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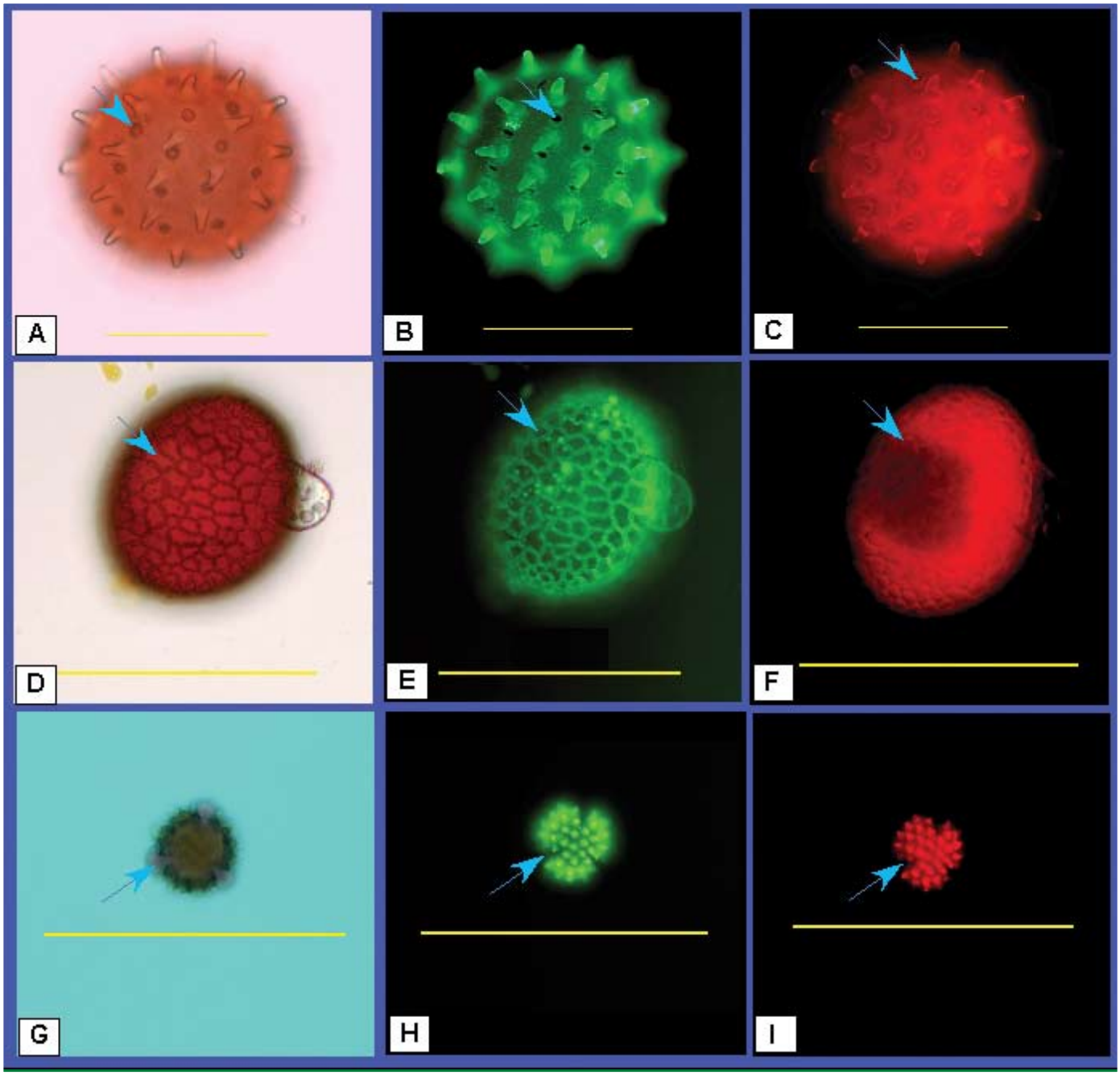

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Camelia G.-A. Maier, Editor
Department of Biology, Texas Woman's University, Denton, TX 76204

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ON THE COVER

Pollen Microscopy - China rose pollen (*Hibiscus rosa-sinensis* L., *Malvaceae*) under bright field (A), with FITC fluorescent filter (B), and with TRITC fluorescent filter (C). Pollen from Easter lily, *Lilium longiflorum* Thund. (*Liliaceae*) is shown under bright field (D), with FITC fluorescent filter (E), and with TRITC fluorescent filter (F). Pollen from Common daisy, *Bellis perennis* L. (*Asteraceae*) under bright field (G), with FITC fluorescent filter (H), and with TRITC fluorescent filter (I). The pollen grains were collected from the stamens of the fresh flower and were teased with a needle for a uniform spreading in Fluorol Yellow 88. This is an azo dye which is derived chemically from anthraquinone. The spectral transmittance is determined on solutions of this dye in mineral spirit. Use of fluorescent stains helps in visualizing the architecture of pollen ectexine. The *Hibiscus* pollen better revealed its concordant pattern of and pores on the ectexine by using the TRITC filter. The *Lilium* pollen revealed the heterobrochate pattern (lattice-like pattern). Colpi and spiny projections on ectexine became more conspicuous in preparations of *H. rosa-sinensis* and *B. perennis* pollen by using the FITC and TRITC fluorescent filters. The micrographs were taken with a BX 40 Olympus microscope equipped with a DP-70 digital camera and processed with the Image Pro Plus software. Bar = 100 µm. **Mandy Whiteside and Nabarun Ghosh, West Texas A&M University.**

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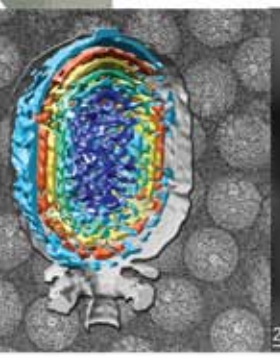

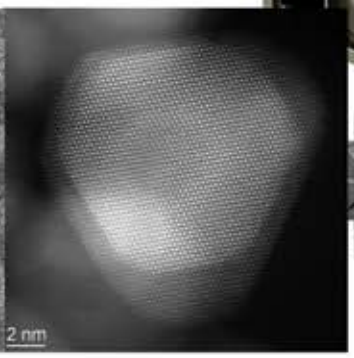

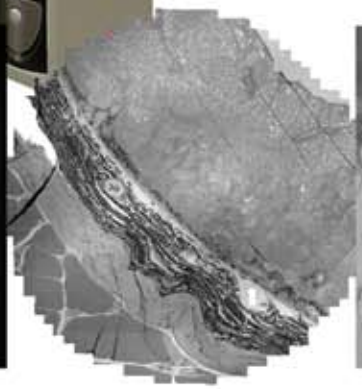

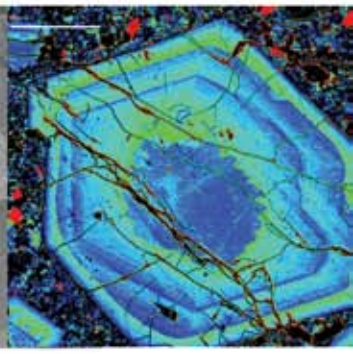

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President's Message

It has been almost a year since we met for our spring meeting in Austin. At that time, the Society decided to adopt one annual meeting with the hope that the attendance and number of presentations will increase. I believe that this was a good decision. The upcoming meeting in Westlake was thoroughly prepared and will offer a workshop and four guest speakers besides the regular presentation sessions. I hope that with the increased attendance and interests towards microscopy and microanalysis we shall be able to bring back the biannual meeting system in the future. The TSM meetings represent our forum, where we meet with friends and make new friends from various fields of microscopy, we exchange ideas, learn new techniques, and establish collaborations. I sincerely hope that we get the chance of more interaction by switching back to the biannual meetings.

My sincere thanks go to our treasurer, E. Ann Ellis, secretary, Mike Pendleton, program chair, Sandra Westmoreland and past president Ernest Couch for their coordinated effort in planning and organizing the spring 2009 meeting. Becky Holdford, our web master has done an excellent job in organizing and updating the TSM web site with the recent issue of the *Texas Journal of Microscopy* and announcements.

I would like to thank Camelia G.-A. Maier, the editor for the *Texas Journal of Microscopy* for her sustained hard work and effort in organizing the scientific articles and maintaining the high quality for the journal. Thanks to Camelia we have the journal continuously getting published and digitally posted on the TSM website.

Paulo Ferreira helped us a lot in organizing the meeting at the UT-Austin campus in the spring of 2008. There were a number of excellent presentations both from the biological and materials sciences. Sandra Westmoreland, Bob Droleskey, E. Ann Ellis, Camelia Maier, Mike Pendleton and Becky Holdford have been helping me all the way to make the 2009 spring meeting in Westlake a success.

The anniversary article on "Wood" by Howard J. Arnott was an excellent article that will inspire future plant anatomists. Dr. Arnott and his students have been contributing to the society continuously by their active participations without which many of the sessions of the society would have not been possible. I always look forward to his elegant and intriguing presentations on varied newer specimens.

I would like to congratulate E. Ann Ellis who was recently honored with the Distinguished Scientist Award at the Southeastern Microscopy Society (SEMS). We mourn the loss of Bob Turner, one of the charter members of the Texas Society for Electron Microscopy, now TSM.

Finally, I would like to thank all of our corporate members whose constant support helped us in organizing the meetings. We need to increase the number of members and involve new groups of researchers, students, and staff who are using various techniques of microscopy and microanalysis in the great state of Texas.

Nabarun Ghosh
President 2008-2009
Texas Society for Microscopy

Call For Papers

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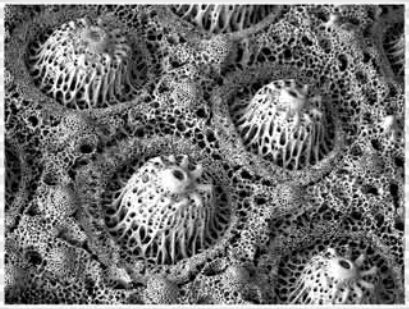
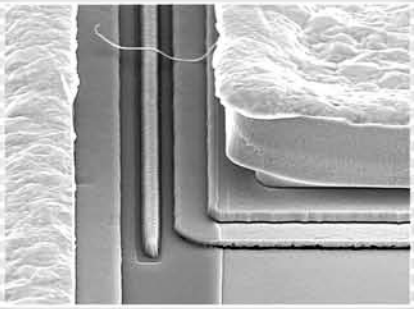
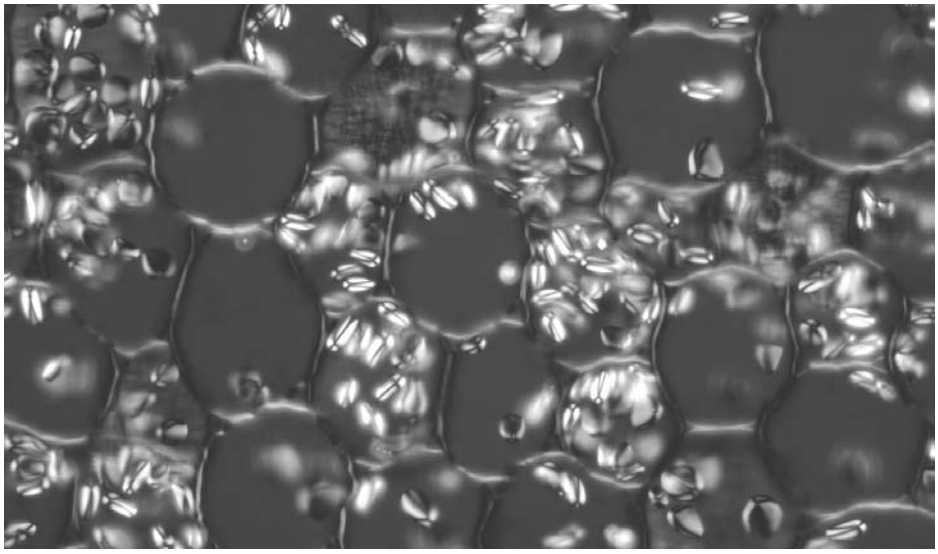


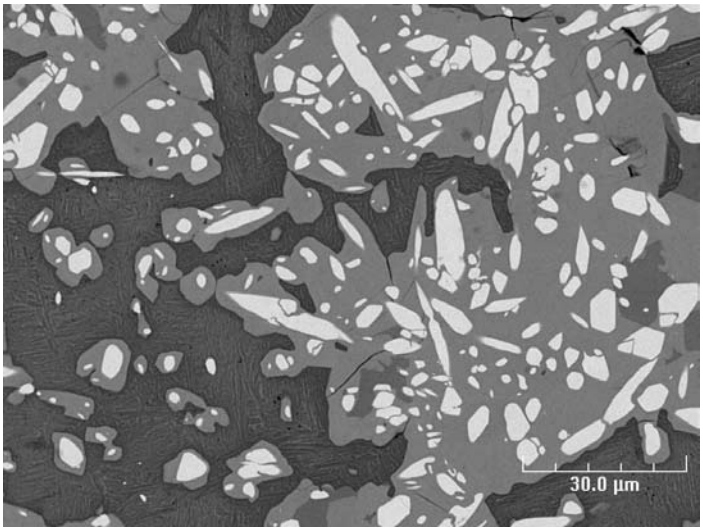


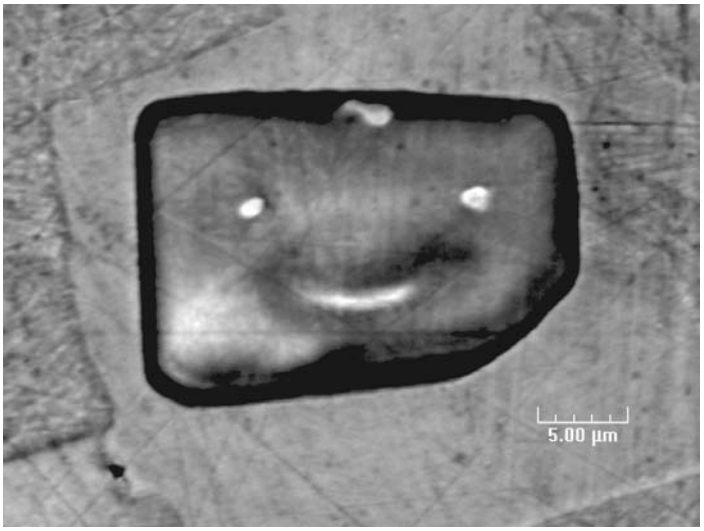
"Answer To What Is It?"



FROM JOURNAL 38:2.
Starch granules under polarized light in plant parenchyma.
Light micrograph by **Dr. Irina N. Gostin**,
Al. I. Cuza University, Biology Department, Iasi, Romania.

What Is It?" *Answer in Next Edition*





Submitted by **Paul Mews**, staff member, Nuclear Engineering
Department, Texas A&M University, College Station, Texas 77843.

Abstracts

BIOLOGICAL SCIENCES
SPRING 2009

USE OF MICROSCOPY AND BIOMETRICS IN FORENSICS. CAITLIN SCHMIDT[1], NABARUN GHOSH[1], NEAL LATMAN[1] AND [2]DON W. SMITH, [1]Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, Texas 79016 and [2]Biological Sciences, University of North Texas, Denton, Texas 76203.

The advancement of forensic tools and refinement of biometrics techniques for the analysis of evidence with the aid of microscopy has improved criminal investigations. Some microscopic biological materials such as pollen, molds and dust are excellent crime solvers because they are virtually impossible to remove from a crime scene or suspect. Observations and analysis of fibers, hairs, dust particles and biological substances like blood, semen, and other bodily fluids and tissues have been used in leading investigations to help solving criminal cases. In this study, various types of fibers, dust, and hair samples were analyzed using a SZ 40 Stereo Microscope equipped with gooseneck fiber optics, DVC camera, and DVC C-View V2.2 Software. Fibers from various sources including clothing, towels, and carpet revealed unique color, length and texture, which helped us determine the sample sources. The fiber length and width were measured using an ocular graticule on the microscope. Fibers analyzed from a towel were dark brown in color and tightly twisted, giving it a thick tubular appearance. Carpet fibers were very thin, straight, and almost translucent with a copper sheen, while the sweater fibers displayed a wired and curled appearance. Biological samples from prepared slides of blood, pollen, and mold samples collected from various sources were also analyzed and identified using the literature. Microscopy and biometric tools are very useful in analyzing evidence to provide conclusive results for criminal cases.

EFFECTS OF DIFFERENT FIXATIVES ON TUNEL STAINING IN RAT TESTES. DIBYENDU DUTTA[1], GLADIS A. SHUTTLESWORTH[2] and NATHANIEL MILLS[1], [1]Dept. of Biology, Texas Woman's University, Denton, TX 76204 and [2]Department of Experimental Radiation Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030.

To quantify apoptosis in tissue samples for diagnostic or experimental purposes, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) is currently a commonly used technique in medicine and research. According to the Organization for Economic Co-operation and Development (OECD) Guidelines, to study testicular toxicity, tissues should be fixed in either Bouin's Fluid (BF) or modified Davidson's Fluid (mDF). The OECD states that modified Davidson's Fluid is better than Bouin's Fluid because it: (1) is safer than BF, (2) maintains comparable morphology and (3) gives better hematoxylin-eosin (H&E) and immune-histochemical staining. Unusually, high positive TUNEL staining in rat testes fixed with BF at room temperature (23°C) were observed and were followed up with a comparison to other fixatives and temperatures of fixation, as follows: (1) BF at 4°C, (2) mDF at 23°C and 4°C and (3) modified HistoChoice (mHC) at 4°C. Morphology, H&E staining and TUNEL staining in adult rat testis and liver were evaluated. All above-mentioned fixatives resulted in similar morphology of both testis and liver. Increased TUNEL background staining of testis fixed with BF and mDF at 23°C was observed, mainly during the type-B spermatogonia and pre-leptotene stages. At 4°C, positive background was reduced in BF-fixed testis compared to BF-fixed tissues at 23°C. However, testis fixed in BF at 4°C still had more false positive nuclei than mDF at 4°C. Modified HistoChoice was used to fix tissues at 4°C and results were similar to those obtained with mDF fixation. With liver, all three fixatives gave similar results, although mDF- and mHC-fixed tissues provided better H&E staining than BF. In conclusion, mDF or mHC fixation at 4°C produced fewer false positive TUNEL stained nuclei compared to BF and fixation of tissues at 4°C reduced DNA breakage observed with all low pH fixatives. Supported by TWU REP Grant and Department of Biology.

EFFECTS OF ATRAZINE ON FRESHWATER PHYTOPLANKTON GROWTH, POPULATION DENSITY, AND COMMUNITY STRUCTURE. GRISELDA ESTRADA, ASHLEY THOMPSON, NABARUN GHOSH, and WILLIAM J. ROGERS, Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, Texas 79016.

Atrazine is the most widely used herbicide in the United States (64-80 million pounds annually). It is used for broadleaf weeds and some grassy weeds and works by inhibiting electron transport in PS II during photosynthesis. Atrazine has been detected in lakes and rivers at levels high enough to be considered a potential health threat to the public (TNRCC, 2001). Many Texas Panhandle ponds and wells are polluted with atrazine. A large fish kill at Lake Tanglewood in Amarillo, Texas, along with a case of illness experienced by a dog after drinking water from the same lake triggered this investigation. There were many assumptions on the cause of this periodic toxicity in Lake Tanglewood. To detect if cyanobacteria were involved in the periodic fish kill, we collected random water samples from the lake and designed an algal growth system in the culture room. We Bottles of used 0.5 L were used to culture the phytoplankton under a 12-hour photoperiod with constant aeration from air pumps. Cell density counts were periodically obtained using a Sedgwick-Rafter Cell Counter and selecting thirty random bright field views at 10X magnification and micrographs were obtained using a BX-40 Olympus microscope with a DP-70 Digital Camera. Three different concentrations of atrazine (0.1 ppb, 1 ppb, 10 ppb) were used to treat the cultures. Statistical analyses using one-way ANOVA and Tukey's test post-hoc were performed. Low atrazine concentrations appeared to have little or no effect in producing a golden algal bloom; however, unexpected population changes were noted in the fourth week with increased turbidity in spite of no nutrients added to the cultures. During this study, a gradual shift in the phytoplankton community from obligatory autotroph to facultative autotroph and finally to a parasitic planktonic community was observed. This gradual shift in planktonic community after application of atrazine may explain the periodic fish kill in Lake Tanglewood. Future studies are aimed at the atrazine's mechanism of action in inducing algal bloom.

LOCALIZATION OF ACC OXIDASE TO MONITOR CELLULAR LEVELS OF ETHYLENE IN PIERCE'S DISEASE IN GRAPE PETIOLES. E. ANN ELLIS[1] and B. GREG COBB[2], [1]Microscopy and Imaging Center and [2]Dept. of Horticultural Sciences, Faculty of Molecular and Environmental Plant Sciences, Texas A&M University, College Station, TX 77843.

Pierce's disease is caused by the bacterium *Xylella fastidiosa* which infects the xylem and results in scorching and premature abscission of the leaves of wine grapes. Ethylene has been demonstrated to play a role in the pathology caused by a number of plant pathogens. The enzyme 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) is the last enzyme in ethylene production and is a biomarker for sites of ethylene production. Colloidal gold based immunocytochemical localization of ACC oxidase was done in petioles of grape leaves infected with *X. fastidiosa* to determine cellular sites of ethylene production in Pierce's disease. In addition, petioles of uninfected plants injected with ethylene were examined for ACC oxidase. ACC oxidase localized in the bacteria, adjacent to the bacteria in the xylem and accumulated in xylem cell walls. There were decreased levels of ACC oxidase in the cytoplasm of parenchyma cells and adjacent vacuoles and cell walls. Localization of the enzyme in cell walls and vacuoles probably indicates cell wall absorption of the secreted enzyme. Ethylene treatment of uninfected plants maintained in the greenhouse resulted in the same leaf scorching and petioles left with the appearance of matchsticks as that seen in plants infected with *X. fastidiosda*. There were high levels of ACC oxidase throughout all cells and tissues of petioles injected with ethylene. These results of ACC oxidase localization in uninfected, ethylene treated grape vines corroborate the results of ACC oxidase localization in naturally infected grape vines and provide a better understanding of the role of ethylene in the pathology of Pierce's disease.

CONFIRMATION OF *E. COLI* INTERNALIZATION IN LETTUCE LEAVES. C. GOMES[1], R. G. MOREIRA[1], E. CASTELL-PEREZ[1], E. ANN ELLIS[2], and M. PENDLETON[2], [1]Biological and Agricultural Engineering Dept., and [2]Microscopy and Imaging Center, Texas A&M University, College Station, Texas 77843.

There has been an increase in the number of foodborne illnesses linked to the consumption of fresh and minimally processed fruits and vegetables. *Escherichia coli O157:H7* is clearly a public health concern since it is the second most important causal agent of outbreaks from ingesting fresh produce. Internalization of bacterial pathogens into the edible portions of plants is of particular concern as these microorganisms are unlikely to be removed by washing or surface sanitization methods. The objective of this study was to understand the mechanisms of pathogen colonization of plants relative to lettuce leaf structures using scanning electron microscopy (SEM) images. Leaves of iceberg, Boston, green leaf, and red leaf lettuce varieties were cut into pieces, submerged in a cocktail mixture of two isolates of *E. coli*, and subjected to a vacuum perfusion process to force the bacterial cells into the intercellular spaces of the leaf tissues. SEM images from the four different varieties of lettuce confirm that the preferential port of entry of *E. coli* internalization to the vegetable leaf is through the stomatal pores. The inoculation method utilized in this study by forcing the infiltration of the bacteria elucidated how this process would occur naturally. Furthermore, the *E. coli* cells were consistently found inside stomatal chambers, at the edges and/or surrounding epidermal stomatal areas in all lettuce varieties. On the other hand, no bacteria were found on the cut edges of leaf pieces. Stomate density and size were measured and no significant differences ($P<0.05$) were found among varieties. This work provides evidence of the potential for lettuce leaves to provide a microenvironment for *E. coli*, where they are protected from removal by decontamination processes such as washing and surface sanitizing since the contamination sites on leafy vegetables are mainly localized to stomates and surface crevices.

PIERCE'S DISEASE AND UNBALANCED XYLEM CHEMISTRY: CAUSE OR CONSEQUENCE? BRENO LEITE, Thermo Fisher Scientific, Madison, WI, 53711 and PETER C. ANDERSEN, University of Florida-NFREC, Quincy, FL, 32351.

Pierce's disease of grapevine is caused by the pathogenic bacterium, *Xylella fastidiosa*. Pierce's disease precludes the successful establishment of high quality *Vitis vinifera* in the southeastern United States, and has caused millions of dollars in losses to the California grape and wine industry. This disease is transmitted by leafhopper vectors, and is characterized by xylem dysfunction and plugging. There is no resistant germplasm within *V. vinifera*, and there is no practical cure. *X. fastidiosa* may be found in the xylem fluid of numerous plant species where it usually is not pathogenic. Xylem fluid is the most dilute plant fluid (10-30 mM) and consists of amino acids, organic acids and inorganic ions. Once bacteria are inoculated in a *Xylella*-susceptible plant, they increase in population and establish a community consisting of bacterial cells (alive and dead), gums and extracellular polysaccharides to form a biofilm. The proliferation of bacteria and biofilm formation is determined, at least in part, by the chemistry of xylem fluid. During this presentation I will discuss a possible relationship between xylem chemistry of grapevine genotypes and susceptibility to Pierce's disease. I will also present Energy Dispersive Spectroscopy data concerning the surface chemistry of xylem vessels of Pierce's-disease infected and non-infected grapevines. Results showed that leaf scorched areas (lesions) accumulated high concentrations of silicon (Si).This metabolic transition is possibly a result of the plant strategy to cope with xylem vessel occlusion due to bacterial multiplication and the reduction of water flow. This is the first work reporting Si accumulation in leaf scorched areas affected by Pierce's disease. Future investigations should reveal the influence of Si on the concentration and availability of calcium (Ca) and magnesium (Mg), as these elements may influence bacterial aggregation and adhesion.

A NOVEL HDAC3-H1 COMPLEX IS LOCALIZED TO MITOTIC POLAR SPINDLE MICROTUBULES. HEMANGI PATIL, MANGALAM SUBRAMANIAN, RHIANNON W. GONZALES, CARRIE WILKS, and MICHAEL BERGEL, Department of Biology, Texas Woman's University, Denton, TX 76204-5799.

Histone deacetylases (HDACs), which remove acetyl groups from core histone proteins, regulate the compaction of the chromatin fiber, thus modulating gene transcription levels. Recently, several HDAC inhibitors (HDIs) have been shown to cause growth inhibition of malignant cells. Although many of these inhibitors have been shown to increase levels of p21 in malignant cells, the targets and the mechanism of action by which HDIs cause growth inhibition of these cells are not clear. It has been suggested that HDIs may target non-histone cellular components to induce the anti-proliferative effect in the malignant cells. Chromatin binding proteins, such as linker histone H1, are also known to cause chromatin compaction. H1 and HDAC3 were independently reported to be involved in the regulation of mitosis. The hypothesis of this study was that H1 and HDAC3 could act together by forming a protein complex involved in chromatin condensation and mitosis regulation. Immunoprecipitation assay results demonstrate the formation of stable complexes between HDAC3 and histone H1 proteins in HeLaS3 (human cervical carcinoma epithelial) cells. Although the amount of complex increased before and during mitosis, the complex was

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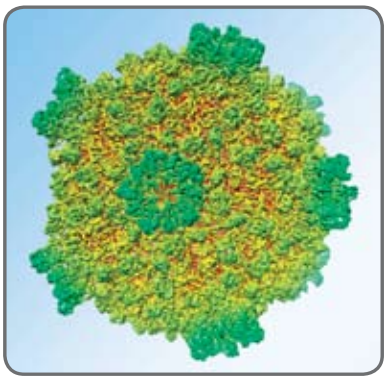

3.88 Å structure of Cytoplasmic Polyhedrosis virus by cryo-electron microscopy

Courtesy of Xuekui Yu, Lei Jin & Z. Hong Zhou, University of California, Los Angeles, USA

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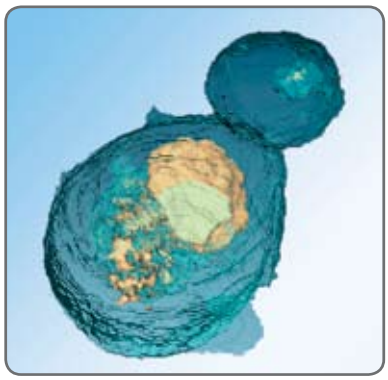

Volume rendering of the three-dimensional architecture of a dividing yeast cell

Courtesy of Sriram Subramaniam, National Institutes of Health, Bethesda, USA

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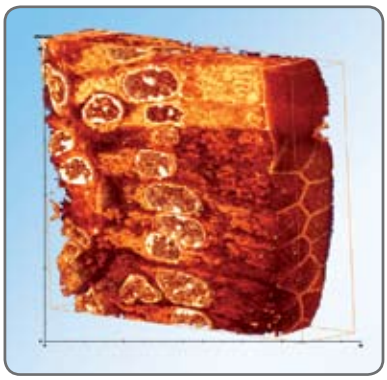

Mouse intestine epithelial tissue imaged 50 x 50 x 10 micron using a pixel size of 25 nm and section thickness of 40 nm

Courtesy of Paul Matsudaira, Dept of Biological Sciences, National University of Singapore

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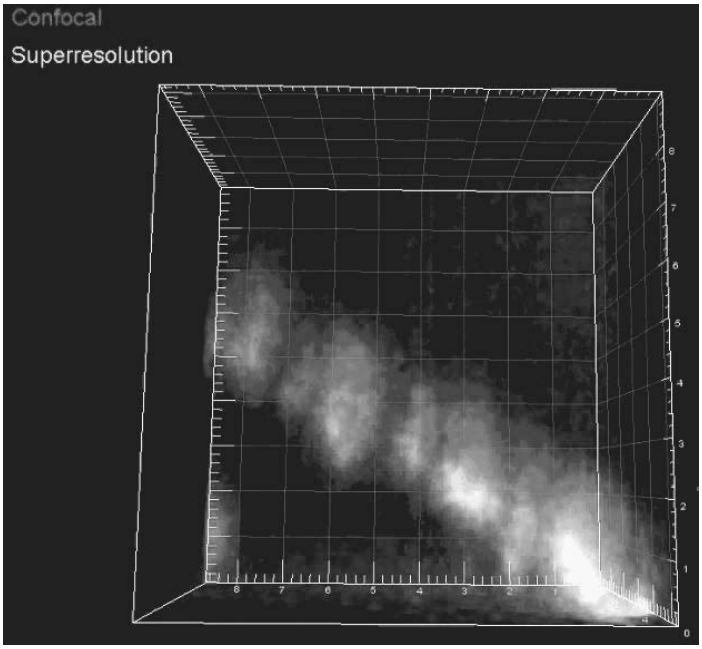
activated for chromatin de-acetylation only in mitosis. Based on our immunocytochemistry and confocal imaging studies, H1-HDAC3 complexes co-localized with mitotic polar microtubules. This is the first report, to the best of our knowledge, in which histone H1 and a class I HDAC are shown to form a complex. This is also the first record of an association of histone H1 to a non-chromatin cellular structure. Currently, siRNA experiments are being conducted in our lab to study the possible functions of the complex in the microtubule dynamics and mitosis regulation.

EFFECTS OF ATRAZINE ON *ALLIUM CEPA* ROOT TIP MITOSIS. CHRISTIAN RIDNER, MAGAN TAYLOR-ALLEN, and NABARUN GHOSH, Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, Texas 79016.

For over 25 years, atrazine has been used as a selective broadleaf herbicide in many capacities, from pre-plant to pre-emergence to post-emergence, depending on the crop and application. Currently, 96% of all atrazine used is for commercial applications in fields of sorghum, corn, sugarcane, pineapple and for the control of undesirable weeds in rangeland. Although, many Texas panhandle wells are polluted with atrazine, the level of pesticide detected in well water samples were not in violation of the state pesticide regulations. In this study we investigated the effect of different concentrations of atrazine on *Allium cepa* (onion), a standard plant test system for studying anomalies during mitosis. We established a control with the *Allium* bulbs grown on hydroponics culture. Different concentrations of atrazine, ranging from .34 ppm to 5 ppm in 4 set treatment were used. Prepared slides of onion root tips sampled after 24, 48 and 72 hours from the initiation of atrazine treatments were used for microscopic observations. Root tips were cut and pre-treated with saturated solution of para-dichlorobenzene (p-DB) for 3 hours, after which they were washed with distilled water and fixed in 1:3 aceto-ethanol overnight. The fixed root tips were stained with 2% aceto-orcein and squashed in 45% acetic acid on microscopic slides. Well-scattered somatic metaphase plates were selected for karyotypic analysis (2n=16). The control set was compared with the atrazine treated sets in terms of mitotic indices and cytological behavior. Various chromosomal abnormalities were observed, such as like sticky bridges, early and late separations, lag chromosomes with higher doses of treatments. Morphologically, the only noticeable effect was the root rot at higher doses of atrazine. Future experiments will employ higher doses of atrazine to detect cellular anomalies in onion and other test systems.

HIGH RESOLUTION FLUORESCENCE MEASUREMENTS OF THE MUSCLE CONTRACTILE APPARATUS. DOUGLAS D. ROOT, Department of Biological Sciences, University of North Texas, Denton, Texas 76203.

A new renaissance in high-resolution light microscopy has recently enabled imaging at beyond the diffraction limit. A novel super-resolution microscopy technique is presented for imaging immuno-labeled myofibrilar proteins using photoactivatable quantum dots (PAQ dots). Previously, photoactivation of cyanine dyes and GFP variants for 2D super-resolution microscopy have been reported, but PAQ dots have sufficient brightness and photostability to enable 3D acquisitions of signals from individual quantum dots using confocal microscopy. The chemical synthesis of Fab-conjugated PAQ dots caused only minor changes in the spectroscopic properties and brightness of the activated PAQ dots relative to unmodified quantum dots as assessed by fluorescence lifetime imaging of single quantum dots. After optimizing conditions so that a balance between photoactivation and photobleaching of the PAQ dots occurred during 3D acquisition in a spinning



disk confocal microscope, 3D images of individual quantum dots were reduced to the 3D center of mass and accumulated until sufficient data for a full image was generated. Initial results demonstrate sub-diffraction resolutions in XY and even more striking resolution improvements in Z. The super-resolution images reveal finer structural details in the myofibrils than conventional confocal imaging. Unlike electron microscopy, all measurements are made in aqueous solutions. Furthermore, the ability to make PAQ dots with a variety of emission wavelengths enables multicolor 3D labeling that can be used for protein mapping at super-resolutions in myofibrils and other samples.

ELECTRON TOMOGRAPHY AND 3D VOLUME AVERAGING OF *GIARDIA INTESTINALIS*. CINDI SCHWARTZ*, SCOTT DAWSON**, and ANDREAS HOENGER**, Boulder Laboratory for 3D Electron Microscopy of Cells, University of Colorado, 347 UCB, Boulder, Colorado 80309 and **Department of Microbiology, UC Davis, 255 Briggs Hall, Davis, California 95616.

Giardia intestinalis is a unicellular, flagellated protist with a two-stage lifecycle that is a ubiquitous intestinal parasite of mammals. Giardiasis is caused by ingestion of the cyst form of the parasite which then excysts in the duodenum into the trophozoite form. Infection with *Giardia* is the result of parasite attachment directly to the intestinal epithelium. The trophozoite form has a highly specialized microtubule (MT) cytoskeleton (four pairs of flagella, the "median body" and the "ventral disc"). Each pair of flagella has a different motility stroke, allowing the trophozoite four different types of movement within the intestine. The mechanism of attachment to the epithelium is not fully understood, but evidence suggests that the ventral disc is a key component as its conformational changes are seen when the cells attach and detach from *in vitro* substrates. In our study, we are combining light microscopy, electron tomography, and cryo-electron tomography of *Giardia* cytoskeletons to determine the structural-functional relationships of the various cytoskeletal components. In some cases, we are able to use our 3D volume-averaging program, PEET (Particle Estimation for Electron Tomography), to find novel structures associated with the ventral disc MTs and flagella. The ventral disc consists of a counterclockwise array of MTs that originate from electron dense bands directly anterior to the caudal flagellar basal bodies. The ventral surface of each MT is attached to the plasma membrane. On the dorsal side of each

MT a microribbon is connected to neighboring microribbons by cross-linkers. In our study, we are combining light microscopy, electron tomography, and cryo-electron tomography of *Giardia* cytoskeletons to determine the structural-functional relationships of the various cytoskeletal components. In some cases, we are able to use the 3D volume-averaging program PEET, to find novel structures associated with the ventral disc MTs and flagella.

EFFECTS OF MULBERRY SEXUAL DIMORPHISM ON THE STRUCTURE AND PROPERTIES OF SILK. PEARLY A. GEORGE and CAMELIA MAIER, Department Of Biology, Texas Woman's University, Denton, Texas 76204-5799.

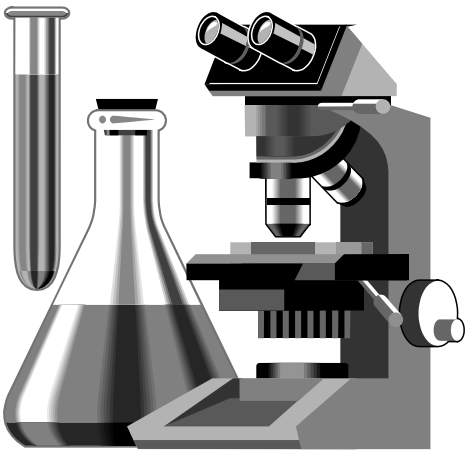
Mulberry (*Morus alba*) is a dioecious species (male and female trees) characterized by sexual dimorphism and constitutes the only source of food for silkworm (*Bombyx mori*). Female plant tissues contain more mineral deposits and free oxalic acid than male plant tissues. It is reported that insect herbivores prefer plant tissue with a low level of mineral deposits. Previous work in our laboratory established that the sexual dimorphism of mulberry affects the development of silkworm caterpillars and the structure of their silk. The purpose of this study is to expand findings of the differences in structure and properties of the silk obtained from mulberry male-leaf fed silkworms *vs.* female leaf-fed silkworms. Silkworm silk is composed of two types of fibrous proteins, sericin and fibroin. The morphology of silk was studied under light and scanning electron microscopes and biochemically by dissolution assays of sericin. Male-fed silk fibers were significantly thicker than the female-fed silk fibers. The dissolution assay results implied that the male-fed silk contains more sericin than the female-fed silk. Aminoacid sequence analyses of both sericin and fibroin will be performed to establish the effect of mulberry sexual dimorphism at the level of molecular silk composition. Silkworms are the only domesticated insects important for the silk fibers used in textile industry. Silk fibers are also used as biomaterials. This study enhances our knowledge of silk structure for practical application of silk as biomaterials.

A NOVEL MICROFLUIDICS TECHNIQUE COUPLED WITH FLUORESCENCE MICROSCOPY FOR ANALYSIS OF AEROALLERGENS. MANDY WHITESIDE AND NABARUN GHOSH, Department of Life, Earth and Environmental Sciences, West Texas A& M University, Canyon, Texas 79016.

Different geographical areas have a diverse plant and fungal allergen species complex whose pollen and spores releases are controlled by the local meteorological conditions. Aeroallergens include pollens, fungal spores, dusts, plant fibers, burnt residues, and plant products like gums and resins. Aeroallergen sampling provides information regarding the onset, duration, and severity of the pollen season. Much of the literature on aerial spore dispersal is based on spore samples collected by the Burkard 7-day volumetric sampler and its progenitor, the Hirst 24-h sampler used in this study. The traditional way of analyzing pollen and spores is performed microscopically. This is not a precise methodology because it has some variability depending on a variety of factors. A more specific aeroallergen analysis method is needed in order to eliminate the variability and allow for a more precise count of the types of allergens in air samples. A novel microfluidics technique coupled with fluorescence microscopy can eliminate some variability in aeroallergen sampling. The Lab-on-a-chip technology utilizes microfluidics, where sample preparation, fluid handling, and biochemical analysis are performed on a microchip. Data processes are analytical and fully automated eliminating the variability seen with other methods. The chips are prepared with either proteins or DNA of different aeroallergens. Fluorescent microscopy shows the detailed morphological and anatomical features of the aeroallergens. These combined techniques highly enhance the identification of aeroallergens.

SUNFLOWER INFLORESCENCES FORM TARGET PATTERNS THAT ATTRACT BEE POLLINATORS. JENNIE WOJTASZEK, FANNY RIVERA, CAMELIA MAIER, Department of Biology, Texas Woman's University, Denton TX 76201.

The language and communication of the honeybees in their colonies have been studied in great detail. This horizontal exosemiotic study focuses on the sign-mediated communication between sunflowers and honeybees. Sunflowers are food sources for bees and bees are pollinator agents for sunflowers. Bees perceive flowers with UV-sensitive photoreceptors in their compound eyes. The sign vehicle between the object (sunflower) and the interpretant (bee) is the target pattern of the sunflower inflorescence based on its morpho-anatomical and biochemical characteristics. Scanning electron microscopic images illustrate the detailed anatomy of the bee eye and pollen basket. Inflorescence images were taken under UV radiation to visualize the bee's perception of the sunflower pigment patterns. Chromatographic methods were used to separate anthocyanins, whose concentration was higher in the flowers at the edge of the inflorescence. These ray flowers formed a white circle under UV radiation, while the disc flowers appeared as a black dot inside the white circle. The disposition and concentration of pigments result in a target pattern directing bees to the nectar and pollen in the center of the sunflower inflorescence. Light microscopic images of the ray flowers show details of epidermal layers and pigment disposition. Scanning electron micrographs confirmed that the adaxial epidermis of the ray flowers has conically shaped cells compared to the flat cells of the abaxial epidermis thus enhancing the perception of the target pattern of the sunflower inflorescence for attracting bees.



MATERIALS SCIENCE
SPRING 2009

COALESCENCE AND SINTERING OF PLATINUM NANOPARTICLES: IN-SITU OBSERVATION BY ABERRATION-CORRECTED HAADF STEM. M.A. ASORO[1], D. KOVAR[1], Y. SHAO-HORN[2], L.F. ALLARD[3], P.J. FERREIRA[1], [1]Materials Science and Engineering Program, University of Texas at Austin, Austin, Texas 78712; [2]Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; [3]Materials Science and Technology Division, Oak Ridge National Laboratory Oak Ridge, Tennessee 37831.

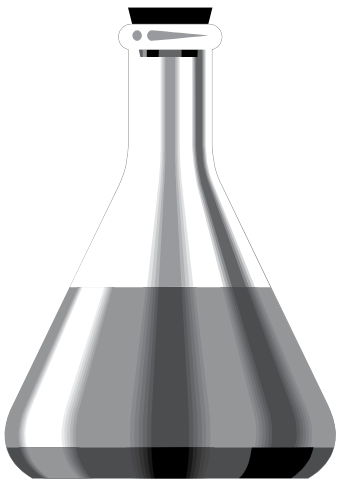
Nanoparticles are currently of great scientific interest due to their large number of possible applications, such as catalysts in fuel cells and delivery vehicles for medicine. However, during processing or usage, nanoparticles have a strong tendency to agglomerate and coalesce over short time scales, even at room temperature, which can lead to significant changes in behavior and performance. In this work, an aberration-corrected JEOL 2200FS scanning transmission electron microscope (STEM), equipped with a high-angle annular dark-field detector (HAADF) was used to monitor the coalescence and sintering of Pt nanoparticles with 2.8 nm nominal size. This in-situ STEM capability is a powerful technique to make direct measurements of fundamental material parameters that are important in understanding how particle size influences coalescence and sintering. Also, the surface diffusivity is determined from measurements obtained from STEM images acquired during the initial stages of sintering. The measured surface diffusivities are in reasonable agreement with measurements made on the surface of nanoparticles, using other techniques. In addition, the grain boundary mobility is determined from measurements made during the latter stages of sintering.

CALCIUM PHOSPHATE COATING OF THREE-DIMENSIONAL TITANIUM SCAFFOLDS VIA ALKALI HEAT TREATMENT. NATHAN J. CASTRO and MALCOLM N. COOKE, College of Engineering, The University of Texas at El Paso, El Paso, Texas 79968-0521.

The focus of this study was to investigate the surface treatment of Ti-6Al-4V (Ti64) titanium porous scaffolds, manufactured by electron beam melting (EBM), using a method of rapid calcium phosphate deposition to produce an osteoconductive substrate. Recent studies have shown enhanced cell adhesion on surface-treated titanium implants by a series of surface modification techniques. The reported method used a commercial βtri-calcium phosphate (βTCP) as the calcium phosphate source for the scaffold surface modification. Ti64 scaffolds were treated in 1M and 5M potassium hydroxide followed by heating to 600ºC to reduce surface morphology alteration while producing an oxide layer for calcium phosphate deposition. Treated scaffolds were post-treated by soaking in 25mM [Ca^{2+}] βTCP solution at 37°C for 4 hours. Scaffolds were seeded with rat bone marrow stromal cells (MSCs), cultured in supplemented media for 24 hours and examined by scanning electron microscopy. Preliminary results show excellent cell adhesion, bridging and spreading of the MSCs, suggesting that the surface treatment process produced a calcium-rich, osteoconductive substrate on the Ti64 scaffolds.

SEM ANALYSES OF PREHISTORIC PAINT PIGMENT USING SECONDARY, BACKSCATTER, AND EDS DETECTORS. MICHAEL W. PENDLETON*, E. ANN ELLIS*, DOROTHY K. WASHBURN**, and BONNIE B. PENDLETON***, *Microscopy and Imaging Center, Texas A&M University, College Station, Texas 77843-2257, **Museum of Northern Arizona, 3101 N. Fort Valley Rd., Flagstaff, Arizona 86001, ***Department of Agricultural Sciences, West Texas A&M University, P.O. Box 60998, Canyon, Texas 79016-0001.

Light microscopy images which included ten painted and ten unpainted areas on an uncoated prehistoric pottery sherd were taken. The same areas were subjected to analysis by energy-dispersive spectroscopy (EDS). Following carbon coating, the same ten painted and ten unpainted areas of the sherd were again subjected to EDS analysis and images were produced by light microscopy as well as by secondary and backscatter scanning electron microscopy. Unpainted areas of the sherd had low counts of iron EDS plots indicating low concentrations of iron present in the pottery material. Painted areas, however, had higher counts of iron EDS plots due to the presence of iron in the paint used to decorate the pottery. The backscatter image of the sherd showed an atomic number contrast due to the iron in the paint applied to the sherd. Areas of high paint concentration were very light in color, while the rest of the image (unpainted pottery area) was dark. An iron EDS map demonstrated the locations of paint on the coated sherd that corresponded to the light colored areas in the backscatter images of the sherd. Light microscope and backscatter images of the sherd provided typographical reference points to locate carbon covered pigment areas prior to EDS mapping. These coated pigment areas are not easily visualized by EDS mapping until after image averaging over time. The results of EDS analyses on the sherd before and after carbon coating demonstrate that carbon coating enhanced the counts of iron EDS plots in both the unpainted and painted pottery locations.



TECHNICAL ABSTRACTS
SPRING 2009

THREE GENERATIONS OF PHOTOGRAPHERS. HOWARD J. ARNOTT, The Department of Biology and Center for Electron Microscopy, University of Texas Arlington, Arlington TX, 76019.

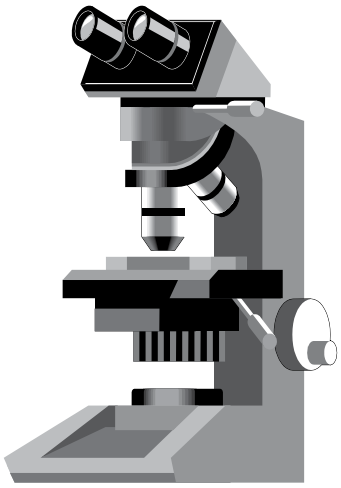
This paper presents the photographic work of three generations of photographers and compares the performances of the cameras they used. All three enjoyed photography, and the latter two earned their living with cameras. **Nellie Leonie Merritt Alderman** was my great grandmother, born in the 1863 during the Civil War; she used a Kodak Brownie camera. My father, **Andrew Hugh Arnott,** born in 1905 used a Speed Graphic camera and I, **Howard J. Arnott,** born in 1928 used and still using various types of cameras, photomicrographic cameras and electron microscopes. The three of us cover a life span of almost 146 years involving three centuries, but only about 115 years of photography. By a stroke of luck many of Nellie Alderman's photographs still exist. Only one of her photos has been published. Since my father was a news photographer for over forty years, hundreds of his photos were published and many are still available in newspaper libraries or online. Many of his news and family photographs have been preserved in our family collection. I formally learned photography at the US Navy Photography School in Pensacola, FL. However, most of my photography was done in my career as a botanist or cell biologist. Several hundred photos, light and electron micrographs have been published in scientific articles, books, magazines and newspapers. In addition, an extensive collection of family photos exists beginning with black and white prints and ending with color and digital images on CDs. Hundreds of additional scientific photos are available in my files. All of us participated in photography long before the "**point and shoot**" era, which began in the 1980's. I still remember the "good old days" when **skill** and **luck** were the major components of the photographic enterprise. I will make use of photos taken by the three of us to illustrate the evolution of photography over more than one century.

X-RAY MICROSCOPY AND TOMOGRAPHY OF LOW Z MATERIALS IN THE SEM. PAUL MAINWARING, Gatan Inc., 5794 West Las Positas Blvd. Pleasanton, California 94588.

The great advantage of SEM-based X-ray microscopy is the ability to image the internal structure of specimens rather than the surface information obtained by standard SEM imaging. The two techniques combine to provide the maximum amount of structural information. An SEM-hosted X-ray ultra microscope (XuM) consists of 1) a series of metallic targets mounted on a high precision software-controlled positioning arm which is placed beneath the electron beam to produce a point source of X-rays; 2) a sample holder combined with a high resolution rotation stage for tomography; and 3) a high sensitivity CCD camera for direct X-ray detection. These components are fully compatible with the SEM working environment and allow switching between SEM and X-ray imaging modes without venting the specimen chamber. Multiple targets in the SEM chamber allow various X-ray energy selections to be made. The desired X-ray energy can be "tuned" to the particular sample by selecting the target material, which will provide optimal intensity and contrast in the images. This is particularly useful for certain low contrast specimens such as biological or polymer samples. X-ray microscopy allows the sample to be always in focus and the great depth of field enables stereo imaging and 3D micro-tomography to be carried out. In general, spatial resolution of 200 – 400nm can be achieved for 2D imaging while tomographic resolution can be about 1μm. Contrast mechanisms available in X-ray images are related to both absorption of the X-rays and to development of phase contrast at the detector. Phase contrast is particularly useful for low atomic number compounds since absorption is minimal as is the corresponding image contrast. Use of a variety of target materials allows a range of 2D absorption images to be created for multi-component samples such as biological specimens. Examples of the use of the X-ray microscope to image low atomic number materials and biological samples in 2-D and 3-D will be given.

METRICS FOR EVALUATING FLUORESCENCE MICROSCOPY IMAGES. MARK N. RAND, Advanced Microscopy Group, Westover Scientific, Inc., 18426 Bothell-Everett Highway, Mill Creek, WA 98012-4022

"Signal to Noise Ratio" is a common metric for fluorescence microscopy image quality but is actually a misnomer, confounding instrument background, sample background, and noise from a variety of sources. Fluorescence microscopy images are often collected with constraints on illumination intensity and exposure time, due to concerns of photobleaching and phototoxicity. These constraints can reduce image quality and increase the variability of intensity measurements. Having consistent, valid metrics for image quality is important for evaluating instrument performance, sample preparation, and lower limits of fluorescence detection. This paper will describe some basic metrics that can be used to evaluate and improve image acquisition in everyday fluorescence microscopy applications.



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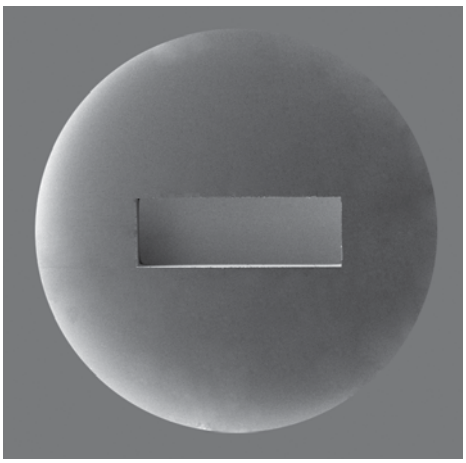

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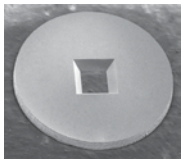
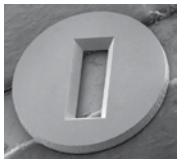


0.5 x 0.5mm

1.0 x 1.0mm



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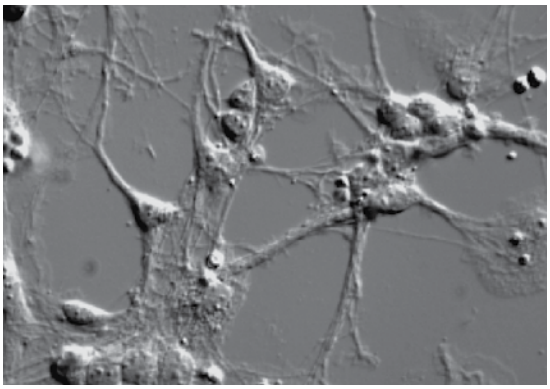

DIC image of hippocampus neurons grown on a silicon nitride substrate by Prof. M. Stowell, et. al., MCDB, CU-Boulder, Colorado

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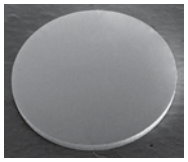

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Meeting Memories

Sheetal Rao from Texas A&M University was the graduate student winner for the biological student competition at the Denton meeting in the fall of 2007 (between Mike Pendleton, TSM Secretary and E. Ann Ellis, TSM Treasurer).

The student in the middle of the group is Kelsey Pendley, Dr. Arnott's student who received the undergraduate student award for her presentation at the same meeting. From left to right: E. Ann Ellis, Sheetal Rao, Kelsey Pendley. Mike Pendelton, and Howard Arnott. (Pictures by E. Ann Ellis)

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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.

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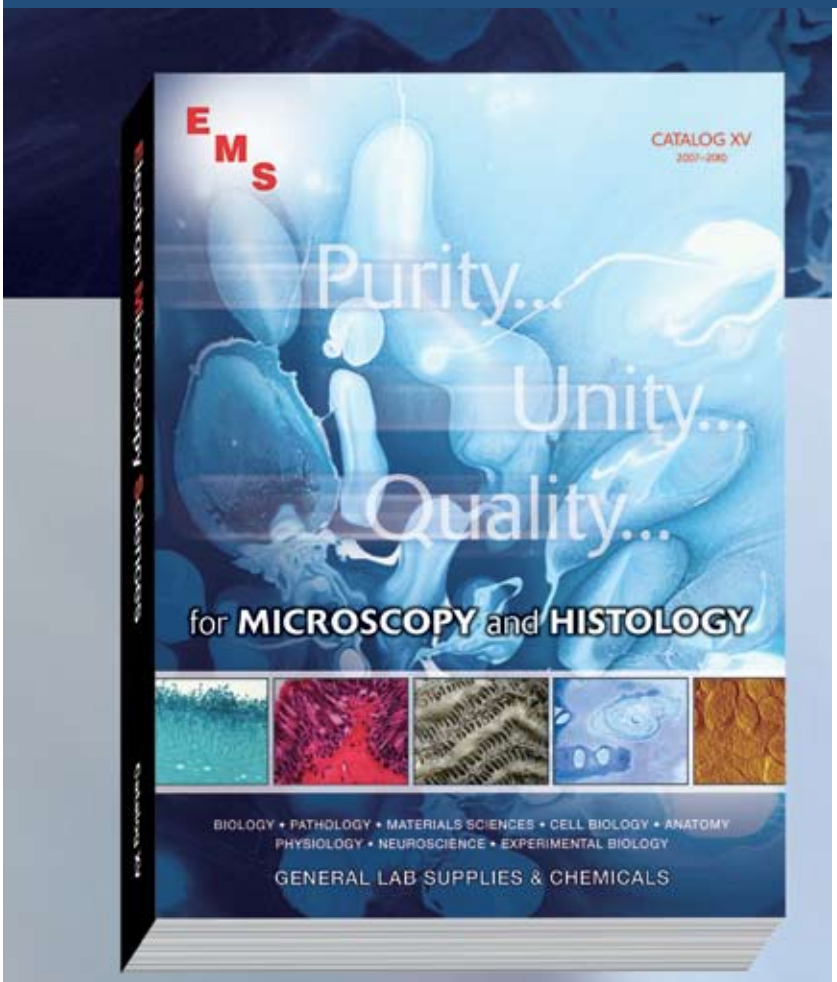

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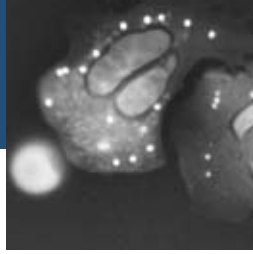
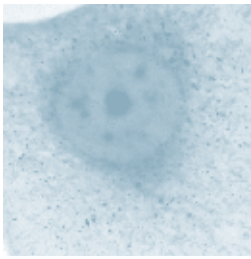
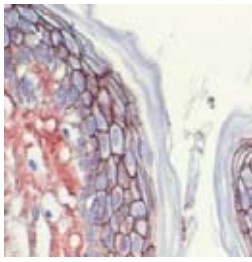
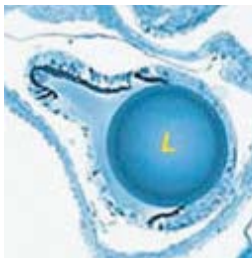
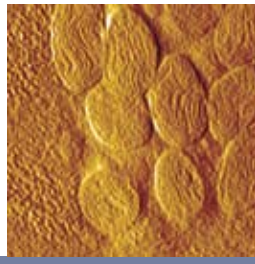

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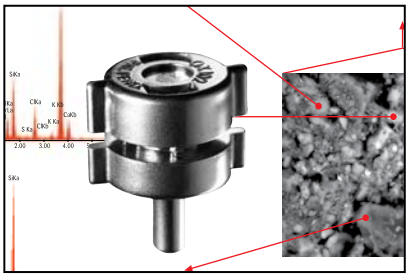
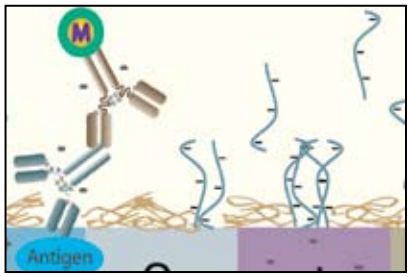

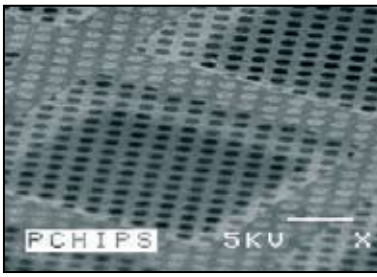

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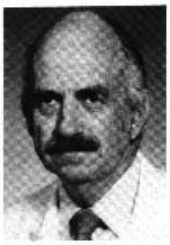
In Memoriam

Bob Turner, charter member of the Texas Society for Electron Microscopy, now known as TSM, passed away on June 28th of last year at the age of 81. Bob was part of the group that founded TSM in 1965 at a meeting that took place at Rice University. Bob was a stalwart supporter of both TSM and MSA serving as President of TSM from 1973 to 1974, TSEM Journal editor from 1977 to 1978, and supporting MSA by serving on several committees including as chair of the local arrangements committee for the 1989 annual meeting. He was a fixture at TSM meetings after the society's inception; even attending meetings after his retirement from Scott & White Hospital in Temple in the late 80's where he served as director of their electron microscopy facility. The below is excerpted from the Twentieth Anniversary Meeting Issue of the TSEM Journal:



Robert A. Turner, President, 1973 — Bob is chief of the EM section of Surgical Pathology at Scott and White Clinic in Temple, Texas. Bob writes the following about TSEM: "It has been a personal satisfaction to see and enjoy the growth of a small society to the country's largest. TSEM is not the oldest in the country but without doubt it is the greatest and the most active. Many local societies have patterned themselves from TSEM; especially their newsletters. It's a fond treasure of memories of all the friends and acquaintances I've made through TSEM". Bob has attended **every** TSEM meeting and has fond memories of all. He did have, however, single out a meeting at the Menger as being very special. This meeting had the first commercial exhibits and apparently the hotel's transformer was "blown" as a result of all the equipment. It is also worthwhile to note that Bob was the first president to be elected on a "milk and cookies" platform!

Although Bob would often come off as a gruff sort of person, beneath that exterior was a caring individual who helped others. On one occasion he took a sick service engineer home with him rather than see him go off to a hotel for the night.

The following are some tributes from some who knew him well:

I first met Bob Turner as the director of the EM lab at Scott and White. As a Professor of Biology we were interested in obtaining a microscope and Bob was most helpful to us in that endeavor. I have some very fond and vivid memories of our relationship. None of us had any idea about the scope and especially the preparation required for sample preparation, so we went to his lab to "learn the technique". The first technique he showed us was to obtain a proper glass knife and Bob started us out the old fashioned way by dropping a glass plate on the floor and having us look for usable knifes. It wasn't after an hour of searching he introduced us to a glass cutter and pliers. Bob was an excellent and patient teacher and we eventually did learn the technique. He was also most generous to allow us and our graduate students to use the scope at Scott and White for research projects. Along with Bob we were able to show the Baylor Administration the value of electron microscopy in modern Biology and we obtained a Phillips 201 EM and designed a room for preparation, microscopy, and an accompanying darkroom. We at Baylor are very sad to hear of his passing, but he will always be remembered for his contribution to education and research in our department. I will personally miss his stories, his warm smile, helping him set up traps in his attic to catch squirrels, and that I knew if I ever needed anything related to science or life in general, Bob would be there for me. *–Dave Eldridge, Professor of Biology, Baylor University*

Bob was there at the very beginning with all of the medical school, hospital, and university departments expressing interest in the development of electron microscopy as a useful tool in research and diagnostics. As a pioneer at Scott and White clinics, Bob recognized the need for trained and innovative technicians and spent many days travelling to those few sites that had active programs and worked with lab personnel to expand techniques and training classes. He worked with Dr. Ward Kischer at Galveston UTMB, with Neurology at MD Anderson, T.C. Hsu in Cell Biology, Bill Brinkley at Baylor, as well as at Texas A&M and SHSU on methods and techniques.

Bob recognized the importance of information exchange and helped establish the three meetings a year for TSEM that lasted through the 70's. Because of these strong and informative meetings, TSEM from 1968 to 1978, was recognized as the most active local chapter amongst MSA local affiliate societies. Bob, with his straight forward manner of speaking and colorful stories was instrumental in helping TSEM attract nationally know figures in research such as Keith Porter, Jean Paul Revel and others to TSEM meetings. Many old timers remember the "Ranch" meetings in central Texas that included both good fun and excellent science. Those were the good old days. *–Terry Hoage, TSEM President, 1974 - 1975*

Bob is survived by his wife Ann.

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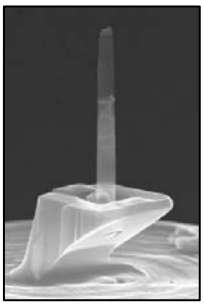

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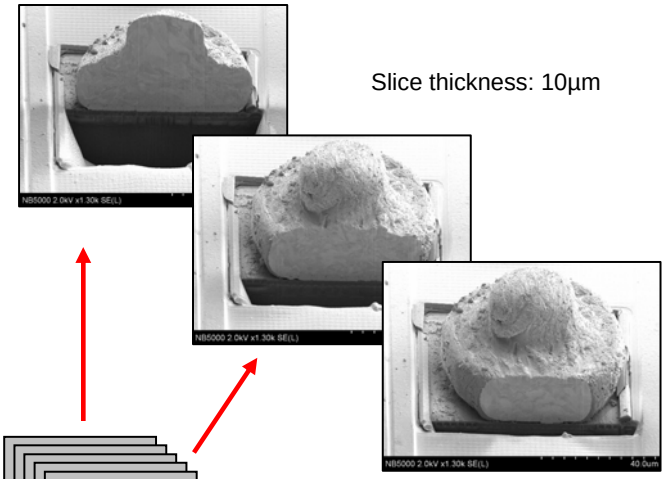
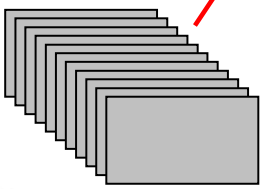


Slice thickness: 10µm



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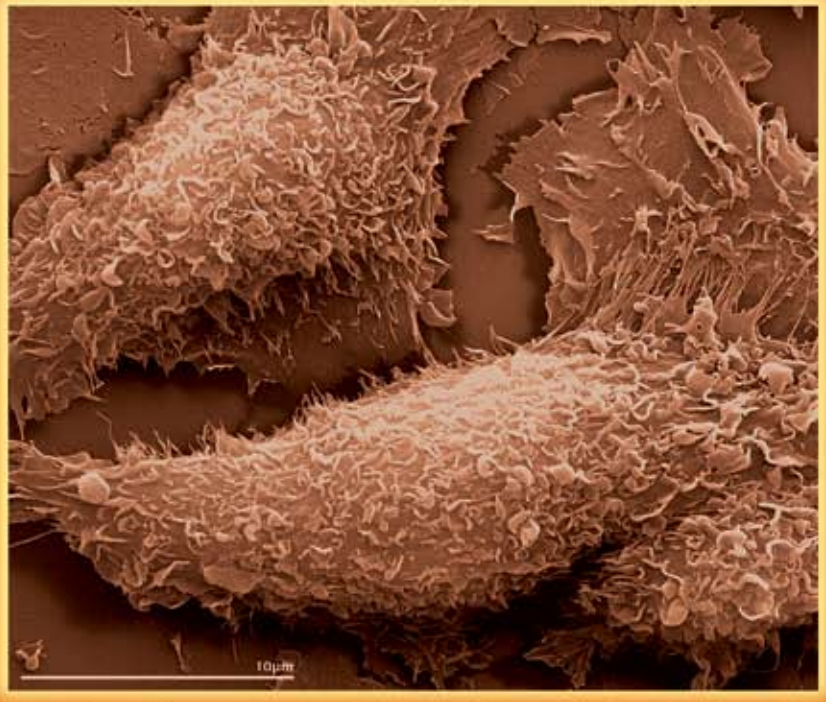

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