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Texas Society for Electron Microscopy

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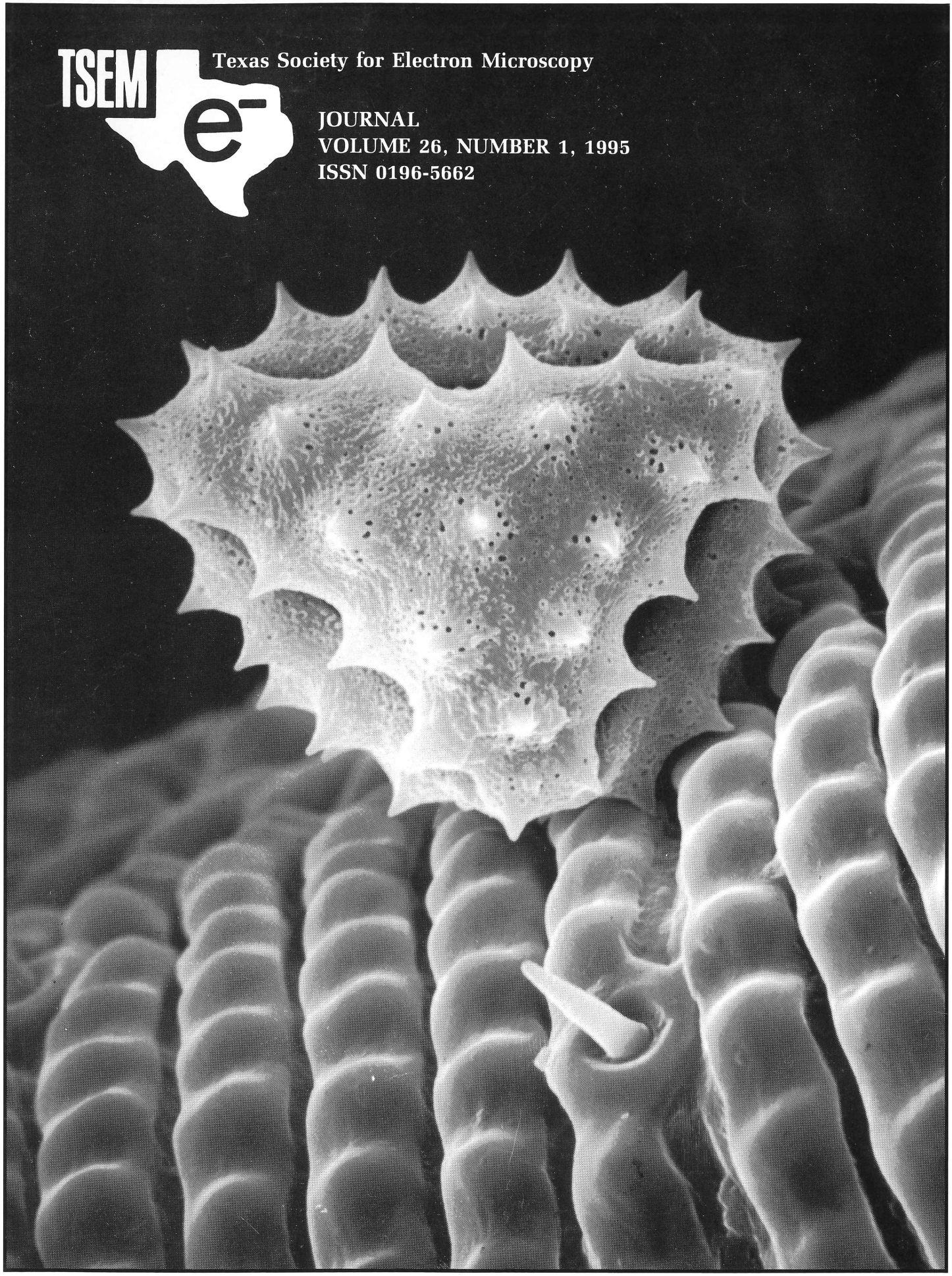

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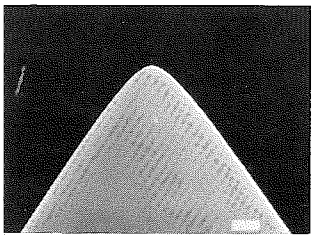
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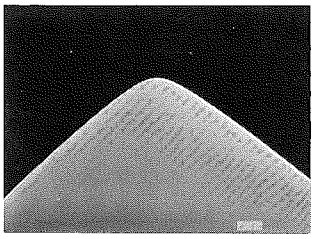
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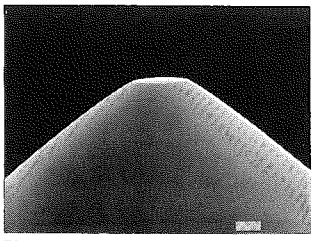
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David C. Garrett, Editor
Department of Biological Sciences, University of North Texas, Denton, TX 76203

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Pollen grain of wildflower on the proboscis surface of the Anicia checkerspot, *Euphydryas anicia* (Lepidoptera: Nymphalidae). Magnification = 3850X. Photo - Dan Petr, Department of Biological Sciences, University of North Texas, Denton, Texas 76203. Current address: Department of Biology, Southwestern Adventist College, Keene, Texas 76059.

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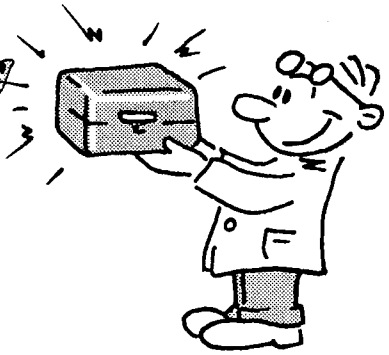

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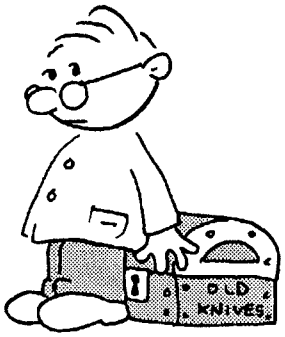

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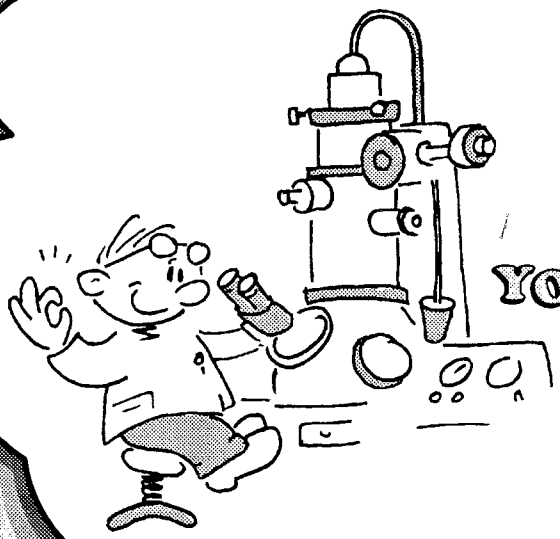

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President's Message

In spite of torrential rains and unprecedented flooding in Texas, the Fall '94 meeting of TSEM proceeded in October with only minor interference. Some members were unable to attend due to the flooding and some attended with great effort in spite of the flooding. Dr. David Cocke, who presented the Thursday workshop on Scanning Probe Microscopies, was stranded in College Station prior to the meeting. He couldn't get out of College Station or into his home city of Beaumont. We are grateful to him for his monumental effort expended to prepare slides from scratch in College Station. It was not possible to get the equipment for the workshop from Beaumont, so the workshop had to proceed without demonstration of the equipment. Many thanks to Dr. Cocke for an informative workshop and to program chair Mitch McCartney for an excellent meeting. There was a lot of interest and enthusiasm generated by our guest speaker, Dr. Steve Duke of the Dental School of the University of Texas Health Science Center at San Antonio, regarding application of various microscopic techniques to dental research problems. I would like to express my personal appreciation to Steve and to my other colleagues from the UTHSCSA who contributed to the program and helped to make it a success.

At the executive council meeting in October, Past-President Hal Hawkins reported from the bylaws review committee. It was decided to not recommend any changes at the present time, per advice from legal counsel. Efforts to resolve legal/tax issues of TSEM have continued and hopefully are near resolution. It is strongly felt by council that the society must change its name to reflect the true range of disciplines that we cover. Until we can vote on the issue and make the resultant bylaws change, it was decided to make unofficial use of a logo on all correspondence and publications: "TSEM - Embracing all forms of microscopy". A second issue that received much discussion was that of going from two meetings per year to one. It was decided to defer that decision and to solicit membership opinion on the officer election ballot in the spring. Ther results will be reported to the executive council and will be taken into consideration in planning meetings in the near future.

Congratulations to ANN GOLDSTEIN, the new President-Elect of our parent organization, the Microscopy Society of America. Ann is Professor of Medicine and Cell Biology at Baylor College of Medicine in Houston and is a Past-President of TSEM (1981-1982). TSEM is proud of her and her accomplishments.

Serving as president of TSEM for 1994-1995 has been a pleasure. I am especially grateful to the officers who have carried on the real work of the society: Mitch McCartney, program chairman; Susan Robbins, secretary; Carolyn Corn, treasurer; David Garrett, journal editor. These are the people who keep the society going. The society is indebted to Mitch McCartney for resolving an unanticipated need by agreeing to organize an additional meeting beyond his elected term. I look forward to the leadership of the new president, Dr. Louis Bragg, who has loyally served the society in the past as program chairman and as journal editor for four years.

Sincerely,

Nancy K. Rodman Smith
President, 1994-1995

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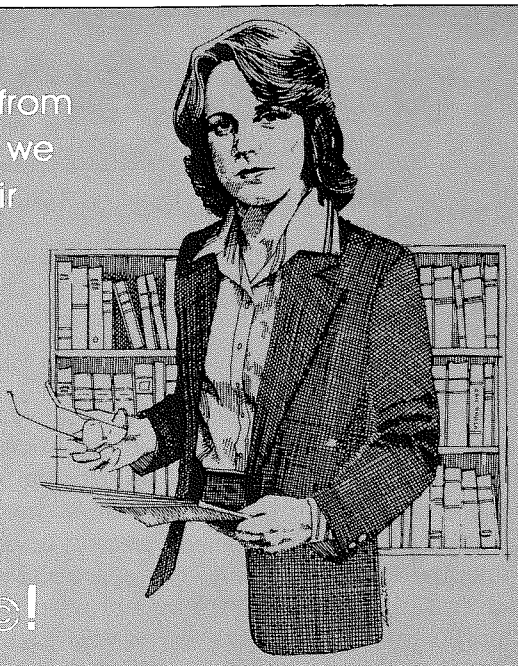

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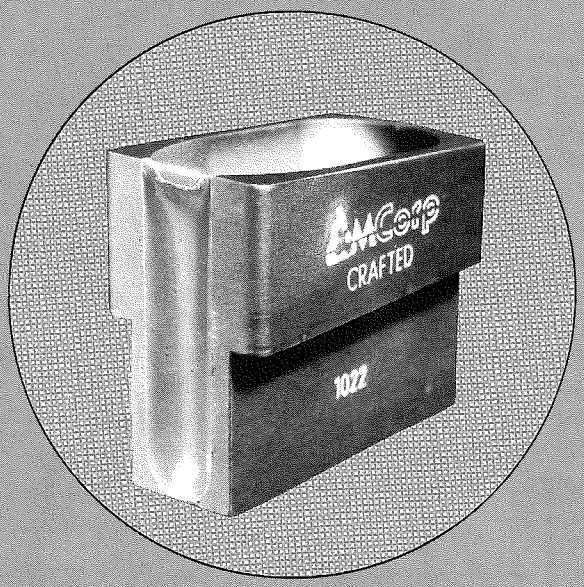

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Certificate of Deposit No. 113515	$3,890.52	
Checking Account No. 70072962	3,720.75	
TOTAL		**$7,611.27**
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Dues	$4,163.00	
Spring 1994 Meeting Registration	1,140.00	
Workshop	205.00	
Exhibitors	600.00	
Donations and Grants	635.56	
Guest	125.00	
Miscellaneous	5.00	
Fall 1994 Meeting Registration	1,450.00	
Workshop	255.00	
Exhibitors	900.00	
Donations and Grants	635.00	
Guest	50.00	
Journal Advertisements 24:2	125.00	
25:1	2,125.00	
25:2	1,625.00	
Miscellaneous	0.02	
Checking Account Interest (Account No. 70072962)	116.25	
TOTAL		**$14,154.83**
Interest, Certificate of Deposit No. 113515	188.85	
EXPENSES:		
Journal, 25:1	$2,261.03	
25:2	1,686.95	
Office Expenses	307.22	
Mailouts	1,600.00	
Spring 1994 Meeting	1,382.82	
Workshop	265.83	
Student Competition/Travel	430.00	
Meeting Refund	30.00	
Invited Speaker	363.00	
Fall 1994 Meeting	3,177.88	
Workshop	344.18	
Student Competition/Travel	0.00	
Invited Speaker	100.00	
Overhead Projector	18.64	
Legal Fees	418.72	
Projector	750.00	
Laser Pointer	57.57	
Bond Renewal	327.23	
Miscellaneous	65.00	
TOTAL		**$13,556.07**
ASSETS AS OF DECEMBER 31, 1994:		
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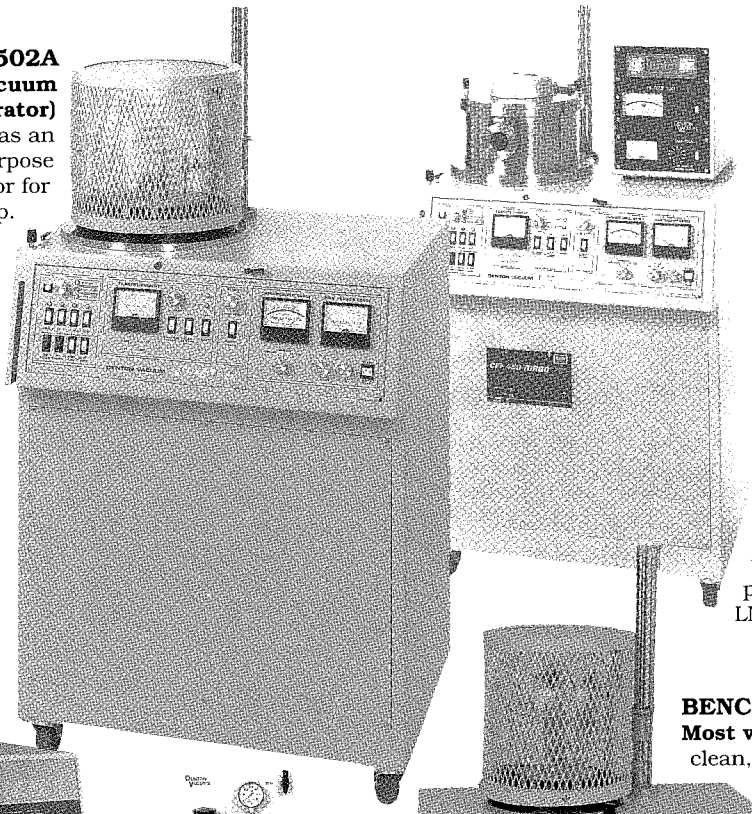

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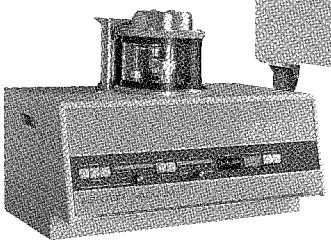

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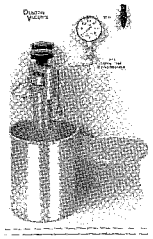

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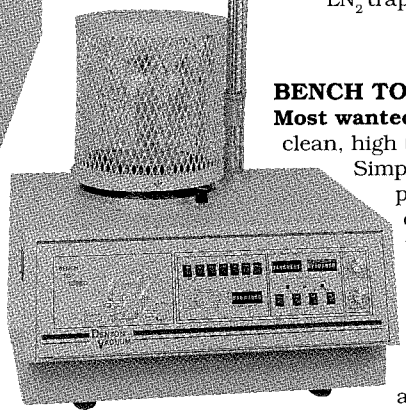

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SCANNING ELECTRON MICROSCOPY OF MINERALS IN SOILS

G. Norman White* and Joe B. Dixon

Soil and Crop Sciences Department, Texas A&M University, College Station, Texas 77843-2474

Soils and sediments are complex assemblages of minerals, organic material, living organisms, water, and gases. The size, shape, and chemical composition of soil minerals may be highly variable. This variability is the result of and one of the principal controls of the chemical, physical, and biological properties of soils. For that reason, observation of soil mineral morphology is very important in the study of soils. Information obtained from mineral morphology and chemistry may reveal the past history of the soil in terms of new minerals formed and modifications of existing phases.

The high magnification and increased depth of field available through the use of scanning electron microscopy (SEM) especially when combined with energy dispersive X-ray spectroscopy (EDS) can provide valuable data in the study of soils and impurities in them. The interpretations obtained by use of a SEM in soil mineralogy studies are in part subjective and rest on the experience of the operator in studying many samples from different locations. The first step in most mineralogy studies is to determine the mineral phases present. The interpretation of SEM data is always aided by knowledge of the bulk mineralogy of the sample. To reduce the number of possible minerals in a sample, the sample is usually pretreated to remove soluble salts, carbonates, organic material, and sometimes Fe oxides (1). These treatments make the sample more easily dispersed so that it can be fractionated according to size prior to X-ray diffraction (XRD). The combination of XRD and particle size fractionation reduces the number of possible phases to a finite population to guide in the identification of individual particles. Almost all of the interpretations of SEM data are based on prior knowledge of the bulk mineralogy of the sample and chemical makeup of the particle determined by EDS and particle morphology.

SAMPLE PREPARATION FOR SEM

Samples used for SEM may be either whole soils or some fraction of a soil (1). The only requirement for preparation of a sample is that it be secured such that it is not going to fall apart in the vacuum system used in the SEM. The sample is usually coated with a conductive material (usually gold, platinum/palladium, or carbon) to reduce charge buildup on the specimen, but this is not always essential. Carbon coating is best for situations where the chemical makeup of the sample as revealed by EDS is important but does not always prove ideal for photography. Platinum/palladium or gold coating a specimen generally proves better for photography than carbon but have EDS peaks which may overlap with characteristic peaks for elements needed for mineral identification.

Two methods of sample preparation are commonly used to prepare specimens for SEM examination. Both methods require samples which have been dried to prevent degassing problems in the microscope.

The first method is to impregnate specimens with some type of resin or epoxy and produce a flat section by sawing and polishing in a manner akin to preparation for optical thin sections. This method is often used in conjunction with optical thin section analysis with the samples being coated for SEM examination following optical microscopy. The principal advantages to this technique are the additional information obtained by optical microscopy and the potential for quantitative X-ray spectroscopy data for single grains in the specimen. Quantitative EDS data can only be obtained from flat specimens. A flat specimen is very important if the features studied are chemically variable (e.g. soil nodules cemented by Fe and Mn oxides) or if the composition of minerals with significant isomorphous substitution is needed. The methods of preparation of flat specimens for quantitative EDS are time consuming, technically complex, and somewhat sample dependent; they normally are not used except when that type of information is required.

The second, more commonly applied method of sample preparation for SEM is to fix single grains or peds (or other stable structural units) onto a SEM stub using conductive tape or glue. Only a very small sample is required for the normal cylindrical sample holder of 1 cm diameter. The sample is mounted on a flat surface of the stub. Larger samples are easily

*Corresponding author.

accommodated by using other sample holders. The potential for problems with sample charging and sample breakage exists if large specimens are used and so usually the sample is as small as possible.

The diagnostic criteria employed for soil samples are given in Table 1 and examples of minerals identified using the criteria are shown in Figure 1.

RESULTS

The chemical composition of a mineral is within distinctive limits. The differences in the chemical makeup at a qualitative level can be used to identify a mineral in a sample assuming some knowledge of the mineralogy of the sample from prior XRD analysis. A known ion saturation also is very helpful when examining samples containing minerals with appreciable ion exchange to avoid uncertainties about the allocation of ions to exchange and structural sites (2). Qualitative interpretation of EDS patterns requires some basis for comparison, usually the ratio of the EDS peaks to each other and to the background. In general, peaks observed in an EDS pattern are directly related to the concentrations of the elements in the area being scanned. The major factors preventing a direct conversion of peak heights to relative molar concentrations are various atomic factors (ionization cross-section, fluorescent yield), detector efficiency, and X-ray absorption. To make the results quantitative, standards and corrections for sample absorption and fluorescence are required with instrumental parameters and acquisition conditions equivalent for the sample and the standards.

It is important when using EDS spectra to identify minerals to be wary of surface coatings. Surface coatings, such as Fe oxides or secondary silica coatings, will cause significant differences in EDS patterns and should be taken into consideration when viewing grains with surfaces which do not appear fresh. Certain minerals do not weather congruently, that is, the EDS spectra obtained may be very much different from the ideal spectrum due to preferential leaching of one or more elements from the mineral surface. Feldspars (3) and pyroxenes (4) are two common minerals which weather incongruently.

Often it is possible to tentatively identify soil minerals by particle morphology alone. This is because the outward appearance of minerals is related to their crystal structure. Care must be taken in identifying minerals using morphology alone, however, because, in some cases such as in the weathering of pyrite, the weathering products of a mineral may preserve the outward appearance of the original mineral yet the composition will change e.g. iron oxide pseudomorphs after pyrite (Figure 1a).

Some minerals, such as the inosilicates, cyclosilicates, diaspores and metal oxides such as rutile, have structures which are made up of repeating units of chains. The linear chains in these minerals cause them to grow or cleave into elongated particles, e.g., needles or long prisms. If the mineral has a distinctive EDS spectrum, the morphology aids in the differentiation from minerals with a similar chemical composition. Other minerals such as halloysite and chrysotile are externally similar but their elongated crystals are the result of a rolled layer structure. Most phyllosilicates, due to their layer structure, have a platy morphology (Figure 1b).

Authigenic phyllosilicate morphology has been the source of intense study by the oil industry because of their effects on oil reservoir properties and their potential as a geothermometer. The most complete study of the SEM observation of authigenic clay is that of Wilson and Pittman (5) which has been used as a standard reference for almost all later work in this area. They found as a result of study of many samples using SEM, EDS, and X-ray diffraction that each major authigenic clay group exhibited a limited number of independent morphologies (pseudohexagonal plates for kaolinite, and laths for illite, for example). Other common minerals have a three dimensional morphology which does not give much aid in their identification. Minerals such as quartz and some feldspars potentially have mineral morphologies which are distinctive enough to allow for their identification but their shape is altered by weathering, transport, or other factors. In these cases, there may be other clues which will aid in their identification. How the mineral is broken by physical weathering or transport can be used to aid in mineral identification. Many minerals such as feldspars and carbonates have good cleavage in one or more directions. Cleavage produces flat surfaces at set interfacial angles which can help in mineral identification. Other minerals such as quartz have extremely poor or no cleavage. That in itself is distinctive as an aid in identification such as the conchoidal fracture of quartz (Figure 1c).

The effects of weathering on a mineral surface may help in the identification of a mineral. Certain feldspars have very distinctive structurally controlled weathering patterns (3). Many K feldspars are perthitic with microscopic intergrowths of Na feldspar. Likewise, the plagioclase feldspars intermediate between albite and anorthite are often demixed into Na rich (albite) and Ca rich (anorthite) domains with regions separated by crystallographic boundaries at a macroscopic size scale. Weathering of these feldspars is almost always incongruent with one type of the feldspar weathered away almost completely leaving the other feldspar appearing pitted but with an EDS pattern characteristic of the end member composition (Figure 1d). Incongruent weathering also is common for many other minerals; for examples, see reference 4.

When particle morphology and qualitative chemical analysis by EDS are used, it is often possible to identify the mineral observed in the SEM with sufficient certainty to conduct meaningful interpretations on the weathering and provenance of minerals in many soils and sediments. The

convenience, efficiency, three dimensional images, and the chemical data make SEM/EDS a very popular and useful pair of research and instructional methods.

ACKNOWLEDGEMENTS

The authors wish to thank the Electron Microscopy Center at Texas A&M University for use of their facilities and cooperation in the performance of the work reported here.

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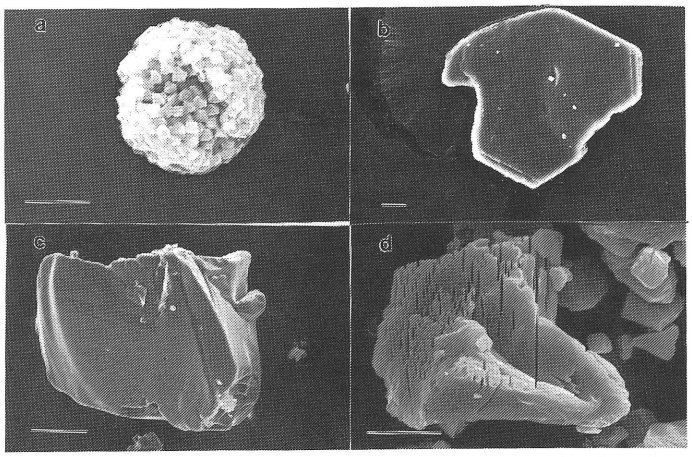


Figure 1. Examples of mineral grains and the criteria used for their identification. **a.** Pseudomorph of Fe oxide after pyrite identified by Fe only EDS peak and pyrite morphology. Scale equals 5 μm. **b.** Muscovite grain identified by combination of EDS pattern and pseudohexagonal platy morphology. Scale equals 10 μm. **c.** Quartz grain identified by Si only EDS pattern and conchoidal fracturing. Scale equals 10 μm. **d.** Weathered feldspar grain identified by EDS pattern and distinctive crystallographically controlled weathering. Scale equals 10 μm.

TABLE 1

Recognition criteria used for mineral identification for soil minerals observed by SEM. Minerals are arranged in an approximate order of decreasing frequency of occurrence in developed soils. Other minerals may occur in soils but their morphology and chemistry are not distinctive enough for use in mineral identification without corroborating XRD data. Elements with atomic number 11 or above (Na) are assumed detectable by EDS. Lower atomic number elements, especially C and O, may be detectable but are not used often because they may result from contamination. It is assumed that the sample is Au coated and examined at 20-25KeV.

Quartz - Si only EDS spectra and morphology.

Muscovite - Al peak almost equal in height to the Si peak, K peak about 0.3 as intense, and platy morphology.

Biotite - Si peak about 3 times K peak with Al, Mg, and Fe EDS peaks; no Na or Ca peaks.

Kaolinite - Al peak almost equal in height to Si peak, platy morphology, and no significant Na, Ca, or K.

Smectite - Very thin platy morphology with minor Na or Ca depending upon cation saturation and stronger Mg, Al, Si, and Fe peaks and little or no K in EDS (assuming cation saturation is known); Usually too small for observation as single crystals but observed as a matrix.

Vermiculite - Thicker platy morphology than smectite with stronger Na or Ca EDS peaks depending upon cation saturation and significant Mg, Al, Si, and Fe peaks and little or no K in EDS (assuming cation saturation is fixed).

Illite - Thin to thick, platy morphology with Na or Ca in small concentrations, Mg, Al, Si, and Fe peaks and K equal or less than that of muscovite or biotite.

Halloysite - Al peak almost equal in height to Si peak, tubular or spheroidal morphology, and no significant Na, Ca, or K.

Chlorite - Platy morphology with varying amounts of Fe, Mg, Mn, Al, and Si in EDS pattern with negligible Na, Ca, or K.

Feldspar - Strong Al and Si EDS peaks with Na, K, or Ca EDS peaks only; often exhibits good cleavage in 2 directions at or close to 90° and preferential weathering based on cationic substitution.

Microcline - K and Al EDS peaks about 0.3 as intense as Si.
Orthoclase - Same composition as microcline, indistinguishable from microcline by EDS.
Sanidine - Like orthoclase with some Na substitution.
Sodic plagioclase - Na peak greater than half Al peak which is about a third as intense as Si in EDS; may have some Ca replacing the Na.
Calcic plagioclase - Ca peak about half the height of the Al peak which is nearly as intense as the Si EDS peak.

Fe oxides - Very hard to distinguish by SEM except in unusual cases where the morphology is distinctive and evident; All show Fe EDS peak only.

Hematite - Rarely has observable platy morphology.
Lepidocrocite - Rarely has observable platy morphology.
Ferrihydrite - Granular morphology almost too small for observation in SEM even as a matrix.
Goethite - Prismatic or acicular crystals sometimes large enough for observation with SEM.

Carbonates - Sometimes have rhombohedral morphology.

Calcite - Ca EDS peak only, may be hard to distinguish from gypsum by EDS alone.
Dolomite - Ca peak nearly twice height of Mg peak in EDS.
Siderite - Fe peak alone or with minor Ca.

Ilmenite - Fe and Ti only in EDS spectra nearly same peak height.

Zircon - Zr and Si EDS peaks.

Rutile - Ti peak only in EDS plus prismatic morphology.

Anatase - Ti peak only in EDS plus nonprismatic morphology.

Gibbsite - Al EDS peak only with platy morphology.

Gypsum - Ca and S EDS peaks nearly equal in intensity, may be hard to distinguish from calcite (Note: S EDS peak overlaps with Au coating peak and may not be observable for this and other S containing minerals).

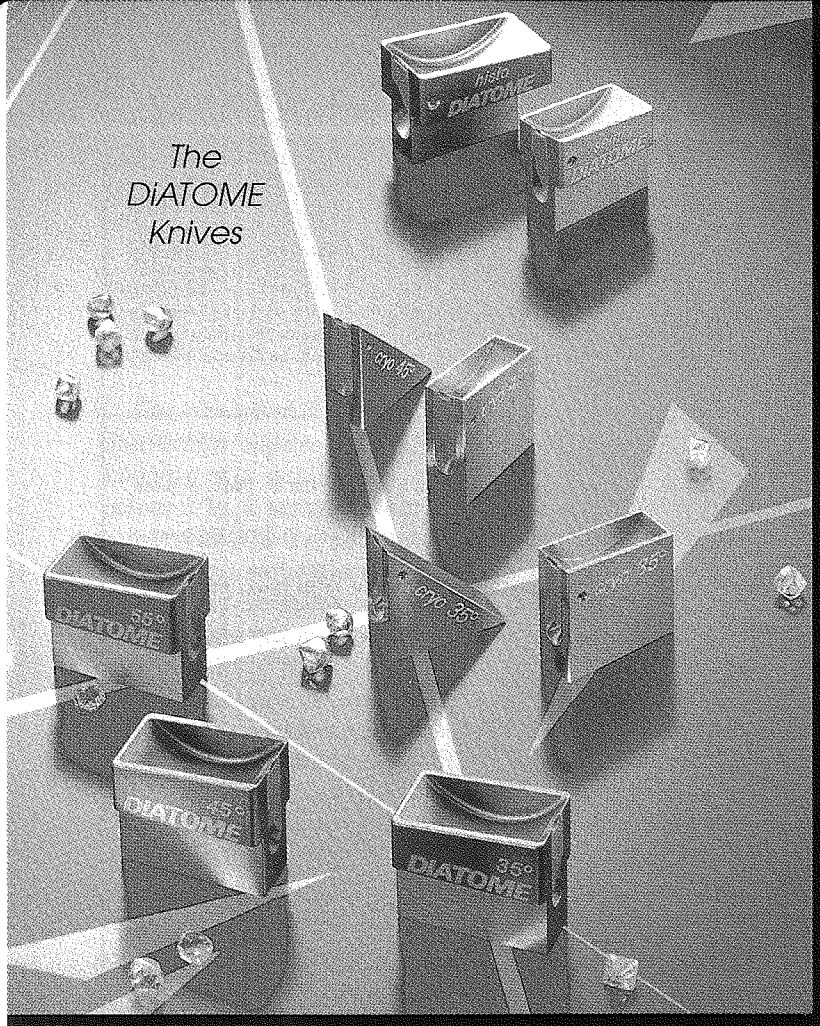
Barite - Ba and S EDS peaks only.

Pyrite - Fe and S EDS peaks usually showing octahedral or cubic crystal faces.

Marcasite - Fe and S EDS peaks with rounded crystal faces and no octahedral or cubic faces.

Halite - Na and Cl EDS peaks often with a cubic morphology.

Sphene - Ca, Ti, and Si in EDS pattern.



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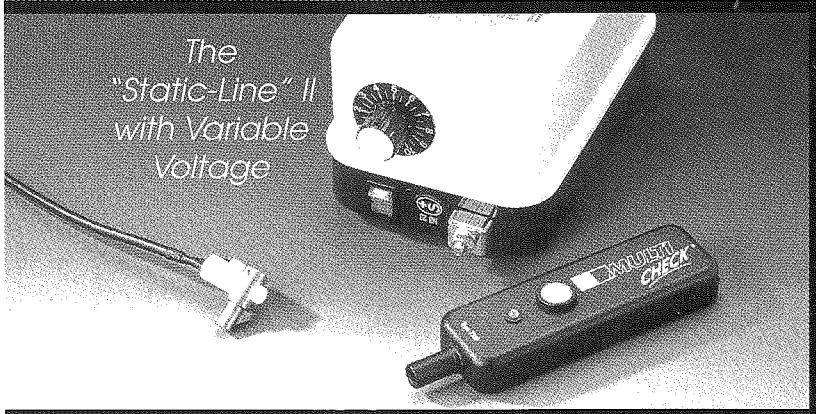

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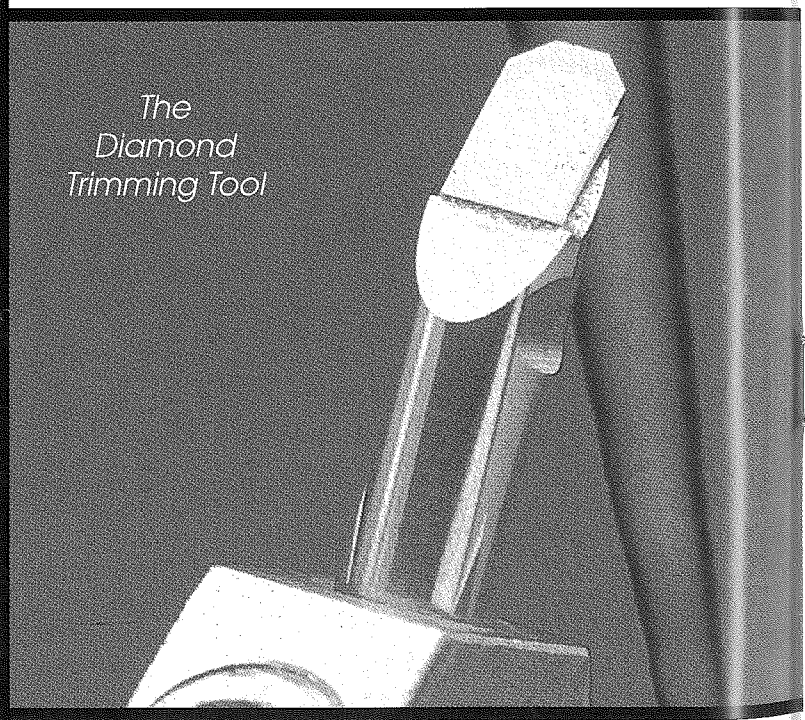

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(3) D. Gabor. Information Theory in Electron Microscopy, in: Quantitative Electron Microscopy. Eds. G.F. Bahr and E. Zeitler (Williams and Wilkins, Baltimore, 1956) 63-68.

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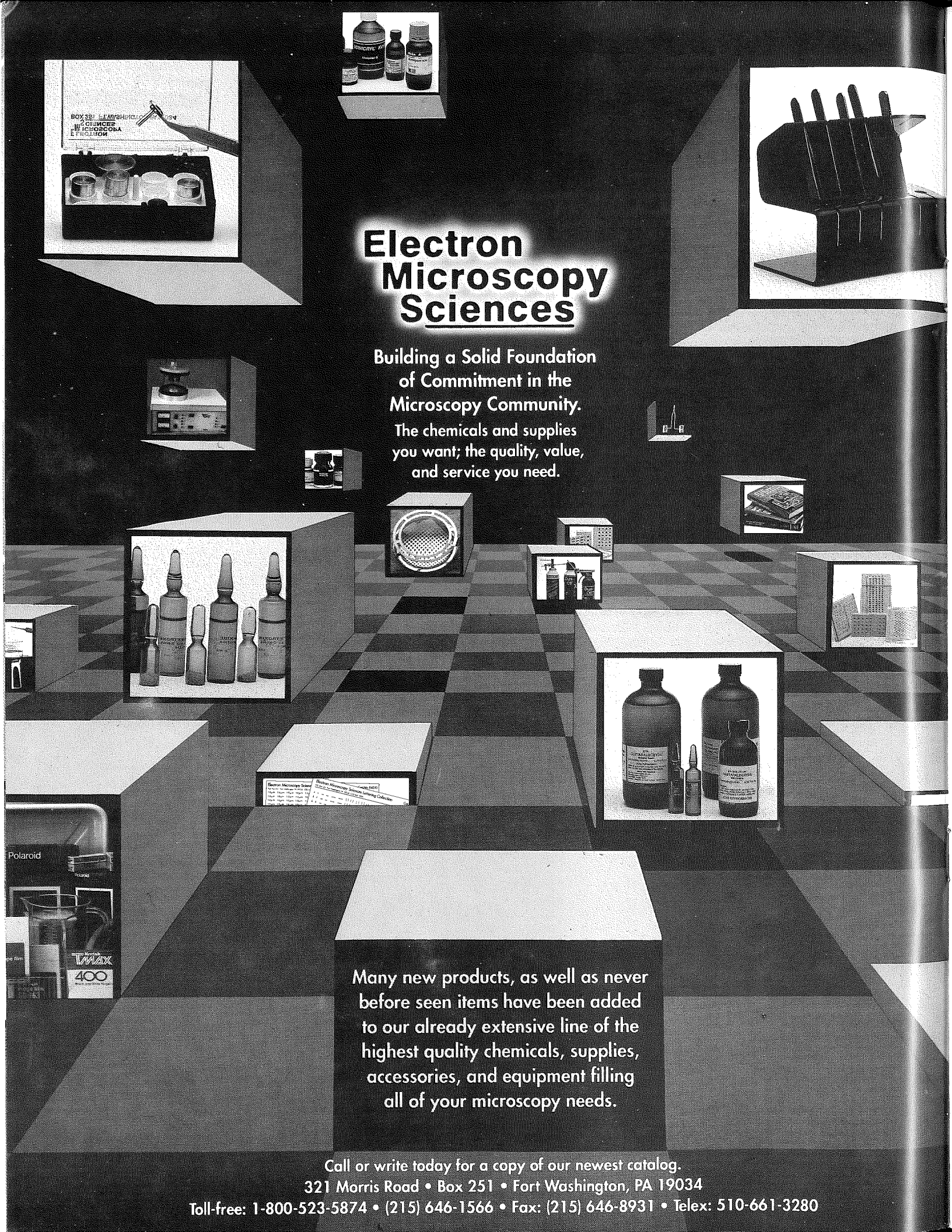
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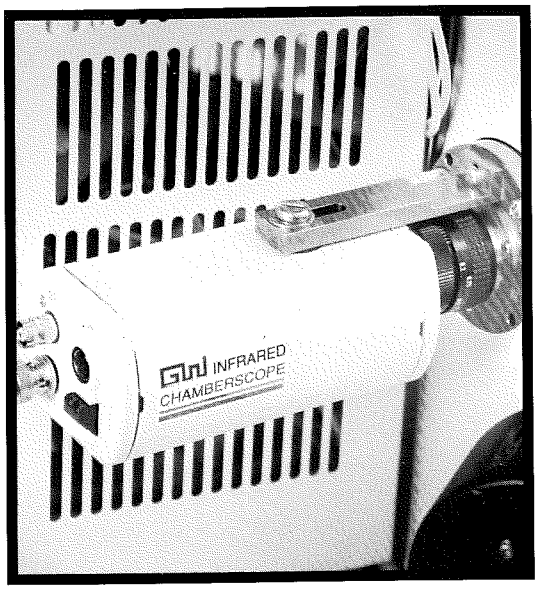

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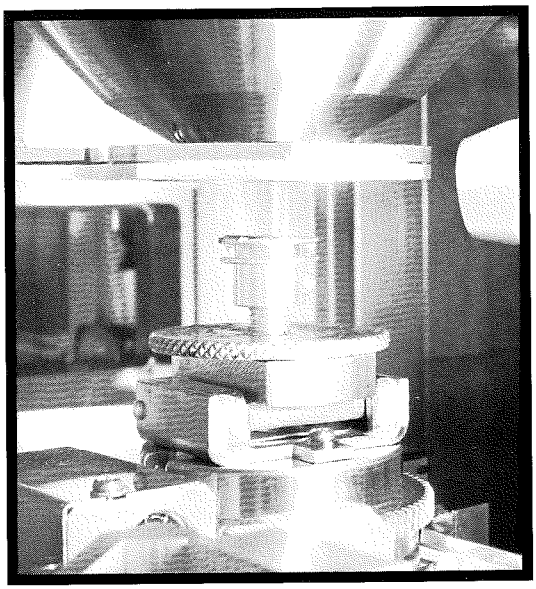

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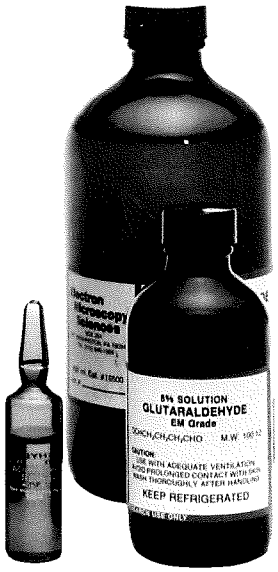

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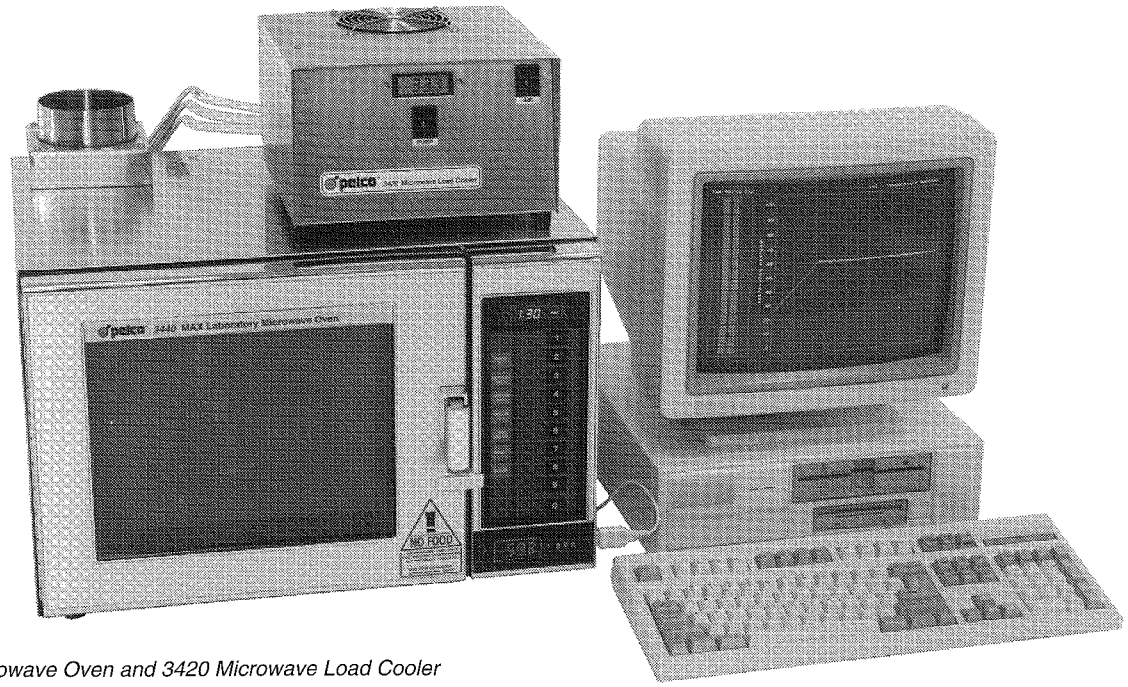

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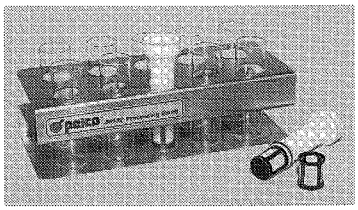

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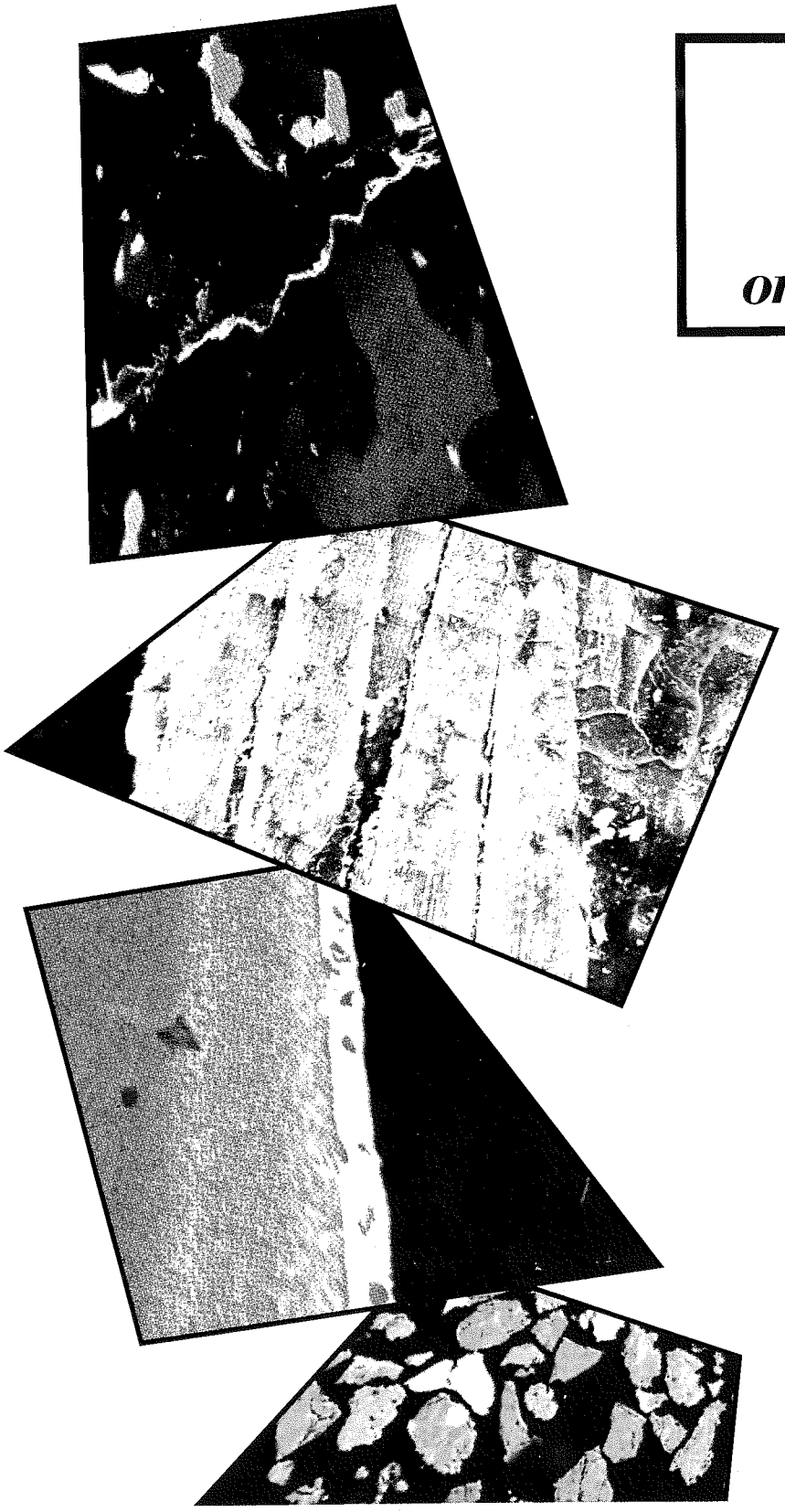

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ADVANCED TEM SAMPLE PREPARATION TECHNIQUES FOR SEMICONDUCTORS. DR. HUN-LIAN TSAI, PHILLIP BRENT BASHAM, JIM WALLER, Texas Instruments Incorporated, Dallas, TX 75243

Increasingly complex design and shrinking geometries necessitate the need to be as creative in the preparation of TEM samples as in the design itself. We have developed a literal arsenal of techniques that help our laboratory to solve problems in production wafers and research devices.

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Fragility of TEM samples is another obstacle to success in the single bit or specific area samples. A combination of earlier techniques developed in our laboratory with recent advances has led us to the Small Hole Technique. This technique produces a sample that is less fragile than earlier methods while increasing the yield of such samples.

COMPARISON OF MICROSTRUCTURES IN STEEL BOILER TUBES: A TEM SEARCH FOR FAILURE MODES AND DEFORMATION MECHANISMS. JOEL DE ALBA,[1] C-S. NIOU,[1] L. E. MURR,[1] P. DIEHL.[2] [1]Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX 79968, and [2]Center for Materials Characterization, University of North Texas, Denton, TX 76203.

Steel boiler tubes in power plants carrying superheat and reheat steam at approximately 540°C fail catastrophically, leading to a cycle of unpredicted replacement of failed tubes that greatly affects the productivity. A portable X-ray device could be used on these tubes to try to predict remaining service life and to help end the unpredictability of tube replacement. Since microstructural changes on failed tubes can provide clues as to the cause of the failure, our objective is to examine failed tubes to try to establish the failure modes and deformation mechanisms using transmission electron microscopy (TEM) as the primary research tool. This approach involves correlating TEM micrographs in different sections in failed tubes corresponding to a range of engineering strains, with corresponding optical micrograph views and X-ray diffraction spectras. This range of strains spans from original, low strain, no-service microstructure to high-strain areas located near the crack tip of a failed tube. Research supported by the Texas Advanced Technology Program (003594-045C) administered by The Texas Higher Education Coordinating Board.

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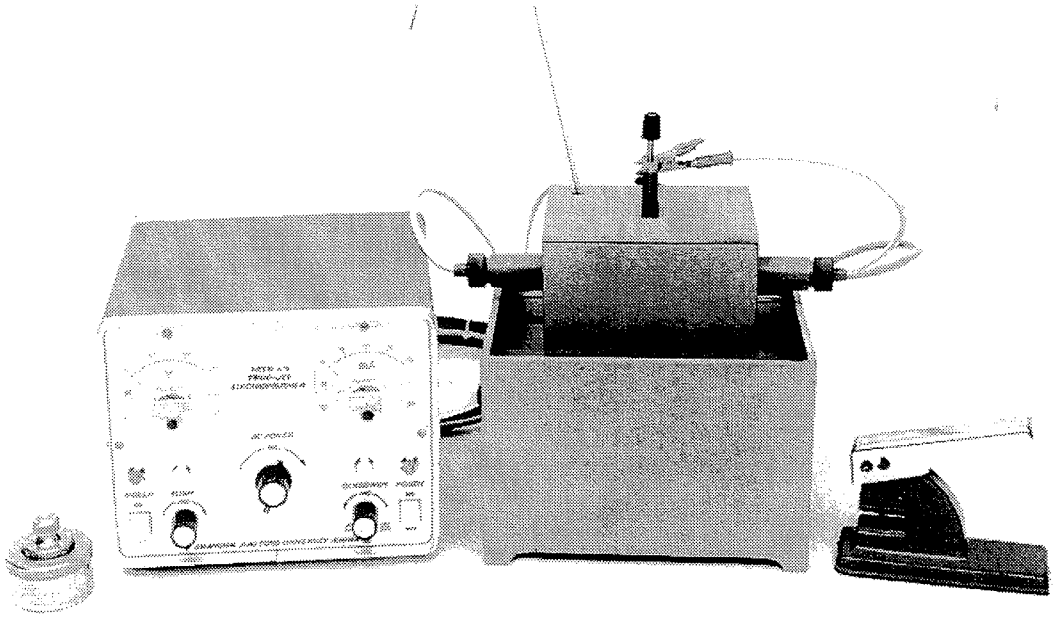

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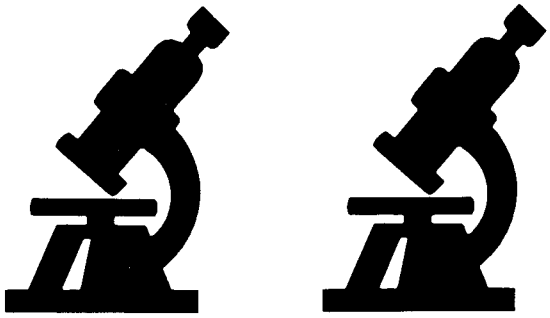
A TEM STUDY OF SENSITIZATION BEHAVIOR OF 304 STAINLESS STEEL DEFORMED AT LIQUID NITROGEN TEMPERATURE. JULIO G. MALDONADO and L.E. MURR. Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX 79968.

Strain-induced α'-martensite, produced in plastically deformed 304 stainless steel, has been observed to form at twin-fault intersections in the microstructure. Upon short-time thermal aging, the strain-induced α'-martensite-rich intersections produce a recrystallized microstructure consisting of fine-grained austenite and martensite. The interfaces of the recrystallized microstructure then become preferred sites for transgranular carbide precipitation since these interfaces have similar energetics as the parent austenitic grain boundaries. In this study, 304 stainless steel was deformed (20% true strain level) at liquid nitrogen temperature (-196°C), and subsequently aged at 670°C for 0-10h. This material demonstrated a drastic reversal in sensitization behavior as compared to a control material deformed to an equivalent strain, but at room temperature. Transmission electron microscopy and optical microscopy were employed to correlate the microstructural changes with the sensitization behavior observed. The electrochemical potentio kinetic reactivation technique was used to quantify the precipitation process. The $\gamma \Rightarrow \alpha$' transformation is almost complete in the liquid nitrogen deformed material, and becomes the dominant feature that provides the necessary impetus for an extensive recrystallization upon a short aging treatment. The extensive recrystallization effectively reduces the grain size of the material (below 1μm) which in turn has an enormous effect on the diffusional paths available. This effect has also been observed to some extent in small grain size (15 μm) 304 stainless steel material deformed at room temperature. The overall effect is then the very rapid sensitization of the material, but also a correspondingly fast desensitization process. J.G. Maldonado acknowledges the financial support received through a Patricia Roberts Harris Fellowship.

MICROSTRUCTURAL EVOLUTION ASSOCIATED WITH IMPACT CRATER FORMATION IN COPPER TARGETS. JESUS M. RIVAS, S. A. QUINONES, E. P. GARCIA, AND L. E. MURR. Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso TX. 79968

When a projectile traveling at high velocity impacts on a metallic target a crater is formed on the target. Laboratory-produced craters have helped to understand some of the cratering process parameters. However, the microstructure of the target has never been considered or related to the crater formation phenomena, or to any computer model that attempts to simulate the cratering process. There has also been a complete lack of information about the residual microstructure of the target material. This investigation is the first detailed microstructural analysis on laboratory-produced craters. Two craters in copper targets produced by 1100 Al projectiles impacting at velocities of 6.73 and 1.4 kms^{-1} were analyzed and compared using optical and transmission electron microscopy. Among the most revealing microstructural features observed are a narrow recrystallized zone adjacent to the crater wall (on the high velocity crater) and a zone that contains extensive regions of microbands which are coincident with {111} planes. Microhardness values were also obtained which corroborated the microstructural observations. Supported by a NASA-Johnson Space Center Grant NAG 9-481 and a NASA Graduate Fellowship (S.A.Q.).



BIOLOGICAL SCIENCES

POSTER PRESENTATION — SPRING 1995

COMPARISON OF QUANTITATIVE LIGHT AND TRANSMISSION ELECTRON MICROSCOPY IN THE EVALUATION OF CHEMICALLY INDUCED FOLLICULAR ATRESIA. R.COX, W.AU, H.HAWKINS, Shriners Burns Institute and Depts.of Preventive Medicine And Community Health and Pathology, University of Texas Medical Branch, Galveston Texas.

Exposure to toxic substances can induce follicular atresia thus reducing female fertility. The established method for evaluating acute follicular atresia is quantitative light microscopy (LM) using serial sections through the ovary. Several criteria are used to define a degenerative follicle, including the percentage of pyknotic nuclei per follicle, irregularly shaped oocytes, loss of contact between the granulosa cells and the zona pellucida (ZP), and inflammatory cell migration into the follicle. In transmission electron microscopy (TEM), additional characteristics of follicular atresia are increased lipid accumulation in the granulosa cells and loss of microvillous projections from the granulosa cells and oocytes into the ZP. In a recent study to evaluate the female reproductive toxicity of ethylene glycol monomethyl ether (EGME) in mice, we used the LM quantitative method, including three animals per dose and evaluating at least 1000 follicles per animal. We also utilized quantitative TEM for the absence of microvillous projections in the ZP, including four animals per dose and evaluating at least 25 ZP per dose. Cyclophosphamide (CP) at 100 mg/kg was used as a positive control. Results of the LM quantitation showed significantly increased follicular atresia after the highest dose of 2700 mg/kg and no statistical increase after the 300 and 900 mg/kg doses of EGME in comparison to control, saline exposed animals. TEM quantitation showed a five to seven fold increase in the incidence of abnormal ZP for all doses of EGME compared to the control animals. Both methods showed significant induced follicular atresia for the CP exposed mice.

These results suggest that: 1) quantitative TEM observation of an abnormal ZP is a more sensitive method of determining follicular atresia than LM quantitation, 2) the retraction of microvillous projections out of the ZP matrix may precede the cellular necrosis and inflammatory cell influx observed by LM.

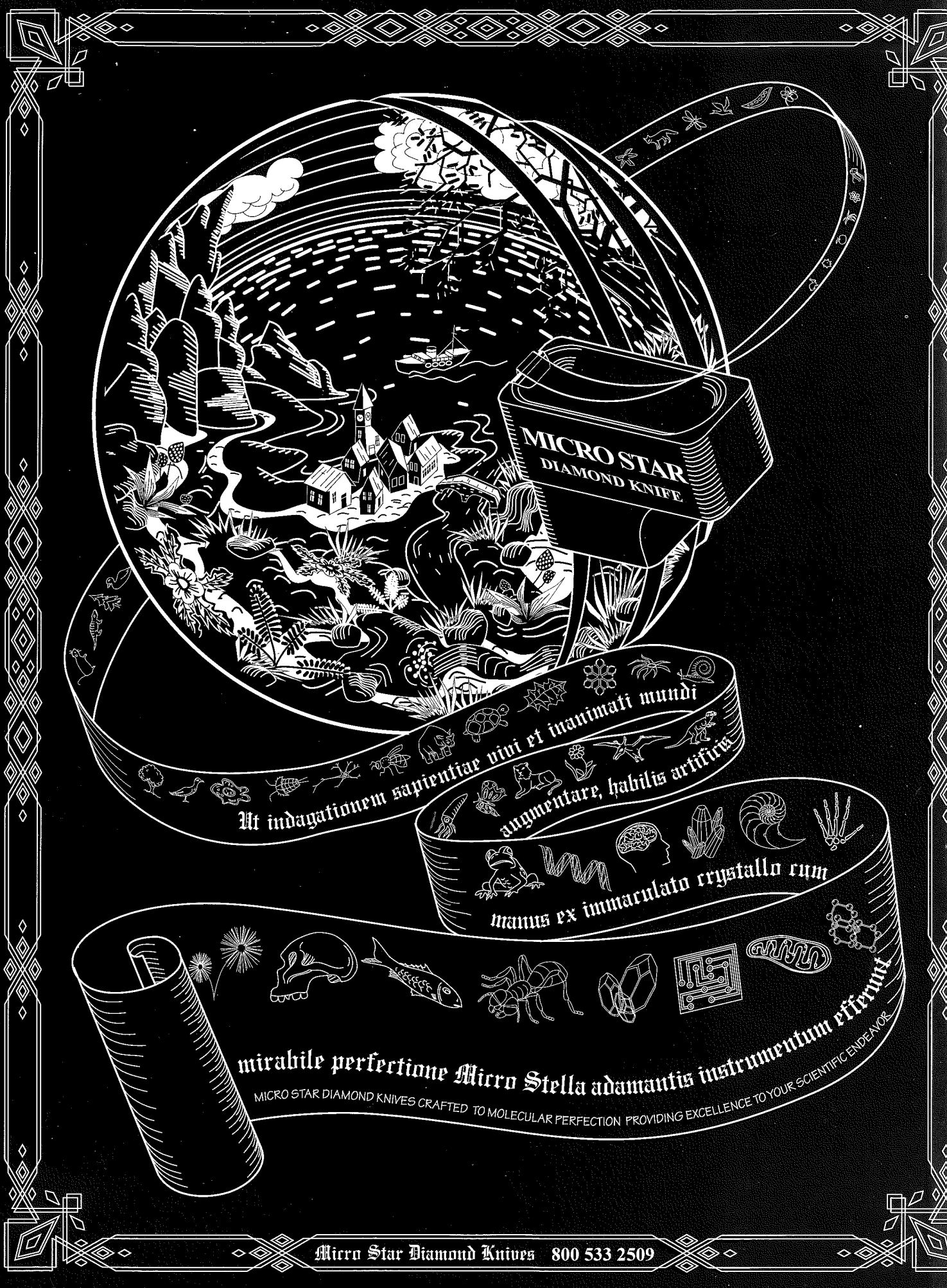
A STUDY OF EARLY HELLADIC II PERIOD WOOD FROM DOKOS, GREECE. GEORGIA FOX and MICHAEL PENDLETON, Department of Anthropology, Texas A&M University, College Station, TX 77845.

This paper focuses on the characterization of wood samples taken from the archaeological site of Dokos, Greece, in August 1991. The Dokos Project, directed by Yannis Vichos of the Hellenic Institute for Marine Archaeology, began in 1989. This site is an underwater shipwreck site dating to the Early Helladic II period, ca. 2200 B.C. The site was originally discovered by Peter Throckmorton in 1975 in 15 to 25 m of water, close to the promontory of Myti Kommeni in the small northern bay of Skindos. To date, the site is possibly one of the oldest shipwreck sites in the Aegean, and the collection of clay vessels from the wreck constitutes one of the largest and most important bodies of Early Helladic material found at one site. Because the area has been subject to seismic activity and local site disturbances, wood samples were taken to determine if the wood fragments recovered at the site were associated with the wreck structure.

In order to attempt to identify these wood fragments, light and scanning electron microscopy were used to observe significant structural features. For light microscopy, wood fragments were embedded in Spurr resin and sectioned with a MT2-B ultramicrotome. Semi-thin sections were stained and observed with a JENA microscope. For scanning electron microscopy, wood fragments were mounted on aluminum stubs with carbon paint, coated with gold using a Hummer sputter-coater and observed with a JOEL T330A. Following these observations, significant features of these wood fragments were entered into the General Unknown Entry and Search System for computer-aided wood identification developed by the North Carolina Agricultural Research Service, North Carolina State University. Evidence of possible wood deformation was observed in some fragments.

ENZYMATIC LOCALIZATION OF CYTOCHROME C OXIDASE IN *CAULOBACTER CRENCENTUS*. K. M. Charrey, T. R. Hoage and H. D. Kurtz, Jr. Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77341.

Caulobacter are aerobic gram negative bacteria characterized by the morphological feature of a single polar stalk. A prostheca (stalk) is an extension of the cell envelope composed of outer membrane, peptidoglycan layer, cytoplasmic membrane and internal core membranes. An adhesive distal prosthecal holdfast renders the cells capable of solid surface attachment. Multitudes of cells attached to a surface results in the formation of a bacterial biofilm. The proximity of the caulobacter prostheca within the biofilm places it in an area of decreased oxygen concentration raising the question, does cellular respiration occur in the prosthecal membranes? This study examines the expression and distribution of cell membrane cytochromes using standard fixation procedures for light microscopy, transmission and scanning electron microscopy. Enzymatic localization of cytochrome c oxidase activity is used to determine the cytochrome distribution within the cell and prostheca of *Caulobacter crescentus*. Free living cells and cells associated within the biofilm are examined.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BIOLOGICAL SCIENCES

PLATFORM PRESENTATION — SPRING 1995

TEM OBSERVATIONS OF SWEET GUM LEAF ANATOMY. J. TAYLOR* and B. VANDOVER, Dept. of Biology, Stephen F. Austin State University, Nacogdoches, TX 75962.

Leaves of sweet gum (Liquidambar styraciflua) were chemically fixed in glutaraldehyde and osmium, embedded in Spurr's resin, sectioned and examined with the transmission electron microscope (TEM). A thick cuticle covered both upper and lower epidermal layers. The epidermis consisted of a single layer of cells. Stomata were present in the lower epidermis. Palisade and spongy parenchyma cells comprised the mesophyll. Parenchyma cells contained numerous chloroplasts with prominent starch grains. The vacuole of many mesophyll cells contained an electron-dense, fibrillar material. Sequential stages in the accumulation of this material were observed. Vascular bundles in the sweet gum leaf were surrounded by a single-layered bundle sheath.

CELLULAR COMPOSITION OF ATHEROSCLEROTIC LESIONS REMOVED BY DIRECTIONAL CORONARY ATHERECTOMY (DCA). M. A. DAVIS AND S. C. WILLIAMS, Department of Biology and Center for Electron Microscopy, University of Texas at Arlington, Arlington, TX 76019.

Cardiovascular disease is the number one cause of death in the U.S. today. Options in the treatment of coronary vessel occlusive disease (atherosclerosis) are currently being applied as alternatives to arterial bypass grafting. Among them is the Directional Coronary Atherectomy device (DVI, Inc.), an instrument that is employed transluminally to resect lesion material. Histopathological studies of resected lesion material can then be performed. Human specimens removed by DCA were fixed in 10% Formalin or 3% gluteraldehyde. For light microscopy (LM), specimens were fixed in formalin, dehydrated in ethanol, embedded in Amerrafin, sectioned to 8-10 μm and stained with hematoxylin and eosin. Scanning electron microscopic (SEM) specimens were fixed in gluteraldehyde, postfixed in osmium tetroxide, dehydrated in ethanol, CO_2 critically-point dried, and coated. Specimens revealed atherosclerotic components including fibrous connective tissue, foam cells, lipid and calcium deposition, hyperplastic smooth muscle cells, and thrombus. Polarized LM revealed birefringence of calcium deposits and areas of collagenous connective tissue.

CHARACTERIZATION OF IN VITRO RAISED CALLUS OF TWO LEGUMINOUS SPECIES WITH LIGHT AND ELECTRON MICROSCOPIC STUDIES. Nabarun Ghosh, Don W. Smith, and A. Chatterjee[1]. Dept. of Biological Sciences, University of North Texas, Denton, TX 76203. [1]CAS, Dept. of Botany, University of Calcutta, India.

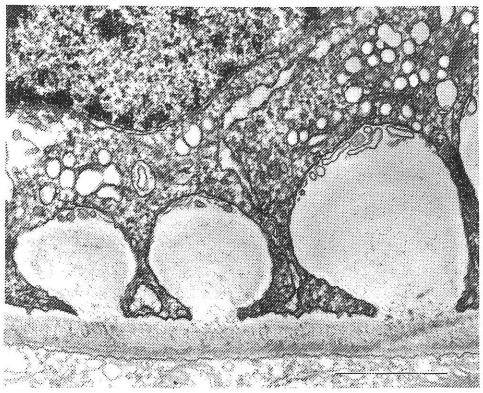
Callus culture of two leguminous tree species was initiated on MS basal medium supplemented with different growth regulators. Cytological studies were carried out after pre-treating the callus with a saturated solution of para-dichlorobenzene (pDB), fixing with 1:3 propionic acid : alcohol mixture and staining with 2% aceto-orcein. Mitotic index, abnormality index and the ploidy level varied with the age and morphology of the callus. A higher mitotic index accompanied by lower abnormality index were noted in the juvenile green callus. The color, texture and morphology of the calli varied with the age of the culture. Light microscopy and Scanning Electron Microscopy were carried out to study the callus surface. Calli were cut into pieces, fixed in 4% gluteraldehyde in 0.1M phosphate buffer, post-fixed in osmium-tetroxide (OsO_4) and dehydrated in ascending concentrations of ethanol and finally in isoamyl acetate. The calli samples were dried at critical point (CPD), coated with gold-palladium and scanned under SEM. The SEM study revealed the varied texture of different types of calli including striations, reticulations and fibrillar nature of the calli. The regenerative callus showed the emergence of shoot buds, globose embryoid-like structures and shootlets. The methodology will provide a technique to differentiate between regenerative and non-regenerative calli and to detect specific type of callus during the in vitro culture.

CONIDIOGENOUS CELL FORMATION IN THE FUNGUS TUBAKIA DRYINA. S. Pursley* and J. Taylor, Dept. of Biology, Stephen F. Austin State University, Nacogdoches, TX 75962.

Nomarski differential interference contrast microscopy, scanning electron microscopy, and transmission electron microscopy have been used to elucidate the type of and mechanisms by which conidiogenous cell formation occurs in the fungus Tubakia dryina. For experimentation, the pathogen was isolated from infected sweet gum leaves and grown in pure culture, thereby providing conidiogenous cells in various stages of development for observation. Holoblastic conidium formation appears to be characteristic of the fungus with conidia forming solitarily or in chains. The conidia can form intercalary or terminal on the mycelial hyphae with conidial succession occurring via the schizolytic pathway.

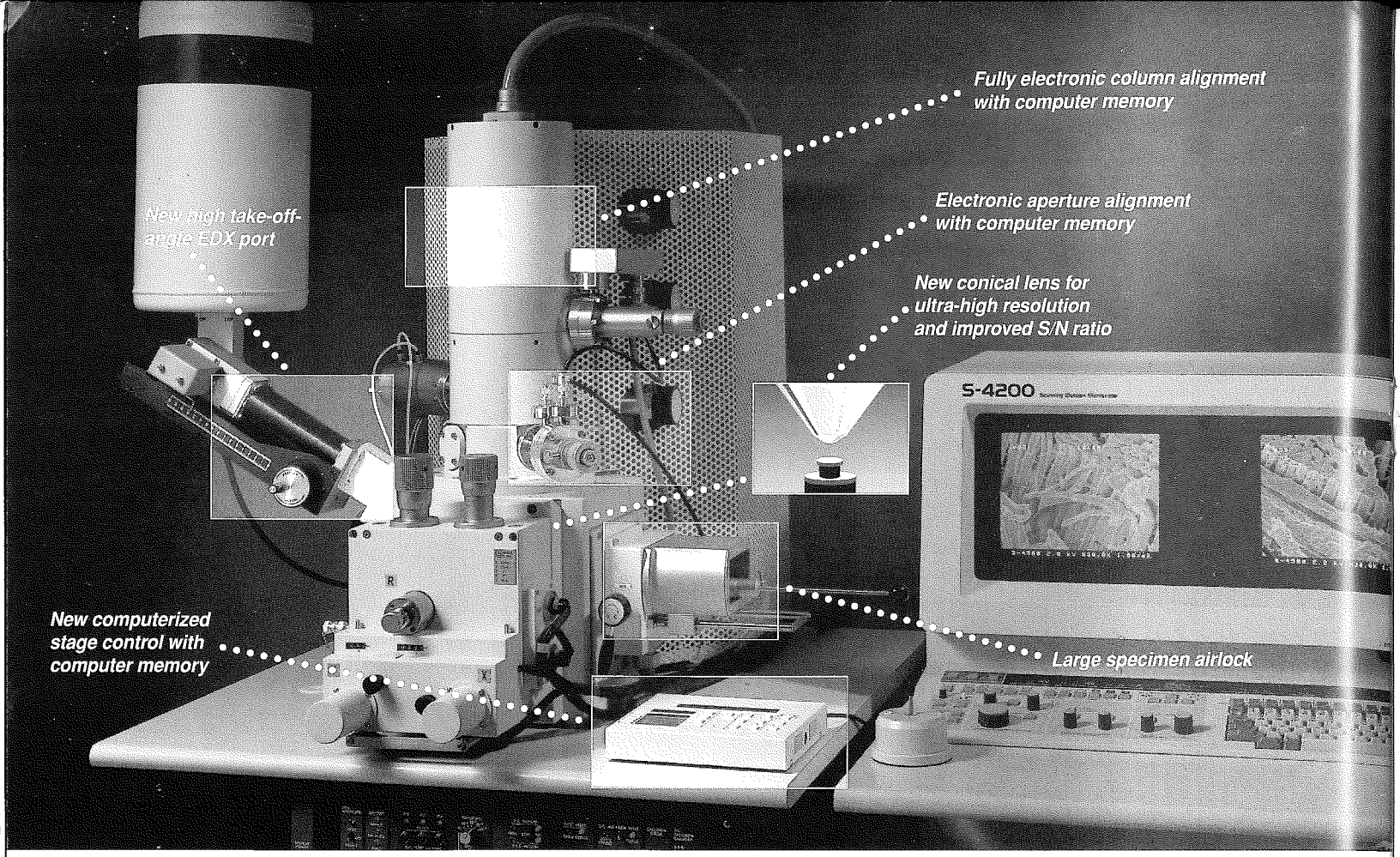
ANSWER TO "WHAT IS IT"

from TSEM JOURNAL 25:2



The micrograph published on the back cover of Volume 25, Number 2, 1994 is a transmission electron micrograph showing parts of two cells in a corn (*Zea mays L.*) root. The upper cell is a developing tracheid (vascular cell) in which four secondard wall thickenings are seen in the process of development. The mircograph shows only a small part of what is a much larger cell. It displays a portion of the nucleus, two or possibly three dictyocomes (Golgi bodies), some ER and many (Golgi) vesicles. Tracheids are devoid of cell contents at maturity and in the non-living condition they function in the transport of water. The secondary wall thickenings help maintain the traceids' shape after death. The lower cell will not develop into a tracheid; it is separated from the upper cell by a compound middle lamella.

Micrograph — H.J. Arnott, Department of Biology and Center for Electron Microscopy, The University of Texas at Arlington.



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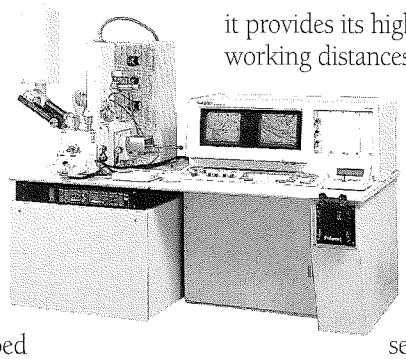

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EVIDENCE FOR PROGRAMMED CELL DEATH IN THE MANDIBULAR ORGAN OF THE LOBSTER, *HOMARUS AMERICANUS*. T.A. MOTLEY, E.F. COUCH, Dept. Biology, Texas Christian University, Fort Worth, TX 76129 and J.K. BUTLER, Dept. Biology, University of Texas, Arlington, TX.

A small population of cells in the mandibular organ of the lobster have been found to be undergoing programmed cell death (apoptosis) with an in situ apoptosis detection kit (Oncor, Gaithersburg, MD). Subsequent tests with a cell death detection ELISA kit (Boehringer Mannheim, Indianapolis) confirmed the presence of apoptotic activity within the gland. The ELISA assays also demonstrated that the apoptotic activity was not uniform throughout the gland. The ELISA studies show that the greatest amount of apoptosis occurs in a lateral region nearest to the "tendon" of the mandibular muscle. Previous work has demonstrated that this gland produces methyl farnesoate, a juvenile hormone-like compound that increases egg yolk production. It is also known that the methyl farnesoate is produced in only approximately one third of the gland. Grossly, the gland appears somewhat like the shape of a partially unfolded fan. The gland itself contains a number cell types, all which appear to be derived from one common stem cell. The stem cells are abundant in the periphery of the gland and are especially common on the latero-anterior aspect of the gland, and area with corresponds with the folds of the fan. These cells are small and have oval nuclei. Deeper into the gland larger and more complex cells are present. Immediately beneath the stem cells is a population of cells containing numerous secretory granules Still further into the gland cells with large masses of smooth endoplasmic reticulum are the dominant cell. Among these cells one frequently finds cells undergoing what appears to be holocrine secretion. However, these cells may be undergoing a form of programmed cell death which might correspond with the histochemical and ELISA data.

AN IMPROVED TECHNIQUE FOR PREPARING PROJECTION SLIDES FROM ELECTRON MICROSCOPY NEGATIVES
Mannie Steglich, Lydia Shanks, and Elsa Ramos, Department of Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

Projection slides of electron micrographs are frequently prepared by directly photographing the print. A black and white negative film used with a reversal process is suitable, but it is more convenient to employ a positive film. With either procedure, the quality of the final slide is directly dependent on the quality and contrast of the print. It is possible to manipulate and often greatly improve the contrast by projecting the electron microscopy negative onto a second, unexposed negative. This method also allows greater flexibility in composing the projection slide. We have found that selection of the type of sheet film is a significant factor in obtaining a projection slide which has good contrast and satisfactory gradation of tones. In the past, the film of choice was in our view DuPont Cronalar Electron Microscope Film. However, several years ago DuPont discontinued production of this film and we began a search for a replacement. After trying numerous combinations of sheet films and developers, we determined that Excelerate 7 Graphics Arts Film manufactured by the 3M Company and developed in Kodak HC-110 diluted 1 part of concentrated HC-110 to 3 parts water yielded excellent results.

A TEM WHOLE MOUNT CELL TECHNIQUE FOR PRESERVING AND IMMUNOGOLD LABELING THE CYTOSKELETON OF CULTURED CELLS. K. WILSON, M.D. MCCARTNEY, S. BROWDER AND A.F. CLARK, Alcon Laboratories, Inc., Fort Worth, Texas 76134

The cytoskeleton of cells plays an important role in numerous cell functions including mobility, phagocytosis and structural integrity. In order to study intact cytoskeletal proteins, a whole mount cell technique combined with immunogold labeling was modified for use with cultured human trabecular meshwork cells. Cultured cells were grown directly on Formvar covered nickel grids prior to being either extracted with 0.15% Triton X-100 in PHEM (PIPES, HEPES, EDTA, Magnesium Chloride) buffer or left untreated. Following aldehyde fixation, all the cells were subsequently osmicated, dehydrated, critical point dried, carbon coated and examined in a Zeiss CEM-902 TEM. The cells, both extracted and non-extracted, showed the typical arrangement of cytoskeletal fibers. In addition, the cellular organelles of the non-extracted cells could be easily visualized. The cytoskeleton was further examined by immunogold labeling of the microfilaments and microtubules. Cells were lightly fixed, extracted, washed in Tris buffer, blocked with 4% non-fat dry milk and incubated with primary antibodies overnight at 4°C. The cells were then incubated with gold labeled secondary antibodies for 2 hours, washed, aldehyde fixed, osmicated, dehydrated, critical point dried, carbon coated and examined using TEM. The cultured cells showed the normal arrangement of microfilament stress fibers and astral arrays of microtubules labelled with the appropriate antibody. This technique has been used to detect subtle changes in the cytoskeleton as well as changes in antibody labeling affinities in response to changes in the culture conditions. The ability of this technique to preserve the intact cell cytoskeleton has allowed it to become an important tool in dissecting the role of the cytoskeleton in the disease process.

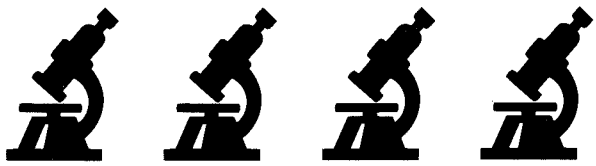
ULTRASTRUCTURAL CHARACTERISTICS OF CELL WALL-DEFICIENT FORMS OF ENTEROBACTER CLOACAE. RENE D. MASSENGALE, THOMAS W. HUBER[1], AND SALLY W. JACKSON, Institute of Biomedical Studies, Baylor University, Waco, TX 76798, and [1]A&M College of Medicine and Olin E. Teague VAMC, Temple, TX 76504.

Enterobacter cloacae 55M is a gram-negative bacterium that constitutively produces beta lactamase. Strains of Enterobacter can be converted to the colony-forming, cell wall-deficient state (CWD) when grown in osmotically-protected conditions in the presence of ticarcillin. Brain heart infustion medium containing 10% sucrose and ticarcillin was inoculated with E. cloacae 55M and incubated under aerobic and anaerobic conditions for periods of 2 and 9 days. Control cultures were prepared by the same method without the ticarcillin. Samples of each of the cultures were prepared for electron microscopy by conventional fixation and freeze substitution. The CWD colonies that grew in the medium containing ticarcillin were compared to the control colonies isolated from the medium containing no ticarcillin.

Control cultures contained cells with the characteristic rod-shaped appearance of E. cloacae. Both the aerobically and anaerobically-grown CWD colonies contained both normal bacilli and CWD cells within the same colony. However, these cell types were mostly confined to separate locations within the same colony. The CWD cells were characterized by a spherical shape and the disruption or absence of the outer membrane (OM). Extracellular material, containing many small blebs and sections of outer membrane, surrounded the CWD cells. This material was found in greater concentrations in the CWD areas of the anaerobic colonies. Furthermore, these blebs and OM segments were present in much lower concentrations among normal bacilli forms in both CWD colonies and control colonies. The presence of the normal bacilli within a CWD colony suggests that some cells revert after a period of exposure to the ticarcillin or that some resistant forms within the colony give rise to normal progeny after ticarcillin levels decrease.

CELLULAR MORPHOLOGY OF THE TERMINAL SPINE OF *AGAVE*. T. S. SHERMAN, H. J. ARNOTT, AND L. E. LOPEZ, Dept. of Biology and Center for Electron Microscopy, Univ. of Texas at Arlington, Arlington TX 76010.

Agave plants are well known as an important source of fibers. These fibers, which consist of phloem fibers and vascular bundles, provide support to these xerophytic plants and converge at the leaf terminus to form a hard and inflexible spine. This research is an attempt to understand the relationship between the cells of the spine and those of the subterminal softer tissues of the leaf. Terminal spines from various stages in leaf development were excised from the leaves and sectioned. These sections were prepared with a standard fixation process using glutaraldehyde/OsO_4 buffered with Sorensen's. Fixed sections were then dehydrated in ethanol, critical point dried, sputter coated with Au/Pd, and mounted for observation with a scanning electron microscope. Significant changes in cellular morphology were visible, especially in the leaf parenchyma cells. Compared to leaf parenchyma cells, spine parenchyma cells in all stages of development exhibited a considerably thickened secondary wall. Spine parenchyma cell walls tended to thicken from the epidermis toward the interior longitudinal axis of the spine; likewise, a similar pattern of secondary wall development was observed from the tip of the spine towards its base. Considerable elongation of parenchyma cells in the spine was observed as well. Vascular bundles in the hard spine were less distinctive than those in the relatively soft leaf because of the thickened parenchyma cells. Phloem fibers were numerous and well defined in the leaf. Phloem fiber wall thickening occurred but was less dramatic than parenchyma wall thickening. Many cells in mature spines exhibited extensive 2° wall development to the point of nearly complete occlusion of the cell lumen. Knowledge of hard tissue development in this plant may aid in the understanding of similar developmental processes in other monocotyledons.



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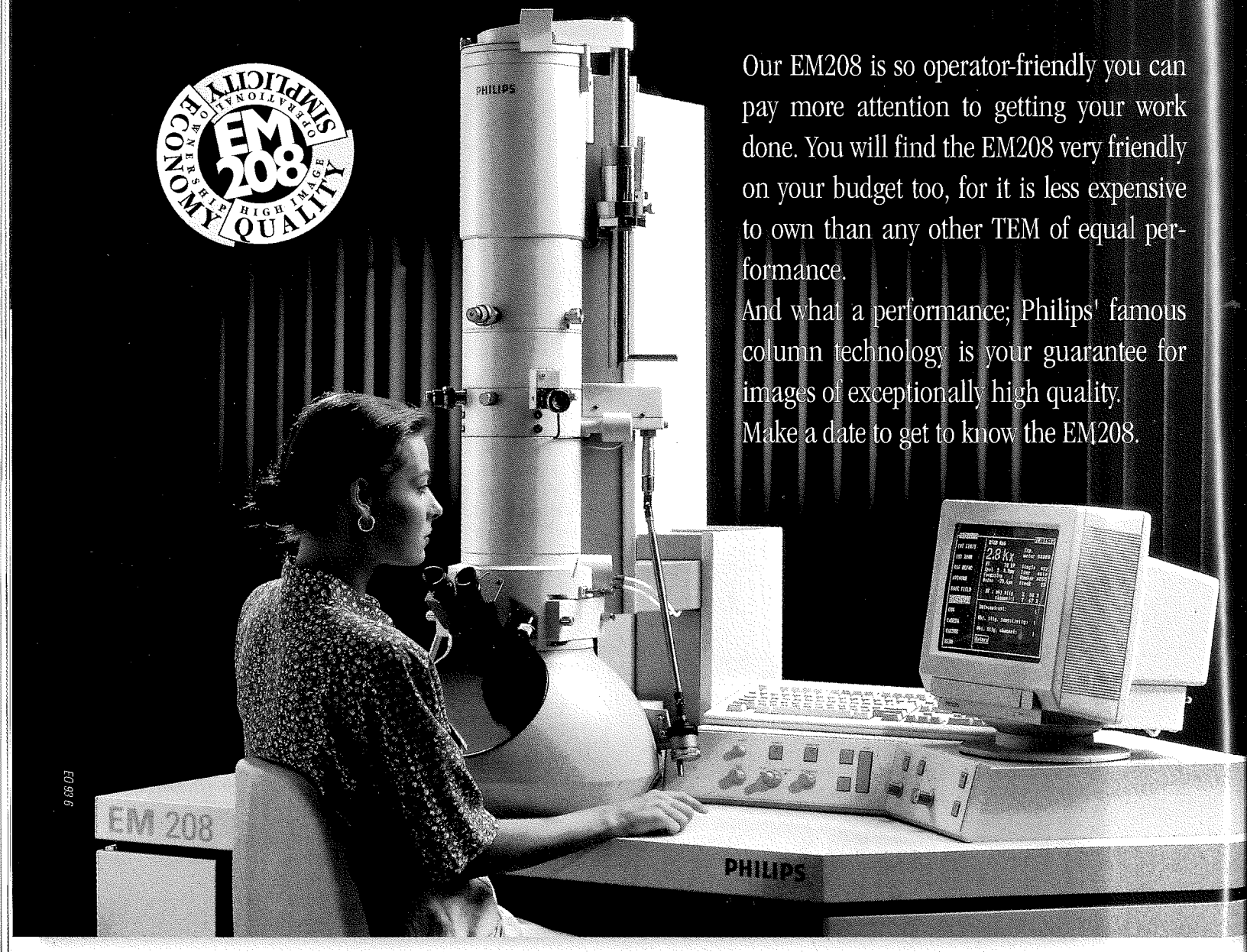

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COMPARATIVE MORPHOLOGY OF SENSILLA STYLOCONICA IN THE PAPILIONOIDEA (LEPIDOPTERA) USING SCANNING ELECTRON MICROSCOPY - A PRELIMINARY REPORT. D. Petr and K. W. Stewart, Department of Biological Sciences, University of North Texas, Denton, TX 76203.

The distal proboscis of many lepidopteran taxa is equipped with peg-like sensilla styloconica which form a brush-like structural arrangement. Although these sensory structures can be seen with a light microscope, their fine morphological detail and diversity can best be studied using the Scanning Electron Microscope. Sensilla styloconica are absent in three representative genera of the Papilionidae, *Papilio*, *Parnassius*, and *Ornithoptera*, suggesting that their presence or absence may run along family lines. This seems to also hold true for representatives of the Danainae in which no sensilla styloconica have been found so far and which are afforded full family status by some. All Nymphalidae genera examined show presence of these sensillae, with tremendous morphological diversity among the species examined. *Heliconius* and *Agraulis* species have a very smooth flattened stylus without any projecting spines or ridges. *Limenitis* species have a smooth flattened stylus with two prominent projections near the sensory papilla, while in *Asterocampa* there is only one projection. In *Nymphalis* we found an orderly ring of seven spine-like projections surrounding the sensory papilla on top of a smooth flattened stylus, and a similar but not quite so orderly arrangement was found in *Vanessa*. Sensilla styloconica in *Cercyonis* are fashioned with seven smooth longitudinal ridges along the entire length of a cylindrical stylus terminating in small processes. *Oeneis* and *Erebia* exhibit serrated, flattened ridges (flutes) on a cylindrical stylus. The number of sensilla styloconica varies with species. The genus *Agraulis* has only a sparse arrangement of 26 sensillae, while a very dense arrangement of 188 was found in genus *Limenitis*. Representatives of Pieridae, Lycaenidae, and Libytheidae have yet to be examined. The morphological diversity of sensilla styloconica points to taxonomic significance and the development of a working nomenclature will be useful in taxonomic work involving Lepidoptera in general. Possible correlation of these sensillae to adult feeding preference will be examined.

A STUDY OF ISOLATED CALCIUM OXALATE CRYSTALS FROM THE LEAVES AND STEMS OF THE VIRGINIA CREEPER (*PARTHENOCISSUS QUINQUEFOLIA*). AMY J. JEFFREYS AND HOWARD J. ARNOTT, The Department of Biology and Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

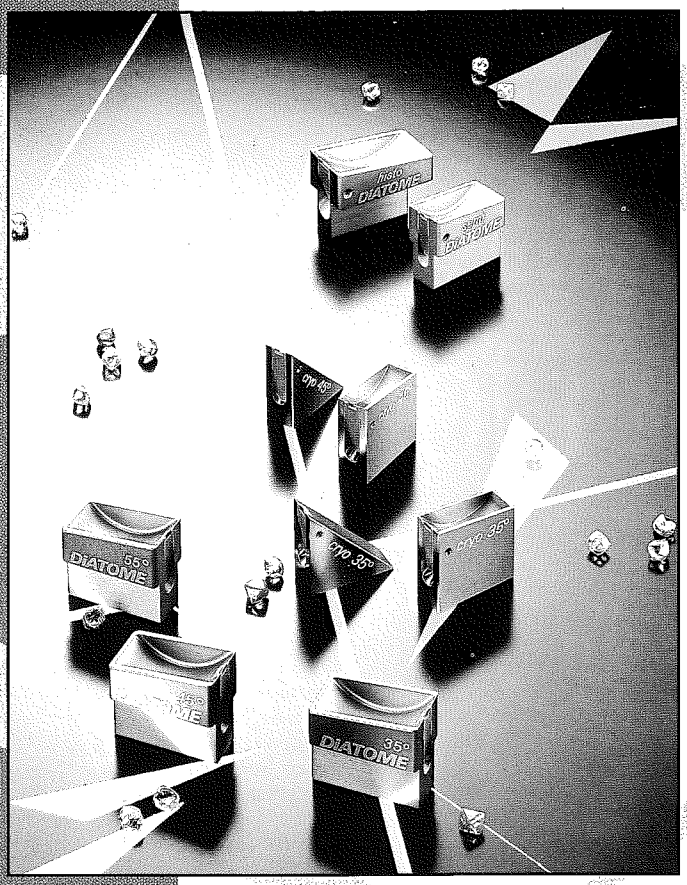
Raphides, druses, and prismatic forms of calcium oxalate were isolated from young and mature Virginia Creeper leaves and stems. Thin, needle-like, monohydrate, raphide crystals are found more frequently in the leaves than other types of crystals, multi-interpenetrant twin druses are found more frequently in the stems, and prismatic crystals are found least frequently in both types of tissues. All types of crystals are birefringent when viewed with polarized light using crossed nicols. Each raphide crystal cell contains an organized bundle which consists of 300-400 twin crystals, each having a bidentate end and a pointed end, enclosed within a water-soluble protein/carbohydrate matrix. The matrix usually extends beyond the length of the crystals and may be thicker at the ends of the bundles. Some bundles exhibit "dimples" which may either be indentations in the outer matrix or extend completely through the outer matrix thus exposing the crystals. It is possible that all bundles have such a dimple, however, since we cannot see the entire surface in SEM preparations, many bundles appear not to have dimples. Druses and prismatic crystals both lack an elaborate external matrix but possess a thin sheath-like covering. In normal raphide cells a bundle of crystals is arranged along a single axis with bidentate and blunt ends facing both directions. In a few crystal cells needle-like crystals are not arranged along an axis but rather extend in many directions forming a "nest-like ring" of needle crystals. We call these cells "nested raphides" and believe they may represent cells in which the normal matrix has not been produced. The lack of matrix and the irregular orientation of the crystals may be evidence that the matrix is directly involved in the process of axial crystal orientiation found in normal raphide crystal cells.

A LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDY OF THE ROLE OF THE PERICARP IN THE DEHISCENCE OF THE FRUIT OF *AESCULUS PARVIFLORA*. S. C. WILLIAMS, Department of Biology and Center for Electron Microscopy, The University of Texas at Arlington, TX, 76019.

The fruits of *Aesculus parviflora* contain one, two, or three seeds that are expelled during dehiscence. Utilizing light and electron microscopy, pericarps of the fruits were examined during the early, middle, and late stages of the dehiscence process. In *A. parviflora* the pericarp is composed of three (occasionally two) valves joined at their margins by regions called sutures; the latter are zones of weakness where the valves break apart during dehiscence. In addition to splitting at the sutures, the pericarp valves also separate. The dehiscence mechanism in the pericarp involves a complicated structure composed of specialized cells and tissues; the physical force responsible for the splitting and separation of the valves is provided primarily by loss of water. Specimens were fixed in FAA, a mixture of formalin, acetic acid and 70% alcohol, sectioned, and stained for light microscopy with fast green and safranin. Specimens for electron microscopic evaluation were freeze-fractured and critically-point dried using ethanol as the transition fluid. Despite the presence of small intracellular air spaces the thick walled cells of vascular bundles remain intact throughout the drying process. However, in the intervening parenchyma, intracellular air spaces are common and represent a beginning point for cavity formation. A trend of cell shrinkage and schizogenous cavity formation begins as the pericarp wall starts to dry, this process increases during the drying process and causes the pericarp to unfold and eventually causes the ejection of the seed from the pericarp. Polarized light microscopy revealed that druse and prismatic crystals of calcium oxalate were present in numerous parts of the pericarp.

A LIGHT AND ELECTRON MICROSCOPIC INVESTIGATION OF THE PERICARP OF CITRUS FRUITS. S. C. WILLIAMS, M . R. JOHNSON, M. A. DAVIS AND H. J. ARNOTT, Department of Biology and Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

The pericarp (peel or rind) of common commercially-grown citrus fruits possess oil glands which contain extremely volatile oils. A major component of this oil is *limonin* which is present in a greater or lesser proportion in most citrus fruits and other members of the Rutaceae. However, the quantity and apparent flammability of these oils differ in magnitude between fruit types. The volatile oils from oranges, tangerines, lemons, limes, grapefruit, and tangelos were tested for flammability. The peels of each of the above were examined using light (LM) and scanning electron microscopy (SEM) to probe the distribution, size, position and other characteristics of the oil glands. Specimens for LM were prepared by 3% glutaraldehyde fixation, dehydrated in ethanol, embedded in paraffin, sectioned and stained. Specimens used for SEM were fixed using 3% glutaraldehyde and osmium tetroxide followed by critical-point drying using liquid CO_2 as the transition fluid. Some specimens were prepared by osmium tetroxide vapor fixation. We found that the pericarp exhibited many stomata on the surface and that the number of oil glands per mm^2 varied between species. The apparent distribution of calcium oxalate crystals also varied, being high in lemon and low in tangerine. When the pericarp was dried or otherwise dehydrated, numerous needle-like crystals could be observed by LM or SEM. These were single and multiple twinned arrays of crystals and according to the literature are an orangic compound, hesperidin. Using LM it was possible to observe the process of hesperidin crystallization.



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THE DEVELOPMENT OF DICTYDINE GRANULES IN *LINDBLADIA*. HOWARD J. ARNOTT, LINDA E. LOPEZ, HAROLD KELLER[1] AND TAKAMI HATANO[2]. Dept of Biology and Center for Electron Microscopy, Univ. of Texas at Arlington, Arlington, TX 76019, [1]Dept. of Microbiology and Immunology, Univ. of North Texas Health Sci. Center, Fort Worth, TX, 76107 and [2]Biology Dept. Mie Univ., Tsu City, Mie Pref. 514, Japan.

Dictydine granules occur in only three genera of the mxyomycetes and are one of the defining characteristics of the family Cribrariaceae (Liceales). As a part of a taxonomic/morphologic study, we have been using LM and SEM to investigate the structure of these granules in the monotypic genus *Lindbladia* in specimens from locations around the world, including the holotype from Sweden. The holotype was collected in Sweden in 1845 and has been maintained in a remarkable state of preservation for 150 years. The dictydine granules, which are colored when viewed with LM, are minuscule bodies associated with both the inner and outer surface of the peridium (sporangial wall). The dictydine granules are spherical or lobed and vary between 0.7 to 1.8 μm in diameter. Close observation shows that they often have a small hole revealing a hollow center. When it is possible to see fractured granules the wall appears to be about 0.2 to 0.3 μm in thickness. We have found associations with the peridium, particularly with pits in the peridium, that appear to be stages in the ontogeny of the granules. One possible mode of development involves the swelling of the peridium through the formation of a "bubble-like" area which subsequently is detached from the peridium to form the dictydine granule. Stages in the detachment have been observed by SEM. Views of transversely fractured peridium show that it is made up of small granules about 0.1 μm in diameter embedded in a matrix. Fractured views of the dictydine granule walls show a matrix/granule makeup similar to that seen in the peridium further substantiating the relationship between the two.

A FRACTURE METHOD FOR PREPARING ENAMEL SAMPLES FROM EXTRACTED HUMAN TEETH FOR S.E.M. EXAMINATION. ROGER D METCALF. The Department of Biology and Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

Extracted human teeth have traditionally been used in training dental students. Since there are blood-borne diseases prevalent that are not easily treated (e.g. AIDS, Hepatitis B and C), there is a valid concern about the safety of using human materials in any sort of training. It has not been conclusively established if there is or is not the possibility of transmission of these types of blood-borne diseases in aerosols such as may be produced by dental procedures. Researchers that investigate the ultrastructure of hard tissues have the additional concern that various sterilization methods may affect the ultrastructure of the tissue they are studying. A method was developed that can be used to produce two specimens that are negative replicas of each other, and will be used by this student in further studies of sterilization methods to test the null hypothesis H_0: the sterilization methods do not cause measurable changes in the enamel rod structure of the human teeth.

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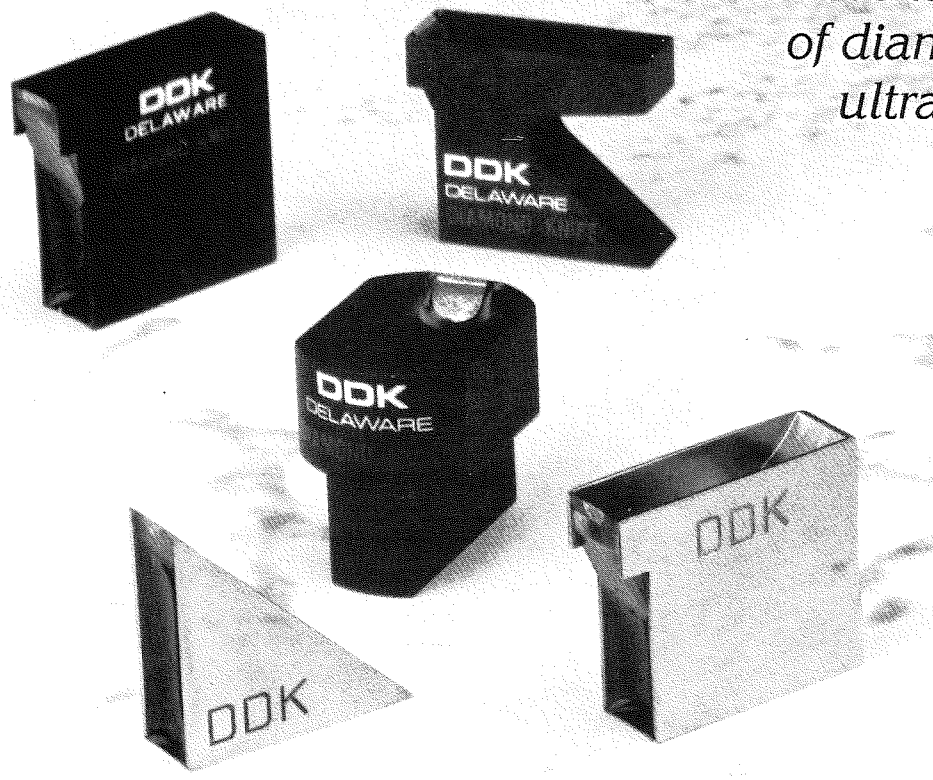

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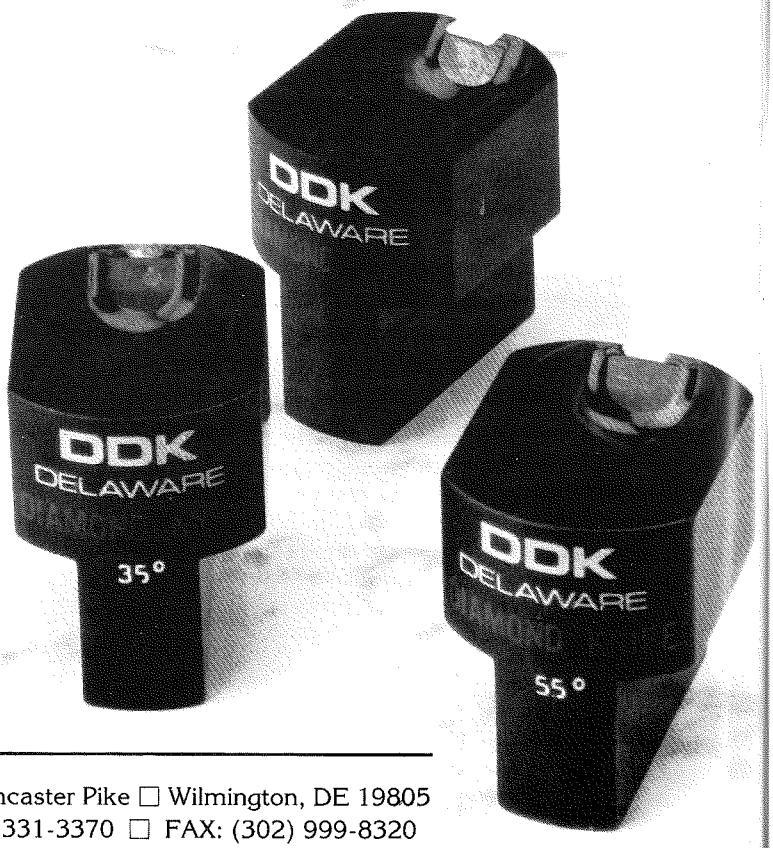

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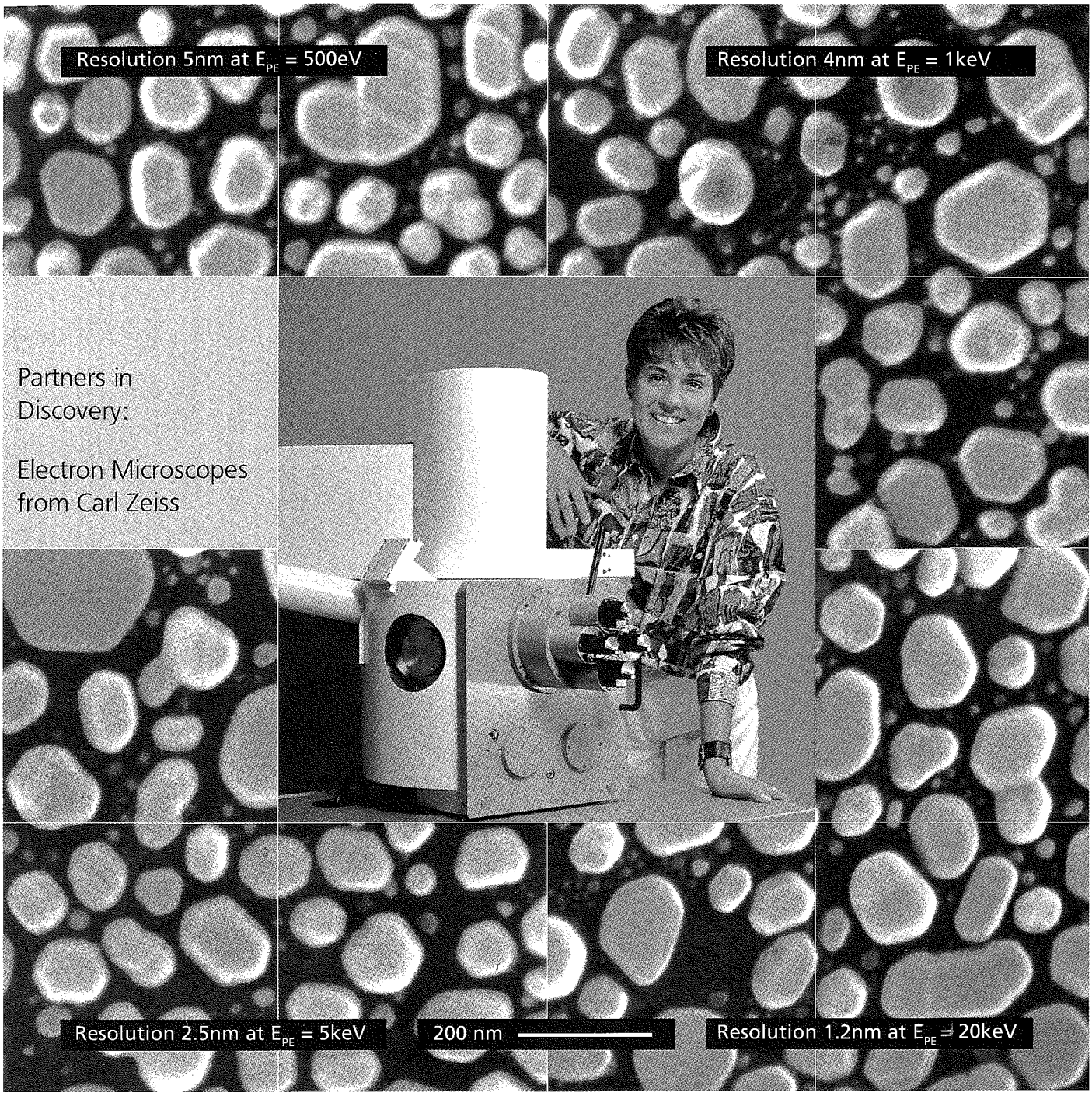

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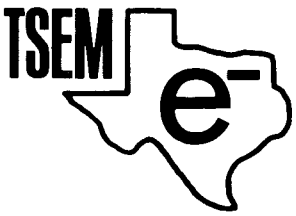

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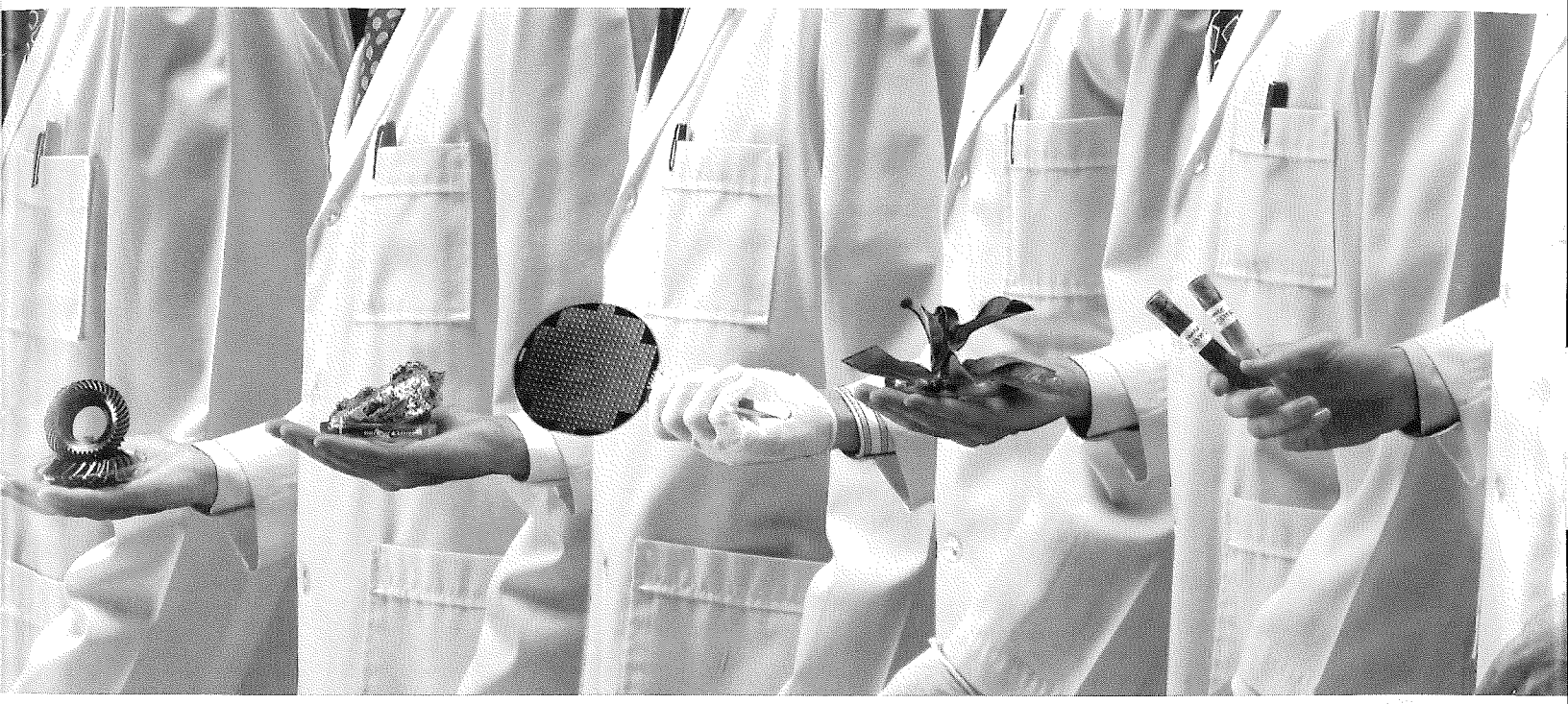

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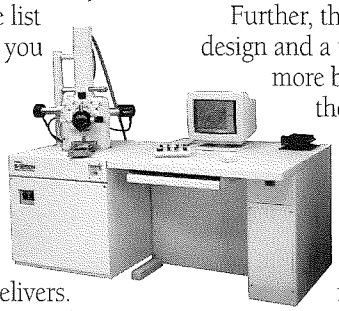

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You also get sharp digital images, with 2048 x 2048, 16-bit pixel resolution on the system's color monitor. Plus advanced image processing power and easy file storage. Simple, mouse-controlled operation. Extensive automation that includes computer-controlled filament saturation and gun alignment. And your choice of models accepting either 6- or 8-inch samples.

Better call or write for details and to arrange a demonstration. See how, with our S-3200N, you'll be more than ready for the next microscopy task. No matter what they throw at you.

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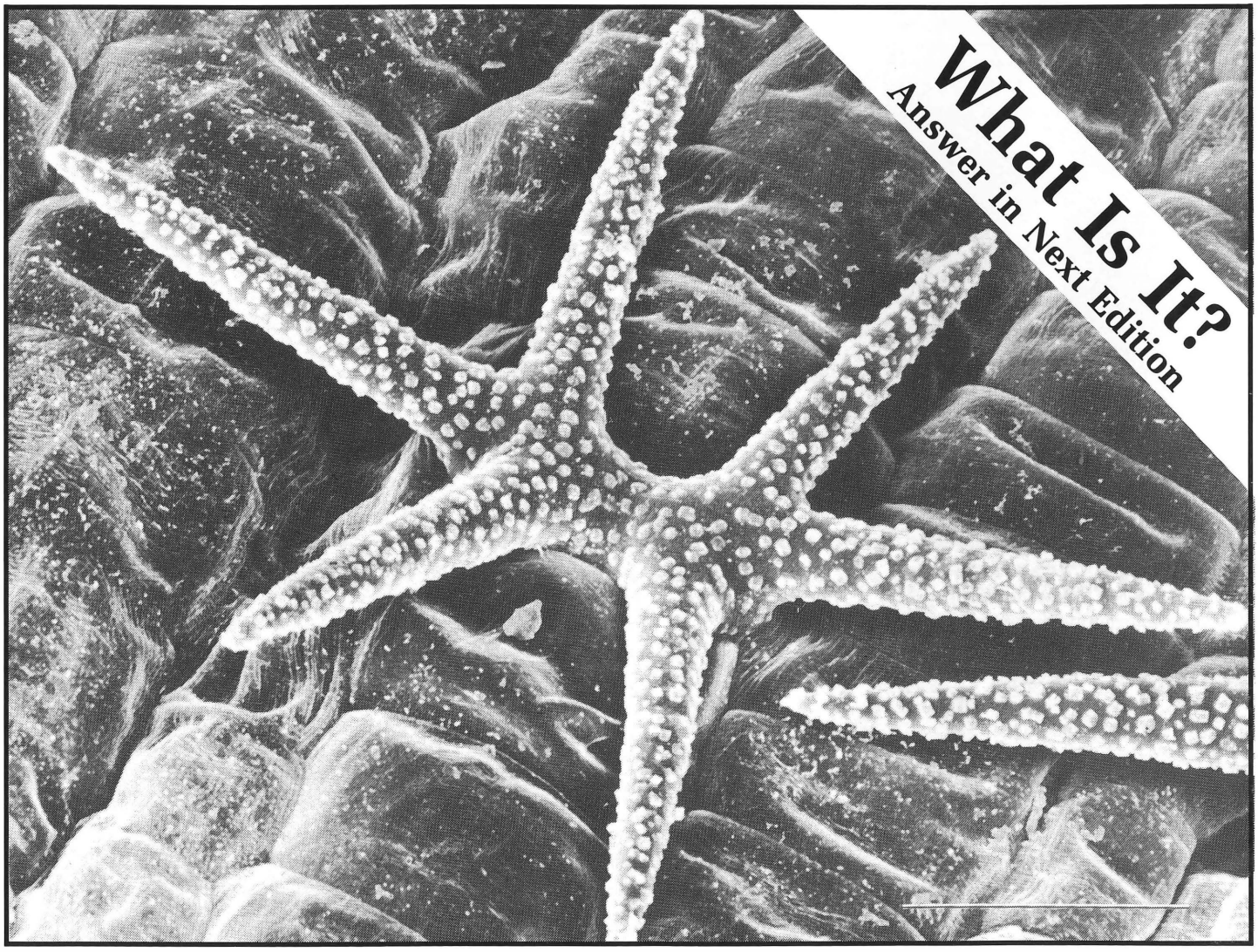

Photo by Michael Pendleton, U.S.D.A. Agricultural Research Service, College Station, TX.Bar = 50μm.