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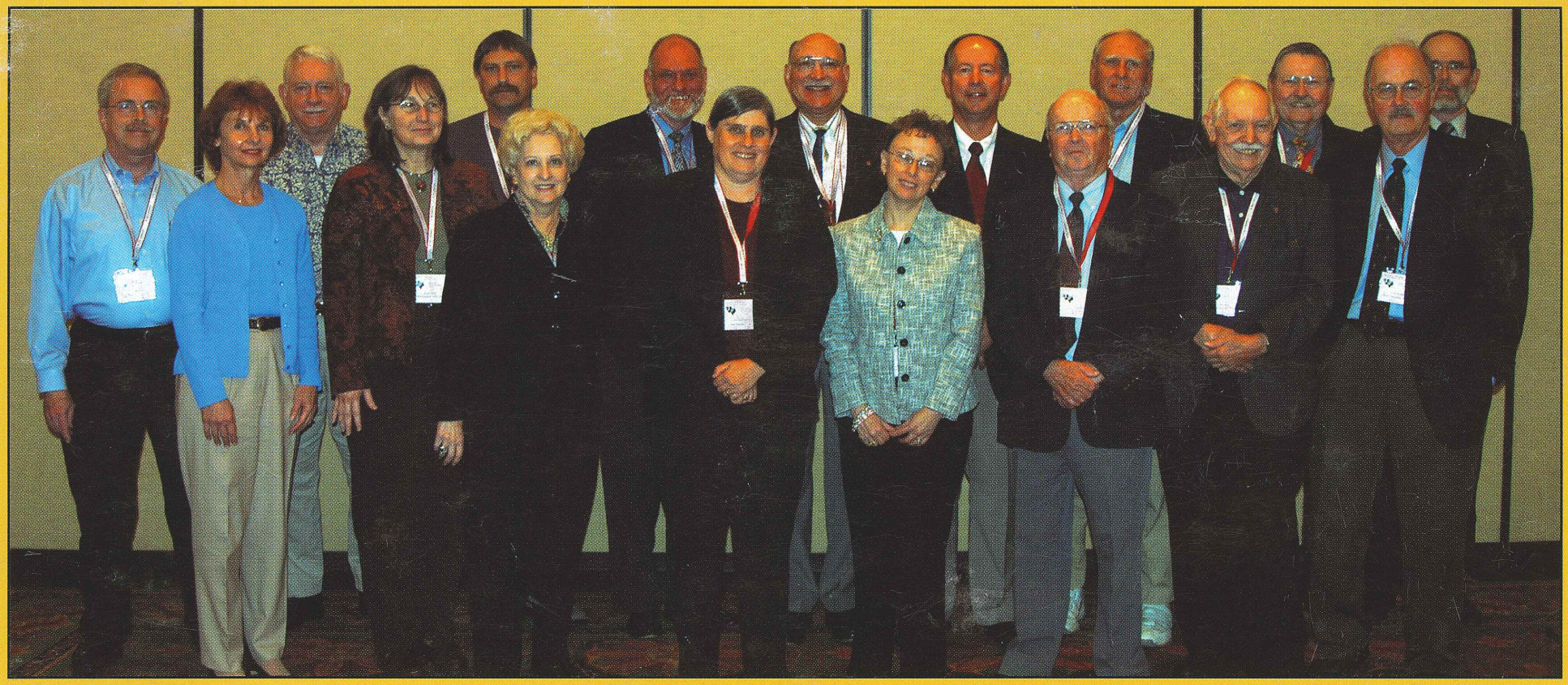

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Camelia G.-A. Maier, Editor
Department of Biology, Texas Woman's University, Denton, TX 76204

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ON THE COVER
The year 2005 marks the 40th anniversary of the Texas Society of Microscopy (TSM), formally the Texas Society for Electron Microscopy (TSEM). The cover is dedicated to TSM presidents present at the spring meeting held at the Dallas Marriott in Las Colinas, April 14 – 16, 2005. Left to right: Hal Hawkins, Sandra Westmoreland, Howard Arnott, Pamela Neill, David Garrett, Ann Goldstein, Mitchell McCartney, Lynn Davis-Gray, Don Hay, Ann Rushing, Charles Mims, Louis Bragg, Terry Hoage, Don Benefiel, Don Smith, Lea Rudee, Bob Droleskey. (Picture provided by Bob Droleskey)

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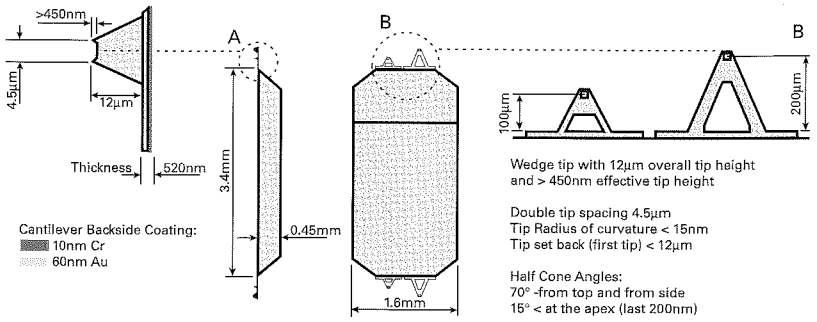

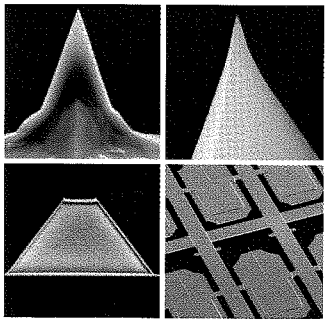

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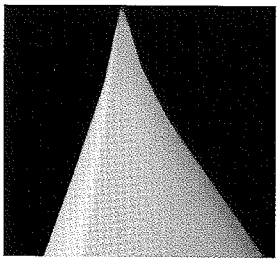

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President's Message

I would like to take this opportunity to thank **Ann Rushing** for her service as president of Texas Society of Microscopy for the last two years. Ann led the Society through four successful meetings, including the Spring 2003 meeting in San Antonio, during which we served as the local host Society for Microscopy and Microanalysis, and the Spring 2005 meeting honoring the 40th anniversary of the Society. Ann's tireless efforts have led the Society in promising new directions. She helped guide the Society toward more healthy financial status and focused energy on recruiting new members from academia and industry, from biological and materials science disciplines. We owe Ann a great debt of gratitude for her efforts to promote the Society during her tenure.

TSM has been served by officers of tremendous talent during the last two years. **Jodi Roepsch and Susan Robbins** performed the duties of program chairmen, setting a high standard of excellence. Their attention to detail has produced well-organized meetings. **Pam Neill**, a past president, has also provided program chair support during Jodi's recent leave of absence. **Bob Droleskey** has attended to the duties of treasurer, putting his talents to excellent use for the Society. Bob has also served as the chairman of the committee to review the TSM by-laws, which will be finalized this fall. **Robert Champaign** has performed excellent service for the Society as secretary, organizing membership information and chairing the nominating committee for new officers. We appreciate the efforts of **Becky Holdford** who, as webmaster, has facilitated our rapid and reliable communication. **Camelia Maier** has served the Society by continuing to produce and update our TSM Journal. We also appreciate the efforts of **Mike Crowley**, corporate member representative, who has contributed many ideas from the corporate perspective to enhance the Society and **Vibhu Bakshi**, a student at Texas Woman's University, for serving as student representative and sharing the student perspective. We welcome as a new officer, **Martha Gracey**, who assumed the duties of treasurer this year. We also appreciate the willingness to assume future duties by President-elect **Joanne Ellzey** and Secretary-elect **Tina Halupnik**.

Sadly, we have lost one of our own this year with the passing of **Ann Burke**. Ann was a past secretary and program-chair elect for the Society. Ann will be missed as a loyal and dedicated member. Both her scientific contributions and her cheerful personality will be a great loss to all of us. We send our thoughts of consolation to Ann's family, who were often the subject of her proud conversations at meetings.

The Spring 2005 meeting honored the 40th Anniversary of The Texas Society of Microscopy. This meeting gave us the opportunity to pay tribute to the history of the Society. We had the chance to meet past presidents and hear some of their stories about TSM meetings from the early years. We truly stand on the shoulders of the giants who founded this Society. In addition, Howard Arnott was honored at the meeting by the formal dedication to him of the student paper award. A reunion of many of his former students provided additional opportunities for us to see that the legacy of the Society includes many accomplished scientists. While honoring the past, we must also look to the future of the Society.

Our direction for the future of TSM must be to embrace the changes that will come to challenge us. We should continue to value our Society as one that is multidisciplinary, with representatives from a variety of organizations in material and biological sciences. As Bob Droleskey said, "This is an eclectic group and we like it that way!" We will continue appreciating and embracing our corporate members and vendors, encouraging their presentations of new technology to us in workshops, displays, and demonstrations. One characteristic of our Society, sustained throughout its history, is that it functions as an incubator for student talent. Therefore, we should and must encourage students to attend our meetings and present their work. These students provide a vital link to our future as a Society. Finally, we need to encourage our colleagues to attend the meetings. The best marketing for the Society is to advertise it to someone whose research you admire. Bring new people in; mentor them to become members of our diverse and outstanding group. I look forward to hearing your ideas about how to ensure the continued success of the Texas Society for Microscopy.

Sandra L. Westmoreland
TSM President 2005-2006

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:

Camelia G.-A. Maier, TSM Journal Editor
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Manuscript deadline is February 17, 2006

AN INVESTIGATION OF MICROORGANISMS ON VARIOUS SPOILED FOOD SOURCES

KELLI BIRD[1], NABARUN GHOSH[1], BOLANLE ENIOLA[1], CONSTANTINE SAADEH[2], MICHAEL GAYLOR[2] AND DON W. SMITH[3]

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[2]Amarillo Center for Clinical Research [2]/Allergy A.R.T.S. 6842 Plum Creek Drive, Amarillo, TX;
[3]Biological Sciences, University of North Texas, Denton, TX

ABSTRACT

Food spoilage is a major concern for both consumers and scientists alike. Liquid and agar cultures of microorganisms associated with food spoilage were obtained and observed microscopically. Fungal specimens isolated from spoiled food were *Rhizopus* sp. and *Aspergillus* sp. from s bread, *Penicillium* sp. from a Dr. Pepper bottle, basidia and basidiospores from various Basidiomycetes from cheese and canned beverages. Bacterial colonies were obtained as follows: Gram-positive bacilli from spoiled pear and strawberry, Gram-positive and Gram-negative bacilli from strawberry and Gram-negative cocci from a Coke can. Most of these food specimens did not exhibit a change in color. Even if no distinct color change or visible deformation of the food material is observed, microbial contamination within the food may still be present.

INTRODUCTION

When food becomes contaminated with fungi and/or bacteria, the process of decay commences. The ingestion and/or inhalation of fungal spores or bacteria can pose a severe health hazard to the community at large. Mead *et al.* estimates that there are 76 million illnesses caused by food-borne pathogens annually in America, and of these illnesses, only 75% are from known pathogens (1).

Fungi grow almost everywhere within a wide temperature range (-5 to 50°C and even greater) (2), although individual species usually grow within a much narrower range. One of the most important physical parameters affecting fungal growth is moisture. A relative humidity over 70% and the water activity of the substrate are the critical parameters for fungal growth. Airborne spores are usually present in outdoor air throughout the year in high numbers and frequently exceed pollen concentrations by 100- to 1,000-fold (3), depending on environmental factors such as water and nutrient availability, temperature, and wind. Most fungi, commonly considered allergenic, are *Alternaria* spp., *Cladosporium* spp., *Epicoccum nigrum*, *Fusarium* spp., and *Ganoderma* spp. Generally, indoor fungi are a mixture of those that have entered from outdoors (4) and those from indoor sources. *Aspergillus* spp. and *Penicillium* spp. are less common outdoors and are usually considered the major indoor fungi (4). Most fungal species act as saprophytes and secrete enzymes to breakdown organic material (5). Fungi and food molds produce waste products that can act as poisons or toxins in the human body causing ill effects such as diarrhea, abdominal pain, and long-term liver illnesses. Microbial growth on food, in high enough concentrations, can also cause food poisoning leading to symptoms similar to intestinal flu (5, 6). Most single-celled fungi can reproduce asexually by fission and budding or fragmentation from a parent mycelium (7). Others either reproduce solely by sexual methods or they have a combination of sexual and asexual phases alternating throughout their life cycle.

Bacteria cause most lethal infections of the intestinal tract. With improved sanitation and clean drinking water, many have become uncommon in developed countries, and some can be treated with antimicrobial agents (8). The majority of bacterial cells are chemoheterotrophs that must consume organic molecules both for energy and as a carbon source. Many of these chemoheterotrophs are saprophytic in nature. Most bacterial genes are carried on a single circular DNA molecule and are continuously being reproduced.

The objectives of this study were to isolate species of fungi and bacteria within spoiled food (fruits, vegetables and processed food sources), determine their morphology, quantify the microorganism colonies, and calculate their growth rates over time. Another objective was to examine if microbial organisms can be detected in the spoiled food even if they do not exhibit discoloration or deformation.

MATERIALS AND METHODS

Preparation of liquid medium and agar plates

Luria Broth (LB) was chosen as a growth medium for culturing and the isolating microorganisms associated with food spoilage. The liquid medium was prepared using 10 g NaCl, 10 g Tryptone, 5 g yeast extract, and 1 liter of deionized water and sterilized by autoclaving before inoculation. Brain heart infusion agar (DIFCO, 52g/L) was chosen for its ability to cultivate fastidious microorganisms and fungi on agar plates.

Sampling and culturing fungi and bacteria from spoiled food specimens

Food items, such as bread, strawberries, grapes, pears, pineapple, soft drinks and Portabella mushrooms, were left exposed to the environment at room temperature for 4 days to initiate the spoiling process. Food specimens exhibiting various degrees of spoilage were used for sampling the microorganisms. All specimens were aseptically inoculated in Luria Broth (LB) and incubated overnight at 37° C to establish cultures. Minute amounts of mold or bacteria were aseptically extracted from the broth with a heat-sterilized looped needle and transferred to agar plates. The agar plates were sealed with parafilm, and placed in an incubator at 37° C for 48-hours.

Gram staining of bacterial slides

On the petri plates the bacteria and yeast colonies (Fig. 1E) could be easily isolated from the fungal colonies (Fig. 1A) based on their overall morphology. The Gram staining technique was employed to stain bacterial slides. With the tip of a sterile loop, a small amount of material from a single bacterial colony was removed and transferred to a droplet of water at the center of glass slide. The slide was flamed for 1-2 seconds and was covered with crystal violet solution for 1 minute. The slide was then rinsed with trickling water until the water was clear and covered with Gram's iodine solution for 3 minutes. The slide was gently rinsed with water, followed by 95% denatured alcohol, allowing the alcohol to flow over the slide for several seconds before rinsing the slide with tap water again. The slide was covered with Safranin O solution for 1 minute and then rinsed with tap water and blotted dry with bibulous paper. The slide was made permanent by adding 3 drops of DPX at the center of the slide and covering it with a cover slip.

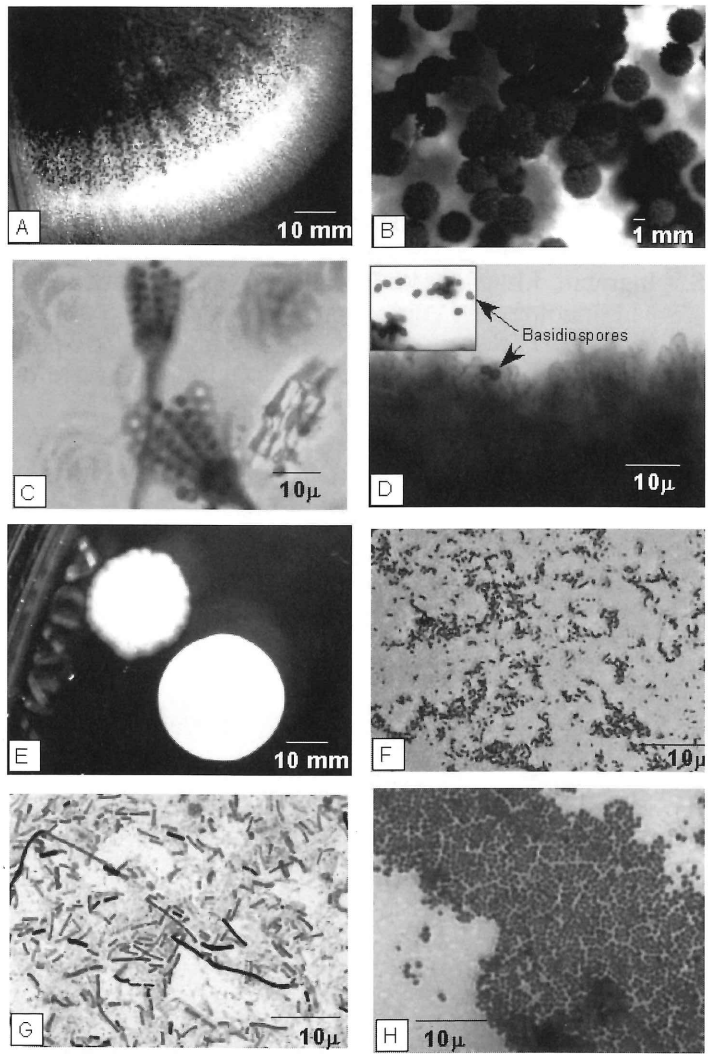

Figure 1. Microscopic observations of microorganisms isolated from spoiled food specimens. **A.** and **B.** *Aspergillus* sp. under SZ-40 stereoscope at 40x and 80x magnifications, respectively. **C.** *Penicillium* sp. from 12-day old open Dr. Pepper container. **D.** Basidia and basidiospores (inset). **E.** Bacterial colonies on agar. **F.** Gram-positive bacilli from spoiled pear. **G.** *Bacillus megaterium* from spoiled strawberry. **H.** Gram-negative cocci from a 6-day old open Coke can.

Preparation of fungal slides

On a clean glass slide, one drop of Lactophenol Cotton Blue stain (Becton, Dickinson and Co.) was added to the center of the slide. With a heat-sterilized needle, a small amount of fungi was extracted from the cultured plate and placed onto the stain. The slide was gently heated by passing through the flame of an alcohol burner for 1-2 seconds, and then allowed to cool for one minute. Using two straight heat-sterilized needles, the fungi were teased carefully in the center of the slide to increase the surface area for a better observation on the fungal morphology. The slide was covered with a coverslip and preserved by sealing it with melted paraffin wax.

Microscopic observations

The cultures in the petri plates were viewed with an Olympus SZ-40 stereomicroscope, which had fiber optics with gooseneck light sources that provides better lighting to microstructures. The prepared slides were viewed with the 4x, 10x, 40x, and oil immersion (100x) objective lenses of a BX-41 Olympus microscope and the micrographs were taken with an Olympus Microfire digital camera attached to the microscope.

Quantification of bacterial colony growth

The bacterial colonies on the agar plates were counted in 4 intervals of 24 h, 48 h, 72 h, and 120 h, using a centimeter grid system. Three sets of petri plates were maintained for each food specimen and from which the average colony number and standard deviation were calculated.

RESULTS AND DISCUSSION

At high magnification, the fungal sporangia were visible with the surface covered with spores (Figs. 1A and 1B). The microscopic slides showed various types of fungi belonging to Ascomycetes, Basidiomycetes and Deuteromycetes. We observed *Rhizopus* sp. and *Aspergillus* sp. on spoiled bread, *Penicillium* sp. (Fig. 1C) in liquid from a bottle of Dr. Pepper that was opened and exposed to air 11 days earlier. Conspicuous conidia and reproductive bodies of *Penicillium* sp., basidia and basidiospores from various Basidiomyceteous fungi (Fig. 1D) were observed on spoiled cheese and beverages. Deuteromycetes often produced mycelial mats and asexual spores. Most of the food materials did not exhibit discoloration or deformation.

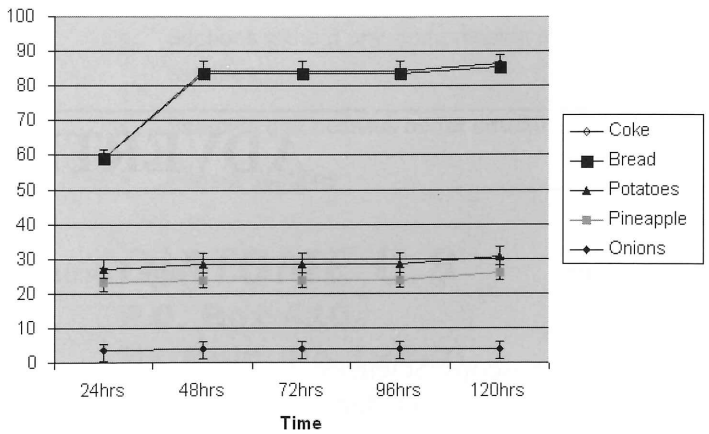

Figure 2. Growth of bacteria isolated from spoiled food specimens over time. The results represent the average number of colonies in three experimental replicates ±SD.

Gram-positive bacteria were observed from spoiled fruits like pear (Fig. 1F), and Gram-positive and Gram-negative bacteria on spoiled strawberry (Fig. 1G). Gram-negative cocci (Fig. 1H) were observed from the liquid in a Coke can that was opened 6 days earlier. Yeast (*Saccharomyces* sp.) cells were found in various beverages within few days after opening the containers. Yeast can grow in any fermented food product easily when not preserved properly or preserved for extended periods of time (9).

According to our observations, illustrated in Fig. 2, there were two noticeable growth patterns of bacterial colonies obtained from various spoiled food sources. Bacteria in processed food, such as soda or bread, exhibited an increased in the number of colonies which peaked at 48 h and entered into a stationary phase thereafter. Bacterial colonies obtained from spoiled fruits and vegetables maintained a constant stationary phase throughout the period of observation. Few bacterial colonies were obtained from spoiled onion, probably due to sulfur compounds present in onion tissues. Much higher number of bacterial colonies was counted for food produce containing sucrose and starch, such as Coke and bread.

From our observation, it is very clear that even if there is no visible change in color, surface, and shape of the food specimens from spoiled canned food and canned beverages, microbial colonies are present after keeping food in open containers in the environment. Canned food and beverages should be consumed as soon as possible after opening the airtight sealing of the cans. It is recommended that that fruits and vegetables be consumed when they are fresh.

Acknowledgments. This research was funded by grants from Killgore Research Center, WTAMU and Allergy A.R.T.S.

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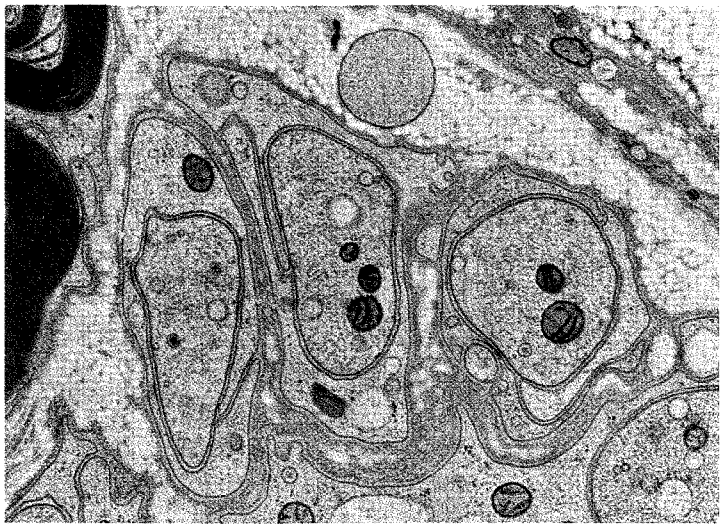

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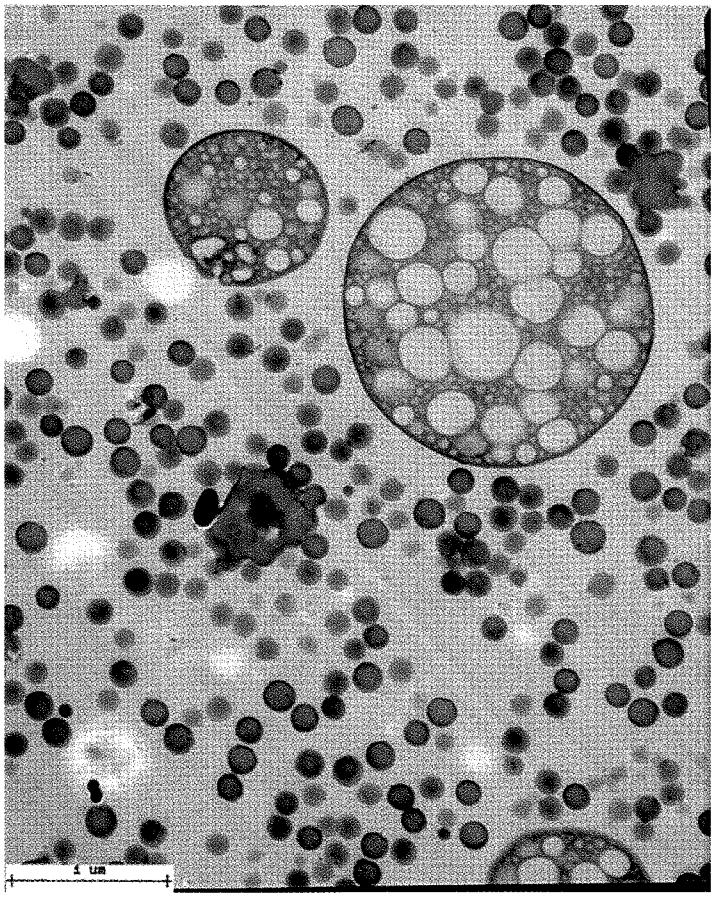

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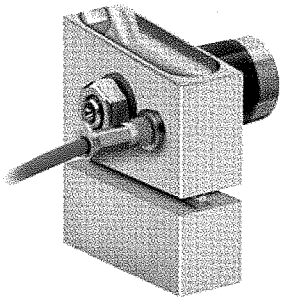

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Abstracts

BIOLOGICAL SCIENCES
FALL 2005

PLANT EPICUTICULAR WAXES AND TRANSPIRATION UNDER VARIED ENVIRONMENTAL CONDITIONS OF LIGHT INTENSITY AND WATER AVAILABILITY. KIMBERLY G. BECK AND CAMELIA G.-A. MAIER. Department of Biology, Texas Woman's University, Denton, TX 76204.

Plant transpiration is regulated through stomates and epicuticular waxes (EW). The synthesis and deposition of EW are affected by environmental conditions. This research project was designed to study the deposition of EW and the rate of transpiration in leeks under varying conditions of water availability and light intensity. Three water availability and three light intensity treatments (low, medium and high) were applied to six leeks per treatment for 21 days. Transpiration increased with treatments of high water availability and light intensity. Plant growth increased in high water availability and low light intensity treatments. Wax deposition was measured by extraction in chloroform. High wax deposition was observed as a gray powder on leaves and under a light microscope for the plants grown in high light intensity and moderate water availability. Light intensity was found to be a better inducer of wax deposition than availability of water.

AN SEM STUDY OF LEAF LITTER FROM AN URBAN ENVIRONMENT. ALBERT WONG AND HOWARD ARNOTT. The Department of Biology and The Center for Electron Microscopy. The University of Texas at Arlington, Arlington, Texas 76019.

Leaf litter from forests and nature preserves contain a wealth of information that may be widely applicable to many fields; including ecology, plant biology, forestry, and conservation biology. Broadly speaking, the study of leaf litter may even be useful in the epidemiology of certain diseases involving plants. However, despite its potential, leaf litter has been a relatively un-studied discipline. Leaf litter has many microscopic aspects worth studying. Cysts, fungi, slime molds, and other interesting microscopic flora and fauna residing in the leaf litter are examples of the many diverse microscopic aspects of leaf litter that have yet to be studied adequately. Due to the size and nature of these aspects of leaf litter, the SEM is the most productive tool for carrying out this study. The leaf litter study presented here was carried out using samples from the 1,300-acre River Legacy Parks in Arlington, Texas. Selected aspects of leaf litter, including fungi, possible slime molds, and other interesting features are presented to provide a visual basis for examining the subject, and the results of analysis of various collected leaf litter samples are also shown. The main goal of this study is to report on the microscopic organisms living in leaf litter. Finally, this study is not meant to be a standalone research project without applicability to other studies: in fact, the study of the River Legacy Parks' leaf litter can serve as a useful standard for comparison to future leaf litter studies carried out at other urban sites.

EXPOSURE OF YEASTS *S. CEREVISIAE* AND *S. POMBE* TO ARSENIC. ANABELLE ARANDA, MARIAN N. VIVEROS AND JOANNE T. ELLZEY. Department of Biological Sciences, the University of Texas at El Paso 79968-0519.

Yi-He Ling *et al.* (2002) found that arsenic trioxide caused polymerization of microtubules and mitotic arrest before apoptosis in human tumor cell lines. Li and Chou (1992) determined that 2.5 μM sodium arsenite for 16 hr caused loss of thick cables of actin filaments in cultured Swiss 3T3 cells. Microtubule organization was slightly affected. The mechanisms responsible for these arsenic effects are not well understood. We have used high pressure freezing substitution for transmission electron microscopy and confocal laser scanning microscopy with time lapse photography to examine the G-proteins, microtubules, microfilaments, and nuclei of two distantly related yeasts, the budding yeast, *Saccharomyces cerevisiae* and the fission yeast, *Schizosaccharomyces pombe.* Western blotting showed no cross-reactivity between the yeast tubulins and the G-protein antibodies, indicating poor cross-reactivity of rabbit and rat antibodies with the yeast genome. We have obtained more specific fluorescent antibodies for yeast cytoskeletal proteins and for detecting apoptosis. It is controversial whether apoptosis occurs in yeasts. Our null hypothesis is that arsenic does not cause structural changes in the cytoskeleton or apoptosis in yeasts. Our alternate hypothesis is that arsenic may cause structural changes and apoptosis in yeasts. Experiments utilizing time lapse photography of cultures growing within a chamber on the Zeiss Pascal confocal microscope with fluorescent antibodies for actin, tubulin, nuclei and apoptotic receptors on yeast cells are underway in the Analytical Cytology Core Facility, the Border Biomedical Research Center supported by the NIH-RCMI grant #5 G12RR008124.

TEM ANALYSIS OF CHEMORECEPTOR ARRAYS IN *E. COLI* INNER MEMBRANES. ROSEMARY S. MCANDREW, E. ANN ELLIS, RUN-ZHI LAI, MIKE MANSON, AND ANDREAS HOLZENBURG. Department of Biology and Microscopy and Imaging Center, Texas A&M University, College Station, TX 77843.

Bacterial chemotaxis is the directed movement of cells in attractant and repellent gradients. *E. coli* cells sense these chemicals with four different chemoreceptors which function as homodimers that span the inner membrane. Ligands bind to the periplasmic domains of the dimers, and the cytoplasmic domains form an active ternary signaling complex with the histidine kinase CheA and the coupling factor CheW. These complexes are located in patches at the cell poles. Their output, the phosphorylated form of the response regulator CheY, modulates flagellar rotation to allow migration in the gradients. The structures of CheA, CheW, CheY, and the periplasmic and cytoplasmic domains of the receptor have been solved, and growing evidence from both *in vivo* and *in vitro* experiments suggests that different receptors act synergistically as mixed assemblages in native membranes. However, the intermolecular contacts in the ternary complex, the architecture of the receptor patch, and the mechanisms that govern the acute sensitivity and broad dose response of chemotaxis are unknown. TEM was used to investigate membrane-associated receptors for serine (Tsr) and aspartate (Tar) in the absence and presence of CheA and CheW, and plus or minus attractant ligand. Receptor-specific gold labeling of ultrathin cell sections and membrane suspensions, and assays for receptor-coupled kinase activity confirmed the association of functional receptors with membrane samples. Negatively stained membranes revealed receptors in clusters of "rosettes", with rosette dimensions that could accommodate a trimer of dimers, ~8 nm and ~2 nm, outer and inner diameters, respectively. Highly ordered two-dimensional arrays were observed in receptor-containing membranes incubated with CheW, CheA, and subsaturating levels of attractant. These reproducibly observed arrays correspond to hexagonal lattices with a = b = 8.2 nm and y = 120°. The presence of rosettes at lattice boundaries suggests that receptors may

assume distinct conformations that are in a dynamic equilibrium. These results provide direct structural evidence of receptor arrays in native membranes that may play an important role in mediating chemotaxis.

MORPHOLOGICAL DIFFERENCES IN SORGHUM GRAINS RESISTANT TO MAIZE WEEVIL, SITOPHILUS ZEAMAIS. MICHAEL W. PENDLETON*, E. ANN ELLIS*, FERNANDO M. CHITIO**, and BONNIE B. PENDLETON**. *Microscopy and Imaging Center, Texas A&M University, College Station, TX 77843-2257, and **Division of Agriculture, West Texas A&M University, Canyon, TX 79016.

The maize weevil, *Sitophilus zeamais* Motschulsky, is a very damaging insect pest of sorghum, *Sorghum bicolor* (L.) Moench. Weevils deposit eggs in kernels of grain and the larva feeds inside the kernel. The goals of this research were to 1) evaluate the resistance to maize weevil to 20 genotypes of stored sorghum grain and to 2) examine the relationship between resistance to weevils and the seed coat morphology observed using scanning electron microscopy (SEM) and light microscopy (LM). Three female and two male newly emerged maize weevils were each put with 5 g of one genus of sorghum grain in a set of 10 vials. Vials of each set of the 20 sorghum genera were evaluated every three weeks for a total of 105 days. During evaluation, grains in the vial sets were checked for damage, numbers of live and dead weevil adults, and grain weight. A scale of 1 – 5 was used to rate damage. Sureno was the most resistant genotype, while SC630-11E11 was least resistant. Before observation by SEM, a razor blade and a small hammer were used to split dry grains of 20 genotypes of sorghum. The split grains were exposed to osmium vapor and coated with gold-palladium using a Hummer sputter coater. The cross-section of the seed coat of each genotype of sorghum was observed by SEM using a JEOL JSM 6400 at 15 KeV, 12 mm working distance, and magnifications of 500-2000x. Seed coats of each sorghum genus were dried, fixed, and embedded in epoxy resin and sectioned for observation by LM using a Zeiss Axiophot at magnifications of 100-600x. The different genotypes of sorghum grain seed coats observed in cross-section with SEM and LM were different in appearance, which may be related to maize weevil resistance.

BIOTIC FACTORS AFFECTING NODULATION IN ALFALFA. [1]MEGAN CANADY, [1]JAY INGRAM, [2]DAVID MUIRHEAD, and [3]JUAN GONZALEZ. [1]North Garland High School, Garland, Texas, [2]Texas Scottish Rite Hospital, and [3]Department of Cell and Molecular biology, University of Texas at Dallas.

After an alfalfa plant begins to grow, signal exchanges occur between the nitrogen fixing bacteria *Sinorhizobium meliloti* and the plant. These signal exchanges, which are called flavonoid signals, stimulate cell divisions in the root hairs of the plant. The root hair curls back from the bacteria and a nodule is formed. After the nodule is formed, the bacteria can enter the plant via a tube called an infection thread. Here, the *Sinorhizobium meliloti* bacteria can fix, or convert, the nitrogen from the air to ammonia, which is a form that is usable by the plant. It seems that if two foreign bacterial strains, *Agrobacterium tumefaciens* and *Pseudomonas fluorescens*, were introduced to this process, nodulation would not occur. Four sets of plants were grown with varying combinations of bacteria. The seeds were germinated and then transferred to petri plates of Jenson's agar, which lacks nitrogen and carbon so that the nitrogen fixation method can occur. All bacterial combinations were diluted to one-part bacteria to one-hundred parts distilled water. After two to five weeks of growth, the alfalfa plants that were inoculated with a combination of *A. tumefaciens* and *P. fluorescens* exhibited no nodulation. The combination of *S. meliloti* and *P. fluorescens* had no nodulation as well. Alfalfa infused with *S. meliloti* and *A. tumefaciens* showed white nodules. The last set of plants was grown with *S. meliloti*, *A. tumefaciens*, and *P. fluorescens*. These plants produced mainly white nodules, but pink nodules did occur in one of the plants. The white nodules that were formed by the *S. meliloti* and *A. tumefaciens* combination suggests that the plant did not receive a sufficient amount of ammonia because the fixation that naturally occurs with *S. meliloti*, exclusively, produces pink nodules. This work proposes that nodulation is possible with the presence of foreign bacteria with the natural *S. meliloti*. Further research will provide an understanding as to what bacteria is invading the nodule and why the combination of all three bacteria produced a small amount of pink nodules.

THE EFFECTS OF ROAD DE-ICER ON PLANT GERMINATION. [1]CAREN COLLINS, [1]JAY INGRAM, [2]DAVID MUIRHEAD, and [3]JUAN GONZALEZ. [1]North Garland High School, Garland, Texas, [2]Texas Scottish Rite Hospital, and [3]Department of Cell and Molecular biology, University of Texas at Dallas.

Road deicers are put onto roads throughout the winter season in order to prevent ice-related accidents from occurring. The effects of a popular road deicer, $MgCl_2$, on radish seeds were experimented on during the process of germination. My hypothesis was that the increasing concentrations of $MgCl_2$ de-icer would stunt, if not kill the seed. Radish seeds were germinated in 1. 5 % agar plates with differing solutions of Miracle Grow, and of $MgCl_2$ concentrations from 1 to 10 percent. After 48 hours, the samples were then dissected and the zone of elongation of the root was fixated into resin using standard fixation protocol. The resin blocks were microtomed after they hardened. The seeds were stained and viewed under the light microscope, and as the amount of deicer present increased, the cells of the root became more oblong, showing dehydration from the $MgCl_2$.

SEM OBSERVATIONS OF STAMINATE FLOWERS OF SOME *VIROLA* AUBL. (*MYRISTICACEAE*) SPECIES. TIANA F. FRANKLIN, Texas Christian University and Botanical Research Institute of Texas.

The *Myristicaceae* is one of the top 10 most important families of flowering plants throughout the tropical rainforests of the world. Inventories of lowland rainforest have shown that *Virola* Aubl., the most diverse genus within the family, usually ranks among the top five to 10 most abundant and often one of the most diverse genera of trees in the American tropics. Yet, there have been few studies of this genus and there has been no thorough systematic treatment since the early 20th Century. The genus *Virola* exemplifies the problems existing throughout the new world taxa: wide distribution, complex, unclear, and myriad species relationships, and little recent taxonomic work. The application of Scanning Electron Microscopy (SEM) techniques to clarify specific problems of taxonomic import in the field of neotropical botany has been investigated. Staminate flower morphology of various species of *Virola* has been evaluated for specific characters of taxonomic significance in species delimitation. This group is dioecious, distinctive in its tiny (~2mm), unisexual flowers. Flowers consist of a whorl of 3-5 tepals united for part of their length. The androecium of the male flowers consists of a group of connate or partially free anthers subtended by a filament column. SEM investigation has allowed more productive attempts in understanding the variations in morphology for various species of *Virola* occurring in Central and South America. General quantitative characters such as size, as well as qualitative characters such as type of tepal pubescence, have been evaluated. Results suggest that although considerable differences were observed between some species based on characters of the androecia, these are not necessarily consistent throughout the genus. This evaluation will contribute to the isolation of characters that may be of taxonomic import in future monographic work for the genus.

THE EGGSHELL STRUCTURE OF RINGNECK DOVES (*Streptopelia risoria*). NATALIE E HUBBARD, Department of Biology, University of Texas at Arlington, Arlington Texas 76019.

The avian egg is a complex structure that is an incubation chamber providing nutrition and protection for the developing embryo. Hens prepare year round for egg production by collecting calcium from their diet and storing the calcium her leg bones until needed for egg production. Mineralization of calcite crystals takes the largest portion of the twenty-four hours development period. Eggshell thickness is only one feature that is involved in the strength of the avian eggshell. Contributing factors also include membrane attachment strength, density and distribution of pores, matrix properties, presence of pigment and concentration of vesicles. Species variations of Ringneck doves, *Streptopelia risoria* and the commercially raised chickens, *Gallus domesticus* are compared in this study. The habitat of the Ringneck doves in the study is a temperature controlled environment and diet of seed and water. Dove eggs were collected, labeled and kept refrigerated in the laboratory prior to examination. Various treatments were used on eggshells to clearly expose the structures. Scanning electron microscopy and Image analysis software were used to study structures of the two species of bird eggs.

DETERMINATION OF EGGSHELL MICROSTRUCTURAL CHARACTERSICTS AND ASSOCIATED PHYSIOLOGICAL PROFILES IN MG-VACCINATED EGG-LAYING CHICKENS. SARAH B. MAY and SANDRA L. WESTMORELAND. The Center for Electron Microscopy, University of Texas at Arlington, Arlington, TX 76019

This experiment determined the effect of vaccination of commercial layers with F-strain *Mycoplasma gallisepticum* on eggshell thickness. Previous studies have indicated that this microorganism alters the tissues of the hen's oviduct. Therefore, it was hypothesized that the thickness of the eggshells would be altered in vaccinated birds. A study was conducted at Mississippi State University under the direction of David Peebles to test this hypothesis. The experiment involved multiple variables in addition to the vaccination, including three different diets and two ages of lay. Three cross section micrographs were taken of each eggshell using the JOEL 35C scanning electron microscope. Multiple thickness measurements were made on each of the three cross sections per egg using Image Pro Plus. A formula was derived to account for the difference in height of the eggshell samples. These data were statistically analyzed using SAS statistical software to determine the effects of the variables on shell thickness.

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF EPICUTICULAR WAXES ON LEEK AND ONION LEAVES. [1]BRANDON ROBERTS, [2]DAVID C. GARRETT, AND [3]CAMELIA G.-A. MAIER. [1]The Selwyn School, Denton, Texas 76207, [2]Department of Materials Science and Engineering, University of North Texas, Denton, Texas, 76201, and [3]Department of Biology, Texas Woman's University, Denton, Texas 76204.

Plants protect their above ground organs against water loss by producing and depositing epicuticular waxes (EW) on epidermal surfaces. Synthesis and deposition of EW are regulated internally, but are also affected by environmental factors, such as availability of water and/or light intensity. The model plants used for this project were leek and onion, since they have abundant deposits of crystalline EW on their leaf surfaces. The plants were placed under two different light intensity levels, 240 μm and 900-1180 μm, and were thoroughly watered at the beginning of the experiment. After 7 days, leaf waxes were extracted in chloroform for GC-MS analyses of their chemical composition. Leaf pieces were also examined under light and scanning electron microscopes. No differences in the growth of the leek plants were observed, but there was a significant difference in the level of evapotranspiration between the plants in the two experimental groups (*t* test, P=0.004, α=0.05). Significant differences in onion plant growth were observed: plants under low light intensity grew taller than the plants under high light intensity. Leek and onion plants under bright light lost more water and deposited more EW than those under a lower light intensity. The direct correlation observed between water loss and EW deposition on leaf epidermal surfaces suggests a role for EW in protection against water loss.

EPIDERMAL ACHENE MORPHOLOGY OF *PSEUDOGNAPHALIUM, ACHYROCLINE,* AND *LAPHANGIUM* SP. (*ASTERACEAE*). ANDREW WALTKE[1] AND GUY NESOM[2]. [1]Department of Biology, Texas Christian University, Fort Worth, TX 76109; [2]Botanical Research Institute of Texas, Fort Worth, TX 76102.

Two primary types of achene epidermal morphology were found among the 17 species of three genera under study, including glandular and eglandular cells, as seen by SEM. *Laphangium* species has characteristic rectangular epidermal cells and 3-celled, elongate, glandular papillae irregularly scattered on its achene surface. *Pseudognaphalium* and *Achyrocline* achenes are eglandular and have elongate, imbricate epidermal cells (distal end overlapping the base of the adjacent cell). The individual epidermal morphologies vary in that the distal ends are raised. The surface may appear (1) *smooth* (distal ends appressed), (2) *papillate-roughened* (distal ends slightly raised above the base of the adjacent cell), or (3) *distinctly papillate* (distal ends greatly raised above the base of the adjacent cell). Both epidermal types are present in the New World species, but the glandular morphology is the most common among a related Old World genus *Helichrysum*. *Laphangium luteoalbum*, which has glandular achenes, occurs in North America but probably is native to Eurasia, where its closest relatives are found. Geographically related species are notably similar, closely sharing both size and epidermal morphology. The implication of these morphologies is that the New World species may be derived from the Old World species.

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THE -6413 FROST RING IS THE OLDEST IN BRISTLECONE PINES

HOWARD J. ARNOTT, *CHRISTINE HALLMAN, *TOM HARLAN and *REX ADAMS

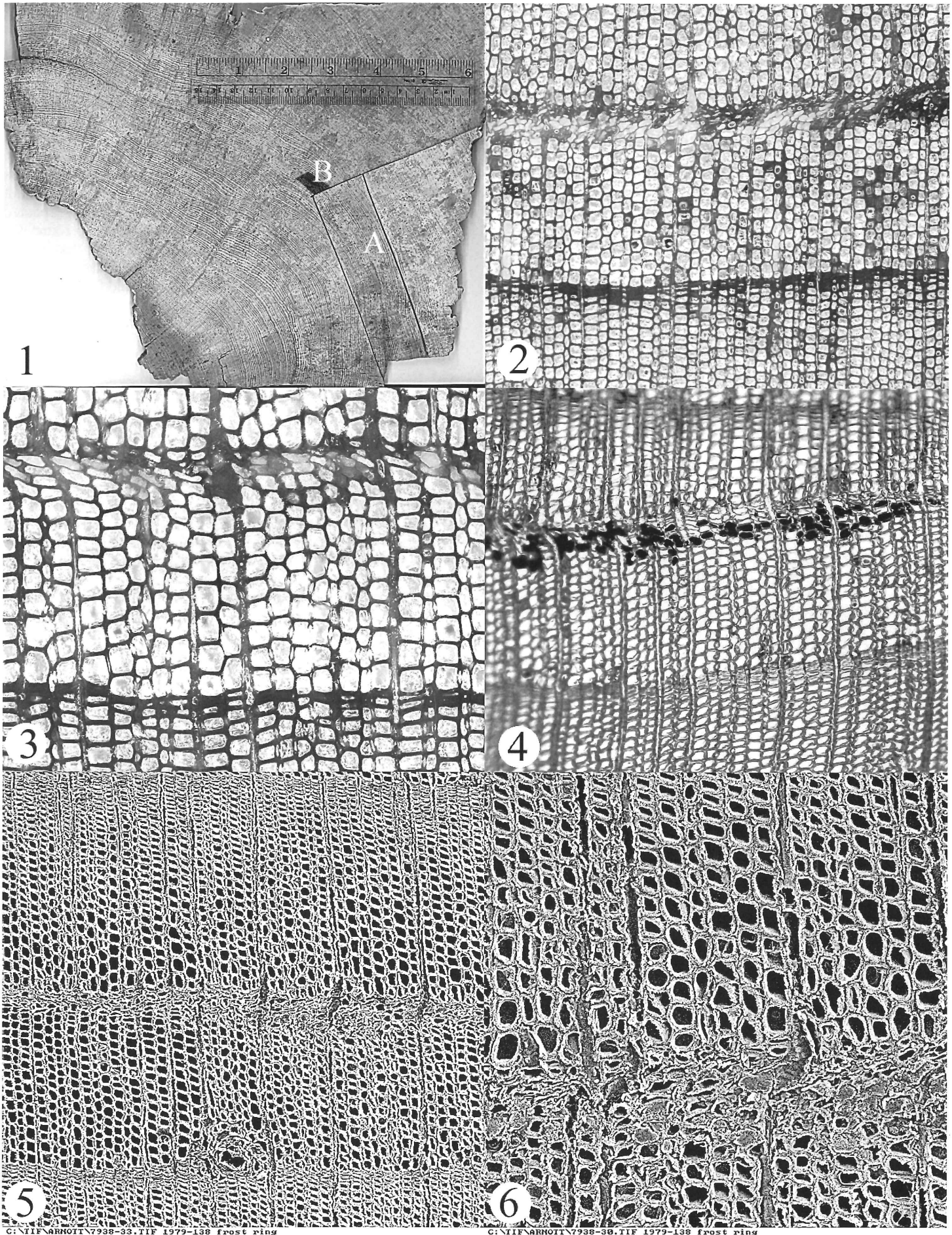
**The Department of Biology, The Center for Electron Microscopy,
The University of Texas at Arlington, Arlington, Texas, 76019,
and *Laboratory of Tree-Ring Research, University of Arizona, Tucson, AZ 85721**

A remnant piece of bristlecone pine (*Pinus longaeva*) wood was collected at about 8900 feet the Methuselah Grove area of the White Mountains in Eastern California. This piece, designated Meth 1979-138, has been in storage at the Laboratory of Tree-Ring Research since its collection in 1979. A slab of that piece was crossdated by Tom Harlan (Fig. 1). The slab extends radially for 30.5 cm and is 30 cm in its tangential dimension; it has 410 annual rings extending from -6609 to -6199. In about the center of this slab, at annual ring -6413 (6414 B.C.), an obvious late frost ring occurs; this frost ring is split for about 6 cm on the left and 2 cm on the right, the remainder, ca 20 cm, remains in tact. Although the possibility of older frost rings in bristlecone pine wood exists, this is currently the oldest frost ring known. This sample is notable also for three other reasons: 1) the sample was collected at a relatively low altitude for bristlecone pines; 2) the frost ring has extensive length, one that provides an extraordinary opportunity for study; 3) although frost rings are common in bristlecone wood from higher altitude, frost rings are rare in bristlecone pines wood from the Methuselah Grove.

A 1200 dpi scanned replica of the slab was made using a Hewlett-Packard 7400C Scanjet. The scan was copied to a CD; thus the exact nature of this rare specimen was effectively documented (Fig 1). Study of the slab showed that the -6413 frost ring formed a semi-irregular arc extending across the entire surface; younger and older rings are on both sides of the frost ring (Fig.1). Three cuts were made to provide a long segment of the frost ring, (Fig 1-A) and to allow removal of a small block for sectioning (Fig 1-B). The small block was hydrated in boiling water and sections were made for LM and SEM using a sliding microtome. Sections for LM were stained with safranin, mounted in resin and viewed and photographed with a Nikon Eclipse 80i microscope (Fig. 4). Additional light micrographs were made from the surface of the segment A using the Nikon microscope with reflected light (RLM) (Figs. 2-3). Sections for SEM were dehydrated to 100% ETOH and placed between two microscope slides, dried in an oven at 60 degrees C., mounted on stubs, and sputter coated with gold/palladium. They were studied using a JOEL 35C Scanning Electron Microscope equipped with a VitalScan digital recording device (Figs. 5-6).

A group of 37 individual RLMs from segment A were merged together to form a long continuous montage of the frost ring. Direct examination of this segment and study of the montage showed that, while the frost ring is continuous, it varies considerably from zone to zone. The characteristic "bending" of the tracheid rows, universally present in late frost rings of bristlecone pines, was in some zones inclined to the left (Fig. 3-4) in others it was inclined to the right (Figs. 2, 5-6) and in still others not "bent" at all. Usually only three to five of the most external tracheids in each row are involved in "bending." In some areas of the frost ring many of the outermost tracheids have a yellow deposit in their lumen when examined with RLM (Fig 2); the density of this material, which may be a resin, varies along zones of the frost ring, being prominent, less prominent and absent in various zones. In sections stained with safranin a "dark" material is localized in tracheid lumina in a manner similar to that seen for the yellow deposits (Fig. 4). Sections of wood as seen in the SEM show many of the characteristic features seen in most bristlecone pine frost rings. The expanded ray cells, usually seen in late frost rings, are seen in Figs. 5-6. The degree of "bending" in both tracheid files and in ray cells can be seen (Figs. 2-6). The results of "renewed" growth and recovery from the frost injury can be observed in Figs. 2-6; note that the ray cells seem to play an important in the organization of "new growth." Wood of Meth 1979-138 did not section well, this may be from minute changes, which occurred as this wood aged through eight millennia (Figs. 5-6).

Figure 1. A scan of Meth 1979-138 illustrating the slab's general shape; the -6413 frost ring runs from the upper left to lower right (scale included). **Fig.ure 2.** A reflected light micrograph showing "bending" of the tracheid rows toward the right, note also the material in the lumen of the "bending" tracheids (100X). **Figure 3.** A reflective light micrograph in which the "bending" of the tracheid rows is to the left, note expansion of ray cells (150X). **Figure 4.** Section of the frost ring stained with safranin showing "black" material deposited in the lumen of many tracheids (90X). **Figure 5.** SEM view of a frost ring showing expanded ray cells (ca. 105X). **Figure 6.** An Electron micrograph showing "bending" of tracheid rows to the right and "expanded" ray cells (ca 230X).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MATERIALS SCIENCE
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TRANSMISSION AND SCANNING ELECTRON MICROSCOPY OF SETEREOLITHOGRAPHY RESINS REINFORCED WITH CARBON NANOTUBES. J.H. SANDOVAL[1], K.F. SOTO[2], L.E. MURR[2], R.B. WICKER[1]. [1] W.M. Keck Border Biomedical Manufacturing and Engineering Laboratory. Department of Mechanical and Industrial Engineering, The University of Texas at El Paso, El Paso, TX 79968 USA, [2]Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX 79968 USA

Stereolithography (SL) is one of the most popular and accurate rapid prototyping (RP) technologies available in the market. The SL system is a layered based manufacturing process, which builds complex 3-D geometries by selectively curing a liquid, photocurable resin by means of an ultraviolet laser. Once the top layer is cured, the part is traversed into the liquid polymer vat and a new layer is built on top of the previous one. This procedure continues until the part is completed. The objective of this research was to investigate tailoring the mechanical and thermal properties of existing epoxy-based resins by dispersing multi-walled carbon nanotubes (MWCNTs) in their polymeric matrix. A novel nanocomposite was created by dispersing controlled amounts of MWCNTs in a commercially available SL epoxy-based resin matrix. Complex 3-D geometries were successfully fabricated by means of a modified SL system, and sample specimens were mechanically tested and characterized by scanning and transmission electron microscopy. Small dispersions of MWCNTs resulted in significant effects on the physical and mechanical properties of the polymerized resin, including increases in mechanical strength and integrity over much wider operating temperatures (allowing for possible use of SL-manufactured parts in end-use applications). Electron microscopy portrayed affinity between the constituents and served as means to measure the impact of this particular nanomaterial on the surface morphology of the samples. Varying concentrations of MWCNTs could be used to tailor existing SL resins for particular applications. Exploiting nanostructured materials' characteristics and properties in stereolithography (SL) may open new markets for unique rapidly manufactured functional devices.

ELECTROSTATIC POTENTIALS WITHIN CORE/SHELL NANOWIRES BY ELECTRON HOLOGRAPHY. JAYHOON CHUNG and LEW RABENBERG. Texas Materials Institute, University of Texas, Austin, TX 78712.

Devices based on semiconductor nanowires are currently being investigated for applications such as transistors and sensors. Engineering useful devices from such nanowire devices will require quantitative measures of the dopant concentrations and potential distributions within them. Electron holography in the transmission electron microscope (TEM) is a technique that records the phase shifts imposed on an electron wave as it propagates through a solid, and is capable of imaging the electrostatic potentials within a solid. In this paper, phase profiles within core-shell nanowires, composed of intrinsic germanium cores and shells of germanium oxide and/or doped germanium, were imaged using electron holography in a transmission electron microscope. Accurate mean inner potentials for germanium and its oxide were determined. Using cross-section analysis, the surface potential, screening length, and doping concentration for the doped germanium shell were determined quantitatively from the two-dimensional potential image. These characteristics were compared with values obtained from a numerical solution of Poisson's equation.

FABRICATION AND OPTICAL PROPERTIES OF ER-DOPED Ge AND SiGe NANOWIRES. JI WU and JEFFERY L. COFFER. Department of Chemistry, Texas Christian University, Fort Worth, Texas 76129.

Germanium (Ge) nanowires (NWs) and SiliconGermanium (SiGe) nanowires (NWs) doped with erbium ions (Er^{3+}) are promising building blocks in fabricating nanoscale devices due to their special electronic and optical properties. Herein, we report a multi-step processes for the fabrication and optical properties of Erbium-doped Ge NWs and SiGe NWs. Ge NWs with high yield are first fabricated by an efficient carbon-assisted vapor transport method. The gold catalyst on the end of the Ge NW confirms the expected vapor-liquid-solid (VLS) mechanism in this synthetic route. Ge NWs of differing mean diameter can be produced by varying the helium carrier gas flow rate and/or reaction time. A thin layer of Er^{3+} ions is then incorporated onto the as-prepared Ge nanowires, which is confirmed by high resolution transmission electromicroscopy (HRTEM) imaging concomitantly with EDX (energy dispersive X-ray) analysis. The as-formed Er-doped Ge NWs do not demonstrate the 1540 nm luminescence associated with Er^{3+}. However, annealing such a sample in air at 600° C results in strong enhancement of this light emission. In the case of Er-doped SiGe NWs, we employ three sequences of Si and Er^{3+} depositions on Ge NWs to acquire three different structures and optical properties. The one prepared by co-depositing Si and Er^{3+} on top of Ge NWs gives the strongest emission at 1540 nm. If the Er^{3+} layer is inserted between Ge and Si layers, this structure shows medium emission intensity. However, if we deposit Er on the top of Ge and Si layer, it will give the weakest emission. HRTEM, EDX mapping, Raman spectrum, scanning electromicroscopy (SEM) and electron diffraction (ED) are employed to characterize the three structures. The effects of annealing condition, thickness of silicon layer and the concentration of Er^{3+} on the photoluminescence are also investigated.

TIN WHISKERS AND THE LEAD FREE INITIATIVE

ROBERT F. CHAMPAIGN

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There is a move currently underway in the electronics industry to use environmentally friendly materials. This includes removing lead (Pb) from electronic assemblies and solder alloys. It also involves removing certain types of flame retardant materials from plastic and encapsulated integrated circuits. The new alternative materials are commonly referred to as halogen free or green materials. Restrictions by environmentally friendly organizations and nations are pressuring electronic manufacturers to develop alternate surface finishes and solder alloys. More expensive solutions are to use nickel/gold/palladium (Ni/Au/Pd) or Ni/Au. Another more cost efficient alternative solution is to use pure tin (Sn) or alloys that are Sn rich [1], [2], [3].

The use of pure Sn poses a serious reliability risk due to the potential for the Sn to form whiskers. Sn whiskers are electrically conductive filaments that can spontaneously grow from pure Sn surfaces. These filaments are single crystal structures whose growth mechanisms are not completely understood. The most compelling theory in the electronics industry is that Sn whisker growth is a compressive stress relief mechanism in the Sn plating. Some identified sources of stress in Sn are plating residual stress, compressive mechanical loading, scratches in the plating surface, intermetallic formation, and mismatches in the coefficient of thermal expansion between the plating and substrate [3], [4], [5]. Extensive studies are still being performed in the industry trying to understand the Sn whisker growth phenomenon.

Sn whiskers have been reported to commonly grow in lengths greater than 60 mils with diameters as much as 10 microns. Some studies report whiskers as long as 394 mils. The shape of the whiskers can vary dramatically from perfectly straight to bent or kinked. The initiation of whisker growth can occur soon after plating or lie dormant for years [2], [4], [5].

Sn whiskers can cause transient or long-term electrical shorts depending on the amount of current available. Metal vapor arcing can also occur in certain environmental and electrical conditions. Whiskers can also easily break loose in components and assemblies generating conductive debris [4], [5].

The Sn whisker issue is a serious reliability concern with space and military electronics. There are many documented cases where Sn whiskers have resulted in the failure of electronic assemblies. Two specific failure analysis cases will be discussed where the main failure mechanism was Sn whiskers. The first involves the failure of hybrid microcircuits when Sn whiskers formed on the Sn plated lids and broke off. The loose conductive whiskers caused electrical shorts to occur in the hybrid circuitry. The second failure to be discussed occurred on a rocket motor initiator when whiskers emanated from the Sn plated internal pins. The whiskers bridged a 10-mil spark gap causing test failures. Various other examples of Sn whisker growth will also be presented from internal experiments performed on Sn plated coupons. Another unique aspect of this paper involves Focused Ion Beam (FIB) cuts into Sn whiskers sites.

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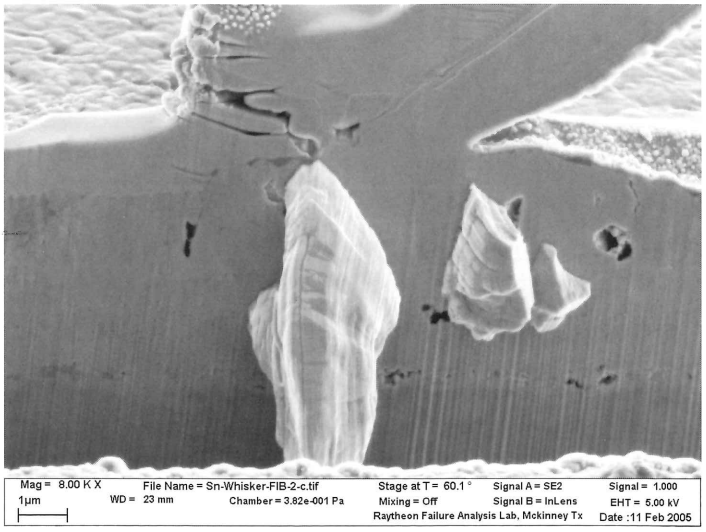

Figure 1: Sn whiskers and nodule initiation sites growing on a Sn plated brass substrate coupon.

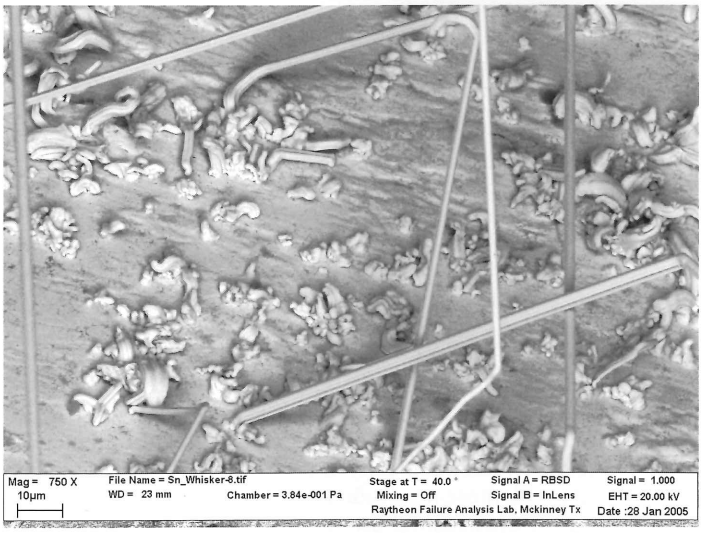

Figure 2: Focused Ion Beam (FIB) cut into a Sn whisker site. Unique to the FIB cut is the fact that several Sn whiskers grew out of the polished FIB cut face in three days time.

TECHNIQUES
FALL 2005

NANOSCALE 3D FIB-SEM TOMOGRAPHY: A PRACTICAL TOOL. E. L. PRINCIPE. Carl Zeiss SMT, Inc., Nanotechnology Systems Division 555 Twin Dolphin Drive, Suite 130, Redwood City California, 94065

The ability to acquire, display and interrogate 3D volumes of image data has been well-established through various scientific disciplines. The medical field, in particular, has exposed the public to tomographic methods through now common medical procedures such as computed axial tomography (CAT) and magnetic resonance imaging (MRI). In an analogous fashion the focused ion beam (FIB) and scanning electron microscopy (SEM) can combine to generate tomographic data. In contrast to established medical procedures, FIB-based tomographic methods are in an early phase of implementation. While less common, the FIB-SEM tomographic method has demonstrated the ability to complete 3D volumetric reconstruction at a resolution of 10nm or better in all three dimensions. Factors that limit wider utilization of FIB-based tomographic methods include the ease, speed and density of raw data collection. Another obstacle is implementing robust, yet versatile data analysis and volume visualization methods suitable for electron imaging. With the advent of high-resolution simultaneous SE imaging during the FIB sectioning process; it is now practical to acquire several hundred SEM image frames in the span of less than one hour in an automated fashion. The live data acquisition method is coupled to a data reduction process that allows convenient display of the high quality volume reconstructions through animated section sequences, exploration of sub-volumes and application of selective transparency. Aspects of data acquisition, data processing and approaches to quantification will be described.

BIODEGRADABLE BIOSILICON/POLYMER COMPOSITES FOR TISSUE ENGINEERING APPLICATIONS. DONGMEI FAN, GIRIDHAR AKKARAJU, JEFFERY L. COFFER, ERNEST F. COUCH, Department of Chemistry and Department of Biology, Texas Christian University, Fort Worth, TX 76129.

Tissue engineering is a relatively new and exciting technique, which has the potential to create tissues and organs *de novo*. It involves the *in vitro* seeding and attachment of human cells onto a scaffold. These cells then proliferate, migrate and differentiate into the specific tissue while secreting the extracellular matrix components required to create the tissue. Natural and synthetic materials are currently used as tissue engineering scaffolds with the necessity of ultra-high porosity to achieve proper vascular and neurological growth. We have recently succeeded in producing microfibers existing as a composite of bioactive mesoporous silicon (BioSilicon) with polycaprolactone (PCL), a non-toxic, biodegradable polymer. The fabrication of a range of PCL/BioSilicon composites of varying polymer solution concentration, BioSilicon concentration, and PCL fiber diameter prepared by an electrospinning technique is demonstrated. Electrospinning is the process of using a high electric force generated between a polymer solution contained in a syringe with a capillary tip and a target. The biocompatibility of these PCL-based scaffolds was tested *in vitro* using human kidney fibroblast cells; results indicate that BioSi-containing PCL-based scaffolds mediate the proliferation of human kidney fibroblast cells at a level comparable to that of cell-only controls. These studies have been expanded to include investigations of viability of another cell line, human mesenchymal stem cells (hMSCs). These cells have the potential for differentiation into a variety of orthopdedically-useful tissues such as cartilage and bone. Overall, these results described in this presentation have implications for the eventual use of these materials in living systems. The SEM images of the cells and scaffolds will be presented in the presentation.

USE OF QUETOL 651 TO LOWER THE VISCOSITY OF EPOXY RESIN EMBEDDING FORMULATIONS. E. ANN ELLIS, Microscopy and Imaging Center, Texas A&M University, College Station, TX 77843-2257

Quetol 651, a low viscosity, Japanese epoxy resin was introduced into the US in the late 1970's and has been used primarily in formulations of Spurr low viscosity resin. The viscosity of a number of more viscous formulations has been reduced by use of Quetol 651. Table 1 shows the formulations for Quetol in a straight chain formulation with LX-112 and substituted for LX-112 in an Epon-Araldite 502 formulation with an anhydride to epoxide ratio of 1.0:1.0. The accelerator for all formulations is benzyl dimethylamine (BDMA). Table 2 shows the viscosity of these formulations compared to the parent formulations and standard formulations such as Epon/Araldite, Luft's Epon and Spurr low viscosity resin. These formulations have been used extensively since 1984 with difficult specimens such as optic nerve and other ocular tissues for studies involving cytochemical localizations and colloidal gold immunocytochemistry. The Quetol 651 formulations allow for improved infiltration, have good sectioning qualities and are stable in the beam.

TABLE 1. NEW EMBEDDING FORMULATIONS USING QUETOL 651

LX-112 FORMULATION		ARALDITE 502 FORMULATION	
DDSA	6.98 g	DDSA	5.66 g
QUETOL 651	1.14 g	QUETOL 651	1.16 g
LX-112	1.88 g	ARALDITE 502	3.18 g
BDMA	0.20 ml	BDMA	0.20 ml

TABLE 2. VISCOSITIES OF STANDARD AND QUETOL 651 SUBSTITUTED FORMULATIONS

EMBEDDING FORMULATION	VISCOSITY [CENTIPOISE] AT 25° C
EPON 812/ARALDITE 502	2500
LUFT'S EPON 812	550
LUFT'S LX-112	340
QUETOL 651/ARALDITE 502	800
QUETOL 651/LX-112	250
SPURR LOW VISCOSITY	60

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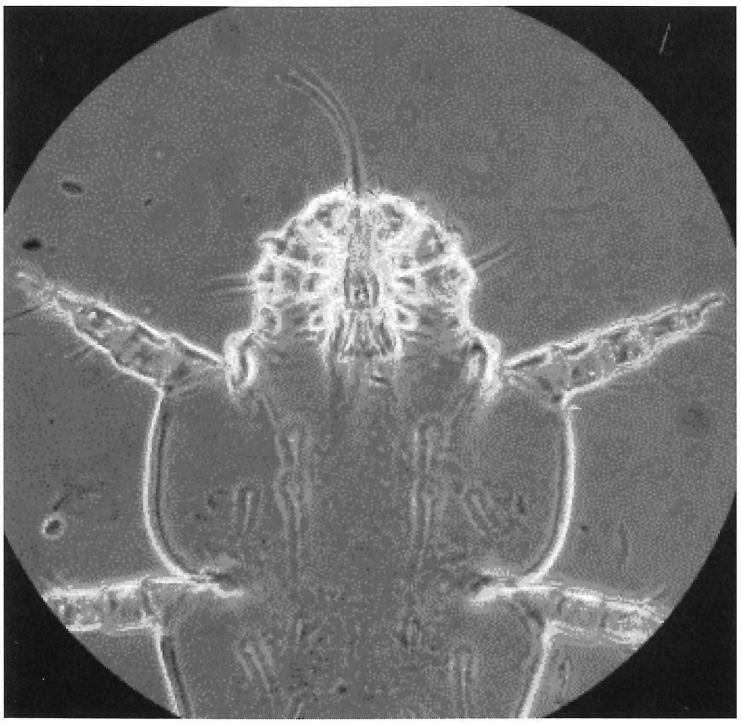
"WETSEM" ELECTRON MICROSCOPY OF FULLY HYDRATED SAMPLES. DAPHNA R. YANIV. Electron Microscopy Sciences, 1560 Industry Road, Hatfield, PA 19440

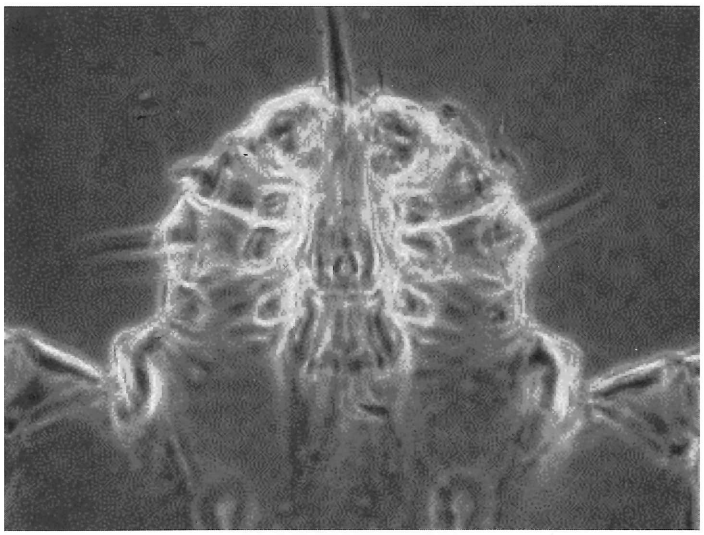
New technology (WETSEM) that allows direct observation of wet samples with scanning electron microscope (SEM), at atmospheric pressure will be introduced. The sample is placed in a sealed specimen chamber, and is separated from the vacuum by a thin, electron-transparent membrane. The partition membrane allows the penetration of electrons to the sample and the collection of backscattered electrons (BSE), while withstanding pressure differences of up to one atmosphere. The technology offers several unique advantages. Sample preparation involves only liquid handling, obviating the need for drying, embedding, sectioning or coating. The simple sample preparation procedures minimize deformations and other artifacts, and allow preservation of labile, hydrated structures. The technology is also compatible with Energy Dispersive Spectroscopy (EDS) measurements of wet samples. Results ranging from life science to material science applications will be presented. Images and EDS measurements of cells, tissues, bacteria, powders, nanoparticles, emulsions, and creams will be demonstrated.

CELL VIABILITY DETERMINATION IN PHOTOCROSSLINKED PEG HYDROGELS FABRICATED WITH STEREOLITHOGRAPHY USING CONFOCAL MICROSCOPY. KARINA ARCAUTE*, BRENDA MANN† and RYAN WICKER*, *W.M. Keck Laboratory, University of Texas at El Paso, El Paso, TX 79968, † Sentrx Surgical, Inc., Salt Lake City, UT 84108.

In the last decade, many researchers have encapsulated cells in scaffolding materials for tissue engineering (TE) applications. Poly(ethylene)glycol (PEG) is a widely used material in cell encapsulation because it can be photocrosslinked to form insoluble, water swollen, 3D hydrogels with tissue-like properties. Simple 3D scaffolds with live cells have been fabricated previously by exposing a PEG-cell suspension contained in a mold to ultraviolet energy. Our research group is exploring the capabilities of the stereolithography (SL) rapid prototyping technology to fabricate complex 3D PEG-based scaffolds in a layer-by-layer fashion to use for the regeneration of damaged tissue. For the cell encapsulation studies presented here, human dermal fibroblast (HDF) cells were encapsulated in PEG hydrogels with and without the adhesion peptide RGDS (as acryloyl-PEG_{3400}-RGDS) in a photopolymerizable aqueous solution of PEG dimethacrylate (PEG_{1000}-dma) and the photoinitiator Irgacure 2959. Cell viability was tested with the Molecular Probes LIVE® assay at 2, 24, 48, 72, and 170 hours. The stained gels containing cells were observed using confocal microscopy with fluorescence capabilities. Results showed a more uniform distribution of cells in the gels without the cell adhesion peptide, while the cells in the gels containing RGDS appeared to group in clumps. Fluorescent images showed that in the absence of RGDS, cell viability dropped to ~20% after 1 week in culture. Fluorescent images of the bioactive gels showed no decrease in cell viability, and significant cell proliferation was observed as the size of the clumps increased over 1 week. In conclusion, HDF cells successfully survived the SL process, remained viable and proliferated in the gels after extended culture periods as long as the cell adhesion peptide RGDS was present in the gel. Consequently, SL is a promising process in TE for the fabrication of bioactive PEG scaffolds containing live cells. Furthermore, confocal microscopy is an easy-to-use technique for the observation of PEG gels with encapsulated cells that does not require special sample preparation and allows the observation of the sample in an aqueous environment.

What Is It? *Answer in Next Edition*





Micrographs sent by Andrew Chen, Research Entomologist with USDA-ARS, Knipling-Bushland US Livestock Insects Research Laboratory, Kerrville, Texas 78028-9184

IN MEMORIAM

ANN SULLIVAN BURKE, 1943–2005

TSM Secretary and Program Chairman-elect
University of Texas Medical Branch and Shriner's Hospital

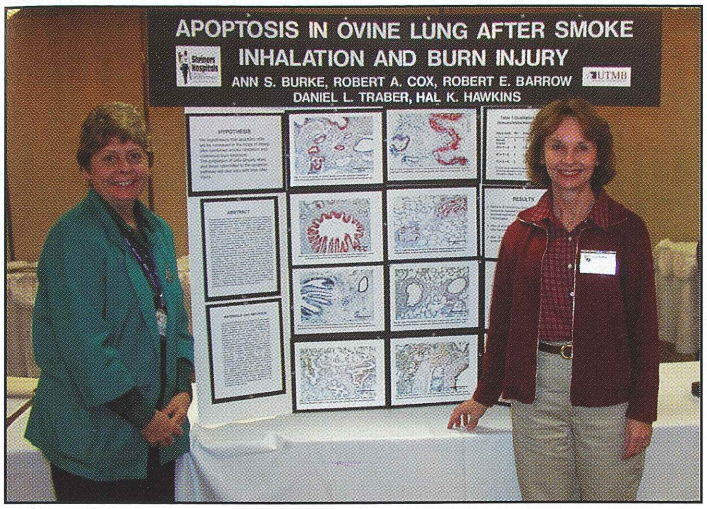
We are mourning the untimely death of Ann Burke on July 13th. Ann became a member of TSM in 1991 and was the Society's past Secretary and Program Chair Elect. She was diagnosed with lung cancer in June, and despite chemotherapy and the help of her friends survived less than a month. It is appropriate now also to celebrate her life and to consider the good times we shared and the gap she has left in our own lives.

Ann greatly enjoyed the TSM meetings and the good times she shared with her many friends here. Ann was Irish, as she delighted to remind us, and was born in Joliet, Illinois. She attended the College of St. Francis in Joliet, Illinois, and continued with graduate work at Purdue University in Indiana. She moved to California and worked for 15 years as a researcher in the Oncology service at UCLA, with Drs. John Wells, David Golde and Charles Haskell. She came with Dr. Wells to UTMB, and did research in the Department of Internal Medicine. In 1989 she joined the Electron Microscopy Department of the Shriners Burns Hospital in Galveston, where she worked, with brief interruptions, until the time of her death. I was privileged to rehire her in that role in 1994 and to be her supervisor since that time.

Since 2004 she has held the title of "Morphologist" at the Shriners Hospital, a position that was created for her. She actually carried out the bulk of the work of the core laboratory. Ann was a tireless and dedicated worker. Her major responsibility was to develop immunohistochemical techniques for new antigens for our research in a sheep model of lung injury. She really became quite expert at development of optimal methods. Her work led to the discovery that tracheobronchial mucous glands probably are important in stimulating airway inflammation. She was a member of the American Burn Association, where she presented many posters, and she recently presented the results of her own work at the national meetings of the Histochemical Society and the Shock Society. She was also a wonderful co-worker, who always took a supportive interest in the lives of her colleagues. One important function of our lab is to provide morphological support for the research of the post-doctoral fellows at the Shriners Hospital, mostly surgeons taking a year or two of research training to further their careers. She served as a teacher and mentor and friend to many of these fellows, and led them into productive studies. After her death I received e-mails of condolence from Brazil, Spain, and Japan as well as all over the USA.

Ann constantly contributed to the life of the Shriners staff by serving on the "fun committee" that planned celebrations and functions. She greatly enjoyed the Galveston Mardi Gras celebrations, and was an active member of the Krewe of Aquarius. She was a dedicated mother who led and cajoled her son into success as an Eagle scout and athlete and scholar. She was also a dedicated Christian who was active in the life of her Catholic congregation. We will all remember Ann fondly as an excellent scientist and a lively, fun-loving friend. For anyone who wishes to contribute to a memorial, her family has requested that a bench be placed at the University of St. Francis in her honor, c/o Patricia McClintock, 500 Wilcox, Joliet, IL 60435.

Hal K. Hawkins
TSM Past President


APOPTOSIS IN OVINE LUNG AFTER SMOKE INHALATION AND BURN INJURY
ANN S. BURKE, ROBERT A. COX, ROBERT E. BARROW
DANIEL L. TRABER, HAL K. HAWKINS
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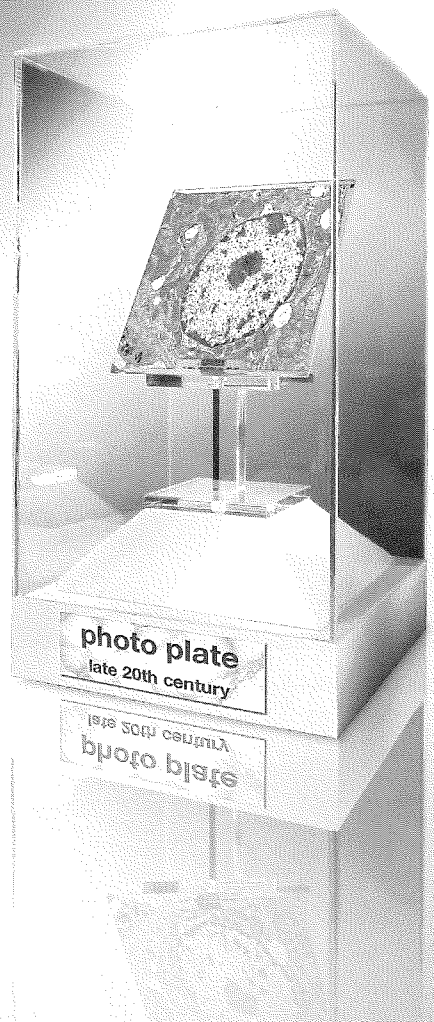

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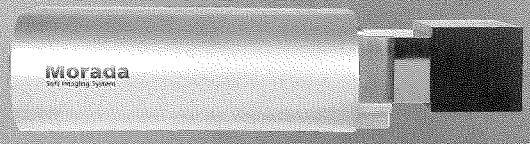

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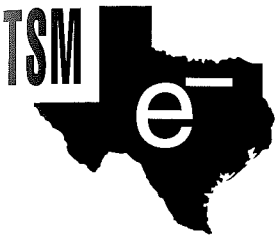

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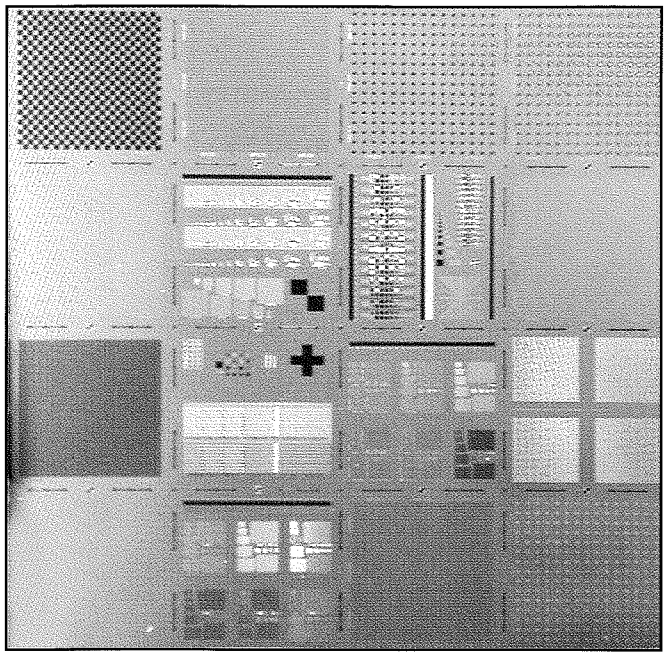

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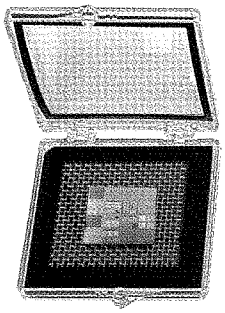

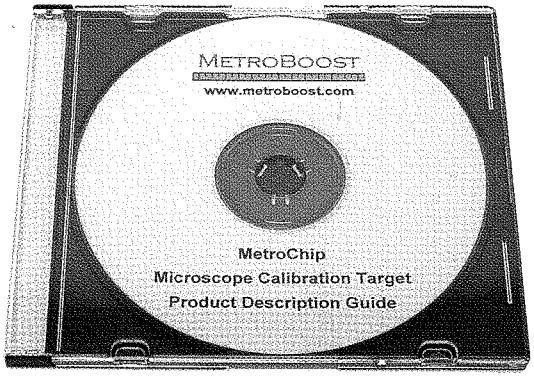

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