TSEM

Texas Journal of Microscopy

Volume 27,
Number 2, 1996
ISSN 0196-5662

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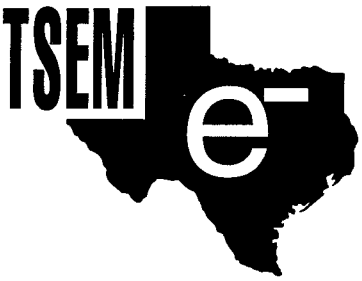

David C. Garrett, Editor
Department of Biological Sciences, University of North Texas, Denton, TX 76203

Official Journal of the Texas Society for Electron Microscopy
"TSEM - Embracing all forms of microscopy."

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ON THE COVER

Sporogonic stage of the protozoan *parasite Hepatozoon canis* isolated from the hemocel of a *Rhipicephalus sanguineus* tick. Arrows indicate junctions of the two plates which constitute the wall of the parasite. Bar=0.5μm. Cover courtesy of Robert E. Droleskey, USDA, ARS Food & Feed Safety Research Unit, College Station, TX 77845

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President's Message

!!Science Funding is in Crisis!!

We have heard this phrase in various contexts for a number of years. Similar to many messages that we hear constantly, the message has lost its impact. Unfortunately, scientists have quit listening either because they have tuned it out or have been beaten down by the all too familiar calls for cut backs in funding, shutting down facilities and unemployment. These situations have moved from the infrequent to the common. Scientists used to be shocked and saddened. We now lower our heads in acknowledgment and tell of other colleagues in the same position.

Reality is that the number of people who practice the art and science of electron microscopy is declining. We have two choices. The first choice is that we can bow our heads and trundle down the road as a defeated army. Alternatively, we can dig in, reach out and convince the powers that be that chemical and molecular composition without morphology does NOT tell the whole story.

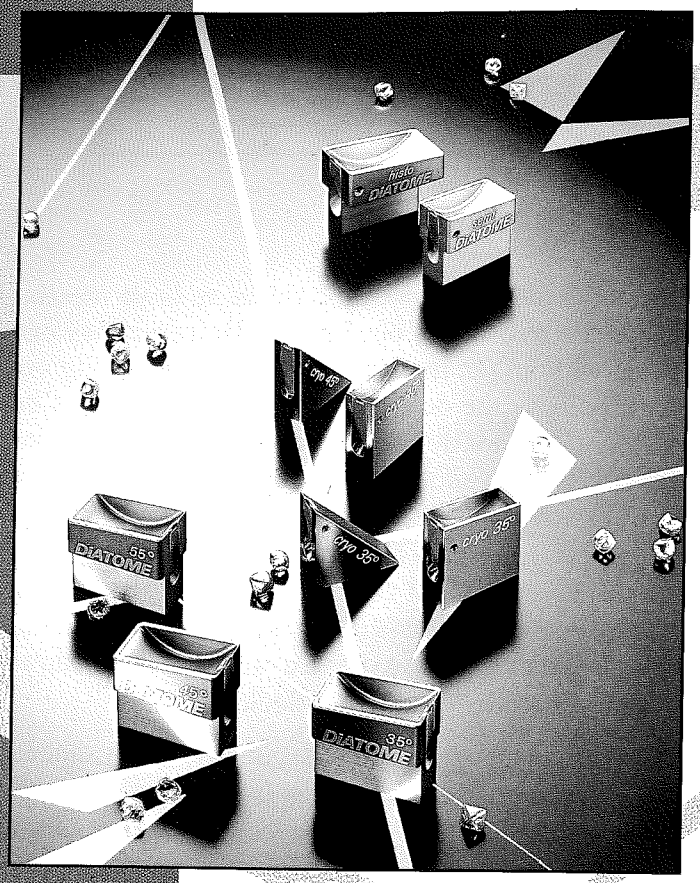
Communication is the key. Communicate with funding agencies and the money controllers within your institutions and tell them how vital your work is. Communicate with your colleagues from other disciplines and show them how morphology and the myriad of other data that microscopists generate will complement and enhance their work. Finally, communicate with the general public. Begin with your neighbors and friends who probably have no idea what you really do. See if the local education facilities know what microscopists contribute to the world. Communication will allow us to continue to practice the art and science that we have devoted a great deal of our lives to.

What has this got to do with the Texas Society for Electron Microscopy? Obviously, I believe it has a lot to do with our Society. Substitute the words, "Texas Society for Electron Microscopy" for Scientific Funding in the title of this column. If we are to survive as a Society, and in the recent past this has been an active topic of discussion, we need to come together and move forward. TSEM is a Society of the members, not just the Executive Council. The Executive Council is elected by the members to make the decisions necessary to make TSEM function. The Executive Council of this Society is a dedicated group of individuals that devote many long hours making it possible for us to have meetings and run the business of our Society. However, they can't do that without the members help. Help is getting your abstracts in by the deadline so that the Program Chair and Journal Editor don't have to start making telephone calls to try to get enough presentations for the meeting. Help is setting an example by having our senior scientists present their work. Help is not only suggesting workshops and speakers but volunteering to give a workshop or be a keynote speaker. Help is attending the meetings in order to share your expertise with your colleagues. Help is being involved in your Society.

I believe that we have scientists in this society that easily rival the expertise in any scientific society in the world. However, we have all become complacent regarding TSEM because of other concerns. The time has arrived for us to lose our complacency. If we don't, in the not too distant future scientists will mention that there USED TO BE a microscopy society in Texas. Accordingly, I challenge all the members of TSEM to come out and support your Society so that when I write my last President's Message I can once again easily speak of a revitalized organization that sees a bright and healthy future.

Sincerely,

Mitchell D. McCartney
President, 1996-97



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Treasurer's Report

TEXAS SOCIETY FOR ELECTRON MICROSCOPY
TREASURER'S REPORT

For Period Ending September 30, 1996

ASSETS ON JANUARY 1, 1996:

Checking Account No. 1882774506	$5,545.28	
Certificate of Deposit No. 1882289323 (Formerly C.D. No. 113515)	$4,079.37	
TOTAL		**$9,624.65**

CHECKING ACCOUNT RECEIPTS:

Dues	$3,421.00	
Spring 1996: Meeting Registration	$1,665.00	
Exhibitors, Donations	$400.00	
Guest	$125.00	
Journal Advertisements 26:2	$500.00	
Journal Advertisements 27:1	$2,375.00	
Checking Account Interest (Account No. 1882774506)	$61.55	
TOTAL		**$8,547.55**
Rollover Interest on Certificate of Deposit No. 1882289323	$205.17	

EXPENSES:

Journal Advertisement: 27:1	$1,890.49	
Office Expenses	$76.55	
Mailouts	$700.00	
New Post Office Box	$58.00	
Plaque	$56.10	
New Student Account	$150.00	
New Sec. Account	$800.00	
President Account	$99.99	
Two Speakers & Travel (Fall '96 Meeting)	$300.00	
Hotel for Speakers	$511.48	
Spring 1996: Student Competition/Travel	$672.00	
Hotel	$1,706.25	
Meeting Miscellaneous & Refreshments	$76.88	
Service Fees & Deposited Item Fee	$10.10	
TOTAL		**$7,107.84**

ASSETS AS OF SEPTEMBER 1, 1996 ..... **$11,269.53**

Certificate of Deposit No. 1882289323	$4,079.37	
Checking Account No. 1882774506	$7,190.16	
TOTAL		**$11,269.53**

Announcing

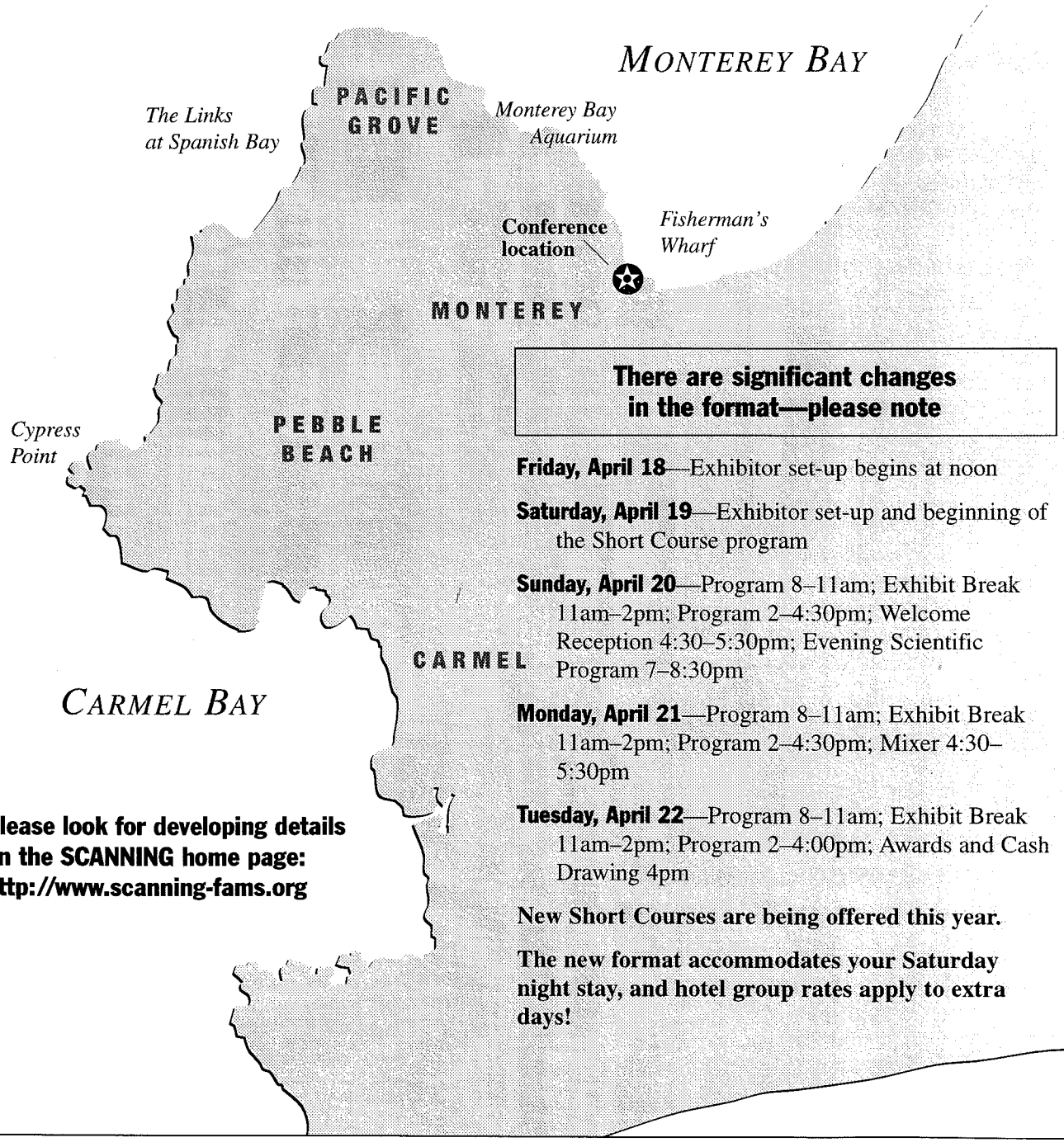
SCANNING 97

April 19–22, 1997

The Foundation for Advances in Medicine and Science, Inc. and *SCANNING, The Journal of Scanning Microscopies* are pleased to announce that the Ninth Annual **SCANNING** meeting will take place in

Monterey, California, USA

at the DoubleTree Hotel at Fisherman's Wharf



There are significant changes in the format—please note

Friday, April 18—Exhibitor set-up begins at noon

Saturday, April 19—Exhibitor set-up and beginning of the Short Course program

Sunday, April 20—Program 8–11am; Exhibit Break 11am–2pm; Program 2–4:30pm; Welcome Reception 4:30–5:30pm; Evening Scientific Program 7–8:30pm

Monday, April 21—Program 8–11am; Exhibit Break 11am–2pm; Program 2–4:30pm; Mixer 4:30–5:30pm

Tuesday, April 22—Program 8–11am; Exhibit Break 11am–2pm; Program 2–4:00pm; Awards and Cash Drawing 4pm

New Short Courses are being offered this year.

The new format accommodates your Saturday night stay, and hotel group rates apply to extra days!

Please look for developing details on the SCANNING home page: http://www.scanning-fams.org

SCANNING 97

April 19-22, 1997 at the DoubleTree Hotel at Fisherman's Wharf, Monterey, California, USA

CALL FOR PAPERS

Abstract Deadline: February 10, 1997

Seefull SCANNING 97 information on the Internet on the SCANNING home page: http://www.scanning-fams.org

The ninth annual SCANNING meeting, sponsored by the Foundation for Advances in Medicine and Science, Inc., and *SCANNING, The Journal of Scanning Microscopies,* will be held Saturday, April 19 through Tuesday, April 22, 1997, at the DoubleTree Hotel at Fisherman's Wharf, Monterey, California, USA. The international conference covers a wide range of topics related to scanning electron microscopy with a forum for the discussion and exchange of information. More that 180 papers will be presented in the areas of confocal microscopy, methodologies and new developments, applications of SEM in forensic science, food structure, cryo-SEM, semi-conductor devices, pharmaceutics and related areas. The program will feature a *new* format accommodating your Saturday night stay, *new* short courses, as well as invited and contributed scientific papers, posters, an extensive exhibit hall showing the most advanced equipment and services available in SEM and related fields, student awards and a $500 cash drawing.

Papers are now being solicited for oral and poster presentation and must be received at SCANNING/FAMS no later than February 10, 1997, via E-mail, mail or fax at the address indicated below. Abstracts will be published in the Proceedings Issue of *SCANNING®, The Journal of Scanning Microscopies*, available at the meeting. In addition, full-length manuscripts may be submitted for peer review to *SCANNING®* for publication in one of the regular issues which appear eight times a year. Presentation for contributed papers will be limited to 20 minutes unless an exception is made by the Program Committee.

For general meeting information and official SCANNING 97 abstract forms, contact:

Mary K. Sullivan at FAMS, Inc.
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Session Topics:

Advances in confocal and related optical microscopies
Applications in marine science
Automated diagnostic microscopy
Biological applications
Biomaterials
Cell surface labelling techniques
Cryo-SEM
Electron/Instrument Interaction Modeling in the SEM
Low Pressure SEM
Food Structure and Functionality
Forensics Applications in SEM
Image analysis, digital processing and stereology
Low-voltage, high-resolution SEM – theory and practice
Materials applications including microstructure of materials
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Abstracts

INVITED SPEAKER

HEALTHY MUSCLE, HEALTHY MICROSCOPY AND TEXAS SOCIETY FOR ELECTRON MICROSCOPY GROWTH. M.A. GOLDSTEIN, Baylor College of Medicine, Department of Medicine, Houston, TX 77030.

Muscle is very important to us. Locomotion is what characterizes animals. Muscles comprise at least half of our body mass, and so it is not surprising that our muscle tone influences our general health. Muscle cells are large, second only to nerve cells in length. They vary in size and shape and composition depending upon the functional demands of the muscle. The pattern of organization of muscle, both at the whole animal level and the cellular level, reflects that we live in a 1 g or 1 gravity environment. Muscle cells are flexible. Muscle cells are adaptable and respond to changes in workload. Muscles vary in their speed of response. Muscle responds not only by increases or decreases in mass but also in the relative composition of the various proteins that make up the muscle cell. Finally, as we learn more about muscle and how it works at the cellular and molecular level, it is possible to train muscles in very specific ways to maximize the functional capacity of the muscle. There is a lot that we do not know about developing muscle or the extent to which the genetic evolution of muscle continues.

Part Two - Healthy Microscopy: Microscopy is and will be in the foreseeable future very important to us. Over half of our brain is devoted to visual processing. The ability to extend our vision using microscopes will continue to be an essential part of global science. We continue to extend the range of vision of microscopes. We continue to harness new kinds of radiation for illumination. There are improvements in lens design and image capture.

Part Three - TSEM Growth: Scientific societies that continue to thrive in our fast paced world of scientific change will be those that are flexible and adaptable, can vary in size and shape, will be highly specialized but at the same time be responsible to multi-purpose situations. The effective metaphor to embrace will be that of seeing a society or organization as a living organism capable of dynamic change in response to different environments and capable of some degree of evolution.

MATERIALS SCIENCES
PLATFORM PRESENTATION—SPRING 1996

TEM CHARACTERIZATION OF MESOSCALE SEMICONDUCTOR STRUCTURES OF QUANTUM-CONFINED CdS ON DNA. Young G. Rho,[1] Yandong Chen,[1] Russell F. Pinizzotto,[1] Shelli R. Bigham,[2] Xin Li,[2] Jeffery L. Coffer,[2] Irma L. Pirtle,[3] Robert M. Pirtle,[3] [1]Materials Science Department, University of North Texas, Denton TX 76203. [2]Department of Chemistry, Texas Christian University, Fort Worth, TX 76129. [3]Department of Biological Sciences, University of North Texas, Denton TX 76203.

Mesoscale semiconductor structures composed of quantum-confined CdS (Q-CdS) nanoparticles were fabricated on polynucleic acids (DNA). pUCLeu4, plasmid and linearized, and ϕX 174 RF II DNA were used as templates to control the overall shape and size. The mesoscale structures were fabricated in three steps. First, Cd^{2+} was mixed with DNA in solution to form DNA/Cd^{2+} complexes. Second, the complex solution was dropped on amorphous carbon films supported by Cu TEM grids. Third, air-dried grids were exposed to H_2S gas to form the desired Q-CdS nanoparticle array. Analytical transmission electron microscopy and high resolution electron microscopy (HREM) were used to characterize the mesoscale structures. Repeated experiments using varying DNA and Cd^{2+} concentrations revealed that Cd^{2+} induces bundling of the DNA structures. However, some isolated DNA/CdS mesoscale structures were also observed. Bright field imaging showed that the size of the DNA/CdS structure was approximately 30% smaller than the size calculated assuming 3.4 Å per basepair. HREM results show that the mesostructures consist of assemblies of Q-CdS nanoparticles with an average diameter on the order of 5 nm. Selected area electron diffraction patterns of the CdS on DNA were consistent with the diamond cubic (Hawleyite) phase. These experimental results demonstrate that mesoscale semiconductor nanostructures of Q-CdS can be fabricated using different DNA sizes and shapes.

TEM AND SEM ANALYSIS OF PARTICLE CONTAMINANTS ON AN INTEGRATED CIRCUIT DEVICE D. Xu, R. F. Pinizzotto, J. A. Sees* and D. Dickson*, Dept. of Materials Science, University of North Texas, Denton, TX 76203. *Texas Instruments Inc., Dallas, TX 75265.

Particle contamination control in the microelectronics industry is a critical manufacturing issue. Even submicrometer sized particles cause yield loss and degrade the integrity of the devices. With its excellent chemical sensitivity and spatial resolution, TEM is an ideal tool for submicrometer particulate contamination analysis. However it is not generally used due to the difficulty of sample preparation. We have developed a technique for contamination analysis using TEM. Particle contamination in integrated circuit (IC) processing chemicals were analysed and the results were presented at the TSEM meeting last Fall. The same technique was recently applied to investigate particle contamination on an integrated circuit (IC) device itself. Device chips were rinsed in ultrapure water, The water was filtered and the particles were collected on a Nuclepore polycarbonate filter. Particles were transferred onto a thin carbon film for TEM analysis. A blank water sample was also prepared to monitor particles in the ultrapure water. The main results are: (1) one type of contaminant consists of clusters of very tiny particles; XEDS shows the main components are Si and /or Al; (2) the second type of contaminant is about 3-10 μm long and 0.4-0.7 μm wide and is composed of light elements. Particles were also examined using SEM, and the results are consistent with the TEM results.

MICROSTRUCTURE AND CHARACTERIZATION OF POROUS SILICON. Yandong Chen[1], Young G. Rho[1], Russell F. Pinizzotto[1], Beata Sweryda-Krawiec[2], and Jeffery L. Coffer[2], [1]Materials Science Department, University of North Texas, Denton, TX 76203. [2]Department of Chemistry, Texas Christian University, Fort Worth, TX 76129.

We have used conventional and high resolution transmission electron microscopy to characterize micropore and mesopore structures of porous Si layers. The porous Si layers are formed by anodic electrochemical etching in HF solution for 30 minutes. After being dried in a stream of nitrogen, two pieces of anodized Si were glued together face-to-face to protect and strengthen the porous layers. The samples were prepared by a mechanical polishing technique using a tripod polisher to prevent chemical contamination and disclose the original structure of the porous layers and interfaces. Structural characterization was carried out using conventional bright field and dark field imaging, and high resolution lattice imaging. This is the first time that the structure entire porous Si layer was seen clearly, from the porous Si/Si interface to the surface. The thickness of the porous Si layers ranges from 2 to 5 μm. Rough interfaces have been observed. Using electron diffraction, the outmost layers were found to be amorphous while the material near the interface is polycrystalline. We believe these results will be useful in understanding the mechanisms of the photoluminescence of porous Si.

THE ROLE OF MICROSCOPY IN IMPROVING THE SUPERCONDUCTING PROPERTIES OF THE MATERIALS. M. K. MIRONOVA, Texas Center for Superconductivity, University of Houston, Houston, Texas 77204 -5932.

The discovery of High Temperature Superconductors (HTS) in several types of ceramic materials have been considered one of the most exciting developments in modern physics, with promising technological applications. In this talk, the brief description of the phenomenon of superconductivity and its characteristics such as the critical temperature T_c, the critical magnetic field H_c and the critical current density J_c will be given, as well as the steps taken to improve HTS properties. One of these steps, the melt-texturing process, is extremely important for achieving the high J_c values in the applied magnetic field. Potential and current application areas of the HTS will be presented. The role of microscopy in improving the superconducting properties of the HTS is discussed using $YBa_2Cu_3O_{7-x}$ (Y-123) superconductor, the most studied material in the HTS family.

For practical applications, melt-textured Y-123 superconductor has to carry currents up to 10^6 A/cm^2 in magnetic fields up to 20 T. This performance can be achieved by introducing high densities of pinning centers into the superconductor. Microstructural features such as twins, dislocations, stacking faults, inclusions and associated defects have been reported to act as pinning sites. In order to determine the effectiveness of these pinning centers, TEM studies of undeformed and deformed melt-textured Y-123 with and without Ag and Y_2BaCuO_5 (Y-211) additions were performed and the computed J_c was correlated with experimental data. According to the obtained results, further enhancement in J_c values can be achieved by a combination of Y_2BaCuO_5 additions and mechanical deformation.

Based on the TEM analysis of the dislocation structure of low-angle grain boundaries in melt-textured Y-123, most of these boundaries are found to be strongly coupled. Short pieces of the boundaries which compensate for large strains between grains are shown to contain several sets of closely spaced dislocations, resulting in a weak link behavior. However, the influence of these pieces on the J_c of the total boundary and thus on the bulk J_c is very small.

MATERIALS SCIENCES
POSTER PRESENTATION—SPRING 1996

JAROSITE AND HEMATITE IN PALAGONITIC TEPHRA FROM HAWAII: MORPHOLOGY AND CHEMICAL COMPOSITION, D. C. GOLDEN[1], D. W. MING[2], AND R. V. MORRIS[2], [1]Dual Inc. and [2]NASA-JSC, Houston, TX

Hematite or jarosite when mixed with unweathered volcanic tephra can simulate the reflectance properties of certain Martian surfaces. Jarosite and hematite are two minerals which are found in some tephra particles on Mauna Kea volcano in Hawaii, thereby making them natural Martian spectral analogs. Hematite occurs as nanometer to micrometer sized particles, and the particle size has an important bearing on the reflectance properties of the mineral. The particle size and mineralogy of nanometer-sized hematite (np-Hm) can best be characterized using high resolution transmission electron microscopy (HRTEM). An example is the HWMK12 basaltic tephra sample which exhibit a well formed palagonitic rind which consists of np-Hm particles. Such a rind can be formed by accumulation of nanometer-sized magnetite particles due to dissolution of the glass matrix and subsequent oxidation of the magnetite to hematite. Jarosite generally occurs as micrometer-sized particles (e.g., HWMK26) can thus be characterized using scanning electron microscopy and electron microprobe. The characteristic morphological differences of jarosite found in different locations of the same basaltic tephra particle may be related to the minor compositional differences of jarosites. Such compositional differences were observed by electron microprobe analysis of the cut and polished surfaces of jarositic tephra particles. Local chemistry of the precipitating solutions can dictate the composition of the precipitating sulfate minerals. The jarosite in these tephra particles have resulted from sulfuric acid attack of the minerals in the tephra particles and not by oxidation of indigenous sulfides as the parent basalt was devoid of sulfide minerals.

MAGNETIC OXIDES IN THE COARSE CLAY FRACTION OF TWO BRAZILIAN SOILS. S. R. TEIXEIRA*, J. B. DIXON and G. N. WHITE, Soil and Crop Sciences Dept., Texas A & M University, College Station, TX 77843-2474.

The coarse clay fractions of two horizons (B23 and B3/C of soils, field classified as Oxisol and Alfisol, respectively) from Presidente Prudente county, São Paulo State, Brazil were examined by x-ray diffraction (XRD) and transmission electron microscopy (TEM) to determine the mineral composition and morphology. High gradient magnetic separation (HGMS) was used to concentrate the magnetic fraction in the coarse clay of sample B3/C. Although both coarse clay fractions have similar mineral suites their concentrations are much different. The Oxisol is very kaolinitic with low concentrations of vermiculite (present only in sample B23), quartz, anatase, hematite, rutile, maghemite, zircon, ilmenite and feldspar. The relative x-ray diffraction peak intensities of the Alfisol sample suggest that anatase and quartz are more concentrated than mica, kaolinite, gibbsite (present only in sample B3/C), hematite, rutile, zircon and maghemite observed in it. Magnetite is a possibility but the setting suggests maghemite. In both samples, hematite and anatase are the most abundant iron and titanium oxides detected by XRD. The morphology of the grains and lattice fringes, observed in photomicrographs confirm the presence of titanium and iron oxides in these samples. Lepidocrocite is confirmed by lattice fringes (~0.63 nm) and by morphology (laths) in several pictures of sample B23. Goethite with lath-like morphology and (010) lattice fringes at ~1.0 nm also was observed. Relative XRD peak intensities suggest that anatase, hematite and quartz (with adsorbed magnetic material or trapped by steel wool) are concentrated in the magnetic fraction, while mica, kaolinite and vermiculite are concentrated in the non-magnetic fraction. The sand and silt fractions of both soil samples consist essentially of quartz. Small amounts of titanium oxides (anatase, rutile and ilmenite), iron oxides (hematite, maghemite and goethite), zircon and feldspar also were detected by XRD and/or TEM.

* S. R. Teixeira is Assistant Professor on leave from Universidade Estadual Paulista - UNESP, Presidente Prudente, São Paulo State, Brazil, and acknowledges the financial support by MEC-CAPES (Grant 0113/95-5).

BIOLOGICAL SCIENCES
PLATFORM PRESENTATION—SPRING 1996

FLOCCULATION BEHAVIOR OF ORGANIC POLYMER - SMECTITE CLAY MIXTURES OBSERVED BY TRANSMISSION ELECTRON MICROSCOPY. J. Y. HWANG* and J. B. DIXON, Soil and Crop Sciences Dept., Texas A&M University, College Station, TX 77843-2474.

Three organic polymers which have been used as flocculants and aggregating agents were mixed with Na-montmorillonite from Wyoming (Volclay, American Colloid Co.). The polymers are the high molecular weight cationic polyacrylamide (494C: 5,000,000 g/mol), anionic polyacrylamide (836A: 15,000,000 g/mol), and moderately low molecular weight (587C: 100,000 g/mol) cationic polymer, which are marketed by CYTEC. The 400 ml suspended solutions consisted of polymer concentration of 200 mg/l and clay of 2g/l were prepared. The solutions were stirred for an hour, and allowed to stand for 24 hours. During the standing period floc formation and settling were observed. After that the solutions were centrifuged for 30 min. at 5,000 rpm. The supernatant was decanted, the clay plugs were washed 2 times with distilled water. Some clay plugs were dried in an oven at 50 °C. Dried and undried samples were investigated by TEM and XRD to determine morphology and d-spacing. Polymer-free clay was included for comparison.

The suspended clays containing cationic polymers flocculated rapidly as soon as they were stirred. The sizes of flocs formed by high molecular weight cationic polymer were much larger than those of moderately low molecular weight. Yet the suspension containing the anionic polymer was not flocculated until after 24 hours. Polymer-free clay was mostly well dispersed thin particles and rarely contained aggregates. Cationic polymer-clay mixtures that were not dried prior to mounting for TEM observation formed large thin sheets 8X25μm and smaller thin particles to 1μm across. Clay treated with cationic polymers and dried and ground to mount for TEM observation were composed of thick (ca. 1μm) aggregates that the 200kV electron beam could not penetrate. The smectite treated with anionic polymer contained relatively few aggregates. Lattice fringes of the polymer-treated smectite were almost all near 1 nm indicating that the polymers were mostly excluded from interlayer space. There were a few 1.1 to 1.2 nm spacings suggesting polymers may have entered some interlayer spaces. XRD data indicated full expansion from 15Å air-dry to ca. 20Å for glycerated smectite. Cationic polymer treatment and grinding produced dense clay aggregates often 1μm across.

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INVESTIGATION OF WATER BEADING ON THE ADAXIAL LEAF SURFACE OF *NELUMBO NUCIFERA*. C. SCHWARTZ AND H. J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

The lotus, *Nelumbo nucifera*, is a waterplant closely related to the water lilies in the family Nymphaceae. The adaxial or upper surface of lotus leaves are constructed in such a manner that water beads and rolls off the surface. As water hits the leaf it forms a bead that is almost spherical, when the beads come together they form even larger spherical beads which generally roll off the leaf. In fact the water behaves almost like quicksilver. This phenomenon does not occur on the abaxial or lower surface of lotus leaves, or on the leaf surfaces of other water lilies such as *Nymphaea mexicana*. Because the interaction of water between the adaxial and abaxial surfaces of lotus leaves is different, these leaves offer a unique chance to study what structural entities are involved in water beading. Light and SEM observations show that the upper epidermis is covered with cells that have short cone like extensions. Technically, each cell would be classified as a trichrome. Similar cells are not found on any other part of the plant. The points formed by these trichrome cones are found to average 15.6 mμ from one another. These cells have a thin cuticle. However, many leaves with a cuticle like that of lotus do not show the beading of water. Currently, we believe beading of water takes place because of a combination of the trichrome shape, the trichrome distribution pattern and the presence of a thin layer of cutin on the surface of these cells. We have treated the surface with acetone and can eliminate the beading of water. However, we do not yet know exactly what effects the acetone has on the surface of the leaf.

A COMPARISON OF DIGITAL AND PHOTOGRAPHIC PROCESSING TECHNIQUES IN DATA PRESENTATION. M. DAVIS, M. JOHNSON, AND H. J. ARNOTT. Department of Biology and Center for Electron Microscopy, University of Texas at Arlington, Arlington, TX, 76019.

The utilities of digital processing techniques are compared with standard photographic preparations in the presentation of light and electron microscopic data. Digital and photographic imaging are analyzed in terms of output quality, ease of manipulation, methods of acquisition, and cost. Issues concerning the advantages and disadvantages of digital versus photographic images are addressed. Methods by which to maximize quality of images as well as enhancement and quantitative analysis will also be presented. Commonly available commercially available hardware and software is used in the preparation of this paper to display costs and benefits of digital imaging utilizing various means. Note: for purposes of this meeting both a 35mm slide presentation and a poster are presented to compare digital and standard photographic techniques.

IN VITRO REPRODUCTION OF *BABESIA* ISOLATES FROM NORTH AMERICAN WILD RUMINANTS. R. E. Droleskey[1], P. J. Holman[2], K. A. Waldrup[1], W. L. Goff[3], L. H. Stanker[1] and G. G. Wagner[2]. [1]USDA, ARS, Food & Feed Safety Research Unit, 2881 F&B Road, College Station, TX 77845; Dept. [2]Veterinary Pathobiology, Texas A&M University, College Station, TX 77845. [3]USDA/ARS, Animal Disease Research Unit, Pullman, WA 99164 USA

Reproduction by the hemoprotozoan parasite *Babesia* generally has been noted to result in the creation of two paired parasites (merozoites) within parasitized erythrocytes of domestic animals. However, recent isolates of *Babesia* from North American wild ruminants produce multiple parasites within a single erythrocyte when cultured in vitro. This observation indicates that reproduction for these isolates may differ from methods previously described for *Babesia spp.* of domestic animals. Accordingly, transmission electron microscopy was used to study the intra-erythrocytic reproduction of cultured *Babesia* isolated from bighorn sheep, elk, caribou, and white-tailed deer. Intra-erythrocytic reproduction appeared to commence from several stages in the life cycle of the parasite. After an initial round of division into two new daughter cells, multiple interconnected parasites could be produced by three distinct pathways. The first resembled budding sensu stricto, i.e. retention of nuclear material by the parent cell for incorporation into daughter cells during subsequent rounds of division. The second involved direct division from paired merozoites and the third was division from paired parasites of apparent trophozoite morphology.

A DEMONSTRATION OF TORSION IN THE RAYS OF *ASTREUS HYGROMETRICUS*. J. M. ULSES AND H. J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

The rays of *Astreus hygrometricus* are involved in the mechanism through which spores are liberated. A hygroscopic mechanism involves the opening and closing of the rays and has been studied by Huffine and Arnott (1993a, b; Tex. Soc. Elec. Micro. J: 24,25; Inoculum 42:41). They showed that the process of sporocarp opening and closing is cyclic and repeatable. They also showed that the bending of the exoperidium (rays) depends on its structure and the presence (or absence) of water (rain in nature). In this study we confirmed that there are four layers in the rays of *Astreus*, although in other earth stars (*Geastrum* sp.) there may be fewer. In *A. hygrometricus* there is a thin mycelial layer on the adaxial surface but this soon wears off. Below that there is a thick fibrous layer, a thin pseudoparenchymatous layer and finally the most abaxial layer that becomes thin and cracked on drying. In this study we demonstrate that the two central layers possess a torsional mechanism which is involved in the opening and closing of the rays. Evidence for this was found using a freezing microtome to cut thin sections of the rays in the transverse and longisectional planes. When the transversely cut ray sections are placed in water they become tightly coiled. However, when rays are cut in longitudinal section the individual sections take on a helical shape. Both types of sections retain their original shape for several days. Clearly, the torsional forces extant in the rays are relieved uniquely; the differences indicate that the long and short axes of the rays are under differential torsion thus when wetted .

The simple " bimetallic strip" explanation of ray bending may have to be abandoned or at least modified.

QUANTITATIVE DIFFERENCES IN GUARD HAIR OF THE WHITE-TAILED DEER *ODOCOILEUS VIRGINIANUS*. C. BOYLES AND H. J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019

The morphologic differences in hair among animal groups is becoming increasingly useful to many areas of science. Macroscopic (length, color, texture) and microscopic (scale patterns, medullary characteristics) features, when analyzed and compared are used extensively in Forensics, Wildlife Management and Taxonomy. However, while much attention has been given to comparisons among animal groups, very little has been addressed to the differences among body areas in a single animal or species. This may be a critical oversight considering the limited information gathered is used as the groundwork for keys and atlases which form the basis for many criminal and Wild Game Management decisions, as well as Taxonomic conclusions. Using hairs taken from different body sites on white-tailed deer, a number of parameters, mainly cuticular and medullary characteristics are compared. Some information, such as the ratio of medullary width to cross-sectional width, supports previously published data. However, the presence of a unique medullary pattern and of some differences in cuticular scale types between dorsal and ventral surfaces and along hair lengths are new observations. This and further research may help to determine if more thorough hair sampling may prove essential and provide information for future studies.

A COMPARISON OF MODERN AVIAN AND FOSSILIZED AVIAN EGGSHELL MICROSTRUCTURE. S.L. WESTMORELAND AND H.J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, University of Texas at Arlington, Arlington, TX 76019.

The modern avian eggshell has a stable multilayer microstructure which has been shown through scanning electron microscope examination to be consistent in all bird orders studied. In this study 22 eggshell samples were examined representing 22 bird species, each of a different avian order. The oldest sample was collected in 1886. Electron micrographs confirm the rigid, calcareous eggshell of modern birds to be composed of four layers: the outermost cuticle layer, composed of organic matter with small calcite crystals embedded in it, the columnar layer, composed of carbonate in the form of calcite crystals perpendicular to the surface, the mammillary layer, composed of numerous conical knobs or mammillae made of noncrystalline minerals, and the inner and outer shell membranes, each a dense fibrous mat of keratin protein. This study supports the earlier findings of Pooley, 1979 (Scanning Electron Microscopy, II: 475-482). A review of scanning electron micrographs of avian fossil eggshells from the study of Hirsch and Packard, 1987 (Scanning Electron Microscopy, I: 383-400) shows a similar microstructure to those of modern birds. The calcified layers of eggshell (columnar and mammillary layers) are preserved in the fossil record, while the organic cuticle and proteinacious membranes are not. Micrographs of fossilized shell identified as "fossil bird, Upper Cretaceous, Mongolia" reveal a mammillary layer with mammillae. The micrographs of fossilized shell identified as "fossil bird, Upper Cretaceous, Montana" also show modern avian-like mammillae. Micrographs of "fossil bird, Eocene, Wyoming" show a column-like layer and mammillary-like layer as the shell is viewed in free-standing cross section.

THE EFFECTS OF RPE CONDITIONED MEDIUM ANTISERUM ON THE DEVELOPING RAT RETINA. T.H. NELSON, H. SHEEDLO , J. TURNER, Dept. of Anatomy and Cell Biology, University of North Texas Health Science Center and The North Texas Eye Research Institute, Fort Worth, TX 76107.

The effects of an antiserum (RPE-SP) against retinal pigment epithilial cell conditioned medium (RPE-CM) on the developing rat retina were investigated by injecting the antiserum and control pre-immune serum into the vitreous of 7 day postnatal rat eyes. The RPE-SP antiserum, recognizes a 67kD protein in both rat and human RPE-CM. This protein has been named RPE-derived retina trophic factor (RPE-RTF). Eyes were examined 7 days after injection at postnatal day 14. The thickness of the inner/outer segments, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer and total retina was measured using light microscopy. Antiserum effects were examined using light and transmission electron microscopy.

A significant decrease in the thickness of the photoreceptor outer and inner segment layer was observed in both the superior and inferior retinal quadrants. In contrast as photoreceptor cells develop in sham injected retinas, the outer and inner segments (IS/OS) continue to elongate. Therefore, RPE-SP antiserum appears to significantly inhibit the development of photoreceptor cells as indicated by reduced IS/OS. Retina development occurs differentially from the central to peripheral areas, with the central region developing earliest and the peripheral retina latest. Therefore, the peripheral retina should be most severly affected upon antiserum administration. In the peripheral retina, antiserum treatment causes a significant decrease in thickness of five of the six layers as predicted as well as a significant decrease in thicknoss of the total retina. Progression from peripheral to central retina indicates that the thinning effect was decreased.

In summary, RPE-SP antiserum injected into the vitreous of 7 day rats prevented further development of the photoreceptor cells and decreased the thickness of the total retina. The protein recognized by the RPE-SP antiserum, RPE-RTF, appears to play a vital role in retinal development .

HISTOLOGICAL AND SCANNING ELECTRON MICROSCOPIC STUDIES ON *IN VITRO* SOMATIC EMBRYOGENESIS OF *ALBIZIA LEBBECK* BENTH.

Nabarun Ghosh[1], A. Chatterjee[2] and Don W. Smith[1]
[1]Department of Biological Sciences, University of North Texas, Denton, TX 76203. [2]CAS, Department of Botany, University of Calcutta, India.

This abstract reports the first observation of somatic embryogenesis of *Albizia lebbeck* Benth.. We used leaflets of young seedlings as explants for initiating *in vitro* culture. The leaf explants responded differently to combinations and concentrations of growth hormones and growth factors. On MS media modified with 6-BAP (6 mg/l), NAA (0.02 mg/l), PVP (0.5%) and coconut milk (5%,v/v) the explants produced proembryoids, other hormonal combinations produced various callus forms. We prepared proembryoids, the regenerative and non-regenerative calli for SEM study following standard techniques: 1. fixation of calli in 4% gluteraldehyde in 0.1 M phosphate buffer, 2. post fixation in osmium-tetroxide(OsO_4), 3. dehydration in ascending concentration of ethanol and finally in isoamyl-acetate, 4. critical point drying, 5. gold-coating. On subculturing on fresh media the proembryoids developed cotyledons. They developed shoots above the surface of the medium and roots below being attached to the mother explant. We cut sections of the developing structures, dehydrated with ascending grades of alcohol and observed under compound microscope after double staining with safranin and light green. We took the photographs under dissecting and compound microscopes with necessary attachments to record the different stages of embryogenesis and plant development.

ELECTRON MICROSCOPY: IS IT A SCIENTIFIC ACTIVITY OR A CULT MOVEMENT? H. J. ARNOTT, Dept. of Biology and Center for Electron Microscopy, The Univ. of Texas at Arlington, Arlington Texas 76019.

A cult is defined as a group with "great devotion to some person, idea, or thing." The cult definition is applicable, at least in some minds, to the practice of electron microscopy. EM practitioners sometimes believe that electron microscopy will bring the final answer(s) to scientific questions. Their critics are quick to label biological electron microscopy as an "art" not a science. An intellectual fad, replete with cult heros. As a case in point, such antagonists are quick to question the process of plant and animal tissue fixation. They point out that no single fixation technique satisfies all "electron microscopists." Diversity is the key word in EM fixation, and they note the following kinds of differences: the type of fixative used, its concentration, pH, molarity, the length and temperature of fixation, the dimensions of the tissues being fixed, pre- or post-fixation treatments, the schedules used for washing, dehydration and in embedding tissues. Unfortunately, the scenarios for fixation almost universally vary and in many cases the details of the fixation scenario are not given. To such criticism, some EM cult members respond that, "minor details in fixation are not important as they probably do not lead to alternative views of structure."

Repeatability is the heart of science. Repeatability of fixation has always been and still is a major problem for EM scientists. Fortunately, an alternative trend seems to be developing in the use of microwave fixation and embedment. These techniques often seem to be devised with replication in mind. Developments in the EM community, which deal positively with the ***repeatability problem,*** will help tilt the opinions toward the side of science and away from the "cult" or "art" conviction often supported by EM critics.

BIOLOGICAL SCIENCES
POSTER PRESENTATION—SPRING 1996

A COMPARISON OF DIGITAL AND PHOTOGRAPHIC PROCESSING TECHNIQUES IN DATA PRESENTATION. M. DAVIS, M. JOHNSON, AND H. J. ARNOTT. Department of Biology and Center for Electron Microscopy, University of Texas at Arlington, Arlington, TX, 76019.

The utilities of digital processing techniques are compared with standard photographic preparations in the presentation of light and electron microscopic data. Digital and photographic imaging are analyzed in terms of output quality, ease of manipulation, methods of acquisition, and cost. Issues concerning the advantages and disadvantages of digital versus photographic images are addressed. Methods by which to maximize quality of images as well as enhancement and quantitative analysis will also be presented. Commonly available commercially available hardware and software is used in the preparation of this paper to display costs and benefits of digital imaging utilizing various means. Note: for purposes of this meeting both a 35mm slide presentation and a poster are presented to compare digital and standard photographic techniques.

THE ULTRASTRUCTURE OF *CORYNEBACTERIUM PSEUDODIPHTHERITICUM* AFTER EXPOSURE TO SUB-MICS OF AMPICILLIN AND TETRACYCLINE. M.R. TRAHAN, S.W. JACKSON, AND A.E. RUSHING, Dept. Biology, Baylor University, Waco, TX 76798.

Sub-minimal inhibitory concentrations (sub-MICs) of antibiotics have been reported to cause morphological and ultrastructural alterations in bacteria. *Corynebacterium pseudodiphtheriticum,* a normal member of the oropharyngeal flora that recently has been recognized as a respiratory pathogen, was examined by transmission electron microscopy after exposure to sub-MICs of either ampicillin or tetracycline. Cells were grown directly on membrane filters placed on brain heart infusion agar for 36 hours until mid-log phase, and then transferred to agar containing one-fourth the minimal inhibitory concentration (MIC) of either ampicillin or tetracycline for 48 hours. Cells were examined immediately after exposure to each antibiotic and after membranes had been placed onto drug-free agar for a 36 hour recovery period. Both ampicillin- and tetracycline-exposed cells contained multilayered membranous invaginations that appeared to be continuous with the cytoplasmic membrane. Invaginations occasionally were associated with the septa of cells in the process of division; however, some were also located adjacent to remnants of previous cell divisions, and at the poles of the cells. These membranous invaginations, which were absent in untreated control cells, resemble those reported in other bacteria after sub-MIC exposures to selected antibiotics. Cells observed after a recovery time of 36 hours contained similar invaginations although with less frequency. Results of this study provide further evidence that antibiotics in concentrations well below the MIC can cause structural changes in bacterial cells.

A COMPARISON OF ULTRASTRUCTURAL DIFFERENCES BETWEEN NORMAL AND ALBINO WATER OAK (*QUERCUS NIGRA*) LEAVES. S. WILSON AND J. TAYLOR, Department of Biology, Stephen F. Austin State University, P.O. Box 13003 SFA Station, Nacogdoches, Texas 75962.

A water oak germination project yielded four albino seedlings out of a total of 3500, an unusually high incidence of genetic defectiveness (0.001%). Normal and albino leaf tissue was prepared using glutaraldehyde/osmium tetroxide fixation, embedded in Spurr's resin, and viewed under a transmission electron microscope (TEM). Tissues were compared to determine the presence or absence of chloroplasts and other essential organelles. No chloroplasts were detected in the albino tissue. However, disorganized thylakoids as well as organelles that appeared to be etioplasts and starch-containing amyloplasts were observed in the albino cells. All other structural components appeared identical in the two samples.

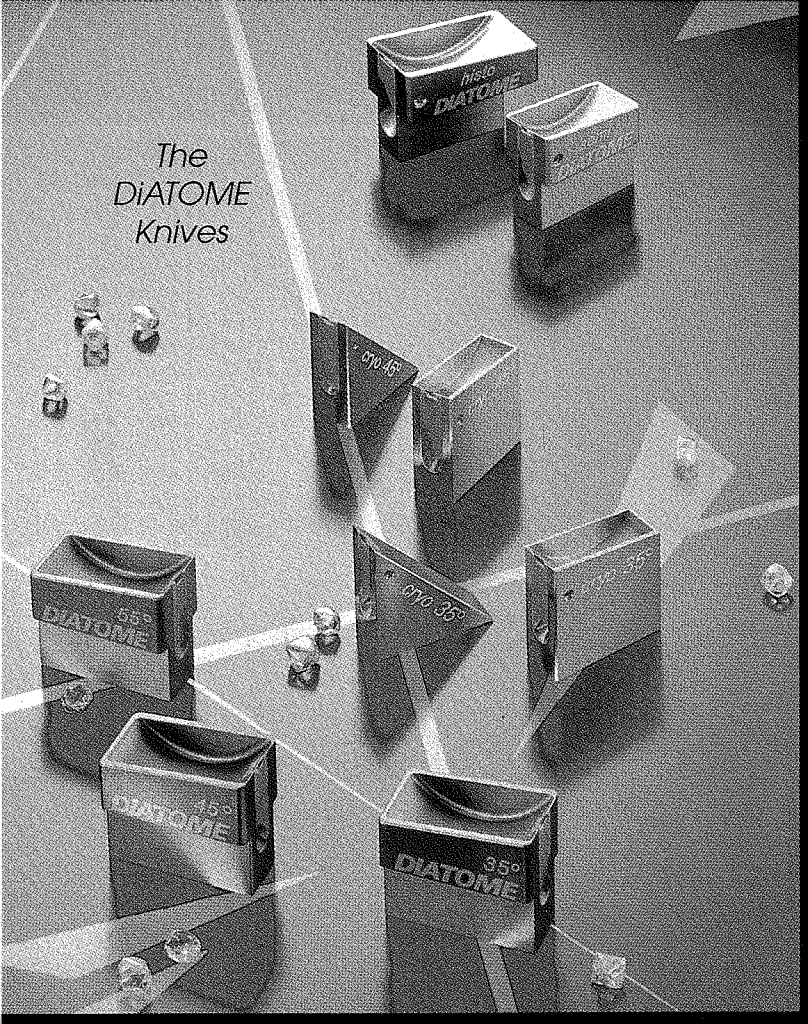
THE PRESENCE OF AN INTERCALATED DISK-LIKE STRUCTURE IN THE HINDGUT MUSCLES OF THE COCKROACH *LEUCOPHAEA MADERAE.* B. J. COOK AND N. W. PRYOR, USDA-ARS-FAPRL, 2881 F&B Road, College Station, TX 77845.

An interfibrillar junction with many ultra-structural features of an intercalated disk was found in the circular muscle of the anterior rectum of the cockroach *Leucophaea maderae.* This junction consisted of a central region with a large amount of electron dense material associated with the I band of the myofibrils, and a zona occludens that extended from the region of the myofilament to the periphery of the cell.

Intercalated disks have been more frequently reported in cardiac muscle. The structures appear to be sites where cell membranes between muscle cells interdigitate extensively. This ensures satisfactory adhesion to prevent separation during contraction and allows exchange of ions through gap junctions for communication between cells.

INFECTION OF ROSE LEAVES BY THE PATHOGENIC FUNGUS *PESTALOTIOPSIS GUEPINII.* V. DHEVAN and J. TAYLOR, Dept. of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

Pestalotiopsis guepinii studies were conducted with *Rosa hybrida,* cv. La Reine. Investigation of the structure and mode of penetration of the fungus was conducted with both scanning and transmission electron microscopy. Fungal germ tubes terminated in penetration structures called appressoria. TEM was used to observe growth of hyphae and ultrastructural changes in host cells. Intracellular penetration of spongy mesophyll cells occurred contradictory to reports that found penetration of epidermal cells only. Intercellular hyphal strands ramified through the mesophyll. Infected cells were highly vacuolate and contained tannin like deposits as a possible defense against the invading fungus. Conidiogenous cells were formed sub-epidermally. This led to rupturing of the epidermal layer and release of mature spores within 7-10 days after inoculation.



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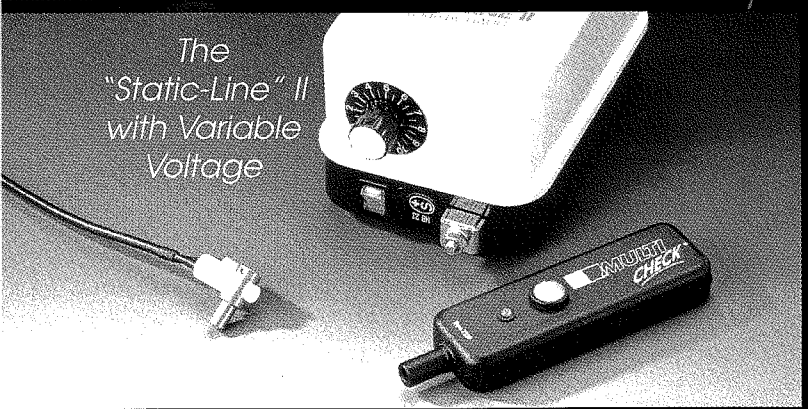

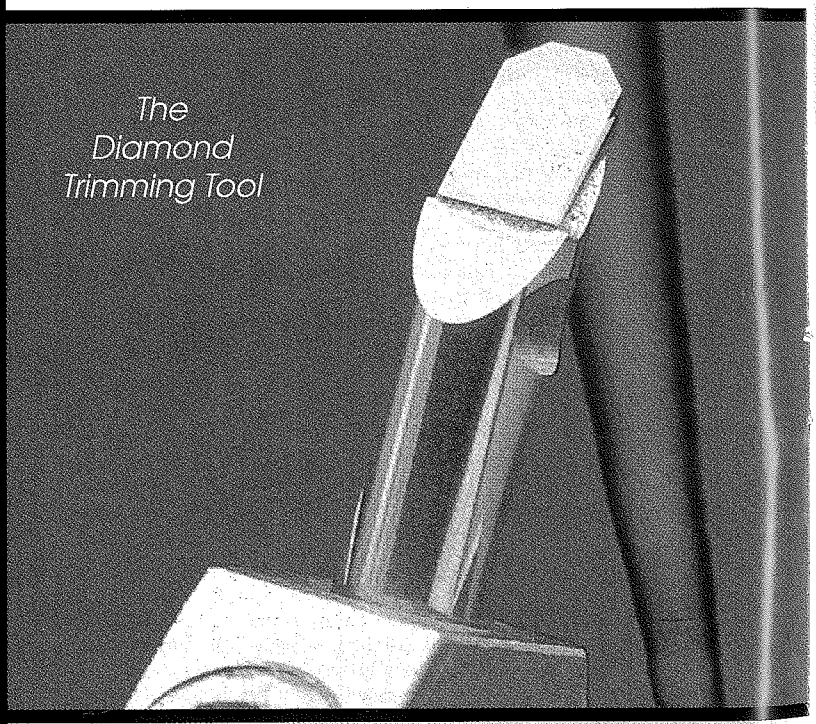

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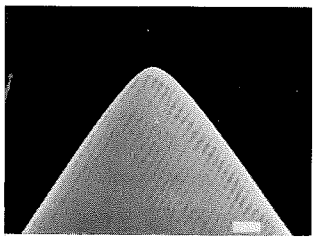

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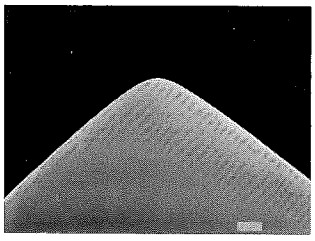

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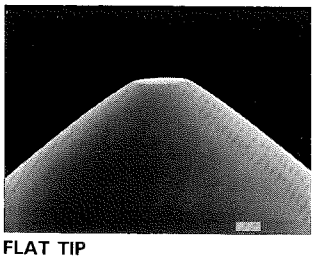

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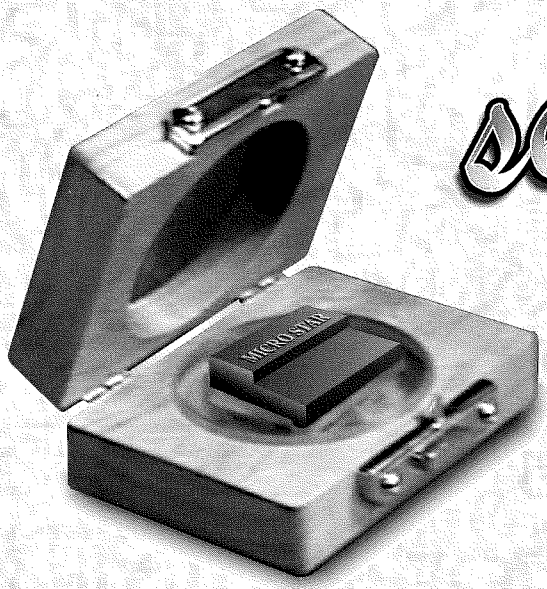

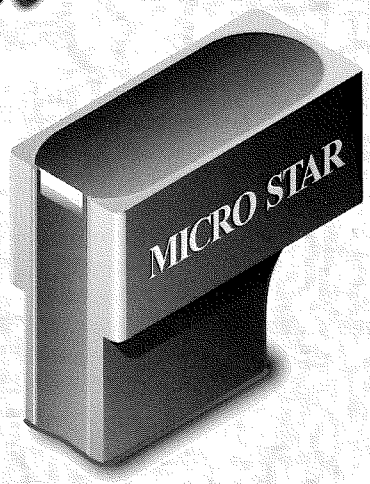

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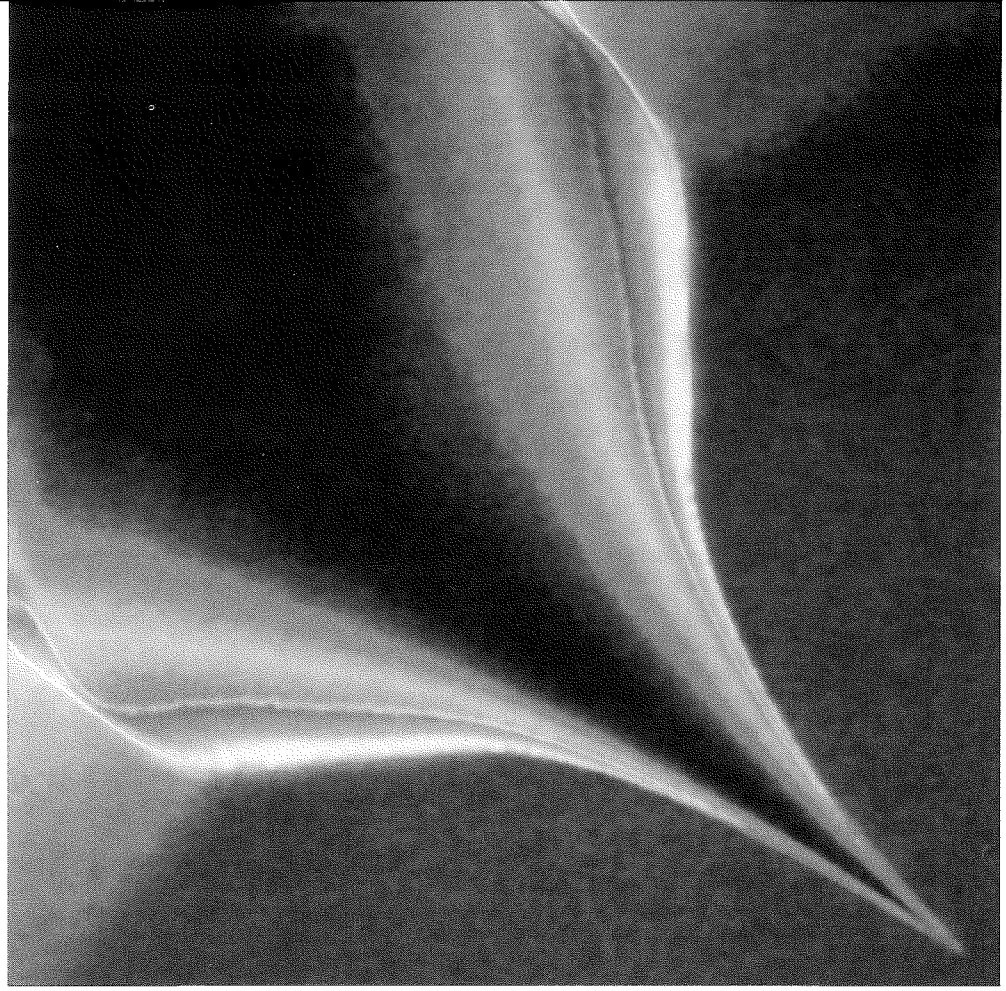

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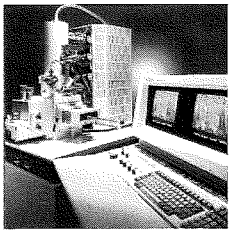

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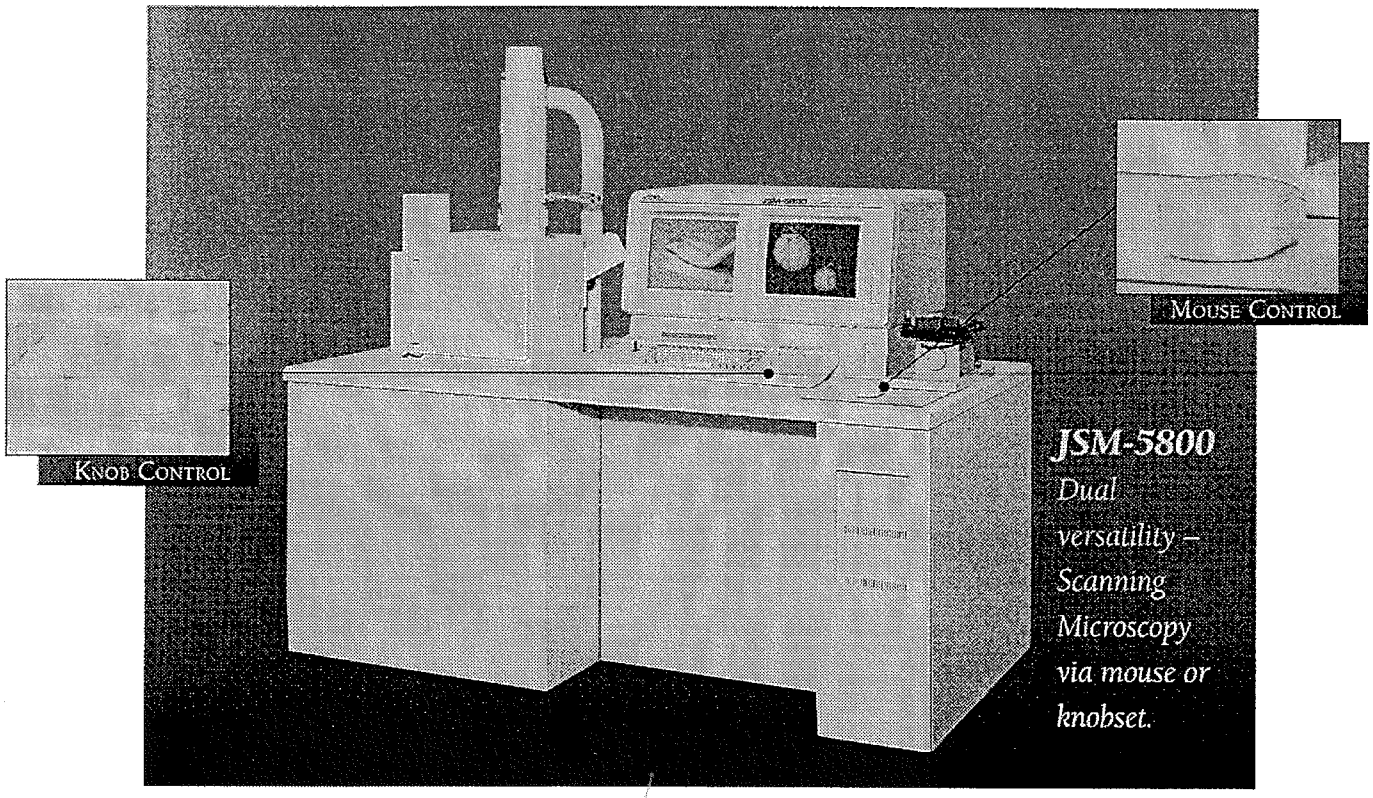

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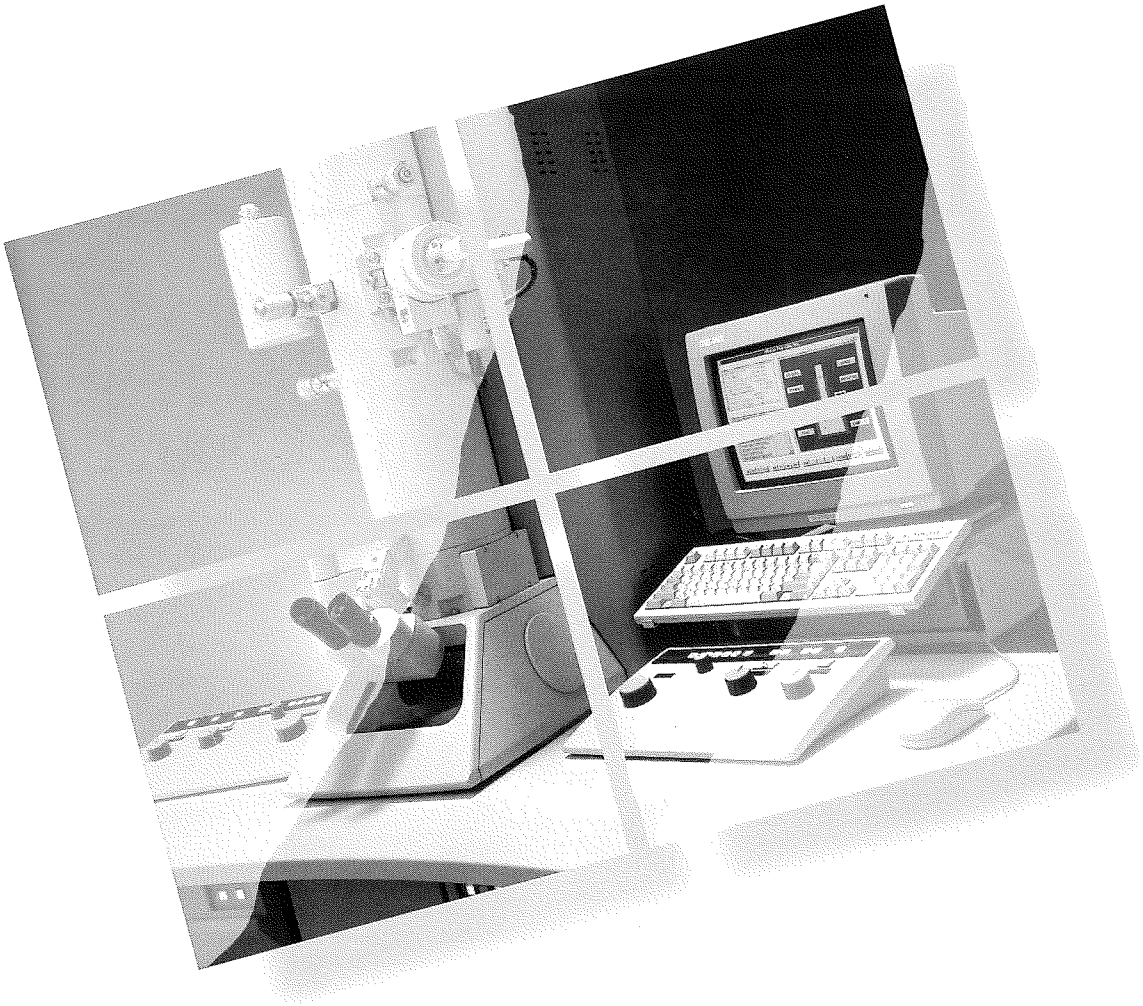

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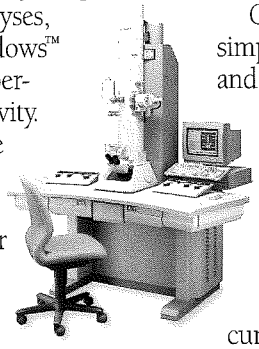

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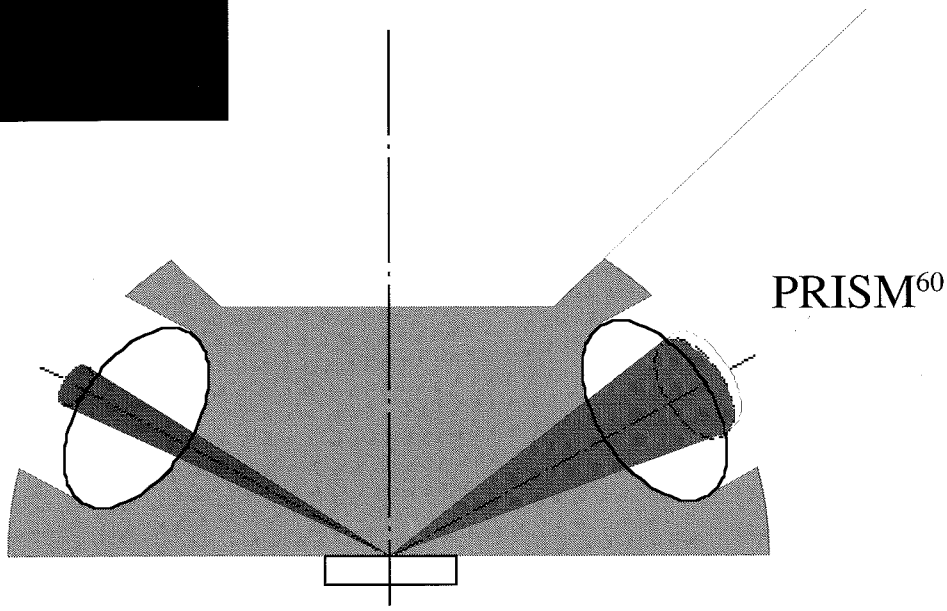

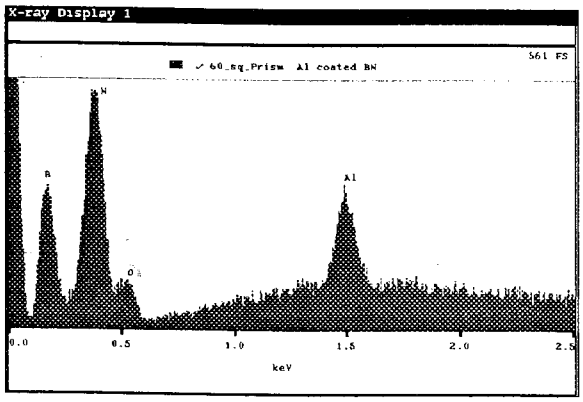

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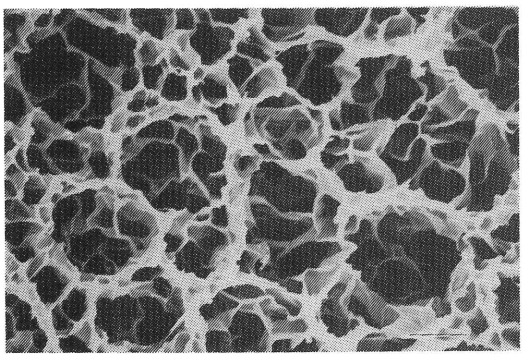

The micrograph published on the back cover of Volume 27, Number 1, 1996, is a SEM view of a co-polymer gel consisting of *N*-isopropylacrylamide (NIPA) and Poly-acrylamide (PAAM). The gel was frozen in liquid nitrogen, fractured, freeze-dried and the gold coated before viewing in the SEM. This micrograph is part of a SEM study for optimizing synthesis conditions of polymer gel networks. Gels with better mechanical properties can be used as soft actuators, controlled drug release devises, and artificial muscles. This study is being conducted at the University of North Texas by D.R. Flanders, Y. Chen, and Z. Hu.

Micrograph - D.R. Flanders, University of North Texas, Electron Microscopy Lab..

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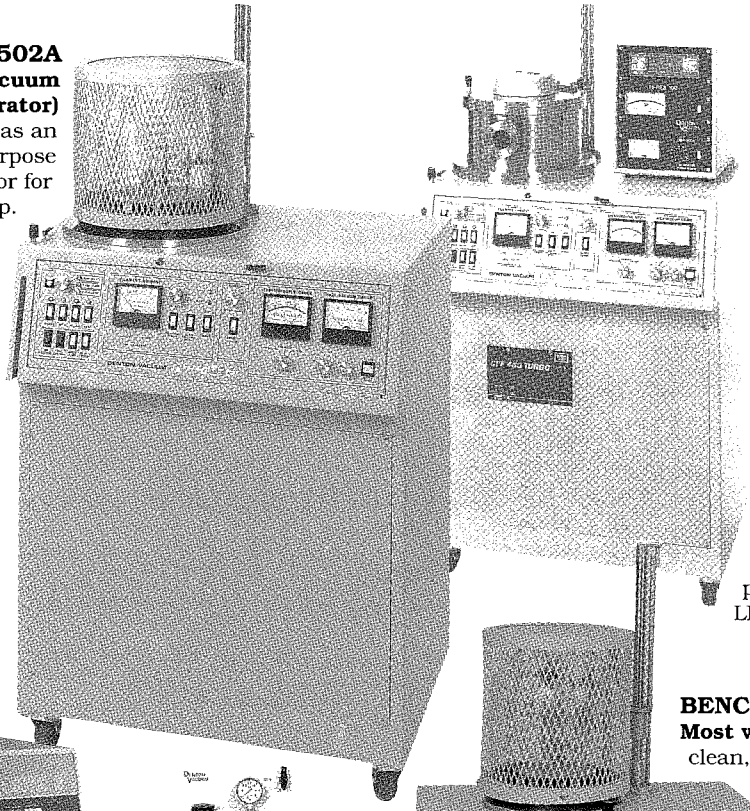

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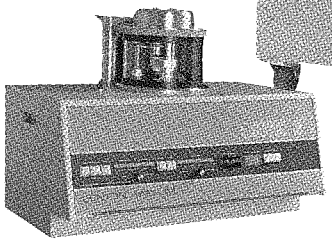

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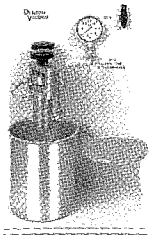

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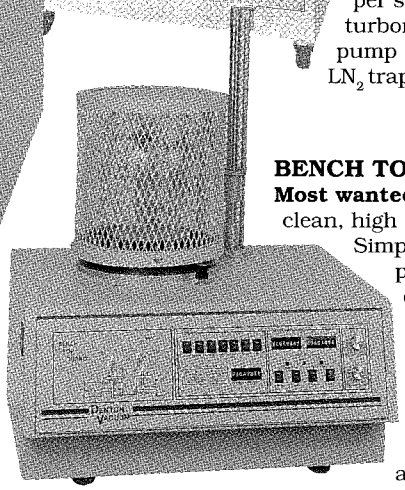

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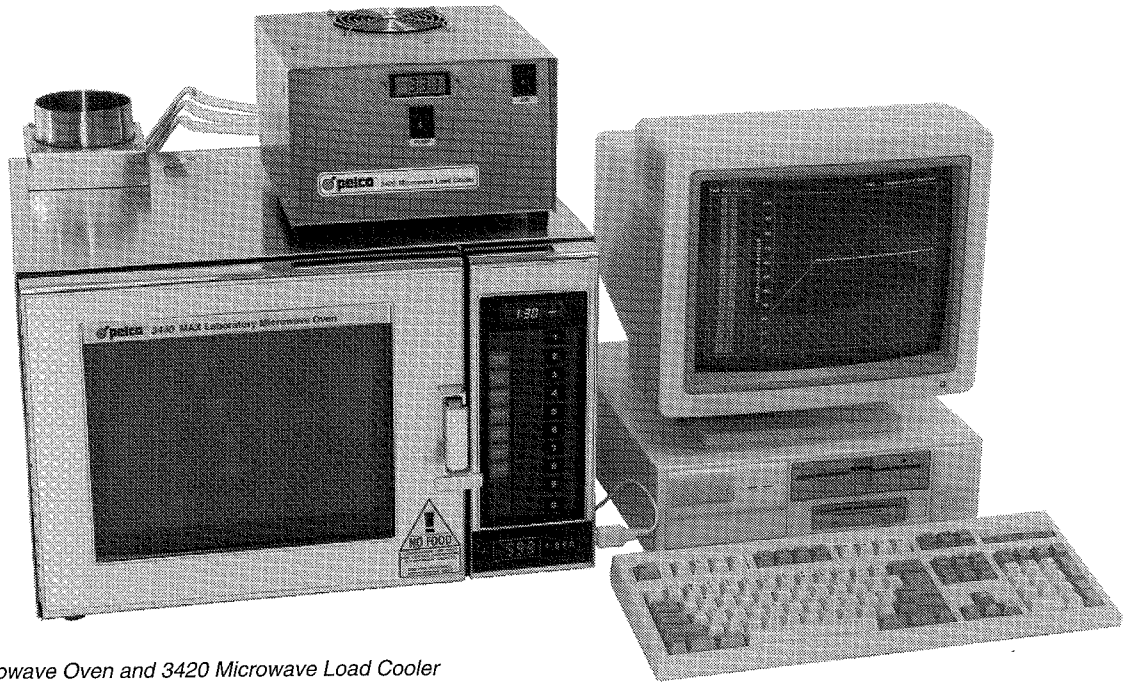

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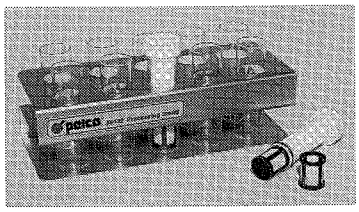

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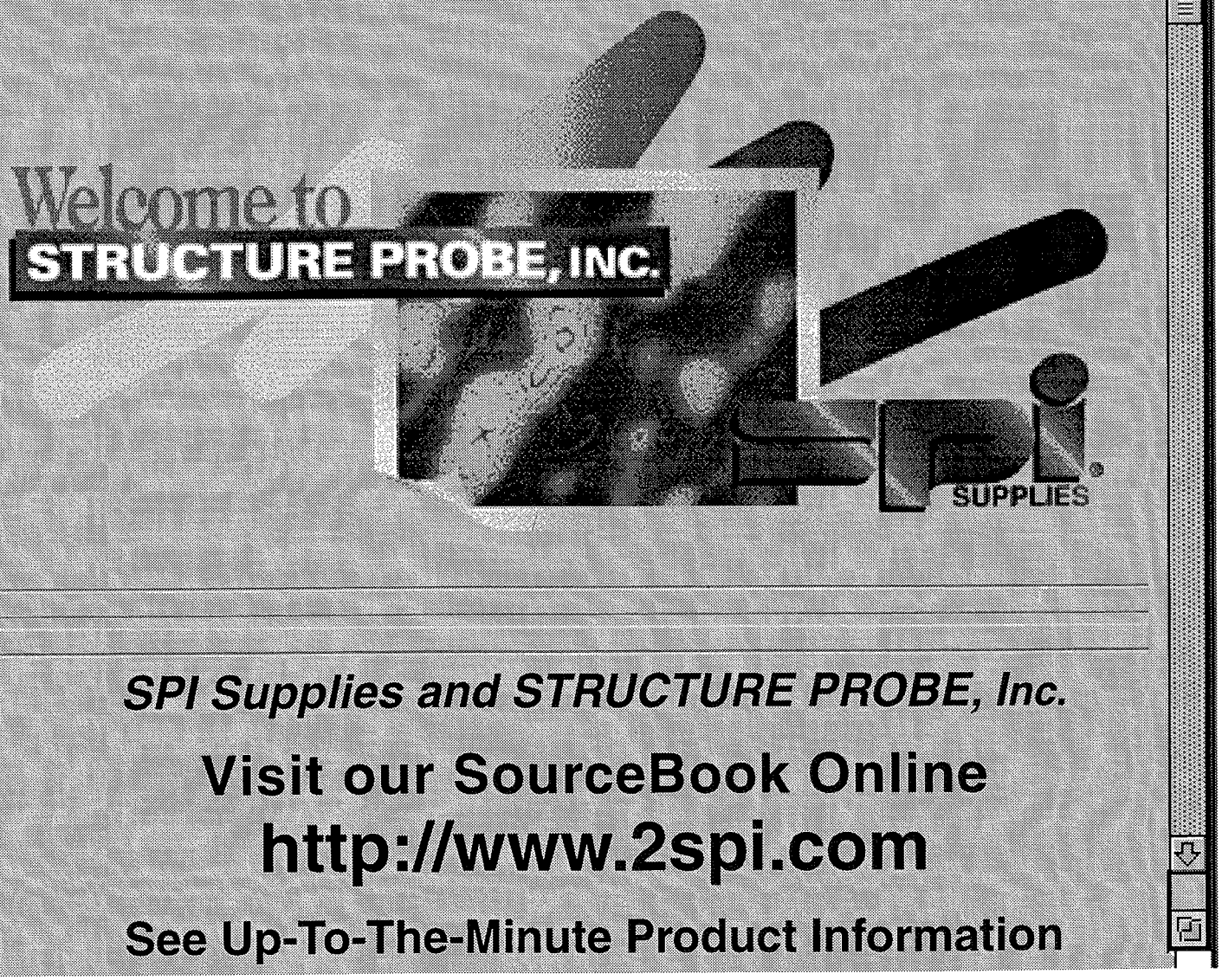

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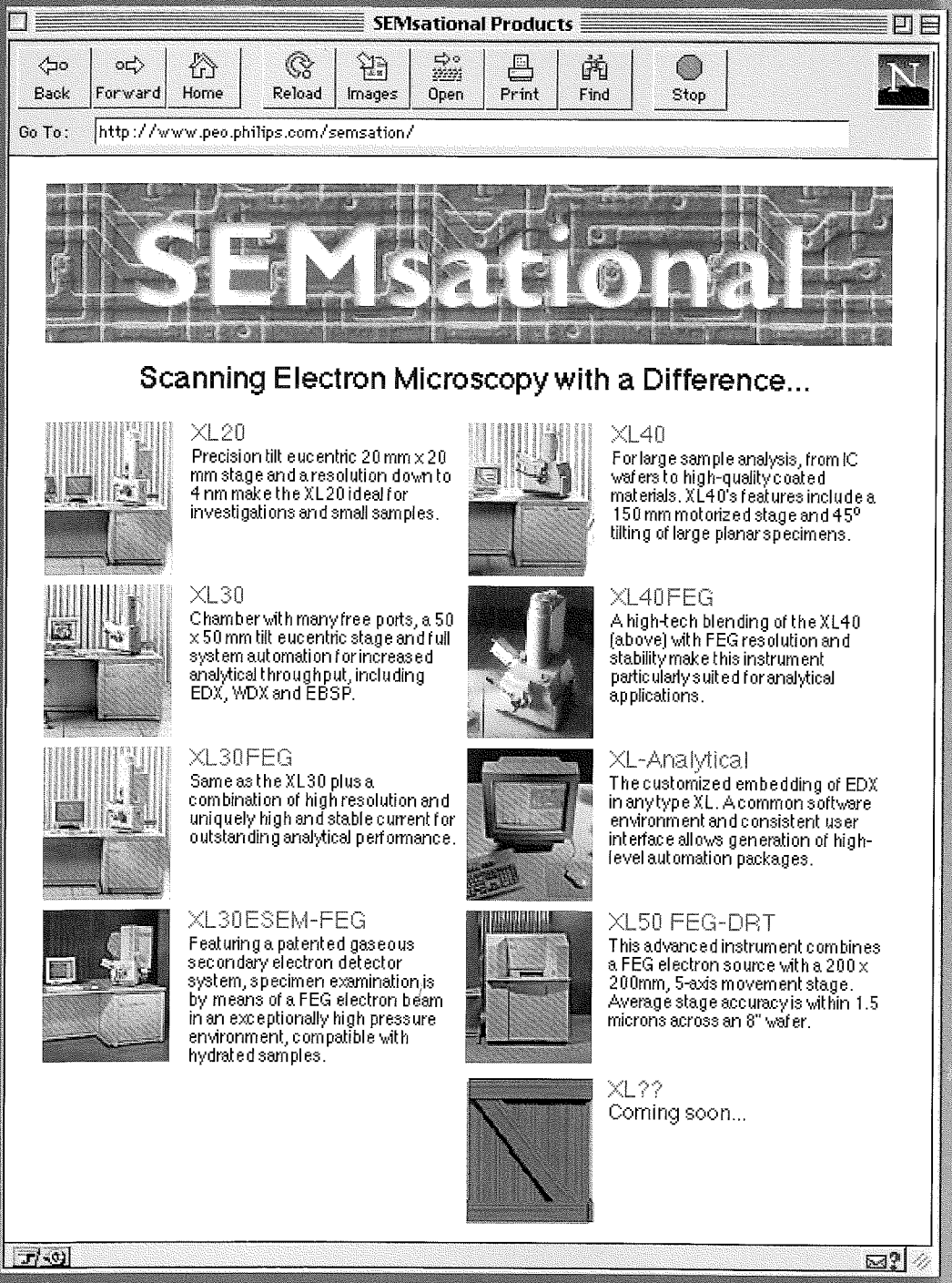

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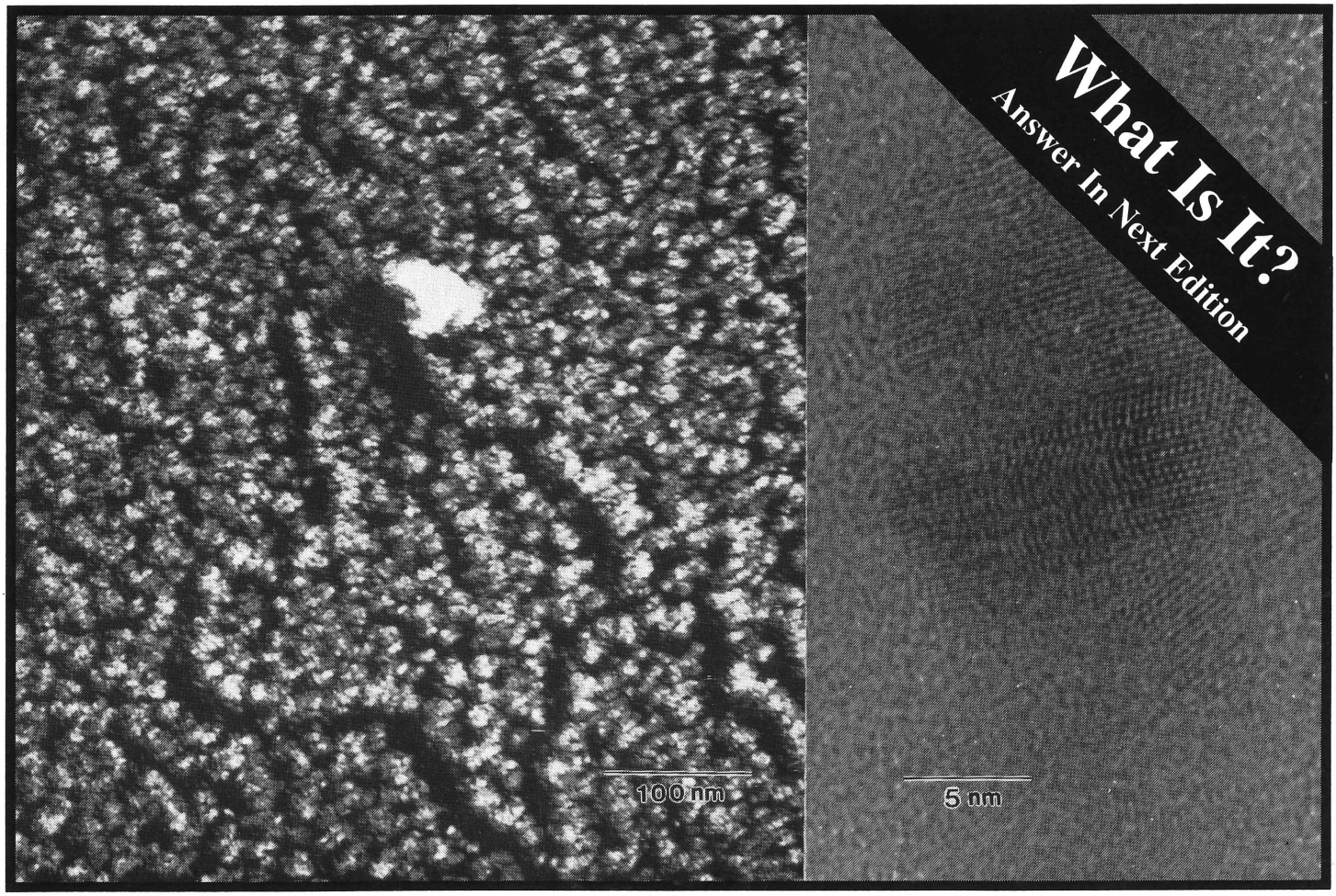

Micrograph by Young G. Rho, Materials Science Department, University of North Texas. Denton, TX 76203