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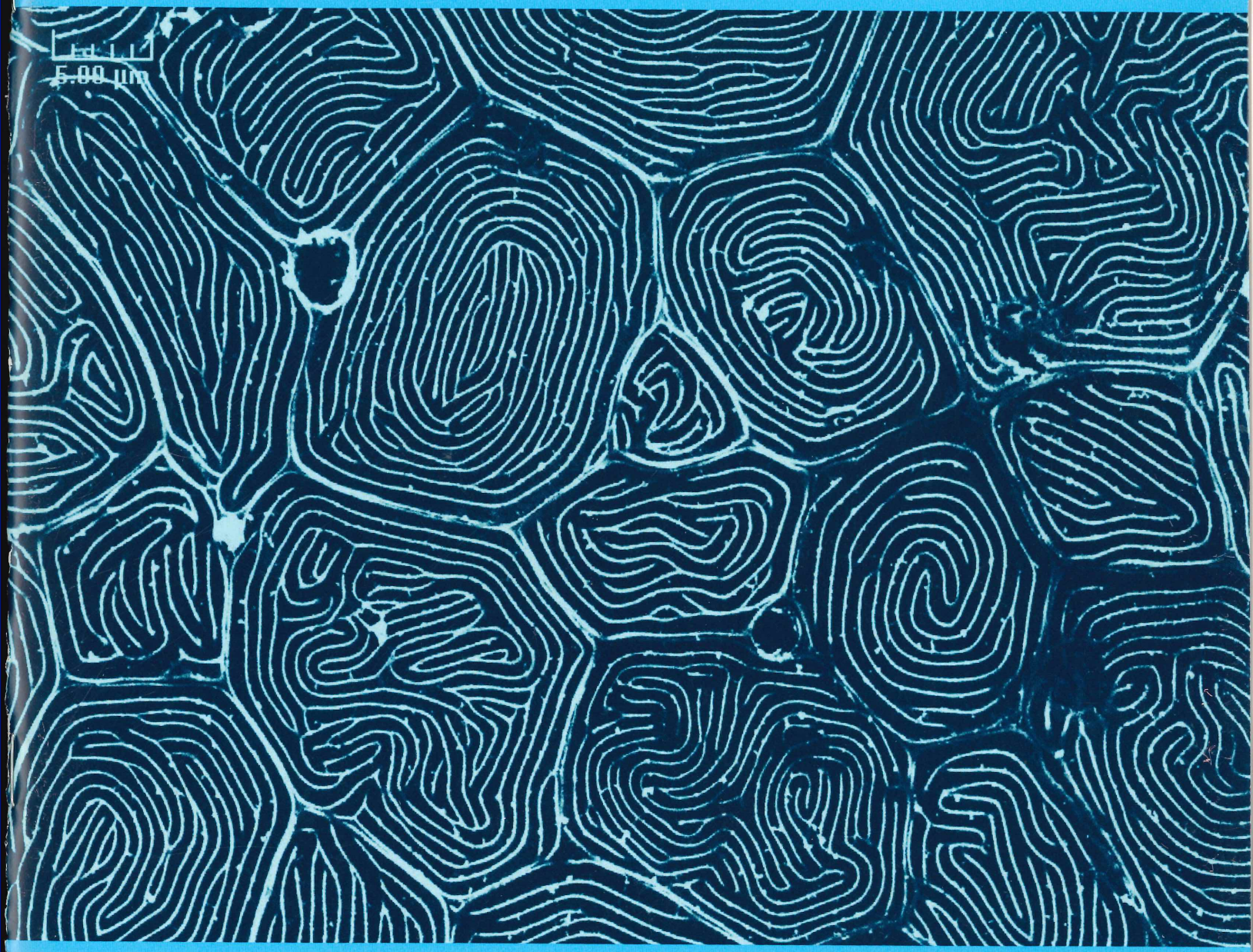

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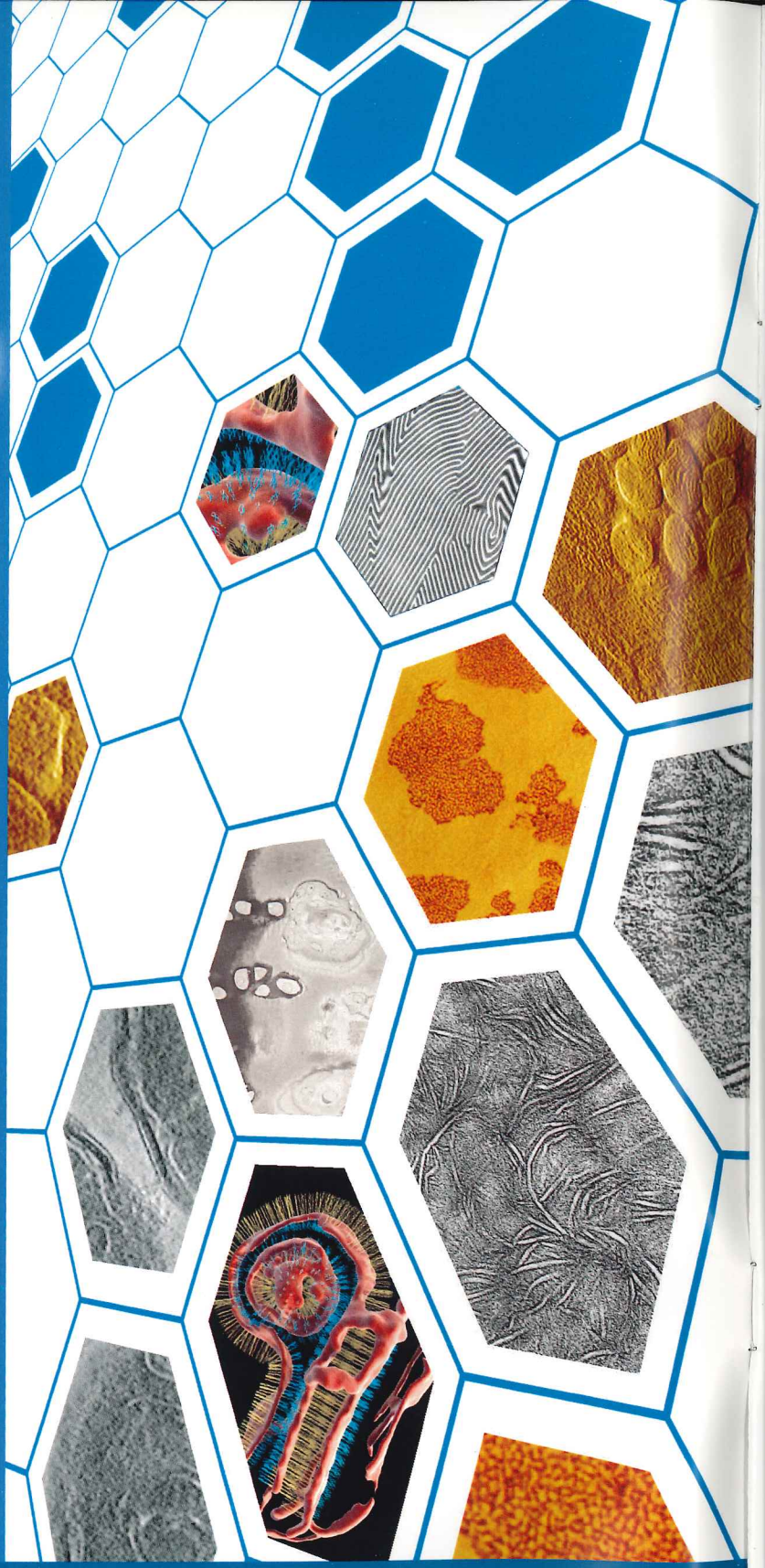

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E. Ann Ellis and Michael Pendleton, Co-editors
Texas A&M University Microscopy & Imaging Center
TAMU MS 2257, College Station, Texas 77843-2257

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ON THE COVER: Scanning electron micrograph of the epidermis of *Sundadanio axelrodi*, a tiny cyprinid fish endemic to the Peat Swamp Forests of South East Asia, showing mucus pores and well developed microplicae on the surface of epidermal cells. Submitted by Dr. Kevin W. Conway, Assistant Professor and Curator of Fishes, Department of Wildlife and Fisheries Sciences, Texas Cooperative Wildlife Collection, Texas A&M University, 210 Nagle Hall, 2258 TAMUS (mailing), 1101 Heep Laboratory Building (office), College Station, TX 77843, Email: kevin.conway@neo.tamu.edu.

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President's Message

Webster's Dictionary defines a volunteer as one who serves in a designated capacity willingly and with no guarantee of reward. The Texas Society for Microscopy would not exist without volunteers willing to serve as officers. I would like to recognize these special people in this President's Message.

I commend Jodi Roepsch, 2009-2010 President of TSM, for her strong leadership and sincere dedication to the Society. Jodi's willingness to "think outside the box" and solicit public school and corporate involvement in TSM resulted in a very successful and first of its kind Student Microscopy Workshop for high school seniors at the Frisco meeting. Her positive attitude toward the future of our group and interest in generating new avenues through which we can serve the scientific community are truly remarkable.

Camelia G.-A. Maier became editor of the Texas Journal of Microscopy in 2000, and served 10 years in this position, publishing volumes 31 – 41. The Society has benefitted tremendously from Camelia's tireless efforts to produce a journal of the highest quality. Camelia stepped down as editor to immediately assume the role of President-Elect. Her commitment to TSM is strong and unwavering.

E. Ann Ellis and Michael Pendleton have taken on the roles of Journal Co-Editors and have begun their work with this issue. This is a time consuming and tedious job which Ann and Mike have taken on with energy and enthusiasm.

David Garrett, Ernest Couch, Bob Droleskey, Alice Stacey, Sandra Westmoreland, and Robert Champaign are all repeating as members of the Executive Council, many having served three or more terms of office. Becky Holdford is our original Webmaster and distributes all of the Society's electronic correspondence to the membership, our major means of communication in modern times. Where would TSM be without the willingness of these loyal volunteers to commit to its future?

Our corporate members continue to generously support the Society, not only with their financial contributions but also by participating in meetings and providing ideas and encouragement for future endeavors. Where would TSM be without this crucial means of support?

This journal will be published in conjunction with our 2011 meeting in Ft. Worth. Two guest speakers will be a part of our program. Dr. Paul Kotula is a Principal Member of the Technical Staff in the Materials Characterization Department at Sandia National Laboratories in Albuquerque, New Mexico. Dr. Kotula was a member of a team of scientists involved in the investigation led by the FBI surrounding the anthrax attacks that killed five people in the fall of 2001. He will speak about the forensics aspects of the anthrax attacks as well as techniques in microanalysis developed and/or applied by Sandia National Laboratories in numerous areas. Dr. Jeffrey D. Cirillo is a professor in the Department of Microbial and Molecular Pathogenesis at the Texas A&M University System Health Science Research Center in College Station. Dr. Cirillo's research interests involve bacterial pathogenesis and host-pathogen interactions at the molecular and cellular level. His presentation will be titled "Emerging Technologies for Imaging Infectious Diseases."

The year 2011 is the 46th year in our Society's history. Our future is in the hands of those willing to volunteer to serve in a leadership role. If you feel so led, please contact any member of the Executive Council. Your participation is needed for TSM to thrive.

Jo Taylor
2010-2011 TSM President

ABSTRACTS
SPRING 2011

BIOLOGICAL SCIENCES PLATFORM PAPERS

SPERMATIC GRANULOMA ASSOCIATED PATHOLOGY IN RAT EPIDIDYMIS FOLLOWING ANDROGEN WITHDRAWAL.

DIBYENDU DUTTA, IN PARK, HIWOT GUILILAT, LAURA HANSON, SAMUEL SANG and NATHANIEL MILLS. Department of Biology, Texas Woman's University, Denton, TX 76204

Depletion of testosterone, vasectomy or chemical insults are known to induce spermatic granuloma (SG) in epididymis. In approximately 40% of vasectomized males SG formation induces abdominal and scrotal pain. Occurrence of spermatic granulomas has been reported after one week of testosterone depletion following castration and in estrogen receptor α knockout mice. Although many theories exist on possible mechanisms for SG formation, its pathology is unknown. We have treated adult male rats with a Leydig cell specific toxicant ethylene dimethane sulfonate (EDS) to deplete testosterone, causing SG formation in epididymis. Depletion of testosterone in serum and testis of EDS treated rats was confirmed by radioimmunoassay and was below the detection limit of the assay (0.05 ng/mL). For reaffirmation of Leydig cells loss, quantification of the mRNA expression levels of two Leydig cell specific markers, *luteinizing hormone receptor* (*Lhr*) and *insulin like peptide 3* (*Insl3*) by quantitative real time PCR (qPCR) was performed. Quantification for *Lhr* and *Insl3* mRNAs in EDS treated rats confirmed 99% elimination of the Leydig cells from the testicular interstitium. Histological evaluation of the epididymis revealed the presence of apoptotic round spermatids in the caput region. Quantification of *Testin* mRNA (a marker commonly used to assess the integrity of blood-testis barrier in testis) by qPCR, revealed a 32 fold increase in EDS treated rats indicating disruption of the blood-testis barrier (BTB). Thus, the presence of round spermatids in the caput of epididymis may be due to disruption of the BTB. Anatomically, the caput tubules were shrunk and between tubules there was massive stroma build up. An increased proportion of tubular epithelial cells in caput were also apoptotic. Immunofluorescent staining showed extensive infiltration of monocytes/macrophages around the periphery of the shrunken tubules and SG as detected by anti-CD11b and anti-CD68 antibodies. $CD4^+$ and $CD8^+$ lymphocytes were also detected inside the tubules and SG in EDS treated rats. This increase in macrophage marker *CD68*, and lymphocyte markers *CD4* and *CD8* were confirmed by qPCR at the mRNA level and they were significantly higher in EDS treated epididymis compared to the controls (19 fold, 3.5 fold and 7.5 fold respectively). After testosterone replacement however, the level of mRNA for *Testin* in testes was restored to the control levels. Expression levels of *CD68*, *CD4* and *CD8* mRNAs were also reduced significantly after testosterone replacement in EDS treated rats. Thus, our data indicates that collapse of the BTB and caput tubules, accompanied by accumulation of apoptotic round spermatids initiates infiltration of macrophages and lymphocytes to generate SG associated pathology.

Support: Texas Woman's University 2008 - 20010 Research Enhancement Program.

STUDIES ON THE POSSIBLE NATURAL HYBRIDIZATION OF TROUT LILY SPECIES NATIVE TO TEXAS.

YEMISIRACH D. GEBETO, NEFSNEGER A. GUILILAT and CAMELIA MAIER, Department of Biology, Texas Women's University, Denton, TX 76204

Two species of trout lily native to Texas, *Erythronium albidum* (4n) and *E. mesochoreum* (2n) are perennial herbaceous plants with white flowers, spotted leaves, and undergrounf corms. It is known that other *Erythronium* species hybridize. Members of the Native Plant Society of Texas reported that some populations of trout lilies have intermediate characteristics between the two native species. The goal of this work was to study the possible natural hybridization of Texas trout lilies. Samples were collected from plant populations at Cedar Ridge Preserve (Dallas) and Spring Creek Preserve (Garland). Morpho-anatomical characteristics were studied for 55-100 plants in order to determine the level of ploidy in the the above populations. Best indicators of ploidy level are chromosome numbers, determined in root metaphases or pollen mother-cell preparation. Leaf dimensions, stomatal chloroplast number, stomatal length and pollen diameter are morphological characteristics also employed for studying the level of ploidy. In this preliminary study, measurements of leaf blade length and width done in the field and stomata length and pollen diameter measured in a Hitachi TM-1000 SEM were compared between the two *Erythronium* populations. There were no significant differences between the averages of leaf length (12.45±2.36 cm *vs.*9.86±2.04 cm), leaf width (2.25±0.84 *vs.* 2.98±0.68 cm), and pollen diameter (64.29±6.94 μm *vs.*62.34±6.04 μm) between the Spring Creek and Cedar Ridge trout lily populations, respectively. These results indicate that both populations are composed of the same

trout lily species. Other morphological characteristics of the leaves and flowers, such asleaf spot color and position. Of the tepals indicated that *E. albidum* plants were present at both sites ubder study. However, a small group of plants at the Spring Creek Preserve had a significantly longer leaf (17.09±1.29 cm), less colored leaf spots and straight yellowish tepals as compared to the rest of the population. This plant may be *E. mesochoreum* or hybrids between the two species. Future work will focus on determining the level of ploidy in the above populations as well as of other trout lily populations by employing molecular techniques. Results will improve our knowledge of taxonomy and reproduction processes in *E. albidum* and *E. mesochoreum* for conservation purposes.

GC-MS AND MICROSCOPIC EVALUATION ON REDUCTION OF VOCs AND AEROALLERGEN ON USING PHOTO-CATALYTIC OXIDATION BY LUNA AIR PURIFIERS.

ALICIA GUZMAN[1], YASEMIN CELIK[1], EDWARD CARAWAY[2], NABARUN GHOSH[1], JAY CHUDASAMA[3] and JEFF BENNERT[4] Life, Earth & Environmental Sciences[1], Department of Agriculture[2], West Texas A&M University, Canyon, TX, Luna US LLC[3], Research and Development, Las Vegas, NV 89120, Air Oasis, Research and Development[4], Amarillo, TX

Luna air purifiers utilize Photo-Catalytic Oxidation (PCO) technology that produces very low doses of negative ions that remove the aeroallergens, dust particles, animal dander, etc. from the air electrostatically. We carried out experiments with Luna at the West Texas A&M University Labs with airborne mold, bacteria and VOCs, at the BSA Hospital with MRSA and at the Coulter Animal Hospital, Amarillo, TX with animal dander, VOCs and airborne molds. Slides with double sticky tape were exposed to room air for 24, 48, 72 and 120 hours before and after running the Luna air purifiers. The exposed slides were stained with 2% Safranin and observed using a BX-40 Olympus microscope, DP-70 digital camera. The images were analyzed with Image Pro 6.0 software. The data were correlated with the aeroallergen index and the frequency of inhalant allergy cases in dogs. Samples were collected from the clinic rooms to analyze the VOC concentrations using the Luna air purifiers to detect the efficiency in reducing the VOCs in the indoor air. The SKC Pocket Pump and thermal desorption tubes were used to obtain the samples from the indoor air. Pumps were set on 200 ml/min as airflow to estimate the concentration of Acetic acid, Isobutyric acid, Butyric acid, Isovaleric acid, Valeric acid, Hexanoic acid, Phenol, p-cresol, 4-ethyl, 2-amino, Indole, and Skatole. GC-MS Spectra analysis data showed a gradual reduction of the VOCs in the indoor air in the clinics after running the Luna air purifiers at different intervals. Analysis of slides and the petri plates exposed at different intervals of 24h, 48h, 72h and 120 hours showed reduction in aeroallergen after running the air purifiers. The high indoor VOC concentrations were reduced on running the Luna Air Purification units. Indoor aeroallergens such as, mold spores, airborne bacteria and animal dander were reduced significantly. . Experiments utilizing the Luna air purifiers established that the use of a negative ion purification system is an effective means of eradicating aeroallergens such as mold and microbes in room air. Allergy indices were reduced considerably on reduction of VOCs and aeroallergens in the room air after running the air purifiers.

MICROSCOPIC EVALUATION ON THE EFFECT OF CRUDE OIL ON THE STOMATAL FREQUENCY OF BASIL, CORN AND WHEAT.

MONIKA JONES-HIGGINS, NABARUN GHOSH, GARY BARBEE AND WILLIAM J. ROGERS, Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, TX 79016

The present investigation covers an evaluation on the effects of crude oil on stomatal frequency and morphology of wheat (*Triticum aestivum*), corn (*Zea mays*), and basil (*Ocimum basilicum*) plants. Environmental changes and toxic chemicals have effects on leaf area, stomatal frequency, stomatal index and pigment content and that are also influenced by other biological and environmental variables. Experimental studies were performed in an attempt to collect an array of data, including plant height, pH and physiological changes. Study on these changes not only helps scientists to assess the environmental impact, but also allows for new hypotheses to form on the physiological impact upon mammals, reptiles and fowl that feed off this same vegetation. For this investigation each plant species was sown in six different concentrations of crude oil by weight, including a control. The concentrations utilized were 0% (control), 0.38%, 0.75%, 1.5%, 3% and 6%. A second set of experiments was performed simultaneously with half of each set of plants sown in sand and half in clay. The slides were prepared by painting the leaf surface of the three species with clear nail polish and then covering them with transparent tapes. The tape was then mounted on a slide and labeled with the source plant, concentration and soil type. An impression was left onto the microscopic slide that was observed using a BX 40 Olympus microscope attached to a DP 70 digital camera. The images were analyzed and captured for recording data on stomatal index for every experimental set. This study revealed, morphological and physiological changes in the guard cells that line the stomatal openings. The stomatal frequency did not appear to be hindered to any great degree; however, the morphological and physiological changes in guard cells suggest a change in plant metabolic activities. Furthermore, the current study implies that the potential effects of crude oil results in devastation of crops in the coastline vegetation as recently evidenced in Louisiana.

COMPUTATIONALLY PREDICTING THE ROLE OF ARP3 RESIDUES IN THE NUCLEATION EVENT AT POINTED ENDS OF ARP2/3 COMPLEXES DURING BRANCHING OF ACTIN FILAMENTS.

AMRUTA C. MAHADIK, HALDAR SOUNICK, D. L. HYNDS and BRIAN W. BECK, Department of Biology, Texas Women's University, Denton, TX 76204

During spinal cord injury, damage to neuronal axons prevents re-growth of neurons at the scar site. Understanding the molecular mechanism that regulates the progression of axonal growth cones may help in developing treatments that promote axon regeneration following injury. Two major protrusive cellular structures, lamellipodia and filopodia, drive the leading edge of the growth cone during axon extension. Filopodia contain linear actin filaments that form bundles, whereas lamellipodia are composed of a meshwork of branched actin filaments. At these branches, Arp2/3 complex, a 220 kDa heptamer, is responsible for barbed-end actin branching, while also increasing the number of initiation points for polymerization. Arp 2/3 complexes are composed of two major subunits, Arp2 and Arp3, along with five accessory units, ArpC1- ArpC5. This complex is intrinsically in an "off" state but is activated in the presence of nucleation promoting factors (NPF's) like WASP and WAVE, ATP, actin monomers, and actin filaments. Using immunocytochemistry, we have determined that Arp3 and WAVE are co-localized in neuronal growth cones. We have characterized the amino acid interactions between Arp3 and WAVE at this interface using molecular modeling and virtual alanine scan analysis. Energetically important residues were identified in the Arp3 subunit whose substitution is predicted to disrupt the interface. These mutants of Arp3 are under construction and are five neuroblastoma cells where their effects on cell morphology are being identified via fluorescence microscopy.

A SCANNING ELECTRON MICROSCOPY INVESTIGATION OF THE RELATIONSHIP BETWEEN STRUCTURE AND FUNCTION OF SCALES IN A VARIETY OF ORGANISMS.

A. MEANS and S. WESTMORELAND, Department of Biology, Texas Women's University, Denton, TX

This study was a science inquiry investigation that focused on the scales found in diverse organisms. Scanning Electron Microscopy (SEM) was used to compare and contrast the structure of scales and how structure relates to function. This study may provide understanding of why unrelated species developed similar structures such as scales. Examination of lepidopteron, ctenoid, cycloid, and reptilian scales was performed.

ELEMENTAL ANALYSIS OF TEXAS NATIVE *EVAX VERNA* (ASTERACE), AN ALUMINUM ACCUMULATOR.

CHINWE O. ORABUCHI and CAMELIA MAIER, Department of Biology, Texas Women's University, Denton, TX 76204

Aluminum is extremely common throughout the world and is not harmful to plants and animals under alkaline or around neutral values of soil and water pH. However, in acidic environments, it can be a major limiting factor to many plants and aquatic organisms. Aluminum is not recognized as an essential nutrient for plants. Hyperaccumulator species of plants may concentrate Al to levels that are toxic to herbivores. We have reported before on the Al accumulation properties of spring pygmycudweed, *Evax verna (Asteraceae)* from one location in Denton. This study presents results on Al accumulation by *E. verna* specimens from two additional sites around the Metroplex, Spring Creek Preserve in Garland and Fort Worth Nature Preserve. Plant organs and soil particles were investigated with a Hitachi TM-1000 SEM equipped for EDS analysis. Aluminum accumulated in all plant organs of all plant specimens from the above locations. In a manner typical of hyperaccumulators, Al was not retained in the plant roots, but was transported to the shoot organs. Aluminum in *E. verna* plants was distributed between the different parts in the following order: roots(16-20%) > soil (2-15%) > mature leaves (3-5%) > floral bracts (2-3%). It seems that *E. verna* is a hyperaccumulator of Al. Although a small plant, it may be possible that this species can be used in phytoremediation of acidic soils or even hazardous waste sites.

A SCANNING ELECTRON MICROSCOPY INVESTIGATION OF THE STRUCTURAL DIFFERENCES BETWEEN NATURAL AND MAN-MADE TEXTILES.

E. A. SAMUEL and S. L. WESTMORELAND, Department of Biology, Texas Woman's University, Denton, TX 76204.

This study focused on the use of the scanning electron microscope (SEM) as an investigative tool in the study of textiles. The structures of natural textiles such as cotton, wool, and silk were compared to man-made textiles such as polyester, acrylic, and rayon. The textiles were examined in order to determine if the structure of the microscopic nature of the textile fibers varied. It was hypothesized that the fabrics such as wool and rayon have noticeable structural features that could relate to the sensitivity of those materials on the human skin. There were noticeable protrusions on the wool and rayon fibers that could contribute to greater sensitivity on the skin. Other fibers such as polyester and cotton were smooth throughout which could explain less sensitivity on the skin.

A COMPARISON OF VISION MECHANISMS OF FLYING VERSUS NON-FLYING ARTHROPODS USING SCANNING ELECTRON MICROSCOPY.

D. SHORT and S.L. WESTMORELAND, Department of Biology, Texas Woman's University. Denton, TX 76204.

The eye mechanisms of six different arthropods were examined using the Hitachi TM-1000 Scanning Electron Microscope. The focus of this investigation was to identify the vision components of arthropods to discover if visual mechanisms differ in the non-flying arthropod versus the flying arthropod. The flying category, by common name: wasp, housefly and mayfly. The non-flying category, by common name: ant, cricket and spider. Several characteristics were easily identified in the compound eye, but no identifiable components were discovered regarding the simple eye. This investigator originally hypothesized that the flying arthropod category would utilize two different eye mechanisms (both simple and compound); whereas the non-flying arthropod category utilized only simple eyes. This hypothesis was quickly rejected within the first few arthropods examined.

EDS TREATED LEYDIG CELLS AT EARLIER TIME POINTS FOR APOPTOSIS BY IMMUNOHISTOCHEMICAL ANALYSIS IN ADULT RATS.

BARKHA SINGHAL, DIBYENDU DUTTA, SAMUEL SANG, IN PARK, and NATHENIAL MILLS, Department of Biology, Texas Woman's University, Denton, TX 76204

Ethane dimethanesulphonate (EDS) is cytotoxic to Leydig cells in the adult rat. This study investigates the onset of apoptosis due to depletion of testosterone (T) by EDS at earliest time points. 30 adult rats were distributed randomly into five groups with testosterone supplement in sesame oil as vehicle, EDS, vehicle only, EDS+T and no treatment (n=5) after two time points of 15 and 24 hours. The tissue from rat testes will be used for immunohistochemical analysis by tunnel assay for apoptosis by confocal microscopy for all groups. The number of apoptotic cells at 24 hours versus 15 hours would increase and therefore the effect of the test supplement might reduce the cytotoxic effect of EDS. Thus, the earlier time points of apoptosis in Leydig cells may establish the involved signaling proteins.

Support: Research Enhancement Program, TWU, 2010.

ANATOMICAL COMPARISONS OF ITALIAN AND RUSSIAN HONEYBEES.

C. TOMOR., Department of Biological Sciences, Texas Woman's University, Denton, TX.

The phenomenon of massive and unexplained disappearances of honeybee colonies since 2006, which is now named 'Colony Collapse Disorder' (CCD), has sparked new interest in honeybees among scientists and the general public alike. There are many factors that influence the behavior and health of individual bees and bee colonies, yet no single agent has shown to be the sole cause of CCD. Two parasites responsible for the majority of bee mortality, and possibly related to CCD, are the trachea mite and *Varroa sp.* mite. While the Italian honeybee has traditionally been the favored species of apiarists, new studies are showing the Russian honeybees to be hardier through winter and more resistant to these parasites. By means of anatomical comparison using scanning electron microscopy, I have investigated some of the selective advantages Russian bees may possess over Italian bees.

CELLULAR CHANGES IN *PROSOPIS PUBESCENS* DUE TO COPPER TOXICITY.

M.N. VIVEROS[1], J.T. ELLZEY[2], Environmental Science and Engineering Program[1] and Biological Sciences[2], The University of Texas at El Paso; El Paso, TX 79968

Urbanization has increased heavy metal contamination that must be remediated (Peuke and Rennenberg, 2005). Phytoremediation is the use of plants to clean up contaminated soils. Locally, mesquite species are being studied for their phytoremediation capabilities. *Prosopis pubescens* (Screwbean mesquite) is a desert plant that we are using to investigate the biological effects of copper tolerance and toxicity.

The primary objectives of this project are to determine how copper is affecting the morphological characteristics as well as the mechanisms of copper transport and tolerance. Structural components have been affected by heavy metal contamination. Light and transmission electron microscopy were used to reveal morphological changes within seedlings of screw bean mesquite. Preliminary results show that starch, phenol, and reserve protein body concentrations are affected in the higher copper concentrations. We continue to see plasmolysis as a result of toxicity. Cell membrane breakage and chloroplast swelling are some of the ultra structural changes observed. Elemental analysis for copper in cross sections of the seedlings is in progress. Copper concentrations were also determined using ICP-OES revealing the highest concentration of copper in the roots. Copper was translocated up to the stems and then to the cotyledons. High copper uptake by screwbean mesquite would label this species as another desert hyper accumulator that may be used for phytoremediation of contaminated soils.

FLORAL PIGMENTS INVOLVED IN THE SUNFLOWER-HONEYBEE MUTUALISTIC RELATIONSHIP.

JENNIE WOJTASZEK and CAMELIA MAIER, Department of Biology, Texas Woman's University, Denton, TX 76420

Honeybees (*Apis mellifera)* are the main pollinators of sunflowers *(Helianthus annuus, Asteraceae).* The mutualistic relationship between sunflower and honeybees reflected in the co-evolutionary adaptations of these organisms to each other. The corolla morphology and pigments of the ray and disk flowers in the sunflower infloresece help form a target pattern which attracts bees. A sunflower inflorescence contains two classes of pigments, carotenoids, and flavonoids. The goals of this study were to anatomically localize and chemically characterize the floral pigments that contribute to the target pattern of the sunflower inflorescence. Morpho-anatomical traits of inflorescence flowers were studied with light, scanning electron and laser confocal microscopes. Chromatographic methods were used to identify sunflower pigments. Ray flower cross-sections were treated with D-boric acid for the purpose of localizing flavenoid pigments. Observations with the confocal microscope showed that flavenoids were Localized in the cell wall and cytoplasm of petal epidermis. The cytoplasmic localization of flavenoids was also observed using the autofluorescence mode on the confocal microscope. Thin layer chromatography of crude flowers resolved several pigment spots, of which one migrated along with a pelargonidin standard indicating that sunflowers contain this pigment. Ray and disc flowers will be extracted with specific solvent systems to separate carotenoid and flavenoid pigments in different fractions that will be used to characterize pigments by employing TLC and HPLC-MS. The results of this study will improve our understanding of sunflower adaptations for insect pollination with possible agricultural applications.

SCIENCE EDUCATION

INTEGRATING SCANNING ELECTRON MICROSCOPY INTO THE ELEMENTARY SCIENCE CLASSROOM.

JOSEPHINE TAYLOR[1], STEPHEN C. WAGNER[1], and ALAN B. SOWARDS[2]. Department of Biology[1] and Department of Elementary Education[2], Stephen F. Austin state University, Nacogdoches, TX 75962

A workshop on scanning electron microscopy (SEM) was incorporated into an ongoing grant project to improve the science content knowledge of in-service elementary teachers. Participants brought materials from their classroom, prepared them for SEM observation and captured images of each item. An image gallery was created for use by all students of the group. In addition, a number of SEM micrographs were produced to illustrate structures in a holiday poem and the teachers developed a lesson plan using the images. Participants are currently integrating the materials developed during the workshop into their science curriculum.

BENCH-TOP SEM AS A STUDY TOOL FOR STUDENTS' FAVORITE PLANTS.

PALLAVI UPADHYAY, JENNIE WJTASZEK, SARAH WEHNER and CAMELLIA MAIER. Department of Biology, Texas Woman's University, Denton, TX

The bench-top SEM is emerging as a very useful tool for students and educators due to its portability and ease of use. One of the assignments for plant biology students at TWU was to study their favorite plants or plant parts employing microscopy techniques under the guidance of their instructors. Roots, stems, leaves floral structures, fruits and seeds from five species in five families of monocotyledons and sixteen species in thirteen families of dicotyledons were studied with a Hitachi TM-1000 SEM capable of elemental data analysis. Students were trained on the microscope for 15 minutes, worked under supervision for a few sessions and the digital images obtained were used for assignment reports and presentation in front of peers. No sample preparation was required for observing surfaces of plant specimens. Cross sections and/or longitudinal sections of roots, stems, leaves and pistils were prepared in order to observe the internal structure of the specimens. Pistil cross sections of hibiscus and Texas sages showed the ovules, young seeds and other ovarian structures. Magnoloa seeds were sectioned and various layers of the seed coat were identified. Pollen samples illustrated the vast diversity of sporopollenin architectural patterns. Students observed the vascularl, ground and dermal tissues in dissected roots, stems, petioles and leaves. Calcium oxalate raphides and druses were observed in sections of peace lily, sunflower, leek and orchid sections. Leaf surfaces and sections showed trichomes, stoma and epicuticular wax crystals. Specialized leaves in Venus flytrap were of great interest among stydents showing triggerhairs, digestion enzyme glands, as well as the presence of phyllosphere microorganisms. Even arthropod pests on leek, Texas sage and lavender and mycorrhizal fungi associated with orchid roots were visualized. Students had the opportunity to learn hands-on about the morphology, anatomy and ecological adaptations of their favorite plants and were very excited about their learning experience on the bench-top SEM. In conclusion, SEM is an effective interactive teaching/learning tool in helping students visualizing the general plant structures on their favorite plant and thus understanding plant adaptations to abiotic and biotic environmental factors.

BIOLOGICAL POSTERS

MICROSCOPIC EVALUATION OF STOMATAL INDEX, WATER USE EFFICIENCY AND SHADE TOLERANCE IN LEGUMES

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The Legume family consisting of all types of beans and peas is critically important for human nutrition due to their high protein content. Shade tolerance is the ability of a plant, to survive and produce at low light. Shade tolerant plants are adapted to be efficient energy-users and grow broader and thinner leaves, to catch more sunlight reducing the cost for yield. Shade tolerant plants are also usually adapted to make efficient use of soil nutrients than shade intolerant plants. A Sorghum-Legume intercropping system showed increased productivity with efficient water usage. We evaluated the effect of shade on legume cropping in terms of productivity and quality in the intercropping system. We compared the water use pattern for required quantity of water for the shaded and direct sun-lighted legumes by determining the stomatal frequency and leaf thickness of some of five leguminous species. Stomatal density is an important data to detect the rate of gas exchange and water loss from the leaf. Stomatal Distribution and Density were determined by counting the number of stomata per unit area of the leaf using a grid (graticule) placed on the eyepiece of the microscope. Nail polish was applied on the leaf surface and allowed to dry. A piece of clear tape was placed onto the leaf surface with nail polish. The tape was removed with the imprint from the leaf surface and transferred to the microscopic slide for viewing under the microscope. The prepared slides were observed using a BX-40 Olympus microscope, DP-70 digital camera and TRITC and FITC filters. We also evaluated the leaf thickness to compare the productivity in the shaded and unshaded cropping systems. We captured images with Digiscope 300 and measured the thickness in situ by using Motic Educator Software. The data were correlated from the observations on different leguminous species. This study improved understanding the water usage in legumes in developing more water-efficient farming.

INDUCTION OF POLYPLOIDY IN *ALLIUM CEPA* TEST SYSTEM USING DIFFERENT DOSES OF 2,4-D AT DIFFERENT TEMPERATURES.

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In recent years, concern over the detection of various chemical pollutants in U.S. waterways and wells has caused the EPA and other regulatory agencies to focus attention on the harmful nature of these chemicals. 2,4-Dichlorophenoxyacetic acid (2,4-D) is a common systemic pesticide/herbicide used in the control of broadleaf weeds. It is the most widely used herbicide in the world, and the third most commonly used in North America. The objective of this investigation was to study the effect of 2,4-D on the cytological stability of a standard plant test system, *Allium cepa* (Green onion). *Allium cepa* has been established historically as the standard test system for plants when studying the effects of chemicals on cell processes, namely mitotic index and chromosomal abnormalities. Scientists preferred it for its high root tip yield, large, well-dispersed chromosomes, low cost, and ease of cultivation (Sharma, 1990). We established a hydroponic culture of *Allium cepa* in the culture room with controlled photoperiod and temperature (25-40°C). *Allium* bulbs were submersed for the chemical treatment at varying concentrations of 2,4-D, 1 ppm, 0.1 ppm and 0.01 ppm. The root tips were then excised at 24, 48, and 72 hours post-treatment. We prepared the root tip squash slides after a pre-treatment with pDB:Aesculin solution for 3 hours, fixation in 1:3 Aceto-Ethanol for 24 hours and staining with 2% Aceto-orcein. We evaluated the mitotic index (MI), and chromosomal abnormalities (AI) at various treatments at different temperatures. A standard karyotype of *Allium cepa* was also erected (2n=16). We found a gradual reduction in the Mitotic Indices and increase in Abnormality Indices with appearance of various types of chromosomal abnormities including early and unequal separations, sticky bridges at 24 and 48 hours, multi-polarity at 48 hours, diplo-chromatids and micronuclei formation after 72 hours of treatment. We observed high percentage of polyploidy at 0.1 ppm dose of 2,4-D after 120 hours of treatment at 40°C. Our investigation shows that specific doses 2,4-D at high temperature can be used for induction of polyploidy in the plant system and may be applied in crop breeding program.

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TESTERONE DEPLETION INDUCES OVEREXPRESSTION OF ANDROGEN RECEPTOR IN ADULT RAT TESTES.

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Testosterone binds with androgen receptor (AR) to induce various signaling pathways required for different physiological functions including spermatogenesis. Bilaterally-castrated prostate cancer patients frequently exhibit testosterone independent AR expression and activation. Overexpression of AR frequently leads to a relapse of prostate cancer. It is unknown whether growth factors or other ligands rather than testosterone are involved in this overexpression of AR. Alternately, the absence of negative regulators secreted from testes may permit constitutive expression of AR after orchiectomy. Here we have investigated the expression of AR in rat testes after depleting testosterone by selectively eliminating adult Leydig cells with the drug, ethylene dimethane sulfonate (EDS). Elimination of Leydig cells was confirmed by quantifying mRNA for Luteinizing hormone receptor (LHR) and insulin like protein (INSL3) by quantifiable polymerase chain reaction (qPCR). Depletion of testosterone was confirmed by radioimmunoassay (RIA) of serum. Our preliminary data indicates an increase in AR mRNA expression in the absence of testosterone in testes whereas testosterone replacement post-EDS treatment lowers the mRNA for AR. Currently we are trying to localize the AR protein in testes to determine the cell type(s) that have a more prevalent increase of AR expression after testosterone depletion.

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COMPUTATIONAL PREDICTION OF ARP3 RESIDUES WHOSE MUTATION WILL DISRUPT ARP2/3 COMPLEX.

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Arp 2/3 complex (**A**ctin **R**elated **P**rotein 2/3 Complex) is an essential regulator in actin polymerization. This heptameric 220-kDa complex is composed of two major subunits, Arp2 and Arp3 along with five accessory units, ArpC1- ArpC5. This complex is intrinsically in an "off" state but is activated in the presence of Nucleation Promoting Factors (NPF's) like WASP and WAVE, ATP, actin monomers, and actin filaments. Interactions between these molecules primes the branching in actin cytoskeleton networks and promotes the formation of cellular structures like lamellipodia at the mobile edge of growing neurons. Using immunocytochemistry, we have previously determined that Arp3 and WAVE are co-localized in neuronal growth cones. We have characterized the amino acid interactions between Arp3: Arp2 and Arp3: WAVE interfaces using molecular modeling and virtual alanine scan analysis and have identified energetically important interfacial residues whose mutations would disrupt the Arp 2/3 complex stability, preventing the formation of trimeric nucleus required for branching of actin filaments. Expression of these mutants with GFP-labels in B35 neuroblastoma cells is underway. Understanding the progression of axonal growth cone at molecular level may help in developing treatments that promote axon regeneration following spinal cord injury.

EXPRESSION OF P-GLUPROTEIN IN ADULT RAT TESTES AFTER ETHANE DIMETHANE SULFONATE (EDS) INDUCED TESTOSTERONE DEPLETION.

IN PARK, DIBYENDU DUTTA, HIWOT GUILILAT, SAMUEL SANG and NATHANIEL MILLS, Department of Biology, Texas Woman's University, Denton, TX 76204

Permeability glycoprotein (P-Gp) is a multi-drug resistanct (MDR) transporter whose overexpression protects cancer cells from chemotherapy and makes tumors drug resistant. Therefore, it is important to understand the regulation of P-Gp expression in different tissues including the testes. Although P-Gp is expressed in different cells of the testes, its regulatory mechanism is unknown. Here we have treated adult male rats with ethylene dimethane sulfonate (EDS), a Leydig cell specific toxicant to deplete testosterone and investigate the role of testosterone in P-Gp expression in rat testes. For gene expression analysis tissues were collected 6 hr, 15 hr, 1 day, 5 days and 7 days post-EDS treatment. Luteinizing hormone (LH) and insulin like protein (Insl3) are regarded as a marker of testosterone depletion in testicular Leydig cells. Analysis of mRNA abundance. was done by quantifiable Real-Time PCR (qPCR). Our preliminary results suggest induction of P-Gp expression after 15 hr and all the later time points. Although EDS treatment leads to testosterone depletion, testosterone replacement had negligible effect on P-Gp expression. Therefore, testosterone may not regulate P-Gp expression in rat testes.

TWU Research Enhancement Program Grant 2008-2010.

USING SCANNING ELECTRON MICROSCOPY AND ENERGY DISPERSIVE SPECTROSCOPY TO DETERMINE IF RESISTANCE OF SORGHUM GRAIN TO MAIZE WEEVIL (COLEOPTERA: CUECULIONIDAE) IS CORRELATED TO THE ARRANGEMENT OF STARCH WITHIN THE SORGHUM GRAIN.

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The resistance of 20 genotypes of sorghum (*Sorghum bicolor* (L.) Moench) grains to maize weevils (*Sitophilus zeamais* Motschulsky) was determined by Chitio [1]. Seven of these genotypes tested by Chitio were examined by a JEOL JSM-6400 scanning electron microscope (SEM) (15 KeV, 15 mm working distance) to produce secondary images of the sorghum kernels in cross section. Spirit software (PGT-Bruker) was used by this SEM to produce energy dispersive spectroscopy (EDS) maps of these kernels. To prepare the kernels for SEM imaging, grains of seven genotypes of sorghum were cut with a razor (1 mm depth), fixed in 2.5% Glutaraldehyde - 1% Acrolein in HEPES buffer (pH 7.3), followed by 1% Osmium in HEPES buffer (pH 7.3). Dehydration by methanol was done at 5% steps, followed by three changes of hexamethyldisilazane (HMDS). After fracturing along the cut area, the grains were mounted on stubs and coated with iodine vapor using the method of Ellis and Pendleton [2] to locate starch. A Cressington 308R evaporative coater (Ted Pella, Inc., Redding, CA) was used to apply a coating of 35 nm of carbon to the kernels before SEM-EDS. Iodine was applied as a vapor [2] over the cross-section of each of the seven genotypes tested. Iodine vapor binds with many forms of starch. An iodine EDS map was used to determine the distance from the seed coat to a band of concentrated iodine (starch) present in each kernel type. A general positive correlation of an increase in distance from the seed coat to the band of concentrated starch and the greater degree of resistance to kernel damage by the maize weevil was demonstrated for these seven sorghum genotypes[3].

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[2] E.A. Ellis and M.W. Pendleton, Micros. Today 15 No. 3 (2007) 44.

[3] This research was supported in part by the Sorghum, Millet and Other Grains Collaborative Research Support Program (INTSORMIL CRSP) sponsored by the United States Agency for International Development.

ADAPTIVE CHARACTERISTICS ON THE TARSI OF ARBOREAL AND TERRESTIAL COCKROACHES.

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This study used the scanning electron microscope (SEM) as a tool to investigate the different adaptive characteristics on the tarsi of several species of cockroach. The species of cockroaches that were selected for this study are: *Blattella germanica*, *Periplaneta americana, Blaberus giganteus, Supella longipalpa, Blatta orientalis,* and *Gromphadorhina portentosa.* These species are common in several regions around the world. This study was designed to compare the *Blaberus giganteus* to the other species of cockroaches. *Blaberus giganteus* is one of the largest species in the world. It comes from South America, Panama, and the West Indies. It is the only species out of the five studied that is arboreal. The other five species are terrestrial. By observing the tarsi of the different species of roach with a scanning electron microscope, the different anatomical characteristics of the roaches were observed in order to determine specific adaptations.

AN INVESTIGATION OF THE MICROORGANISMS FOUND IN DOMESTIC BEDDING MATERIALS USING SCANNING ELECTRON MICROSCOPY.

S. TAYLOR-WHITLOW and S. L. WESTMORELAND, Department of Biology, Texas Woman's University, Denton, TX 76204

The goal of this project was to determine what types organisms exist in bedding material, especially pillows and mattresses. This knowledge will help people decide when they should discarded bedding to avoid allergies, breathing difficulties, and other health or sanitation issues that could be directly or indirectly related to the organisms in the bedding. Samples have been gathered from new and used feather pillows, as well as from used mattresses of a variety of ages. Scanning electron micrographs were taken of the samples and compared to relevant literature for determination of the identities of any organisms found in the samples. Organisms resembling dust mites and molds have been found. The types of health concerns caused by microorganisms found in bedding material will also be discussed in this presentation.

MATERIALS SCIENCE PLATFORM PAPERS

IN-SITU TEM OBSERVATION OF SOLID TO VAPOR PHASE TRANSITIONS IN SILVER NANOPARTICLES.

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In-situ transmission electron microscopy (TEM) heating of silver nanoparticles was performed in a TEM equipped with a novel "Aduro™" heating stage designed by Protochips Inc. in order to directly observe the solid to vapor phase transitions that occur in nanoparticles. The silver nanoparticles were heated *in-situ*, from room temperature to a temperature where the particles undergo a phase transition. We observed that the nanoparticles remain crystalline, without melting, until they transform directly from the solid to the vapor phase. The sublimation process occurs in steps, as evident from the shrinkage and subsequent disappearance of a nanoparticle with time. The smaller nanoparticles evaporated at lower temperatures while larger nanoparticles evaporated at higher temperatures similar to the size-dependent melting behavior observed in nanoparticles. This work shows that *in-situ* TEM heating experiments on nanoparticles gives us information on the thermal stability of nanoparticles and the temperature ranges over which these nanoparticles can be used.

SIMPLE PREPARATION OF HYDROGELS FOR SEM AND TEM.

E. ANN ELLIS, Microscopy and Imaging Center, Texas A&M University, College Station, TX 7784

Hydrogels, polymers with a high water content are used in many biotechnical and biomedical preparations. The high water content necessitates cryo preparations for examining hydrogel specimens with transmission or scanning electron microscopy. Modified techniques have been developed for examining these specimens without cryostages. Small pieces of hydrogels can be dispersed on glow discharged carbon coated grids followed by rapid freezing in liquid nitrogen and then drying by plunging the grid into methanol followed by chemical critical point drying by plunging the grid into hexamethyldisilizane (HMDS). Grids can then be examined in the TEM. Specimens can also be fixed in high concentrations of aldehyde (10-20% vol/vol), followed by washing and dehydration in closely graded steps of methanol (5% vol/vol steps) to HMDS. After at least four changes of HMDS, including an overnight change of HMDS, critical point drying can then be completed in the fume hood. Specimens mounted on stubs can then be exposed to ruthenium tetroxide vapor before examining the hydrogel specimens in the SEM.

AUTOMATED ANALYSIS OF TEXTURE AND LOCAL STRESSES IN NANOSCALE COPPER INTERCONNECTS USING D-STEM AND PRECISION MICROSCOPY.

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The demand for higher processing speeds has resulted in the downscaling of copper interconnect (CI) lines, which exhibit grain sizes at the nanoscale. Knowledge of texture and grain boundaries in such narrow CI lines is critical to address reliability issues like stress induced void formation and electromigration. In the current work, a recently developed Diffraction Scanning Transmission Electron Microscopy (D-STEM) technique is integrated with a novel precession system to obtain orientation information from grains as small as 3 nm in 120 nm CI lines by a completely automated procedure. This technique represents a major leap in the characterization of CIs, as Electron Backscattered Diffraction (EBSD), currently the most used method for obtaining grain orientation in an automated fashion, is limited to approximately 30 nm grain size. By employing the aforementioned technique, we determine the texture of CIs with line widths of 180 nm and 120 nm. The CI lines are under residual stresses after thermal cycling due to a mismatch in the coefficients of thermal expansion between copper and the underlying silicon substrate. Finite element modeling of the CI microstructure is performed to investigate local stresses in these interconnect lines.

MATERIALS POSTERS

GEOMETRIC CHARACTERIZATION OF SURFACE NODULES AND IDENTIFICATION OF CONTAMINATIONS IN THIN FILM OPTICAL COATINGS WITH FIB-SEM AND EDX.

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Nodular defects are commonly found in optical coatings. In optics used with high power IR lasers, these defects are believed to trigger laser-induced damages which lead to failure of the optics. In this study, a Ge/ZnS multilayer optical coating was studied by scanning electron microscope (SEM), and nodules with a density of 10^6 cm^{-2} were observed on the surface. The nodules have a dome shape on the surface with various sizes, ranging from less than 1 μm to 3 μm. A TESCAN Lyra focused ion beam – scanning electron microscope (FIB-SEM) system was

used to examine the cross section of the nodules. It has been revealed that a typical nodule has an inverted cone shape buried in the multilayer coating under the dome. Compositional changes within the coating were analyzed by energy dispersive x-ray (EDX) spectroscopy. The results suggested that submicron size contamination introduced during the coating process act as the nuclei necessary for the formation of nodules.

INNOVATIVE TECHNIQUES PLATFORM

REPLACEMENT OF POLAROID INSTANT FILM USED FOR RECORDING SEM IMAGES WITH A DIGITAL SLR.

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With the discontinuance of Polaroid instant film, users of older scanning electron microscopes who had not converted to a digital image acquisition system were left with limited options for recording images. While single sheet film is still available from Kodak, it has the same limitations that traditionally led researchers to use Polaroid instant films in the first place. Historically, a few individuals have adapted 35 mm SLR cameras using conventional film for recording SEM images. Utilizing today's available technology, a digital SLR can be substituted for the 35 mm SLR camera. One such system is commercially available to fit most SEM's for an investment of approximately $5,000. Before investing in that sum of money we decided to see if it was possible to adapt our laboratory's Nikon D100 digital SLR to the 4X5 camera mounting system on our Hitachi 7110, and then determine if the recorded images were of suitable quality. A mounting adapter was fabricated and the camera fitted with a 35 – 85 mm f2.8 lens. Usable images were obtained in all recording formats; however, due to the prolonged exposure required to record images, the use of the camera's internal noise suppression software was mandatory. Although there are limitations with the system as presented, it none-the-less offers a viable alternative.

James Long (Thermo Fisher Scientific) checks his phone messages at the 2010 TSM meeting in Frisco.

Scanning Electron Microscopy Used as a Tool to Facilitate Open Inquiry in Pre-Service Secondary Science Teachers

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Abstract

Science teachers are encouraged by national and state guidelines to use inquiry in designing and implementing science instruction. With little prior experience in using the inquiry method, many pre-service teachers may be confused about the meaning and application of inquiry science to their classrooms. The purpose of this study was to determine the impact of an open inquiry research experience using scanning electron microscopy (SEM) as a research tool on the attitudes of pre-service middle school and high school teachers toward inquiry science. Participants in this study were all pre-service teachers in a master's program for science teaching certification at a public university in the southern U.S. Participants were enrolled in a 16-week independent study course in which they were taught to use the SEM and then allowed to engage in open inquiry by each selecting an investigable question on a topic of their choice. Students proposed hypotheses, designed investigations, took digital micrographs, and prepared posters to present their findings at a professional state microscopy meeting. Student attitudes were assessed by their reflective journals and their final reflective papers. The students were initially very uncomfortable with planning their own research projects. They found the lack of direction unsettleing as they had never experienced the freedom to create their own investigations. After an initial struggle to locate an appropriate topic, their confidence began to grow and they became enthusiastic about their personal inquiry experiences. After an initial struggle to locate an appropriate topic, their confidence began to grow and they became enthusiastic about their personal inquiry experiences. Students developed a sense of pride in their investigations and learned the satisfaction of owning their discoveries. As one student put it, "That is what is so special about inquiry; it motivates you from the inside instead of the outside." This study advocates the involvement of pre-service teachers in authentic inquiry experiences while using reflective journaling to help them to understand the inquiry process.

Introduction

In response to concerns about the lagging achievement of US students in science, national standards have been developed that advocate the use of inquiry-based learning (NRC 1996; NRC 2000). Advocates of inquiry promote it as a means of helping students learn science concepts, think scientifically, and understand the nature of science (Marshall, Horton, and White 2009; Nadelson 2009). In addition, inquiry is promoted as a means of developing higher order thinking skills: analysis, synthesis, and application, as opposed to rote memory which may result from direct teaching methods. Inquiry teaching/learning can take on various forms, depending on the roles of the teacher and the students in the investigation. For example, in structured inquiry, the teacher provides students with a question, methods, and materials; the students determine the answer to the question from the activity. In guided inquiry, the teacher provides the question and the materials for the investigation, allowing students to construct their own investigation to answer the proposed question. In open inquiry, students are given much autonomy. They are allowed to identify the question, devise the investigation, and interpret the data to answer the question (Coburn 2000, Martin-Hansen 2002). It is the responsibility of the science teacher to determine when and how to use these various forms of inquiry in guiding their students toward more independence and higher levels of responsibility for their own learning. However, many pre-service teachers have limited experience in using the inquiry method and are unsure how to implement it in their classrooms (Windschitl 2002). Past studies have shown that reflection can assist teachers in developing a deeper understanding of the inquiry process (Moseley and Ramsey 2008). According to Bencze *et al.* (2006), development of positive attitudes in pre-service teachers toward inquiry may be fostered by giving them challenging open-ended scientific experiences and asking them to reflect on those experiences. In this paper we propose that pre-service science teachers derive benefit from having an authentic open-inquiry research experience and using reflective journaling to help them better understand the process of inquiry. What this study proposed to accomplish was to reveal pre-service teachers'

attitudes about inquiry and to foster their understanding of the inquiry process.

Materials and Methods

The participants in this study were three graduate students enrolled in a master's program with state teaching licensure for middle school or high school science. Two were Caucasian; one was Indian. All three had science degrees: one in biotechnology, one in botany, and one in dental hygiene. In the prior semester, all were enrolled in a science teaching methods course in which the inquiry method of teaching using the 5E lesson format was modeled and practiced. The students were concurrently enrolled in a course, "Scientific Communication," in which they developed skills in reading, writing, and presenting information on scientific topics.

Students in this study were enrolled in a 16-week independent study course which met for three hours each week. Students were taught to use the Hitachi 1000 Tabletop SEM and were encouraged to bring samples of interest to view. After initial practice, each student was asked to think of an investigable question narrowing the scope to a project appropriate for the limited time. Students designed and executed an investigation, and documented their findings with electron micrographs. Students created posters and presented their findings at a professional state microscopy meeting, as well as at the university research symposium. Students used journals to record their scientific data and their impressions of their experiences in the class. In addition, students wrote a reflective paper answering the following questions: What is inquiry science? What are the potential benefits of inquiry science? How could inquiry science be implemented in the classroom? What is your attitude toward inquiry science and how was it changed by this experience?

In this qualitative study, journals and reflective papers were reviewed for evidence of students' attitudes about the inquiry experience. Journal entries were transcribed and correlated by date of entry to discern patterns of attitude toward inquiry science. Reflective papers were reviewed to determine how student attitudes about inquiry had changed during the semester.

Results

Students in this study moved through three stages of attitude shift during the course of the inquiry experience. At the beginning of the inquiry, students reported that they felt uncomfortable, isolated, and frustrated by the open inquiry experience. In the quoted remarks below from journals and reflective papers, students are coded S1, S2, and S3 for privacy.

"[I'm] not really sure where to go. I have many snippets of ideas swimming around but nothing concrete. I'm not really used to being this unsure about something. It just feels unnatural. I think that was the part that worried me about this class, having to come up with my own ideas. I guess I'm just used to people telling me what to do." S3

"My initial feeling of this inquiry experience was frustrating, as there was no defined curriculum and no textbook to follow. However, [my professor] encouraged me to explore and think about the research question without any fear of [a] good or bad question." S2

"I knew what interested me, but I really couldn't think of an investigable question…For me, this was the most frustrating part of the entire class." S1

Secondly, as students chose topics and became focused on designing their investigations, their anxiety abated and they became intent upon collecting and interpreting data.

"I finally have a direction to go for my project. It feels like a huge weight has been lifted off of my chest…" S3

"With a sigh of relief that decision was finally made; I could finally move forward with my research project. With a goal in mind my time with the microscope quickly became more focused and efficient." S1

Finally, as students presented their results to audiences at professional meetings, they developed a sense of pride in their investigations and learned the satisfaction of owning their discoveries.

"Who would have thought this would be one of my favorite classes even though it scared me…I think I enjoyed it so much because I was able to do it over something I chose." S3

"I realized that inquiry learning helped me take ownership of the learning and made me confident in my ability to modify the existing activities for my students." S2

"That is what is so special about inquiry; it motivates you from the inside instead of the outside." S3

"The most important area of growth in the class, however, was the experience I gained in learning through inquiry. Because it was a topic that I chose and something that I was interested in and cared about, learning was more fun. I looked forward to going to class and seeing what I might discover under the microscope that day…All in all it was a very rewarding experience that allowed me to live through inquiry learning and see the joy that it can bring back to learning." S1

This qualitative study allowed for a better understanding of the students' attitudes toward inquiry science as a result of an open inquiry research experience. The students' attitudes moved through phases of first anxiety and frustration, followed by relief and focus, and finally confidence and pride as they completed their inquiry investigations. The reflective journals and reflective papers helped students to further understand the inquiry research process. These understandings will also

inform their future teaching as they understand the attitudes of their own students experiencing inquiry learning.

Discussion and Conclusions

This study suggests opportunities for using other scientific research tools to involve pre-service teachers in open inquiry investigations, while using reflective writing to reveal the attitudes of the participants. These data will be useful to educators of science teachers to determine how pre-service teachers experience an open inquiry research investigation. Future studies will include additional cohorts of pre-service and in-service teachers. Information will also be obtained in the future on how the open inquiry experience impacted teachers as they designed and implemented science instruction with students in their own classrooms.

President Jo Taylor and winners Carmen Ayala (L), undergraduate student winner, and Paul Gray (R), graduate student winner, of the student competition at the TSM meetings in 2011 in Frisco.

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Synthetic Calcite Particle Size Using Scanning Electron Microscopy and Laser Diffraction

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Abstract

Much of our current scientific knowledge regarding mineral and contaminant interactions in the environment is derived from the study of model mineral systems. Calcite is a naturally occurring mineral of relatively high abundance and may be part of a complex system that determines the transport and fate of contaminants in the environment. The particle-size distribution of calcite is of particular importance since it affects the reactivity of calcite whereby a larger reactive surface area to volume ratio occurs as the size of the particles become smaller. The determination of particle size distribution can be estimated using a variety of techniques with each having unique accuracy based on the size range of the particles of interest. Laser-diffraction particle-size analysis (LPD) is the most commonly employed particle measurement technique in the low micron size range. The accurate characterization of micron-sized calcite can provide valuable information for further studies which assess the model mineral and its interactions with contaminants in laboratory experiments. The objective of this study was to analyze two different lots of synthetic calcite by LPD, compare the results to qualitative interpretations of particle size by scanning electron microscopy (SEM), and compare all results to the particle size listed on the manufacturer's certificate of analysis (COA). Two lots of synthetic calcite were obtained from the same vendor each with a micron particle size listed on the COA. The particle size of each evaluated by SEM appeared to be smaller than that determined by laser-diffraction particle-size analysis (LPD); although, particle aggregation was evident by viewing the SEM images whereas LPD requires assumptions regarding the complete dispersion of individual crystal particulates. Both SEM and LPD were in close agreement when compared to the manufacturer's COA. The LPD method coupled with detailed electron microscopic investigation allows for better estimation and characterization of calcite particle size than either method used exclusively, and should be used in lieu of accepting a manufacturer's COA for particle size on a *prima facie* basis without further study.

Introduction

Sedimentary carbonates form in the environment by chemical, biological and detrital processes that occur at the ocean floor. Carbonates, in the marine subsurface, are predominantly created from biogenic processes and their formation depends greatly on biological activity (Ham and Pray, 1962). These sediments are composed principally of skeletal remnants, calcareous excrement of marine organisms and microbially-mediated lime mud. Depositional and diagenetic processes acting on these sediments over time form carbonate rocks. Carbonate rock classifications are based on depositional texture and porosity, sedimentary structure, fabric, and biological composition (Dunham, 1962; Choquette and Pray, 1970; Embry and Klovan, 1971). Calcite can be a major component of the chemical and mineralogical matrix of the sedimentary, carbonate rock that is formed. Calcite ($CaCO_3$) is the second most abundant mineral at the Earth's surface next to quartz. Calcite in soil can be derived from parent material, from transport processes of water, wind or ice deposition, or from pedogenic processes of dissolution and precipitation that can translocate carbonate minerals spatially within the soil profile. Calcite derived from parent material can be distinguished from pedogenic calcite since it usually exists as larger, sand-size crystals, compared to pedogenic calcite of typically smaller, silt-sized crystals (Doner and Lynn, 1989). The different types of carbonate minerals in soils are classified based on differences in particle size, morphology and crystal arrangement (Doner and Grossl, 2002). Calcite particles are termed micrite, microspar or spar, with sizes of < 5 μm, 5 – 20 μm and > 20 μm, respectively. Particle size affects the reactivity of calcite since a larger reactive surface area to volume ratio occurs as the size of the particles become smaller.

Materials and Methods

Reagent grade calcite lot A and lot B were obtained from Alfa Aesar (Ward Hill, MA). Particle-size analysis was performed using the Beckman Coulter (Fullerton, CA), LS 13 320, laser-diffraction particle-size analyzer with a 750 nm laser beam. The instrument measures the particle-size distribution from 0.045 – 2000 μm. The laser beam accurately measures particles > 0.4 μm but uses the polarization intensity differential of scattered light to measure particle sizes from 0.045 – 0.40 μm. The natural calcite was ground using a mortar and pestle before analysis. Dry calcite samples were weighed and then prepared as suspensions in 10 ml of deionized water before introduction into the instrument. The calcite suspension was mixed thoroughly and quickly added to the sample chamber to assure a representative composition. Sample

Table 1 **Trace Element Content of Calcite Lot A and Calcite Lot B**

ANALYTE	Calcite Lot A[1]	Calcite Lot B[1]	ANALYTE	Calcite Lot A[1]	Calcite Lot B[1]
ASSAY (metals basis)	**99.99%**	**99.99%**	**Iron**	**5 ppm**	**5 ppm**
Barium	15 ppm	2 ppm	**Chlorine**	*	42 ppm
Magnesium	8 ppm	7 ppm	**Sulfur**	*	12 ppm
Sodium	10 ppm	32 ppm	**Arsenic**	*	*
Strontium	13 ppm	58 ppm	**Phosphorus**	*	*

[1]Data from Alfa Aesar (Ward Hill, MA) certificate of analysis
*** Not detected**

was added until the required light intensity was achieved for sample analysis. Dispersion of the calcite particles was achieved by ultrasonication for 10 min at the highest energy setting within the aqueous liquid module before the particles were introduced into the sample chamber for analysis. The Mie theory optical model (Eshel *et al.*, 2004) with a calcite refractive index of 1.6583 was used to calculate particle-size distribution, and the results were reported as a percent of the total volume of solid. Electron microscopy was performed using the JEOL (Tokyo, Japan), JSM-6400, scanning electron microscope. Samples were prepared by fixing dry calcite to an aluminum sample stub with carbon conductive tape. The sample was then sputter coated with gold-paladium (60/40) using a Hummer I coater to reduce charge buildup on the sample particles. Digital images were captured using the secondary electron imaging mode. The images were generated using a digital resolution of 2056 dpi. The image magnification ranged from 2300 to 15000x.

Results and Discussion

Two different lots of synthetic calcite were analyzed for particle-size distribution by laser diffraction and by scanning electron microscopy. The COA for calcite lot A provided an assay of 99.99% calcium carbonate on a metals basis with a 20 – 30 μm average particle size and 99.95% on a metals basis with a 5 μm particle size for calcite lot B. The trace elemental analysis for each lot is listed in Table 1.

The particle-size distributions for calcite lot A and B as determined by LPD are shown in Figure 1 and Figure 2, respectively. The mean particle diameter of calcite lot A was 6 μm, with 2.9 % of the particles by volume in the range from 0.4 – 0.6 μm, 92.8% of the particles in the range from 1.8 – 11.8 μm, and 4.2% of the particles in the range from 14.3 – 22.7 μm. The mean particle diameter of calcite lot B was 24 μm and the range of particle distribution was a minimum of 4.6 μm and a maximum of 47.9 μm. The distribution of calcite lot B particle sizes appears Gaussian with slight tailing toward the smaller particle sizes. The SEM images of calcite lot A (Figure 3 and 5) indicate that the individual calcite particles are considerably smaller than 10 μm particle diameter and qualitatively confirm the LPD results. The SEM images for calcite lot B (Figure 4 and 6) indicate that the particle-size for calcite lot B is smaller than that for calcite lot A; although the aggregate size for calcite lot B is larger.

The particles in the SEM image for calcite lot B appear to be more highly aggregated that those in calcite lot A. The surface structure for calcite in air, when the relative humidity is greater than 60%, is identical to the surface structure of calcite in water and consists of hydroxyl groups and water that can hydrogen-bond to other calcite particles to result in interparticle bonding and aggregation (Fenter *et al.*, 1999). The SEM images of calcite in air show that calcite lot B has a higher degree of interparticle bonding and aggregation than calcite lot A under the same atmospheric conditions. The LPD distribution of calcite in lot B is much greater than the particle size results from the SEM image. This comparison substantiates that the particles in calcite lot B have a higher degree of aggregation than those in lot A. The ultrasonic treatment for 10 minutes at the highest energy setting was not sufficient to disperse aggregates in lot B for LPD analysis. The enhanced interparticle bonding and aggregation of calcite lot B could have been impacted by its higher anion content, sulfate and chloride, as summarized in Table 1.

The assay of contaminants in calcite could be an important factor that affects particle aggregation and the results of these analyses. The trace elemental content of the calcite lots used in this study suggests that sulfate content might have impacted the affinity of calcite particles to bond together and form aggregates. Previous studies (Celi *et al.*, 2000) have shown the impact of anion adsorption to the surface of calcite and its impact on particle-size distribution and aggregate stability. Tung *et al.* (2004) used scanning electron microscopy images to show the increased aggregation of calcite particles due to the inclusion of sulfate. The consequences of this phenomenon should be considered when assessing calcite particle-size distribution using laser diffraction.

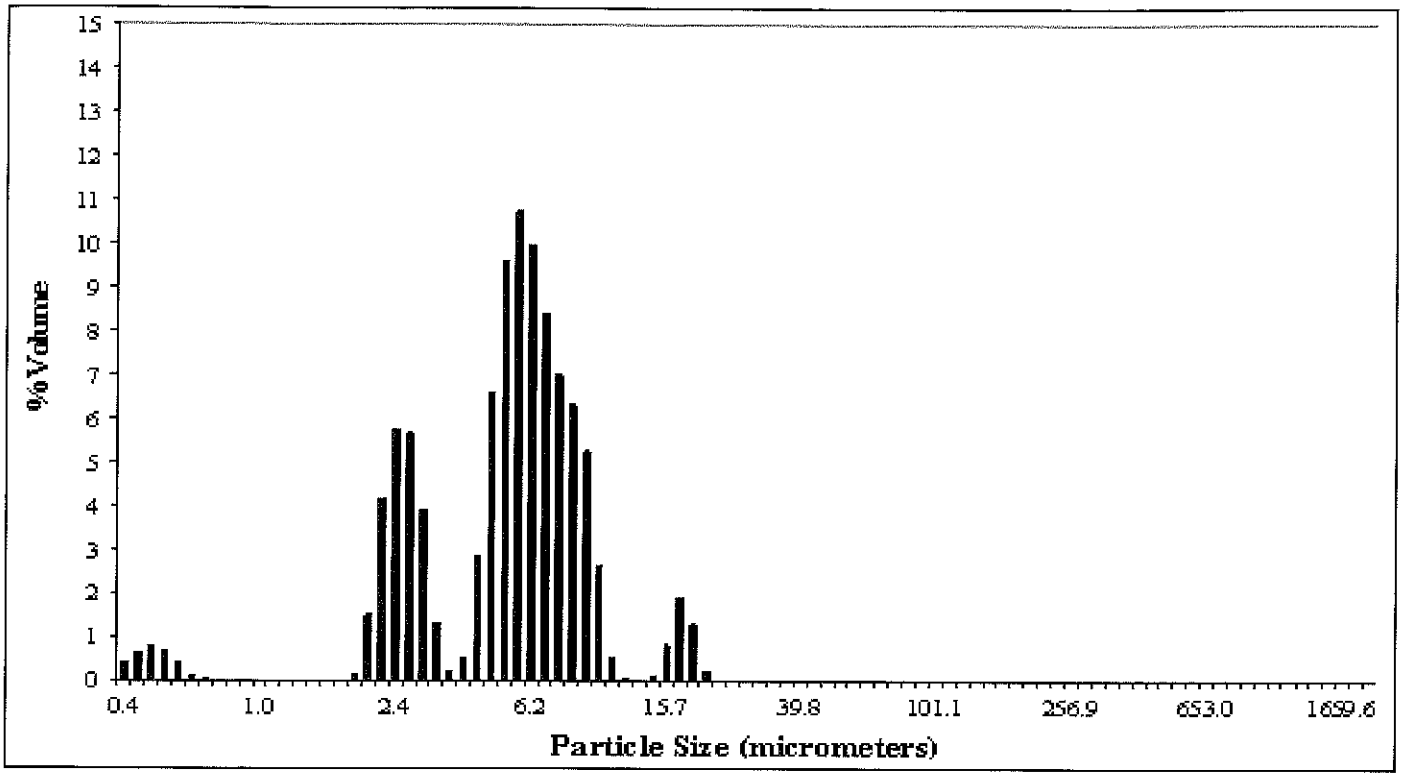

Figure 1. Particle size distribution of calcite lot A as determined by laser diffraction. The mean particle diameter is 6 μm.

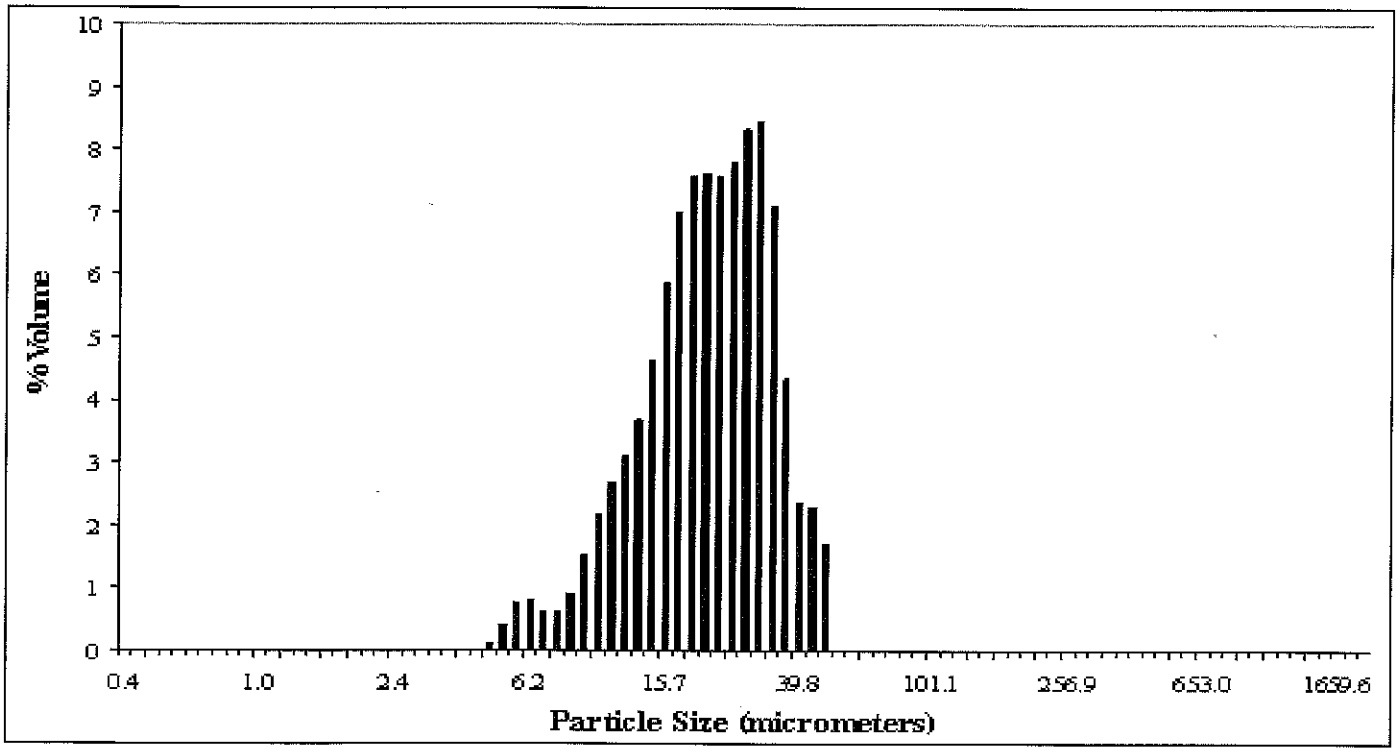

Figure 2. Particle size distribution of calcite lot B as determined by laser diffraction. The mean particle diameter is 24 μm.

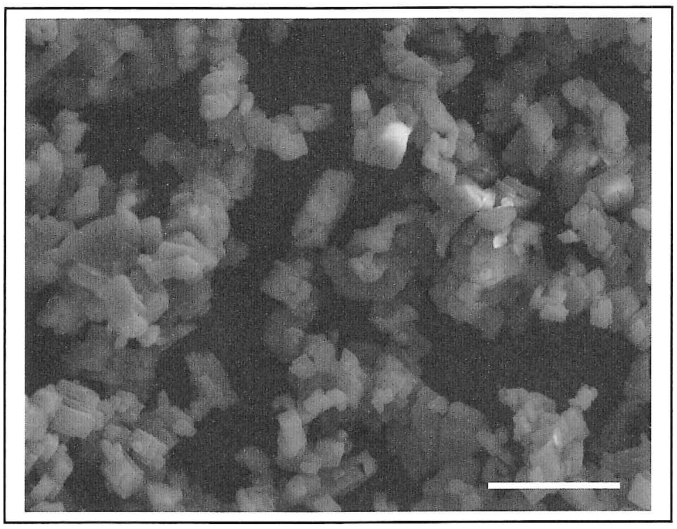

Figure 3. Scanning electron microscopy image of calcite lot A. Scale = 10 μm.

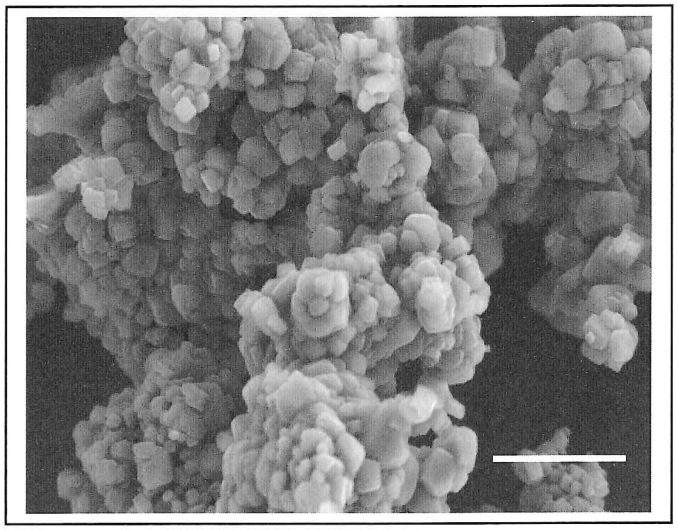

Figure 4. Scanning electron microscopy image of calcite lot B. Scale = 10 μm.

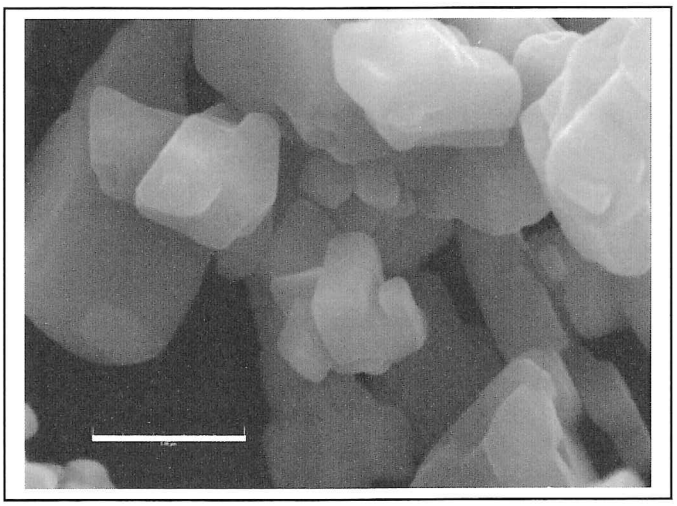

Figure 5. Scanning electron microscopy image of calcite lot A. Scale = 2 μm.

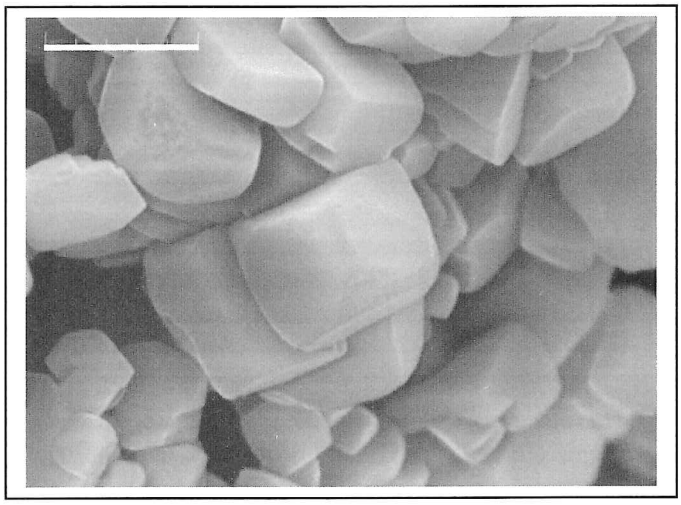

Figure 6. Scanning electron microscopy image of calcite lot B. Scale = 2 μm.

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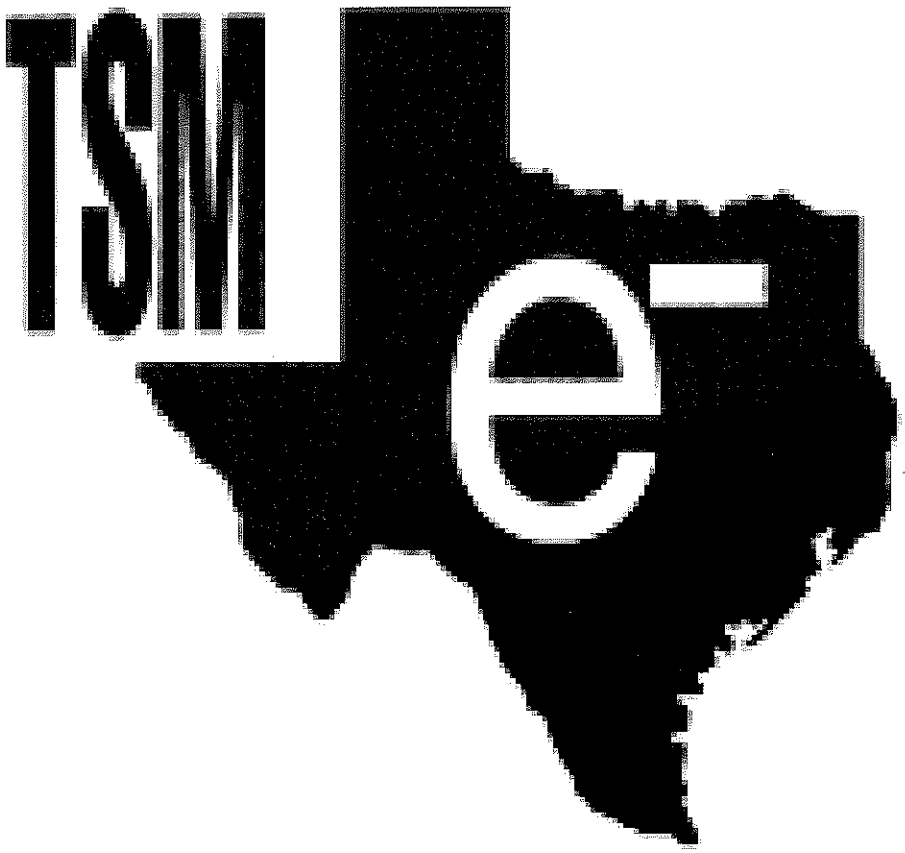

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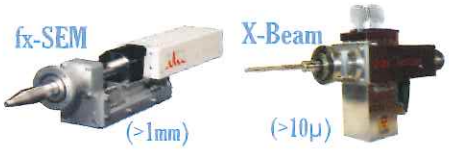

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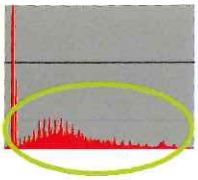

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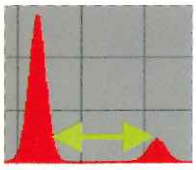

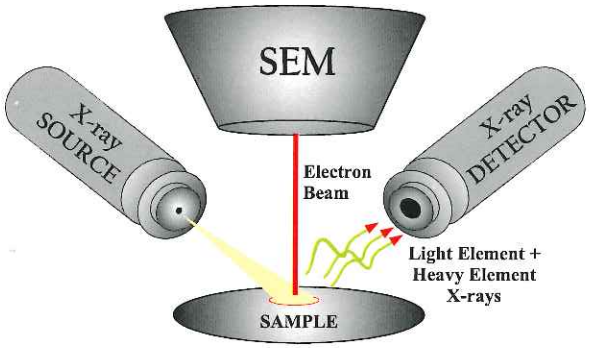

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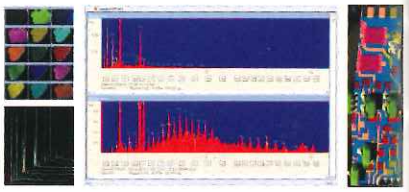

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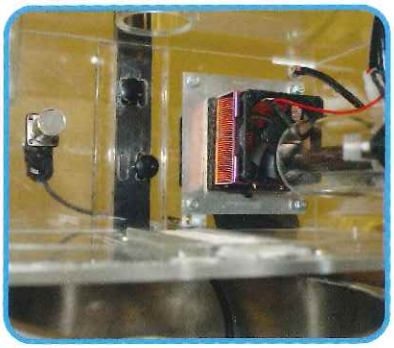

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