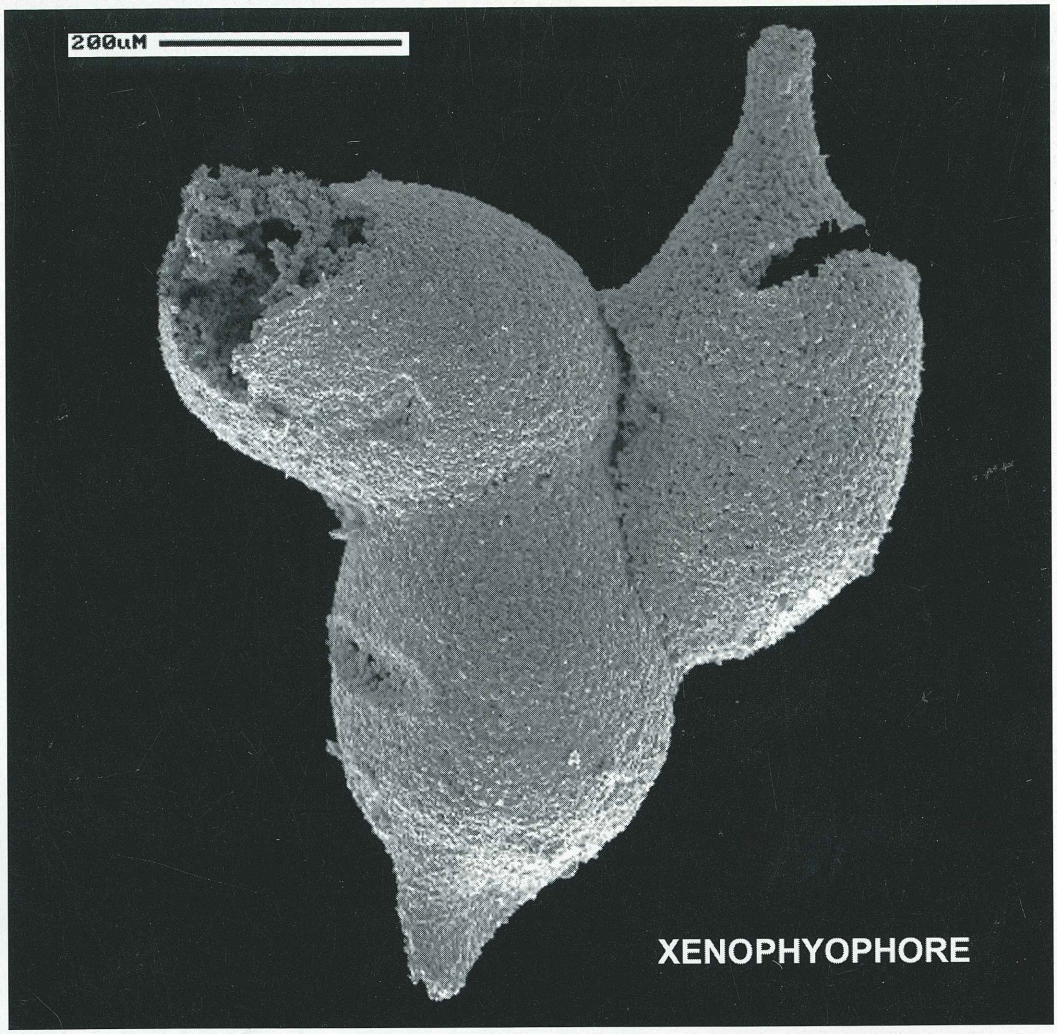
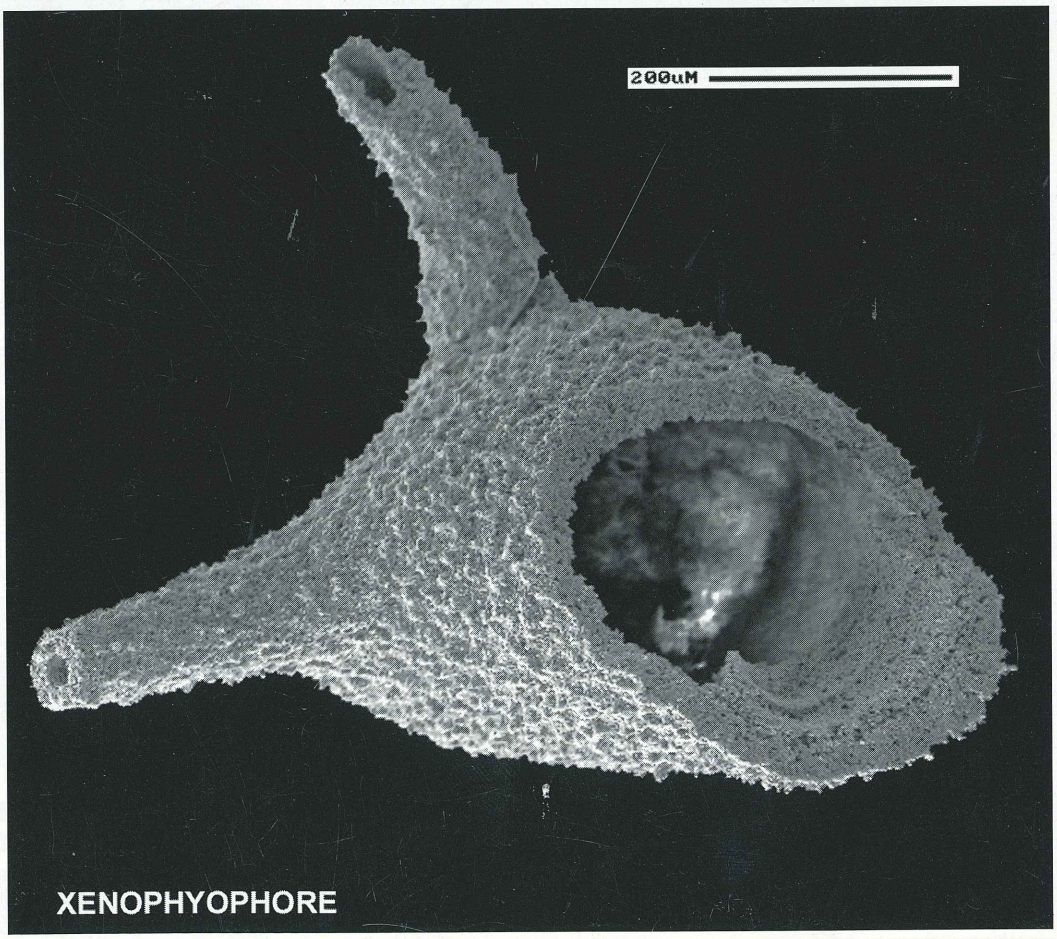
Texas Journal of Microscopy





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Camelia G.-A. Maier, Editor
Department of Biology, Texas Woman's University, Denton, TX 76204

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ON THE COVER

Xenophyophores are marine benthic protozoans, named after their test consisting of "xenophyae", a collection of foreign particles in an organic matrix. Xenophyophores are known to be the largest protozoans with sizes ranging from 1 mm to 25 cm. For more details, see the corresponding abstract on page 43 by G. Nestell, M. Nestell, and M. Gracey. SEM provided by **Martha Gracey**, The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, Texas 76019.

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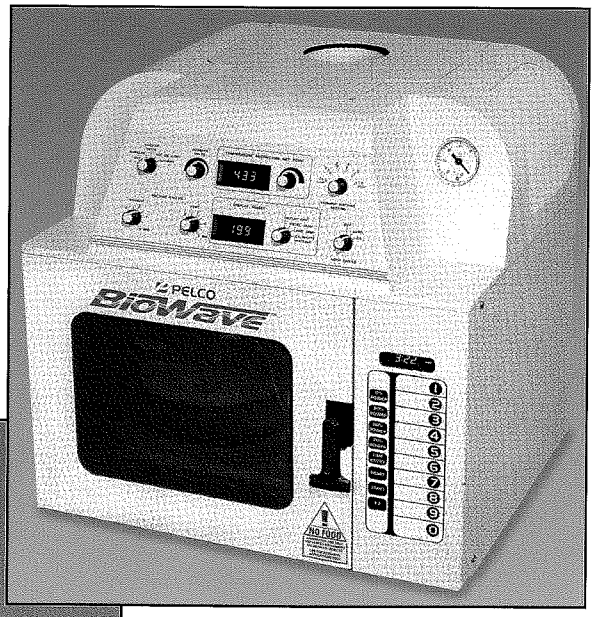

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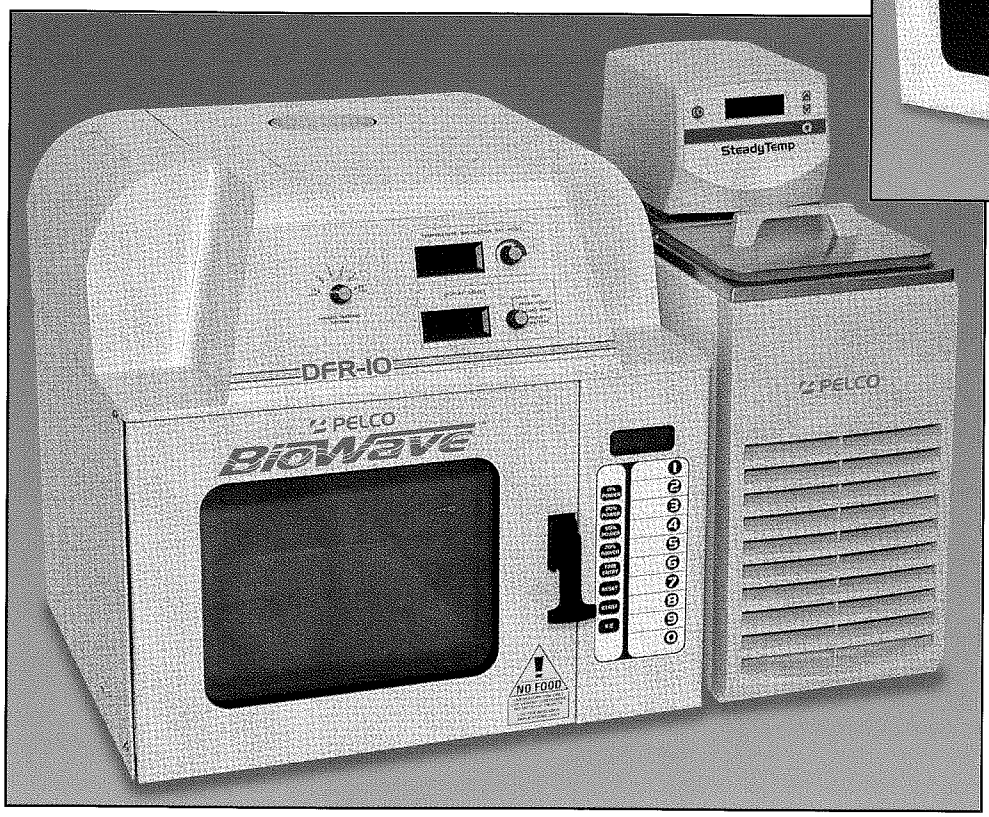

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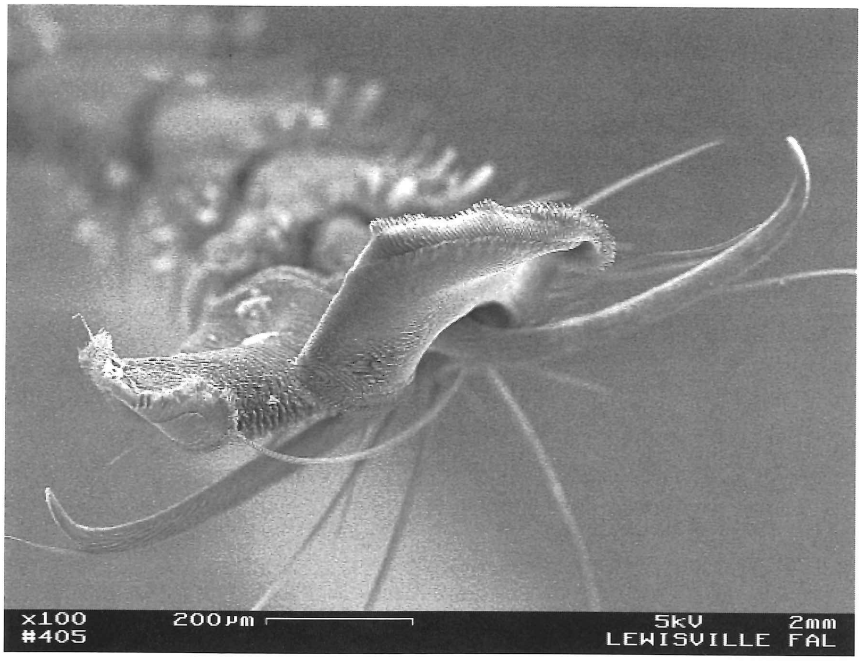
Answer to "What Is It?"

from Texas Journal of Microscopy 35:1



Jodi A. Roeipsch, Raytheon Network Centric Systems, used SEM to visualize the foot of a fly (*Musca domestica*). The fly was coated with gold prior to imaging at 20 keV in a field emission SEM. The proximal segment of the fly's leg shows 'nails' and hairs.

EDITORIAL POLICY

LETTERS TO THE EDITOR

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

MICROGRAPHS AND COVER PHOTOS

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

EMPLOYMENT OPPORTUNITIES

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

TECHNICAL SECTION

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

PUBLICATION PRIVILEGES

The right to publish Abstracts in the TEXAS JOURNAL OF MICROSCOPY is restricted to TSM members or to those whose membership is pending. A membership application form can usually be found in each issue of the TEXAS JOURNAL OF MICROSCOPY. Membership dues are as follows: student $10.00; regular members $30.00; Corporate members $300.00. Research articles are accepted from both members and non-members. Individuals who belong to TSM by virtue of a corporate membership are invited to participate in Journal submissions as are our regular or student members. However, papers of a commercial nature, either stated or implied, will not be accepted for publication as a Research Report or Techniques Paper. Such papers may be acceptable as advertising copies.

President's Message

Let's get fired up! The executive committee has done a tremendous job over the past year, promoting the society and preparing for the future. Our spring meeting in Houston was well attended, with informative presentations by members and invited speakers. Thanks to Program Chairman Susan Robbins for coordinating this successful meeting. Thanks also to Journal Editor Camelia Maier for putting together such an informative and attractive issue of the Texas Journal of Microscopy. Congratulations to Rebecca Johnson from Texas Women's University who received our first Howard J. Arnott Student Presentation Award for her co-authored presentation "Development and performance of silkworms (*Bombyx mori*) as a function of mulberry sexual dimorphism." As always, our student presenters were enthusiastic and inspiring.

Our next two meetings will take place in the Dallas-Fort Worth area. Program Chairman Jodi Roepsch, Secretary Robert Champaign, and Corporate Member Representative Mike Crowley, have been extremely dedicated to promoting the Materials Sciences portion of the Society. Their enthusiasm is contagious. With a workshop and guest speaker in the materials area at the fall meeting, we hope to build on their enthusiasm and attract new members to the Society. I particularly appreciate the efforts of Jodi Roepsch, who took over immediately as Program Chairman without the traditional year-in-training. Susan Robbins, our past Program Chairman, has provided invaluable support and advice during this transition.

Our Spring 2005 40th anniversary meeting is quickly approaching. We have committees that are locating past officers of the society and assembling the history of the society. We hope to have many of our past officers, particularly our Past-Presidents, in attendance. We also are focusing on both regular and corporate membership recruitment. Encourage your colleagues to visit our web site and consider participating in the Society. If you would like to contribute to any of these committees, please volunteer. I urge you to get involved in the activities of the Society. Your efforts will be greatly appreciated.

Ann E. Rushing
TSM President 2003-2004

Call For Papers

Manuscripts are needed for the next edition of the Texas Journal of Microscopy. Please send your work as short communications, full articles or review articles in biological sciences, material sciences or education to:

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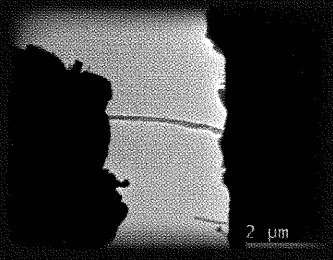
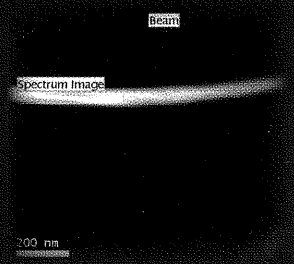
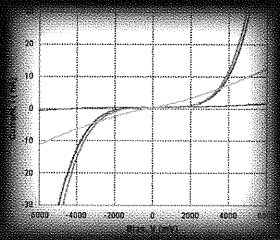


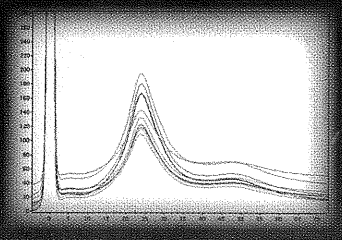
TEM image of the boron nanowire bridging the gap between STM tip and substrate



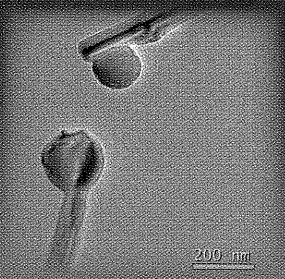
STEM DF image used for EELS SI



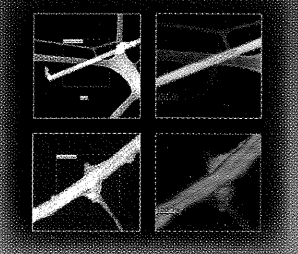
I-V curves acquired from the boron nanowire



EELS SI acquired from boron nanowire



TEM image of the melted boron nanowire at bias of 30V



In-situ nanocharacterization EELS STEM spectrum image

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Abstracts

BIOLOGICAL SCIENCES
FALL 2004

A MICROSCOPIC STUDY OF THE NUTMEG (*MYRISTICA FRAGRANS*) SEED. HOWARD J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019

The seed of the nutmeg (*Myristica fragrans* Houtt.) is a football-shaped brown/black colored oval between 2 to 4 cm in length and having numerous surface grooves. The seed is ruminate, meaning that parts of the seed coat extend into the central perisperm or endosperm (the available literature is not clear about perisperm vs. endosperm). Recently (2002) a nutmeg seed was studied by high-resolution X-ray computed tomographic scanner (X-ray CT), which detailed the complicated nature of the seed coat invaginations and showed the position of the embryo (www.digimorph.org/specimens/Myristica_fragrans/). The current study investigated the cellular nature of the seed using light and scanning electron microscopy. For this study we used seeds of the "current crop" and two (remnant) seeds approximately 20 years old. In both age groups the invagnations of the seed coat are composed of large parenchyma cells that average 180μm in diameter and which have modest cellular contents. The cell walls of these cells appear orange in LM. The invaginations, which make up a substantial part of the seed, are attached to the seed coat and expand as they "move" toward the center of the seed where they appear to branch and anastomose. The tissue between the invaginations is composed of smaller parenchyma cells averaging about 100μm in diameter. Unlike the cells of the invaginations, the latter are packed with "storage products" many of which are starch grains which stain blue with IKI. The starch grains occur as single, double, and multiple grains; the single starch grains are round and average about 9μm in diameter. The single and double starch grains produce "normal" birefringence patterns using polarization optics. However, the multiple grains have bizarre patterns. These cells also contain many small birefringent crystals, unstained by IKI, as well as larger bodies which stain pale yellow in IKI. Some individual cells in this tissue contain large orange pigment granules. Cell walls seen in profile have small secondary wall thickenings extending a short distance into the cell interior.

LOSS OF NORMAL MORPHOLOGY AND EXPRESSION OF FLAGELLA BY *CAMPLYOBACTER COLI* RESULTING FROM CONTINUOUS PASSAGE *IN VITRO*. ROBERT E. DROLESKEY, RICHARD L. ZIPRIN, MICHAEL E. HUME, CYNTHIA L. SHEFFIELD, KATHLEEN ANDREWS, ROGER B. HARVEY and DAVID J. NISBET. USDA, ARS, Southern Plains Agricultural Research Center, College Station, TX 77845

Campylobacter coli cells maintained by continuous *in vitro* passage are routinely evaluated by scanning and transmission electron microscopy before they are utilized in both *in vitro* and *in vivo* experiments. Examination of cultures prior to one experiment revealed the presence of a culture composed of cells that lacked the normal spiral-helical morphology of *C. coli*. Cells at this sampling were uniformly present as straight rods with flagella. Bacteria from subsequent passages, in addition to having the appearance of straight rods, lacked expression of flagella. For comparison purposes, a culture of the isolate in question was initiated from cryopreserved cells frozen at the time of its original isolation ,and evaluated using both SEM and TEM. Cells from the reestablished culture displayed normal spiral-helical morphology with polar flagella. Polymerase chain reaction analysis of specific genomic sequences and Riboprinting™ analysis of ribosomal RNA sequences from both the original isolate and the altered strain were performed to document the relatedness of the two cultures. Analysis by these techniques indicated that both cultures were of the same linage. Repeated observations of the altered *C. coli* culture from subsequent passages have shown that these bacteria possess a stable change in morphology and flagellar expression. Additionally, our findings suggest caution when relying strictly on morphology as a sole criterion for the identification of suspect *C. coli* isolates.

HISTOLOGICAL, ULTRASTRUCTURAL AND MICROARRAY COMPARISONS BETWEEN C3H MICE FED SODIUM ARSENATE IN DRINKING WATER. LUCIA E. GODINEZ, DONNA M. BYERS, JAIME B. VIGO and JOANNE T. ELLZEY. Biological Sciences, The University of Texas at El Paso, El Paso, Texas 79968-0519.

There is currently a debate concerning the acceptable levels of arsenic in drinking water. C3H mice (12 males and 12 females) were subdivided into six groups corresponding to sex and treatment, and supplied with drinking water containing 0 ppm (controls), 5 ppm, or 150 ppm sodium arsenate for nine weeks. The liver and kidneys of each mouse were fixed in glutaraldehyde and osmium tetroxide,

dehydrated and embedded in plastic for histological and ultrastructural analyses. The cerebellum and basal forebrain were dissected and preserved in RNAlater™ (Ambion) for gene expression analysis. Gene expression was analyzed in the diencephalons by DNA microarray hybridization. A total of 75 genes were differentially expressed in control versus 5 ppm arsenic exposure, while 43 genes were differentially expressed in control versus 150 ppm arsenic. In the differentially expressed genes, the low dose group genes were generally up-regulated and the high dose group genes were generally down-regulated. Characteristically, organelles such as mitochondria, peroxisomes and the smooth endoplasmic reticulum (SER) are known to swell under adverse conditions, resulting in an increase of the amount of cytoplasm occupied by the organelle (volume density). ANOVA results showed no significant differences in volume density among these groups with regard to mitochondria, peroxisomes and smooth endoplasmic reticulum. Blood glucose levels and body weight were recorded weekly, and though trends were detectable, no significant changes were observed. cDNA microarray analysis of the diencephalon, a center of metabolic control, was performed for each animal, and 4 genes among the most highly affected by arsenic exposure were confirmed by qPCR.

FUNGAL ALLERGENS OF THE TEXAS PANHANDLE: A RISK FACTOR FOR ALLERGIC RHINITIS. DEB MAHAPATRA[1], EDWARD CARAWAY[1], NABARUN GHOSH[1], CONSTANTINE SAADEH[2], MICHAEL GAYLOR[2] and DON W. SMITH[3]. [1]Department of Life, Earth and Environmental Sciences, West Texas A& M University, Canyon, TX 79016. [2]Amarillo Center for Clinical Research/ Allergy A.R.T.S. 6842 Plum Creek Drive, Amarillo, TX 79124. [3]Biology, University of North Texas, Denton TX 76203.

Besides pollution due to ever-increasing industrial growth, aeroallergen has become one of our prime concerns since the last few decades. A wide gamut of aeroallergens has been implicated to cause serious respiratory ailments mainly in young adults such as allergy and asthma, affecting millions of people each year. Among them, various types of pollen (tree, weed and grass) have enticed the scientific community and us in the past. In our present study, special emphasis was given on the previously ignored fungal allergens. Sensitivity to fungi is a significant cause of allergic diseases, and prolonged exposure to fungi is a growing health concern. Sampling of the aeroallergens was done with a Burkard Volumetric Spore Sampler (Burkard Scientific, U.K.), installed on the roof of the Agriculture and Natural Sciences building at West Texas A&M University, Canyon. The most significant aeroallergens found during this study were fungal spores from *Alternaria*, ascospores from Pezizales, *Dreschlera*, *Cladosporium*, *Curvularia* and *Pithomyces*. On careful analysis of data, the fungal spore count was found to have a positive correlation with wind speed, rainfall, maximum and minimum temperature and most vital of all incidence of patients at clinics with allergic rhinitis.

CHANGES IN JAPANESE QUAIL EGGSHELLS EXPERIENCING SPACE FLIGHT. SANDRA WESTMORELAND*, TINA HALUPNIK*, and PATRICIA HESTER**. *The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, Texas 76019; **Purdue University, Department of Animal Science, Lafayette IN 47906.

As part of a program of study on the effects of space flight upon the incubation of Japanese quail embryos, we received thirty-two eggshells harvested from fertile eggs interrupted at approximately twelve days of incubation. There were five experimental groups. Ten of the eggshells had gone into space aboard Space Shuttle STS-108 on December 5, 2001, a flight lasting eleven days. Two experimental groups were created from the flight eggs: four eggs were exposed to zero gravity conditions, and six were spun in a centrifuge at a constant 1 G. A special incubator, developed for the space shuttle, was programmed to bring the eggs to incubation temperature after a stable orbit had been achieved, maintain a constant humidity, and turn the eggs 180 degrees once an hour. The third and fourth experimental groups contained nine eggs that had been placed in a ground incubation unit identical to that flown on the space shuttle. Six of these eggs were kept stationary at 1 G, and the other three were spun, resulting in variable Gs of force. The final group of thirteen eggs was incubated in a standard laboratory. We predicted that none of the eggshells would exhibit evidence of calcium loss when viewed with scanning electron microscopy. Our previous SEM study of Japanese quail eggshells had not revealed any visual evidence of calcium loss until day fourteen of incubation. To our surprise, all of the examined eggshells exhibited some degree of calcium loss from their mammillary cones. Since all of the eggs, flight and ground controls, exhibited apparent calcium uptake by the embryos, it was not possible to assign any spaceflight effects to the appearance of the mammillary cones in the day twelve eggs.

A STUDY OF THE PELAGE OF THE *MAMMUTHUS PRIMIGENIUS*. MARTHA GRACEY. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, Texas 76019

Wooly mammoths, or *Mammuthus primigenius* is a well-known cold climate resident. Equipped with a thick shaggy coat and a layer of thermal fat for insulation against the bitter winter weather, mammoths roamed the frozen northern portions of the world grazing on tundra vegetation. The *primigenius* was smaller than most mammoths, measuring about nine feet high where others, particularly the Columbian mammoth, *Mammuthus columbi* measure in at roughly eleven to fourteen feet high. The precursor to modern elephants, mammoths have been located in Russia, South Dakota, and Canada to name a few locations. A small sample of mammoth hair from Taimyr, Siberia near the Artic circle region of Russia, was obtained and three types of microscopy were performed on it in the most nondestructive method possible. Nothing conclusive can be determined from this experiment but there was the pleasure of looking at hair that was about 10 to 20,000 years old.

XENOPHYOPHOREA (PROTOZOA) FROM THE UPPERMOST MIDDLE PERMIAN OF THE APACHE MOUNTAINS, WEST TEXAS. GALINA NESTELL, MERLYND NESTELL, and MARTHA GRACEY. Departments of Geology and Biology, The University of Texas at Arlington, and The Center for Electron Microscopy, Arlington, Texas 76019.

The xenophyophores are marine benthic protozoans presently living in deep zones of the oceans of the World. They are characterized by various shapes of their body: spherical, discoid, dendritic, and foliaceous. Some resemble a friable sponge. The xenophyophores are the largest protozoans known and their size varies from 1 mm to 25 cm. The body of the xenophyophores is organized as a multinucleate plasmodium enclosed by a branched tube system of organic origin ("granellare"). Their tests consist of "xenophyae" – a collection of foreign particles held together by an organic substance. These xenophyae can be composed of foraminiferal tests, radiolarian skeletons, sponge spicules or mineral grains. Documented findings of the xenophyophores in the fossil record are rare. There are some data about their presence in the Upper Devonian of Missouri and Illinois of the USA, and the Zechstein deposits (Upper Permian) of Germany. Tests of xenophyophores were recently discovered in strata of the uppermost Middle Permian (Capitanian Stage) from Seven Heart Gap, Apache Mountains, West Texas. The specimens found are referable to the genus *Aschemonella* Brady, 1879 of the family Syringamminidae Tendal, 1972 of the order Psamminida Tendal, 1972. Their test consists of a series of elongate and rectangular chambers arranged in a single row or branching series. Each chamber has one or two long necks with a rounded aperture at the ends. Tests are attached or free. These xenophyae consist completely of quartz grains. The xenophyophores are indicative of deep sea environments. The Middle Permian strata in which they are found in the Apache Mountains are clearly turbidites deposited on a down slope part of the Delaware basin.

RELATIONSHIP BETWEEN MORPHOLOGY OF COWPEA GRAIN AND RESISTANCE TO COWPEA WEEVIL. F. M. CHITIO[1], B. B. PENDLETON[1], T. C. STEPHENS[2], and M. W. PENDLETON[2]. [1]Division of Agriculture, West Texas A&M University, Canyon, TX. 79016-0001, [2]Microscopy and Imaging Center, Texas A&M University, College Station, TX. 77843-2257.

In the tropics and subtropics, the cowpea weevil, *Callosobruchus maculatus* F., is an insect pest of cowpea, *Vigna unguiculata* L. Cowpea pods (in the field) and cowpea grain (in storage) may be infested by this weevil, which attaches eggs to the outer surface of the grain. After hatching, larvae chew into the cowpea grain for two weeks to six months. Following development, larvae chew from the interior of the grain to an area just below the grain's surface, leaving a transparent layer. After pupation, the adult emerges through this layer. Six or seven generations of adult weevils are produced each year. Cowpea weevils can damage 100% of stored grain, causing a loss of as much as 60% of grain weight. The objective of this research was to compare the amount of damage caused by the weevil to the different morphological traits of the seed coat outer surface and to the palisade of malpighian cells of different varieties of cowpeas by SEM. Resistance of cowpeas was determined by placing five newly emerged cowpea weevils with 10 g of a single variety of cowpea grain in each of 10 vials. The vials were evaluated for damage every three weeks for five times (105 days total). Factors determining resistance to damage included number of eggs produced, weight loss, and number of emergence holes in the grains. Before observation by SEM, a razor blade and a small hammer were used to split six varieties of non-damaged dry cowpea grains along the boundary between the cotyledons. The split grains were exposed to osmium vapor overnight in a fume hood and then coated with gold-palladium using a Hummer sputter coater. The cowpea structures were observed using a JEOL JSM 6400 SEM at 15 KeV, 12 mm working distances, and magnifications of 500 to 2000x. The structures of the cowpeas observed by SEM were very different in appearance, and this difference was related to resistance to the cowpea weevil. This research was supported by INTSORMIL (International Sorghum and Millet Collaborative Research Support Program), US-AID (United States Agency for International Development), and INIA (Mozambique National Agriculture Research Institute).

ELECTRON MICROCOPY OF FULLY WET SAMPLE. A. Chausovsky. Quantomix, P.O. Box 4037, Nes Ziona 70400, Israel.

Electron microscopy is the prime tool for the investigation of biological ultrastructure. However, its routine use in cell biology and histology is hampered by lengthy and arduous sample preparation procedures. Additionally, a number of artifacts may be introduced during such process. The Wet SEM is a recently developed enabling technology solution that allows direct observation of native samples in conventional scanning electron microscope. The sample is placed in a sealed specimen capsule, and is isolated from the vacuum by a 100 nanometers thick, electron-transparent partition membrane. The Wet SEM technology allows sample imaging to a depth of a few micrometers using backscattered electrons. As a result, wet, un-embedded cells, tissues and non-biological specimens are visualized, and there is no need for thin sectioning and other lengthy preparation steps. We will present a broad spectrum of existing applications and scientific results in a number of independent systems. This enabling technology allows broad application in different fields of tissue engineering, material science, cell biology, diagnostics and numerous aspects of quality control operations.

ASSESSMENT OF AIR QUALITY IN THE PUBLIC SCHOOLS OF THE TEXAS PANHANDLE

EDWARD CARAWAY[1], DEB MAHAPATRA[1], NABARUN GHOSH[1], CONSTANTINE SAADEH[2], MICHAEL GAYLOR[2] and DON W. SMITH[3]

[1]Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, TX 79016. [2]Amarillo Center for Clinical Research/Allergy A.R.T.S., 6842 Plum Creek Drive, Amarillo, TX 79124. [3]Department of Biology, University of North Texas, Denton TX 76203.

Children in Amarillo and Canyon, Texas spend at least 1260 hours per year in school. The occurrence of allergy and asthma in children is on the rise (1). Indoor Air Quality (IAQ) testing can provide data to recommend prevention of the increasing rate of respiratory ailments such as asthma, influenza, rhinovirus or colds associated with air quality and to ensure a healthy environment at the public schools (2). There are many airborne pathogens and allergens that can be quantitatively measured.

Sample Collection and Staining. All the methods of sample collection have their positive and negative aspects. The surface sampling was the method employed in this study, but other methods such as dust sampling, calibrated air instrument sampling and culture sampling are all acceptable (3). Samples of aeroallergens were collected from the air vents, air ducts, ceiling and other surfaces where particulate matters settle. Prior to sample collection we prepared slides with the double-sticky tape by placing a 25mm x 15mm pieces of tape (Scotch brand, Office Max) on the microscope slides and placed them in a sealed box to avoid prior contamination. To obtain samples, the exposed tape area of the slide was placed onto the target area. Each school was considered as one experimental unit (EU) consisting of 10 sub units collected from different areas. The frosted side of each slide was labeled with data (date, place). Collected samples were placed in a slide box and preserved for staining, observation and analysis.

A mounting chemical Gelvatol (Burkard Corporation, UK) was mixed with two drops of 2% safranin and was applied to the surface of a coverslip (22x60mm, VWR). The Gelvatol smeared side of the cover slip was placed carefully onto the exposed tape of the microscope slide having aeroallergen samples. Care was taken to avoid the formation of any air bubble and to ensure uniform smearing. Slides were cured for 24-48 hours before observation.

Microscopic analysis. The slides were observed at different magnifications and images were captured using the Microfire digital camera attached to a BX-41 Olympus microscope. Aeroallergens including pollens, fungal spores, dusts, plant fibers, burnt residues, insect debris and plant products like gums and resins were observed (Fig.1A-B). Tapes were analyzed to determine the mean aeroallergen concentration. The mean aeroallergen concentration was determined mathematically by taking a sum total of all traverses and multiplying this sum by a correction factor. Correction factors are microscope-objective specific and are determined prior to counting that could be expressed as the total area sampled divided by the graticule width (4, 5). The most significant aeroallergens recorded from the prepared slides were the fungal spores like *Alternaria* (Fig. 1C), *Dreschlera* (Fig.1D), *Stachybotrys* and *Cladosporium*. *Alternaria* conidia were present in greater quantities in all the slides observed. To our surprise we also found several pollen grains attached to the surface of the ceilings and the air vents. We observed the pollen like grass pollen (Poaceae) (Fig.1F), Short Ragweed (*Ambrosia artemisiifolia*) and Lamb's Quarters (*Chenopodium album*) (Fig.1E). Specific data on aeroallergens that are present in the public schools could reduce diagnosis time and improve treatment and comfort measures for school age children who suffer from asthma and allergies.

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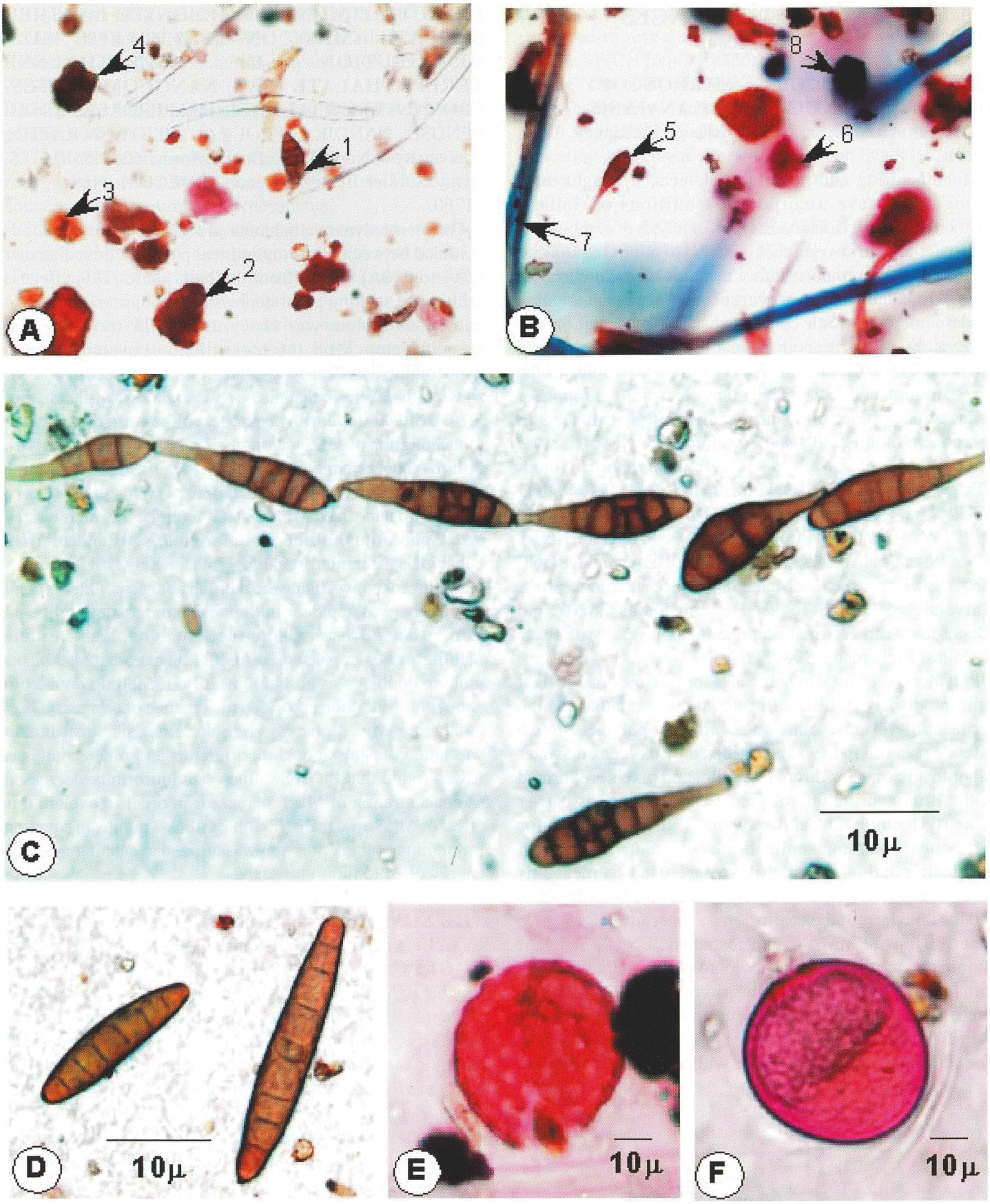


Fig. 1A and B: General views of a slide at 40X showing mold spore (1), gums (2), resins (3), plant debris and burnt materials (4). *Alternaria* conidia (5), gums (6), plant debris (7) and burnt materials (8). **Fig. 1C:** showing a chain of *Alternaria* conidia. **Fig. 1D:** two mature spores from *Dreschlera* sp. **Fig. 1E:** Lamb's Quarters (*Chenopodium album*) pollen (40X). **Fig. 1F:** Grass pollen (*Poaceae*).

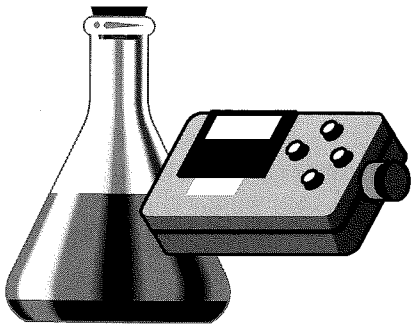
MATERIAL SCIENCES
FALL 2004

THE ROLE OF MICROSCOPY IN MICROELECTRONIC FAILURE ANALYSIS. JAMES IZZO, Failure Analysis Lab, Raytheon, McKinney, Texas 75071

Manufacturing and processing defects, if caught early enough, can save a corporation millions of dollars. Furthermore, fault isolation in failure analysis of components and materials can also guide production to minimize failures. Microscopy is an important tool that aids the failure analyst in quickly and reliably identifying defects or anomalies within modern microelectronic components. This presentation will give a brief background on the fundamentals of microelectronic failure analysis as well as examples of the use of microscopy to detect microelectronic component failures. Examples of vendor defects that can lead to component failure will also be examined.

STUDY OF COATING ON POLYMER IN IMPROVING THE BARRIER PROPERTIES FOR FLEXIBLE DISPLAY APPLICATION. LAXMI K SAHU[1], UNNAT BHANSALI[2], NANDIKA ANNE D'SOUZA[1] and BRUCE GNADE[2]. [1]Department of Materials Science and Engineering, University of North Texas, Denton, Texas 76207 and [2]Department of Electrical Engineering, University of Texas at Dallas, Richardson, TX 75083.

Polymer can be used as substrates for flexible organic light emitting display (OLED). But the major limitation is high permeability of gases and water vapor in polymer. Active cathode material in LED gets oxidized by oxygen and water vapor that permeates through the film and pixels are formed in the LED and lifetime of the LED reduces. This barrier property to moisture and oxygen is also important for different packaging application. Different organic and inorganic coating and multilayer are used to meet this requirement. The barrier property is governed by the quality and the thickness of the coating on polymer substrate. In this paper, 30 nm of PMMA is spin coated on PEN and 30 nm of alumina were sputter deposited onto it. The quality of this transparent barrier film was investigated by optical microscopy, scanning electron and atomic force microscopy (AFM). Defect density and surface roughness was quantified. The defect density was correlated with the barrier properties.



EFFECT OF MONTMORILLONITE LAYERED SILICATE (MLS) ON SPHERULITIC SIZE DISTRIBUTION IN POLYETHYLENE TEREPHTHALATE (PET) NANOCOMPOSITES-FROM INTERFACIAL ENERGY APPROACH. SIDDHI PENDSE[1], NANDIKA D'SOUZA[1], and JO ANN RATTO[2]. [1]University of North Texas, Denton Texas 76203-5310, [2]U.S. Army Soldier Systems Center, RDECOM, Natick, MA 01760.

The thermodynamic behavior of fluids and organic solids confined between glassy cylindrical pores of finite diameter (20Å to 500Å) differs from the bulk phase. This effect is seen as the melting point depression of confined phase. An analogous system was observed in PET (polyethylene terephthalate)- MLS (Montmorillonite Layered Silicate) nanocomposites. Transmission electron microscopy (TEM) showed the average distance between the MLS platelets of 50Å to 39Å depending on the concentration of MLS (concentration of MLS was varied from1% to 5% by weight). Polarized optical microscopy (POM) was used to determine the morphology of PET nanocomposites.

The hard nanosized MLS platelets confine the micron sized PET spherulites to alter its morphological and thermal behavior. Differential scanning calorimeter (DSC) analysis was utilized to investigate the thermal changes. Melting point depression was apparent in PET nanocomposites compared to neat PET. Broad melting transition and doublet in the melting peak was observed in PET nanocomposites. This melting doublet was absent in neat PET. Melting doublet in nanocomposites corresponded to presence of two phases i.e. bulk and confined phases. The spherulites size distribution was studied using optical micrographs and image analysis. It was found that the spherulites size uniformity decreased as concentration of MLS increased. In order to explain this effect of MLS on size distribution, Gibbs-Thompson equation for melting point depression was utilized to calculate surface energy of solid liquid interface.

THE SCANNING ACOUSTIC MICROSCOPE, AN INVALUABLE NON-DESTRUCTIVE ANALYTICAL TOOL. C. TODD SNIVELY, Failure Analysis Lab, Raytheon, McKinney, TX 75071.

Manufacturing and processing defects, if caught early, can save a company millions of dollars. Furthermore, fault isolation in failure analysis of components and materials can also guide production to minimize failure. The scanning acoustic microscope (SAM) is an analytical tool that can quickly and reliably provide an acoustic image of a material to help identify defects and anomalies within the bulk as well as evaluate bond lines in bulk materials and microelectronics. This presentation will give a brief background on the fundamentals of acoustic imaging as well as examples of materials and assemblies examined. Examples of material process defects as well as vendor defects that can lead to component failure will also be examined.

RELATION BETWEEN SOLDER VOIDS AND HELE-SHAW FLOW. KIRK L. WIGGINS. Failure Analysis Laboratory, Raytheon Systems Company, McKinney, Texas 75071

Solder voiding is often observed in thin joints between parallel surfaces.

The problem to be solved is the formation of voids in very thin joints formed by solder solidification. This report concerns some preliminary experiments.

The joints were formed by melting lead-tin solder between two copper plates to form a thin joint. The joints were intentionally insufficient, i.e., there was not enough solder to form a complete joint. Therefore, when the solder solidified, an equilibrium condition was known to exist between pressure and surface tension. In all cases a complex intermetallic formed as the solder front proceeded. The solder joint was then fractured to reveal a snapshot of solder flow and void formation. Intermetallic formation could also be observed as part of the solder flow process.

It was found that solder flowed in a manner analogous to viscous fluids in a Hele-Shaw cell.

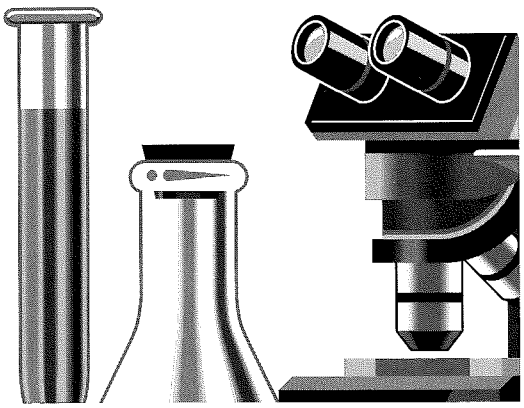
CHARACTERIZATION OF CARBON NANOTUBES AND NANOTUBE /EPOXY COMPOSITES USING TEM AND SEM. Z. YANG, N. A. D'SOUZA*[1] and J. BAHR[2]. [1]Department of Materials Science and Engineering, University of North Texas, Denton, Texas 76207 and [2]Carbon Nanotechnologies Inc., 16200 Park Row, Houston, Texas 77084.

Transmission electron microscope (TEM) was used to characterize the morphology of pure single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). Scanning electron microscope (SEM) was employed to reveal the fracture surfaces of both rigid and flexible SWCNT/epoxy nanocomposites. SEM micrographs revealed the pullout of MWCNTs out of rigid and flexible matrix after tensile test while SWCNTs were only pulled out of rigid matrix and not pulled out from flexible matrix indicating a much stronger interfacial bonding with flexible matrix.

GETTING A COMPLETE SAMPLE DESCRIPTION WITH EDS. PATRICK CAMUS, Thermo Electron Corporation, 5225 Verona Road, Madison, WI 53711

Electron microscopy is providing an unparalleled amount of information on ever decreasing size of sample features. However, the detected electron signals provide little or no information about the chemistry of the material. Modern EDS-acquisition techniques like Spectral Imaging provide unprecedented levels of chemical information, but extremely large data sets. Beyond simple x-ray maps, advanced post-acquisition data analysis techniques are required to completely extract chemical information about the sample. Example analyses will be presented which show the advantages of post-acquisition techniques of quantitative elemental mapping, advanced PCA analyses and automated phase analyses.

Material Sciences continued on page 50



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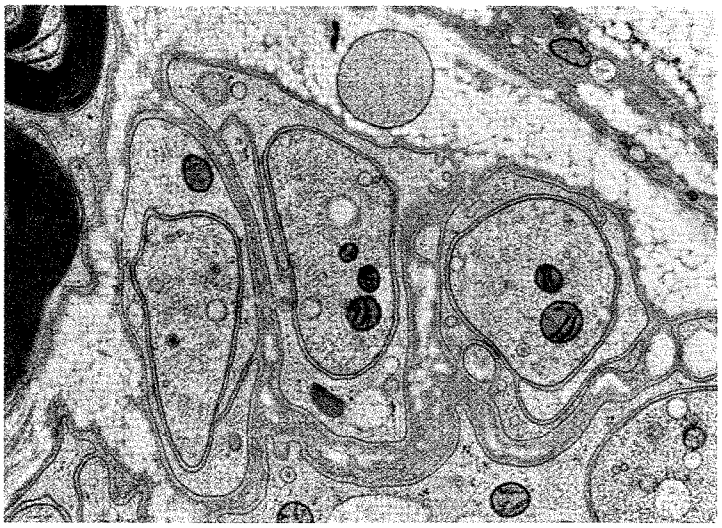
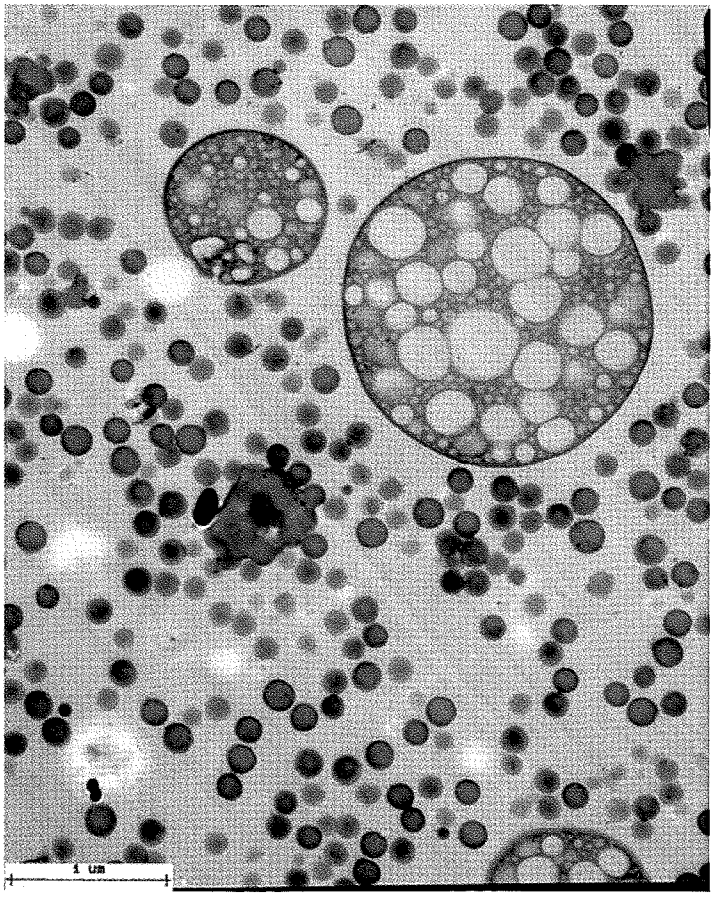


Peripheral nerve (rat), HP frozen, freeze substituted, Epon embedded, cut with the *ultra sonic* knife, section thickness 50nm.



ABS stained with osmium tetroxide, sectioned at room temperature with The *ultra sonic* knife, section thickness 50nm. Note the almost perfect spherical shape of the larger rubber particles and the preservation of the inclusions inside. Also the smaller dense rubber particles are well preserved

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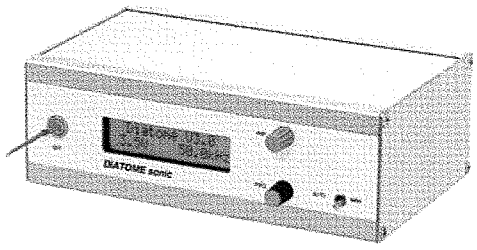

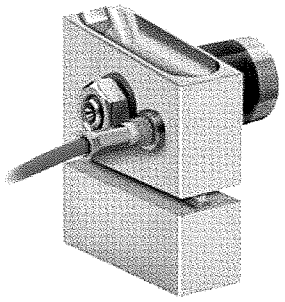

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MECHANICAL POLISHING METHODS OF METAL SAMPLES FOR ELECTRON BACKSCATTER DIFFRACTION

DANIEL FLATOFF[1], SHANE ROBERTS[1] and BRIAN TRUE[2].

[1]South Bay Technology, Inc., San Clemente, CA 92673, [2]EDAX/TSL, Draper, UT 84020.

Electron Backscatter Diffraction (EBSD) is a powerful technique that has gained momentum in materials science research over the last ten years. Recent advances in electron microscope technology and automation has made EBSD a more viable and routine analytical tool. Sample preparation is a key component in the use of EBSD due to the nature of the backscattered signal. Pattern formation is from the top 10-50 nm of the sample surface, and therefore mechanical damage remaining from sample preparation will result in a poor quality signal. Several methods of sample preparation have been investigated that vary in abrasive material, preparation time, and material system. These methods have yielded acceptable results but proved to be extremely time consuming. A simple approach of mechanical polishing utilizing standard metallographic processes has been used on a wide variety of materials to produce EBSD quality samples. The technique uses a semi-automatic polishing machine combined with a precision lapping fixture to control the polishing process. Thickness control of each step is critical in the ability to eliminate mechanical deformation of the sample and to ensure that a high quality EBSD pattern will be obtained. Metal samples of unknown compositions of brass, stainless steel, and copper were prepared with quality images and EBSD patterns resultant.

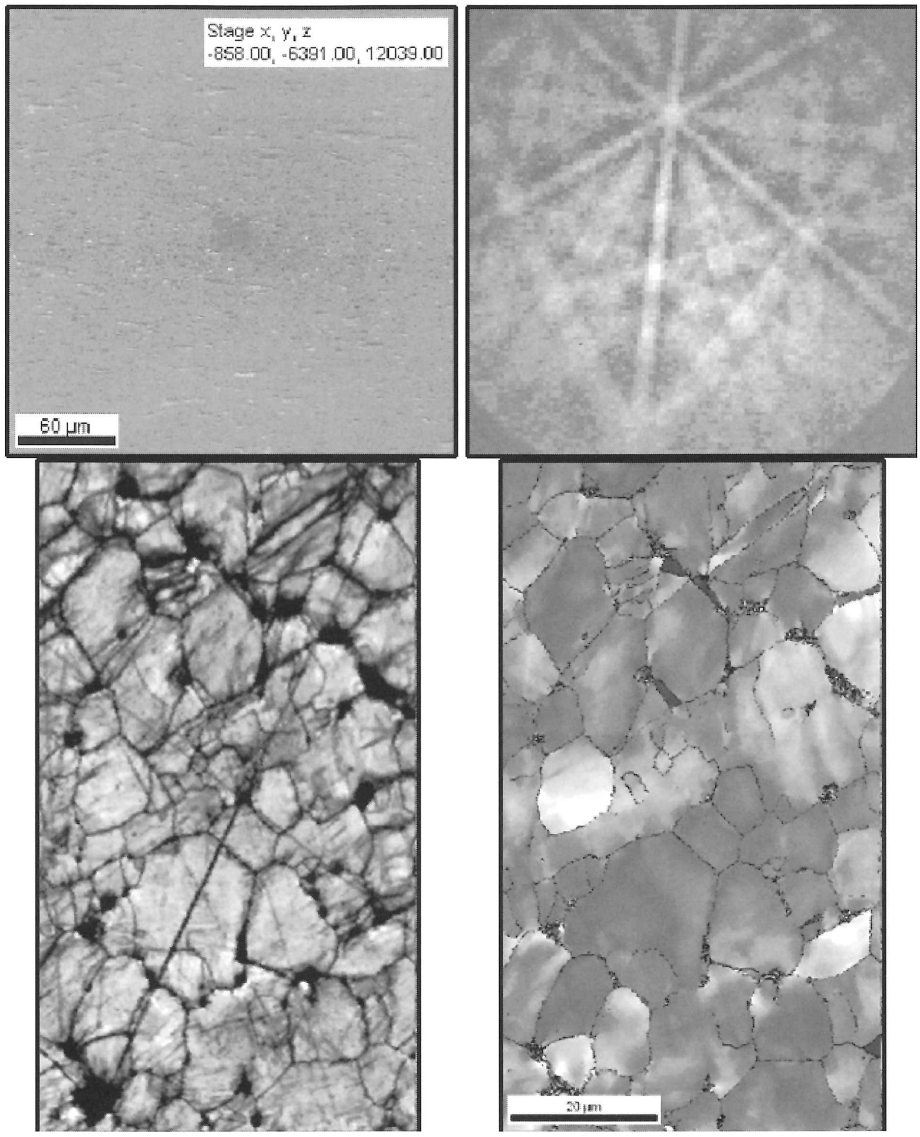


Figure 1. ***Upper left:*** Surface image of the brass sample taken using SEM. ***Upper right:*** EBSD pattern acquired from the sample. ***Lower images:*** Image quality maps demonstrating a good, well-polished specimen.

TIN WHISKERS

ROBERT F. CHAMPAIGN

Raytheon, Engineering Shared Services, Failure Analysis Lab
2501 W. University, McKinney Texas 75071

There is a move currently underway in the electronics industry to remove lead (Pb) from electronic assemblies and solder alloys. Restrictions by environmentally friendly organizations are pressuring electronic manufacturers to develop alternate surface finishes and solder alloys. One cost efficient alternative solution is to use pure tin (Sn) or alloys that are Sn rich.

The use of pure Sn poses a serious reliability risk due to the potential for the Sn to form whiskers. Tin whiskers are electrically conductive filaments that can spontaneously grow from pure Sn surfaces. These filaments are single crystal structures whose growth mechanisms are not completely understood. The most compelling theory in the electronics industry is that Sn whisker growth is a compressive stress relief mechanism in the Sn plating. Some identified sources of stress in Sn are plating residual stress, compressive mechanical loading, scratches in the plating surface, intermetallic formation, and mismatches in the coefficient of thermal expansion between the plating and substrate. Extensive studies are still being performed in the industry trying to understand the Sn whisker growth phenomenon.

Tin whiskers have been reported to commonly grow in lengths greater than 60 mils with diameters as much as 10 microns. Some studies report whiskers as long as 394 mils. The shape of the whiskers can vary dramatically from perfectly straight to bent or kinked. The initiation of whisker growth can occur soon after plating or lie dormant for years.

Tin whiskers can cause transient or long-term electrical shorts depending on the amount of current available. Plasma arcing can also occur in certain environmental and electrical conditions. Whiskers can also easily break loose in components and assemblies generating conductive debris. The Sn whisker issue is a serious reliability concern with space and military electronics. There are many documented cases where Sn whiskers have resulted in the failure of electronic assemblies. Two failure analysis cases will be discussed where the main failure mechanism was Sn whiskers.

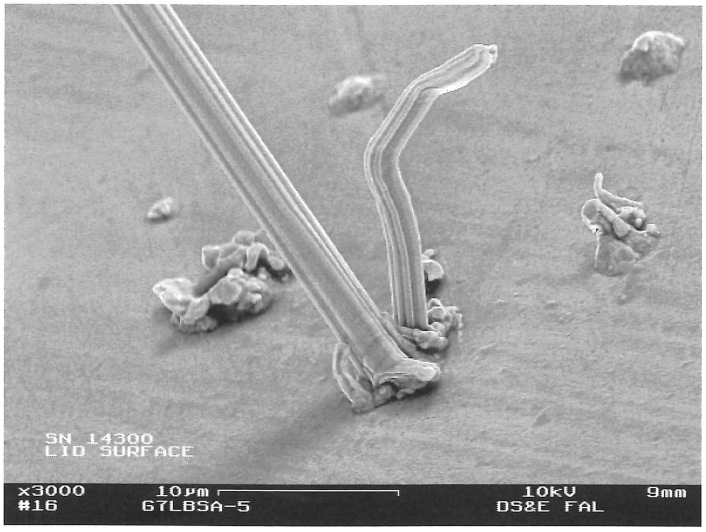


Figure 1: Surface of a Sn plated coupon showing a dense coverage of Sn whisker initiation sites and multiple Sn whiskers varying in length and width.

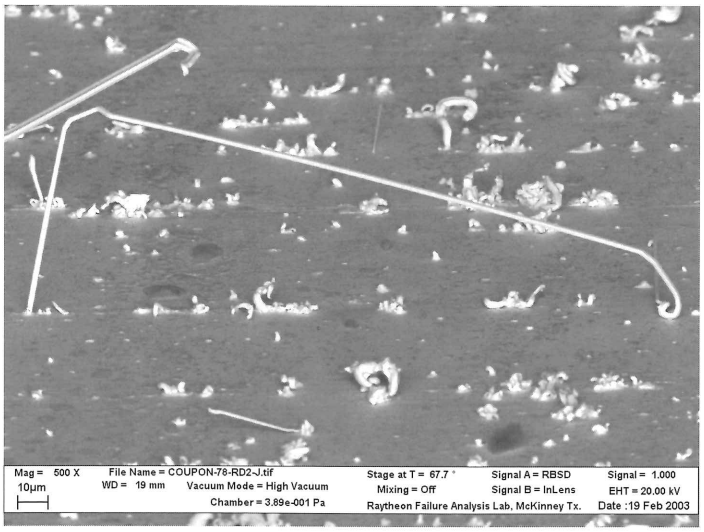


Figure 2: Sn whisker growing from the surface of a Sn plated lid on an electronic module.

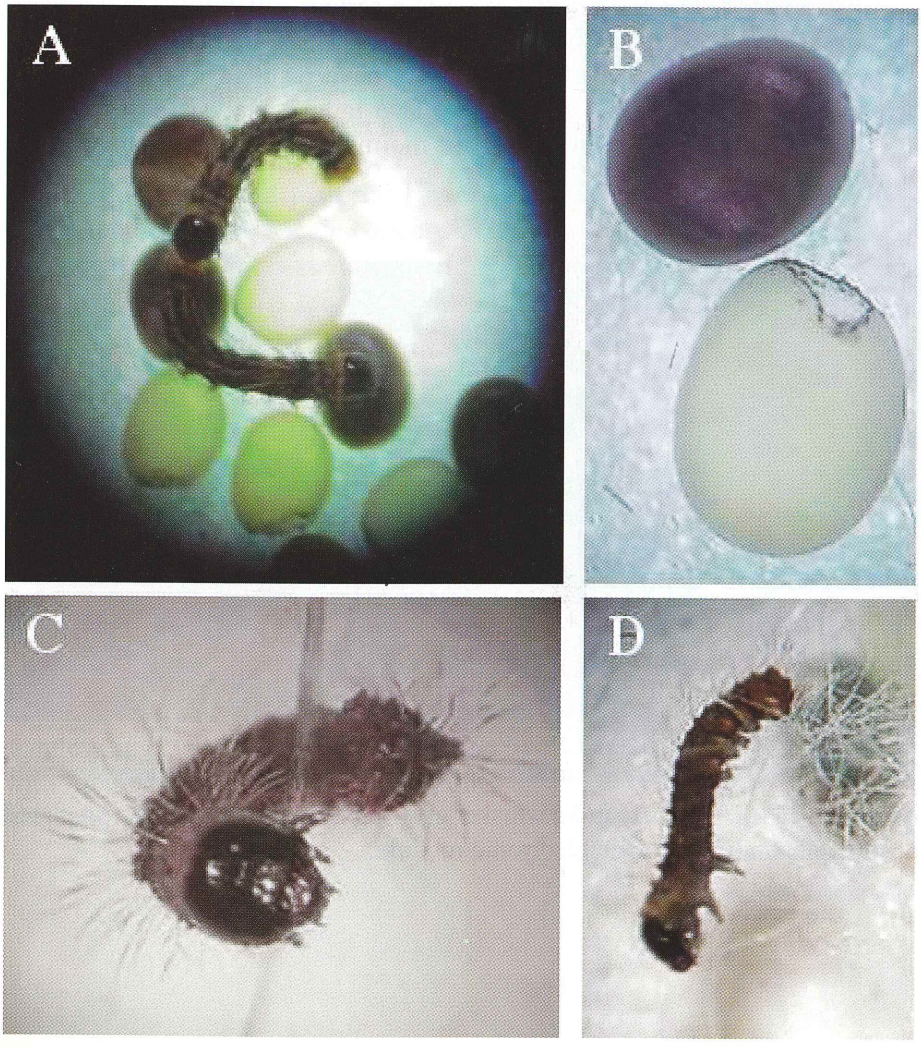
EDUCATION
FALL 2004

DIGITAL PHOTOMICROGRAPHY FOR USE IN TEACHING A PLANT ANATOMY LABORATORY. **HOWARD J. ARNOTT.** The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, Texas 76019

We are using two digital photo micrographic work stations in the current laboratory presentation of Plant Anatomy. Each work station consists of the following equipment*: a Nikon Eclipse E200 student microscope with 4X, 10X, 40X and 100X objectives; a Nikon DXU1200F Digital Camera (12 mega pixels); a Dell Optiplex GX270 desktop computer using the Windows XP Professional operating system and a 17 inch flat panel monitor; Nikon Act-1 version 2.63 microscope control program; Photoshop CS and other programs; Epson R200 inkjet printers. With a few minutes of explanation, and using ordinary prepared slides, the students were able to make "professional micrographs." Using these work stations they can supplement the drawings and notes accompanying each area of subject material with micrographs printed on paper. The quality of the printed micrographs is of course dependent on the paper used, but even when using "plain" paper, the micrographs are of sufficient quality to capture the images with faithful representations: using glossy photo paper produces images better than those seen in many text books. Pedagogically, the use of these photo micrographic work stations, provide each student with the opportunity to use modern digital equipment as a part of their plant anatomy learning experience. It not only provides them with a chance to illustrate their current work, but it also introduces them to the feasibility of using photo micrographic descriptions as a part of their future course and/or research studies as well as their future professional activities. Obviously, such a system could be effectively used in any course in which microscopy is a part of the routine understanding of the subject matter.

*Listing of equipment does not represent an endorsement of these products by any member or component of The University of Texas.

OUR STUDENTS



Light microscopy of newly hatched silkworm larvae by **Corina Moraru**, working with Dr. Camelia G.-A. Maier at Texas Woman's University. For documentation purposes and informal lab presentations, Corina took pictures using a Sony Mavica digital camera positioned directly at the ocular of a Ken-A-Vision stereoscope with no attachments.

A. Two newly hatched silkworms (few minutes old) moving on top of eggs in searching for food.
B. Unhatched eggs, containing the embryo, are black. Larva chews its way out of the egg living behind the white shell.
C. A 2-day old silkworm hanging on a silk fiber.
D. Profile of a newly hatched silkworm entangled in silk.

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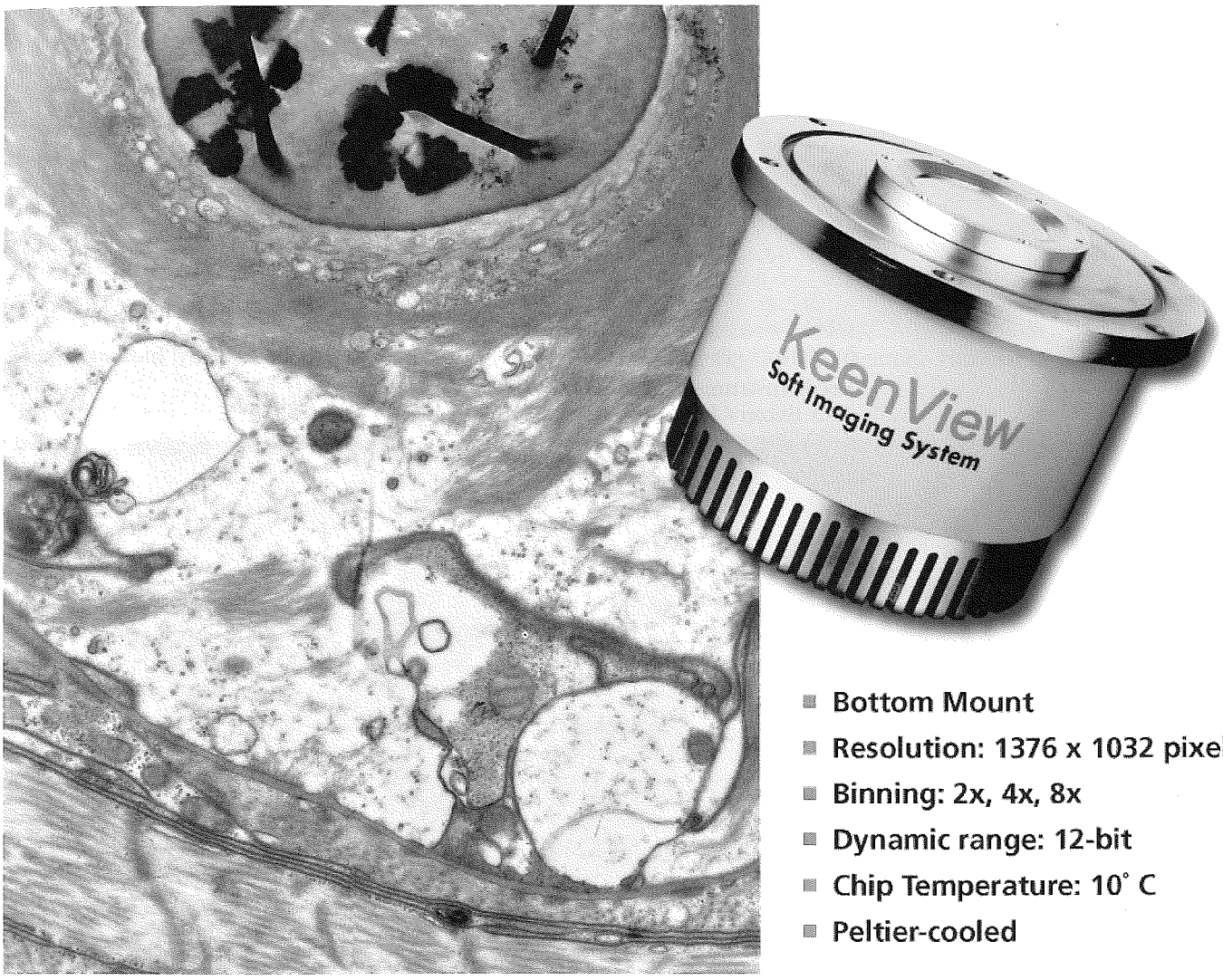

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Meeting Memories

Audience in the meeting room, spring meeting 2003 in Denton. Howard J. Arnott and Martha Gracey in the first row.

Vendors' Exhibit at the spring meeting in Houston. Former TSM President Don W. Smith and Program Chairman Susan Robbins visit with corporate members. (Photos courtesy to Nabarun Ghosh, former TSM Treasurer)

Meeting report by TSM President Ann E. Rushing at the spring meeting 2004 in Houston.

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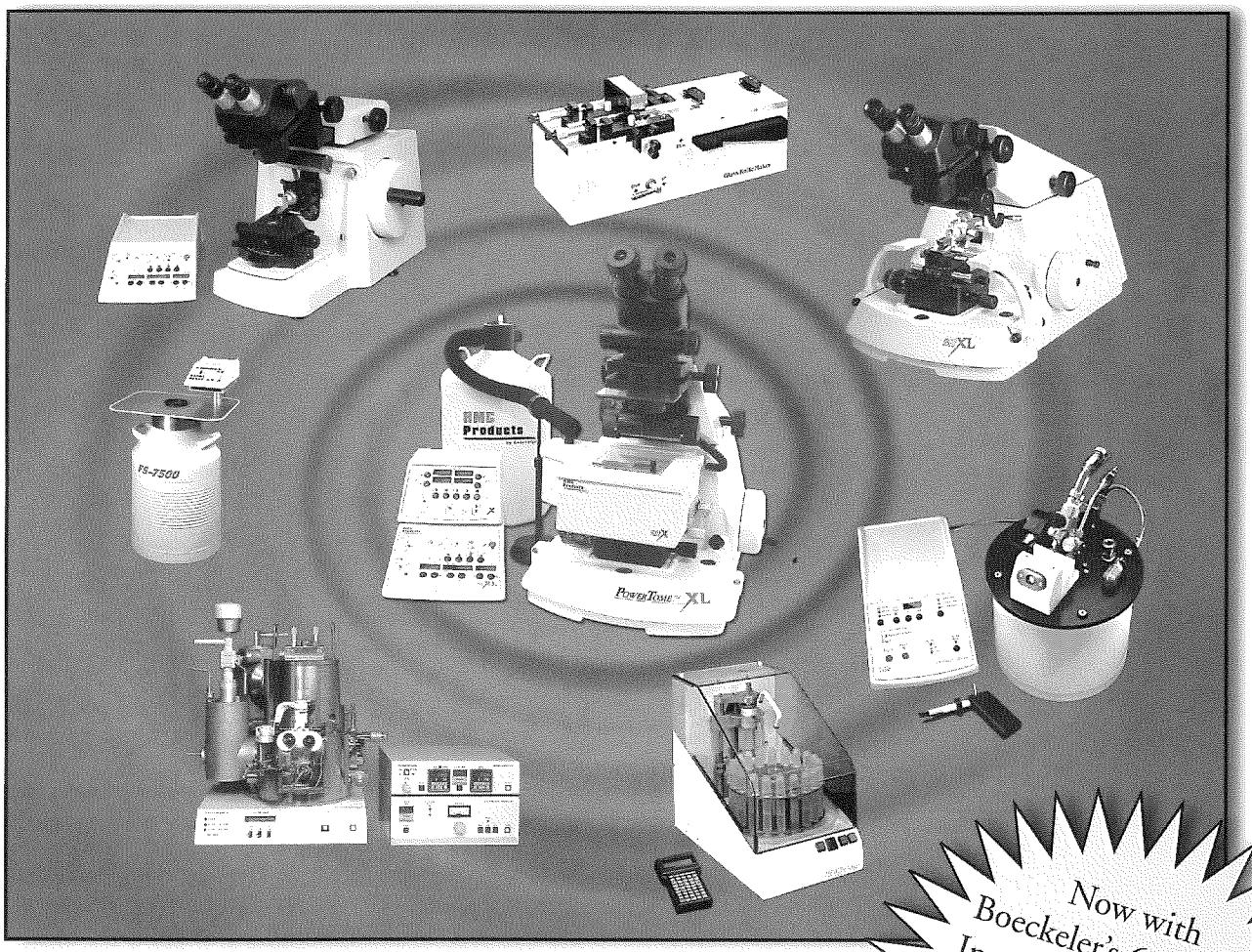

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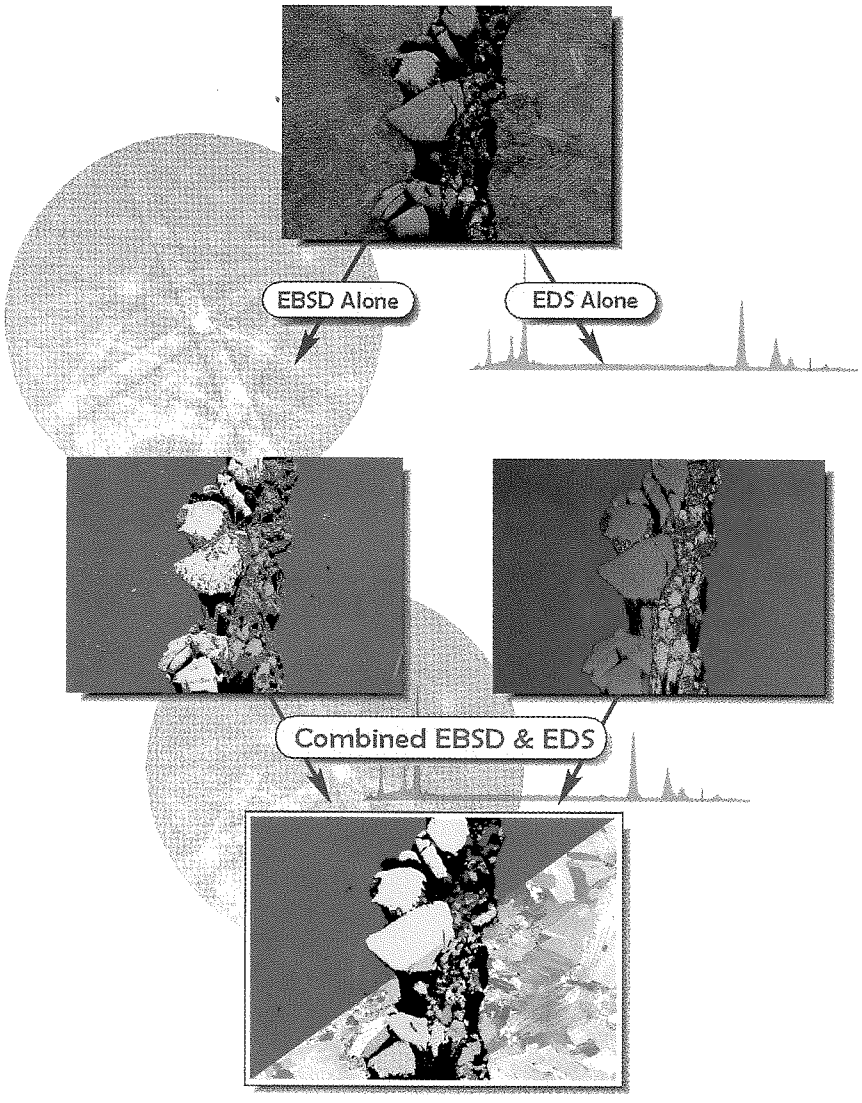

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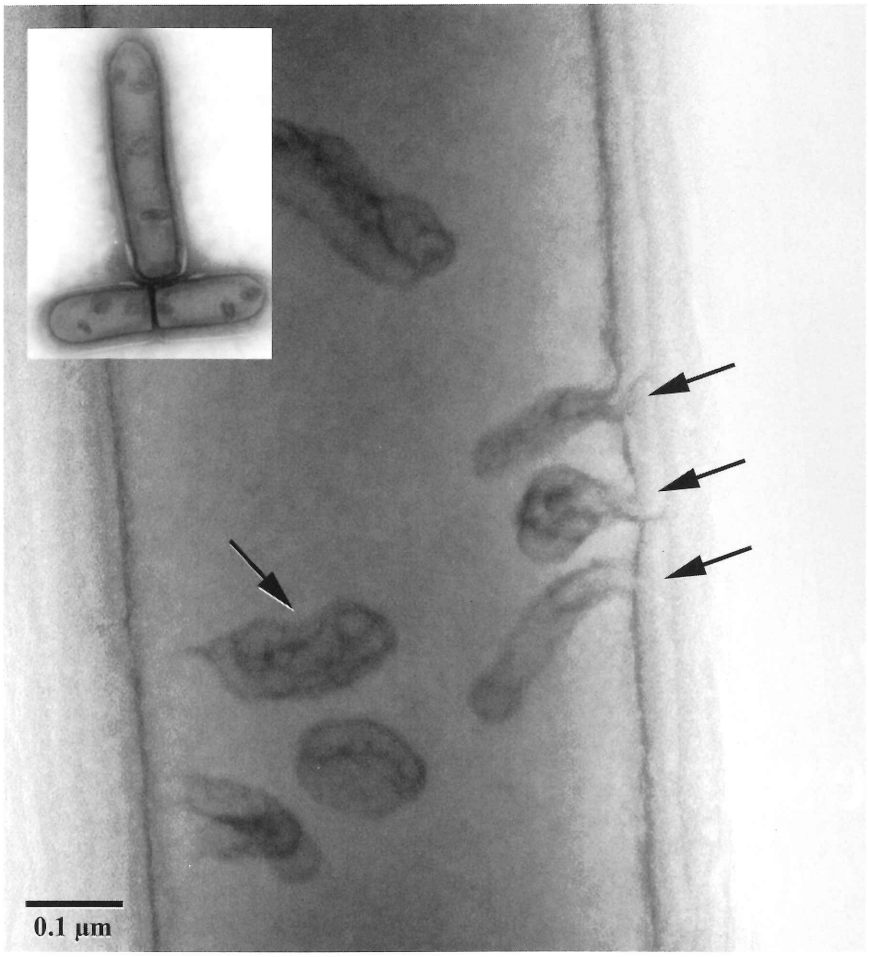

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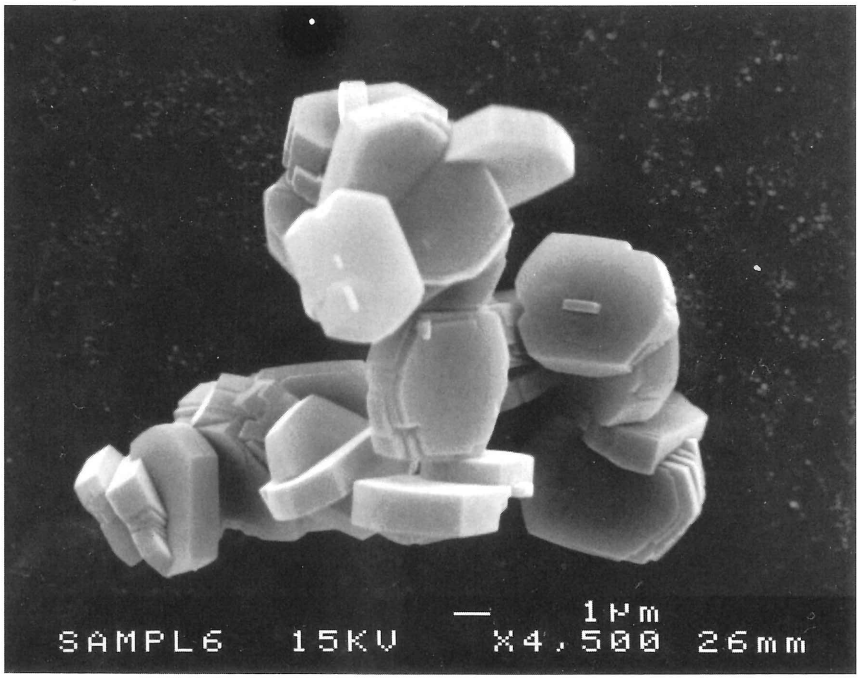

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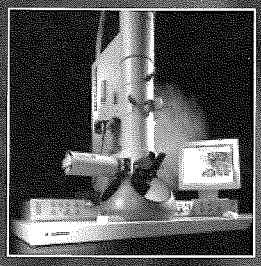

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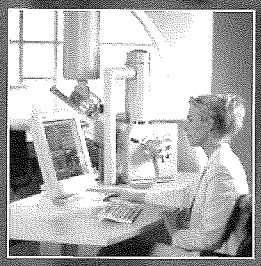

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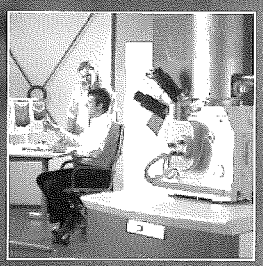

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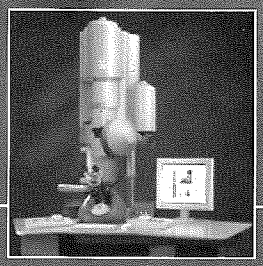

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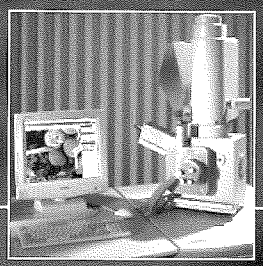

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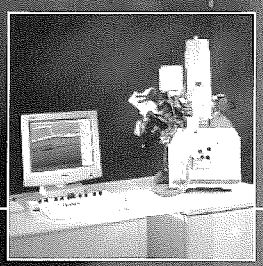

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