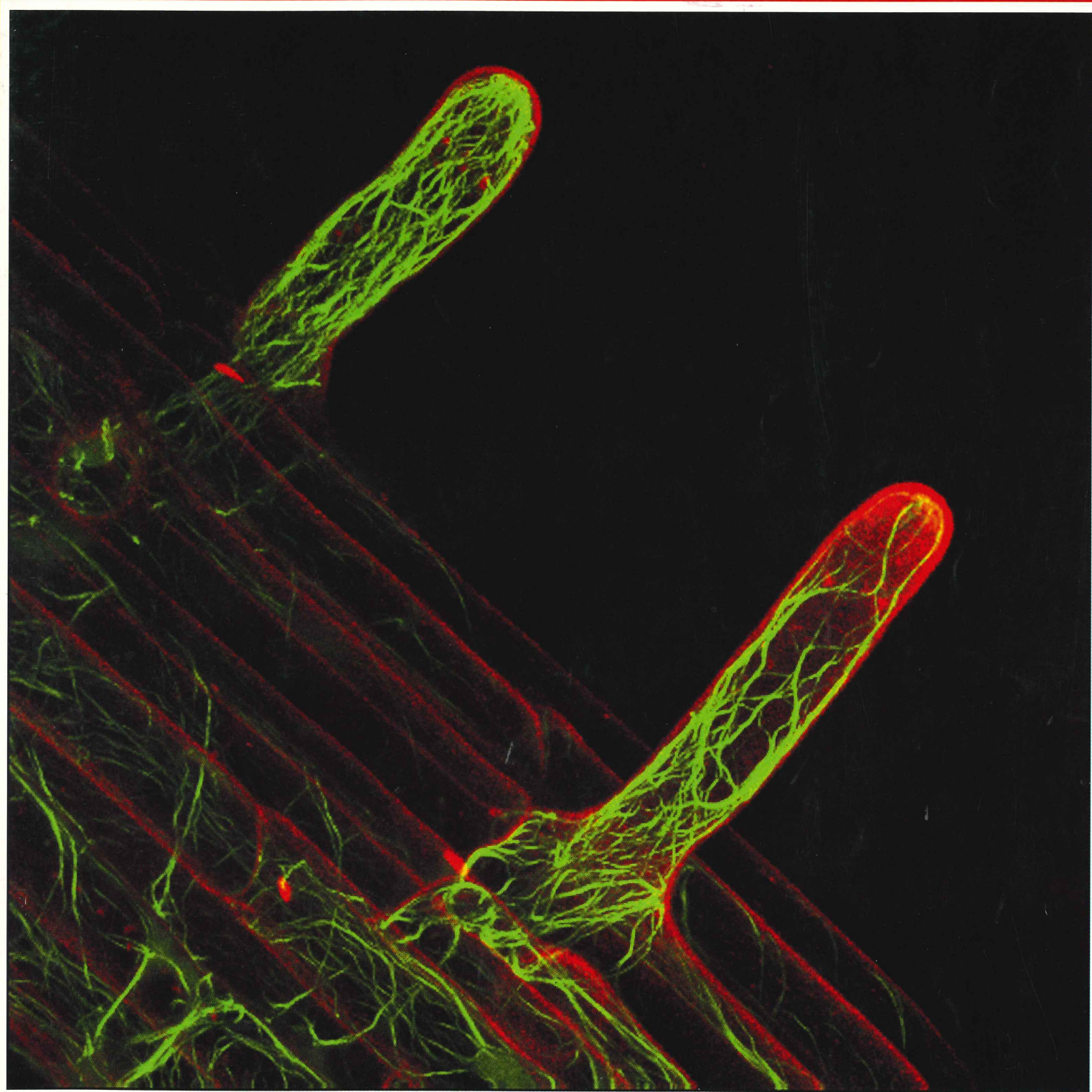




Texas Journal of Microscopy



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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

This image shows two root hairs of transgenic *Arabidopsis thaliana* plants expressing a green fluorescent protein (GFP) fused to the actin-binding domain of talin (an actin binding protein from mouse). This fluorescent protein fusion decorates the actin filaments (colored green) in living plant cells. The image was taken with a laser scanning confocal microscopy (Bio-Rad 1024 ES) and represents a projection of 30 optical sections taken at 0.3 micron intervals. The root was stained with propidium iodide to outline the cell wall (colored red). The use of green fluorescent proteins and its variants have allowed imaging of a variety of plant cellular structures *in vivo*. Deepti R. Mohamalawari and Elison B. Blancaflor, Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK 73401.

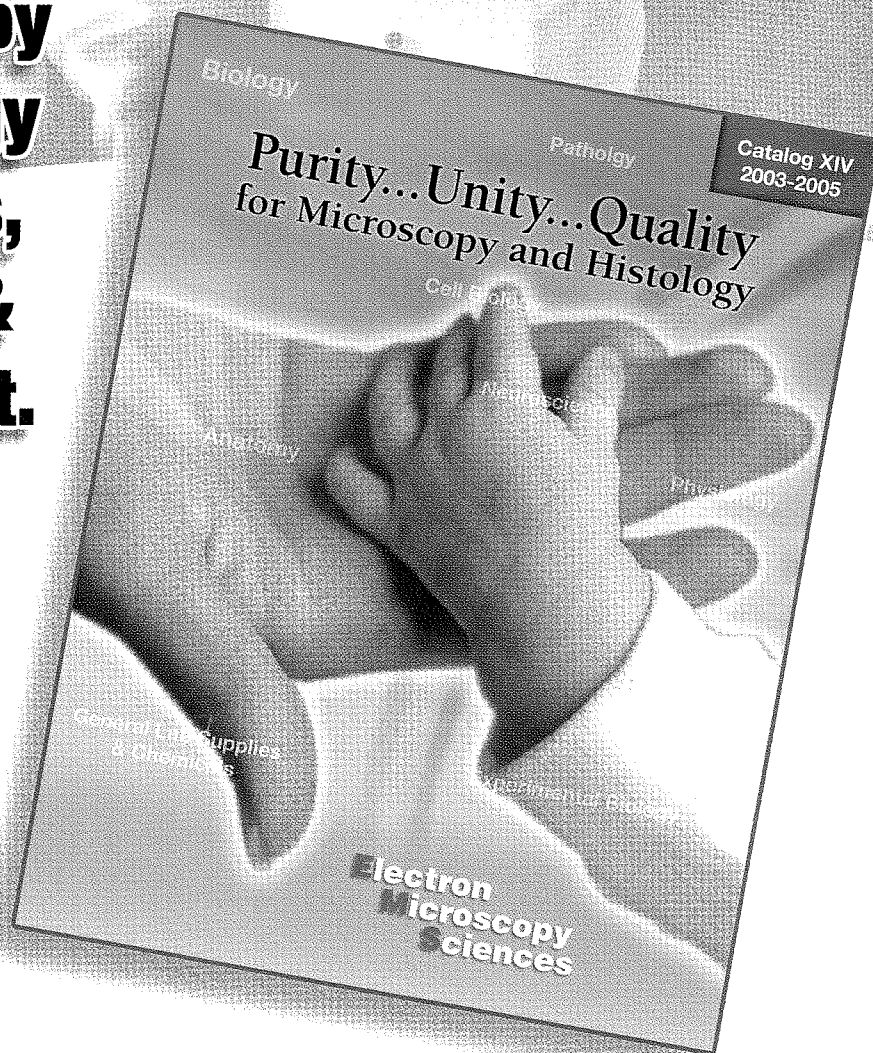
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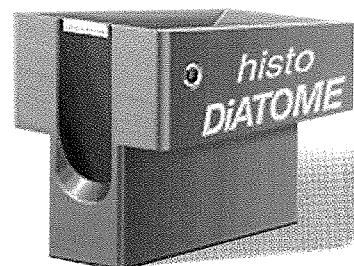
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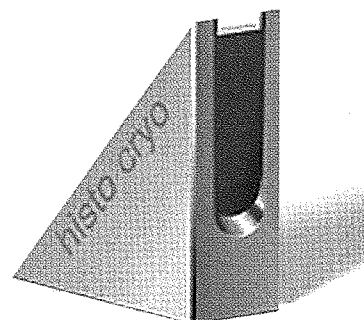
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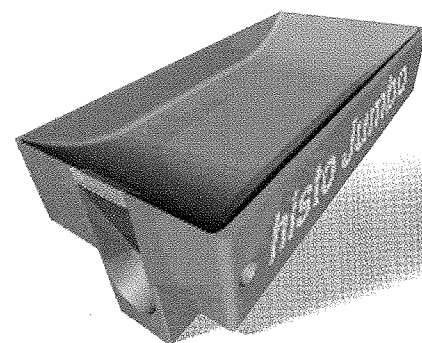
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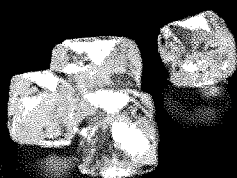
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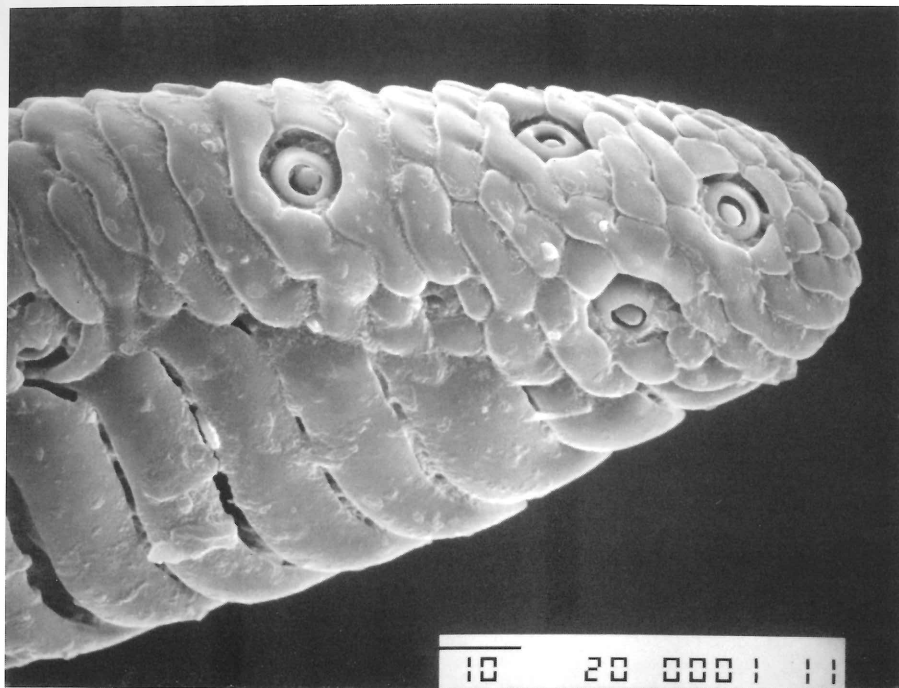
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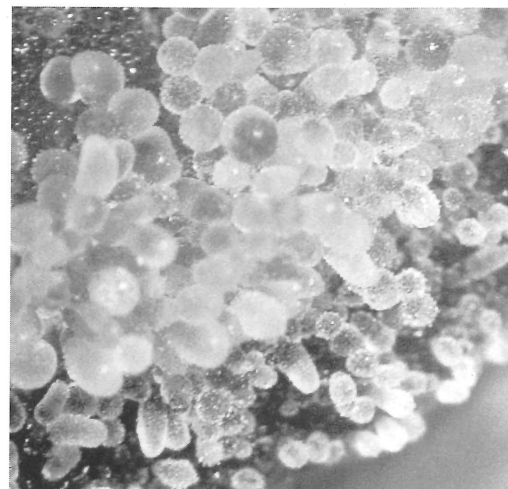
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Answer to “What Is It?”

from Texas Journal of Microscopy 33:2



This SEM by Daniel Petr, Department of Biology, Southwestern Adventist University, represents the distal part of proboscis tip of a non-nymphalid butterfly showing absence of prominent sensilla styloconica. Small sensilla basiconica are present, which represent chemoreceptors only. The butterfly is the giant swallowtail, *Papilio cresphontes*.



This light micrograph by Camelia G.-A. Maier, Department of Biology, Texas Woman's University, represents *Aloe vera* leaf callus. *A. vera* young leaf sections were placed on Murashige and Skoog medium in the dark. Callus cells on the surface of the leaf section are mostly globular and some are also elongated, showing rough cell walls.

ADVERTISER'S INDEX

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President's Message

Time has slipped away again and here we are getting ready for another great meeting. The Austin meeting was well attended by regular, student, and corporate members. We even had high school student participants who presented serious microscopy work and became members of our Society at the Austin meeting (see 'Education' on page 22 for more details). A big thank you to David Muirhead who guided North Garland High School students' work, to Graham Bird, their sponsor, and to students' accompanying instructors to the meeting. Thank you to all members for giving the high school students a warm reception at the Fall 2002 meeting. There was some serious recruiting by our academic members after the student presentations.

I want to welcome the members and corporate sponsors to the Spring 2003 meeting in Denton. We will be meeting on the 100-year old Texas Woman's University (TWU) main campus, in Hubbard Hall, a historic building. The University's high-rise residence halls and academic and administrative buildings dominate Denton's skyline. Historic buildings such as the TWU first building, Old Main, and the Little Chapel-in-the-Woods, inaugurated more than 50 years ago by Eleanor Roosevelt blend with modern buildings. Other special features of the Denton campus are the exhibits in the Blagg-Huey Library, the DAR Museum containing the gowns of Texas' First Ladies, the Pioneer Woman statue, and the Botanical Gardens. Bring the family and tour the campuses of TWU and its neighbor, University of North Texas.

I am looking forward to the microwave workshop, co-sponsored by Pelco, the presentation by the MSA speaker, Dr. Lucille Giannuzzi, University of Florida, on focused ion beam (FIB) specimen preparation, and our members' poster

or podium presentations.

I would like to take this time and remind the members of some of the advantages of being an active member of TSM. This is a wonderful group to get acquainted with. You will interact with your peers, exchange ideas, enjoy dinner and social gatherings, and become more than a name on a list. TSM is your society. Come and join the exchange of ideas. Start planning to attend the next meeting and present papers and/or posters. Encourage your students and technicians to participate and help recruit new members.

Thanks to the corporate sponsors who have supported our society and been active in coordinating workshops representing basic and new technologies at the previous meetings. We were given the opportunity to bring our samples to work with during many of the workshops. What a great way to try new technologies on own target projects/specimens before investing. Support your corporate members when you consider equipment and supply purchases. I also want to thank the officers who have made my job easier this past year. We had good leadership and it has been a privilege to work with them and represent TSM as your president.

In closing, there has been much discussion about the fall 2003 meeting. You have probably gotten information from MSA about the 2003 national meeting in San Antonio. The TSM council knows you will be going to M&M when it's in beautiful San Antonio so we will have our fall 2003 business meeting during M&M. Watch for more details on our website www.texasmicroscopy.org for time and location. We will be planning the Spring 2004 meeting in the Houston area. More details in the next few months.

Pamela Neill
TSM President 2002-2003

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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Manuscript deadline is July 15, 2003

Treasurer's Report

Treasurer's Report For Period beginning Sept. 1, 2002 and ending March 5, 2003

ASSETS AS OF SEPTEMBER 1st, 2002:

Checking Account No. 005772227833 (Bank of America) \$ 764.98
Certificate of Deposit No. 1882289323 (Bank One) \$4079.37

Total **\$4844.35**

Income:

Fall Meeting Registration \$4431.10
Dues \$ 910.00

Journal Advertisement Revenue:

33:2 \$3000.00

Interest:

Checking account \$ 1.27
CD \$ 231.44

Total Income **\$8573.81**

Expenses:

Fall Meeting \$4571.15

Journal Printing:

33:2 \$3059.07

Office Expenses:

Bond \$144.59
President's Plaque \$ 71.99
Bank Re-statement Charge \$ 5.00
Checking Account Service Charges \$ 48.00
Web Fees \$129.80

Total Expenses **\$8029.60**

ASSETS AS OF MARCH 5, 2003:

Checking Account No. 005772227833 (Bank of America) \$1077.75
Certificate of Deposit No. 1882289323 (Bank One) \$4310.81

TOTAL **\$5388.56**

Treasurer's Report

Treasurer's Report 2002 Year End

ASSETS AS OF JANUARY 1, 2002

Checking Account No. 005772227833 (Bank of America) \$ 567.30
Certificate of Deposit No. 1882289323 (Bank One) \$ 4079.37

Total **\$ 4646.75**

Income:

Spring Meeting Registration \$ 1515.00
Fall Meeting Registration \$ 4431.10
Dues \$ 2365.00

Journal Advertisement Revenue:

32:2 \$ 1410.00
33:1 \$ 1850.00
33:2 \$ 2600.00

Interest:

CD \$ 476.21
Checking Account \$ 2.37

Total Income **\$14649.68**

Expenses:

Spring Meeting \$ 2865.99
Fall Meeting \$ 4571.15

Journal Printing:

32:2 \$ 1234.74
33:1 \$ 1306.68
33:2 \$ 3059.07

General Mailings \$ 775.00

Office Expenses:

Bond \$ 144.59
President's Plaque \$ 71.99
Bank Re-Statement Charge \$ 5.00
Checking Account Service Charges \$ 132.00

Total Expenses **\$14166.21**

ASSETS AS OF DECEMBER 31, 2002

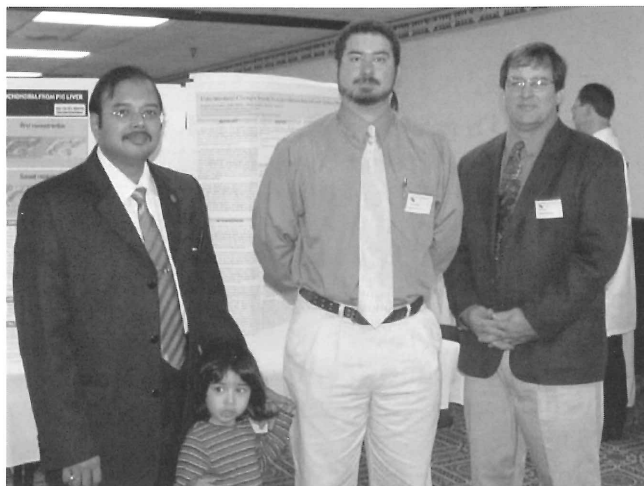
Checking Account No. 005772227833 (Bank of America) \$ 819.41
Certificate of Deposit No. 1882289323 (Bank One) \$ 4310.81

TOTAL **\$ 5130.22**

Meeting Memories



The Thermo Man, Graham Bird, at the Fall 2002 Meeting in Austin. Graham is the president of ASI and the sale representative for many other companies in Texas.



Nabarun Ghosh with his students, Brit Patten, Greg Lewellen, and his daughter Monica in the poster room, at the Fall 2002 Meeting in Austin.



Ann S. Burke, the TSM Secretary at her poster, with Sandra L. Westmoreland, Past Secretary. Fall Meeting, 2002 in Austin. (Pictures taken by Howard J. Arnott)

Abstracts

BIOLOGICAL SCIENCES SPRING 2003

SEM STUDY OF THE EFFECTS OF TEMPERATURE ON SHELL MORPHOLOGY OF *PHYSA ACUTA* JUVENILES. MAJ ANGARANO, Department of Biology, The Center for Mollusca, The University of Texas at Arlington, Arlington, Texas 76019

Gastropods are known to exhibit environmentally induced interpopulation variation of shell morphology attributed to ecophenotypic influences and recently, evidence supports intrapopulation variation as well. However, studies on several gastropods have contradicted these findings; correlations between specific environmental factors explored previously were absent. Hypotheses for the discrepancies include the existence of some other yet unknown environmental factors. Also to be considered is the possibility of the changes in shell morphology occurring at a time prior to collection and thus failing to correlate to the environmental factors measured at the actual time of collection. Conceptually, environmentally induced morphometric shell changes of the juvenile mollusk may change the morphology of the future adult shell. Based on this second hypothesis, the effects of rearing temperatures on just hatched juveniles oviposited by a single parenteral *Physa* individual at 20, 25, and 30 degree C temperatures were studied. Newly hatched juveniles (<1mm length) were collected using a fine art paintbrush, fixed in 70% and subsequently dried in a 60 degree C incubator. Specimens grouped by temperature treatments were mounted aperture side up on a stub, sputter coated and examined in the SEM. Micrographs of each shell were analyzed with imaging programs to measure aperture length (AL) and width (AW). The calculated shell morphometric ratio AW/AL was used to determine mean ratios for each temperature group and subjected to ANOVA analysis. Results of the study indicate that there are no significant effects of rearing temperature on the juvenile aperture shape.

THE OCCURRENCE OF SPHERICAL STRUCTURES IN MAMMILLARY CONES OF AVIAN EGGSHELLS. DIANE B. NGUYEN and SANDRA L. WESTMORELAND, Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, Texas 76019

Calcium is an essential mineral required by birds for proper hatchling development and eggshell formation. The most readily available source during embryogenesis is found in the eggshell in the form of calcium carbonate. Biomineralization in the eggshell is the process in which inorganic crystals and crystalline aggregates are formed and deposited. Initiation of biomineralization of the avian eggshells occurs at the tips of the mammillary cones during the 18-20th hour of egg production, and is complete once the cones of calcium carbonate arrange into a calcified layer.

As calcium is deposited, the mammillary cones widen and grow upwards forming knob-like projections until they converge, giving shape to the eggshell. Accurate development and expansion of the mammillary cones is essential, since the shape of the avian eggshell is crucial for proper physiological development of the hatchling during embryogenesis. However, the exact location from which the calcium is absorbed and transferred to the developing embryo has yet to be determined. Mammillary plastic casts of five different orders of birds (*Causariiformes*, *Gaviiformes*, *Ciconiiformes*, *Pelecaniformes*, and *Galliformes*) were prepared and examined with a Joel 35C scanning electron microscope. For comparison SEM images of each type of shell were taken using Vital Scan Imaging. Plastic casts of eggshells of all orders showed similar spherical structures, which were noted to be most frequent in *Causariiformes* (Emu) at 42%. These spherical structures were situated just under the tips of the mammillary cones, and were intermingled with the shell membrane fibers located there. Of those samples, in which sections were taken from the three regions of eggshells (air-space, equator and blunt end), spherical structures were noted to be most frequent on the equator region. Since shells of all bird orders studied contained these spherical structures in the mammillary cones, these structures may be vital components during biomineralization and embryogenesis.

TRACKING CYTOSKELETAL REORGANIZATION IN PLANTS USING GREEN FLUORESCENT PROTEIN AND ITS VARIANTS. DEEPTI R. MOHAMALAWARI and ELISON B. BLANCAFLOR, Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK 73401

The green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* is widely used as a marker to study cellular events in a non-invasive manner. Point mutations in GFP have led to the development of other fluorescent protein variants such as cyan and yellow fluorescent proteins (CFP & YFP). Another exciting development is the discovery of DsRed, a 28kDa protein from *Discosoma* sps, which has the ability to produce fluorescence brightness comparable to rhodamine dyes. In order to investigate the utility of GFP, CFP and DsRed as cytoskeletal reporters, fusions were made to the actin-binding domain of talin. Transient expression of the GFP, CFP and DsRed-talin by particle bombardment in leaf epidermal cells of tobacco and inner epidermal cells of onion bulbs revealed filamentous patterns typical of the pattern of F-actin observed in fixed material. GFP fusions were made to transmembrane domain of a rat sialyl-transferase protein that targets to Golgi bodies. A close association of actin filaments (labeled with DsRed) with Golgi bodies could be observed using confocal microscopy. Co-bombardment of a GFP-MAP4 construct and the DsRed2-Talin in a variety of cell types allowed the simultaneous visualization of microtubules and actin. We are testing our fluorescent protein constructs in a variety of plant systems such as *Medicago truncatula* hairy roots, *Arabidopsis thaliana* and BY2 to-

bacco suspension cells to study *in vivo* patterns of cytoskeletal reorientation in response to microbes, hormones and other environmental factors (Supported by NASA grant number NAG 2-1518).

POND SNAILS AND PENISES: AN EXAMINATION OF MORPHOLOGY IN THE GENUS *Physa* USING SCANNING ELECTRON MICROSCOPY. DAVID K. BRITTON, Department of Biology, The University of Texas at Arlington, Box 19498, Arlington, Texas 76019

Freshwater pond snails in the genus *Physa* (Gastropoda: Pulmonata: Basommatophora) are distinguished by variations in the shape of their shells. Approximately 40 species are currently recognized. Recent studies have revealed, however, that shell shape in *Physa* is not a genetically fixed. It is subject to environmental influences such as temperature and the presence or absence of predators. Consequently, the validity of species' designations within this genus has been questioned. Despite the recognition of numerous *Physa* species, anatomical comparisons have revealed that virtually all are anatomically indistinguishable. The most remarkable non-conchological differences within this group belong to the snails' penises. Among all of the numerous species examined, only three different penile morphologies have been described for this genus. It is likely that only three species actually exist. Only stylized illustrations of *Physa* penile anatomy exist in the primary literature. Thus, the objective of this study was to generate and compare scanning electron micrographs of several different Physid species' penises, including *Physa virgata*, *Physa acuta*, *Physa microstriata*, and *Physa hendersoni*. Additionally, penises representing several different Physid populations in Texas were compared to address whether, perhaps, more than one species occurs in Texas. Two different morphologies were clearly distinguishable in the species examined: *P. microstriata* and *P. hendersoni* shared one penile morphology, while *P. virgata* and *P. acuta* shared another. All snails examined from Texas had the same penile morphology, failing to provide evidence of more than one species within this state.

A COMPARITIVE STUDY OF PREPARATION TECHNIQUES FOR IMAGING AVIAN EGGSHELL. SANDRA L. WESTMORELAND, Department of Biology and The Center for Electron Microscopy, University of Texas at Arlington, Arlington, Texas 76019

Imaging avian eggshell is a difficult task. No one preparation technique or microscopy method can present a complete picture of its complex biomaterial. In general, microscopists obtain different perspectives of eggshell based on the method of examination. Only by piecing together information obtained using various techniques can the true picture immerge. The avian eggshell, as represented by the shell of the White Leghorn chicken, is composed of calcium carbonate crystals entwined with a protein matrix. The external shell surface is covered with an organic cuticle, while the shell interior is lined with organic shell membranes. Shell structures support shell functions: physical protection of the embryo and provision of a source of calcium for the growing embryo. Microscopic pores penetrate the shell allowing for gas exchange with the egg interior without allowing desiccation or microbe invasion. By using various physical and chemical treatments in conjunction with light (LM), scanning electron (SEM) and transmission electron microscopy

(TSM), various shell components have been exposed and examined in this study for better understanding. Scanning electron microscopy of untreated shell provided a basis for comparison to treatment methods. Ground sections of epoxy-embedded eggshell, studied under LM, allowed a view of the shell interior including the calcium reserve assembly, the site from which calcium is removed during embryogenesis. Microtomed sections of embedded shell were viewed with both LM and TSM. These sections provided information about the structure of shell membranes and the interrelationship of shell crystals and shell matrix. Changes in the crystalline structure of the eggshell were effected using buffered solutions of pH 6.6-7.6, revealing the protein matrix for imaging with the SEM. Eggshell was also treated with a baffle furnace at 750°C, exposing the crystalline structure. Polishing and chemical treatments were used to remove the shell's cuticle, uncovering "pore plugs" in the mouths of shell pores. High-pressure epoxy-embedded eggshell was used to prepare casts of shell pores and interior shell surfaces for imaging with SEM. The comparison of micrographs obtained using various forms of microscopy and preparation techniques has confirmed the structure of and added new data to enhance our understanding of avian eggshell.

CORRELATION BETWEEN THE METEOROLOGICAL CONDITIONS WITH THE AEROALLERGEN CONCENTRATION IN THE TEXAS PANHANDLE. NABARUN GHOSH¹, RENE CAMACHO¹, ELIZABETH J. SCHNIEDERJEN¹, CONSTANTINE K. SAADEH² AND MICHAEL C. GAYLOR² ¹Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, TX 79016 ²Amarillo Center for Clinical Research Ltd., 1901 Medi Park, St. 40, Amarillo, TX 79016

Aeroallergens are known to cause serious allergic and asthmatic reactions, the concentration of which varies widely with the meteorological conditions like temperature and precipitation. This investigation was carried out to determine any possible correlation between the daily pollen and spore indices with the meteorological conditions of the Texas Panhandle. We previously reported the major types of aeroallergens that were prevalent in the Texas Panhandle area. The spore trap, containing a drum on which Melinex tape coated with paraffin wax, suctions air at a rate of 10 liters per minute capturing aeroallergens. The collection of tape was done every day at 10:00AM CDT. The tape was taken out of the drum and placed on a slide containing water and an emulsion Gelvatol was then added to the cover slip. A drop of 2% Safranin was added to the emulsion to facilitate observation of aeroallergens. After mounting, the slide was observed using a BX-40 Olympus microscope. Tapes were analyzed with a minimum of five latitudinal traverses, and daily concentration was assessed. These five counts also provided the data on pollen counts at the five different intervals of the day. The concentrations were multiplied with a predetermined correction factor of 2.899 to have the aeroallergen index. This report contains the data on the aeroallergen count that we collected from September 2002 to February 2003. We noticed a gradual reduction of the pollen and spore counts with the advent of winter. With the freezing temperature the pollen and spore counts reached '0'. With the return of warm dry weather conditions the dry air spora increased that including *Alternaria*, *Cladosporium*, *Curvularia*, *Pithomyces* and many smut teliospores. Diurnal levels of these spores

usually have peaks during the afternoon hours under conditions of low humidity and maximum wind speeds. By the end of February the mold count reached moderate level and weed pollen count reached high since the weather conditions promoted the production and dispersal of fungal spores and weed pollens with a few centimeter rain and warmer weather.

EVOLUTION OF SUBCAUDAL MICROSTRUCTURE IN ARBOREAL VIPERID SNAKES. JESSE M. MEIK, Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, Texas 76019

Arboreality has evolved independently on multiple occasions in snakes of the family Viperidae. Unique characters such as slender habitus, green coloration, and elongated prehensile tails are characteristic features of each of these lineages and appear to have evolved convergently in response to arboreality. I examined coarse and fine microstructure on the subcaudal surfaces of selected arboreal and terrestrial viperid species using scanning electron microscopy and report for the first time the presence of prominent coarse microstructure that is present only in the arboreal species examined. Interestingly, four arboreal genera (*Trimeresurus*, *Atheris*, *Bothriopsis* [= *Bothrops*], and *Tropidolaemus*) exhibited coarse subcaudal microstructure while one arboreal genus (*Bothriechis*) did not. The types of coarse microstructure observed were highly variable and unique to each genus (from prominent longitudinal ridges in *Bothriopsis* to reticulated craterlike pits in *Atheris*). The potential adaptive value of these microstructures is discussed in the context of the comparative method based on phylogenetic hypotheses of the Viperidae.

THE GENUS *ISOETES* (ISOETACEAE) IN TEXAS. WALTER C. HOLMES¹, ANN E. RUSHING¹, and JASON R. SINGHURST², ¹Department of Biology, Baylor University, Waco, TX 76798 and ²Wildlife Diversity Program, Texas Parks and Wildlife Department, Austin, TX 78704

Isoetes (Isoetaceae), a fern ally commonly known as quillwort, consists of about 150 species of worldwide distribution. Twenty-four species occur in North America north of Mexico. Of these, two (or three) species currently are recognized in Texas. Features of leaf morphology do not readily delineate the Texas species. The most useful taxonomic characteristics for distinguishing species are size and surface features of the megaspores. Scanning electron microscopy examination of Texas specimens reveals differences in megaspore ornamentation patterns of the proximal and distal surfaces. Our studies of megaspore features support the recognition of four species of *Isoetes* in Texas.

FUNGAL LEAF SPOT PATHOGENS OF *CAMPTOTHECA ACUMINATA* IN EAST TEXAS. MELISSA M. LONG¹, JOSEPHINE TAYLOR¹, and SHIYOU LI², Department of Biology¹ and Arthur Temple College of Forestry², Stephen F. Austin State University, Nacogdoches, TX 75962

Camptotheca acuminata Decaisne is a member of the family Nyssaceae and is native to China. In the 1950's it was found that camptothecin isolated from *C. acuminata* was a potential source of anti-cancer drugs. Presently, in the United States there are many different analogs of camptothecin that are in various phases of testing and two have been approved by the FDA to treat several types of cancers. Leaf spot is one of the most common fungal diseases of *C. acuminata* in China. The purpose of this study was to identify leaf spot

pathogens of *C. acuminata* in East Texas, providing future researchers with information needed to develop disease control strategies. Sections of *C. acuminata* leaves exhibiting leaf spots were plated onto Rose Bengal agar. Fungal hyphae growing from each leaf section was transferred to potato dextrose agar to obtain a pure culture. Conidia were used to identify each isolate to the lowest taxon possible, and to inoculate healthy leaves. Lesions that formed on artificially inoculated leaves were used to re-isolate each pathogen, thus completing Koch's postulates. *Epicoccum nigrum*, *Alternaria alternata*, and *Pestalotiopsis guepinii* were found to cause leaf spot, whereas *Fusarium avenaceum* failed to produce symptoms on artificially inoculated leaves.

A COMPARISON OF PORE SIZE IN EGGSHELLS MEASURED USING THREE METHODS: WATER VAPOR GAS CONDUCTANCE, COMPUTER IMAGE ANALYSIS, AND VITALSCAN. SANDRA L. WESTMORELAND, Department of Biology and The Center for Electron Microscopy, University of Texas at Arlington, Arlington, Texas 76019

Gas exchange, which occurs by diffusion through the eggshell pore system, is critical to the optimum growth of the avian embryo. Eggshell porosity must be appropriate to accommodate the embryo's needs, allowing for adequate gas exchange but preventing the occurrence of desiccation. This study compares data collected with three methods of pore measurement to determine if the values obtained are consistent. Eggshell of White Leghorn chickens of the Hyline-19 breeding line was used for this study. The most commonly used method of measuring eggshell porosity for the last 30 years has been that developed by Ar (1974). Ar quantified shell porosity by studying the loss of water from eggs stored under conditions of known humidity. Ar stated that water vapor gas conductance (G_{H_2O}) through pores is a function of both the "total functional pore area" (A_p) and shell thickness (L). Using Ficke's first law of diffusion, Ar derived a formula for calculating functional pore area using G_{H_2O} values. The method used in this study for determining eggshell porosity as measured by water vapor gas conductance is that described by Arad and Marder (1982). Water vapor conductance (G_{H_2O}) and functional pore area (A_p) were calculated according to the method of Ar, et.al., 1974. Pore surface area openings were measured using an image analysis computer program macro developed using Image Pro Plus software. The outer surfaces of shell fragments, which had been treated for cuticle removal, were digitally imaged using a Vanox light microscope and Olympus camera attachment. The pore surface area data were collected and analyzed. In addition, pore dimensions were taken from the cross-sections of eggshell fragments using scanning electron microscope images and Vitalscan computer software, which has a calibrated measuring feature. The pore measurements from the three different techniques were found to be complementary. The "functional pore area," determined with the gas conductance method was found to be approximately equivalent to the narrow pore width at the shell interior. Direct measurement of the pore dimensions using Vitalscan provided data that were consistent with that collected by the other two methods of pore measurement.

A MICROSCOPIC STUDY OF FROST-RINGS IN *PINUS PONDEROSA*

HOWARD J. ARNOTT

The Department of Biology and The Center for Electron Microscopy,
The University of Texas at Arlington, Arlington, TX 76019

In the woody stems of many plants abnormal annual rings termed "frost-rings" are occasionally formed. Studies of frost-rings in bristlecone pines relate frost-ring "chronologies" with volcanic eruptions that may have had major effects on climate (LaMarche and Hirschboeck, 1984; Hirschboeck and Hallman, 2003). Anatomical studies, which might provide an understanding of frost-ring development are rare in recent times. However, in the first part of the last century investigations into both "the timing and the cellular changes" involved in the ontogeny of frost-rings were published (Rhoads, 1923; Bailey, 1925; Glock, 1951). Until now the techniques and increased resolution of scanning electron microscopy (SEM) have not been widely applied to the study of frost-rings. The present SEM study involves transverse slabs from three trees of *Pinus ponderosa*, the yellow pine, growing at about 8000 feet in the Santa Catalina Mountains of Southern Arizona. The specimens were kindly provided by Dr. Ed Wright of The Laboratory of Tree-Ring Research at The University of Arizona. Prior to examination in the SEM, the well-finished slabs were carefully inspected with the dissecting microscope and various features were noted. Subsequently, the slabs were scanned using a flat bed scanner. These scans were made to insure that after parts of the slabs were cut apart during SEM preparation, the position and structure of all rings could be established. For examination in the SEM, appropriate portions of the slabs were sectioned by hand, attached to aluminum stubs, sputter coated, and then studied in a JEOL 35C SEM with a VitalScan unit allowing the collection of digital images. For purposes of this presentation the frost-rings of only one tree, WU3, will be described. The first growth ring in WU3 was produced in 1961; it was harvested in 1998. At the level examined, WU3 was 37 years old and the stem had a diameter of ca. 10 cm. This tree exhibited three frost-rings, which occurred in 1967, 1969 and 1971. The frost-rings varied in apparent intensity with the 1971 frost-ring being the most abnormal (Figs. 1-4). Almost all annual rings of WU3 showed false rings characteristic of the summer monsoon climate found in the Tucson, Arizona area. Each of the three frost-rings found in WU3 had similarities in structure. However, none of them showed the lateral displacement of tracheid files characteristic of frost-rings in *P. longaeva*. Likewise, little "crushing" of tracheids was found in these frost-rings (Figs. 1-4). Although not illustrated, some "inflated" tracheids possessed circular bordered pits characteristic of the radial cell walls of tracheids in normal wood. The mode of cell change which produces frost-rings has been attributed to the freezing of water in one or more of the compartments in the vascular cambial region. Many authors (Glock, 1951 for example) recognize the recently formed phloem as the most probable "compartment" to be involved. Still others (Leuschner and Schwingruber, 2001) relate the ontogenetic changes that engender frost-rings to the formation of a callus; a structure similar to a callus formed during mechanical wounding. The undifferentiated cells characteristic of a callus would help explain how the highly modified cells found associated with frost-rings may differentiate. Whatever the specific cause of frost-ring structure, unless the tree or branch is killed, new xylem and phloem will form in a normal centrifugal growth pattern. However, the manner in which the vascular cambium is reestablished and generates ordered files remains a significant mystery.

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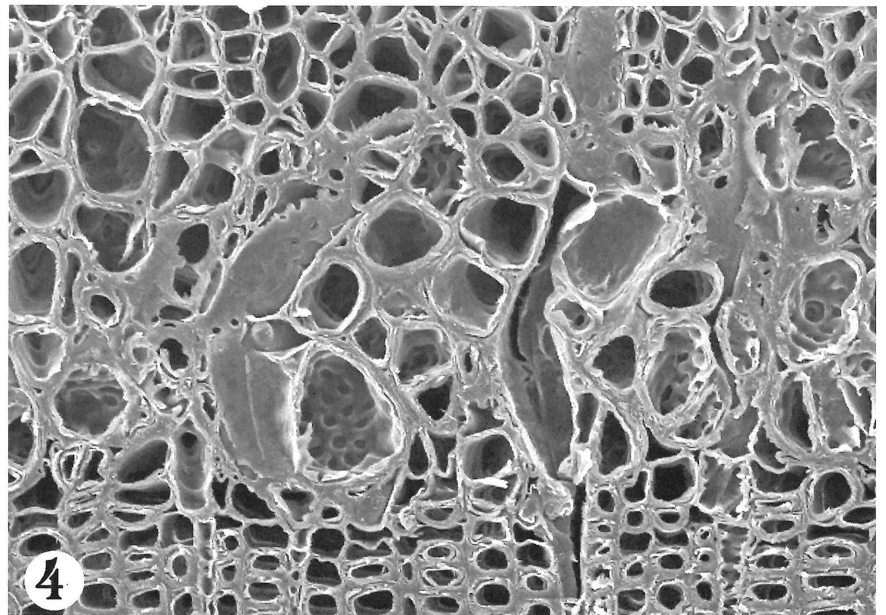
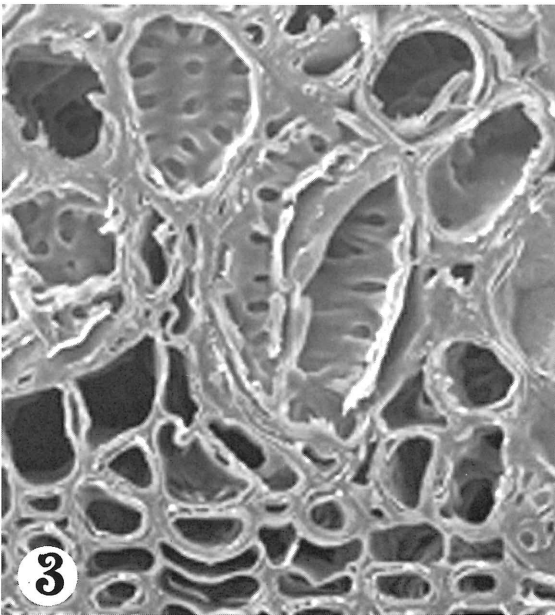
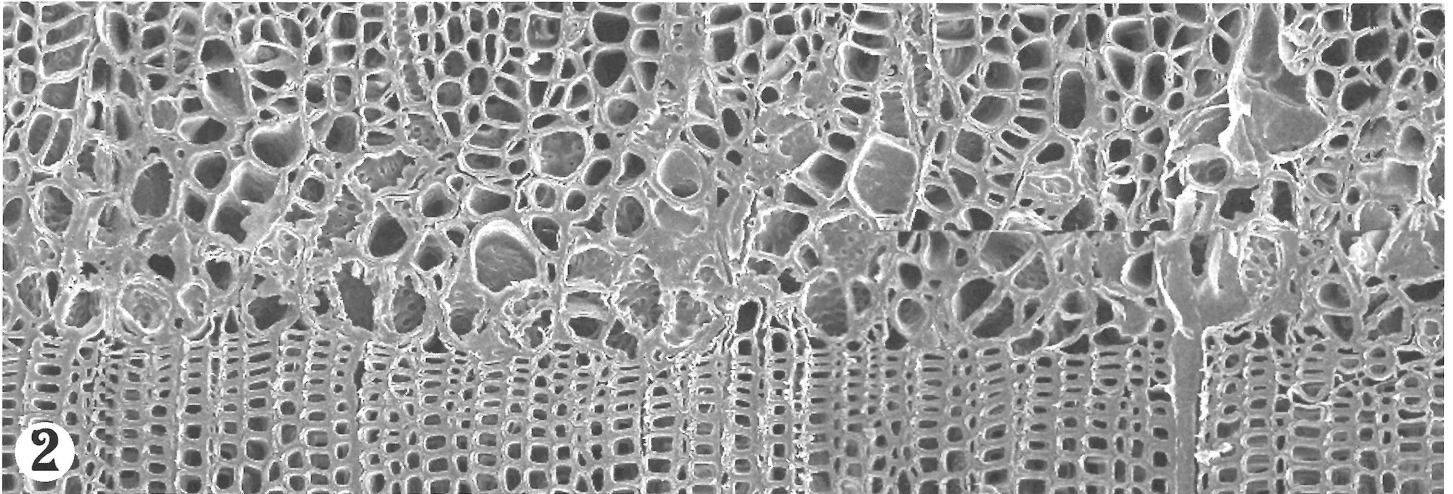
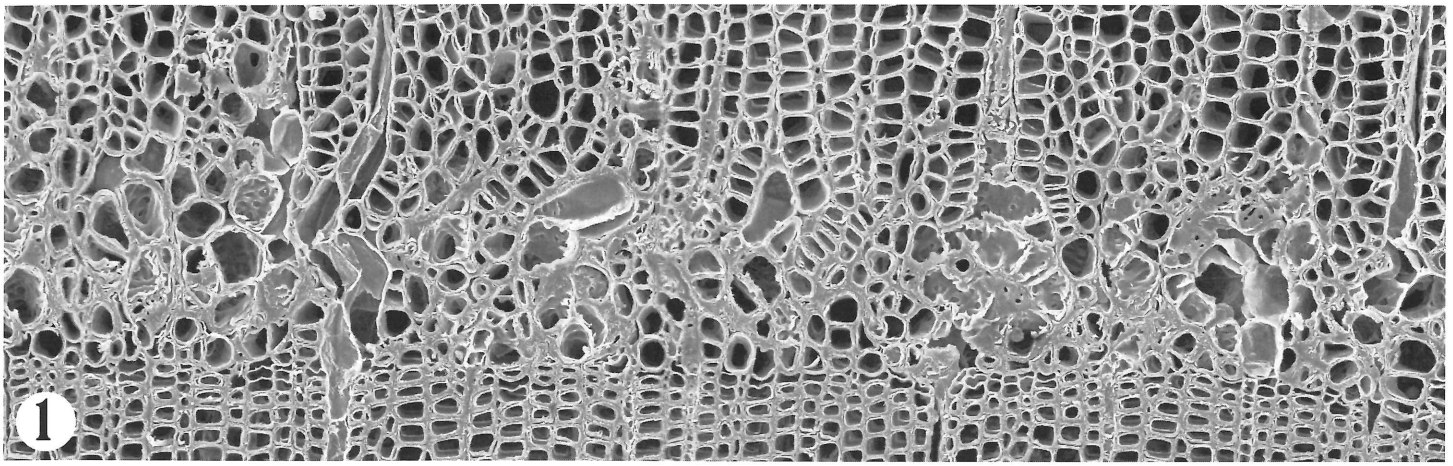


Figure 1. A montage illustrating a portion of the 1971 frost-ring in *Pinus ponderosa* WU3 from the Santa Catalina Mountains of Arizona. Note the continuity and cellular modification of the rays which is especially well shown in the left half of this figure. Bar = 100 μ m. **Figure 2.** A montage of the 1971 frost-ring in *P. ponderosa* WU3 was sectioned at a different level of cut than in Figure 1. A small degree of lateral displacement in the files of the frost-ring is shown in this case. Bar = 100 μ m. **Figure 3.** A portion of the 1971 frost-ring showing details of the highly modified "tracheids" found in associated with frost-rings in this case. Note the pitting displayed in the cells in the center of this figure. Bar = 50 μ m. **Figure 4.** Another area of the 1971 frost-ring showing the cellular details involved in the growth response. Note that there is little lateral displacement of the cell files unlike the case in the bristlecone pine, *P. longaeva*. Bar = 75 μ m.

GEOLOGY

SPRING 2003

A MICROSCOPIC STUDY OF BLACK SAND FROM HAWAII. HOWARD J. ARNOTT and MARTHA I. GRACEY, Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019

Black sand from three sites in Hawaii was studied using standard SEM techniques supplemented by light and laser scanning confocal microscopy, flat bed scanning and image analysis. The first sample was collected from Anaeho'omalu Beach on the island of Hawaii and consisted of small volcanic pebbles. The pebbles had numerous interconnected "bubble spaces" with bits of olivine both on the surface and internally. The second sample was collected at Hana Bay Beach, Maui, appears to be representative of many black sand beaches. It consisted of a mixture of angular glassy materials with bits of coral, olivine and other particles. Some particles in this sample show well-rounded profiles resulting from the constant agitation of wave action. The third sample was collected from a smoking urn found on the third floor of The Sheraton Maui Hotel, located on the West side of Maui near the town of Lahaina. The latter is representative of black sand sold throughout the United States by janitorial supply houses, e.g., Cleansweepsupply.com. This type of "black sand" is manufactured when molten glass derived from the burning of coal is quenched in water. The resultant product is called boiler slag and is sold under trade names such as "Black Beauty" or "Black Pearl." This product has many uses (in sand blasting, as an asphalt aggregate, for water filtration, and as a component of roofing shingles), however, its use in smoking urns is only of minor economic importance. Obviously this "black sand," boiler slag, is not derived from the black sand beaches of Hawaii or any other area. However, the volcanic process which forms the black sand beaches of Hawaii is essentially the same as that involved in the formation of boiler slag. Molten lava (or glass) is quenched by water causing formation of a hard, black, angular, glassy material. Older black sand beaches such as that at Hanna Bay still show some of this angular material. We have "duplicated" this process in the laboratory and found particles similar to those in the urn sand and also like those found in the Hana Bay sand.

SEM EXAMINATION OF RADIOLARIA IN THE LAMAR LIMESTONE OF THE PERMIAN BASIN, APACHE MOUNTAINS, TEXAS. MARTHA I. GRACEY, GALINA NESTELL and HOWARD J. ARNOTT, Department of Biology and The Center for Electron Microscopy (MIG, HJA) and The Department of Geology (GN), The University of Texas at Arlington, Arlington, Texas 76019

Limestone from the Apache Mountains in west Texas contains many interesting objects. In addition to the typical minerals, Lamar limestone contains some "pretty neat fossils." The fossils selected from the rock and presented here will be *Radiolaria*, members of the Phylum Protista. They are unicellular and form their shells (tests) from silica in the water in which they live. Radiolarians are marine plankton and many living species can still be found today. Specimen preparation begins with about two kilograms of rock broken down into small pieces and placed in a solution of 5% formic acid and left to dissolve. The resulting sludge is wet sieved, washed and dried resulting in a powdery residue called "fractures." The fractures are dry sieved and inspected for the quality specimens. The actual selection of the samples is tedious and time consuming. A perforated tray and a 00000 sable paintbrush are used to collect the specimens. Under high magnification, the *Radiolaria* can be seen along with an abundance of sponge spicules and residual rock particles. Many specimens disappear during the collecting process, apparently due to the static charge on the brush, they "jump" out of the field of view. The fractures examined for collection were sized as sixty mesh, one hundred mesh and residual material. Radiolarians are picked out and placed on a stub, sputter coated and examined and identified in the SEM. Several types (species?) were found in the Apache mountain samples and will be presented here. Montages and stereo pairs were made of many specimens in order to increase the resolution and understanding of individual fossils



ELECTROLESS NICKEL/IMMERSION GOLD (ENIG) SURFACE FINISH AND BLACK PAD DEFECT

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Electroless Nickel/Immersion Gold (ENIG) surface finish has become widely used in the electronics industry. The plating technique is ideal for fine pitch packaged devices such as Ball Grid Array (BGA) assemblies and Surface Mount components. The plating finish is also used on Printed Circuit Boards (PCBs) and most recently on under-bump Flip Chip assemblies. The finish offers many advantages for fine pitch features such as co-planarity, the ability to survive multiple soldering cycles, solderability, wear resistance, corrosion resistance, diffusion barrier characteristics, conductivity, and the ability to wire bond to the surface.

The ENIG plating finish is performed by initially applying electroless nickel (phosphorous/nickel) plating, usually over copper. This is followed by the immersion gold plating step, which can be best described as a galvanic displacement process. The gold ions in solution accept electrons from the nickel due to the difference in galvanic potential. The nickel atoms on the surface get replaced with gold atoms, this is a self limiting nickel diffusion process. The resulting gold layer is usually very thin, in the 2 to 8 microinches range. The gold provides a porous free protective barrier for nickel to prevent oxidation until soldering of the component or board is performed.

A defect that can occur with the ENIG plating process has been termed 'Black Pad'¹. This defect is the result of a hyperactive galvanic attack to the electroless nickel during the immersion gold plating process. Black pad occurs when excessive nickel is removed and replaced with gold leaving behind a phosphorous rich layer at the nickel to gold interface. The defect results in a phosphorous rich surface that can affect solder joint reliability. In its most severe state, the defect can cause de-wetting or non-wetting of the solder.

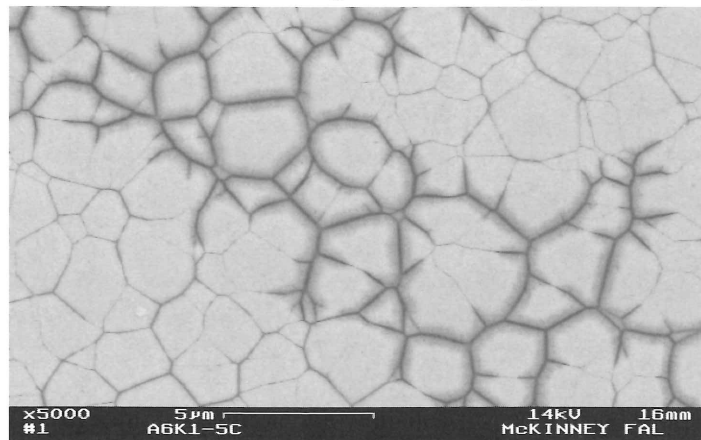


Figure 1. Electroless nickel surface after chemical removal of the immersion gold plating. The image shows the mud-cracked appearance of the nickel and the phosphorous rich zones initiating at the grain boundaries.

Other reliability concerns associated with the defect include weakened bond interface and susceptibility to interfacial fractures. The defect derives its name from the visual dark gray to black appearance of the nickel surface. Another identifier of black pad is the mud-cracked appearance of the surface, as the defect initiates at the nickel nodule grain boundaries. As the severity of the defect increases, deep spreading of a phosphorous rich zone will occur along with penetrating deep crevice/corrosion spikes that initiate along the grains.

¹Nicholas Biunno, A Root Cause Failure Mechanism for Solder Joint Integrity of Electroless Nickel/Immersion Gold Surface Finishes, IPC Printed Circuit Expo, Long Beach, CA Session 18-5-1, March 14-19, 1999, pp. 1-9

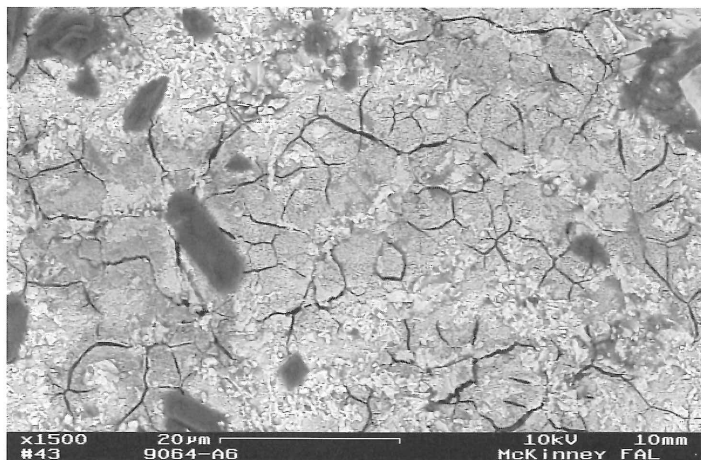


Figure 2. Another example of black pad defect and the mud-cracked surface. This pad failed solderability testing, the solder would not wet to the phosphorous rich surface.

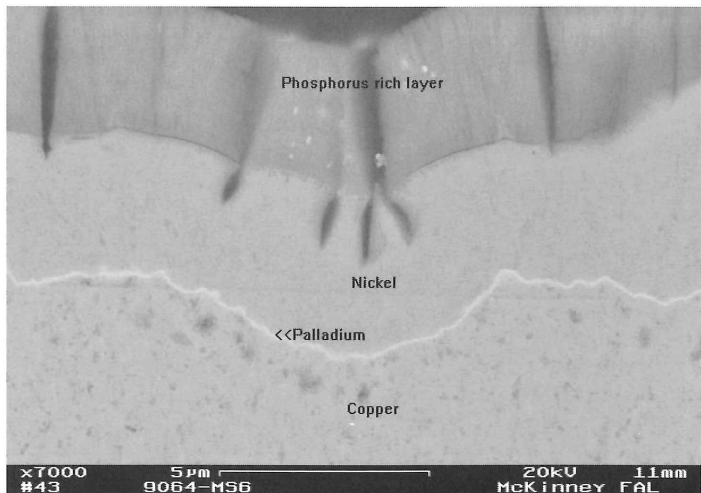


Figure 3. Microsection of a black pad defect region showing the darker phosphorous rich layer and the characteristic crevice/corrosion spikes protruding into the nickel.

FAILURE OF ALUMINUM TO GOLD BONDS DUE TO PURPLE PLAGUE

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The growth of gold/aluminum intermetallics is a phenomenon that has created havoc in the semiconductor industry for many years. Occurrence of excessive intermetallic growth between aluminum and gold may optically appear purple thereby given the name purple plague. This defect can weaken the joint, ultimately leading to joint failure either electrically and/or mechanically. It has been reported that gold will diffuse more quickly into aluminum than the aluminum into the gold.¹ This will cause voiding at the intermetallic to gold interface, which can lead to joint failure. Evidence has also been reported in the past that contamination in the gold will affect purple plague. It has been determined that as the aluminum and gold interdiffuse to form the intermetallic, contaminants in the gold remain behind. These contaminants concentrate in a layer as the intermetallics grow and eventually reach a critical point in which precipitation occurs. The contamination layer will inhibit gold diffusion to further allow for the intermetallic growth. Small Kirkendall voids will occur which eventually cause failure of the joint.²

The bonds between the aluminum bondwires and gold pads are commonly achieved ultrasonically. This bonding process uses vibration to breakdown the oxide layer on the aluminum which otherwise would interfere with the adhesion to the gold. This process produces energy in the form of heat, contributing to the intermetallic growth.¹ Intermetallic growth is expected and necessary in the formation of a good joint and only in extreme cases will result in failure. Subsequent processing and environmental exposures will continue to influence intermetallic growth, which is time and temperature dependent making latent failures a possibility.

Failure analysis was performed on a device in which the failure cause was determined to be purple plague. Aluminum bondwires, ultrasonically bonded to a gold pad, were found to be lifted from the pad. The separation occurred be-

tween the Au/Al intermetallics on the underside of the aluminum bondwire and the pad. Voids were observed in the gold remaining in the vicinity of the bondwire. A well formed around the bondwire due to the diffusion of gold while the intermetallics were being formed. The intermetallics were unique in appearance with the growth occurring in a columnar fashion. The device was examined optically as well as by Scanning Electron Microscopy (SEM). Elemental data was obtained by Energy Dispersive Spectroscopy (EDS). Investigation into the failed device demonstrates how severe gold/aluminum intermetallics, purple plague defect, can cause joint failure.

¹ Charles H. K., Electronic Materials Handbook, Volume 1 - Packaging, ASM International, pp.224-235, 349-350, 1989

² Horsting C. W., 10th Annual Proceedings Reliability Physics, Purple Plague and Gold Purity, pp. 155-158, 1972

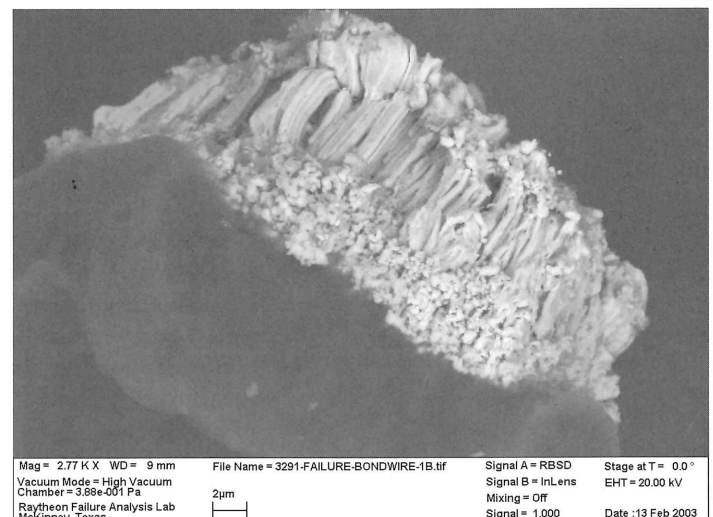


Figure 2. Columnar features of the Au/Al intermetallics that resulted in joint failure.

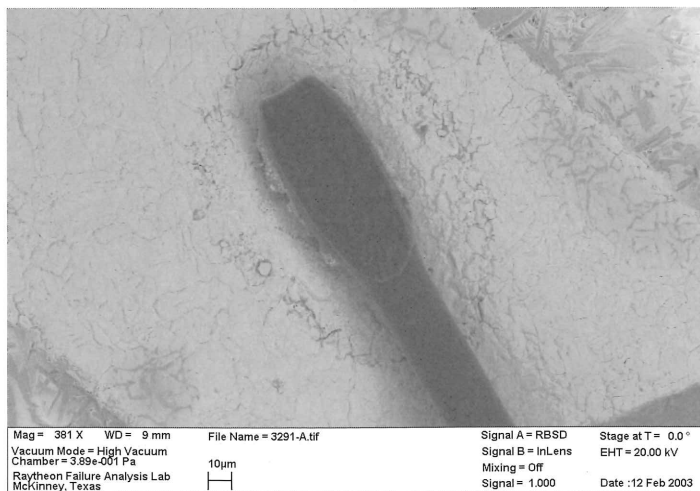


Figure 1. Image of well in the gold pad surrounding the aluminum bondwire.

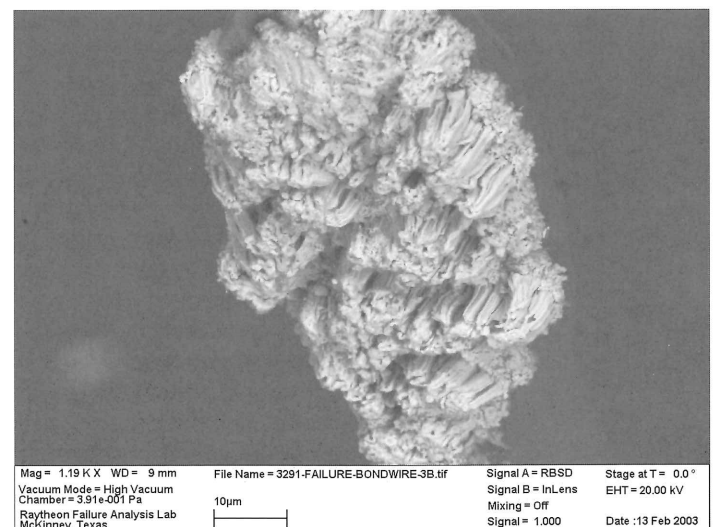


Figure 3. Underside of aluminum bondwire with Au/Al intermetallics.

PLASMA-ETCH CLEANING OF CONTAMINATED STRIP APERTURES: A PRELIMINARY REPORT.

ROBERT E. DROLESKEY¹ and JACK MARLOWE², ¹USDA/ARS, Southern Plains Agricultural Research Center, 2881 F&B Road, College Station TX 77845; and ²Hitachi High Technologies America Inc., 5100 Franklin Drive, Pleasanton, CA 94588

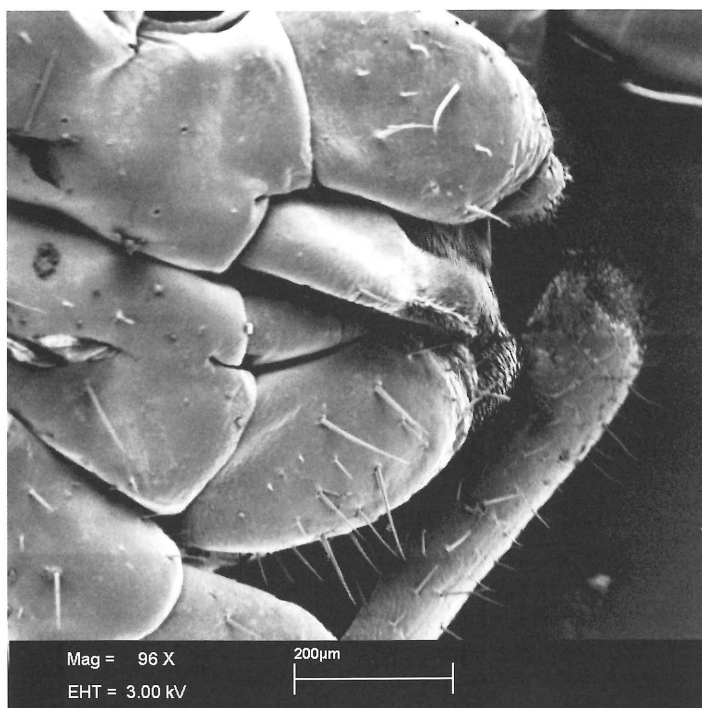
Contamination of apertures in electron microscopes is initiated by the deposition of organic compounds on their surfaces within the microscope column. Sources of these organic compounds include vacuum oils used in the pumping system, gaskets and O-rings used to seal the vacuum system, greases used to lubricate O-rings, and finally the sample itself. Subsequent exposure of the deposited organic molecules to the electron beam results in the formation of a carbon film over the aperture surface. With time, accumulated contamination may hinder the ability of the user to compensate the microscope's condenser and objective lenses leading to either the replacement or cleaning of the contaminated aperture. Traditionally, contaminated strip apertures have been cleaned either by heating to a cherry red hot state within a vacuum evaporator, or by direct heating with a propane torch. Heating to such an extent can lead to excessive heat build up which can distort the shape of the aperture strip as well as destroy welds used to hold multi-plate strips together. Subsequent to a recent TSM workshop on plasma-etch cleaning of SEM samples prior to observation in the microscope, we undertook to determine the suitability of using a plasma cleaner to remove the deposited carbon contamination layer from used condenser and objective strip apertures. Contaminated strip apertures were cleaned for 15-30 min in a Fischione Instruments plasma cleaner operating at 100 watts in a mixture of 75% argon and 25% oxygen. Prior to and after cleaning, strips were inspected and photographed using a conventional light microscope. Images generated from inspections before and after cleaning were compared for each aperture hole. Results indicate that plasma-etch cleaning effectively removed the contaminating carbon layers without distorting the aperture or affecting the dimensions of holes in the strip aperture. Further experiments will determine the minimal time and energy needed to effectively remove deposits from contaminated apertures.

AUTOMATED ELECTRON TOMOGRAPHY. MICHAEL K. MIZELL and STEVE KIM, Emispec Systems, Inc., 2050 S. Cottonwood Dr., Tempe AZ 85282

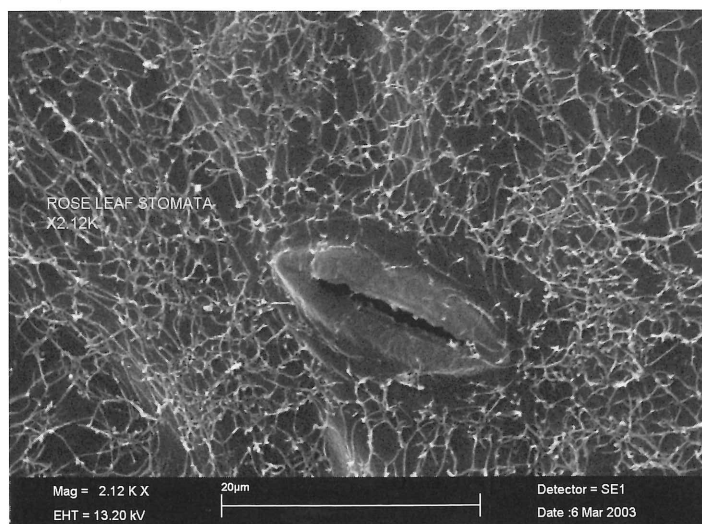
Advancement of computerized interfaces in current electron microscope (EM) instrumentation has opened new possibilities in the areas of integration and automation. Enhanced control of the microscope and its detectors has shifted the process of acquiring and analyzing data from being "technique oriented" to "results oriented". This shift is marked by improvements in the areas of usability, expandability, customizability, speed, and accuracy. Emispec's new platform for EM, Cynapse, has been designed to perform customized, automated and results-oriented microscopy using Windows PC's. Cynapse is compatible with a wide variety of EMs and detectors, and provides all the building blocks for data acquisition, analysis, archiving, and presentation. We have used Cynapse to implement automated electron tomography. Our main design goal was to dramatically reduce the total time needed to acquire and reconstruct the dataset. To accomplish this, we performed streamline acquisition, reconstruction and visualization. The Cynapse tomography module fully automates stage characterization and data collection. Separating these two tasks dramatically reduces the amount of time required to collect a high-quality tilt series. The stage characterization is carried out using a CCD camera at suitable microscope magnification and stage tilt angles. After calibrating the x, y and z stage movements as function of tilt angle, multiple tilt series can be acquired very quickly either in TEM or STEM operation. Any detector or imaging technique could be used for acquisition. Common detectors include CCD cameras and BF, DF or HAADF detectors. Possible imaging techniques include standard TEM, standard STEM, energy filtered TEM, and electron holography. These techniques allow for 3-D characterizations sensitive to local mass-density, chemical structure, and electric or magnetic field strength. The Cynapse tomography module includes reconstruction. Retrieval of the experimental data and initiation of the reconstruction process is straightforward, since all functionality is implemented using the Cynapse platform. Here, speed is being achieved by parallel processing using any number of networked Windows PC's. Final visualization and analysis of the reconstructed volume can be performed on Windows or UNIX platforms through 3rd party software that is dedicated to this purpose. Cynapse exports data in formats compatible with these visualization packages. The strategies incorporated in the software allow scientists to obtain results within 30 minutes. The quick turnaround can be used, for example, to improve experimental conditions or to survey more areas of the sample.



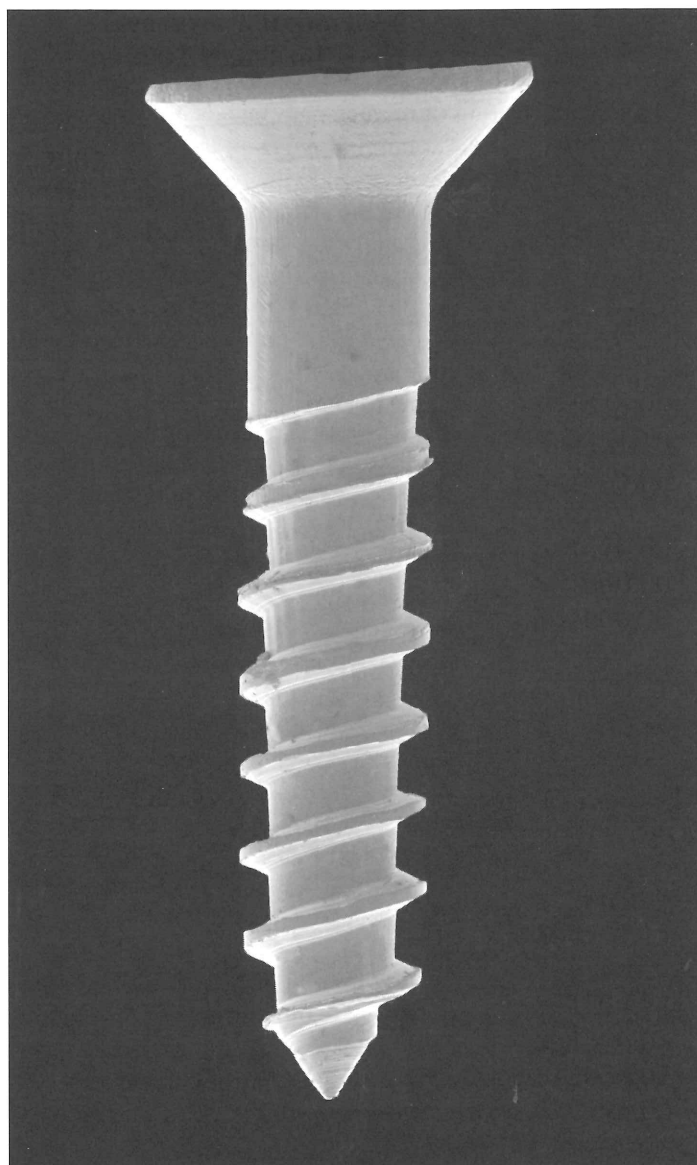
OUR STUDENTS



SEM by **Mary Yanez**, student at **The University of Texas-Pan American** in Edinburg, Texas, showing the mouthparts of an immature American cockroach.



This SEM represents a the surface of a rose leaf, showing a stomate and epicuticular wax formations. Photographed with LEO VP 435 SEM by **Chakavak Sara Farhangi**, student at the **University of Texas-Pan American**, Edinburg, Texas.



SEM "artwork" of a wood screw. This is a montage of a number 2X, ½ inch brass wood screw. The montage consisted of thirty-nine images taken at 50X assembled in Photoshop and was a part of the curriculum in a course on Scanning Electron Microscopy. The original micrographs were taken and put together by Nicole Conaway, student at **The University of Texas at Arlington Center for Electron Microscopy** under **Dr. Howard J. Arnott's** supervision.

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Education

Electron microscopy techniques can be successfully taught to high school students, as David Muirhead proved at the last TSM meeting. North Garland High School students presented ten projects and they prepared them as PowerPoint presentations as well as posters. Some of the projects were dealing with the ultrastructural effect of copper on the brain and liver of the *Carrasius auratus*, the symbiosis between alfalfa plant roots and *Sinorhizobium meliloti* bacteria, the lung development of *Gallus domesticus* under hypoxic and normoxic conditions, the ultrastructure of fibroblasts and endothelial cells grown in two dimensional cell cultures, the ultrastructural changes in the N-gene tomato plants infected with tobacco mosaic virus (TMV), and others.

David, originally from Scotland, worked in several foreign countries before arriving from Australia to the United States to become the coordinator of the Electron Microscopy Facility in the Department of Cellular Pathology at the Texas Scottish Rite Hospital for Children, in Dallas. High school student exposure to modern microscopy techniques was made possible by David's collaboration with Soft Imaging System Corp (SIS), a provider of digital imaging software and cameras for Light Microscopes, Transmission Electron Microscopes, and Scanning Electron Microscopes.

SIS donated a MegaView II system to North Garland High School, where David is using it to teach a course introducing

interested students to electron microscopy techniques as well as to modern digital image analysis and acquisition software. This experience has created a tremendous amount of enthusiasm from students at North Garland High School. Their presentations at the last TSM meeting in Austin were very well received by the TSM members, proving the success of David's two years old initiative. David hopes to keep the younger generations interested in science, expanding their knowledge in the area of microscopy, exposure not really offered in many other high schools across the country. Only a few high schools have functional electron microscopes for students with no digital analysis equipment attached to them.

The Editor

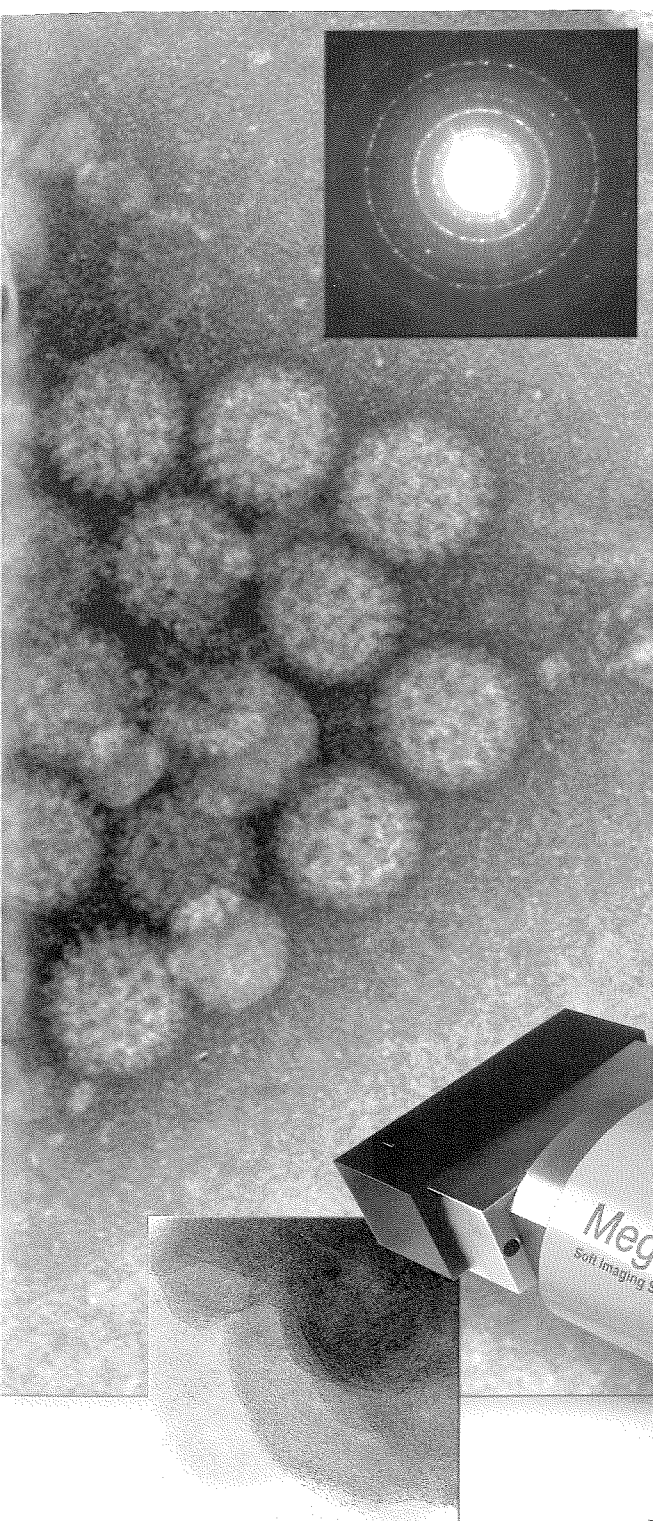


David Muirhead and Don W. Smith, former TSM President.



North Garland High School students presenting their projects and answering questions at the 2002 fall meeting in Austin.





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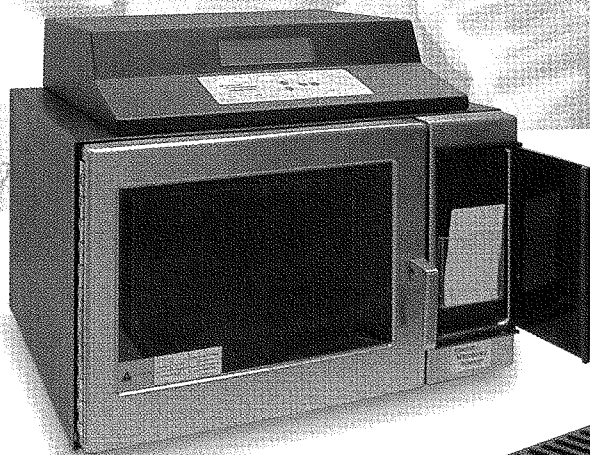
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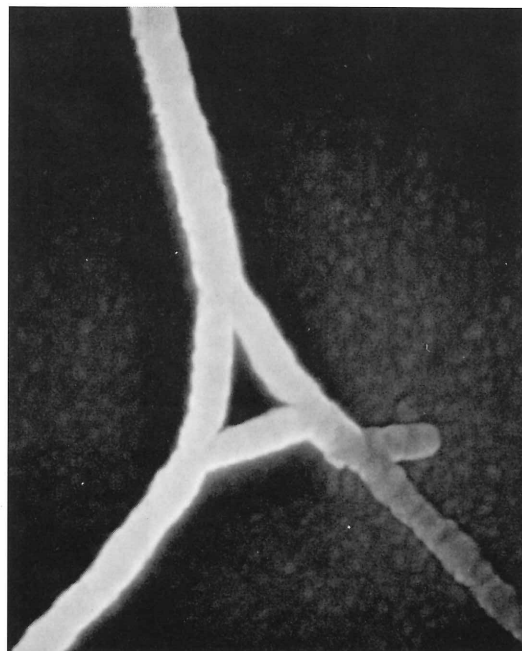
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