Osmium vapors readily leak out of many containers as is evidenced by the black preceipitates on inside refrigerator walls in many E.M. laboratories. A convenient way to prevent such leakage is to store the osmium solutions in glass vacuum-type blood collection tubes. The osmium vapors will not penetrate past the rubber stopper and contaminate the surrounding area.

MATERIALS AND DISCUSSION:

The "red-top" tubes which have no silicone coating and no additives are the best choice for this purpose. The tube top should have no needle punctures. Up to about 12 ml of solution can be stored per tube. To add or remove solution, simply remove the stopper and replace it when you are done. It is not necessary to evacuate the tube. We currently use Monoject brand (Sherwood Medical, St. Louis, MO) tubes but any brand used in clinical labs should suffice. If large quantities of osmium stock are to be made up, the osmium can be aliquoted into the tubes for easy storage and transport. The tubes can be grouped together and stored in a jar or other sealed container for safety. An "indicator" for leakage can be made by soaking a piece of filter paper in corn oil (or other unsaturated lipid) and placing it in the jar with the osmium tubes. This set-up is also ideal for transport to remote locations. There is little danger of leakage (even if the tubes get inverted) unless the tubes get broken. We have stored osmium in the same tubes for many months without any detectable leakage using this method. Regardless of how you store or transport osmium, however, corn oil or some other agent for denaturing the osmium should be on hand.

ANSWER TO "WHAT IS IT"

Scanning EM view of rat trachea after treatment with EDTA solution, which removes the ciliated and nonciliated epithelial cells, leaving predominantly basal cells attached to the basal lamina. The pavement-like pattern of basal cells is visible at the bottom, while the ciliated and Clara cells are present on the top. (Magnification X 1500)

Micrograph — Robert A. Cox, Shriners Burns Institute, Galveston, TX 77550

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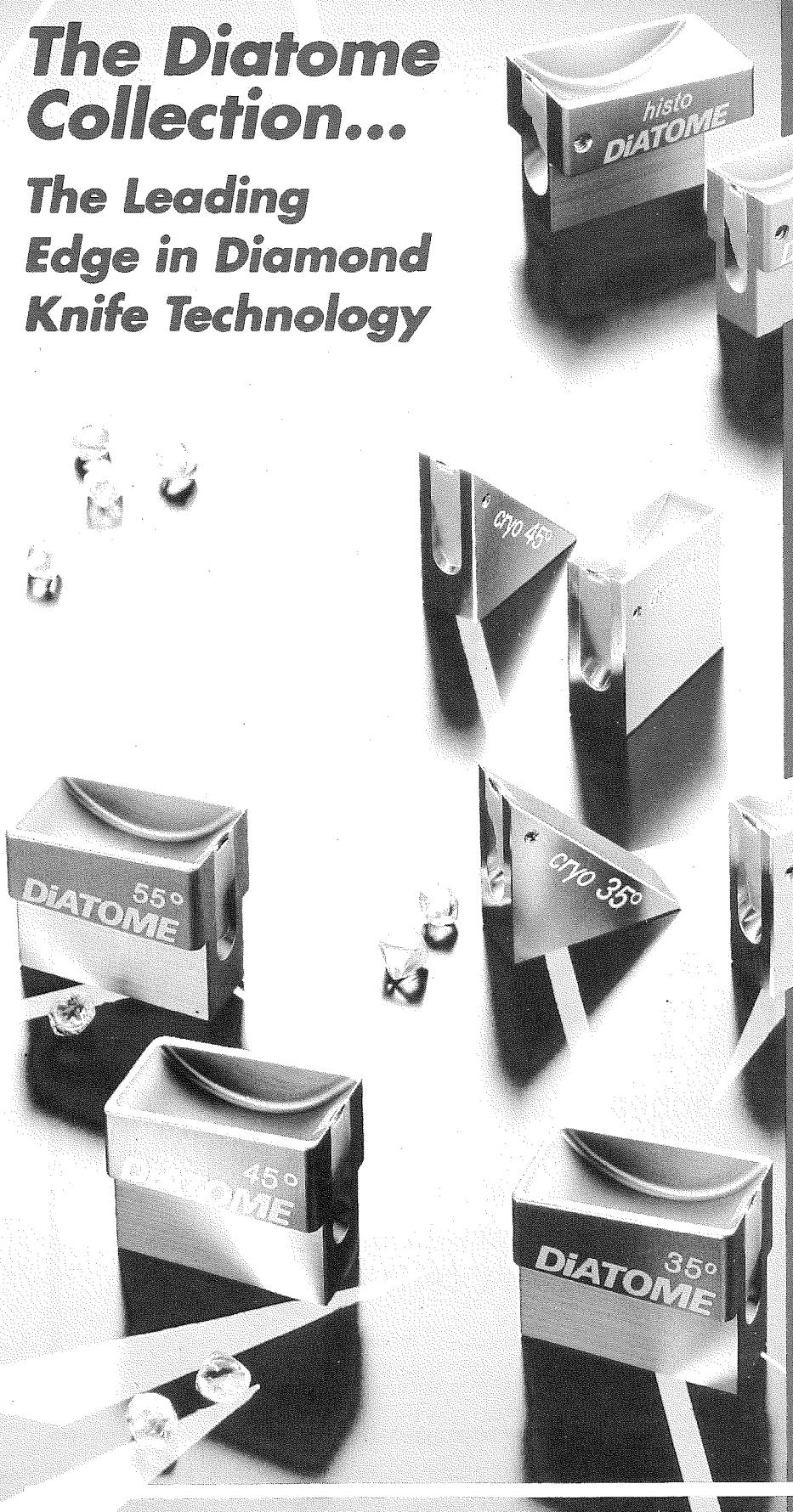

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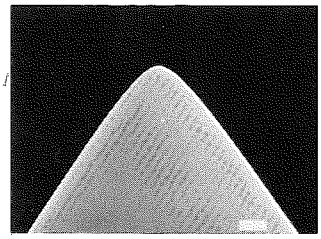
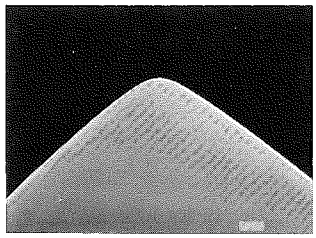
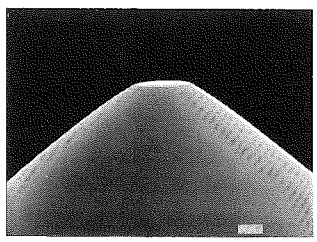

SHARP TIP


ROUND TIP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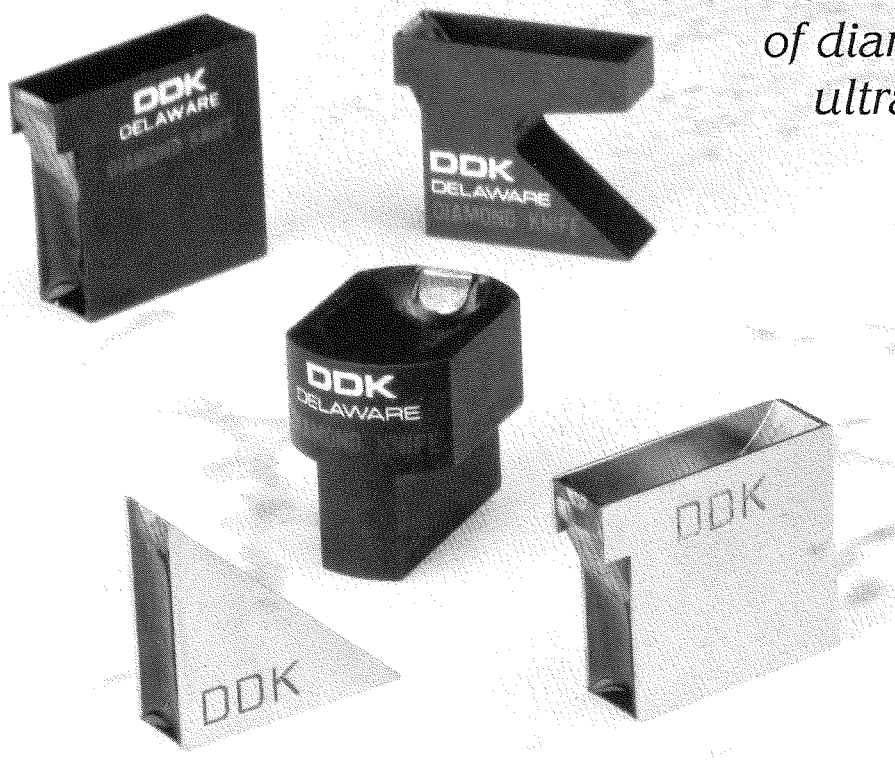

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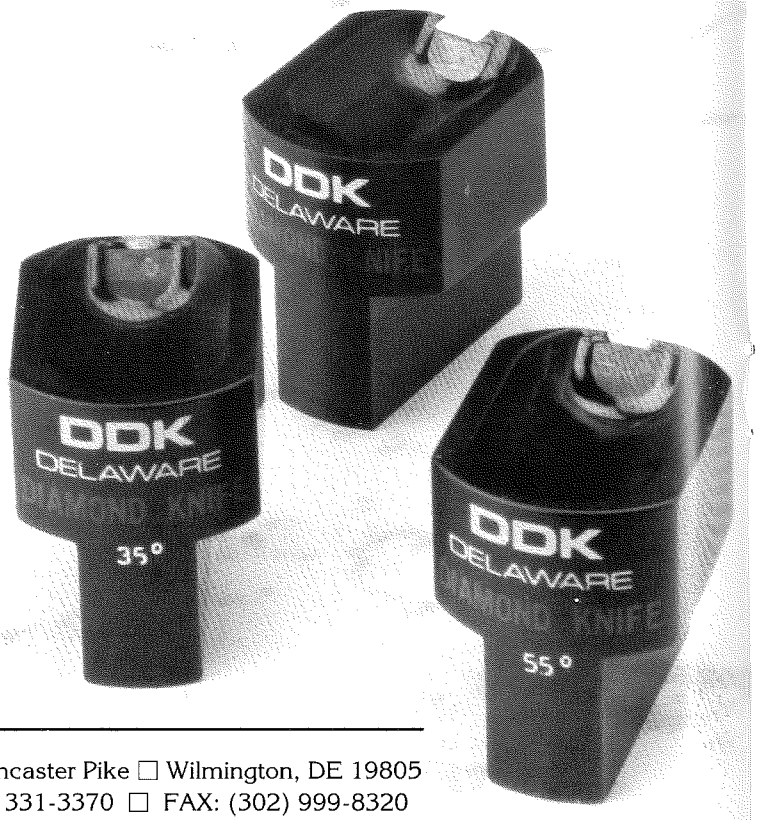

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Letters to the editor are printed as they are received in the order of their arrival. These letters reflect the opinion of the individual TSEM member and do not necessarily reflect the opinions of the editor or the society. The content of the letters should be concerned with the philosophical or operational aspects of the TSEM, the Journal and its contents, academic or national policies as they apply to TSEM and/or its members and electron microscopy in general. Editorial privilege may be evoked to insure that the LETTERS SECTION will neither be used as a political forum nor violate the memberships' trust.

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Micrographs submitted for cover photos should be marked as such. The choice of photographs will be made by the editor. Photograph receipt and/or dispensation will not be acknowledged. Photographs will not be returned. Electron micrographs to be used for cover photos and text fillers are welcome and should be selected with some attention to aesthetic appeal as well as excellence both in technique and in scientific information content.

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News items should be submitted through the regional editor in your area and made to conform to the standard format used by the regional news section. Regional contributions should be sent to the Regional News Editor. Editorial privilege may be executed for the sake of brevity or to preserve the philisophical nature of the TSEM Journal.

The JOB OPPORTUNITIES section will be comprised of a "Jobs Available" and a "Jobs Wanted" sub-section. Anonymity of individuals listing in the Jobs Wanted or Jobs Available sub-sections may be maintained by correspondence routed through the Regional News Editor's office.

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The Technical Section will publish TECHNIQUES PAPERS, HELPFUL HINTS, and JOB OPPORTUNITIES. The TECHNICAL PAPERS will describe new or improved methods for existing techniques and give examples of the results obtained with methods. The format of the Technique Papers will be the same as that used for regular research reports. HELPFUL HINTS will be in the form of a brief report with an accompanying illustration, if required for clarity. Helpful Hints should embody techniques which will improve or expedite processes and/or procedures used in EM.

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The right to publish in the TSEMJ is restricted to TSEM members or to those whose membership is pending. A membership application form can usually be found in each issue of the TSEMJ. Membership dues are as follows: student $2.00; regular members $15.00; Corporate members $75.00. Individuals who belong to TSEM by virtue of a corporate membership are invited to participate in Journal submissions as are our regular or student members. However, papers of a commercial nature, either stated or implied, will not be accepted for publication as a Research Report or Techniques Paper. Such papers may be acceptable as advertising copy.

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REQUIREMENTS:
You must be the sole author, personally present your paper from the platform, and submit a student competition application signed by a regular T.S.E.M. member, if possible your supervising professor. Two abstracts must be submitted by the designated deadlines; a regular T.S.E.M. abstract following normal procedures submitted to the current *Journal* editor, and an EMSA style two page abstract with an application for student travel submitted to the current secretary. Since it is assumed that your professor has supervised your work and others may have contributed in various ways, you must acknowledge these contributions on your application as well as in your platform presentation.

SPECIAL ABSTRACT FORMAT

1. The paper must be two pages each 8½" by 11". Margins should be 1" top and bottom and ¾" left to right. Text should be 12 characters per inch IBM LETTER GOTHIC or 11 point TIMES ROMAN with 12 point spacing each font at 6 lines per vertical inch.
2. The first page will have text only. Title on first line in all capitals except chemical symbols, single spaced if more than one line is needed. Leave one line of space; then your name and address skipping one line between each. Leave one line blank and start text with no indentions and skip one line between paragraphs. Group all references at the end on the text before illustrations.
3. Page two will include pictures and text. Micrographs should be numbered, have an appropriate scale marker, and be trimmed to form a retangle with no gaps. Figure captions should follow the micrographs and come last.
4. Examples and additional guidelines may be found by consulting an EMSA call for papers.

AWARDS:
Up to 3 awards (0-3) may be given at each Fall meeting. These awards may be cash or prizes as determined by the Executive Council. The top award that can be given is substanial support towards competing in EMSA's Presidential Student Award program. This award can only be given if you meet EMSA qualifications and compete at the next EMSA meeting.

JUDGING:
Judging will be by a panel of regular T.S.E.M. members. You will be judged 50% on the quality of your special abstract and 50% on the quality of your presentation, including how well you answer questions from the audience. The regular abstract you submit for publication in the *Journal* will not be judged. Because of additional demands of disclosure each entrant will be given an additional 5 minutes of podium time.

TSEM STUDENT COMPETITION APPLICATION

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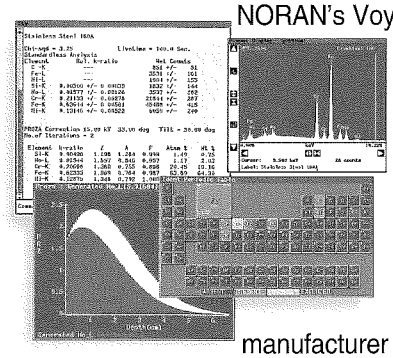

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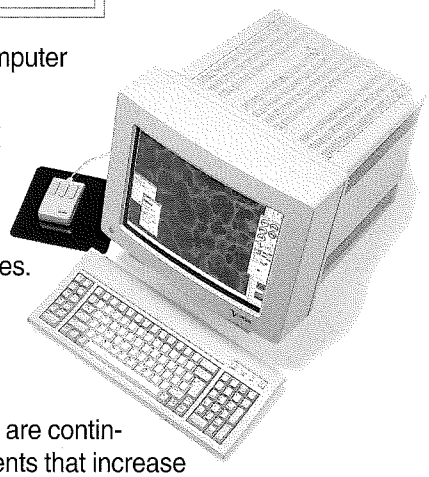

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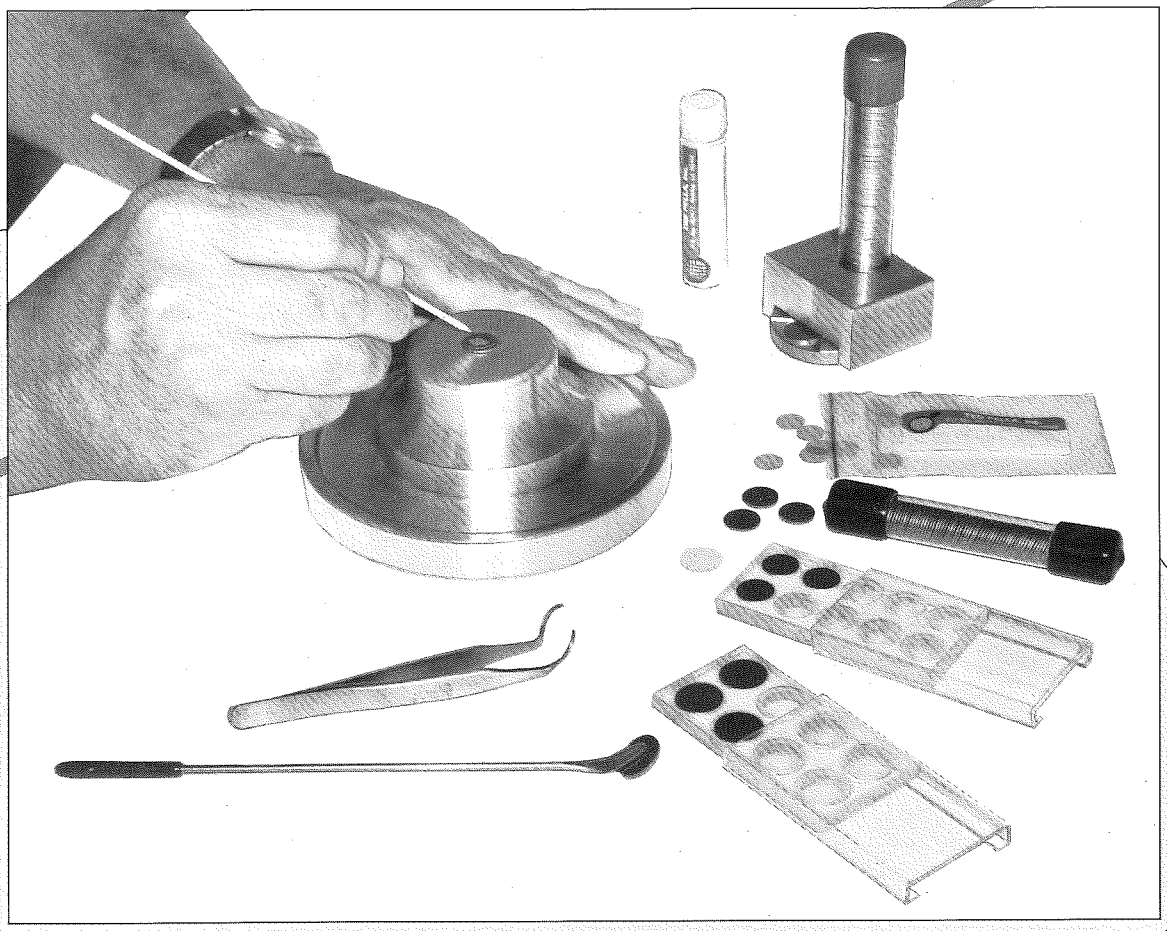

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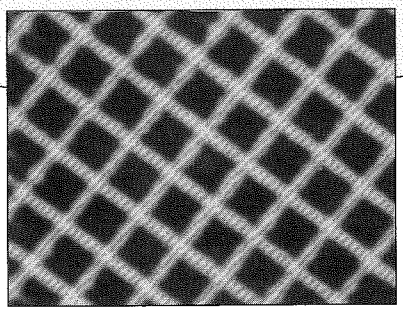

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Information for Authors

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FORMAT: Submit an original and two copies of the entire manuscript, typed, double-spaced, on 8½ x 11 white paper, leaving ample margins. Number each page and identify the article by placing, at the top left of the page, a shortened form of the title, followed by the last name of the first author.

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d. Full name, telephone number and address of the author to whom reprint requests are to be sent.

SECTIONS: The text of each original article and technical report should be divided into four major sections entitled INTRODUCTION; METHODS AND MATERIALS; RESULTS; AND DISCUSSION.

Historical and current reviews and case reports do not need to be divided into the aforementioned sections.

ABSTRACT: Summarize the article in no more than 150 words. This takes the place of a final summary paragraph.

REFERENCES to other work should be consecutively numbered in the text using parentheses and listed at the end, as in the following examples:

(1) A. Glauert, Practical Methods in Electron Microscopy. Vol. 2 (North-Holland. Amsterdam, 1974) 82-88.
(2) P.S. Baur, Jr., G.F. Barratt, G.M. Brown and D.H. Parks. Ultrastructural Evidence for the Presence of "Fibroclasts" and "myofibroclasts" in Wound Healing Tissues. J. of Trauma. 19 (1979) 774-756.
(3) D. Gabor. Information Theory in Electron Microscopy, in: Quantitative Electron Microscopy. Eds. G.F. Bahr and E. Zeitler (Williams and Wilkins, Baltimore, 1956) 63-68.

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NOMENCLATURE AND ABBREVIATIONS: Journal abbreviations used should be those listed by the "Index Medicus." Nomenclature abbreviations should be similarly standardized.

ACKNOWLEDGEMENTS should appear as a footnote which will appear at the top of the first page of the article.

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1. The Microscopy Society of America (MSA) has established a scholarship fund for undergraduate research. The purpose of the scholarship is to further educational and research potential in full time undergraduate students intent on pursuing electron microscopy as a career. Applications in all areas of electron microscopy will be accepted for review.
2. Scholarships will be awarded to full time undergraduate students. Maximum total dollar amount awarded each year will be $10,000.00.
3. When possible at least one scholarship will be awarded to an under-represented minority applicant.
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6. Awarded funds must be used within a designated time period not to exceed one year from the award date. Students are eligible to receive an award only once.
7. Applications must be received by 15 November of each year to be awarded by 1 March of the following year.
8. Complete application forms should include the following items:
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   - **B.** A research proposal not to exceed 3 pages in length. The proposal should include a brief introduction, a short methods section, and itemized goals of the study.
   - **C.** A budget proposal detailing how awards will be utilized. If the proposed project will exceed the requested amount, a statement should be provided indicating sources of additional funding.
   - **D.** Two letters of reference from academic and/or industrial personnel familiar with the student's competence are required.
   - **E.** A letter from the laboratory supervisor where the proposed research will be performed indicating the applicant will be accepted in the laboratory to work on the proposed project. A laboratory must be designated in the proposal for funds to be awarded.
   - **F.** A cirriculum vitae detailing previous education and/or experience in electron microscopy, and a brief statement of career goals.
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Include a detailed budget with sources of additional support for project on a separate page.

12. Name, title, and addresses of two academic or industrial personnel familar with the student who will provide letters of reference. Applicant is responsible for having all letters sent before the application deadline. Letters must be received before application is considered for funding.

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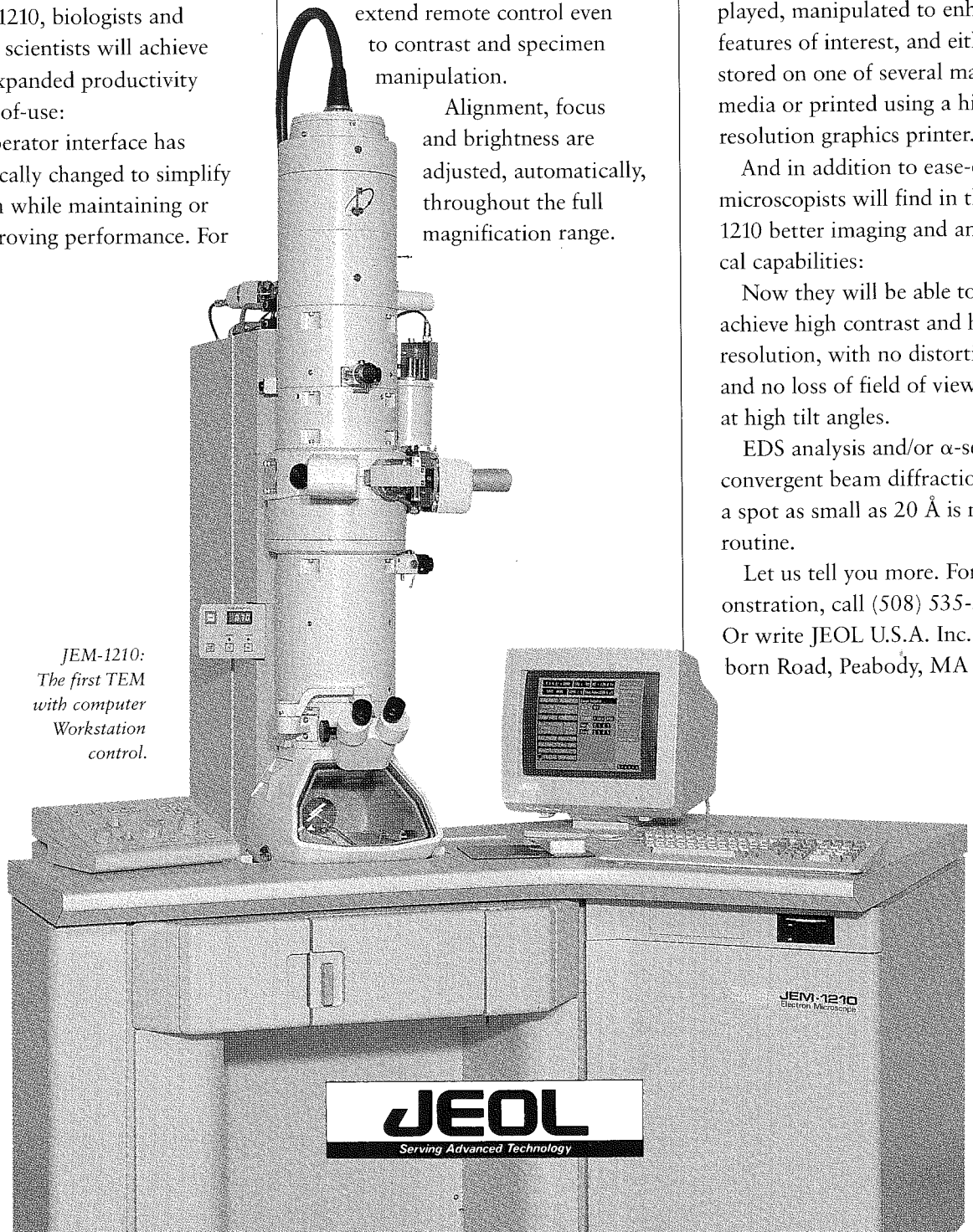

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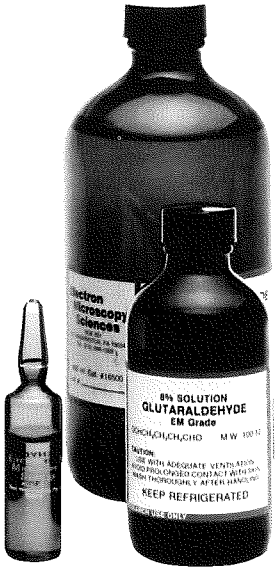

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Electron Microscopy Sciences

Abstracts

BIOLOGICAL SCIENCES

PLATFORM PRESENTATION — FALL 1993

FREEZE-FRACTURE METHODS FOR THE ANALYSIS OF CULTURED HUMAN TRABECULAR MESHWORK CELLS TREATED WITH DEXAMETHASONE. D. CANTU-CROUCH, M.D. MCCARTNEY, S.T. MIGGANS, A.F. CLARK, Alcon Laboratories, Inc., Fort Worth, TX 76134

The aqueous humor is produced in the eye and travels through a filter-like structure known as the trabecular meshwork before exiting the eye. Glaucoma is a disease that is characterized by an increase in intraocular pressure and is thought to be the result of increased resistance to aqueous outflow. Dexamethasone (DEX), a glucocorticoid, has been shown to induce a similar rise in intraocular pressure and produces a disease remarkably similar to glaucoma. In the present study, cultured trabecular meshwork cells (TM) were treated with DEX and compared to control TM cells using freeze-fracture. Human TM cells were cultured and grown to confluence on Millipore HA filters or glass coverslips. TM cells were then treated for 14 days with 0.1 µM of dexamethasone dissolved in ethanol. Control cells were treated with an equivalent amount of ethanol for 14 days. The cells were then aldehyde fixed and processed for freeze-fracture and electron microscopy. The intramembrane particle density was similar, and gap junctions were numerous on control and DEX treated TM cells. Vesicle fusion sites were found on both apical and basal membranes of DEX treated and control cells. On the apical surface, vesicle fusion sites were randomly arranged and few in number. Basal vesicle fusion sites were arranged in linear rows and in large patches, with areas of clear membrane interspersed. However, the density of vesicle fusion sites was significantly higher in the DEX treated human trabecular meshwork cells. These finding support our previous findings of increased secretory activity in dexamethasone treated human TM cells and appears to correlate with in vivo biochemical and ultrastructural data.

A TECHNIQUE FOR PRESERVING TISSUE SURFACE MUCIN: IMPROVEMENTS IN TISSUE FIXATION. J. BEAIRD AND M.D. MCCARTNEY, Alcon Laboratories, Inc., Fort Worth, Texas 76134

The mucin layer forms an important part of the protective barrier on ocular and other tissue surfaces. However, routine electron microscopy processing techniques remove this layer completely. Previous studies have shown that the mucin layer can be preserved by the addition of cetylpyridinium chloride (CPC) which stabilizes mucous glycoproteins. In order that this technique could be used in our laboratory, a series of experiments were performed to improve the preservation of the tissue while maintaining the mucin layer. Three variations of a fixative containing 0.5 % CPC ± tannic acid were used to preserve the tear film mucin on rabbit corneas. Tissue was processed without en block staining with uranyl acetate, dehydrated through an ascending series of alcohols and embedded in a Polybed/Araldite mixture. In addition, samples were divided at various stages of the fixation process to determine if the preserved layer was indeed mucin or artifact. All of the fixative combinations preserved the tear film mucin. However, primary fixation followed by a fixative containing CPC caused severe cracking of the superficial epithelial cells. Fixation with CPC but without the addition of tannic acid caused poor visualization of the mucin layer and low tissue contrast. Superior preservation of the corneal epithelium with a uniform layer of darkly stained mucin was achieved with a fixative containing 2.0% paraformaldehyde, 2.5% glutaraldehyde, 0.5% CPC followed by a second glutaraldehyde fixation with the addition of tannic acid. No edge effect was noted with any of the fixation methods confirming that the staining was not an artifact. This method provides direct electron microscopic visualization of tissue mucin and is potentially useful for investigations in the gastrointestinal and respiratory systems.

ULTRASTRUCTURAL STUDY OF BORON DEFICIENCY IN ROOT TIPS. CAMELIA G.A. MAIER, DAVID C. GARRETT, AND DON W. SMITH, Biological Sciences Dept., University of North Texas, Denton, TX 76203.

Tomato (*Lycopersicon esculentum* Mill.) and black-eyed pea (*Vigna sinensis*) seedlings were grown in a complete medium for 96 h and subsequently transferred to a medium deficient in boron. Samples of root tips were taken at 3, 24, and 48 h intervals and processed for transmission electron microscopy observations.

An increase in cell wall thickness and irregularity was observed as early as 3 h without boron in tomato seedlings. There was no difference in the ultrastructure of black-eyed pea roots with and without boron at 3 and 24 h. Increased vesicle formation at the wall-plasmalemma interface was observed in tomato root cells at 24 and 48 h. Mitochondria in both tomato and black-eyed pea showed hypertrophied, more electron dense cristae than those of control cells at 24 and 48 h. In boron-deficient tomato cells, myelin-like arrangement of membranes were observed to be associated with mitochondria. Golgi bodies in some of the boron-deficient tomato root cells showed damaged cisternae and vesicles at 48 h. These cells appeared to be in the early stages of senescence.

Black-eyed pea cells responded more slowly to the removal of boron than did tomato cells. A few visible changes in the ultrastructure of cell wall, mitochondria, and cytoplasm were observable only after 48 h without boron. The primary action of boron deficiency is probably not evident at the ultrastructural level. Many biochemical changes occur before they are expressed in the cell structure. Tomato cells were more sensitive to boron deficiency. The first symptoms were visible as early as 3 h after the removal of boron from the culture medium.

SEM STUDY OF THE DEVELOPMENT OF PROSOPIS GLANDULOSA VAR. GLANDULOSA SEEDS AND FRUITS. R.S. WESTOVER AND L.H. BRAGG, Dept. Biol., University of Texas, Arlington, TX 76019.

Prosopis seeds and their corresponding fruits were examined at various developmental stages using morphological characters to separate seed development into three stages.

Stage I seeds have an absence of mature seed surface features. In transverse section, future palisade cells are immature and osteosclereids have not developed. Seven to eight layers of undifferentiated parenchyma cells are present.

During Stage II a pleurogram develops. In surface view rugae begin to appear. In transverse section, palisade cells have elongated, osteosclereid precursors are evident and initiate differentiation into hour-glass cells. Five to six rows of parenchyma cells remain. A light-line becomes visible on the palisade cells.

In Stage III, cracks form on the seed surface. Rugae mature into a rugose pattern. In transverse section, osteosclereids (hour-glass cells) complete differentiation and parenchyma cells become sclerified. Endocarp cells (inner fruit wall) appear to be flattened by pressure from seed enlargement. An "imprint" of the seed's pleurogram is evident on most post-pleurogram endocarps.

THE DUAL SYSTEM OF CALCIUM OXALATE CRYSTAL FORMATION IN THE LEAVES OF *GINKGO BILOBA*. HOWARD J. ARNOTT, Dept. of Biology, The University of Texas at Arlington, Arlington, TX 76019.

The bilobed leaves of *Ginkgo biloba* are relatively unchanged from those found as fossils. Therefore, it is interesting to note that the production of calcium oxalate in modern *Ginkgo* leaves is not only quite substantial but involves at least two entirely separate systems of crystal production. This study used light microscopy (LM) and scanning electron microscopy (SEM) to examine the characteristics of this dual system. In this so called "living fossil" the distribution of calcium oxalate crystals in the various leaf tissues was examined by LM; both sections of the leaf and leaf clearings were important in demonstrating the number and placement of the crystals. The structure of both isolated calcium oxalate crystals and calcium oxalate crystals *in situ* were studied with LM and SEM techniques. Calcium oxalate monohydrate crystals are found in two tissue systems in *Ginkgo* leaves. In the phloem many relatively small druses, ca. 25 micrometers in diameter, are found associated in longitudinal files of cells; generally 6 to 10 crystal cells are in each phloem file. The crystals are produced in phloem parenchyma cells found between the phloem conducting cells (ray-like cells); the individual phloem druses are quite uniform in size. In the second system much larger druse crystals are formed in mesophyll cells that are closely associated with the vein surfaces. These crystals may have diameters up to 130 micrometers and are much more variable in size than those found in the phloem. Their volume may be as much as 250 times that of those found in the phloem. In some of the large mesophyll druses it is possible to see a non-crystalline core. The general function of both systems is presumed to be concerned with the regulation of calcium, however, the possible function(s) of the individual systems is not clear.

LUMINANCE ANALYSIS OF ELECTRON BEAM DAMAGE IN SECTIONS OF CALCIUM OXALATE MONOHYDRATE CRYSTALS PRODUCED IN THE LEAVES OF *VITIS VULPINA*. HOWARD J. ARNOTT AND LINDA E. LOPEZ. Dept. of Biology, The University of Texas at Arlington, Arlington, TX 76019.

The leaves of *Vitis vulpina* contain large numbers of cells that each produces packets of several hundred twin crystals of calcium oxalate monohydrate (COM). Both the crystals of calcium oxalate dihydrate (COD) and COM crystals are unstable in the electron beam. Because of this, most investigators who have used transmission electron microscopy (TEM) to study calcium oxalate production in either animals or plants must be satisfied with either irregular holes or zones in which the crystals have been replaced with plastic. The use of scanning transmission electron microscopy (STEM) provides an opportunity to examine these beam sensitive crystals because the electron beam passes each point of the crystal quickly. Because there is little or no damage, depending on beam parameters, the structure of these crystals can be examined. However, in attempting to inspect these crystals by dot-map x-ray analysis the rastering system will cause the beam to dwell on individual points in the crystal. Even though the dwell time may be quite short, e.g. 10 msec, the electron beam will often damage the crystals. In our experiments we have controlled the electron beam's dwell time and the spacing between beam points of interaction. Thus, we produced a series of regularly spaced beam damage spots on the sectioned crystals. The spot series have been produced in both the longitudinal and transverse planes of the calcium oxalate twin crystals. We will present measurements of both the diameter and the luminance of the spots in these series. These observations show that the spots are not uniform, that is, individual spots differ substantially from the norm.

CALCIUM OXALATE DRUSE CRYSTALS IN THE STEM OF *VITIS VULPINA*. TAMMY HANCOCK NELSON AND HOWARD J. ARNOTT, Dept. of Biology, The University of Texas at Arlington, Arlington, TX 76019.

Calcium oxalate druse crystals were observed utilizing both light and electron microscopy. Examinations were made of both fresh and fixed sections of the stems of *Vitis vulpina* collected on the campus of The University of Texas at Arlington. Both single-isolated crystal cells and longitudinal files of crystals were found to be present in the stem; the files of crystal cells were found associated with the phloem. Examination of the crystals extracted from this plant by x-ray diffraction showed them to be calcium oxalate monohydrate (COM). Both light and electron microscopy show that the druse crystals have a central core. Under crossed nicols in the light microscope this central core is dark and not birefringent. However, surrounding this dark core there is a series of sharp, highly birefringent COM crystals extending outward. It is these crystals which make up the bulk of the material present in each druse; each druse is a compound structure made up of many COM crystals. Energy dispersive x-ray analysis shows that the central core of the druse has a different elemental composition from that found in the COM crystals which have a strong calcium peak. Each sharp crystal forming the bulk of the druse appears to have a sheath around its exterior. It is not clear whether this sheath is equivalent to the "crystal membrane" found in many other calcium oxalate producing systems in plants. In addition to this sheath, in some cases it appears that cell wall-like material grows around the druse thereby separating it from the remainder of the crystal-containing cell. The calcium oxalate crystals found in files in the phloem seem to occupy most of the volume of each cell, however, those found in the cortical cells seem to occupy much less of the total cell volume. It is interesting to note that while raphide producing crystal cells are common in the leaf of *V. vulpina* they are not common in the stem.

ANALYSIS OF CELL STRUCTURE AS IT PERTAINS TO THE HYGROSCOPIC ACTIVITIES OF THE EXOPERIDIAL RAYS OF *ASTRAEUS HYGROMETRICUS*. M. S. HUFFINE AND H. J. ARNOTT. Dept. of Biology, The University of Texas at Arlington, Arlington, TX 76019.

When dry the exoperidial rays of *Astraeus hygrometricus* overarch and protect the endoperidium which contains the spores of this fungus. When hydrated these exoperidial rays bend outward and expose the endoperidium allowing spore release. The process of opening and closing is cyclic and repeatable. The bending of the exoperidium depends only on its structure and the presence or absence of water. In this study we used light microscopy, scanning electron microscopy and time-lapse video to characterize the mechanism of ray bending; we designate the longitudinal axis of the exoperidial rays as the "X-axis ", the width of the rays as the "Y- axis" and the ray's thickness as the "Z- axis." Sections of the exoperidial rays showed that they were composed of four distinct layers of hyphal cells as viewed in the X- or Y-axis. On the adaxial surface one finds a series of loosely attached cell clusters, the **cell cluster layer**. Below that thin surface layer is a thick **dense layer**, consisting of tightly packed hyphal cells whose long axes run parallel to the Z-axis of the rays; in the **dense layer** the cells are closely fused together and there very few "airspaces." Below the **dense layer** is a third layer, the **accommodation layer**, which is composed of loosely interwoven hyphae with many "airspaces". Below the **accommodation layer** is the **compact layer** which extends to the abaxial surface and is composed of tightly interwoven hyphae that run in various planes and contain few "airspaces." Based on the structure of the exoperidial rays, bending can be explained on a differential swelling (or drying) basis. When the **dense** and **compact layers** swell differentially bending is caused; the layers remain attached together because of the loose arrangement of hyphae in the **accommodation layer**.

PARTIAL CHARACTERIZATION OF A CYTOSKELETAL - ASSOCIATED PROTEIN IN MUSCLE. S. R. Jeffcoat, K. A. Price, *S. K. Maholtra, and J. R. Koke. Department of Biology, Southwest Texas State University, San Marcos 78666 and *Department of Zoology, University of Alberta, Edmonton, Canada T6J 2E9.

We have recently isolated and characterized a cytoskeletal protein which is recognized by a monoclonal antibody designated G3.5 raised in mice against human multiple sclerosis plaque material taken at autopsy. The G3.5 antigen has a molecular weight of 100,000 Da as determined by western blot analysis, and immunofluorescence studies have shown that the antigen co-localizes with the intermediate filament desmin. After homogenization in 10 mM phosphate with 0.5M NaCl, 1.0% non-ionic detergent, and various protease inhibitors, the antigen was found in the 4,000g pellet. Treatment of the pellet with 6.0M urea solubilized the antigen and after dialysis against phosphate-buffered saline (10 mM phosphate, 5.0 mM KCl, 0.15 M NaCl) the antigen remained in solution. These results indicate that the G 3.5 antigen is tightly bound to desmin or another intermediate filament and that the antigen possesses similar solubility properties as those of intermediate filaments.

We hypothesize that determining the amino acid sequence of the G 3.5 antigen will suggest its function. Experiments in progress include partial digestion of the purified protein with formic acid followed by analysis by Applied Biosystems 477A sequencer and screening skeletal muscle cDNA libraries. Preliminary results suggest the G3.5 antigen has sequence homology with ∂-actinin, and a relation to ∂-actinin is also suggested by our previous immunolocalization studies and the similar molecular weight of G3.5 antigen and the ∂-actinin monomer (100,000 Da). Experiments are currently in progress to differentiate between the G 3.5 antigen and ∂-actinin. Because ∂-actinin is an actin binding protein and the G3.5 antigen binds to intermediate filaments, the G3.5 antigen may be an ∂-actinin isoform which cross links intermediate filaments and actin.

BIOLOGICAL SCIENCES

POSTER PRESENTATION — FALL 1993

INTEGRIN EXPRESSION IN AIRWAY EPITHELIAL CELLS

Q. ZHU, A. BURKE, M.J. EVANS, R. COX and H.K. HAWKINS, Shriners Burns Institute and the University of Texas Medical Branch, Galveston, Texas 77550

Integrins are glycoprotein complexes responsible for non-junctional adhesion of airway epithelial cells to one another and to the extracellular matrix. Such adhesion is necessary for anchorage, migration, growth and differentiation of airway epithelium. Immunocytochemistry of intact trachea has identified the integrins expressed in airway epithelium, but information is lacking on the specific integrins expressed on the various cell types present in airway epithelium. The purpose of this investigation was to examine in dissociated cells from different species, the integrins expressed on the basal, ciliated and secretory cells in the airways.

Results of these studies showed that basal cells exhibited integrins α1, α2, α3, α6 and αV. Integrin α5 was detected with only trace staining. All species exhibited the same integrins for basal cells except the rat, which lacked α1.

Secretory cells also expressed all integrins except α6, although trace staining of α6 was expressed on rabbit secretory cells.

Expression of integrins on ciliated cells was less than on the other cell types. Readily detectable staining was found only for α3 and only on sheep and rabbit cells. α1 and α6 integrins were not expressed although trace staining was found for α2, α5 and αV.

These results indicate that integrin expression is quite distinct in different cell types and shows only slight variation among species. Consistent features include expression of α6 on basal cells and α3 on basal, ciliated and secretory cells of all three species examined.

DETERMINING POLLEN CONCENTRATION USING LUBRA-GLIDE® DRIL-BEADS®. A. RAYMOND[1], D. NEWTON[1], M. PENDLETON[1], and J. BIRD[2], [1]Dept. of Geology, Texas A & M Univ., College Station, TX 77843, [2]Sun Drilling Products Corporation, 1500 N. Post Oak, Suite 190, Houston, TX 77055.

The interpretation of microfossil and modern pollen data requires knowledge of microfossil and pollen concentrations. These concentrations are usually determined by adding tablets containing known quantities of *Lycopodium* spores to samples prior to pollen extraction. The relationship between the quantity of microfossils and pollen to the quantity of *Lycopodium* spores in a sample will determine pollen and microfossil concentration levels.

Currently a laboratory in Sweden produces *Lycopodium* concentration tablets but this product suffers from: 1) uncertain supply and availability and 2) great variability from batch to batch in spore number and standard error.

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Scanning electron microscopy was used to evaluate the damage to these beads generated by chemicals (HF, HCl, CH_3COOH, $ZnBr_2$, H_2SO_4) and procedures (centrification, sonication, and exposure to temperatures over 100°C) commonly utilized to separate pollen grains from their soil sample matrix.

Scanning electron microscopy was performed at the Texas A & M Electron Microscopy Center. Lubra-Glide® Dril-Beads® provided by Sun Drilling Products Corporation. This research is being considered for funding by the Texas Higher Education Coordinating Board, Advanced Technology Program.

SOME OBSERVATIONS ON THE PLANT MITOCHONDRIAL CRISTA. HILTON H. MOLLENHAUER, Electron Microscopy Center, Texas A&M University, College Station, Texas 77843-2257

Isolated mitochondrial cristae from plant root cells were examined by negative staining. The procedure was as follows: Tips of roots from bush bean (var. Top crop) and maize (var. Golden Bantam) were cut off about 1 mm from the end of the root and discarded. The remaining root stub was then dragged through a micro-drop of phosphotungstic acid (PTA) which had been placed on the edge of a Formvar-coated grid, and then over the central part of the grid. This disgorged cell contents and mixed them with the PTA -- all in a total time of less than 10 seconds. Conventionally fixed and embedded cells were used as controls.

Many negatively stained cell constituents could be recognized, including mitochondria. Mitochondrial form varied from intact to completely disrupted, but cristae could be identified at all stages of mitochondrial disintegration. Mitochondrial matrix substances were solubilized by the PTA, but membranous structures such as cristae remained intact. The results verified that cristae were rather uniform tubules 30 - 50 nm in diameter with a length of ¼ - ¾ μm. The ends of each crista tapered rapidly in a short transition zone to a diameter of <20 nm. Most isolated cristae were interconnected end-to-end to form strings of up to twelve cristae. A small fragment of inner mitochondrial membrane was often interposed between two interconnected cristae. These results suggest that cristae are connected to the inner mitochondrial membrane through transition zones of unique character.

A PROCEDURE FOR TRIPLE-LABEL IMMUNOELECTRON MICROSCOPIC STUDIES P.J.G. Neill and K.R. Fry. Alice R McPherson Laboratory of Retina Research. The Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX 77381.

Multiple-label immunoelectron microscopic studies using antibodies raised in the same species have been hampered by inherent problems with crossreactivity of the secondary markers. In addition, it is essential that the marker used for each antibody must have a distinctly different appearance at the electron microscopic level. In the past, this laboratory has overcome this problem in double-label studies by combining a pre-embedding immunoperoxidase label with a post-embedding immunogold label. This double-label paradigm has been used to study the synaptic relationships of a subpopulation of retinal ganglion cells utilizing γ-aminobutyric acid as a neurotransmitter (Neill and Fry, TSEM Spring Meeting 1993). The current study describes a methodology for introducing a third labeling procedure, i.e. a triple-label, by performing a second post-embedding immunogold labeling step after silver enhancement of the first immunogold complex (Beinz, et al. 1986). This silver enhancement procedure increases the gold particle size and at the same time blocks the antibody site, thereby allowing for labeling with a second antibody raised in the same species. The silver particles are uniform in shape and their size can be controlled with development time. This triple-label paradigm is being used in this laboratory to study the various neurotransmitter-specific synaptic relationships between GABAergic amacrine cells, glycinergic amacrine cells, glycinergic bipolar cells, and ganglion cells (GABAergic and non-GABAergic). Preliminary observations have demonstrated synaptic relationships from glycinergic amacrine cell processes to GABAergic amacrine cells, other glycinergic amacrine cells and ganglion cell processes; and from glycinergic bipolar cell processes to GABAergic amacrine cell processes. (Supported by NIH EY06469 and the Retina Research Foundation)

MATERIALS SCIENCES

PLATFORM PRESENTATION — FALL 1993

DIFFUSION BEHAVIOR OF TIN IN GOLD COMPOSITE SOLDER, YUJING WU, Center for Materials Characterization, University of North Texas, Denton, TX 76203-5308.

Intermetallic Cu_6Sn_5 and Cu_3Sn form and grow at the interface of Sn/Pb solder and a copper substrate. The addition of Au particles to eutectic Sn/Pb solder strongly affects the diffusion behavior of Sn, and therefore affects Cu-Sn intermetallic formation. The activation energies for the formation of Cu_6Sn_5 and Cu_3Sn for 4 wt% Au composite solder are 0.65 and 0.85 eV, respectively, which are smaller than the values of 0.84 and 1.63 eV for the eutectic solder alone. To determine the mechanisms of how Au particle additions affect Sn diffusion and intermetallic growth, the microstructure of the Au composite solder was studied using SEM, TEM, and XEDS. The diffusion data in the literature were also examined. Au rapidly diffuses by an interstitial mechanism in the Au-Sn system. All Au particles in the solder matrix react completely with Sn to form $AuSn_4$ during soldering. Initially, the amount of Sn that reaches the solder/copper interface is reduced and the thicknesses of Cu-Sn intermetallics after the soldering operation are thinner than for the eutectic solder alone. The $AuSn_4$ particles are distributed uniformly in the solder matrix. XEDS in STEM mode shows that at $AuSn_4/AuSn_4$ grain boundaries or $AuSn_4$/Sn phase boundaries, the intensity ratios of Sn to Au are much larger than inside the $AuSn_4$ grains. This is strong evidence that these boundaries act as enhanced diffusion pathways for Sn. Since Sn diffuses easily through these boundaries after soldering, the Sn supply to the solder/copper interface is increased, and the activation energies for the formation of Cu-Sn intermetallics at the Au composite solder/copper substrate interface are reduced relative to the eutectic solder alone.

A DARK FIELD TEM STUDY OF THE MICROSTRUCTURE OF $BaTiO_3$ ON Si AND Si/Ge, Y.G. RHO, E.G. Jacobs, R.F. Pinizzotto, S.R. Summerfelt* and B.E. Gnade*, Center for Materials Characterization, University of North Texas, P.O. Box 5308, Denton, TX 76203. *Materials Science Laboratory, Texas Instruments Inc., Dallas, TX 75243.

The microstructures of BaTiO3 (BT) thin films deposited by pulsed laser ablation onto substrates of (100) Si with and without 0.3 μm thick Ge barrier layers were studied using dark field TEM. The samples were fabricated under various temperature and O_2 overpressure conditions. Our previous studies indicated that BT deposited on Ge has a strong preferred orientation, whereas BT deposited directly on Si does not. The plane view dark field studies showed that as much as 40% of the BT on Ge is aligned with the same orientation as compared to only 10% of the BT on Si. Using cross-sectional TEM, it has been observed that extensive areas (> 50%) of the BT on Ge consist of large, highly-oriented grains with embedded smaller grains. These results may have important implications for "ferroelectric domain engineering" control.

INTERMETALLIC PHASES OF EUTECTIC Ag/Sn SOLDER. D.R. FLANDERS, E.G. Jacobs, R.F. Pinizzotto, Center for Materials Characterization, University of North Texas, Denton, TX 76203.

Copper strips approximately 2mm wide and 10mm long were soldered with eutectic 4Ag/96Sn solder to form sandwich-like structures. The goals of this study are to characterize the intermetallic phases formed at the Cu/solder interface, to study the diffusion behavior of Sn, and to calculate the activation energies for intermetallic formation. Samples were annealed in dry box ovens at 110, 130, 150 and 170°C for 0, 1, 2, 4, 8, 16, 32 and 64 days. This gave an overall sample matrix of 32 samples. The solder and intermetallic phases were examined using SEM/XEDS. The intermetallic layers at the Cu/solder interface were Cu_3Sn, Cu_6Sn_5 and Ag_3Sn. Average thicknesses of the intermetallic layers were calculated by encoding a minimum of 100 thickness measurements into a PC for data analysis. Diffusion coefficients, D, for each intermetallic layer were calculated at each temperature assuming a rate equation of the form $x=\sqrt{Dt}$. Using this data, the activation energies of the Cu_3Sn and Cu_6Sn_5 layers were determined.

ASSESSMENT OF MATERIALS COMPATIBILITY WITH MOLTEN SOLDER IN LIQUID METAL JET SYSTEM. S.L. RANSOM. E.G. Jacobs, CMC UNT Denton, TX, J. Priest, P. Dubois, ARRI UTA Fort Worth, TX.

Liquid metal jetting, similar to ink-jet printing, is a promising alternative for applying solder to printed circuit boards. A system for jetting molten solder is being developed at the Automation and Robotics Research Institute (ARRI). The technical challenges of producing a reliable jet stream and consistent drop formation are intensified by the operating temperatures ($\geq$ 400 °F) and the chemical reactivity of molten metal with the system components. Several factors were studied, including clogging of the jet orifice, the effect of placing a filter in the jet stream, impurity content of starting and filtered solder, and droplet formation. Orifices (50 μm in diameter), clogged during jetting of eutectic Pb-Sn solder, were examined by SEM/XEDS. Only Pb and Sn were found in the clogged material. Type 316 steel sintered filters, placed in the flow stream to filter impurities and inclusions from the starting materials, were examined by SEM/XEDS. Inclusions (105-10 μm diameter) containing Fe, Ni, and Cr were found at the filter/filtered solder interface. These inclusions are large enough to clog or partially clog the orifice and interrupt the jet flow. Wet chemical analysis of the starting and filtered solder showed that the Ag, Cu, and Sb contents decreased after filtering. However, the Ni content was 4 times greater in the filtered sample. These results suggest that solder is picking up impurities from the jet system. This project demonstrates that using initially clean materials and preventing the introduction of impurities into the molten solder are essential to successful operation of the liquid metal jet.

This work is funded by the Texas Advanced Technology Program.

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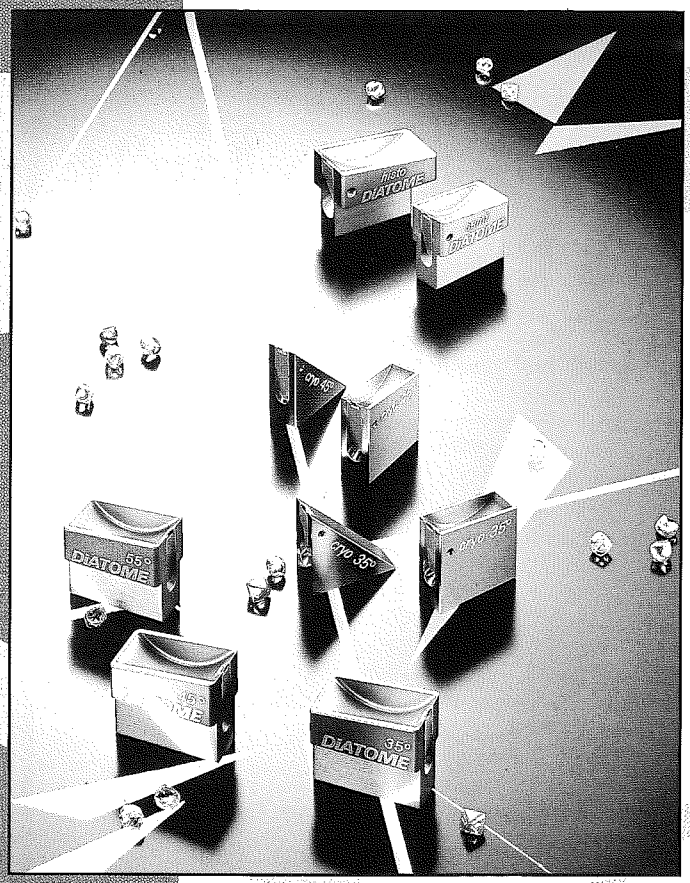

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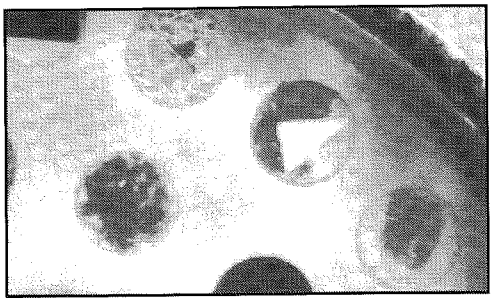

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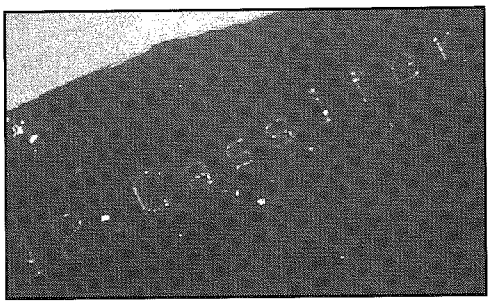

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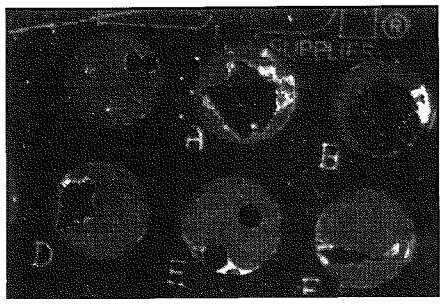

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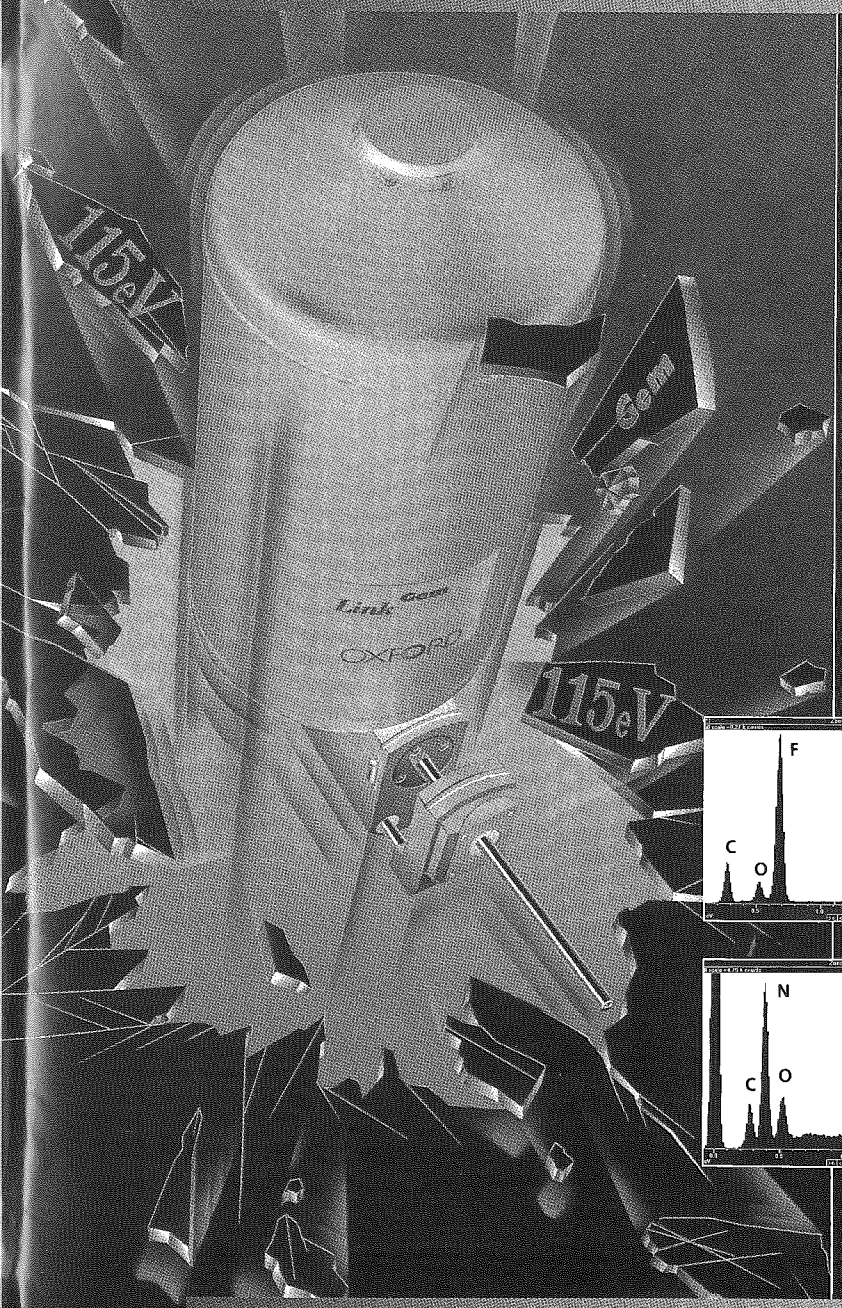

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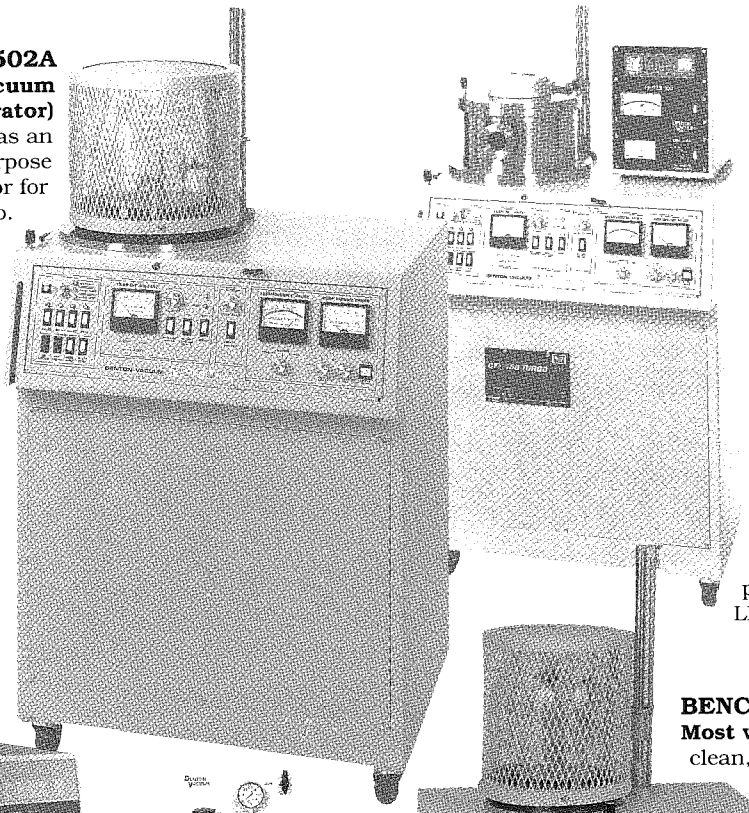

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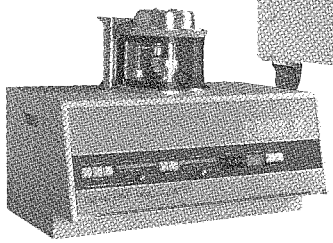

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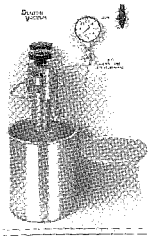

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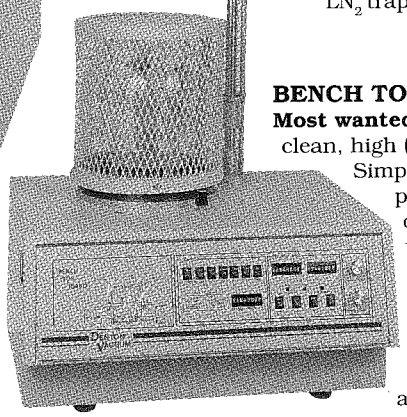

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