TSEM
Texas Society for Electron Microscopy

JOURNAL
VOLUME 22, NUMBER 1, 1991
ISSN 0196-5662

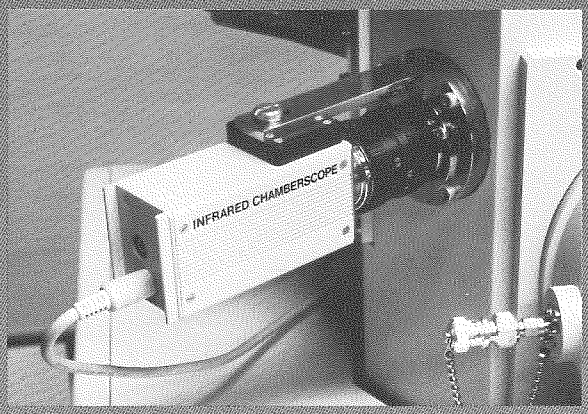
In this issue . . .

- TSEM 25th Anniversary Photos
- Article — The China Experience
- Article — Epoxy Resin for Tissue Embedment

SEM CHAMBER VIEWING CAMERA

INFRARED CHAMBERSCOPE

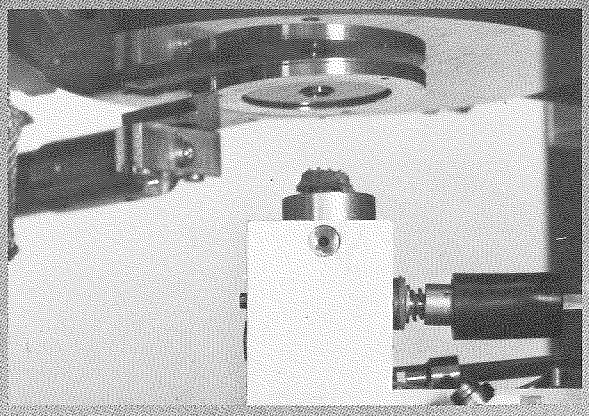
The Infrared Chamberscope (IRCS) provides a continuous view of the specimen and interior of the specimen chamber on a standard television monitor.



Infrared Chamberscope mounted on a microscope column.

IRCS BENEFITS:

- Orient specimen to best advantage for SEM imaging by various detectors.
- Locate desired specimen when several specimens are mounted on a single stub.
- Avoid damage to the objective lens, X-Ray or Backscattered Electron Detectors, or the specimen itself especially when observing large or awkward specimens.
- Observe specimen and stage position to avoid damage when opening chamber.



Photographic rendering of an SEM chamber.

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Contents

TEXAS SOCIETY FOR ELECTRON MICROSCOPY JOURNAL
VOLUME 22, NUMBER 1, 1991
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

SEM micrograph of fracture face of plastic.

Photo — Douglas A. Hayworth, Department of Biology, The University of Texas at Arlington, Arlington, TX 76019. (Magnification approximately 3200X; Bar = 0.01mm)

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Our new JEM-1210 is the first to incorporate modern 32 bit computer technology into a state-of-the-art TEM. The resulting instrument offers biologists and materials scientists new levels of microscope performance.

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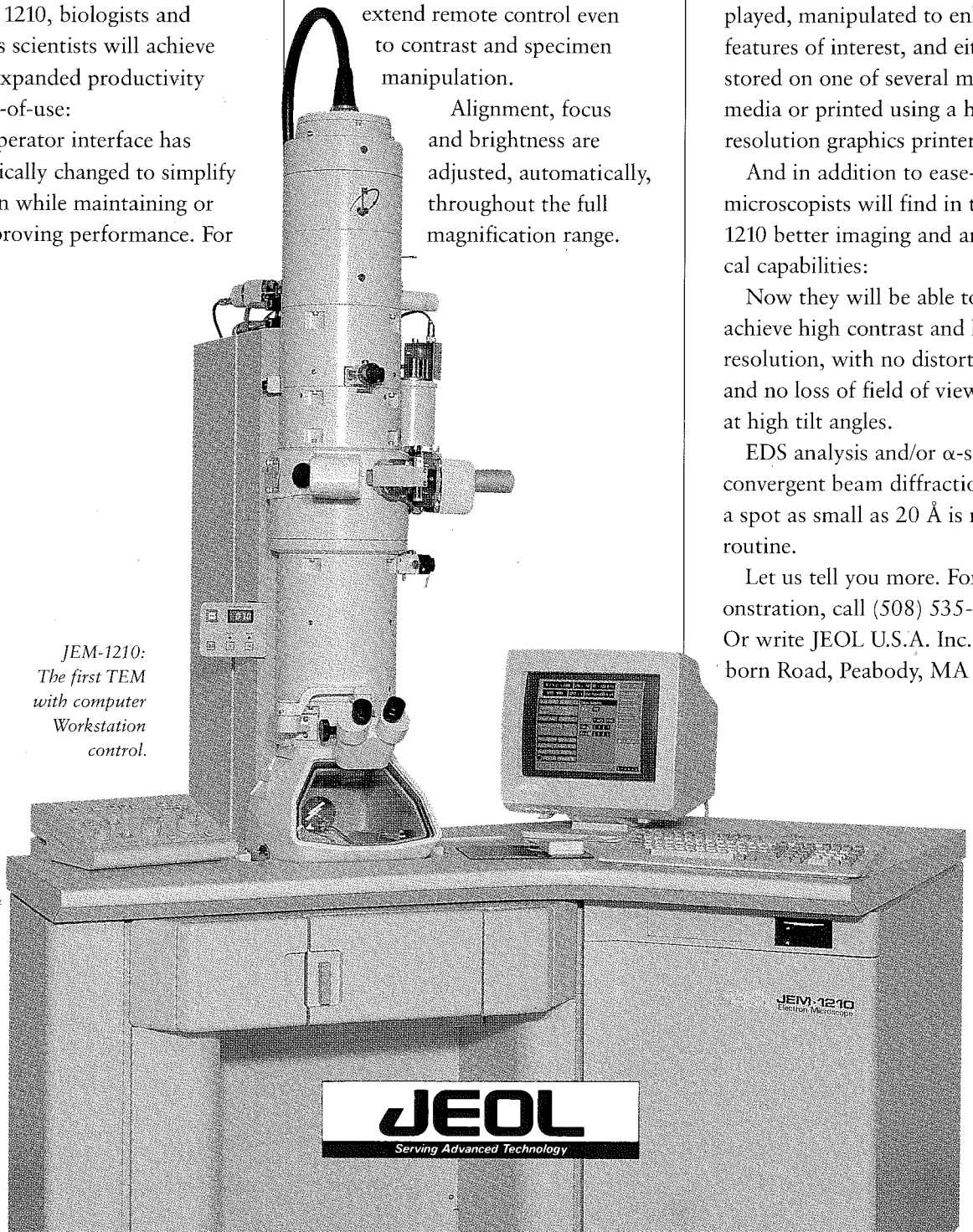


JEM-1210: The first TEM with computer Workstation control.

President's Message

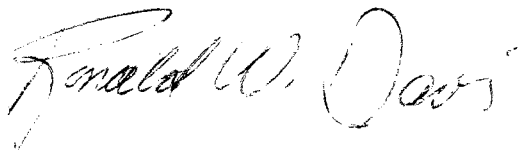
The first thing I want to do is thank all the people that attended the 25th TSEM Anniversary meeting last October in Galveston. I was very pleased with the way it turned out and must say that that success was due primarily to the efforts of Lynn Gray, the Program Director. As with our past experience with the Tremont House, the rooms, food and service were excellent.

For those of you that could not be there, you missed an opportunity to meet a lot of people that have been associated with TSEM for a long time. That included a fair number of the TSEM Charter Members. One very special attendee and former TSEM president, Dr. Larry Thurston, was there and started the meeting by announcing the first paper of the opening season. We hope to see him at more meetings in the future.

One nice feature of the last several meetings has been afternoon workshops. The workshop conducted by Hal Hawkins on conjugation of proteins to colloidal gold was extremely interesting and well attended. Hal and friends put in a lot of work. I believe that workshops will continue to be part of TSEM meetings in the future, although we may not be able to have one at every meeting.

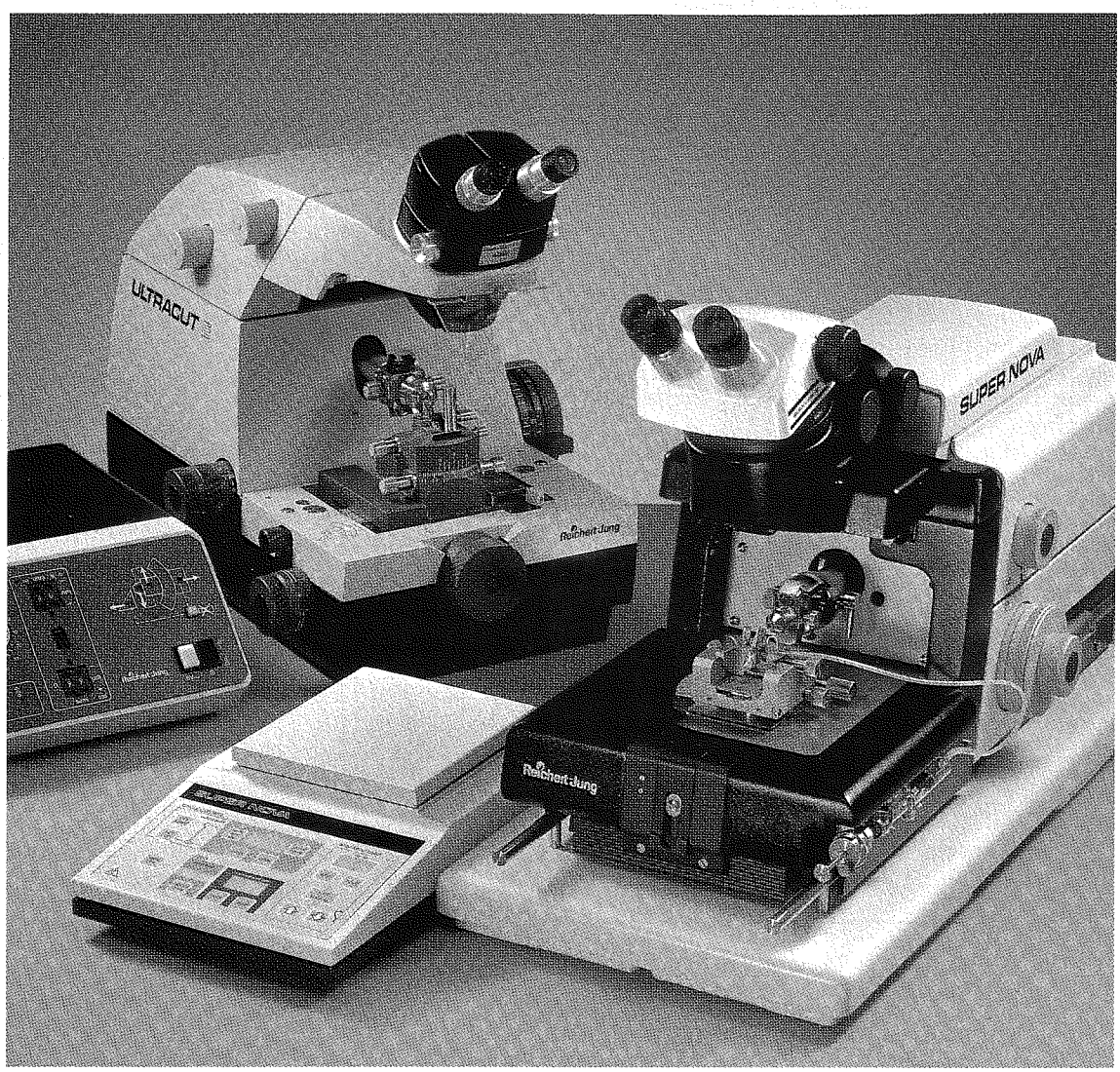
As you read this we will be either conducting or have already conducted our joint meeting with the Oklahoma Society for Electron Microscopy (OkSEM) in Arlington, Texas. Again, Lynn Gray has done a superb job of planning a logistically complex meeting.

Other people that need to be thanked are Nancy Smith, our secretary, who consistently does a super job albeit with a computer program that seems to satisfy the law of maximum human unhappiness. Mannie Steglich, treasurer, who has kept our finances in order and has no problem saying, "No, you can't buy that." Louis Bragg, Journal Editor, who is doing a fine job. Carl Duffner, of the TAMU Electron Microscopy Center, who almost single-handedly has tried to organize TSEM physical scientists. If Carl and the rest of TSEM can carry through with this very important project it will help satisfy the challenge of our former president, Howard Arnott, to develop a physical scientist membership and make TSEM "not the best **little** EM society in the USA," but the **best** EM society in the USA!"



Ronald W. Davis
President, TSEM

ULTRAMICROTOMY BY REICHERT-JUNG



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Treasurer's Report

TREASURER'S REPORT
For Period Ending 31 December, 1990

ASSETS ON 1 JANUARY, 1990:		
Certificate of Deposit No. 177576	$3,241.48	
Certificate of Deposit No. 10-0003893	2,492.19	
Checking Account No. 01600041996	1,626.76	$ 7,360.41
RECEIPTS:		
Dues	$3,688.00	
Arlington Meeting Exhibitor Fees	130.00	
Kerrville Meeting Registration	910.00	
Kerrville Meeting Exhibitor Fees	1,255.00	
Electron Diffraction Workshop	240.00	
Galveston Meeting Registration	2,320.00	
Galveston Meeting Exhibitor Fees	1,050.00	
Colloidal Gold Workshop	850.00	
Journal Ad Revenues 20:2	745.00	
Journal Ad Revenues 21:1	2,500.00	
Journal Ad Revenues 21:2	2,375.00	
Checking Account Interest	221.75	
Certificate of Deposit Interest	383.76	
Donations	1,872.21	$18,540.72
EXPENSES:		
Journal Editor Expenses	$ 93.26	
Treasurer's Expenses	100.00	
Professional Services	$ 100.00	
Kerrville Meeting Expenses	3,180.33	
Student Travel	525.00	
Honorarium — Kerrville Meeting	200.00	
Electron Diffraction Workshop	203.76	
Galveston Meeting Expenses	5,151.77	
Colloidal Gold Workshop Expense	150.00	
Honorarium — Galveston Meeting	400.00	
Journal Printing 21:1	2,014.10	
Journal Printing 21:2	2,191.58	
General Mailout / Secretary's Expenses	2,287.05	
Registration Refund	25.00	
Checking Account Service Charge	85.87	$16,707.72
ASSETS AS OF DECEMBER 31, 1990:		
Certificate of Deposit No. 177576	$3,241.48	
Certificate of Deposit No. 0014483028	1,500.00	
Certificate of Deposit No. 0014483036	1,500.00	
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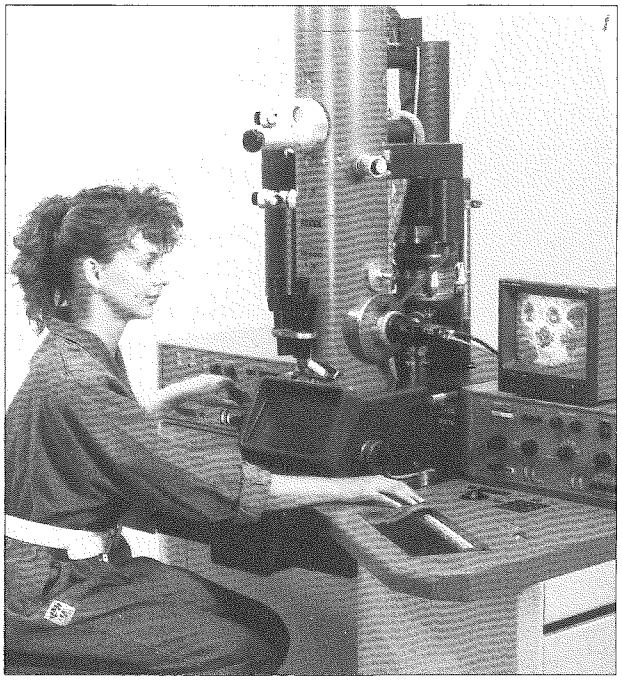

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Letter to the Editor

A REMINISCENCE OF TSEM AT THE 25th ANNIVERSARY MEETING

The babe known as TSEM was conceived in a Volkswagen on the Gulf Freeway in early 1965. Proud parents, Bill Philpott and Lea Rudee, held a christening party at Rice University shortly thereafter and surrounded the baby with lots of loving uncles, aunts and godparents. The babe grew in stature and was fed a steady diet of electron microscopy washed down not too infrequently with healthy quaffs through the slim neck of a Budweiser. The babe was schooled all through Texas and met its sister many times in New Orleans.

When just learning to walk, the babe came under the social guidance of Uncle Bobbo, the Grand Poo Bah of TSEM. The Bandera Ranch taught it that "all work and no play makes TSEM a dull boy". It passed puberty (in more ways than one) at Waterwood, thanks to Uncle Terry; and, who can forget how the babe came of age and lost its innocence with this exchange between Uncle Ivan and Dean (of the Graduate College at UTMB) Page, a recent NIH Director:

Uncle Ivan: *How can NIH justify awarding grants to applicants who have already done the research they propose?*

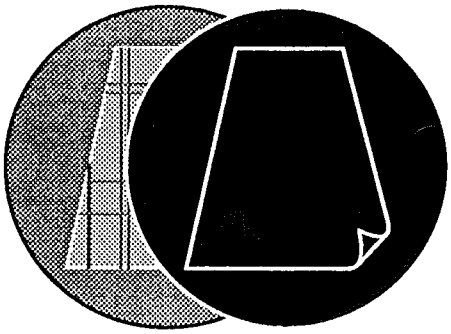
Dean Page: *Why, I have never heard of that!*

Uncle Ward inherited the babe's diary from Uncle Carl. He believed others should know what the babe was up to and prepared its history in the form which is now a Journal. All this time others were watching. The babe's history became the model for the EMSA Bulletin. The babe started the Local Society Section of EMSA and contributed mightily to the advance of e.m. knowledge, and threw a bash for the grandparents in San Antonio.

So many contributed to the stature of this babe, not to mention the tender touch of Aunt Ann, who taught it some civility. And, who can forget the "Iowa State Connection": Uncles Bill, Terry, Ward, Larry and Jerry. Maybe there is something to the midwestern work ethic. They all pretty much succeeded one another and in that period the babe grew its biggest and strongest. From all appearances it has retained its strength and visibility. The family looks good.

But, for all present and those to come, don't forget how the babe came to be and the tremendous efforts put into its schooling. All else forgotten, remember, the Grand Poo Bah is watching. If ever necessary he would rally to your side all the uncles, aunts, and godparents of long ago.

Ward Kischer



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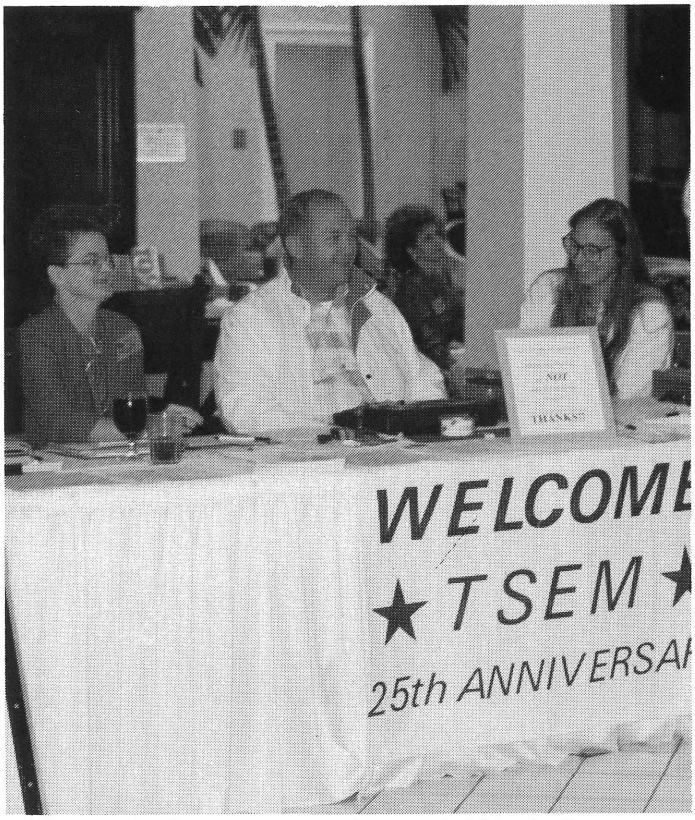
TSEM CELEBRATES ITS 25th ANNIVERSARY

Lynn D. Gray, Ph.D.
Program Chairman, TSEM
Dept. of Cell Biology and Environmental Sciences
The University of Texas Health Center at Tyler
P.O. Box 2003
Tyler, Texas 75773

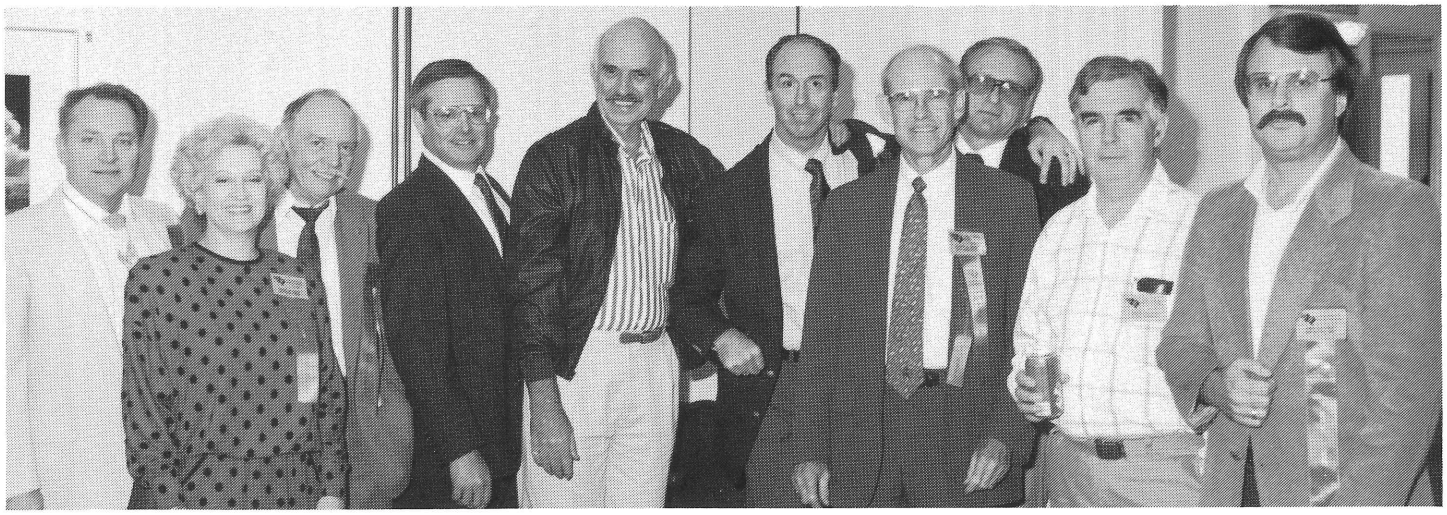
TSEM proudly celebrated its 25th Anniversary in Galveston on Oct. 11-13, 1990 at the elegant Tremont House in Galveston's Historical District. The meeting featured a ''Colloidal Gold Workshop'' taught by Dr. Hal Hawkins (Texas Childrens' Hospital, Houston) with Dr. Joiner Cartwright (Baylor College of Medicine), Mr. Rick Giberson (Ted Pella, Inc.) and Ms. Gena Kranig (Life Cell Corp.). Invited speakers included Mr. Rick Giberson (Ted Pella, Inc.), Dr. Alice Johnson (The University of Texas Health Center at Tyler), Dr. David MacMurray (Texas A&M College of Medicine) and Mr. Nathan Little (Philips Electronic Instruments, Inc.). Their topics included silver enhanced gold for light microscopy, basic cell culture methods, development and selection of antibodies for immunogold labeling and PC based scanning electron microscopy, respectively.

All past presidents and charter members of TSEM were issued special invitations to the meeting and a reception was held in their honor on Friday at noon. We were fortunate that so many of these special people could attend. In all, over 100 registrants and their families attended the meeting. There were 22 platform presentations, 4 invited presentations, 3 poster presentations, 1 workshop and 22 commercial exhibits.

Corporate exhibitors included: AMRAY, EMS, Diatome-US, Gatan, Hitachi, JEOL USA, Kevex Instruments, Leica, Life Cell Corp., Link Analytical, Meyer Instruments, MicroEngineering, Ted Pella, Inc., Philips Electronic Instruments, Photo/Graphic Concepts, RMC, Sarastro North America, Scanning/FAMS, Scien-Tech, SPI, Tracor Northern and Carl Zeiss Electron Optics.



Registration for the over 100 attendees was held in the Tremont House foyer.

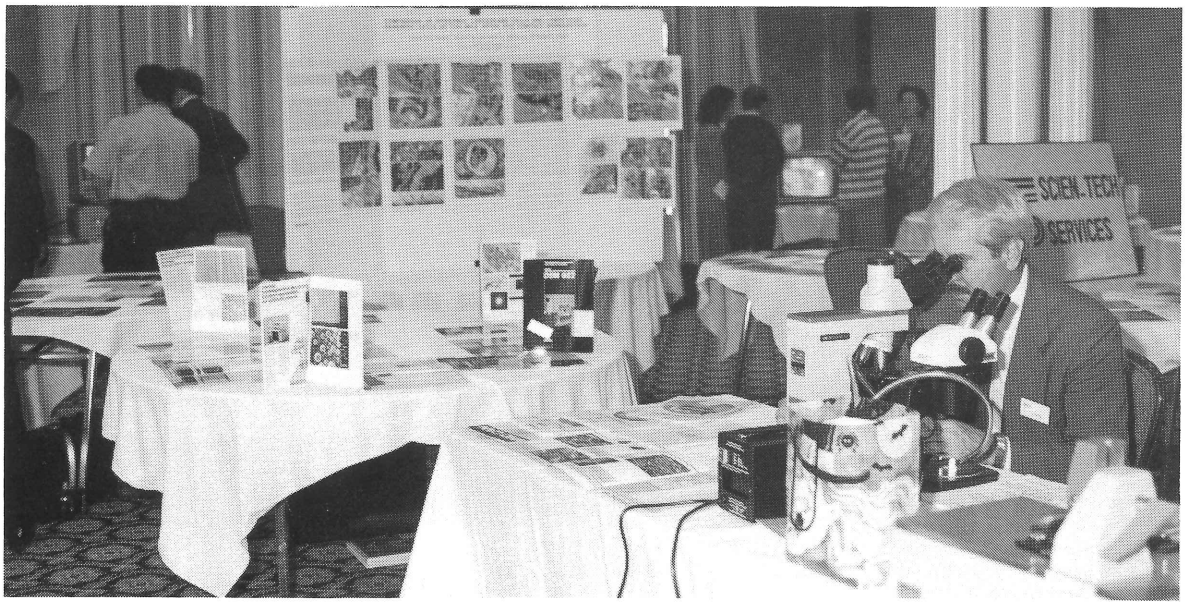


Past Presidents attending the reception were as follows (from left to right): Ward Kischer, Margaret Ann Goldstein, Don Benefiel, Jerry Berlin, Robert Turner, Larry Thurston, Robert D. Yates, Terry Hoage, Bruce Mackay and Joiner Cartwright, Jr.

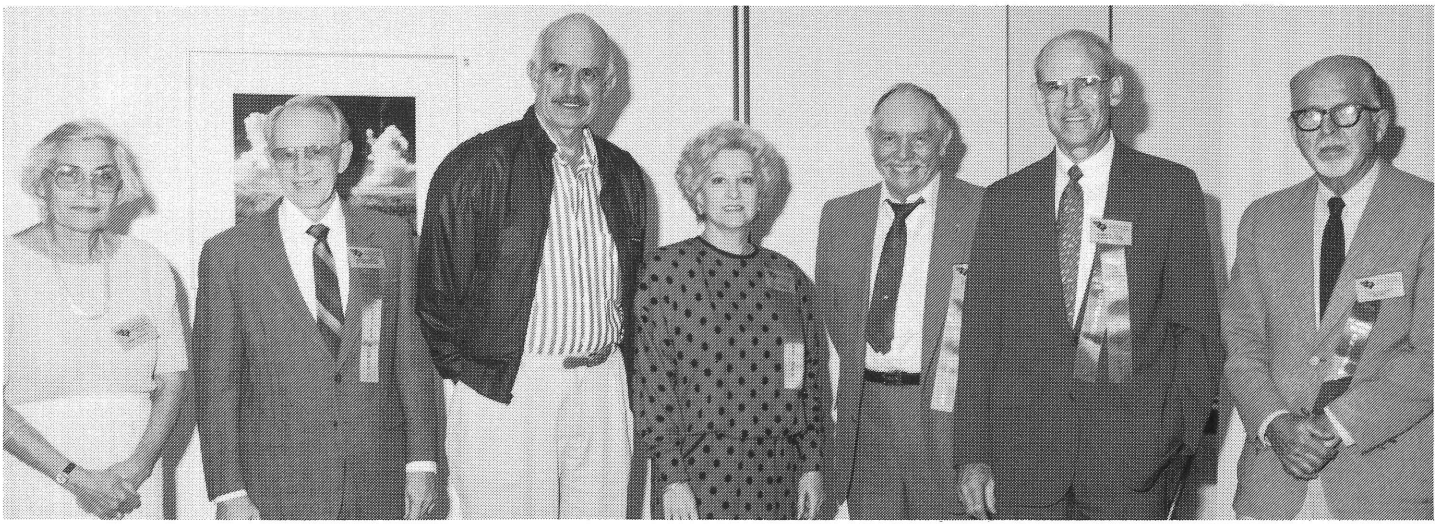
TSEM gratefully acknowledges our corporate members for their sponsorship of meeting events: **Workshop** — Nikon, Ted Pella, Inc.; **Thursday Evening Social** — Leica; **Audiovisual Equipment** — AMRAY; **Continental Breakfasts** — Hitachi, RMC; **Breaks** — Kevex Instruments, MicroEngineering, Tracor Northern; **Friday Evening JEOL Hospitality Suite** — JEOL USA; **Presidents/Charter Members Reception** — Gatan, Meyer Instruments, Philips Electronic Instruments, Scien-Tech and Carl Zeiss Electron Optics. Thanks to Leica for the 25th Anniversary cake served at the Social!

Thanks to all who helped with registration, slide projection and all the countless details that go into the making of a great meeting!

The banquet had lots of happy faces looking forward to a delicious seafood buffet in the Topgallant Room over the Wentletrap Restaurant.



Twenty two corporate tables and 3 posters were displayed in the Exhibitors' room.



Charter Members in attendance at the reception were as follows (from left to right); Liane Jordan, Marion Campbell, Robert Turner, Margaret Ann Goldstein, Don Benefiel, Robert D. Yates and Carl Tessamer.

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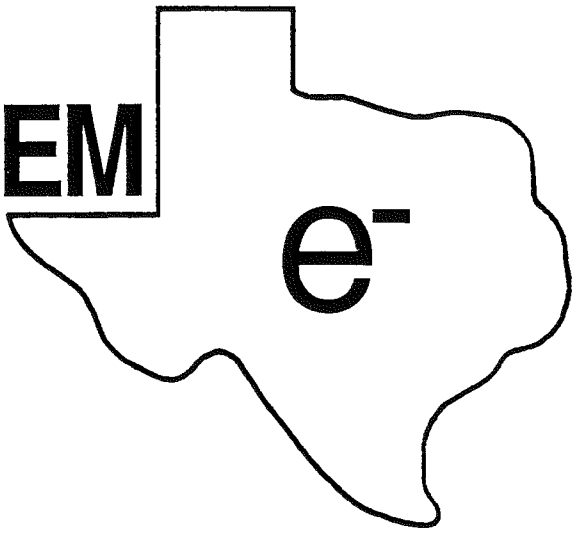
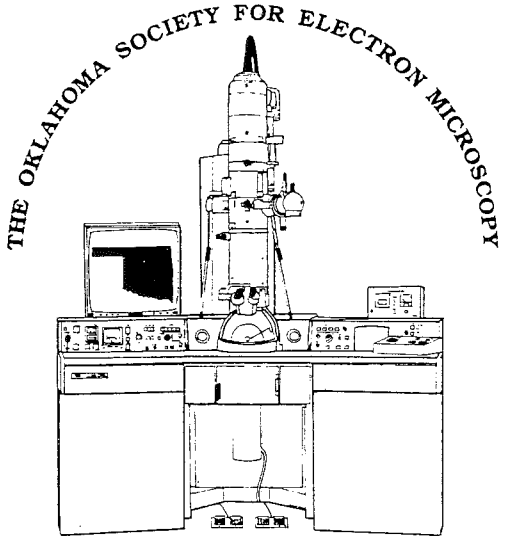
Joint Spring Meeting

TSEM





THE BEAM IS ON!

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Lee Peachey, U Penn., "Correlated IVEM and Confocal Laser Microscopy"
Mark Ellisman, UC San Diego, "3D Reconstruction of Alzheimer Pathology"
Larry Murr, UT El Paso, "Contemporary Applications of TEM in Materials Sciences"

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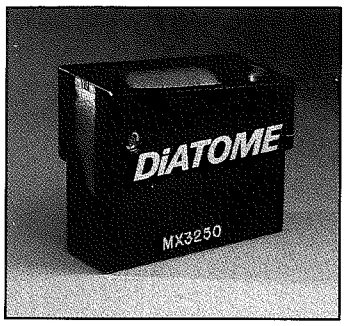

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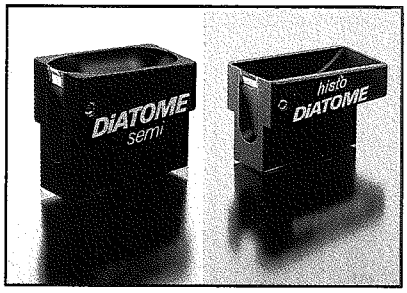
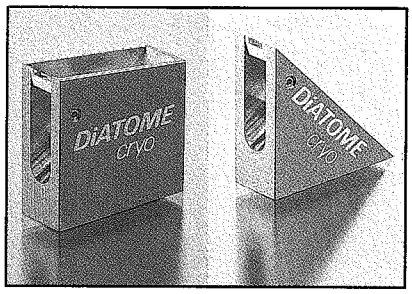


Semi thin 0.1-1µm, Histo 0.2-5 µm

35° for better stuctural preservation



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

Signature of applicant: _____ Date _____

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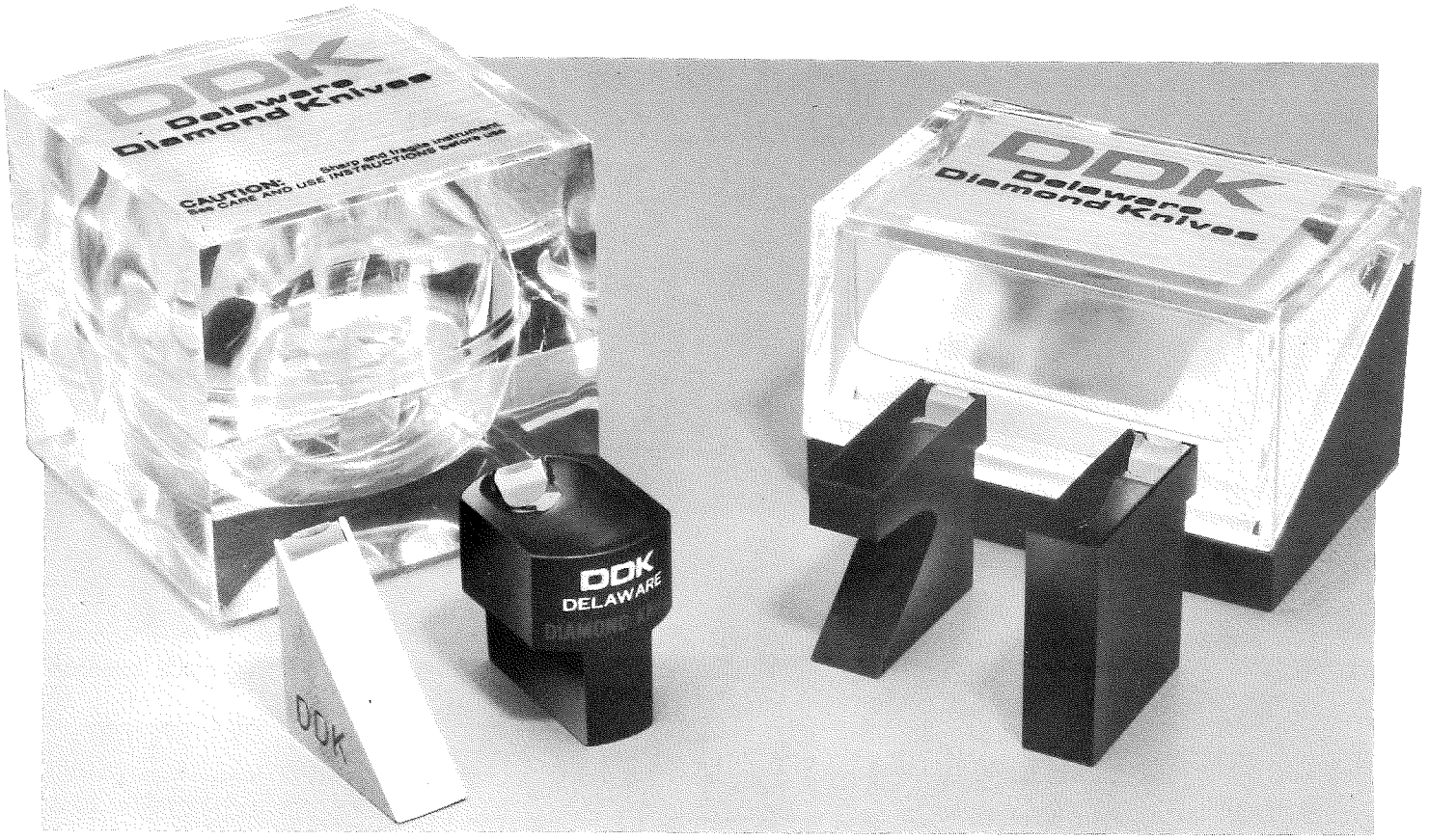
ANSWER TO "WHAT IS IT"

Surface of part of bee's head showing pollen grains among hairs.

Photo — Douglas A. Hayworth, Department of Biology, The University of Texas at Arlington, TX 76019. (Magnification approximately 1875X; Bar = 0.01mm)



Now Delaware Diamond Knives quality comes in four boat styles

Since we acquired the Du Pont Diamond Knife business, we've successfully provided our customers with unique personalized service and improved quality. We offer quality Delaware Diamond Knives in your choice of four boat styles:

Standard Boat with its large oval reservoir has been very popular since it makes specimen retrieval easy. It is supplied on all new knives unless otherwise specified.

Old-Style Boat was supplied with Du Pont Diamond Knives for 20 years. It is compact and has an 8 x 25 mm trough.

All Delaware Diamond Knives can be used for cryoultramicrotomy. Two boats have been designed for specific cryo applications.

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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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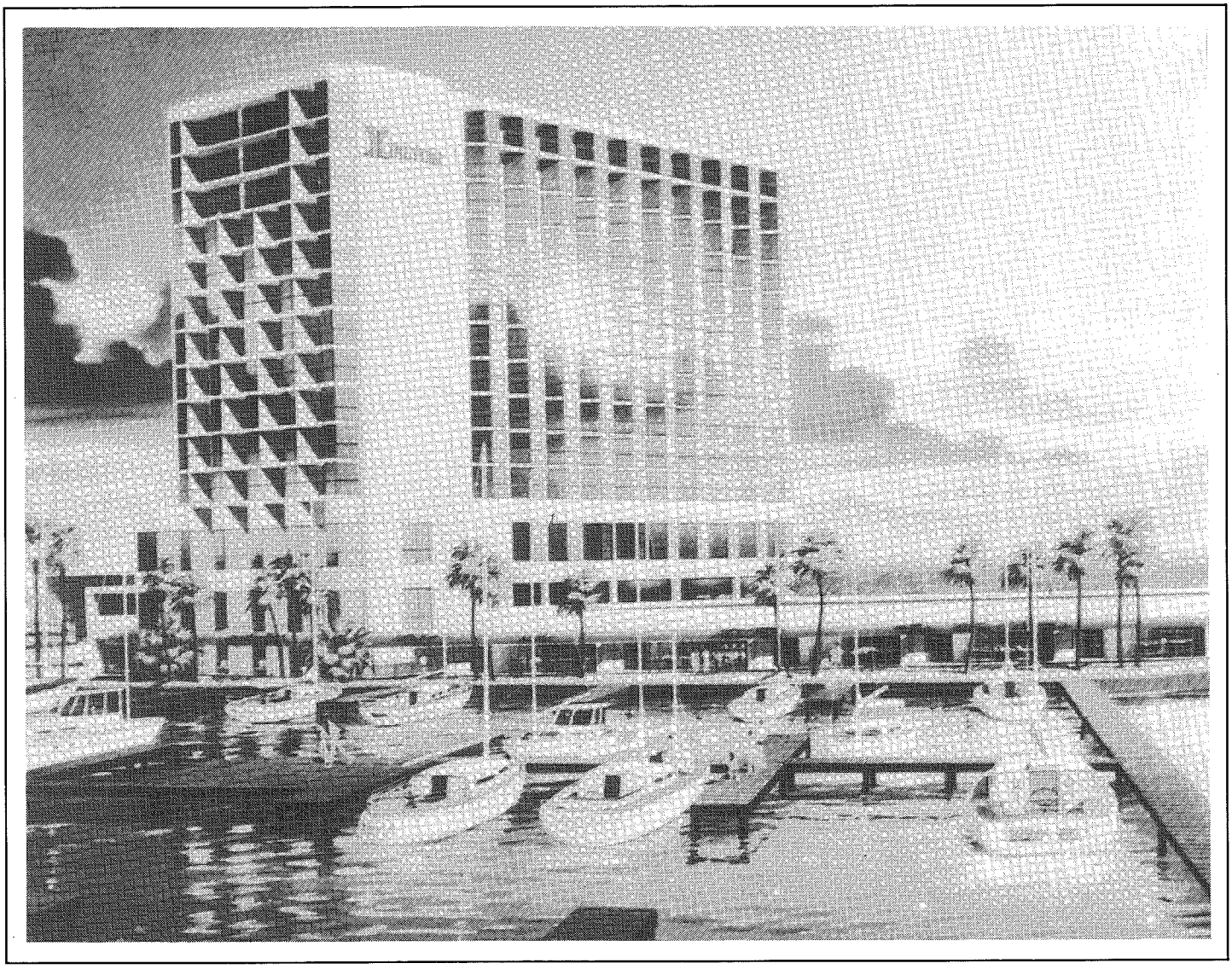

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DENKA now offers an even wider selection of LaB6 cathode styles to suit your specific electron beam applications

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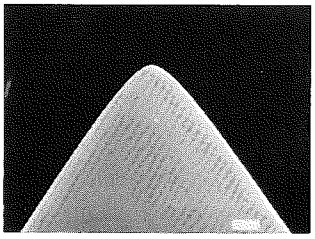
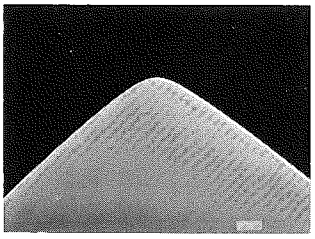
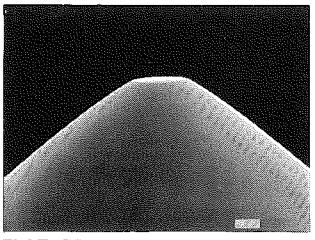

SHARP TIP


ROUND TIP


FLAT TIP

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In October, 1990, Drs. Crang, Harris, and Mollenhauer visited Shanghai and Beijing, (The People's Republic of China) where they presented a series of workshops on various aspects of specimen preparation for electron microscopy. The following report, prepared by Dr. Richard F.E. Crang for the Midwest Society of Electron Microscopy, is a summary of this experience.

THE CHINA EXPERIENCE

Report of
The 1990 Visit of U.S.A. Electron Microscopists
Participating in Biological Workshops
and the
10th Annual Chinese Electron Microscopy Society
Meeting in the People's Republic of China

MSEM Participants:
Richard F.E. Crang
Joseph B. Harris
Hilton H. Mollenhauer

I. Preliminary and organizational plans: In 1990, the China Exchange visits previously sponsored by the Electron Microscopy Society of America (EMSA) were turned over to the EMSA Local Societies to engage their representatives in exchange activites. Notably, the Midwest Society of Electron Microscopists, Inc. (MSEM), was invited as 1990 sponsors to participate in sending individuals for both biological and physical sciences delegations. This report is specifically on the activities of the biological sciences delegation of MSEM.

Dr. Richard Crang was contacted by Dr. Judy Murphy, EMSA China Exchange Coordinator to organize on behalf of MSEM a delegation to participate in a special session of the 10th Annual Chinese Electron Microscopy Society (CEMS) meeting in Beijing, and to work with Chinese counterparts in the organization of two pre-meeting workshops. The first formal contact with the Chinese organizers occurred in March, 1990, and plans quickly developed for the workshops to be held in Shanghai and in Beijing. Background information and advice from previous EMSA-sponsored visits was kindly provided by Dr. Murphy, Ms. Caroline Schooley (founder of the program), and by Mr. Joe A. Mascorro, a 1988 EMSA participant.

By summer of 1990 Drs. Joseph Harris and Hilton Mollenhauer, both MSEM members, were identified as the other participants for the trip along with Dr. Crang. Contact was made at the XII International Electron Microscopy meeting at Seattle, WA is August with three of the Chinese individuals involved in the

meeting and workshop arrangements. At that time the dates of the workshops were set, the topics for presentation were finally established, the numbers of students to participate in the workshops was agreed upon, and the organizational plans for the workshops were finalized. Letters of invitation arrived from the China Association for Science and Technology and visas were duly processed.

Travel arrangements were made through a Chinese specialty travel bureau in Chicago, which arranged flights from Chicago to Shanghai via Tokyo and Osaka. Since many of the laboratory sessions were to involve low-temperature specimen preparations, arrangements were made in advance to have Mr. Walter Bilek, product manager of Leica Instruments electron microscopy division (Vienna, Austria), and Mr. Simon Lam, Leica Hong Kong microscopy representative, to be present and to bring equipment for specimen freezing, freeze-substitution and cryo-ultramicrotomy to the workshop sites in Shanghai and Beijing.

II. Itinerary: After belated changes, the following itinerary was organized for the MSEM biology delegation:

Oct. 9 . . . . . Arrive Shanghai
Oct. 10 . . . . . Depart Shanghai for Hangzou by train
Oct. 10-11 . . . Tour Hangzou and Environs; Stay at Medical College
Oct. 11 . . . . . Return to Shanghai by train
Oct. 12-15 . . . Conduct workshop in Shanghai at Second Military Medical University
Oct. 16 . . . . . Travel by air to Beijing; Stay at Zi-Wei Hotel
Oct. 17-19 . . . Conduct workshop in Beijing at the Institute of Military Basic Medical Sciences Instrument Analysis Center for E.M.
Oct. 20-21 . . . Participate in the 10th Annual China Electron Microscopy Meeting
Oct. 22 . . . . . Tour Great Wall and Ming Tombs
Oct. 22-23 . . . Return to Shanghai by overnight train
Oct. 25 . . . . . Leave Shanghai

III. Design of workshops: The same plan was followed in both Shanghai and Beijing for the pre-meeting workshops. Mornings were devoted to lectures (from 8:30 until noon) giving time for three approximately 1-hour lectures. Luncheon time ran from noon until approximately 1:30. Afternoon laboratory workshops time extended from 1:30 until 5:30 with three sessions each running approximately 1 hour and 15 minutes. Enrollment in the morning lecture sessions was unlimited and usually had about 65-75 people in attendance. Some of these were interested local faculty or staff individuals. The afternoon workshops were limited to a total of 45 students, 15 in each of three laboratory sessions. The afternoon students (also a part of the morning enrollment) were comprised of individuals both locally in each city as well as from throughout China. Some individuals came from as far as 2,000 km to attend.

The lecture topics presented by Dr. Crang were:

A. Cryo-techniques in Biological Electron Microscopy
   Part I. Freezing
B. Cryo-techniques in Biological Electron Microscopy
   Part II. Cryo-ultramicrotomy and Bulk Specimens
C. X-Ray Microanalysis of Biological Specimens
D. Montaging Electron Micrographs (Shanghai only)
E. Artifacts in Biological Scanning Electron Microscopy.

The lecture topics presented by Dr. Harris were:

A. Simplified Heavy Metal Staining Techniques Demonstrated with Fast Plant Leaf Tissue
B. Preparation of Higher Plant Tissues for TEM: Special Techniques
C. An Efficient Design for the Undergraduate Electron Microscope Laboratory Experience.

The lecture topics presented by Dr. Mollenhauer were:

A. Some Practical Aspects of Using Epoxy Resins
B. Artifacts Associated with TEM and Epoxy Embedding Resins
C. Thin Sectioning and Section Poststaining
D. Ultrastructure of the Golgi Apparatus with additional subjects covered on "Freeze Substitution Using a Simple Home-built Apparatus", and "The Photographic Process in TEM".

Each of the above topics constituted a morning lecture and/or an afternoon laboratory session. The equipment provided by Leica, and the active participation of Mr. Bilek and Mr. Lam constituted a significant contribution to the overall workshop experience by the participants. It is also noteworthy that Dr. Harris' paper on "Simplified Heavy Metal Staining Techniques Demonstrated with Fast Plant Leaf Tissues" (and co-authored by T.G. Guilliams and J.A. Schultz) was also submitted (and accepted) for publication in the Chinese journal "Cell Research" (Vol. 2).

The afternoon laboratory sessions were conducted in "round-robin" format with 15 students in each group. Chinese laboratory assistants and interpreters were present for all sessions during morning lectures and afternoon laboratory workshops. In addition to cryo-electron microscopy equipment, Apple and IBM-compatible personal computers were obtained for tutorial work and simulation studies in the afternoon workshop sessions.

IV. Participation in the CEMS meeting: In Beijing, the primary scientific and social function was the tenth annual meeting of the China Electron Microscopy Society. Since this constituted a significant event, speakers had been invited from America, Europe, and other Asian countries including

Taiwan (R.O.C.). Each of the MSEM delegates were invited to present a paper in a special morning symposium on problems in biological specimen preparation for electron microscopy.

Dr. Crang's presentation was "Problems and Techniques in Biological Scanning Electron Microscopy".

Dr. Harris' presentation was "Application of Plant Preparative Techniques to TEM Study of Leaf Aging in Tobacco".

Dr. Mollenhauer's presentation was "Epoxy Resins for Tissue Embedment".

All three of the presentations were prepared (10-20 pages) in camera-ready style and submitted for publication through World Scientific Publishing Co., Ltd. (Singapore) as a part of the proceedings. At present, all three papers are in press. Approximately 175 people attended the morning biological symposium which was made up of presentations by the three MSEM participants. The Shanghai interpreters generously assisted in the presentations.

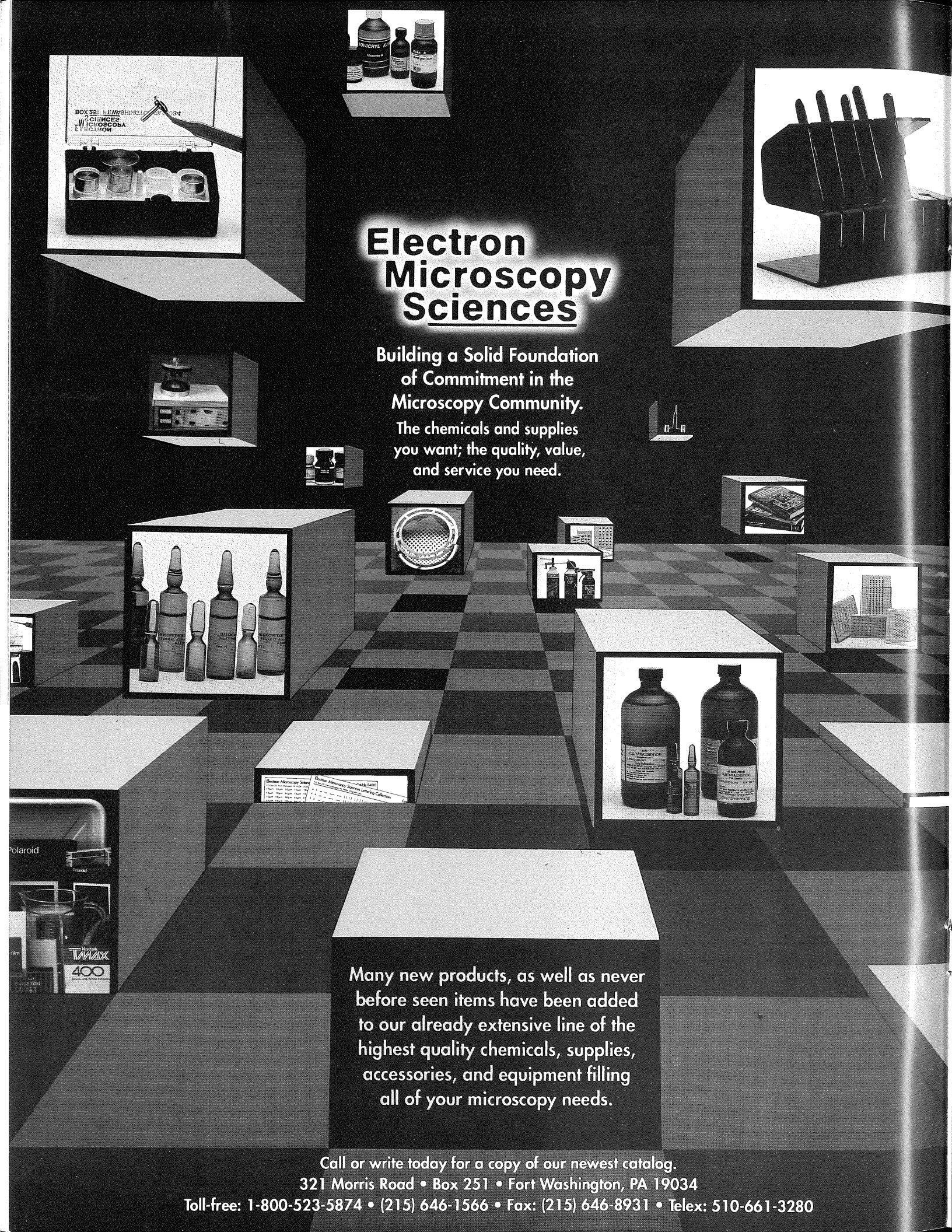
V. Adjunct travels and visits: The limited time away from the meetings (approximately four and one-half days) were spent in traditional tourist visits and, at least one very special visit relevant to electron microscopy. While in Shanghai, we learned of a factory which manufactures both transmission and scanning electron microscopes and arranged a visit. The visit was accomplished and it was possible to use a scanning electron microscope at the factory, and to observe stages in the assembly of transmission instruments. The Chinese admit that they are "catching-up" in the production of electron optical equipment, and indeed many mechanical parts of the instruments still appear crude in their operation, but optically the instruments nearly compete with the corresponding Japanese instruments. In fact, we learned that some of the components for Japanese instruments are actually manufactured in China.

From our site in Beijing, the perfunctory visit to the Great Wall next to Inner Mongolia, the Ming Tombs in Ding-Ling, and the Forbidden City adjacent to Tiananmen Square in central Beijing were made. Limited time was also available for shopping, riding the metro, and Dr. Crang had an appointment at Beijing Hospital dental clinic for reconstruction of a tooth partially lost while enjoying Beijing roast duck.

VI. Future activities: It is difficult to fully assess the prospect for future activities in China, but it is clear that the Shanghai Society would like to establish a "sister-society" with MSEM. If this happens, it may well lead to the establishment of literature exchanges, up-dates in the respective newsletters, and possible invitations of scientists to be hosted by each Society. It is also likely, that the 1992 Asian Electron Microscopy Meeting, scheduled for Beijing, will be an event for which American participants from each of the exchanges which has occurred will be invited, and which may offer the opportunity for additional representatives to attend and participate. Our future with the Chinese depends as much on our own initiatives as it does on the actions of our counterparts in China. Precluding political actions of an adverse nature, we can fully expect a far greater role with the Chinese in the near future (5-10 years) both scientifically and in most aspects of international relations.

VII. An assessment: Such relationships with the People's Republic of China naturally bring to the fore questions about the political advisability of such exchanges and the possible consequences in today's world. We feel that these concerns carry a certain validity, but must be also addressed by a consideration of the long-term benefits to science and international relations. Although it cannot be said with certainty, all signs indicate that China is on the threshold of emerging in our life-time as a world power both politically and economically. Such emergence can be facilitated in some small measure by our contacts and scholarly exchanges. "To maintain scientific contact with Chinese colleagues does not contradict in any way with support for human rights or the future democratization of China." (Quote from Hans Ris.)

The immediate rewards felt by all members of our delegation, and members of other electron microscopy delegations with whom we have discussed, have been the many genuine expressions of gratitude from the Chinese for our efforts, and the evident sincere interest with which the students responded in our classes. Such feelings no doubt have to be experienced to be fully appreciated.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CALENDAR OF MEETINGS (continued)

LeHIGH SEM/AEM SHORT COURSES

SCANNING ELECTRON MICROSCOPY AND X-RAY MICROANALYSIS

June 10-14, 1991

ANALYTICAL ELECTRON MICROSCOPY AND THIN SPECIMEN PREPARATION

June 17-21, 1991

SEMICONDUCTOR DEVICE CHARACTERIZATION BY SEM ADVANCED IMAGING TECHNIQUES IN SEM X-RAY MICROANALYSIS OF BULK, PARTICLE AND THIN FILM SPECIMENS

June 17-20, 1991

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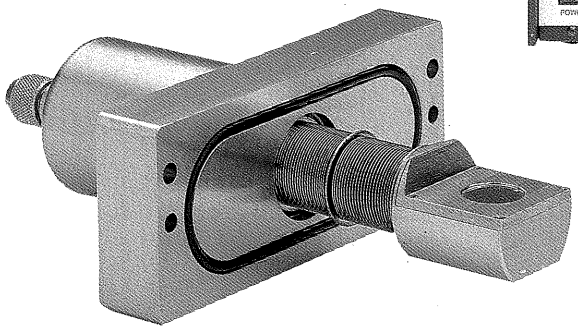
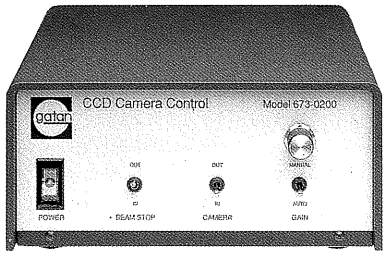
LeHigh University, Whitaker Lab #5 • Bethlehem, PA 18015 • Phone: (215) 758-5133

Model 673 Mk3
Wide Angle TV Camera for TEM





Model 673 Wide Angle TEM TV Camera and Controller

Application

The Model 673 was designed to improve on the performance of the large phosphor viewing screen of the transmission electron microscope (TEM). The initial goal, which has now been accomplished, was to present clear, high contrast images that could be viewed on a TV monitor under normal room light conditions by several researchers at the same time and to serve as an aid to teaching and discussing research results.

Large Field of View

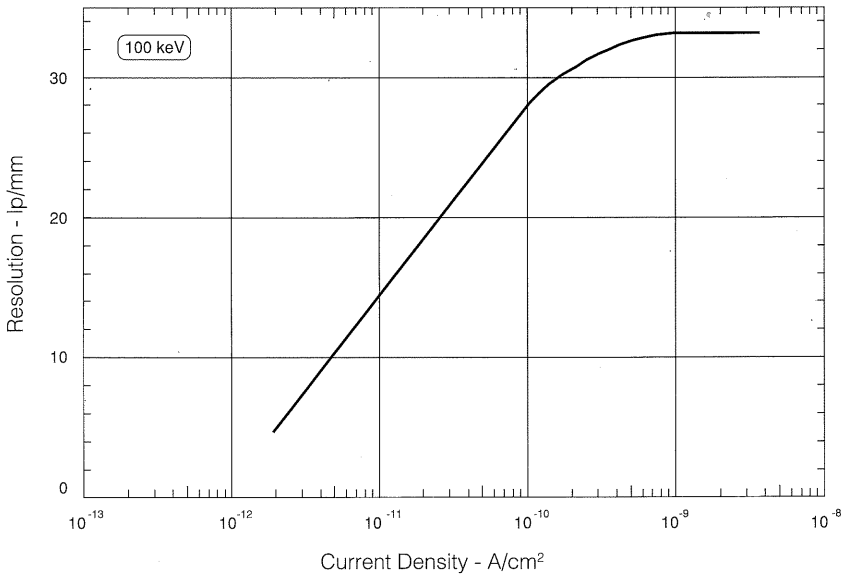
The Gatan Model 673 Wide Angle TV System mounts in one of the 35 mm roll film camera ports at the top of the TEM projection chamber, leaving the second port free for other attachments, such as the Gatan Model 689 slow scan camera or Model 673-04 pneumatically controlled beam stop. Because it is mounted high in the projection chamber the Model 673 covers a large part of the TEM image field and has a similar field of view to the cut film camera. However, when the image from the Model 673 is displayed on a 9" monitor it is approximately 6x larger than the image on the camera plate.

High Efficiency Optics

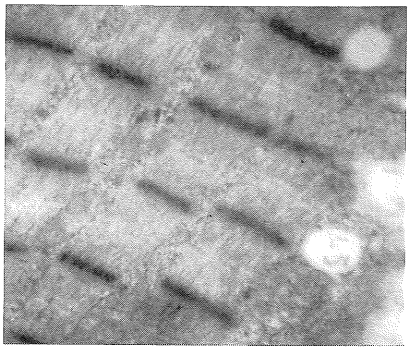
A thin yttrium aluminum garnet (YAG) transmission scintillator converts the electron image to a light image which is then transferred to a CCD sensor through a fiber optic plate. This direct coupling minimizes light losses where they matter most – between the scintillator and the first light sensor, and provides superior performance compared to camera systems using lenses and mirrors. The fiber optic coupling in combination with the CCD sensor also has the advantage over lens coupling that it produces images with negligible distortion. This is especially important when processing diffraction patterns.

Effective Over a Wide Range of TEM Magnifications

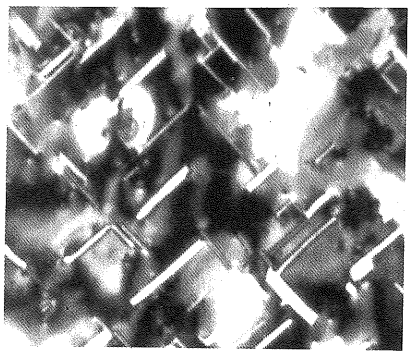
High linearity, resistance to blooming and high sensitivity ensure that the Model 673 produces good quality, high contrast images over a wide



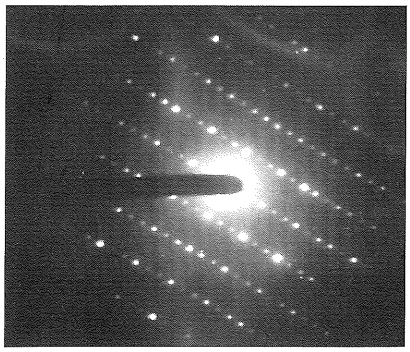
Resolution vs current density performance of the Model 673 Mk3 wide angle camera.



TV monitor image of stained human heart muscle viewed at 50,000x magnification.



TV monitor image of vapor deposited gold viewed at 50,000x TEM magnification.



TV monitor image of a diffraction pattern from asbestos.

range of TEM operating conditions. At comfortable illumination levels the image resolution is >330 line pairs/cm at the YAG screen (see graph).

The Model 673 is particularly effective in the microscope magnification range 5,000x to 100,000x but will produce good images (e.g. the graphite lattice is clearly resolved) at TEM magnifications >300,000x.

Smooth Pneumatic Drive
A pneumatically driven arm, powered by the compressed air system of the TEM, moves the camera head quickly and reproducibly into or away from the electron beam. Metal bellows prevent TEM vacuum degradation when the drive is actuated. Careful design ensures that the motion of the camera causes no vibration of the microscope column and high resolution images may be recorded on the cut film camera directly after moving the camera out of the electron beam.

CCD Technology
The Model 673 uses the latest low noise, buried channel charge coupled device (CCD) technology and has an active optical area of 6.6 x 8.8 mm. A connector on the control unit allows direct interfacing to and control by a computer. The camera operates on either the EIA format (493 x 768 elements) or the CCIR format (581 x 756 elements).

Other Gatan Cameras
While the Model 673 Wide Angle TV is ideal for TEM applications at lower and intermediate magnifications, the Gatan Model 622 camera (brochure available) is preferable for low light level, high resolution imaging at TV rates. For high resolution imaging in conjunction with the Gatan PEELS™ electron energy loss spectrometer, the Model 676 TV camera is recommended. Gatan also provides a range of slow scan cameras (SSCs) which offer the ultimate quality in electronic image recording and are especially suitable as an alternative to the microscope film camera.

The following table lists the Model numbers and functions of all Gatan TV and slow scan cameras:

Function	TV	SSC
Wide angle	673	689
High resolution	622	679
PEELS compatible	676	690

Compatibility and Ordering
The Model 673 is compatible with all STEM detectors, EELS and all other Gatan cameras except in some cases the Model 689. A free port is required at the top of the TEM projection chamber for mounting the Model 673 (**Note:** this port is not present on certain TEMs). When placing your order, please state the model and manufacturer of the TEM on which the camera is to be mounted. An optional 9" monitor and pneumatically operated beam stop can be supplied if required.

Gatan Inc. 6678 Owens Drive, Pleasanton, CA 94588, USA Tel (415) 463 0200 Fax (415) 463 0204
780 Commonwealth Drive, Warrendale, PA 15086, USA Tel (412) 776 5260 Fax (412) 776 3360
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Gatan Ltd. 3 Saxon Way East, Corby, Northants, NN18 9EY, UK Tel (0536) 743150 Fax (0536) 743 154

EPOXY RESIN FOR TISSUE EMBEDMENT

By

Hilton H. Mollenhauer

U.S. Department of Agriculture, Agricultural Research Service
Food Animal Protection Research Laboratory
College Station, TX 77840
and
Department of Pathology and Laboratory Medicine
College of Medicine, Texas A&M University
College Station, TX 77843, USA

ABSTRACT

Epoxy resins are the principal embedding medium for the preservation of tissues to be sectioned and examined by transmission electron microscopy (TEM). Their primary advantages are good ultrastructural preservation, little or no shrinkage, ease of sectioning, and reasonably good stability in the electron beam. However, epoxy resins also have some disadvantages; e.g., they are toxic, they may mask antigenic sites, and they do not penetrate tissues as well as less viscous media. In this paper, some simple methods for mixing and handling epoxy resins are presented. Also presented are some unusual characteristics of epoxy resins that may be revealed, for example, in the shrinkage of organelles, as problems with post-staining sections, and as instability of sections in the beam.

INTRODUCTION

Epoxy resins were introduced in the 1950's by A. Glauert[1] and have become the principal medium for tissue preservation. Their primary advantages are (1) excellent tissue preservation, (2) ease of sectioning, and (3) reasonably good stability in the beam. Since their introduction, two categories of epoxy resin formulations have evolved which are best described simply as high and low viscosity resins. The first resin formulations (e.g., Araldite,[1] Epon 812,[2] Maraglas[3]) were all medium to high viscosity resins of 150-6,000 cp. The first low viscosity resin formulation was developed by Spurr[4] (7.8 cp) primarily to infiltrate plant tissues which are notably difficult to embed. Several other low viscosity formulations were subsequently introduced, most notably Quetol[5] and a formulation by LADD Industries, Inc. (Burlington, VT 05401, USA). The purpose of this paper is to review some of the characteristics associated with epoxy resins and some protocols for their use.

In reading this paper, two considerations must be kept in mind; first, that the methods presented here represent opinions of the author and may not be applicable to the work of others and second, that the examples of artifacts presented in this work are extreme representations of very common occurrences.

SAFETY

All epoxy resins are toxic; all are mutagens; some are carcinogens.[6,7] Additionally, epoxy resins or components of epoxy resin mixtures may be highly allergenic to some individuals. In general, low viscosity resins are more toxic than high viscosity resins. Moreover, components of the low viscosity resins are volatile, particularly at the elevated temperatures used to polymerize them. Therefore, it is essential that embedding ovens be separately ventilated or placed in rooms with good ventilation.

Gloves should be worn when handling any epoxy resin mixture, or any component of the mixture. However, gloves do not offer long term protection and cannot be considered reliable for periods longer than ½-1 hour.[6,7] Skin contamination with resin should be cleansed with soap and water and not by solvents such as acetone which will accelerate penetration of resin into the skin.

MIXING AND STORAGE

It is, perhaps, best to store all stocks of resin components in the refrigerator or freezer over long periods of time. However, for times of 1-3 months, epoxy resins and associated components are quite stable on the bench at room temperature. For these short-time applications, the author has found that plastic dispensing bottles of the type illustrated in Fig. 1 are particularly convenient for storing resin stocks. Three bottles are required; one for the epoxy

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resin(s), one for the hardener(s), and one for the accelerator. Additives such as dibutyl phthalate can go in either the resin or hardener stocks whereas lecithin is best added to the accelerator stock.[8]

Mixing of the resin and hardener stocks is best accomplished in disposable 50-100 ml plastic beakers (Tri-Pour, Monoject Scientific, St. Louis, MO 63101, USA or equivalent); the components being dispensed into the beaker by weight rather than volume. A top loading balance (Fig. 1) is almost a necessity.

The accelerator can be dispensed either by weight or by measured drops into the other resin components. For example, the author uses 1 drop of accelerator (DMP-30) for each gram of resin mixture. However, the number of drops of accelerator must be determined by trial and error for each resin mixture and operating environment that is used.

Although stirring is generally recommended for mixing the several ingredients of the formulation, shaking may be a more reliable approach. The weighed stock solutions can be mixed by capping and shaking the Tri-Pour beaker vigorously. However, the beaker lids often do not fit very well and care must be exercised to prevent spilling of resin from the beaker or leakage of resin through (or around) the lid of the beaker. Alternatively, the stocks can be mixed by ''sloshing'' the mixture back and forth in the beaker with a rotating action of the wrist. The high viscosity resins may need to be heated slightly to reduce viscosity before shaking or sloshing. For these resins, it is best to weigh and then heat the resin and hardener stocks and then add the accelerator just before mixing. This retards the start of polymerization a few minutes longer. A fresh resin mixture should be prepared for each infiltration step. Storage of mixed resins in the refrigerator or freezer is not recommended.

An example of resin preparation using the freeze-substitution mixture of Howard and O'Donnell,[9] is given below:

Stock A ... 6.2 g Epon 812 (or Polybed 812)
8.1 g Araldite 506
1.4 g dibutyl phthalate

Stock B ... 7.5 g DDSA
7.5 g NMA

Stock C ... DMP-30

Use 1 part by weight of Stock A; 1 part by weight of Stock B; 1 drop Stock C per gram of resin mixture.

Either 125 ml or 250 ml bottles are used for storing Stocks A and B and a 30 ml bottle for Stock C. Stocks with more than one component are shaken before dispensing contents to prevent separation of the resin components during storage.

DEHYDRATION SOLUTIONS

The most common dehydration solutions are ethyl alcohol (ETOH), propylene oxide, and acetone. These

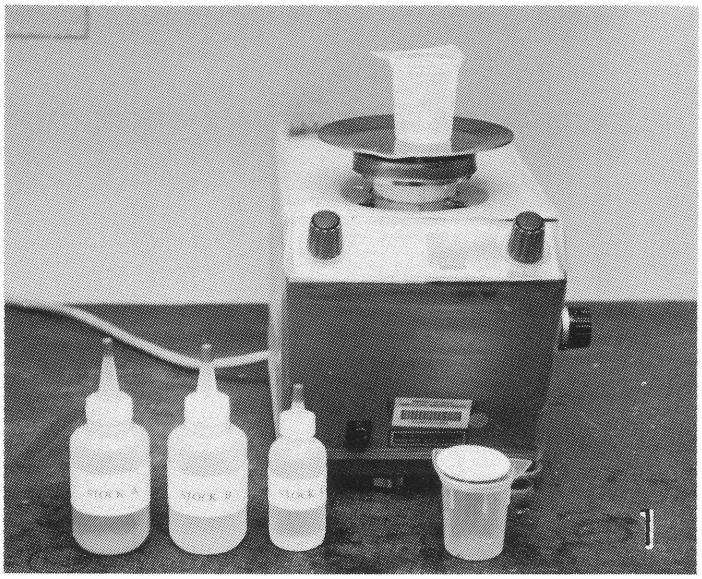


Fig. 1. This figure illustrates the types of dispensing bottles used for the short-term storage (i.e., 1-3 months) of the resin components, the disposable beaker for mixing the resin, and the top loading balance for weighing out the resin components.

may be used singly or in combination depending on the applications. For example, ETOH (only) may be required when dehydrating cells cultured in Tissue Culture Chamber/Slides (Miles Scientific Laboratories, Inc., Naperville, IL 60566), the walls of which are soluble in acetone. On the other hand, acetone alone (or preceded by ETOH) is generally preferred since acetone is easier to remove from tissue than is ETOH. It should be emphasized that complete removal of solvents before polymerization of the resin is very important; i.e., any solvents left in the tissue or resin will result in a soft block or a block that is soft on the upper surface and brittle on the bottom surface.

Acetone is probably the best compromise of the dehydrating agents considered. ETOH is particularly difficult to eliminate from resin, and residues of ETOH are a common cause of soft blocks. Because of its toxicity, propylene oxide is seldom used even though it is easy to eliminate from the resin and is compatible with epoxy resin so that soft blocks seldom occur even if minor residues or propylene oxide are left in the resin.

RESIN CHOICE, INFILTRATION SCHEDULE, AND EFFECTS OF VISCOSITY

Many protocols for resin infiltration have been published but there are many variables and the problem of selecting the best is not simple. Some points to consider before deciding on a protocol are listed:

1. It is intuitive that the resin should not polymerize during infiltration as this would impede its

penetration into the tissue. Yet, it is almost impossible to prevent this from occurring unless infiltration is done at very cold temperatures or by formulating the resin mixture with reduced levels of accelerator. Unfortunately neither of these options are acceptable because (a) cold temperatures increase viscosity which hinders penetration and (b) reduced accelerator will prolong polymerization beyond reasonable limits.

2. Solvents must be removed before the resin polymerizes. Failure to do this will lead to soft blocks.
3. The rate of infiltration is approximately inversely proportional to viscosity; i.e., low viscosity resins penetrate tissue faster than high viscosity resins.
4. Each component of the resin mixture will penetrate at a rate that is approximately inversely proportional to its viscosity. If all resin components have the same viscosity, then the resin mixture will penetrate *in toto* without component separation. If component viscosities differ, then the components of the resin mixture will separate as they infiltrate the tissue and the resin within the tissue will not be correctly formulated. This problem can be minimized by using resin mixtures with components of similar viscosity and by infiltrating only very small, or thin, pieces of tissue.
5. The final characteristics of the resin block after polymerization are somewhat dependant upon the epoxy/anhydride ratio and the temperature of the oven at which polymerization occurs.[10,11] Preservation of tissue, ease of sectioning, and stability of a section in the beam may all be influenced by these factors. However, for most work, considerable deviations from optimum can be tolerated. A simple rule of thumb for determining (or checking) the proper epoxy/anhydride ratio is that most of the resin formulations in common use are light yellow when polymerized. With excess hardener, the polymerized resin will become whitish; with too little hardener, it will become more intensely colored.
6. The rate of resin polymerization in the oven is dependent upon the type, as well as amount, of accelerator used in the mixture. For example, polymerization of the Spurr mixture with DMAE begins slowly and then accelerates whereas Epon mixtures with DMP-30 seem to begin polymerization almost immediately. Thus, the Spurr mixtures have a relatively long period in which infiltration can occur at low viscosity, without excessive polymerization of the resin.
7. Almost all polymerized resin blocks probably contain unreacted resin components which remain either because the resin formulation was not correct of because polymerization of the resin was not complete. Furthermore, any resin additives such as dibutyl phthalate or lecithin may also result in unbound constituents in the block. These unbound or unreacted constituents may diffuse out of the block during sectioning to contaminate the water in the boat and the knife edge, or diffuse onto a section surface where they interfere with post-staining and degrade image quality.[12,13] Section stability in the beam may also be reduced by these unbound resin constituents which do not contribute to the structural integrity of the polymerized block.
8. Exchange of fluids is very slow, especially when cells and tissue elements are interposed between the fluids and it seems unlikely that complete homogeneity of resin within the tissue can be achieved in the infiltration times usually used.
9. Tissue elements dilute the resin mixture and may substantially change its properties when polymerized. These effects may be expressed as sectioning difficulties, as instability of a section in the beam, and as swelling and/or shrinkage of some tissue elements. Moreover, epoxy resins do not bind very tightly to some tissue elements causing sections to split at these junctures. Lack of resin binding is particularly noticeable in the cuticular materials of plants and insects. Epoxy resins and formulations vary considerably in their ability to bind and glue tissue elements together, and it is sometimes possible to minimize the problem by trying several resin mixtures and selecting the most suitable. Sectioning parallel with, rather than across, a surface that is prone to split may also help reduce separation.

Thus, when one considers all of these points, it becomes clear that there is no "perfect" formulation or infiltration procedure. In attempts to prolong infiltration times before the onset of polymerization, the author has tried heat-activated accelerators (i.e., accelerators that become active only when heated to their activation temperature) to retard polymerization but the results were not encouraging. However, this still seems a viable approach and, perhaps, should be reconsidered as time permits.

A DEHYDRATION/INFILTRATION PROTOCOL

The following dehydration/infiltration protocol is the one most commonly used by the author. However, it must be stressed again that other protocols may be more appropriate for requirements that differ from those of the author.

Fixation/Blockstaining: 0-4°C

Water Rinse: 4-5 times, 10-15 min. each, 0-4°C

Dehydration: 25% ETOH, 10-15 min., 0-4°C
50% ETOH, 10-15 min., 0-4°C
75% ETOH, 10-15 min., 0-4°C
95% ETOH, 10-15 min., 0-4°C

100% acetone, 10-15 min., 24°C (room temp)
100% acetone, 10-15 min., 24°C
100% acetone, 10-15 min., 24°C

Infiltration: 50/50 acetone/resin, 1-2 hr., 24°C
25/75 acetone/resin, 1-2 hr., 24°C
100% resin, 1-2 hr., 24°C
100% resin, 0-1 hr., 24°C

Polymerization: Oven, 60-70°C

After the resin/tissues approach oven temperature (5-10 min.), they are evacuated briefly using a 2-stage mechanical vacuum pump, primarily to remove any remaining residues of solvent. Evacuation proceeds until most bubbles cease to form; usually no longer than 10-15 min. The oven is then returned to atmospheric pressure and the resin allowed to polymerize.

Several points need to be considered:

1. Some components of the low viscosity resins are volatile at oven temperature and especially under vacuum, which may result in significant formulation changes. Thus, the low viscosity resins should be evacuated only briefly or not at all.
2. The author uses flat embedding in 24 mm diam. x 3 mm deep plastic lids which allow easy evaporation of residual solvent. For high viscosity resins such as Epon, the lids need not be covered during resin polymerization since neither the resin nor its components are volatile and none of the components are affected by air during polymerization. However, with low viscosity resins such as Spurr, the lids need to be covered since the resin and several components of the mixture are volatile. Without a cover, the resin may set up soft on top and brittle on the bottom. The reasons for this are not clear; however, a simple cover such as a petri dish or even a cardboard box lid is sufficient to prevent this problem from occurring.

SOME CHARACTERISTICS OF POLYMERIZED EPOXY RESINS

A number of problems may be associated with epoxy resins and a few of them are discussed briefly. The examples shown are more extreme than might normally be observed; however, they do illustrate problems that probably occur to some extent in all specimens and all EM preparations.

Epoxy Resins are Fluid: The surfaces of tissue blocks immediately after sectioning are mirror smooth. Yet, the same surfaces several months after sectioning, may be rough with outlines of cells and cell constituents (e.g., Fig. 2).

What happens is that either tissue elements or resin (or both) move with respect to each other to reveal the outlines. Thus, the polymerized resin acts as though it were a viscous fluid in which the tissues float. Within the block this represents no problem since the deformed surface of the block is usually trimmed from the block and seldom appears as part of the section

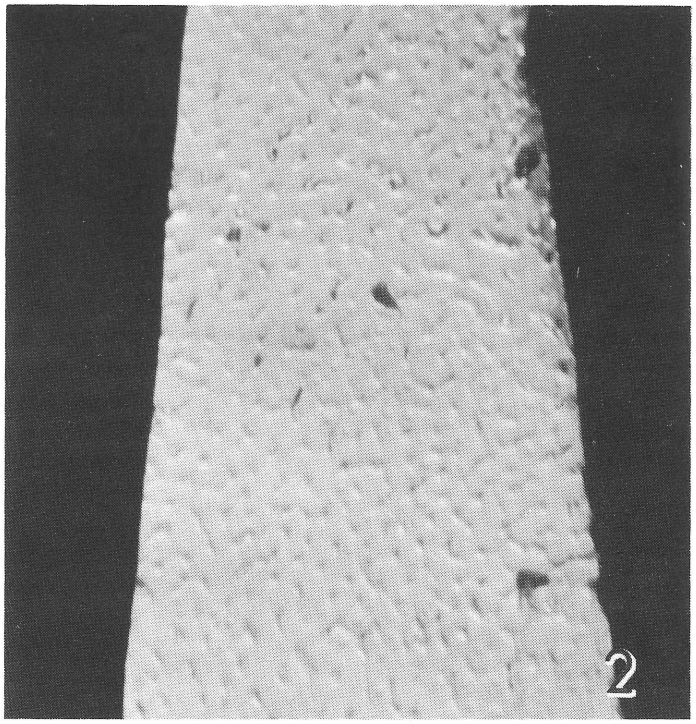
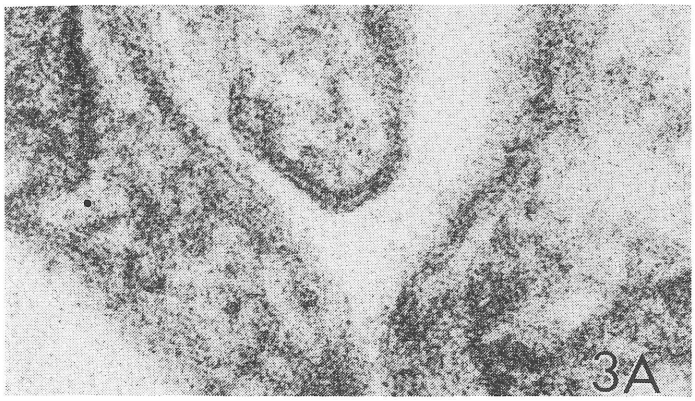


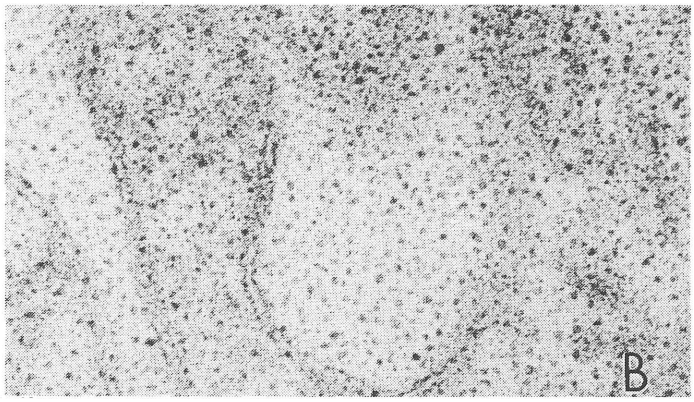
Fig. 2. Photomicrograph of a part of the face of a tissue block in which *Euglena* are embedded. The block had been sectioned to a smooth surface about 6 months before this photograph was taken. During the 6-month interval, the *Euglena* moved with reference to the face of the block and became highly visible. (From: Aldrich and Mollenhauer[12] and Mollenhauer[13]).

used for acquiring data. However, such is not the case in sections with two closely-apposed surfaces that cannot restrict the movement of tissue elements. For example, cell movements as great as 0.01-0.1 nm per hour probably occurred in the cells illustrated in Fig. 2. Thus, sections have a finite life which may vary from a few hours to many days depending on the stability of the resin used and the accuracy of tissue preservation required. Movement of tissue elements is seldom blatantly obvious in sections even when it occurs. However, the images from sections where movement has occurred may be ''less sharp'' than one might expect or the microscope may appear as though ''it was not functioning as well as it had in the past''.

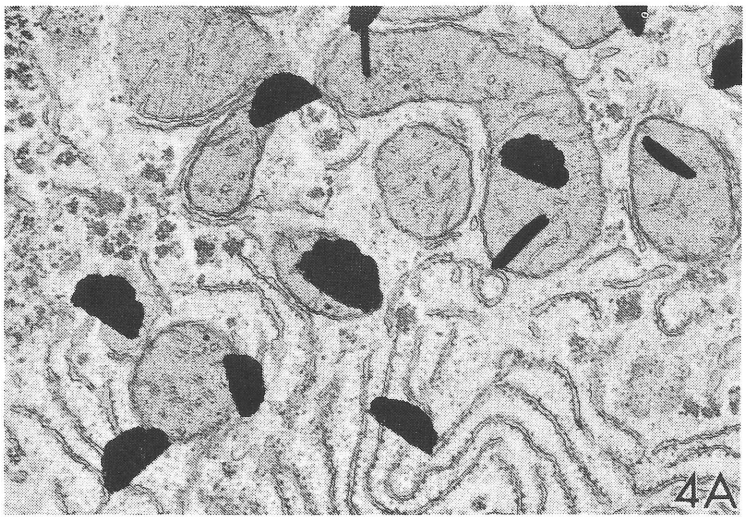
Another problem related to resin instability is illustrated in Fig. 3. Here, unpolymerized or unbound components of the resin or tissue have migrated within the section or to the surface of the section where they have contributed to the formation of pepper and lowered image contrast.

Post-staining Solutions Penetrate Tissue, not Resin: It is generally assumed that poststains penetrate sections even as thick as 1-2μm. However, this is not necessarily true as the following micrographs will illustrate (Figs. 4A-C). These micrographs are from

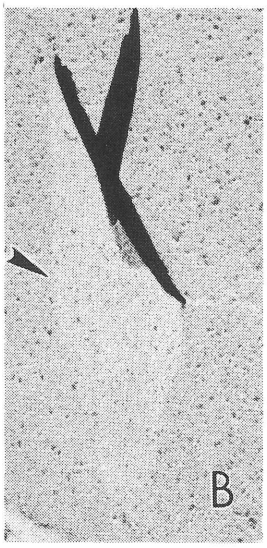


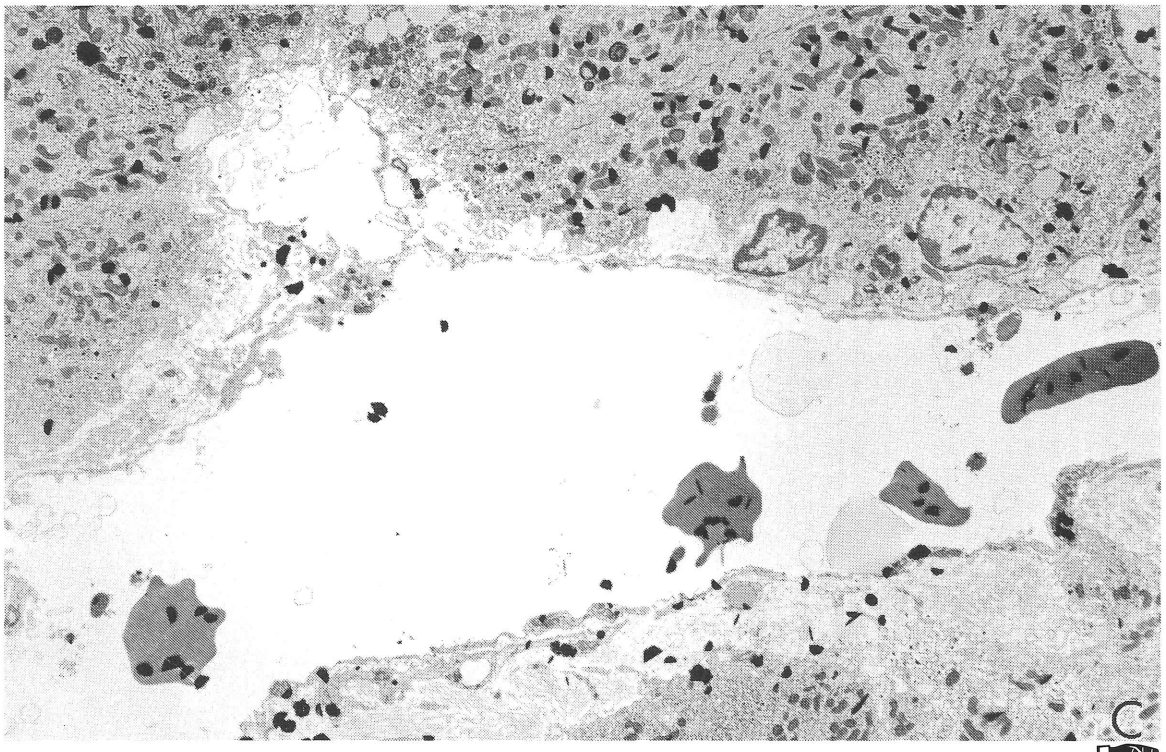


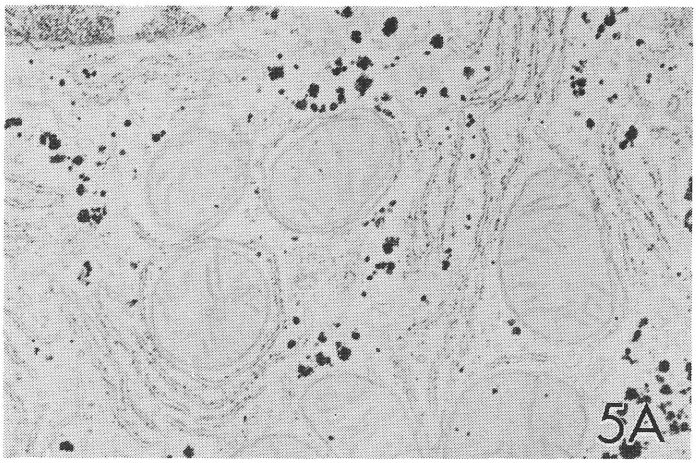
Figs. 3A, B. Section of rat liver fixed in glutaraldehyde and osmium tetroxide and embedded in Spurr resin. The sections were treated similarly except that the one in (A) was cut, stained, and examined immediately and the one in (B) was stored 1 month before it was stained and examined. (From: Aldrich and Mollenhauer[12] and Mollenhauer[13]).

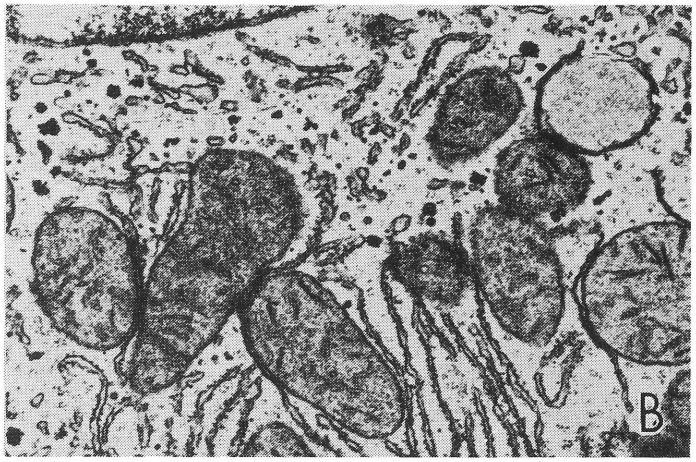


Figs. 4A-C. Sections of rat liver fixed in glutaraldehyde and osmium tetroxide, and embedded in Spurr resin mixture. Sections were stained by floating them on the surface of a drop of lead citrate. (A) Crystals were a prominant contaminant of the section. (B) The crystals were on the upper (dry) side of the section as determined by shadowing the section surfaces (see shadow at the arrowhead). This demonstrates that the stain penetrated the section. (C) This low magnification view of the section shows that the crystals were over tissue, but almost absent from those parts of the section that contained no tissue. Thus, although the stain readily penetrated the tissue, it barely penetrated the resin if at all.









Figs. 5A, B. Rat liver fixed in glutaraldehyde and osmium tetroxide; dehydrated in ETOH and acetone; poststained with lead citrate. (A) Embedded in LADD resin mixture. (B) Embedded in Spurr Mixture. All imaging and photographic procedures were standardized so that the micrographs represent

sections of Spurr-embedded rat liver poststained by floating the sections on drops of lead citrate. During poststaining, crystals formed on the upper (dry) surfaces of the sections (Figs. 4A, B), thus verifying that the sections were permeable to the poststaining solutions. However, the distribution of the crystals was not uniform over the surfaces of the sections; almost all being associated only with those parts of the sections that contained tissue. Very few crystals were over those parts of the section that contained only resin. Thus, stain penetration occurred only on those parts of the section that contained tissue but ***did not occur on those parts that were only resin.*** Therefore, it is conceivable that tissue elements completely within the section (e.g., particles such as ribosomes) would either not be stained or would be stained less than tissue elements that were directly exposed to the staining solution. However, the amount of stain penetration probably varies considerably depending on resin formulation (e.g., see Fig 5).

Image Contrast May Vary Between Resins: Contrast can be expressed most easily in terms of differences between the light and dark areas of an image. In TEM, these differences in light/dark are proportional to specimen density. Therefore, contrast may be considered as ratios between dense and less-dense regions of the specimen. The greater the density differences, the greater the image contrast.

Image contrast (i.e., specimen density differences) may vary markedly even in sections of tissue that are comparably fixed, but embedded in various resin formulations (e.g., Figs. 5A, B). There are several reasons why this could occur; namely (1) there are inherent differences in the density of the embedding resins, (2) the poststains do (or do not) penetrate the section, (3) the poststains do (or do not) react with the specimen, and (4) some of the polymerized resin is evaporated by the beam.

Differences in resin density (Item 1 above) probably do not affect contrast since polymerized epoxy resins are all very close to the same density (Mollenhauer, unreported data).

However, the degree of poststain penetration into a section, and its reaction with tissue elements (Items 2, 3 above) almost surely play significant roles in determining image contrast. Similarly, loss of resin under the beam (Item 4 above) must also play a significant part in establishing contrast. Thus, these last three factors collectively may significantly moderate image contrast.

Some Post-staining Artifacts May Be Related To The Embedding Resin: Amongst various post-staining artifacts, two have stood out as definable entities that are capable of either being understood or at least correctable. These are, fixation pepper[12,13,15] and embedding pepper.[13,16] Only the latter will be discussed in this report.

Embedding pepper is illustrated in Fig. 6A. The name was coined because this form of pepper is believed to be caused by a defect in tissue embedment. Specifically, it is believed that resin components separate during infiltration of the tissue causing some organelles to be poorly embedded. These poorly embedded organelles either trap poststain, or otherwise react with the poststain to leave residues of lead that appear in the form of pepper. The problem is particularly severe if sections are floated on the lead stain rather than being submersed into it.

Fortunately, this form of pepper can be essentially

prevented or eliminated by using a resin mixture whose components do not separate during infiltration, or by trying to embed only very small or thin pieces of tissue. Also, the pepper can be significantly reduced by submersing a section into the post-staining solution rather than floating the section on the surface of the stain.

Embedding pepper can be removed from a section by pretreating the section with 0.5% HCl or 1% EDTA for 1-3 minutes before staining it with the uranyl acetate and/or lead citrate (Fig. 6B).

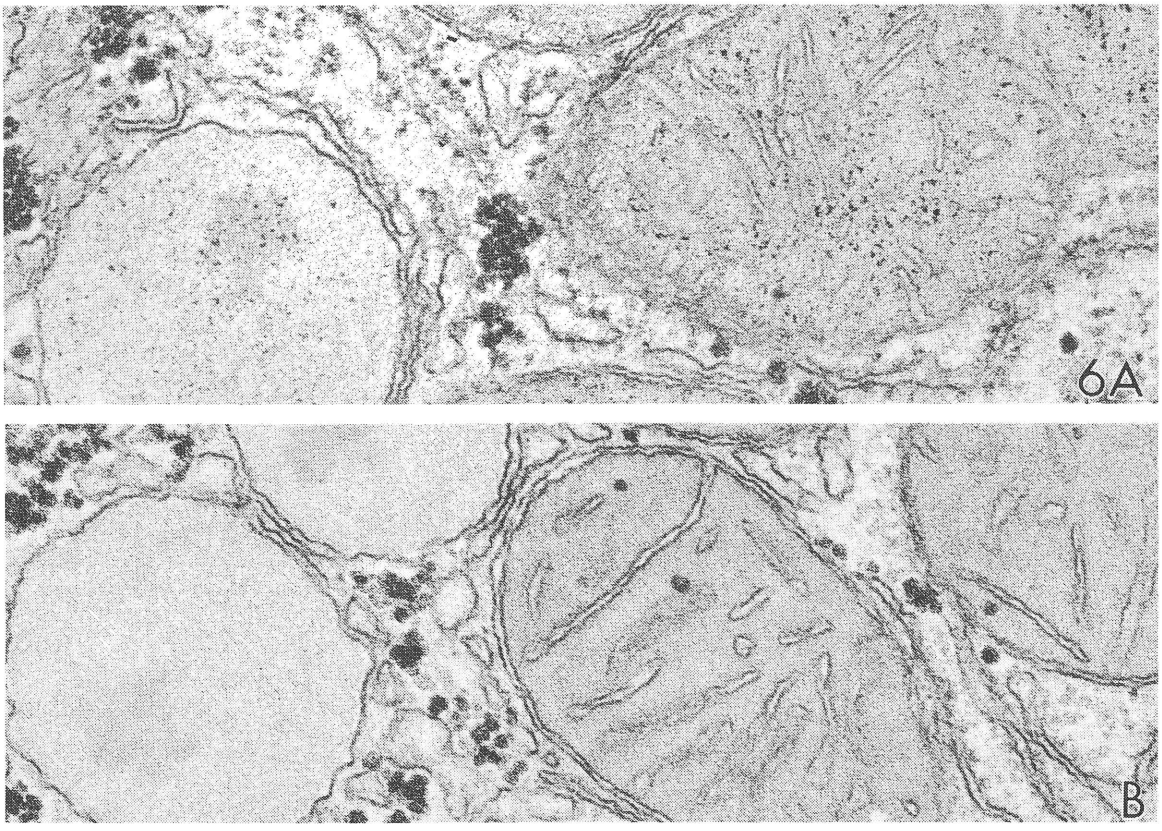
Some Tissue Elements May Shrink During Resin Polymerization: The volume of epoxy resins changes little during polymerization. In tests done with several common embedding resins, linear shrinkage was less than 2%. Yet, in tissues embedded in these same resins, a linear change in size of cell organelles of 15% has been noted.[14] These results are illustrated in Figs. 5A, B. It is believed, but without verification, that the organelles embedded n the Spurr mixture have shrunken whereas those embedded in the LADD mixture are close to their *in vivo* size. The reason for this appears to be related to an improper resin mixture; i.e., the shrunken organelles are embedded in a resin that does not have the proper mixture of components. The reason for the improper resin mixture is thought to be due to the separation of resin components during tissue infiltration. This appears to be more prevalent in formulations with hardeners and resins of widely differing viscosities. In this example (illustrated in Figs. 5A, B) which uses the Spurr mixture, it is thought that the medium viscosity hardener (NSA, 117 cp) did not penetrate the tissue as rapidly as did the very low viscosity resin (VCD, 7.8 cp). The problem was not apparent in the LADD mixture, but here, the hardener is HXSA which has a relatively low viscosity.

This effect can be minimized by using resins whose components have approximately the same viscosity or by embedding very small pieces of tissue so that the components have less opportunity to separate as they infiltrate the tissue.

BEAM-SPECIMEN INTERACTION

It is abundantly clear that the electron beam must interact with the specimen to produce an image. The amount of interaction is proportional to the density of the target and is moderated by the accelerating potential used and other characteristics of the microscope. Although this interaction is sufficiently intense so that the specimen always suffers some degree of damage or disintergration[17,18] it is also influenced by the embedding resin. This damage takes three forms; (1) movement and/or removal of atoms and/or molecules from the sample, (2) ionization of atoms and/or molecules with the specimen, (3) heat.

The removal of atoms or molecules (Item 1 of previous paragraph) from a sample, or the introduction of electrical charges (Item 2) into the specimen may result in the redistribution of



Figs. 6A, B. Sections of rat liver embedded in Spurr resin mixture. (A) Section was submersed into the lead citrate stain for 3 minutes, washed, and dried. Pepper was primarily associated with mitochondria, peroxisomes, and red blood cells. (B) Section was pretreated with 0.5% HCl for 1 minute before staining with the lead citrate as above. Pepper was eliminated.

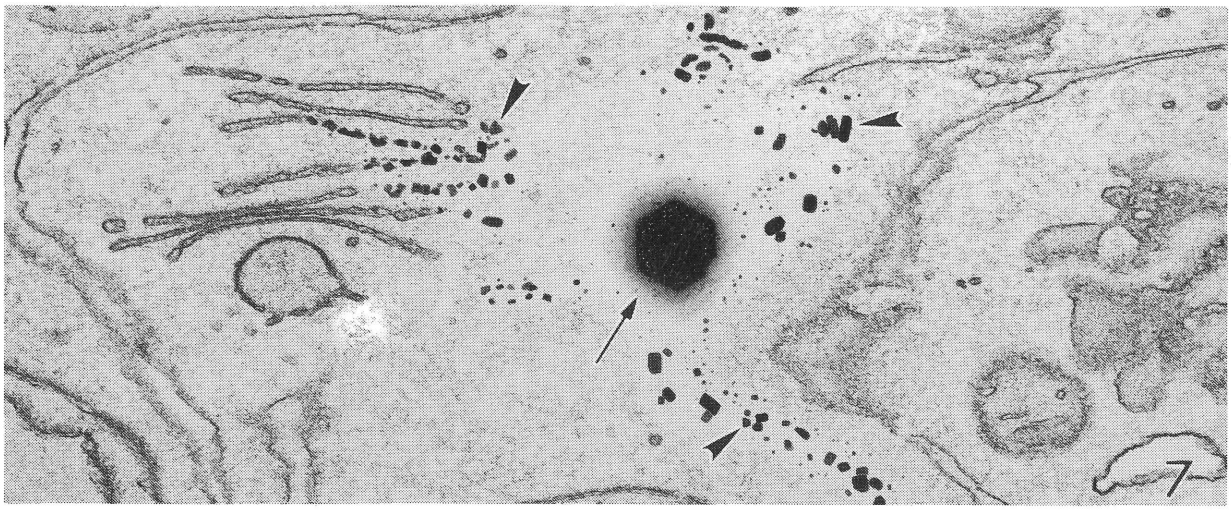


Fig. 7. Section of maize root cell with contaminant (arrow) on the surface of the section. The contaminant was vaporized by the beam and redeposited over the section (arrowheads). The reason that the vaporized material deposited along membrane outlines was due to the low shadow angle (i.e., almost zero degrees; Muhlethaler, personal communication, 1961).

molecules within the specimen with an effect far removed from the site of the beam/specimen interaction (e.g., many nanometers). Heat (Item 3 above) is induced by the transfer of energy from electrons trapped within the section, and may be sufficient to vaporize components of the tissue and part of the embedding resin. Maximum energy transfer occurs, quite obviously, in those regions that trap the most electrons; namely those regions of maximum density. Thus, the greatest heat transfer (and greatest tissue damage) is associated with those tissue elements stained with osmium tetroxide, uranyl acetate, lead, or any other dense material that may have been used or acquired as a contaminant (Fig. 7).

When a section is first placed in an electron microscope, it is an insulator which, when exposed to the electron beam becomes charged. These charges produce interacting forces within, and on, the section which may be sufficient to tear an unsupported section apart. At the very minimum, there is likely to be significant movement of tissue elements under the beam, especially if the section is not well supported. With continued exposure to the beam, however, the section will become heated and thus conductive (i.e., most insulators become conductors when heated), the volatile elements of the sample and resin will be dissipated, and the remaining parts of the section will be burned. These effects occur whether the section is supported or not, but are much more intense in unsupported sections.

Loss of resin from a section may be quite large in some instances depending to a great extent on the characteristics of the resin used. These losses of resin can result in significant increases of image contrast (e.g., Figs. 5A, B) which, for many biological materials may be a useful attribute. However, loss of resin is also loss of the supporting matrix that maintains specimen integrity and this may contribute to image artifacts.

A secondary effect of beam/specimen interaction is a blurred micrograph caused by specimen movement while recording the image on photographic film. Stabilization of sections with carbon is, perhaps, the best remedy for this but making supporting films is time consuming and adds some problems (like folds in the section) when the sections are retrieved from the knife boat. Formvar support films offer similar advantages, but to a lesser extent and with a greater loss of image contrast. Although supporting films may be beneficial, they are often not warranted for the results desired.

CONCLUSIONS

Epoxy resins are the best embedding matrixes available to electron microscopists when considering good tissue preservation, low shrinkage, and ease of sectioning. However, they are plagued with their own problems including high viscosity, toxicity, infiltration, and masking of antigenic sites.

ABBREVIATIONS

cp — Centipoise
DMP-30 — Tri(dimethyl amino methyl) phenol
DDSA — Dodecenyl succinic anhydride
EM — Electron microscopy
ETOH — Ethyl alcohol
HXSA — Hexenyl succinic anhydride
NMA — Nadic methyl anhydride
NSA — Nonenyl succinic anhydride
VCD — Vinylcyclohexene dioxide

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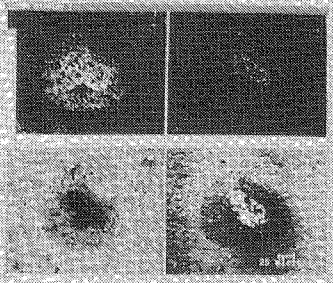

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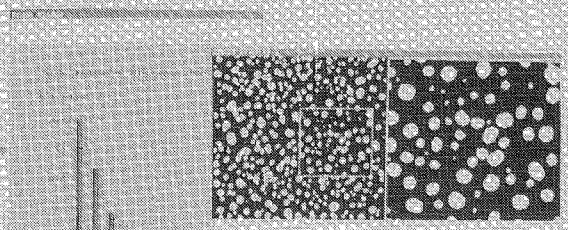

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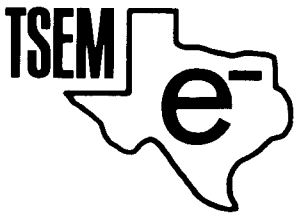

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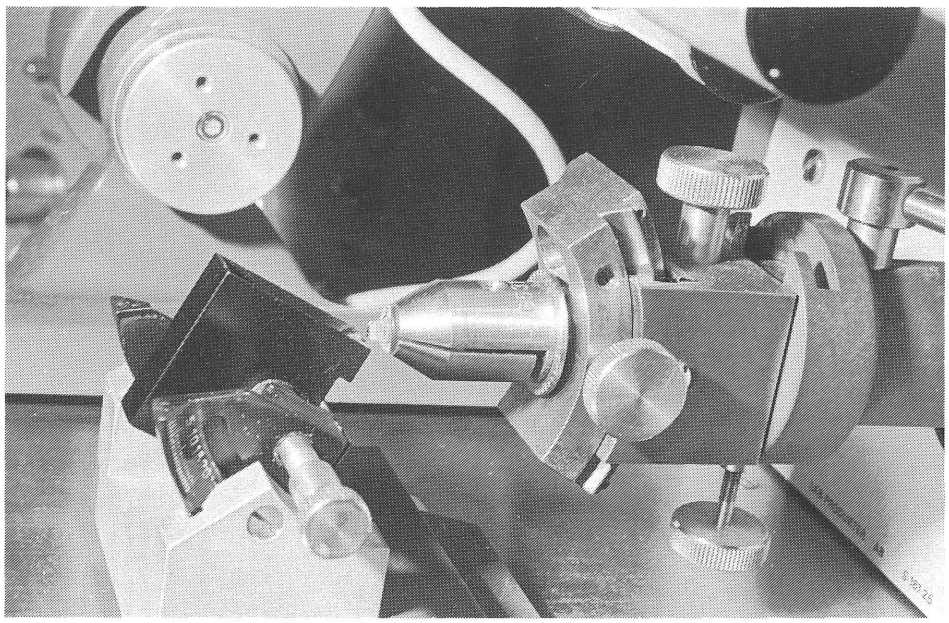
Questions & Answers

By Hilton H. Mollenhauer

Q *How critical is the clearance angle of a knife in the microtome?*

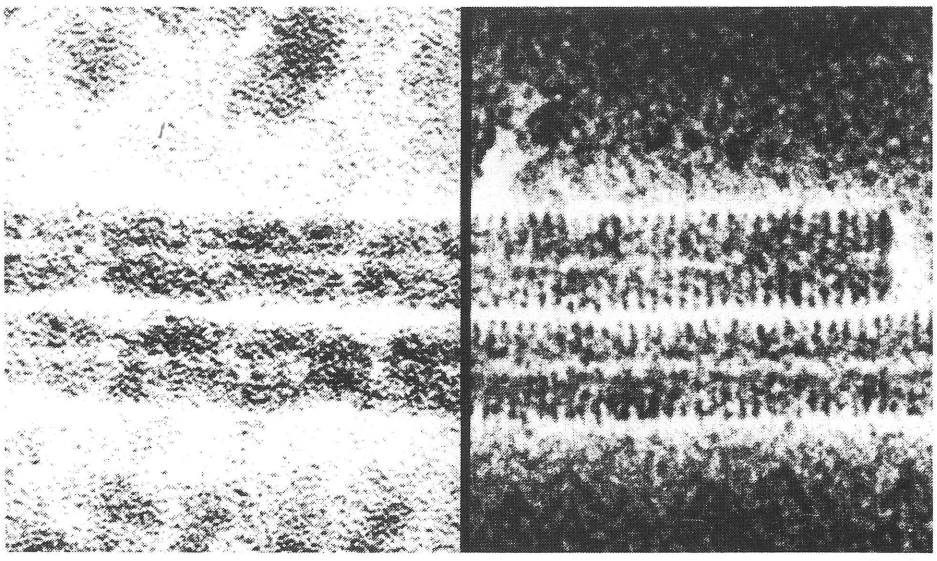


A The angle is not very critical for sectioning **if the knife is good.** This example shows a clearance angle of almost 25° that still allowed the acquisition of usuable sections. However, the angle **is** critical in minimizing stresses to the knife edge. The angle should be low (about 6° is a good average value) to minimize the forces across the edge of the knife. Diamond knifes are extremely brittle and chip easily. On the other hand, there **must** be some clearance so that the block does not drag on the back face of the knife; i.e., too little clearance angle will create a leverage force directly across the edge of the knife.

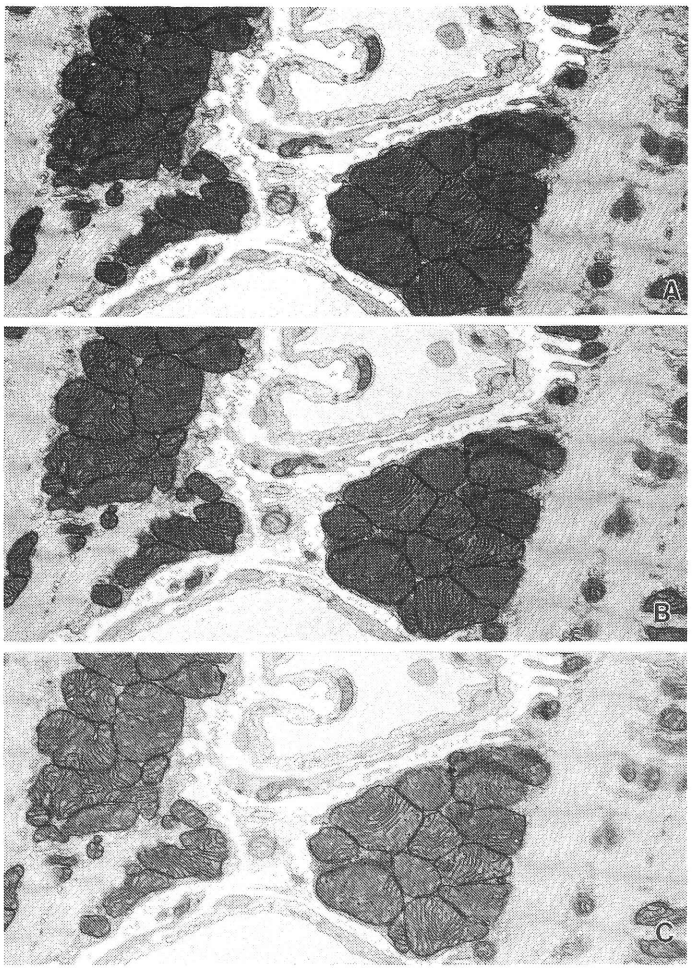
Q *The difference in these two micrographs of a plant virus are due to the way in which the viruses were photographed in the electron microscope. What were these differences?*

This micrograph was reproduced from a slide kindly supplied by Dr. O.E. Bradufute, Ohio State University, OARDC, Wooster, Ohio.

A This is an illustration of beam-specimen interaction and resultant specimen damage. The virus on the left was first viewed on the flourescent screen of the microscope, focused, and then photographed. However, the time required to do this was too long and the virus was damaged by the beam before the exposure could be completed.



The virus on the right was photographed by low-dose photography which minimizes radiation damage. In the low-dose mode, selection of the virus was done quickly and at very low beam intensity. The beam was then moved off the selected virus and onto an adjacent virus for focusing. As soon as the virus was focused, the beam was shifted back to the selected virus just long enough to make the exposure. This mode of operation is available as a computer program on most modern microscopes.



Q *These three pictures were all printed on Grade-1 paper to approximately the same background density. Development was for 45 seconds in Kodak Dektol developer diluted 1:2 with water. How was the contrast changed?*

The tissue is diaphragm from a rat treated with the ionophore lasalocid. Ionophores such as lasalocid and monensin are feed additives commonly used to promote weight gain in cattle and control coccidia in chickens. If given in excess, they strongly affect the mitochondria of striated muscle and often cause mitochondrial condensation (as illustrated here) or swelling (not illustrated). Condensation of mitochondria is probably a prelude to swelling, but this remains to be conclusively demonstrated.

A There are occasions where it is necessary to reduce print contrast below that of the lowest grades of paper commercially available. In this example of contrast adjustment, the paper (Grade-1) was exposed briefly to white light* during printing. This brief exposure (or "flashing") adds a background grey level to the print which can effectively reduce paper contrast as much as 2 grades without adding too high a level of background grey. **Figure A** was not flashed. **Figure B** was flashed 3 seconds with a weak white light*. **Figure C** was flashed 6 seconds with the same light.

*The light was from a 7.5 watt bulb located approximately 10 feet from the enlarging easel.

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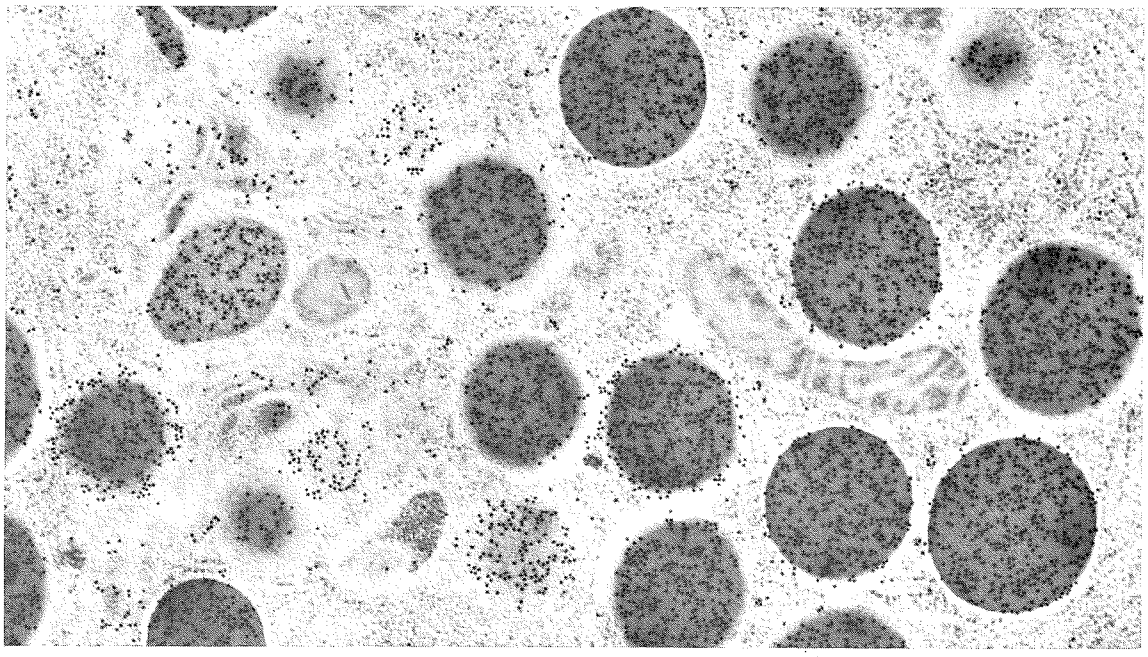

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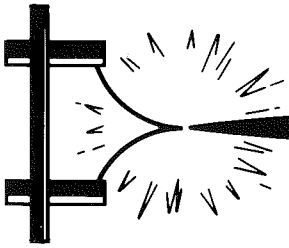

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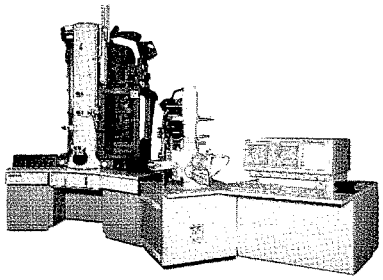

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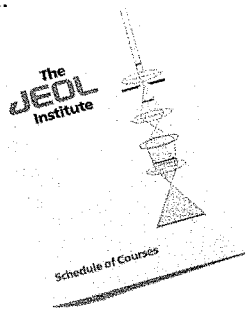

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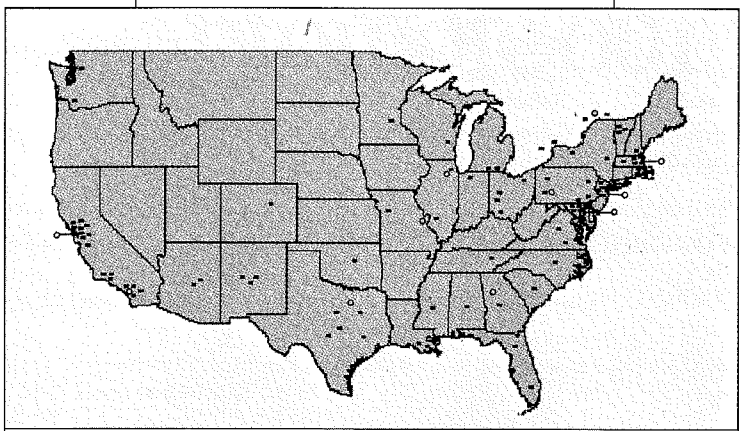

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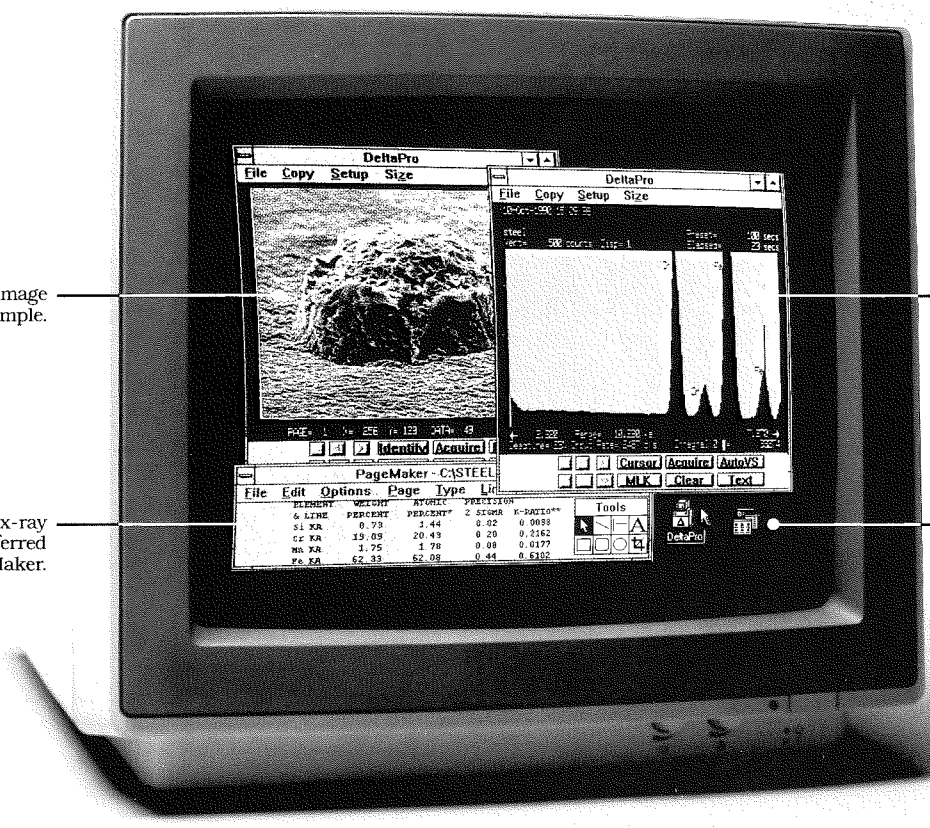

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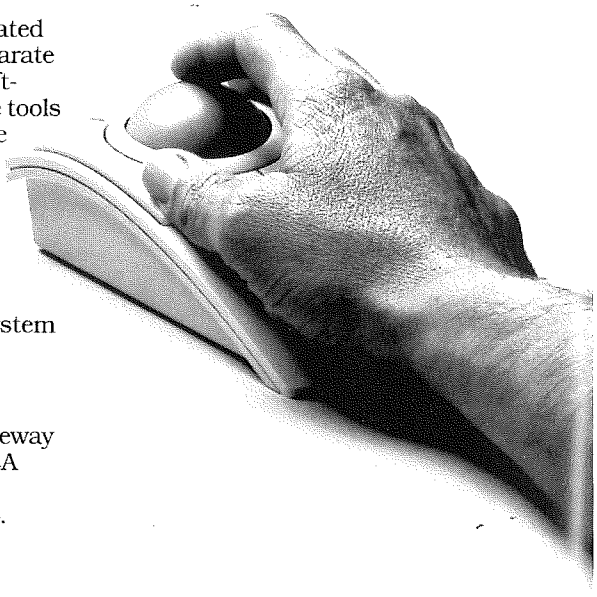

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Abstracts

BIOLOGICAL SCIENCES

PLATFORM PRESENTATION — SPRING 1991

THE EFFECTS OF DROUGHT AND GREENBUG FEEDING ON THE ULTRASTRUCTURE OF SORGHUM. MIJITABA HAMISSOU, PAUL E. RICHARDSON, AND GLENN W. TODD. Department of Botany, Oklahoma State University Stillwater, OK 74078.

Insects and allied pests constitute one of the major obstacles to food production. It is estimated that losses due to insects in the United States amount to $5 to $6 billion annually and that agricultural pests cause up to $1.8 billion in losses. One of the most important insect pests of sorghum is the greenbug Schizaphis graminum (Rondani). Damage caused by this aphid is accompanied by a yellowing of the feeding site followed by necrosis of the tissue and eventually stunting or death of the plant. Another obstacle to agricultural production is naturally occurring drought during the growing season. Little is known about the combined effects of drought stress and greenbug feeding on sorghum ultrastructure. Sorghum responds to drought by producing surface wax and by leaf rolling which minimize water loss. These plant responses involving structural modifications may impede normal mesophyll function since accessible intercellular spaces are necessary for gas exchange. Responses of the plants to aphid feedings are not fully understood. The major objective of this study is to determine the effects of drought combined with greenbug feedings on sorghum plant ultrastructure. Two near isogenic lines of sorghum were grown and maintained in a growth chamber with 14 hours light, 10 hours dark and constant temperature of 28°C for 2 weeks. Drought was simulated by withholding water for 7 days. Relative water content of the plants was measured the 7th day. Leaf samples were taken and processed for electron microscopic observations. There were 69.74% and 63.64% water content reduction in plants under drought stress. Aphid feeding and drought produced similar effects which included thinner cell wall and clustering of cytoplasmic contents.

CORRELATIVE LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL STUDY OF MATERNAL IMMUNOGLOBULIN ACCUMULATION IN THE JEJUNUM OF NEWBORN PIGS.
L.G. Kömüves[1], B.L. Nichols[1] and J.P. Heath[1,2]
USDA/ARS Children's Nutrition Research Center[1],
Department of Cell Biology[2],
Baylor College of Medicine, Houston, TX 77030

Immunoglobulins (IGs) secreted into the colostrum are absorbed from the lumen of the small intestine of suckling neonates and transported across the epithelium into the circulation. We are studying this process using correlative light and electron microscopy in newborn piglets. The jejunum of suckling piglets was processed for Spurr's and LR White embeddings. Consecutive thin sections and 0.5 μm thick sections were labeled using rabbit anti-pig IgG and goat anti-rabbit IgG conjugated to 10 nm gold. The thick sections were silver intensified. No IGs were detected in the jejunal epithelial cells of nonsuckled newborn piglets. After 1 hr of suckling, IG containing granules can be seen in the apical cytoplasm. These granules are formed from endocytotic vesicles originated in the apical tubulo-vesicular system, characteristic to the neonatal enterocytes. Sparsely distributed between the granulated enterocytes, another cell type can be detected in the immunolabeled, silver intensified thick sections. These cells do not contain granules, but are characterized by a strong uniform cytoplasmic staining. Their importance in the IG transport, however, remains to be determined.

ORIGIN OF DIMORPHIC SPERM CELLS IN THE ANGIOSPERM PLUMBAGO ZEYLANICA. SCOTT D. RUSSELL AND GREGORY W. STROUT. Department of Botany & Microbiology and Samuel Roberts Noble Electron Microscopy Laboratory, University of Oklahoma, Norman, OK 73019

Formation and morphogenesis of the generative cell of Plumbago zeylanica begins with an eccentric division of the microspore forming a lenticular cell. The founder population of heritable organelles in the generative cell cytoplasm includes an average of 3.88 plastids, 54.9 mitochondria and 3.7 vacuoles (far less than typical somatic cells). Initially, the generative cell is attached to the interior of the intine; however, during its maturation, the cell separates from the intine and becomes highly polarized in form and distribution of its organelles. Multiplication of organelles occurs with increases of 600% in numbers of plastids, 250% in mitochondria and 4300% in vesicles. As the generative cell separates from the intine, polarity of organelles is reversed, with plastids migrating to the end of the generative cell most distal from the vegetative nucleus. A cellular protuberance forms at the end of the cell; this faces the vegetative nucleus and elongates into a long, slender projection that occupies complementary grooves in the surface of the vegetative nucleus. Mitochondria and vesicles are displaced toward the projection end of the cell, whereas the generative nucleus and plastids are displaced towards the opposite end. As the generative cell divides, the organellar constituency is fixed into two dimorphic sperm cells: the S_{vn}, which is associated with the vegetative nucleus, and the S_{ua}, which is unassociated with the vegetative nucleus. These sperm cells have different organelles and fates. The mature S_{vn} contains an average of 256.2 mitochondria, 0.4 plastids, 158.9 vesicles and 0.36 microbodies, whereas the mature S_{ua} contains 39.8 mitochondria, 24.3 plastids, 91.1 vesicles and 3.18 microbodies. The S_{ua} preferentially fertilizes the egg, whereas the S_{vn} preferentially fuses with the central cell to form the endosperm.

CYTOSKELETAL ORGANIZATION AND MODIFICATION DURING FERTILIZATION IN PLUMBAGO ZEYLANICA. BING-QUAN HUANG AND SCOTT D. RUSSELL*. Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019

The organization of the cytoskeleton was investigated in isolated embryo sacs (ES) of Plumbago zeylanica prior to, during and after fertilization using immunofluorescent probes, observed with confocal laser scanning and epifluorescence microscopy. Triple staining was employed to co-localize the nucleus using DAPI fluorescence, microtubules (MT) using FITC-conjugated anti-tubulin, and actin using rhodamine-phalloidin. Because these fluoresce at different wavelengths, they can be visualized together or separately. Three distinct MT regions were identified in the unfertilized egg: (i) a cap-like organization of MT at filiform apparatus (FA); (ii) parallel arrays of MT in the lateral region; and (iii) a random arrangement of MT in the perinuclear region. Actin filaments were organized as a distinct network in the egg that appears to wrap around its nucleus. When the pollen tube (PT) arrives at the FA and penetrates into the ES, dense MT and actin arrays surround the PT and seem to restrict its growth to a defined pathway determining the site of sperm cell discharge. At fertilization, MT are densely localized near the two deposited sperm as they approach the nuclei of egg and central cell respectively. Actin is localized in an apparent circumferential band separating the two sperm cells. MT and actin are largely depolymerized or present in single fibers near the path of PT, at the gametic fusion stage and only later reorganizes acropetally around the periphery of the zygote. Cytoskeletal elements in the embryo sac appear to play a role in helping the sperm cells to approach the target cells.

ANALYSIS OF FLORAL MORPHOLOGY IN NAMA (HYDROPHYLLACEAE). DOUGLAS A. HAYWORTH, DEPT. OF BIOLOGY, UNIVERSITY OF TEXAS AT ARLINGTON, ARLINGTON TX 76019

Replicate flowers of four species, separated into corollas with adnate stamens and receptacles with gynoecia and sepals, were examined using scanning electron microscopy. Characters compared include those previously described such as filament insertion, and those which are described first here such as trichome type and distribution and corolla cell topography.

FINE STRUCTURE STUDIES OF THE HUMAN OSTEOARTHRITIC SYNOVIUM AND COLLOIDAL GOLD LABELLING OF INTERMEDIATE FILAMENTS. W.D. MEEK, Department of Anatomy, College of Osteopathic Medicine-Oklahoma State University, Tulsa OK 74107.

The synovial lining cells (SLCs) possess unique morphological features, not explained in earlier normal cases or in patients with osteoarthritis (OA) and rheumatoid arthritis (RA). SEM reveals what appear to be cell processes, with several topographical features (microvilli, blebs, and ruffles), emanating from the synovium surface as it faces the joint cavity. TEM clarifies that these are indeed cell processes, in addition to microvillous-covered cell bodies. The cell processes may extend over 30 micrometers from cells deeper in the synovium. The processes contain a prominent cytoskeleton of intermediate filaments (IFs), microtubules, and microfilaments. The IFs label with protein A gold directed against a monoclonal antibody to vimentin. The tissue, embedded in Lowicryl and polymerized by UV light in a specially built chamber, is negative for keratin and desmin. Controls are also performed on a cultured human amnion cell line known to contain both vimentin and keratin IFs. Antibodies are also tested with immunofluorescent techniques. Cell types and junctions are quantitated, with both OA and RA having an increase in Type B cells, and OA synovial lining cells having more gap junctions than both RA and normal. Hopefully, these results better explain the morphology of the normal synovium and the influence of the disease process. Supported by Grant #89-09-045 from the American Osteopathic Association.

DOPAMINE SULFUR AND IRON IN THE PRIMATE VENTRAL MESENCEPHALON JG Wood, Department of Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104 USA

Microscopic studies have described the various neuronal subtypes of the ventral mesencephalon (VM), and the fluorescent detection of dopamine (DA) has been effected. Intraneuronal DA has been demonstrated with a cytochemical method incorporating chromium (Cr) which has been verified by X-ray microanalysis. X-ray microanalysis has also shown that a relationship exists between sulfur (S) and Cr in certain VM neurons. The present investigation utilizes light microscopy, histochemistry and cytochemistry, electron microscopy and microanalysis using both energy dispersive spectroscopy (EDS) with STEM and wave length spectroscopy (WDS) methods employed on the Cameca Microprobe. The WDS method has some advantages over the STEM in that larger brain stem section areas can be scanned and DA, S and other elemental sites can be mapped. Light microscopy reveals VM neurons with large inclusions which can be visualized with ordinary light, phase and dark field microscopy. Transmission EM reveals neurons with different types of inclusion bodies of various shapes and densities. EDS shows, that in some instances DA positive bodies within perikarya are also positive, WDS demonstrates both DA and S indicates traces of iron (Fe). At times there is overlap of DA, S and/or Fe. These findings indicate that the neurons contain DA and it may also be possible that DA and S are in the same organelles. Fe has long been known to be a component of the VM, but its ultrastructural location has not been established. Detailed TEM and EDS studies of the SNAR should reveal the Fe loci. This study illustrates the benefits of combining histo-cytochemical methods with various types of analytical microscopy in revealing components of brain stem nuclei. (Supported by grants BSRG-1187607 and Presbyterian Foundation-5198501).

URTICATING HAIRS IN FOUR SPECIES OF THEARAPHOSID SPIDERS (ARANEAE, THERAPHOSIDAE). A.M. PATTERSON, Dept. of Biology, Univ. of Texas at Arlington, Arlington TX 76019

Urticating hairs were found on four species of Theraphosid spiders, commonly known as tarantulas. Only Type IV was not found. Hairs were found on both the ventral and dorsal section of the abdomen, as well as on the spinerettes. The only species of spider not exhibiting two types of urticating hairs was Rhecostrica hentzi, a local Texas species. The other three species were collected from localities in Malaysia, Guatemala, and Surinam. It has been proposed that urticating hairs are part of a unique defensive strategy allowing species with urticating hairs to be less aggressive and less venomous than species lacking the hairs.

CHONDROGENESIS OF LIMB MESENCHYMAL CELLS CULTURED ON MICROCARRIER BEADS. E. DAANE, P.J. DUKE, M. CAMPBELL, Dental Branch, Orthodontics, DSI, University of Texas, Houston, TX 77225

Currently our laboratory is preparing micromass cultures of embryonic mouse limb mesenchyme for flight on the space shuttle Atlantis in December 1991 in an effort to determine whether microgravity (which is known to alter chondrogenesis in vivo) affects chondrogenesis in cell cultures. Because it is argued that the special conditions of cells cultured during spaceflight (i.e. no settling, no convection currents, etc.) are responsible for the observed changes in behavior of cells flown in space, and because microgravity simulation is difficult to achieve with attached cultures, the decision was made to attach the cells to microcarrier (mc) beads so that they could be flown in a bioreactor in space or exposed to simulated microgravity in a STLV (slow turning lateral vessel). Micromass cultures were prepared and $1x10^{7}$ cells were innoculated into a culture vessel containing a suspension of collagen coated Cytodex 3 beads (Pharmacia) in attachment medium. After 3 days a sample of the culture was removed and fixed in 1% glutaraldehyde in Sorenson's buffer. A portion of this sample was stained with alcian blue and we observed single cells had formed matrix and extended numerous filopodia. The remainder of the sample was postfixed with 1% OsO_4 for SEM and TEM studies. SEM micrographs revealed chondrocytes with a more rounded and "ruffled" appearance than chondrocytes cultured on a flat surface. Matrix was apparent on these micrographs as well, showing chondrogenesis in cells attached to mc-beads. TEM studies were inconclusive because of sectioning difficulties due to the insolubility of dextran beads during processing. Future studies will use polystyrene beads.

MORPHOLOGY, STRUCTURE, AND ANALYTICAL PROBLEMS WITH MANGANESE OXIDES IN SOILS AND WEATHERED ROCK. J.B. DIXON, Dept. Soil & Crop Sciences, Texas A&M University, College Station, TX 77843-2474.

Manganese is essential to plants and Mn oxides are important visual indicators in soils and they have selective cation adsorption of pedological and geochemical importance. Yet Mn is less than 0.9% of the Earth's crust and Mn oxides are difficult to identify in soils and weathered rocks. Electron microscopy methods have proven effective in solving some of these analytical problems. Todorokite has a fibrous morphology and has a trilled twinning pattern that is diagnostic. Also, it has large lattice image spacing that is easy to identify by transmission electron microscopy. Lepidocrocite is the Mn oxide of importance in many soils and it forms crystals that are large enough to characterize with the scanning electron microscope. Birnessite is the Mn oxide that is most difficult to identify and describe in soils. The crystals are small, thin, and often scarce. Certain fungi form delicate crystal masses of buserite in laboratory preparations. Characterization of these crystal masses by SEM has led to speculations that they are the precursors of the birnessite in soils. The combination of morphological, structural, and chemical data obtained with electron microscopes holds some promise for identifying and describing this fascinating group of compounds.

THE MICROSTRUCTURE AND EVOLUTION OF SCALE SURFACES IN XENOSAURID LIZARDS
Michael B. Harvey, Dept. of Biology, University of Texas at Arlington, Arlington, TX 76019

A varied and phylogenetically informative morphology characterizes the scale surfaces of the xenosaurid lizards. Scanning electron microscopy demonstrated the presence of polygonal cells and a subcellular surface microornamentation of a type previously known only from the distantly related Iguania. The genera and species of xenosaurids differ in polygonal cell size and in the types of scale organs and microornamentation present. For Shinisaurus crocodilurus, an ontogenetic change in scale surface morphology is here reported for the first time in a lizard species. Data from a supplementary histological study demonstrated the enervation and structural characteristics of the scale organs and further aided in the description of the polygonal cells and flaps seen in SEM photographs. Polygonal cells result from folds in the alpha and beta layers of the epidermis and are not monocellular Oberhautchen structures as previously suggested.

STATISTICAL ANALYSIS OF COMPUTER-GENERATED MEASUREMENTS FROM MANUALLY-OUTLINED POLLEN PERFORATIONS. E. L. VEZEY, H-S. YU and J. J. SKVARLA, Dept. of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma 73019.

The repeatability and accuracy of manual outlining (segmentation) of perforations on SEM micrographs and computer-generated patterns were investigated using image-analysis measurements and ANOVA. This study was conducted because (1) automated operators were unable to correctly outline features of interest on SEM micrographs and (2) semi-automated pointing devices were not available with our image-analysis configuration. In this investigation, groups of seven individuals made multiple tracings on plastic of real pollen perforations and computer-generated black-on-white perforations. Perforation size and ink color used to make tracings (black and red) were varied in different experiments. Tracings were digitized and image-analysis measurements of perforation diameter and perimeter were examined using ANOVA. Results indicate very small variation within individuals and statistically significant but tolerable variation between individuals. ANOVA results were very similar for real vs. artificial perforations, small vs. large perforations and red vs. black ink. Finally, measurements from tracings of computer-generated images were compared to measurements from the original black-on-white image. Black-ink tracings produced more accurate measurements than red-ink tracings. (Measurements from red-ink tracings were uniformly smaller than the correct value.) Results of this study provide a basis for comparison with semi-automated and automated segmentation techniques.

MATURATION AND SENESCENCE OF THE TRANSFER CELL ZONE IN CORN CARYOPSES. RONALD W. DAVIS, DEPARTMENT OF HUMAN ANATOMY AND MEDICAL NEUROBIOLOGY, COLLEGE OF MEDICINE, TEXAS A&M UNIVERSITY, COLLEGE STATION, TX 77843.

The transfer cell zone in maize is composed of a large number of cells at the base of the endosperm. They are characterized by numerous invaginations of the cell wall and are thought to function in transportation of nutrients passively in the apoplast and/or actively in the symplast. It has been previously shown that in field corn differentiation of the endosperm cells into transfer cells begins as early as 4 days post pollination and appear to be fully formed by about 15 days post pollination. As the embryo and scutellum enlarge, endosperm cells are degraded and crushed. In some cases apparent degradation of the transfer cells can be seen as early as 26 days post pollination. By approximately 39 days post pollination the transfer cells are crushed and appear as if they are no longer physiologically active. By this time a clear "black layer" or "closing layer" is usually present in the pedicle below the nucleus. The nature of the development of this layer and its composition are not clear. It appears that among the final steps of caryopsis maturation is the degradation and destruction of the transfer cells. This suggests that the process of grain filling is terminated by an internal physical process.

QUALITY CONTROL AND QUALITY ASSURANCE IN DIAGNOSTIC ELECTRON MICROSCOPY. Mannie Steglich, Lydia Shanks and Elsa Ramos. The University of Texas M.D. Anderson Cancer Center, Houston.

Currently there is a strong emphasis on quality control and quality assurance in diagnostic pathology. As they specifically pertain to diagnostic electron microscopy, these disciplines impact on the procurement, documentation, processing, evaluation and reporting of tissue specimens from patients. We have addressed each issue as it applies to the technical aspects of electron microscopy in the pathology department of a cancer center. A critical factor is obtaining tissue of good quality for ultrastructural study: within the hospital, this requirement can be controlled by setting up an efficient system and monitoring it diligently, but problems are frequently encountered in procuring suitable specimens from procedures performed outside the hospital, and are more difficult to resolve. The universal technical artifacts associated with processing and sectioning tissues are compounded in a hospital setting by the variety of types of specimens submitted for diagnostic electron microscopy and the need to process them in different ways. An important step is evaluation of semi-thin sections and this should be performed jointly by the technician who will trim and thin-section the blocks and the staff pathologist who will perform the ultrastructural study. It is necessary to expedite the diagnostic procedure, and rapid photographic techniques are used in order that micrographs can be furnished along with the written report of the findings. Documentation methods must take into consideration the need for correlation with other pathologic diagnostic procedures on the same specimen or patient, and provide for rapid and selective retrieval of records.

BIOLOGICAL SCIENCES

POSTER PRESENTATION — SPRING 1991

ROLE OF PROTEIN KINASE C (PKC) SUBTYPE II (β) IN TRANSMEMBRANE WATER TRANSPORT IN TOAD URINARY BLADDER EPITHELIAL TISSUES. A.J. MIA, Jarvis Christian College, Hawkins, TX 75765, L.X. OAKFORD AND T. YORIO, Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

Mezerein (MZ), a non-phorbol activator of protein kinase C (PKC) increases transmembrane aqueous flow across the toad urinary bladder when added to the mucosal surface. Mezerein stimulation was found to be associated with degranulation of secretory granules and an increased incidence of cytosolic aggrephores and membrane fusion events at the apical membrane. Cytochemical detection of protein kinase C (PKC) for subtype I, was carried out using specific monoclonal antibody and protein A-gold particles following 60 min exposure of bladder tissues to MZ. Protein A-gold labelled PKC isozyme I was distributed singularly and in discrete isolated patches in cytosol as well as in apical membrane sites. Distribution of clustered gold particles over diffused cytoplasmic bodies suggest an association of PKC subtype I with cytoplasmic aggrephores. Similar studies carried out using specific monoclonal antibody against protein kinase C (PKC) subtype II and protein A-gold probes as reported here indicated similar distributional patterns of gold particles over the cytosolic domain as well as in the apical membrane sites, suggesting a possible role of protein kinase C (PKC) isozyme subtype II in transmembrane water transport process. Parallel studies with antidiuretic hormone (ADH) revealed remarkably similar distributional patterns of protein kinase C (PKC) isozyme subtype I and II as that of MZ-exposed tissues. Control bladder tissues, lacking antibodies, exposed to 0.1% BSA and protein A-gold probes showed no binding of gold particles. These results coupled with biochemical studies of osmotic water flow suggest that PKC activation for aggrephore translocation from cytosol to apical membrane sites may be a necessary precondition for enhanced water flow mediated by ADH. Supported by NHLBI grants K14HLO1730 (AJM), and T35HLO7465 (TY) and a Texas Research Enhancement Grant (TY).

ACROLEIN FIXATION REVISITED. HILTON H. MOLLENHAUER AND ROBERT E. DROLESKEY, USDA-ARS, Route 5, Box 810, College Station, Texas 77845-9594.

Acryl-aldehyde (acrolein) as a fixative for electron microscopy was first described by Luft (1959, Anat. Rec. 133,305). Its primary attributes are that it is capable of penetrating large pieces of tissue, causes little shrinkage, and gives a reasonably good ultrastructural image. Unfortunately, acrolein has a strong acrid odor and irritating vapors so that it may be very unpleasant to use unless the work area is well ventilated. Nonetheless, it does have at least some merit as indicated by the three projects listed here. [1] Whole ceca of 1, 2, and 3 day old chicks were stabilized in an acrolein/glutaraldehyde fixative so that the distribution of *Salmonella* could be determined without the distortions that occur when tissues are cut into the small pieces most suitable for electron microscopy. The ceca were about 5 mm in diameter and 30 mm long. Fixation was consistent throughout the ceca and was relatively good. [2] In a study of latex formation, whole stems and leaves of *Ipomoea batatas* (sweet potato) were fixed intact in an acrolein/glutaraldehyde fixative to stabilize the latex and prevent its discharge from the latex ducts when the tissues were dissected into pieces small enough for glutaraldehyde fixation. Lettuce roots, which also contain latex ducts and are difficult to fix intact, were subjected to the same procedure with excellent results. [3] Whole chicken hearts from 1-3 day old chickens were excised and fixed initially in acrolein/glutaraldehyde, further dissected into pieces small enough to facilitate further processing, and then fixed in osmium tetroxide in the usual manner. The basic problem is that chicken tissues often do not fix well with glutaraldehyde. Tissue preservation was improved significantly with acrolein-based fixatives. In these three examples, acrolein/glutaraldehyde fixatives were better than glutaraldehyde as a primary fixative, and cell preservation was consistent throughout the blocks of tissues. Thus, acrolein may be useful for stabilizing and partially fixing large pieces of tissues for electron microscopy.

ULTRASTRUCTURAL LOCALIZATION OF HAPTEN IN ANTIGEN PRESENTING CELLS INVOLVED IN CONTACT SENSITIZATION.

Junmin Tang, Corazon D. Bucana, Kenneth Dunner Jr., and Margaret L. Kripke, Departments of Immunology and Cell Biology, The University of Texas, M.D. Anderson Cancer Center, Houston, TX, 77030

Exposure of C3H mice to the contact sensitizer, FITC, was characterized by an increase in dendritic cells (DC) in the draining lymph node (DLN). Many of these DC were fluorescent and were identified by electron microscopy as Langerhans cells. Previous studies indicated that fluorescent dendritic cells are antigen presenting cells that participate in the initiation of the contact hypersensitivity response within the regional lymph node. The purpose of this investigation was to identify the fluorescent DC by electron microscopy to determine the ultrastructure localization of FITC. C3H mice were exposed to FITC by painting the shaved abdomen with a solution containing 0.5 % FITC in a solvent composed of equal volumes of acetone and dibutyl phthalate and DLN were removed 18 hours later. Lymph nodes from unexposed animals were used as controls. Lymph nodes were fixed and cut in half; the tissues were processed for post-embedding labeling. Dendritic cell suspensions were prepared from DLN and enriched for DC by Ficoll-Hypaque centrifugation. Cell pellets of DLN cells and lymph node tissues were embedded in Lowicryl K4M and thin sections were immunostained using polyclonal antibody to FITC. FITC-positive dendritic cells were identified by morphology and by the presence of gold label inside the cell. The majority of the anti-FITC label was inside the cell in the perinuclear area. Gold particles were present in clusters in a vesicle(endosome?) and some were arranged in a linear array. Gold particles were also associated with mitochondria and lysosomes. Only a few gold particles were associated with the outer plasma membrane. Because the fixation protocol does not allow for enhancement of membranes, it was not possible to identify Birbeck granules in the dendritic cells. However, parallel samples showed that dendritic cells of similar morphology contain Birbeck granules. Serial sections immunostained with polyclonal anti-thy1.2 antibody showed gold label only in lymphocytes. The negative controls showed only background labeling. Similar results were observed in lymph node tissue. These results demonstrate that most hapten is internalized and associated with cytoplasmic organelles in antigen presenting cells, suggesting that presentation of hapten involves action processing and internalization, rather than binding of hapten to surface proteins.

THREE-DIMENSIONAL RECONSTRUCTION AND QUANTITATIVE CYTOLOGY OF POLLEN OF AN ORCHID, CYMBIDIUM GOERINGII. HONG-SHI YU & SCOTT D. RUSSELL. Department of Botany & Microbiology, University of Oklahoma, Norman, OK 73019

The organization of the generative cell (GC) and the vegetative nucleus (VN) was examined in pollinia of Cymbidium goeringii Rchf. at both a young stage (1 week before anthesis) and later (at floral anthesis) using transmission electron microscopy, computer-assisted serial section reconstruction and quantitative cytology. At the young stage, the GC has a spherically-shaped main body, occupied by the large nucleus, and 1 to 4 cytoplasmic extensions with a length that may be over 10 μm. At this stage, the VN is characterized by a large groove which encloses one side of the main body of the GC. Through the complementarity present between the groove in the VN and the main body of the GC, a close association is established. By the time of anthesis, some GCs lose their cytoplasmic extensions and become spherical while others retain their extensions. The surface of the GC becomes highly wrinkled during maturation as the GC, VN and pollen grain lose up to 38%, 18% and 44% of their previous volume, respectively. The distance between the GC and the VN increases during this stage and in some cases the two entities separate completely. Numerous enucleated cytoplasmic bodies (ECBs) are distributed on the surface of the GC and near the tips of cytoplasmic extensions of the GC. All ECBs are located on the side of the pollen grain in which the GC is located. The close distribution of ECBs with the GC and their similar ultrastructure indicate that ECBs originate from the GC, thereby resulting in a reduction of male cytoplasm during development. In light of observed gradational stages in the degeneration of ECBs and their stability in average surface area and volume, it appears that the creation and degradation of ECBs are counter balanced. Measurements of volume and surface area of 7 cellular and nuclear features indicate that statistically significant differences exist between the two developmental stages. These changes occurring during maturation appear to be related to the dehydration of the pollen.

DRAFTING MADE EASY. J.R. SCOTT, Electron Microscopy Center, Texas A & M University, College Station TX 77843-2257.

Drafting is the art form most often used in illustrations for objects to be made by skilled workers. Without the technical skills and tools, a useful, drafted copy of the work may be too time consuming to consider. The print can allow the use of the many skilled craft people in aiding when equipment or replacement parts are needed. A modern aid is at hand in most offices... the copy machine.

The presentation will show the use of the copy machine for a recent application in the Electron Microscopy Center at Texas A&M University.

T-MAX AND TRI-X: COMPARISONS OF SHEET FILM EMULSIONS FOR USE IN SEM PHOTOGRAPHY.* J.C. Long, Electron Microscopy Center, Texas A&M University, College Station TX 77843.

The potential benefits of Kodak's Tabular Grain Emulsion Films (brand name: T-MAX) has resulted in our lab considering a switch from Kodak TRI-X 4x5 sheet film to Kodak T-MAX 4x5 sheet film for use in SEM photography. The current regime is TRI-X sheet film developed in D-19 diluted 1:2. Processing is at 20^{o}C in large (4l) tanks with N_2 burst agitation. The procedure used for this study was T-MAX and TRI-X sheet film processed in T-MAX TS developer; using the same large tank, and N_2 burst system, but at temperatures ranging from 20^{o}-24^{o}C. The potential benefits are: 1) Improved image quality; tabular grain emulsions are purported to have improved sharpness, a finer grain at a given film speed, and better tonal separation than previous emulsions. 2) Ease of use; T-MAX TS is a highly miscible liquid concentrate which can be used at 20^{o}-24^{o}C. The primary temperature recommendation is 24^{o}C; this would reduce the need for chilled water at our facility.

The initial comparison was made using Kodak TRI-X and Kodak T-MAX 400 sheet film. Exposures were made using a JEOL T330A equipped with a high resolution photo CRT and waveform monitor (WFM). Brightness settings were determined using the WFM in the procedure described by J. Holm. Negative density was determined using a Zeiss Axiophot equipped with a MPM 20 Photometer. The percent value of transmitted light (%T) was converted to diffuse density (D) using formula D-Log 1/T. The calculated diffuse density was plotted against the brightness line position on the WFM. Granularity was examined by visual comparison using 12x enlargements from T-MAX and TRI-X negatives of similar density.

MATERIALS SCIENCES

PLATFORM PRESENTATION — SPRING 1991

SLOW SCAN CCD IMAGING SYSTEMS AND THEIR APPLICATIONS IN ELECTRON MICROSCOPY. O.L.KRIVANEK, G.Y.FAN, P.E. MOONEY, K.V.TRUONG, M.L.LEBER, Gatan R&D, 6678 Owens Drive, Pleasanton, CA 94588

Slow Scan CCD (SSC) imaging systems for transmission electron microscopes (TEM) are now commercially available with a variety of possible configurations. Due to their large dynamic range, superior linear response, excellent sensitivity and resolution, and the convenience of producing high-quality images without the dark room process, these systems promise to completely replace the photographic film currently used in electron microscopy. At the present, SSCs based on three astronomy-grade CCD chips (576x384, 512x512 and 1024x1024 pixels) are available, and they can be mounted either on the bottom, in front of an energy loss spectrumeter, or in the 35-mm camera port of a TEM. The SSC signal is presently digitized to 12-bits, and is typically calibrated so that one digital count corresponds to two primary electrons. Electronic noise is less than one digital count r.m.s., giving a detection threshold close to a single electron and a dynamic range of 4000. The non-linearity is about 1% from zero dose to saturation, making it easy to extend the dynamic range further by combining several images taken with different exposures. The software supporting the SSC can control most electron microscopes currently in production, and is capable of computing and displaying two 256x256 FFTs per second, and of fully on-line control and alignment of the microscopes. The unique ability of the SSC makes it highly desirable in many fields which use image data quantitatively, such as in quantitative electron diffraction, electron holography, 3D reconstruction, matching simulated high resolution images and low-dose imaging. The SSC also promises to be highly useful as the front end to computerized image archiving systems.

HIGH RESOLUTION ELECTRON MICROSCOPY AT THE UNIVERSITY OF NORTH TEXAS, R.F.Pinizzotto, J.Villalobos and H.Yang, University of North Texas, Center for Materials Characterization, P.O.Box 5308, Denton, TX 76203-5308.

The University of North Texas has recently installed a Hitachi H-9000 High Resolution Transmission Electron Microscope in the Center for Materials Characterization. This 300 kV instrument has produced lattice images with a resolution of 0.1 nm and structure images with 0.17 nm point-to-point resolution. A Gatan video imaging system is interfaced with an Imaging Technology Image Processor, a Mercury Array Processor and two Apollo computer workstations for digital image acquisition and processing. The NUMIS software package developed at Northwestern University is used for instrument alignment and multi-slice image calculations, and SEMPER VI is used for additional electron microscopy specific data processing.

Current applications of HREM at UNT include the study of the development of MnS and carbide particles in steam turbine rotor steels, the morphology of thin films used as optical coatings, the formation of buried oxide silicon-on-insulator, and structure images of organic molecules.

This presentation will include a description of the HREM facility and discussion of typical results. In particular, the methods used to obtain theoretical and experimental structure images of metal-phthalocyanine molecules, which are a particularly good resolution test for the entire HREM system, will be presented.

FORMATION OF BURIED OXIDE LAYERS IN SIMOX. H.YANG and R.F.PINIZZOTTO, Center for Materials Characterization, University of North Texas, P.O.Box 5308, Denton, TX 76203-5308 and F.NAMAVAR and E.CORTESI, Spire Corporation, Patriots Park, Bedford, MA 01730.

In the SIMOX process, the high temperature anneal following oxygen ion implantation is an important step. Parameters such as annealing temperature and time directly affect the quality of the material. Cross-sectional transmission electron microscopy has been performed to study the effect of annealing time on the microstructure of SIMOX, with emphasis on the kinetics of the formation of the buried oxide layer. Oxygen ions were implanted into single crystal Si substrates to a total dose of 4 x $10^{17}/cm^2$ with a current density of 30 $\mu A/cm^2$. Samples were annealed at 1300°C from 0 to 6 hours. Results show that at this sub-stoichiometric dose, the damage introduced by implantation can be completely removed by the high temperature anneal. A defect-free top Si layer was obtained in the sample annealed for 6 hours. By tracking the microstructural development of the buried oxide layer as a function of post-annealing time, a diffusion controlled process, governed by $L = (Dt)^{1/2}$, is found to explain the growth and coarsening of SiO_2 precipitates, and the formation of the buried SiO_2 layer. A model based on classical dendritic growth is proposed to explain the observed morphological development.

USE OF THE TWO-FILM PREPARATION OF THIN FILMS FOR TEM STUDY OF CHEMICAL REACTIONS IN BINARY ALLOYS: II. OBSERVING CROSS-SECTIONS FOR ACTUAL INTERFACES. D.C. Dufner, Electron Microscopy Center, Texas A&M University, College Station TX 77843.

In the previous presentation (TSEM Fall Meeting 1990), a method of preparing thin-film binary metal alloys for TEM study was described. This described method was effective in allowing chemical reactions resulting from interdiffusion to be observed by high-resolution TEM. However, observations of the actual reaction interfaces were not possible with the present configuration. A modification of the two-film preparation will be discussed in this presentation.

Thin film samples were prepared by the two-film method introduced by Shiojiri, et al (J. Crystal Growth 52, 883 (1981)). In the case of the Pt-Sn system, the preparation consisted of the formation of a few hundred nanometers of Pt by vacuum-evaporation onto holey carbon films, thus producing "holey" Pt films. In a separate deposition, Sn films with an average thickness of 20 nm were produced by evaporation at rates of 1.5-3.0 nm/sec onto air-cleaved KBr substrates. The Sn films were wet-stripped and collected on the holey Pt films. This combination of Pt and Sn films formed a solid-solid interface through which interdiffusion of Pt and Sn can take place and allowed for structural and compositional changes to be observed in the TEM.

The two-film couples mounted on TEM grids were embedded and microtomed to form cross-sections. These cross-sections were examined in the JEOL 2010 200 kV TEM to elucidate the nature of the actual Pt-Sn interface.

METALLURGICAL ANALYSES OF TURBINE ROTORS, H. MALLELA, V. GOVINDARAJU, G.E. SEETON, R.F. PINIZZOTTO, *T. THOMPSON, *D.W. BRASWELL and *B.M. HOLMAN, Center for Materials Characterization, University of North Texas, Denton, TX 76203, *Texas Utilities Electric Company, 400 N.Olive Street, L.B. 81, Dallas, TX 75201.

Samples were obtained from the rotors of low pressure steam turbines used for electric power generation at several TUE power plant locations. Metallurgical analyses were performed using optical microscopy, scanning electron microscopy and transmission electron microscopy. Studies of the compositions of these samples were performed using X-ray Energy Dispersive Spectroscopy (XEDS) and ion coupled plasma emission spectroscopy (ICP). The matrix conformed to ASTM A470 Class 7 specifications. However, within the matrix, MnS inclusions of different sizes were observed to occur preferentially near the grain boundaries. Particle size and distribution analyses were performed in an attempt to correlate the inclusion morphology with the temperature and trace impurity profiles along the longitudinal axis of the rotor.

CHARGE-INDUCED SCANNING MICROSCOPE EVALUATION AND TESTING (CISMET), T.J. Aton, S.S. Mahant-Shetti, M.H. Bennett-Lilley, K.A. Joyner, M.G. Harward, C.H. Blanton, Semiconductor Process and Design Center, Texas Instruments Inc., Dallas, TX 75265

We report a novel method of testing semiconductor microstructures using electron beams. We use the charging induced by an ordinary Scanning Electron Microscope (SEM) beam to generate a "voltage contrast" between microstructure elements that are connected to or isolated from a separate region of the microstructure held at fixed potential. The method is simple and fast, requiring only collection of a regular SEM micrograph. It provides proof of isolation between elements with resistances exceeding 2E11 ohms. The technique highlights only those elements that fail the isolation test. It is especially suited to in-process or in-situ testing because it does not require completely fabricated wafers or mechanical probing. It can be applied to a wide range of microstructures, including p-n junctions.

Submicron manufacturing techniques require high isolation over small distances. Conventional electrical testing methods require a large area of wafer space to test only a few values of spacings, are "pass-fail", and provide no information on the number or density of defects. An SEM beam provides an alternate probe that both generates the electrical drive for our test structure and collects the test information. To illustrate, consider a group of islands of conductor located near another conducting element. This second element is held at some fixed potential (ground). If the islands are isolated from the grid (ground), they change gray scale because the deposited charge induces a voltage contrast. Connected islands retain the gray scale of the grid. Using the high spatial resolution of the SEM, the isolation of thousands of nodes can be verified in this immediate, parallel, and graphic fashion. Also, since the accelerating voltage of the electron beam is variable, it is necessary to only switch from low kV to high kV to reverse the charging from positive to negative. In this way, p-n junctions can be tested for leakage with either the p or n region being exposed to the beam.

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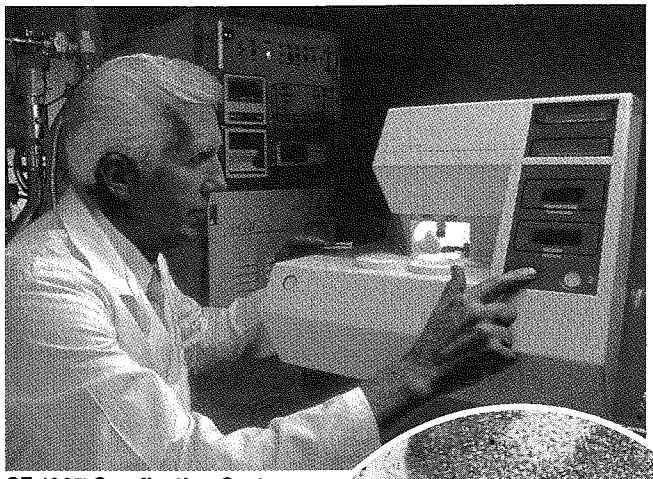

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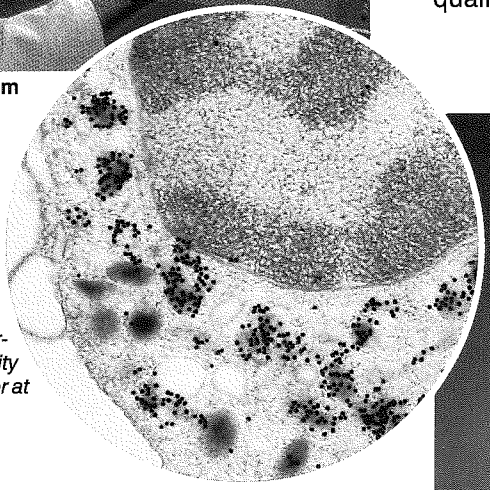

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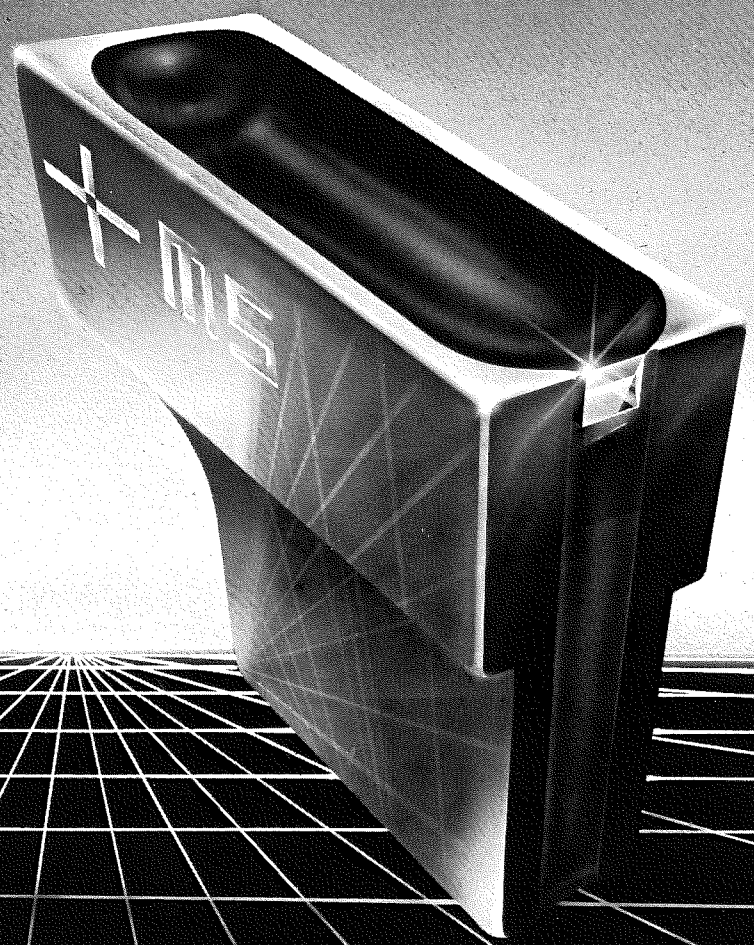

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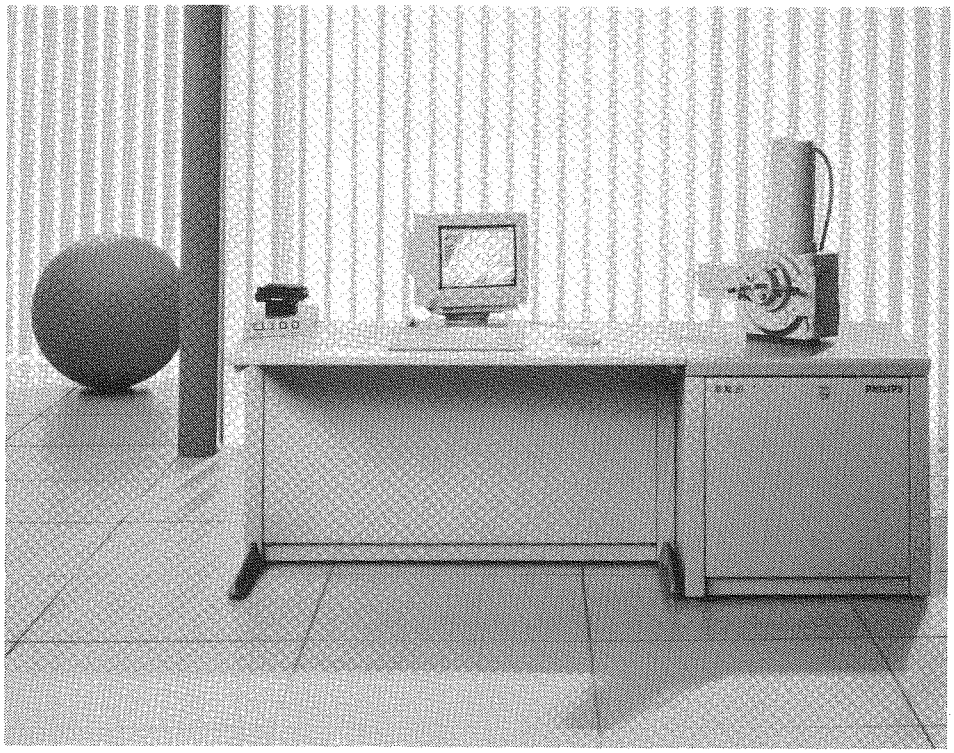

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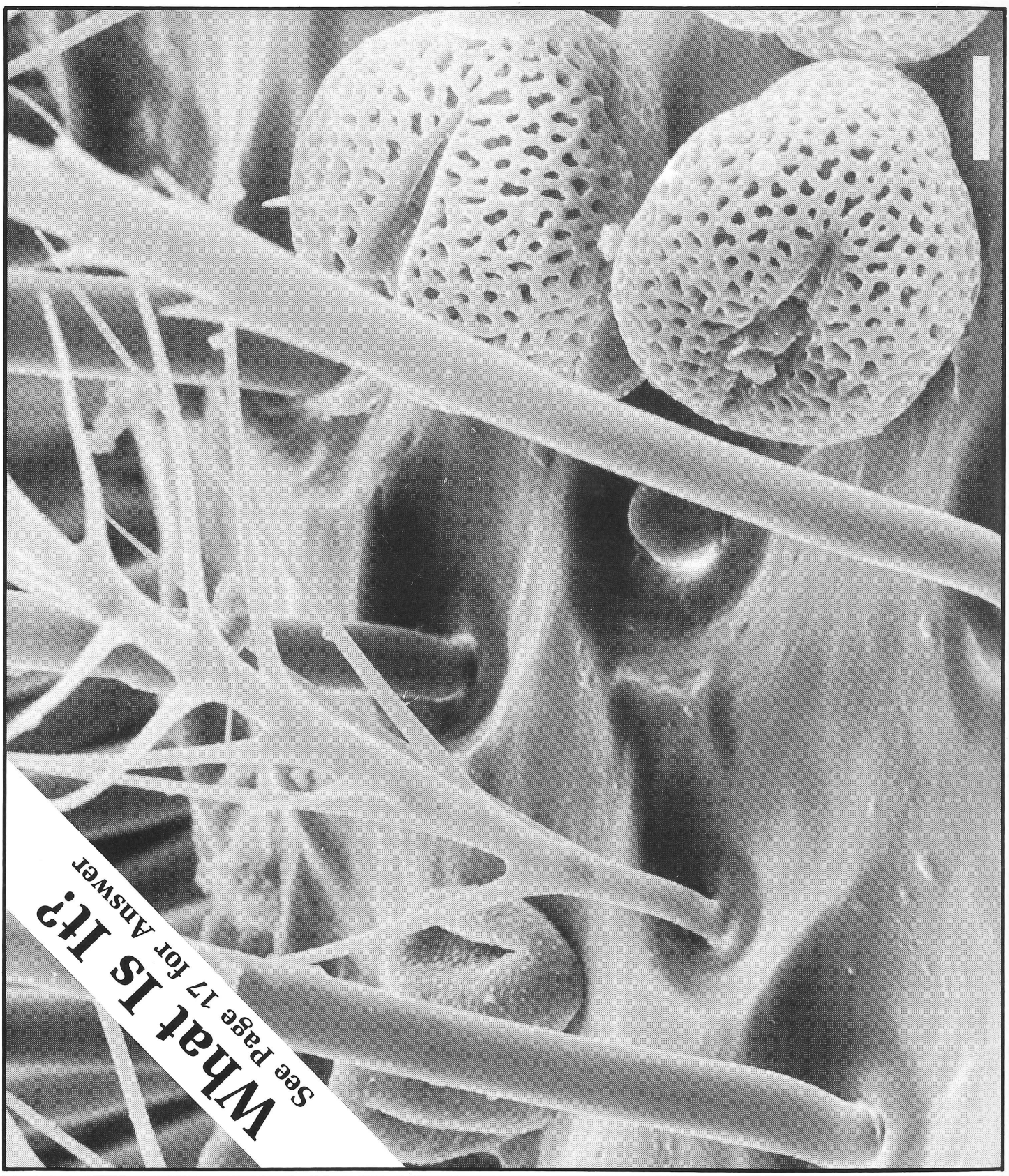

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