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Camelia G.-A. Maier, Editor
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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). Two anniversary articles, one by **Donald Duncan**, charter member and the other by **Howard J. Arnott**, Past President of the Society can be found on pages 24 and 27, respectfully. The series of anniversary articles will continue in the next issue of the journal.

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President's Message

My term as president has gone by quickly. As I reflect back on the past two years, I am proud of our accomplishments. The entire executive committee has been outstanding, offering new ideas and working tirelessly whenever asked. We have all pulled together to chart a course for the future while still respecting our past. I am particularly grateful to Bob Droleskey, current treasurer and also past president, who has watched over the finances of the society during the past two years. Robert Champaign, current secretary, and Ann Burke, immediate past secretary, always answered every request I made for membership lists, updated addresses, and other random pieces of misplaced information. Pam Neill, past president, has been a valued advisor and friend. Program Chairmen Susan Robbins and Jodi Roepsch have made sure that our recent meetings were both enjoyable and cost effective. Journal Editor Camelia Maier has given a fresh and inviting look to the Texas Journal for Microscopy. I extend my sincere thanks to everyone who has served on the executive committee over the past two years.

Our participation as local host society for the 2003 Microscopy and Microanalysis meeting in San Antonio was a significant event that energized everyone who attended. Personally, I felt a renewed sense of obligation to the Texas Society for Microscopy that has driven me during the past two years. Our business meeting in San Antonio was my first as president and substituted for our annual fall meeting in 2003. The spring 2004 meeting in Houston, organized by Susan Robbins, was enthusiastically attended. Jodi Roepsch took over as program chairman for the fall 2004 meeting and is graciously serving for two years. For the fall meeting in Allen, Jodi recruited heavily among her materials science colleagues, with workshops and speakers designed to attract scientists in both materials and biological science. Congratulations to Lucia E. Godinez and Jaime B. Vigo who received the Howard J. Arnott Student Presentation Award for their presentation, "Histological, ultrastructural and microarray comparisons between C3H mice fed sodium arsenate in drinking water," coauthored with Donna M. Byers and Joanne T. Ellzey, all from the University of Texas at El Paso. Our planning for the 40th anniversary meeting began in earnest during the fall 2004 meeting.

This 40th Anniversary spring 2005 meeting brings together both the past and the future. We welcome many past officers of the society and hope that seeing colleagues will remind us all of the original intentions of the founders of the society in 1965. We will have a highly contested student presentation competition and a photo contest for all members. The future of the society is in the hands of the students we mentor. We also honor Dr. Howard J. Arnott for his many years of service to the society and formally announce the naming of the student presentation award in his honor. Many of his former students and colleagues will join us in celebrating his accomplishments. A special session on the history of the society will include presentations by several former presidents who are traveling to Texas especially for this event. Pam Neill, our immediate past president, has taken on the task of converting journals to electronic format. If not finished for this meeting, the compilation will be available in the future.

During the past two years, I feel that many of our members have made a renewed commitment to the future of the society. We have notably improved our financial position. No longer can we afford to spend freely as in the past but must be vigilant with the finances of the society. Our renewed emphasis on pre-registration allows for better meeting planning and particularly helps to avoid financial loses associated with hotel and catering expenses. We must seek ways to increase donations to the society and must request funding from the Microscopy Society of American whenever possible. We have expanded our membership in the materials sciences, largely due to the efforts of Jodi Roepsch and Robert Champaign. We must continue to seek members to form a diverse base. On several occasions, I have sent letters to biology departments in colleges and universities around the state. We must continue to recruit among our academic colleagues and well as expanding our contacts in industry. Personal contacts are our most effective means of recruitment. We have strengthened our corporate relationships thanks to Mike Crowley, our corporate representative from Oxford Instruments, Inc. We appreciate the prospective he brings to the executive committee. He has offered many suggestions and championed the idea of seeking corporate hosts for our workshops. We must continue to seek the input of our corporate members if we are to have their support in the future.

As I turn over the gavel to Sandra Westmoreland, our next president, I challenge each of you to work diligently on behalf of the society. I ask myself if the society is better now than when I became president in 2003 and I believe I can say that it is. The combined efforts of the entire executive committee and others have inspired me. Let us all be motivated to promote the Texas Society for Microscopy among our colleagues. The city of Fort Worth is bidding to host the Microscopy & Microanalysis Meeting in 2011. If their bid is successful, we will be the local host society. I look forward to our continued success as we anticipate showcasing the Texas Society for Microscopy again at a national meeting.

Ann E. Rushing
TSM President 2003-2005

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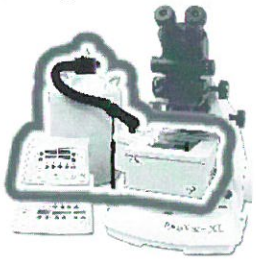

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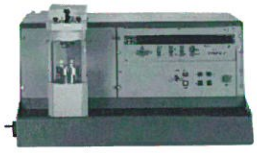

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OBSERVATIONS ON NUCLEOLI OF *MELOIDOGYNE* (NEMATODA) INDUCED GIANT CELL NUCLEI

R. J. WIGGERS* AND D. A. GRAVATT

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ABSTRACT

Nematodes of the *Meloidogyne* genus induce the formation of large, multinucleate giant cells in their hosts. These giant cells have elevated metabolic processes and supply the developing nematode with all necessary nutrients. Long known to possess elevated DNA content and chromosome counts, this study examined the nucleoli of giant cell nuclei. As compared to root tip nuclei from uninfected plants, giant cells at one and two weeks post inoculation, but not three weeks, had a significantly percentage of their nuclei occupied by nucleoli. These disproportionately enlarged nucleoli may be required to enable the giant cells to meet increased metabolic demands made by the maturing nematode.

INTRODUCTION

Nematodes of the genus *Meloidogyne*, commonly called the root-knot nematodes, induce the formation of giant cells in suitable host plants. Giant cells, which develop from vascular or provascular parenchyma cells (1), are large, multinucleate cells, which function as a source of nutrients for the developing nematode (2, 3). Giant cell development is correlated with nematode development; they reach maximal size and metabolic activity as the female nematode is producing eggs and giant cells senesce as nematode life cycle completes (2). The metabolic increases observed in giant cells is that of a general increase of metabolism,• rather than an increase of specific metabolic pathways (1) and has been suggested to be mediated by a wide scale de-repression of many genes simultaneously (4). It has been documented (5, 6) that proper giant cell development is absolutely required for the nematode to complete its life cycle.

A wealth of data exists pertaining to the genetic conditions of the giant cell nuclei. They are known to be multinucleate, and have elevated chromosome counts and DNA content. Various authors have reported that the number of nuclei per giant is highly variable, with counts ranging from 26 per giant cell in *Pisum sativum* (7) to 150 nuclei per giant cell in *Glycine max* (8). The multinucleate conditon is believed to arise via repeated mitotic divisions without cytokinesis (1). Some authors have found the chromosome counts to follow strict euploid progressions (9) while others have documented the presence of both euploid and aneuploid nuclei (10). DNA content of individual giant cell nuclei has been shown to vary by as much as 15 fold when compared to normal root tip nuclei (10), although no specific gene sequences have been found to be selectively amplified (11).

The nucleolus was discovered as the site of ribosomal rRNA sysnthesis in the early 1960s (12). Recent studies suggest that the nucleolus may be involved in many more aspects of gene expression, such as the processing and export of certain mRNA's, transcription of both the signal recognition particle and telomerase enzyme RNA components, as well as the processing of tRNA intermediates (13). Giant cell nuclei have been documented to possess enlarged nucleoli (14), however no quantitative study has been undertaken to ascertain whether or not this enlargement is simply due to the overall increase in DNA content or is disproportionate in nature. In light of this growing role of the nucleolus in gene expression, we have examined the nucleoli in giant cells for evidence of selective enlargement.

MATERIALS AND METHODS

Seeds of *Pisum sativum* cv. Little Marvel were germinated and inoculated with approximately 50 *Meloidogyne arenaria* juveniles when they had reached one inch in length. Seeds were germinated and inoculated using the ragdoll method (15) to achieve synchronous infection by all juvenile nematodes. Infected plants were allowed to grow for three weeks post inoculation, at which time adult egg producing females were observed, indicating the life cycle was complete. Root tips were taken from uninfected plants to serve as control nuclei (only one set of controls was taken, to be compared against all giant cell nuclei) and root sections containing giant cells were taken excised from infected plants at one week, two weeks, and three weeks post inoculation. All specimens were fixed in a 1:1 (v:v) solution of 5% gluteraldehyde: 0.1M potassium phosphate buffer at 4 C overnight. Samples were rinsed with 0.05 M potassium phosphate buffer and then immersed in a 1:1 (v:v) 2% osmium tetroxide : 0.1 M potassium phosphate buffer and held at 4 C for two hours. After rinsing with distilled water, specimens were immersed in an aqueous solution of uranyl acetate at 4 C overnight. All samples were dehydrated via a graded ethanol series at room temperature before being immersed in 100% acetone. The specimens were placed in 70% acetone:30% Spurr's resin (16) and infiltrated overnight at room temperature. A second room temperature overnight infiltration was performed in 30% acetone:70% Spurr's resin before the specimens were immersed in 100% Spurr's resin for 8 hours. Thin sections (100 nm) of giant cell and root tip nuclei were placed on copper grids and stained with aqueous uranyl acetate and lead citrate (17) and allowed to dry completely. All samples were examined and photographed using a Hitachi HS-9 transmission electron microscope (Hitachi, Ltd., Tokyo, Japan).

All negatives of giant cell and control nuclei were scanned using a HP Proscan 7400c (Hewlett Packard Co., Palo Alto, CA.). Digitized images were analyzed using Adobe Photoshop 6.0 (Adobe Systems Inc., San Jose, CA). Twenty nuclei of each type (control, and one, two, and three week giant cell) were examined. The size, in pixels, of each nucleus was determined, as well as the pixel size of any and all nucleoli. The percentage of each nucleus occupied by the nucleolus was determined by dividing the total number of pixels occupied by nucleoli by the total nuclear area.

Statistical analyses were performed using PC-SAS statistical software (SAS Institute Inc., Cary, NC). All data was checked for homogeneity and normality and an arcsine transformation was performed. One-way analysis of variance was used to test the significance of differences for the percentage of nucleus occupied by nucleoli. When statistical significance was found, Tukey's procedure was used to make pairwise comparisons of the means. A P value less than 0.05 was accepted as statistically significant.

To determine whether a given thin section was a good representation of a nucleus, serial sections of uninfected root tip nuclei were prepared. At least two sequential thin sections (89 nm) of a nucleus were prepared, five more 89 nm sections were removed and discarded, followed by the collection of at least two more sequential sections. Percentage of nucleus occupied by the nucleolus was calculated as previously described and the data was subjected to the same statistical analysis as previously described.

RESULTS AND DISCUSSION

The percentage of nucleus occupied by nucleoli for control root tip nuclei and giant cell nuclei is shown in table 1. Images of control and giant cell nuclei are shown in figure 1. Giant cell nuclei at one and two weeks post inoculation had significantly higher percentage occupied by nucleoli than uninfected root tip control nuclei (Figure 1B & 1C; Table 1). By three weeks differences in nucleolar percentage had disappeared (figure 1D, table 1). This is consistent with the observation that giant cell development is tied to nematode development – by week three, the female has completed her maturation process and the giant cells are beginning to senesce. When serial sections of root tip nuclei were analyzed, no significant differences between sections were observed ($P > 0.1$), indicating that any given section was representative of the nucleus as a whole.

Table 1. Nucleolar data for control and giant cell (G.C.) nuclei. For each category, 20 nuclei were examined.

	Nucleolus as a % of the nucleus
Root tip control	8.2%
One week G.C.	12.1%*
Two week G.C.	10.9%*
Three week G.C	7.3%

* Significantly different from uninfected root tip control nuclei.

The only previous study to look at giant cell nucleoli (14) had reported enlarged nucleoli but it was not determined if the nucleoli occupied significantly larger percentages of the nucleus. It appears, then, that in the early stages of giant cell formation, nucleoli are disproportionately enlarged, perhaps to keep pace with the increased metabolic demands made on the giant cell. If there is indeed a large scale de-repression of many genes, as previously suggested (4), then additional nucleolar volume could be required to keep up with increased processing of mRNA or to produce enough rRNA and ribosomes to meet the cells increased translational output. It remains to be determined how enlarged nucleoli are generated. Several possibilities, including the selective amplification of the rRNA genes, the selective increase of chromosomes 4 and 7, on which the rRNA genes reside (18), increased transcriptional activity of the rRNA genes along with increased ribosomal assembly, or increased processing of mRNA and tRNA, exist. It has previously been demon-

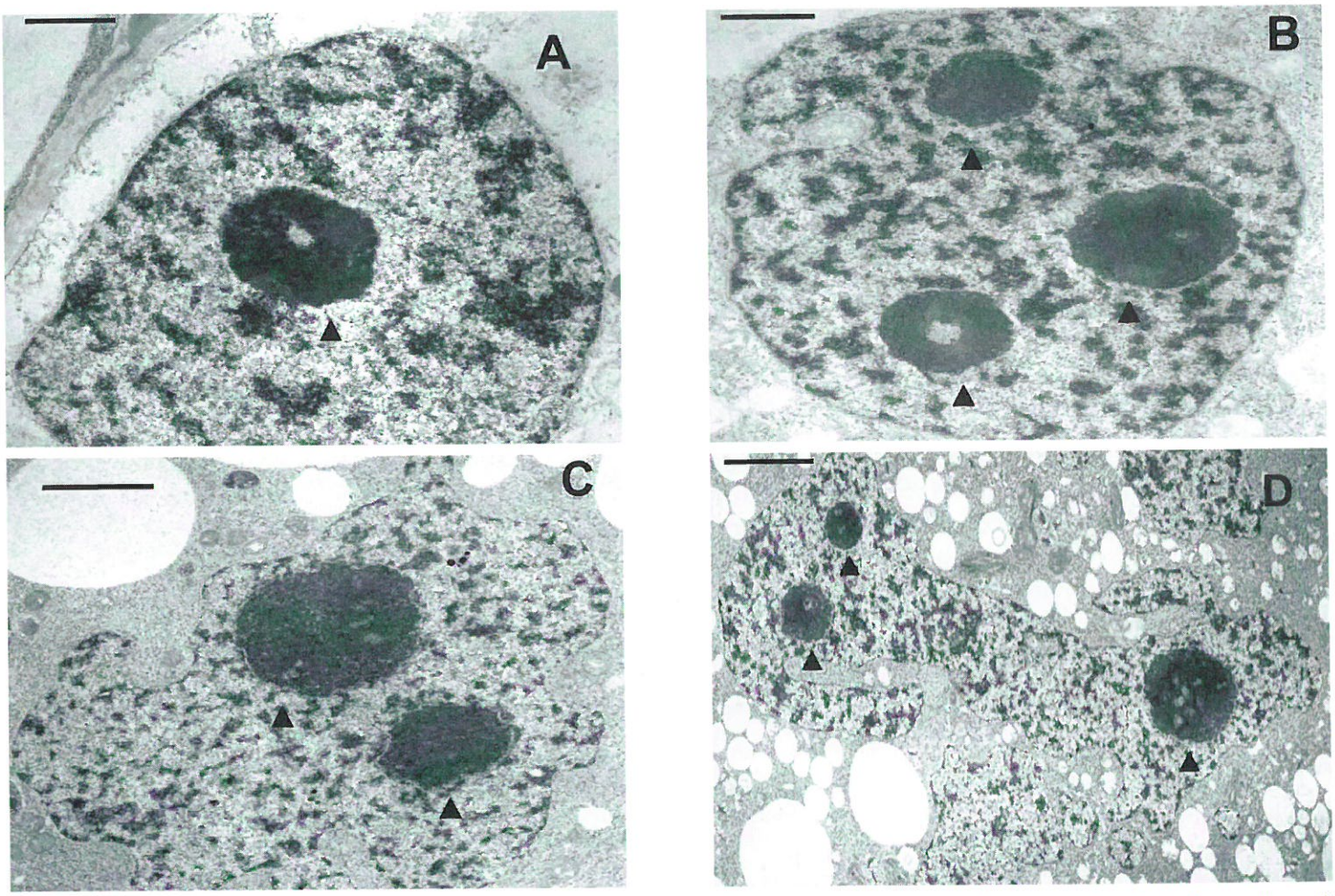

Figure 1. Electron micrographs of giant cell nuclei and nucleoli. (A). Control nucleus from uninfected root tip. Note the single well formed nucleolus indicated by arrowhead. (B), (C), and (D). Giant cell nuclei from one-week, two-week, and 3-week old giant cells, respectively. Note the multiple nucleoli indicated by arrowheads. In all photographs, bar = 1μm.

strated that giant cells do not selectively amplify the rRNA genes (11). This would also suggest that giant cell nuclei do not selectively increase the numbers of chromosomes 4 and 7. The most likely explanation is that nucleolar activity is increased substantially as compared to normal cells, and this increase leads to a disproportionate enlargement of the nucleoli. It remains to be determined whether the increased activity involved the transcription and processing of the rRNA genes or the processing of other RNA species.

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Abstracts

BIOLOGICAL SCIENCES
SPRING 2005

ELECTRON MICROSCOPY OF FULLY HYDRATED SAMPLES. DAPHNA R. YANIV, Quantomix, P.O. Box 4037, Nes Ziona 70400, Israel.

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.

NITRIC OXIDE AND BASEMENT MEMBRANE THICKENING IN DIABETIC RETINOPATHY. E. ANN ELLIS[1] and MARIA B. GRANT[2], Microscopy and Imaging Center[1], Texas A & M University, College Station, TX 77843, and Department of Pharmacology and Therapeutics[2], College of Medicine, University of Florida, Gainesville, FL 32610.

Diabetes is a disease of vascular complications and capillary basement membrane (CBM) thickening is an ultrastructural hallmark of diabetic retinopathy. In diabetes, hyperglycemia stimulates increased lecels of superoxide which can uncouple nitric oxide synthases (NOS) and reduce availability of nitric oxide (NO). Pharmacological inhibition of inducible NOS (iNOS) results in coronary vascular remodeling in rats and interstitial fibrosis is exacerbated in kidneys of mice lacking the gene for iNOS. In non-insulin dependent diabetes there is reduced availability of NO in the vascular endothelium. We investigated the role of NO in CBM thickening in galactose induced diabetic retinopathy in three strains of mice: wild type C57BL/6J (WT), endothelial $NOS^{-/-}$ [(eNOS knockout) eNOS KO] and $iNOS^{-/-}$ [iNOS knockout (iNOS KO). Eyes were enucleated, fixed and embedded for transmission electron microscopy after cytochemical localization of NADH oxidase, a marker for superoxide. Localization of nitrotyrosine, a marker for peroxynitrite, was done by colloidal gold based methodology. Measurements of CBM thickness were done on capillaries in the retinas of galactosemic (diabetic) and non-galactosemic (non-diabetic) mice by the orthogonal intercept method at the start of and after 12 months of galactose feeding. At 3 months, CBM thickness for normal diet and galactose fed eNOS KO mice were 1.8 fold thicker than those of WT or iNOS KO miced. After 12 months of galactose feeding, CBM thickness of WT and iNOS KO mice were comparable to the initial CBM thickness of the eNOS KO mice. Increased CBM thickness in eNOS KO mice without galactosemia indicates a role for NO in vascular homeostasis. The increased CBM thickness in glactosemic WT and iNOS KO mice points to the long term effect of reduced availability of NO in the galactose induced model of diabetic retinopathy as a result of removal of NO by reaction with superoxide to form peroxynitrite as detected by nitrotyrosine.

HISTOLOGICAL, ULTRASTRUCTURAL AND BIOCHEMICAL EFFECTS OF ARSENIC ON MOUSE (*MUS MUSCULUS*) KIDNEY, LIVER AND PANCREAS. JAIME B. VIGO, LUCIA E. GODINEZ and JOANNE T. ELLZEY. Biological Sciences Department, The University of Texas at El Paso, El Paso, Texas, 79968-0519.

There is a debate concerning toxicity from arsenic in drinking water. Recently, arsenite (As (III)) exposure was linked with the onset of characteristics of diabetes mellitus-2 in adipocytes (Walton, et al., 2004). In our study, thirty-six C57BL/6J mice were subdivided into three groups and supplied with drinking water containing 0 ppm, 5 ppm, or 75 ppm of sodium arsenate (As (V)) for 12 weeks. Part of the liver, kidney, and the pancreas of each mouse was fixed, dehydrated, and embedded for histological and ultrastructural analyses. Measurements of body weights and food consumption showed significant differences between the control and the high dose treatment group. The 75 ppm mice lost weight, but consumed more food than the control mice. Bi-weekly measurements of blood urea nitrogen (BUN) showed a significant trend between week and BUN, with an increase of BUN over time. A marginal statistical difference between the control and the 75 ppm group was observed for aspartate amino transferase (AST), an enzyme indicative of liver dysfunction. No significant differences between the groups were observed for blood glucose, insulin, alanine amino transferase (ALT), triglycerides, liver to body weight ratio, kidney to body weight ratio, or water consumption. A blind study of the histology of the liver, kidney and pancreatic tissues from these mice showed no pathology. Currently, measurements of the nuclear contour index (NCI) of hepatocytes and the lipocyte/hepatocyte ratios are underway.

HUMANS ARE MORE IMAGE CONSCIOUS: IMPLICATIONS FOR SCIENTISTS IN THE 21ST CENTURY. MARGARET A. GOLDSTEIN, Department of Medicine, Baylor College of Medicine, Houston, TX 77030.

Technological, social and cultural influences are bringing about a shift from word to image. This shift in consciousness of images has profound effects on our brains as well as on our communication. Experiments in the knowledge-vision loop and visual context will be described. New findings in human visual processing suggest new ways to extract information from image and to communicate more effectively with images. Describing images with words is less precise than mathematical expressions or symbols, yet presenting images without words is hard. The increased use of scanners and computer programs such as Photo Shop and Power Point provides more options for extracting and presenting information. We are so closely involved in these changes, that we may forget we are part of an even bigger shift in consciousness as observers. Images of brain activity are extending our knowledge of visual processing and of various levels of consciousness. The question remains. Do we tell people to see or do we help them discover what they see?

ULTRASTRUCTIRE AND DISTRIBUTION OF TYPE VIII COLLAGEN IN L450W COL8A2 FUCHS CORNEAL DYSTROPHY. JOHN D. GOTTSCH, MD, Center for Corneal Genetics, Cornea and External Disease Service, The Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD.
PURPOSE. Genetically and clinically distinct phenotypes of Fuchs corneal dystrophy have been identified. Here the unique ultrastructure and distribution of collagens, fibronectin and laminin in Descemets membrane of early onset L450W COL8A2 and late onset Fuchs corneal dystrophies (FCDs) are described.

METHODS. Descemets membrane of a button with early onset L450W COL8A2 (L450) FCD and three buttons with late onset disease without COL8A2 reported mutations were studied with antibodies to fibronectin, laminin collagens Type I, II, III, IV, V, VI, VIIIA1, VIIIA2, and IX. Transmission electron microscopy was performed on the L450W COL8A2 corneas. Control buttons studied included keratoconus, aphakic bullous keratopathy and two eye bank corneas with no known pathology. A portion of each button was prepared for transmission electron microscopy.
RESULTS. Distribution of COL8A1 and A2 distinctly stain the anterior fetal membrane in Descemets membrane in L450W COL8A2 FCD in contrast to little to no staining in late onset disease. There is little COlA1 or A2 staining in the anterior banded portion of the L450W; however, A2 staining is intense in the posterior banded portion of late onset disease with swirls of extension into the anterior banded portion. There is a linear demarcation of the anterior and posterior Descemets with both A1 and A2 staining of L450W corneas. The posterior corneas diffusely stain with COL4 in L450W corneas; however, stain only the posterior aspects of the excrescences in late stage disease. Similar staining patterns are noted for fibronectin and laminin. The ultrastructure of L450W Descemets membrane was distinctly different from previous studies of FCD with a thickened anterior fetal membrane (4.6μm) and a homogenous and uniformly markedly thickened second layer measuring 7.9 microns in thickness containing multiple foci of wide-spaced collagen, especially in the posterior aspect. The third fibrous layer measures 6.7 microns in thickness and is composed of irregularly oriented collagen fibrils of 20 nm.
CONCLUSION. There are distinct differences in the distributions of fibronectin, laminin, COL4, and COL8A1 and A2 antibody staining between early L450W COL8A2 disease and late FCD. The grossly thickened Descemets membrane in L450W disease may represents the accumulation of COL8A1 and mutant A2 polypeptides that are more resistant to digestion and resorption Accumulations of COL8 in late onset FCD suggests a role in the pathogenesis of this disease.

DEVELOPMENTAL AND PHYSIOLOGICAL CHARACTERISTICS OF ASEXUAL PROPAGULES OF A TEXAS SUCCULENT PLANT, *SEDUM WRIGHTII*. DENNIS A. GRAVATT and JOSEPHINE TAYLOR, Stephen F. Austin State University, Department of Biology, Nacogdoches, TX 75962.

In a desert rock outcrop, features of the environment limit reproduction and growth of plant species. *Sedum wrightii* is a succulent plant species adapted to survive in the hot, dry environment of a desert rock outcrop. However, sexual reproductive success may be limited to certain years when favorable conditions for seed germination exist. The purpose of this study was to gain an understanding of how, and to what extent, greenhouse grown *S. wrightii* reproduces using vegetative propagules. Detached leaves of *S. wrightii* produce plantlets on the basal portion of the adult leaf in association with callus tissue from what appears to be thinned-walled parenchyma and epidermal cells of the petiole. Shoot primordia appear first, emerging over lateral regions of the callus tissue by about day 5, with the roots emerging several days later. After 120 days following detachment, leaf survival was 80%, with 98% of those leaves developing propagules. By day 120 of the experiment, detached leaves maintained malate accumulations that were 36% of levels recorded on day 0, indicating that the detached leaves remained physiologically active during the study. The formation of vegetative propagules, derived from existing detached leaves, may be a more efficient and successful mode of reproduction given the hostile environment in which this plant species is found.

MINERAL DEPOSITS IN DIOECIOUS OSAGE-ORANGE, *MACLURA POMIFERA* (*MORACEAE*). A. M. HAMMETT[1], DAVID C. GARRETT[2], and C. G.-A. MAIER[1]. [1]Department of Biology, Texas Women's University, Denton, Texas 76204, [2]Department of Materials Science, University of North Texas, Denton, Texas, 76201.

Mineral deposits, especially calcium oxalate and calcium carbonate, are common in plants but little is known about their functions and how they form. The goal of this study was to characterize mineral deposits in *Maclura pomifera* organs at different developmental stages. The morphology, abundance, and distribution of mineral deposits were determined by light microscopy in stems, nodes, leaves, and inflorescences of male and female trees starting in February 2005. Two types of calcium oxalate deposits, prismatic crystals and druses, were found in both male and female *Maclura pomifera* tissues. More prismatic crystals than druses were found in stems and nodes of both sexes. There is no significant difference in the total calcium oxalate crystals in stems of both sexes; however there is a tendency for the male to have more crystals than the female. Also, significantly more druses were found in female than male stems (*t*-Test, $P<0.05$). Male and female leaves contain druses and few prismatic crystals. Significantly more total crystals were found in the female than male leaves (*t*-Test, $P<0.05$). Male inflorescences contained only druses. These results indicate that calcium deposition abundance is a sexual dimorphism characteristic in *M. pomifera* as it is in mulberry species (*Morus* sp., *Moraceae*), previously shown by our lab. Moreover, the data obtained for Osage-orange suggest a trend in the formation and abundance of calcium deposits which correlate with seasonal development as seen before in mulberry species as well. Compared to mulberry, however, Osage-orange contains no $CaCO_3$ deposits. Chemical composition of the crystals and the level of free oxalate will be determined for the purpose of understanding their possible deterrent effect on herbivores.

TRAPS OF WILD TYPE AND MUTANT CARNIVOROUS PLANTS. MICHELLE A. SAVOLAINEN[1], LEA C. SAVOLAINEN[1], DAVID C. GARRETT[2], and CAMELIA G.-A. MAIER[1], [1]Department of Biology, Texas Woman's University, Denton, TX 76204, [2]Department of Materials Science, University of North Texas, Denton, 76201.

Carnivorous plant species are flowering plants which live in acidic, especially nitrogen-deficient soils. They use various techniques, such as hair-triggered traps, pit fall traps, sticky leaves, etc. to capture mostly insect prey as a source of nitrogen. The traps of *Dionaea muscipula* and *Nepenthes fusca* (Sarawak) were dissected and fixed for electron microscopy, to study the mechanisms responsible for the capture of prey. Mutants of *Dionaea* (*cup trap, dentate,* and *fused tooth*) were compared to wild type plants from the point of view of the trap morphology and anatomy, to determine preying efficiencies. It was observed that wild type traps have long teeth that are equidistant along the traps' outer edge, while mutant traps display randomly placed, modified teeth. Digestive glands and trigger hairs observed appeared to be similar in mutant and wild type plants, suggesting that trapping efficiency is determined by leaf-edge tooth morphology.

SILKWORM PERFORMANCE AS AFFECTED BY MULBERRY GENDER. CORINA MORARU[1], DAVID C. GARRETT[2], AND CAMELIA G.-A. MAIER[1], [1]Department of Biology, Texas Woman's University, Denton, Texas 76204 and Department of Materials Science and Engineering, University of North Texas, Denton, Texas 76201

Dioecious mulberry (*Morus alba*) is the only source of food for silkworm caterpillars (*Bombyx mori*). Mulberry leaves contain mineral depositions and oxalate, which are thought to function as deterrents against herbivores. Previous studies in our laboratory showed that: 1) mulberry female leaves contain significant higher levels of both oxalate and calcium carbonate than the male leaves; 2) silkworms prefer mulberry male leaves and develop better and sooner on male leaf food compared to female-fed caterpillars. The purpose of this study was to establish differences in the performance of two populations of silkworms, one fed with mulberry male leaves, the other one fed with female leaves. Electron microscopy examinations of the cocoons showed differences in the external aspect of silk cocoons but not for the internal face. Cocoons originated from male-fed silkworms have thicker silk fibers on the outside surface then those from cocoons originated from female-fed silkworms. Dissolution analysis of silk showed that male-fed cocoons contain more sericin than the female-fed cocoons. Analy-

sis of carbohydrates and proteins in mulberry leaves showed characteristics of sexual dimorphism in this species, in that higher levels of sucrose in male leaves and of proteins in female leaves were found. It is possible that silkworms prefer male leaves to female leaves due to a lower concentration of mineral deposits and oxalate and/or a higher level of sucrose. Spring and early summer leaves should be analyzed for levels of carbohydrates and proteins before we can conclude with confidence that mineral deposits are responsible for the differences in development and performance between male-fed and female-fed groups of silkworms.

"THE ACTION OF WOODS ON PHOTOGRAPHIC PLATES IN THE DARK" . . . A PAPER REVISITED. MARTHA I. GRACEY. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019

In a paper dated June 16, 1904, the technique utilized in this paper was described. Efforts to replicate the technique are currently taking place in the lab. Wood, even if old and dry, placed on photographic plates will react in such a way that when development of the film is performed, a clear picture of the wood structure is reproduced. The results were purported to be enlargeable with and very good detail. Conifers resulted in some of the best results. This experiment will incorporate several types of woods, not to exclude bristlecone and *Pinus sylvestris*. Exposure times vary ad will be from one half hour to forty eight hours. Quite possibly it is the hydrogen peroxides in the woods that yield the final image. Speculation suggested that the radioactive compounds in the resins might be responsible for the images, but the original author William J. Russell disproved that theory. Resins around knots were reported to give very small if any reactions. If the results are as the author of the original research reports, then a secondary method of imaging wood may come to light one hundred years after the fact.

THE USE OF HIGH PRESSURE FREEZING/FREEZE SUBSTITUTION FOR BIOLOGICAL TEM. C. W. MIMS[1] and E. A. RICHARDSON[2], [1]Department of Plant Pathology, University of Georgia, Athens, GA 30602, [2]Department of Plant Biology, University of Georgia, Athens, GA 30602

High pressure freezing (HPF) is a fixation technique that uses liquid nitrogen applied at 2,100 bar to prepare biological samples up to 0.5mm in thickness for ultrastructural study. Since its introduction in the1980s, HPF followed by freeze substitution (FS) has become the gold standard for preserving biological samples for TEM. Not only does HPF/FS give outstanding preservation of most membranes, organelles and other cellular inclusions, the extremely rapid immobilization of structures and events, afforded by the technique, greatly facilitates studies that attempt to correlate structure with function. HPF/FS also facilitates gold-labeling experiments due to the improved preservation of antigenicity in cryofixed samples. There are, however, some challenges as well as a few disadvantages relating to the use of HPF/FS. Most challenges relate to the handling of specimens before and after freezing and resin infiltration problems involving thick-walled samples. Disadvantages include physical damage to samples during freezing, extraction of lipid bodies, an increase in time required for sample preparation, the need for a high pressure freezing machine and an increase in the cost of conducting research. This presentation is designed to introduce HPF/FS to those who have not used the procedure. Topics to be discussed include the handling of specimens before and after freezing, the use of cryoprotectants and resin infiltration. Results obtained for various types of samples will be discussed and illustrated.

AN INVESTIGATION ON MICROORGANISMS FROM VARIOUS INFECTED FOOD SOURCES. KELLI BIRD[1], NABARUN GHOSH[1], CONSTANTINE SAADEH[2], MICHAEL GAYLOR[2] and DON W. SMITH[3]. [1]Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, TX 79016. [2]Amarillo Center for Clinical Research /Allergy A.R.T.S. 6842 Plum Creek Drive, Amarillo, TX. [3]Biological Sciences, University of North Texas, Denton, TX 76203.

Food spoilage is of paramount concern to both consumers and scientists alike. The spoiled food materials used in this study were bread, strawberries, grapes, pears, soft drinks and Portabella mushrooms. We prepared the agar plates using Brain heart infusion agar for replication and isolation of microorganisms associated with food spoilage. All specimens were aseptically inoculated to the agar plates and incubated overnight at 37°C. We followed Gram staining for the bacterial slides and Lacto-phenol Cotton Blue staining for preparing slides with the fungal materials. The petri plates were viewed with an Olympus SZ-40 stereomicroscope attached to a fiber optics gooseneck light source that helped us to differentiate the fungal culture from the bacterial culture. Micrographs were also taken at different magnifications using a BX-41 Olympus microscope attached to an Olympus Microfire digital camera. We observed fungal species included *Rhizopus* sp. and *Aspergillus* sp. from infected bread, *Penicillium* sp. from a bottle of spoiled Dr. Pepper, basidia and basidiospores from various Basidiomyceteous fungi from molded cheese and beverages. Gram-positive bacteria were observed from infected fruits like pear, Gram-positive and negative bacteria from strawberry and Gram-negative *Cocci* from a can of coke that was opened 6 days earlier. Most of these food materials did not exhibit a change in color. Even if there is not a distinct color change or visible deformation of the food material, infection within the food may still be present, especially in canned foods and bottled beverages. It is recommended that that the fruits and vegetables are consumed when they are fresh. Canned foods and beverages should be consumed as soon as possible after opening the airtight sealing. This research was funded by grants from Killgore Research Center, WTAMU and Allergy A.R.T.S.

SHIP COMPONENTS, CARGO, OR CONTAMINATION: DETERMINING THE ORIGIN OF WOOD FRAGMENTS RECOVERED FROM AN UNDERWATER SHIPWRECK SITE. MICHAEL W. PENDLETON[1], BONNIE B. PENDLETON[2], GEORGIA FOX[3], E. ANN ELLIS[1], AND TOM C. STEPHENS[1], [1]Microscopy and Imaging Center, Texas A&M University, College Station, TX. 77843-2257, [2]Division of Agriculture, West Texas A&M University, Canyon, TX. 79016-0001, [3]Museum of Anthropology, California State University, Chico, CA. 95929-0400.

The characteristics of wood samples recovered from an underwater shipwreck site are described using a scanning electron microscope (SEM). The site was located in the Bay of Skindos in the Aegean Sea and was dated to 2200 B.C. The wood samples were analyzed to determine if they were associated with the wreck structure, or the wreck cargo, or were modern debris brought into the site by ocean current activity.

The wood samples were transported from the wreck site in vials of seawater prior to critical point drying with a Denton CP-1. After drying, the wood samples were coated with 600 angstroms of gold-palladium using a Hummer II sputter coater and observed using a JEOL JSM-6400 SEM. Features of the samples were entered into the General Unknown Entry and Search System (GUESS) program for computer-aided wood identification.

When recovered from the wreck, the outer surface of each wood sample was covered with debris. To obtain images of the dried wood surfaces which were free of debris, the samples were split and the cleaved surfaces were examined. Those wood samples with debris and/or crystals in the vessels observed on cleaved surfaces may have been associated with the shipwreck as structural timbers or cargo. Wood samples having no debris and/or crystals observed in vessels on cleaved sections are probably modern wood fragments that have been washed into the shipwreck site.

PERIDERM DEVELOPMENT IN *ULMUS ALATA* MICHX. TIFFANY B. FOWLER* AND ANN E. RUSHING. Department of Biology, Baylor University, Waco, TX 76798.

Ulmus alata produces a protective periderm that replaces a trichome-covered epidermis during the first year of stem growth. Scanning electron microscopy reveals a circumfluent tissue of approximately 4-7 layers of phellem or cork cells in addition to the phellogen. The production of winged extensions of the periderm is initiated by uneven enlargement of cells in localized regions of these peridermal layers. It appears that radial expansion of existing cork cells initiates wing formation. Expansion of these cork cells results in a protrusion that often ruptures the original epidermis early in development. Additional layers are added to the wing by normal cell divisions in the phellogen (cork cambium), followed by radial expansion of the newly produced cells. During formation of the winged extensions of the periderm, the cork cambium does not appear to be active in the remainder of the stem. Typically, wings are oriented laterally on opposite sides of the stem, although additional wings may be produced in the same areas. Similar wing development has been observed in *Ulmus crassifolia* and has been reported in *Acer*, *Quercus*, and *Liquidambar*. *Euonymus alatus* produces wings prior to full periderm initiation by localized growth of the cork cambium.

MICROGRAVITY'S EFFECT ON MAMMILLARY CONES OF JAPANESE QUAIL EGGS. S. L. WESTMORELAND*, T. HALULPNIK*, J. WALKER* AND P.Y. HESTER**. *The University of Texas at Arlington, Arlington, Texas 76019. ** Purdue University, W. Lafayette, IN 47907.

A study was conducted in cooperation with NASA, National Aeronautics and Space Administration, to determine if normal incubation of fertile eggs of Japanese quail could occur in microgravity. Our role was to examine the eggshells using scanning electron microscopy (SEM) to establish whether normal calcium loss had occurred. Based on a previous baseline study with Japanese quail, we predicted that the earliest calcium loss from mammillary cones of shells would be at 12 d of incubation. There were 5 experimental groups in the NASA study. The eggs of all groups were incubated at 37 ° to 37.5 ° C and turned hourly. Eggs in groups 1 and 2 were incubated in space in an Avian Development Facility (ADF) unit aboard the Orbiter Endeavour flight STS-108 for 12 days. Group 1 was in 0 g; group 2 was spun in a centrifuge at 1 g. Groups 3 and 4 were incubated in the ground control laboratory in an ADF unit. Group 3 was stationary at 1 g, while group 4 was spun vertically in a centrifuge, resulting in variable force from 0 to 2 g. Group 5 was incubated on Earth in a standard laboratory incubator. The incubation was interrupted at days 4, 7, and 12 in each of the test groups. The day 4 and 7 shells were injected with 4% paraformaldehyde in 50 Mm Cacodylate buffer, pH 7.4 to terminate incubation. Incubation of day 12 eggs was terminated by opening the shells; no preservative was used. Eggshell samples from each group were treated with 6% sodium hypochlorite for removal of shell membranes. The SEM examination revealed that calcium loss had occurred in some mammillary cones from each group, regardless of treatment or length of incubation. Fixative most likely played a role in the dissolution of Ca from the mammillary cones of shells from embryos incubated for 4 and 7 d. An ash analysis of the Ca content of the day 12 shells obtained from live embryos showed no significant difference among treatment groups. These results indicate that embryos incubated in microgravity for 12 d utilized Ca from the shell during development.

DEVISING A PLAN TO DOCUMENT THE EFFECTS OF F-STRAIN *MYCOPLASMA GALLISEPTICUM* ON THE EGGSHELLS OF COMMERCIAL LAYERS. SARAH B. MAY and SANDRA L. WESTMORELAND. The Center for Electron Microscopy, University of Texas at Arlington, Arlington, Texas 76019

This experiment was designed to determine the effects of F-strain *Mycoplasma gallisepticum* on eggshells of infected chickens. Previous studies have indicated that this microorganism alters the tissues of chickens' oviducts. Our hypothesis was that eggshell structure and thickness would also be altered in infected birds. In addition, several other factors were included in this controlled study, which may also affect the eggshell characteristics. These included diet and age of the bird at lay. In order to document the effects of these variables, we will be making scanning electron micrographs using the JOEL 35C and shell thickness measurements using Image Pro Plus for image analysis. Collecting data for this experiment requires careful organization of micrographs and measurements. In order to accomplish this, we have devised a spreadsheet on which we will record information, allowing us to identify the eggs, micrographs, and shell thicknesses. All variables will be noted in a manner that will allow shell thicknesses to be statistically analyzed.

GROWTH OF *QUERCUS STELLATA* SEEDLINGS FOLLOWING MYCORRHIZAL INOCULATION: A POTENTIAL PROMOTER FOR SURVIVAL IN AN URBAN ENVIRONMENT.

Mycorrhizal hyphae act as extensions of the root system, allowing the root system to effectively increase its surface area. In addition, mycorrhizal mycelia are more effective in nutrient and water absorption than roots themselves. Compared to non-mycorrhizal roots, mycorrhizal roots are able to utilize significantly more phosphorous, iron, manganese, copper, and zinc. Much is known of the mycorrhizal association. However, little is known of the benefits of the mycorrhizal association within an urban environment and the challenges unique to the urban environment. Urban soils often have an elevated pH due to concrete rubble, crushed limestone, or calcareous subsoil intruding into the root zone. Furthermore, urban soils suffer from increased compaction, high levels of inorganic and organic contaminants, elevated soil temperatures, mixed soil profiles, disrupted hydrologic flow, and interrupted nutrient and carbon cycles. The problem of soil compaction has been a problem in the urban environment and in managed forests since the advent of the bulldozer. Our project hopes to demonstrate the growth benefits of mycorrhizal inoculation in *Quercus stellata* (post oak) seedlings. This majestic tree of the southwest often suffers from stress, induced primarily by urban development. However, very little has been written about this tree and virtually nothing exists concerning post oak mycorrhizae.

FORTY YEARS LATER: HOW DO PLANTS MAKE CRYSTALS? MARY ALICE WEBB, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

Plants produce calcium oxalate crystals in species-specific patterns of morphology and distribution. These crystals form inside plant cells where they develop in intravacuolar membrane compartments, termed "crystal chambers" (Arnott and Pautard, 1965; Arnott, 1966). Forty years ago Howard Arnott presented for the first time images of crystal chambers viewed in the transmission electron microscope. This was followed by many studies examining the structure and differentiation of crystal chambers in a variety of plants, documenting their essential role in crystal initiation and development. In addition, similar membrane compartments and other organic matrices are now known to be critical in the ability of organisms to control mineral deposition in most biomineralization systems. Nonetheless, forty years after Arnott's descriptions of crystal chambers in plants, very little is known about their composition or how they control developing crystals at the molecular level. This talk will summarize our studies characterizing proteins associated with raphides, needle-shaped crystals of calcium oxalate, in grape. Using an immunological/molecular approach, we have identified and characterized several cDNAs putatively encoding crystal chamber proteins. One of these encodes a co-chaperone that we have shown to be a calcium-binding protein. Another encodes a kinesin that may be involved in moving and organizing crystals within the cell. Recently, we have developed a novel method that allows us to assay a mix of proteins extracted from isolated plant crystals and to identify individual proteins within the mixture that promote or inhibit crystal growth *in vitro*. Preliminary results using this method to identify both promoting and inhibiting proteins in grape will be presented.

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TRAUMATIC RESIN DUCTS IN OLD WOOD OF WHITE SPRUCE

HOWARD J. ARNOTT[1], ANGELIKA CLEMENS[2] AND DAVE MEKO[2]

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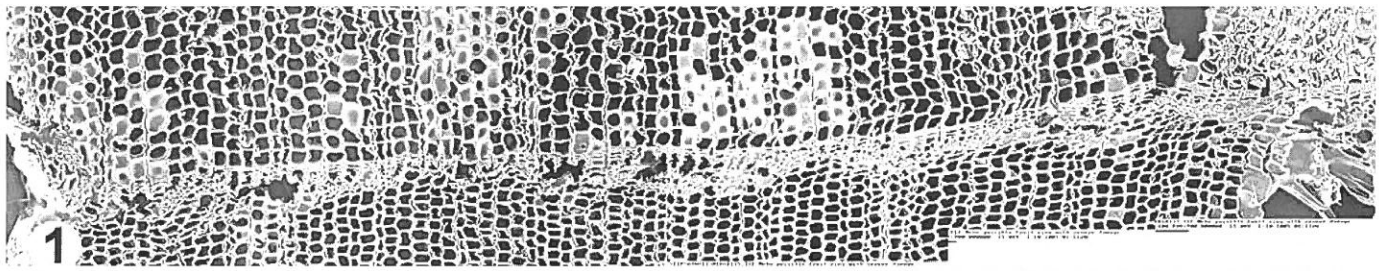
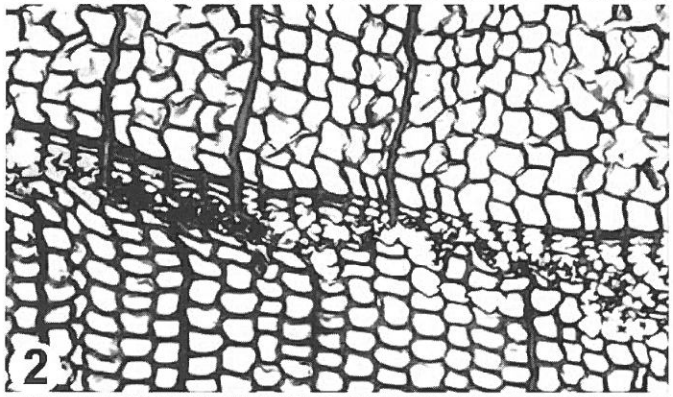
Cores from white spruce (*Picea glauca*) collected near Lake Athabasca, Saskatchewan, Canada were examined by both Light (LM) and Scanning Electron Microscopy (SEM). Two cores (QF507A and QF507B), collected in 2001 were carefully examined for abnormalities in growth patterns such as frost rings. Core QF507A contained 212 rings extending back to 1789 and core QF507B had 198 rings extending back to 1803. The cores were mounted in the usual manner and sanded to produce useful surfaces. The cores were scanned at 2400 dpi in order to provide a permanent record of the growth rings in each core. Two promising segments from QF507A and a single segment from QF507B were identified. Using a fine hack saw blade, the cores were cut down to their center, segments were removed and glued onto wooden blocks. This technique retains half of the original core *in situ* should the need of more material be necessary. Thin sections of the cores were made for LM and SEM using a sliding microtome. Sections for LM were stained with safranin, mounted in resin and viewed in a Nikon microscope. Sections for SEM were dehydrated in 100% ETOH and placed between two microscope slides, dried in an oven at 60°, and subsequently mounted on stubs. The specimens were sputter coated with gold/palladium and were studied using a JOEL 35C Scanning Electron Microscope equipped with a VitalScan digital recording device.

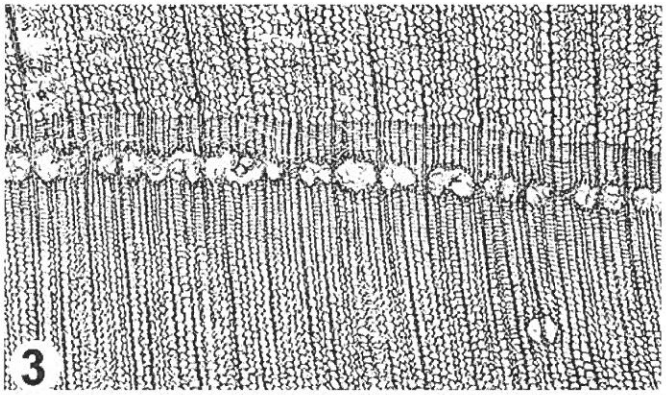
Possible frost rings were seen in QF507B in 1809 and 1829 annual rings, however, none were identified in QF507A either in 1809, 1829 or any of the other 210 annual rings. In QF507B, the late frost ring of 1809, viewed only by LM, shows a typical bending pattern in the tracheid rows similar to that seen in the late frost rings of bristlecone pine wood. The 1829 "frost ring" extended across the entire core but was difficult to define (Fig. 1). The possible "frost ring" begins just a few cells before the thin layer of cells forming the late wood of the 1826 annual ring. In this "frost ring" many tracheids seem to be broken or distorted and cavities can be seen in several areas (Fig. 2). This pattern does not match the characteristics of other frost rings. Alternatively, this anomalous structure may be the result of some type of growth trauma or perhaps may be an artifact of the collection and mounting processes.

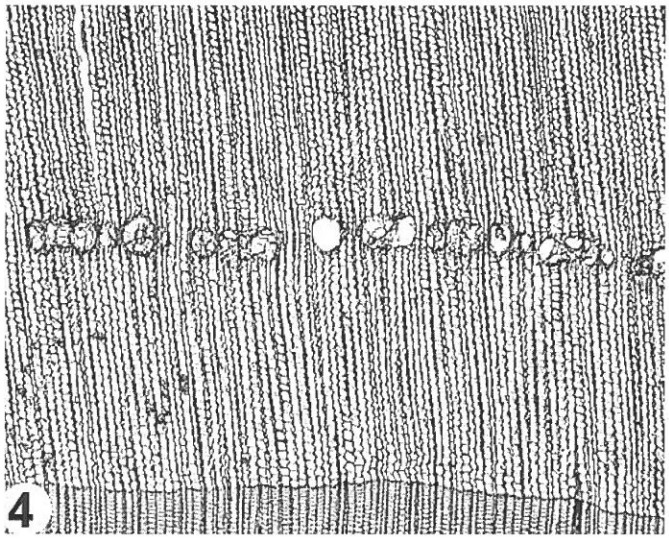
Several tangential rings of resin ducts were found in core QF507A. These rings consist of numerous resin ducts forming an almost continuous line across the entire core (Fig. 3-4). The lines may contain as many as 26 resin ducts and averaged 23.8 ducts in the four "complete" lines of resin ducts found. "Complete" resin duct rings were found in the annual rings of 1792, 1808, 1820 and 1975, a partial resin duct ring containing 15 resin ducts was found in 1789 (Fig. 6). Of course, there is no way of knowing how extensive the resin duct rings were in the entire tree, and the use of "complete" only referrers to the core. The set of resin ducts forming rings were most often in the central part (midyear portion) of the annual ring. However, the resin duct ring of 1975 (QF507A) occurred very late in the season, only a few cells from the end of the growing season in an annual ring of normal thickness (Fig. 3). Most annual rings (those without resin duct rings) had less than three resin ducts randomly distributed in the ring; many annual rings had no resin ducts (Fig. 6). Individual resin ducts forming the rings, often have rays separating them, tracheids were usually not found between them. Occasionally resin ducts are appressed to each other in the line. Individual resin ducts are more or less circular in shape and are about 150 to 200 Fm in diameter; occasionally rings are oval rather than circular (Fig. 5). Little difference was seen between the resin ducts in the tangential resin duct rings and the randomly distributed resin ducts in other annual rings.

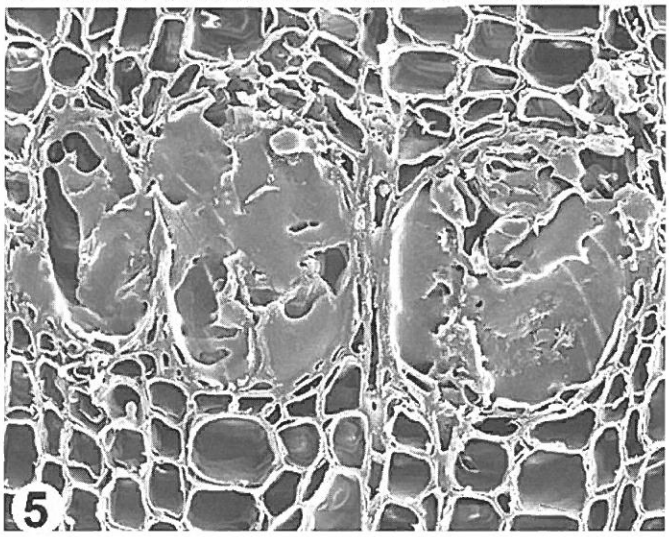
Are the resin duct rings shown here the same as the traumatic resin ducts reported in the literature: Berryman, 1972 (Bioscience 22:598-602); Fahn et al, 1979 (New Phytol. 82:537-544); Alfaro, 1995 (Can J For Res 25:1725-1730); Bryn McKay et al, 2003 (Plant Physiol. 133:368-378)? These reports indicate that traumatic resin ducts formation (resinosis) can be induced by mechanical wounding, insect attack, or by certain elicitor molecules (See Nagy et al 2000, Am J Bot 87:302-313) for the development of traumatic resin ducts induced by methyl jasmonate. In Sitka spruce Bryn Mckay et al found a partial ring of tramautic resin ducts, almost exactly like those seen here, four months after being induced by mechanical drilling . The simplest explanation for the resin duct rings reported here, is that they were caused by insect attack; perhaps even caused by the White Pine Weevil, which has been shown to produce traumatic resin ducts by Alfaro 1995 (Can J For Res 25:1725-1730). It is interesting to note, that the tree of core QR507A was free of resin duct rings for 155 years, that is, between 1820 and 1975. Prior to that four "complete" resin duct rings occurred in the 31 years between 1789 and 1820.











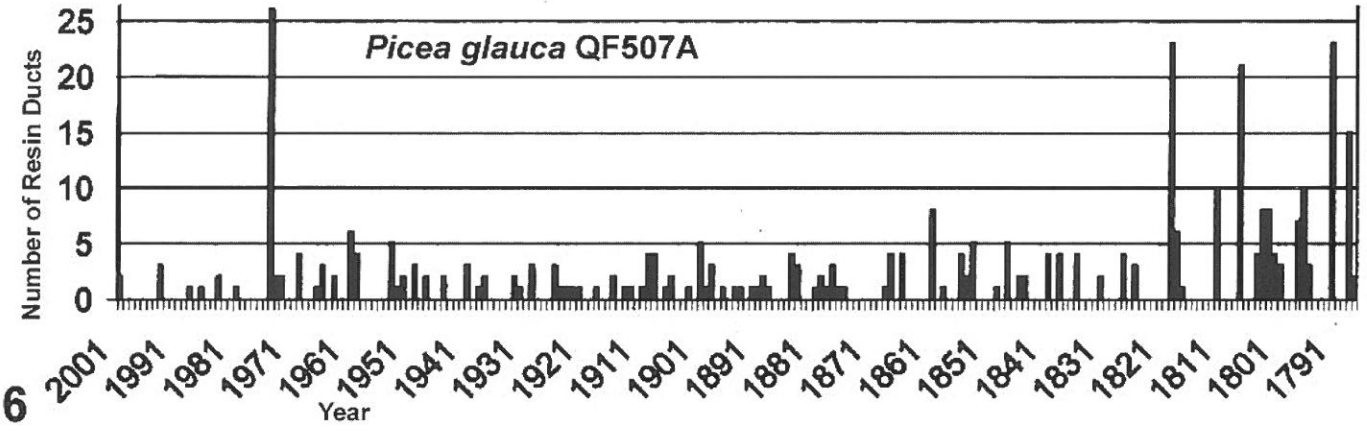


Figure1. Montage of SEM micrographs showing a possible frost ring in QF507B in 1829. 23X. **Fig. 2.** LM showing area of the 1829 possible frost ring with torn and broken tracheids. Note the strong bending of rays. 200X. **Fig. 3.** LM showing resin duct ring in the late wood part of 1975 annual ring, the ring is almost "complete" with occasional rays passing through the line. Note that many resin ducts are filled with tyloses-like cells. 45X. **Fig. 4.** LM showing resin duct ring in the center of the 1808 annual ring. Note that many resin ducts are filled with tyloses-like cells. 45X. **Fig. 5.** SEM micrograph showing structure of the resin ducts of 1808 annual ring. Individual resin ducts are filled with tyloses-like cells inside the epithelial layer. 360X. **Fig. 6.** Graph showing the number of resin ducts found in each of the 212 rings found in QF507A. Four annual rings have more than 25 resin ducts which in each case form an almost "complete" ring crossing the entire core.

MATERIAL SCIENCES
SPRING 2005

INVESTIGATION OF CATASTROPHIC FAILURES IN HIGH-POWER MOSFET DEVICES. JOSEPH COLANGELO, Failure Analysis Lab, Raytheon, McKinney, TX 75071.

MOSFET power transistors have been in widespread use as high-power switches and in power supply applications. The transistors operate with high efficiency and require only basic drive circuitry. This presentation will review the various techniques that were employed to investigate random catastrophic failures of power MOSFETs in an airborne radar system. The results from IR imaging, SEM/EDS, microsectioning, X-ray, SAM, and electrical test will be discussed. The evidence pointed to physical degradation of the transistor package as the most likely cause of failure.

DISPERSION OF MONTMORILLONITE LAYERED SILICATES (MLS) IN BULK AND THIN POLY ETHYLENE TERPHTHALATE POLYMER FILM. LAXMI SAHU, NANDIKA ANNE D'SOUZA AND DAVID C. GARRETT, Department of Materials Science and Engineering, University of North Texas, Denton, TX-76207

Polymer nanocomposites based on dispersion of clays such as montmorillonite layered silicates (MLS) have shown promise as organic-inorganic hybrids with the potential to improve properties compared to pure polymer. The magnitude of improvement of different properties depends on size and dispersion of MLS. Dispersion of the MLS is studied both in bulk and thin film. Thin films of polymer and nanocomposite are formed by spin casting the solutions onto Si wafer. Bulk films are processed by extrusion method. Transmission electron microscopy is used to observe the dispersion of MLS in bulk film and optical microscopy is used for thin film. Effect of MLS dispersion is correlated with mechanical and barrier properties in bulk film and thermal properties such as glass transition and thermal expansion in thin film.

DETERMINATION OF METAL PENETRATION IN LOW-K DIELECTRIC THIN FILMS USING EFTEM, STEM-EDS, AND Z-CONTRAST IMAGING. BRIAN P. GORMAN and RICK REIDY, Department of Materials Science and Engineering, University of North Texas, Denton, TX 76201

Metal interconnect penetration into porous, low-k dielectric thin films can have a large impact on the electrical performance of microelectronic devices. Penetration into the dielectric can change capacitance values by changing the geometry of the layers, and ultimately increase RC losses. Penetration depths vary on the nanometer scale, depending upon material parameters such as pore size and surface functionality. Characterization of metal penetration on this length scale requires advanced electron microscopy techniques. In this work, high resolution transmission electron microscopy techniques were developed to differentiate the average metal penetration depths.

Cross-sectional TEM samples of blanket silsequioxane thin films on Si substrates were prepared using a dual beam focused ion beam (FIB, FEI Nova 200) and *in-situ* liftour (Omniprobe Autoprobe). Energy filtered TEM and STEM-EDS profiles were used to determine penetration depths using material chemistries in an FEI Tecnai F20ST high resolution TEM equipped with a Gatan Tridiem energy filter/PEELS spectrometer. Z-contrast and high-resolution imaging were used to directly image changes in crystallography and atomic number on the nanometer scale. Using combinations of these techniques, metal penetration depths down to 1nm were determined.

GRAIN BOUNDARY STUDIES IN $CuInGaSe_2$ USING EBSD IN THE DUAL BEAM FIB. BRIAN P. GORMAN, Department of Materials Science and Engineering, University of North Texas, Denton, TX 76201

Chalcopyrite structured II-IV-V_2 and I-III-VI_2 semiconductors are important materials for polycrystalline thin film photovoltaics. Specifically, $CuInSe_2$ and $CuInGaSe_2$ (CIS and CIGS) have a high tolerance for defects and stoichiometry changes. Unlike other optoelectronic semiconductors, CIS and CIGS have shown stable polar <112> facets. This results in Cu vacancies or interstitials, which has been suggested to be responsible for the enhanced grain boundary conductivity. These facets have been observed experimentally using AFM and XRD, but grain boundary normals (defining the structure of the grain boundaries) have not been examined on the localized scale. In this study, EBSD was used to study the frequency of formation of these <112> grain boundaries. 1μm thick CIGS samples were grown using CVD on Mo-coated glass substrates. Uncoated samples were imaged in an FESEM at 20keV. A Digiview EBSD system (EDAX/TSL, Inc.) was used to map the grain normals of these samples, from which maps of the grain boundary trace normals could be determined.

In order to determine the grain boundary normals (3-D *vs.* 2-D grain boundary traces), EBSD experiments were conducted in an FEI Nova 200 Nanolab dual beam FESEM/FIB. Initially rough surfaces were milled using a 5keV accelerating voltage on the ion column, and subsequent mills allowed for 3-D examinations of the grain boundary trace normals. Once <112> grain boundary trace normals are determined, TEM samples can be made normal to the grain boundaries and directly examined in the TEM.

USING MICROSCOPY AS A STOCK SCREENING TOOL FOR DEFECTIVE INTEGRATED CIRCUIT COMPONENTS. JULIAN HARRIS and JAMES IZZO, Failure Analysis Lab, Raytheon, McKinney, TX 75071.

Manufacturing and processing defects, if caught early, can save a company millions of dollars. Furthermore, fault isolation in failure analysis of Microelectronic components can also guide production to minimize failures. Microscopy is an important analytical tool that can quickly and reliably provide detailed information to aid in identifying defects. This presentation will give a brief background in the process Raytheon uses to screen microelectronic components purchased from non-franchised distributors and as well as examples of recently found non-conforming components. Examples of vendor defects that can lead to component failure will also be examined.

EFFECTS OF MAGNESIUM AND MANGANESE ON THE PRECIPITATION AND MECHNICAL PROPERTIES OF MODIFIED 319 ALUMINUM ALLOYS. J.Y. HWANG[1], H.W. DOTY[2], L.A. DEMPERE[1] AND M.J. KAUFMAN[3]. [1]Department of Materials Science and Engineering, University of Florida, Gainesville, FL 32611; [2]GM Powertrain, Metal Casting Technology, Milford, NH 03055; [3]Department of Materials Science and Engineering, University of North Texas, Denton, TX 76203.

The influence of Mg and Mn additions on the structure and properties of Type 319 aluminum casting alloys has been examined using a combination of optical, scanning and transmission electron microscopy. The baseline 319 alloy (Al-7wt%Si-3.8wt%Cu) is widely used for automotive engine blocks and heads due to its combination of castability, density and mechanical properties. It is shown that these modified alloys respond favorably to the aging treatment and that these alloying additions enhance the strength considerably without too great of a loss in ductility. TEM analysis is used to follow the precipitation behavior in the matrix while optical microscopy and SEM are employed to follow any changes

the interdendritic phases in the casting. The combined results are used to explain the measured properties and their dependence on thermal history. This work is supported by General Motors Powertrain Division. The use of the facilities in the Major Analytical Instrumentation Center at the University of Florida and those at the Center for Advanced Research and Technology at the University of North Texas are gratefully acknowledged.

A NOVEL USE FOR THERMAL IMAGING: MICRO-CURCUIT DEVICE DESIGN. C. TODD SNIVELY, Failure Analysis Lab, Raytheon, McKinney, TX 75071

Thermal imaging has been widely used in industry as a tool to identify thermal gradients on systems such as electrical substations to areas small enough to reside on a silicon die. Police forces use thermal imaging to track suspects at night, as do firefighters in the search for hot spots in burning buildings. The application of thermal imaging provides information regarding thermal characteristics of a desired object. This presentation will provide a basic explanation regarding the use and theory of thermal imaging as well as provide typical examples of its use. Furthermore, a detailed look into how thermal imaging was able to validate a circuit design will be presented.

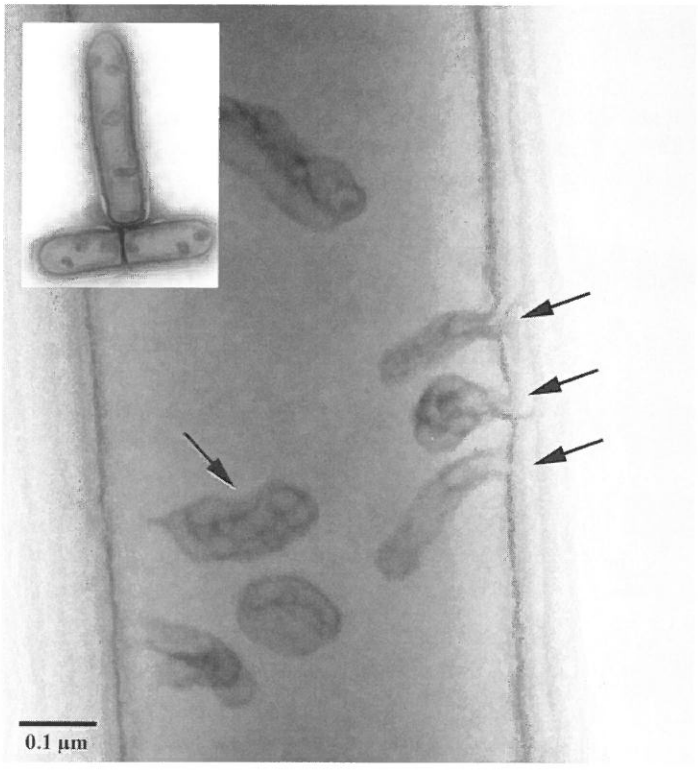
EFFECT OF TITANIUM INCLUSIONS ON CRACK PROPAGATION IN 321 STAINLESS STEEL. KIRK WIGGINS, Failure Analysis Laboratory, Raytheon Systems Company, McKinney, Texas 75071

Austenitic corrosion resistant steel is subject to corrosion sensitization because of chromium carbide formation. To counteract this, manufacturers add 0.4% titanium to 304 stainless steel. Applied titanium preferentially forms titanium carbide which does not result in sensitization. However, titanium exists as cubic inclusions which influence the propagation of cracks under some conditions. Strain controlled fatigue tests were performed to predict the life of parts made from 321 stainless steel. Both SEM and optical images were obtained for fracture surfaces and sections. The propagation of cracks in the presence of titanium inclusions is documented.

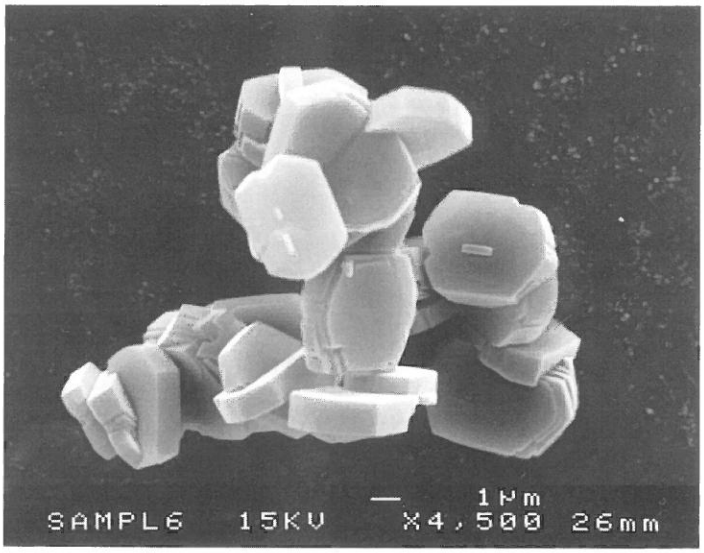
Material Sciences continued on page 20

Answer to "What Is It?"

from Texas Journal of Microscopy 35:2



Negative stain preparation of unfixed *Bacillus licheniformis* cells. Arrows point to invaginations of the cytoplasmic membrane, referred to as mesosomes. Mesosomes are thought to be either biochemically active components of the cell, repositories of new membrane for use during cell division, or artifacts produced during staining and or fixation.
TEM by Bob Droleskey, USDA/ARS/SPARC, College Station, Texas 77845.



Zeolite crystals.
SEM by Michael W. Pendleton, Microscopy & Imaging Center, Texas A&M University, College Station, Texas 77843-2257.

BOND FAILURES RELATED TO PLATING REWORK PROCESS

JODI A. ROEPSCH

Raytheon NCS Shared Services Failure Analysis Lab, McKinney, TX 75071

An investigation of non-stick gold bonds was carried out to determine root failure cause. During thermasonic bonding of a gold bondwire to a gold pad, non-sticks were occurring. This resulted in the gold plating pulling up, exposing the underlying electroless nickel plating. (Image 1) The plating stackup is electroless nickel, immersion gold followed by electroless gold. The immersion gold provides a good surface on which the electroless gold can plate.

During this study, two vendors of similar boards were compared. Vendor A had failures at the Ni surface while Vendor B did not. Examination of the failures determined an oxide layer is present between the nickel and gold plating layers. (Image 2) The corresponding coupons to the failed boards also had an oxide layer at the nickel plating surface. The failures from Vendor A were from panels that had been reworked. The data suggests the rework process is resulting in an oxide layer at the nickel surface. The oxide layer affects the adhesion between the nickel and gold plating layers. Gold bondwires were subsequently pulled up from the failed board and analyzed. The data shows the electroless gold from the board is sticking to the gold bondwire and that some of the nickel from the oxide layer is pulling up with the gold.

A stock screen was carried out on coupons associated with each panel of boards still in stock. The gold was etched and the Ni surface examined by SEM. A total of about 70 coupons were examined. None of the coupons had the oxide layer identified on the failed boards. However, about half of the coupons had a black tarnished appearance. Although black pad defect has been identified on the coupons and boards examined to date, the discoloration does not appear to be the result of this defect. EDS analysis at 5keV indicates increased levels of C and O in the discolored areas. The tarnished appearance seems to stem from a thin contamination layer on the surface. To date, the tarnished discolored appearance of these coupons has not been associated with a failure.

It was discovered that the boards from Vendor A were put through a different cleaning process than Vendor B. Vendor A was having a problem with measling if put through the standard cleaning process. The difference in the cleaning process may be contributing to the failure mode and is still under investigation.

The solder mask from Vendor A and Vendor B was also studied. Vendor A has some coupons with a dark green solder mask. These coupons did not have the tarnished appearance. Other coupons from Vendor A were a light green. The light green coupons were either tarnished or had a thick oxide layer that resulted in adhesion failures. Vendor B always has a dark green solder mask. The light and dark green solder masks were compared with no significant differences found. The techniques used for this investigation include Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS), Raman Microscopy, and Gas Chromatography/Mass Spectroscopy (GCMS).

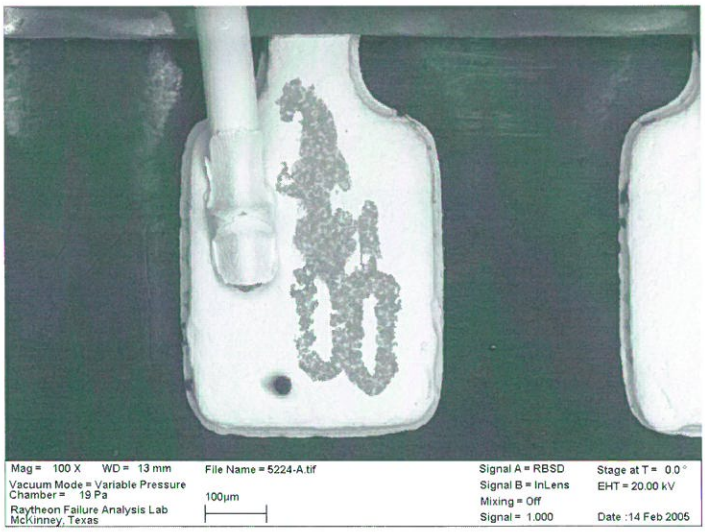


Image 1: Low magnification image of gold plated pad with exposed Nickel plating. The bonding was attempted two times before it adhered to the pad.

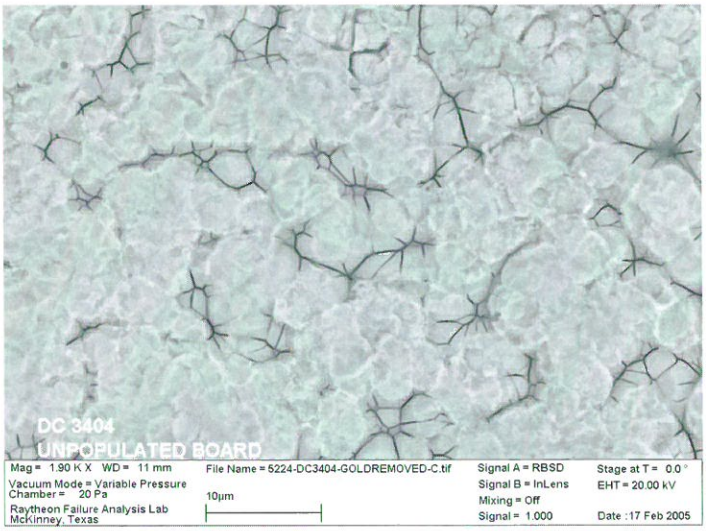


Image 2: Area of exposed nickel plating. The majority of the surface contains an oxide layer with some nickel grains exposed.

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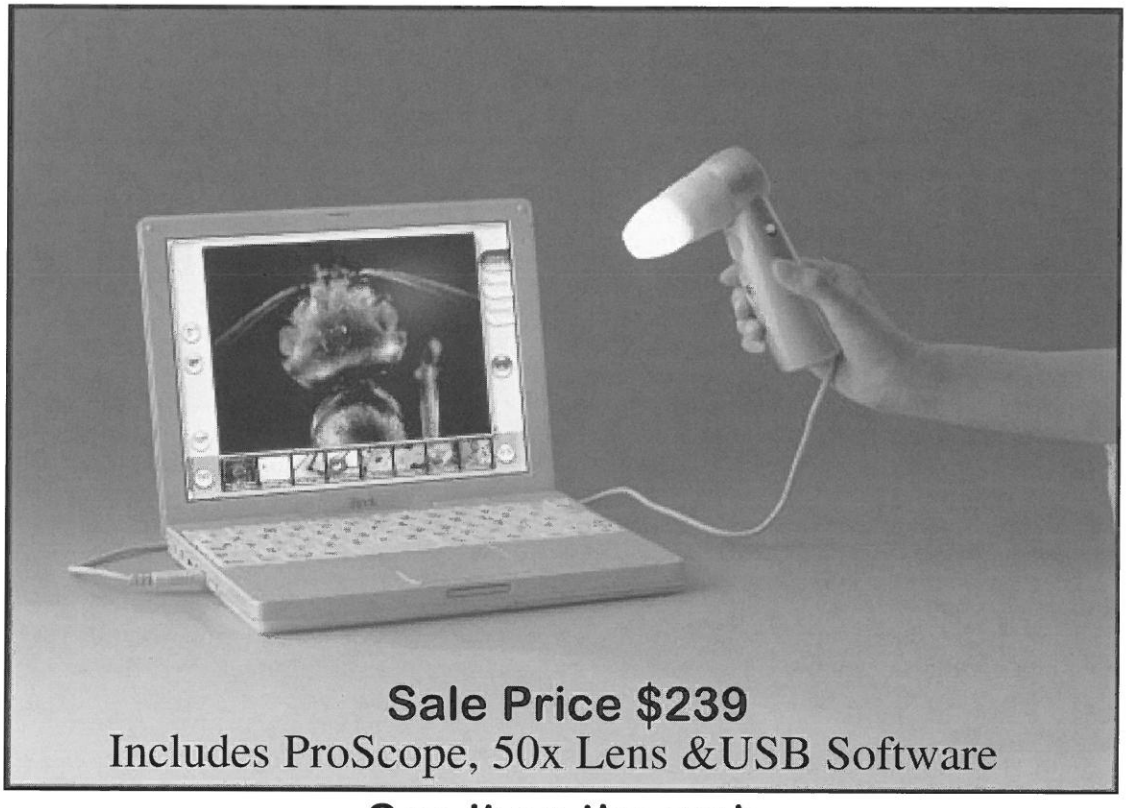

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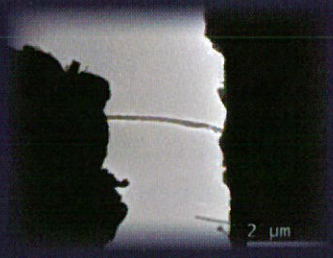
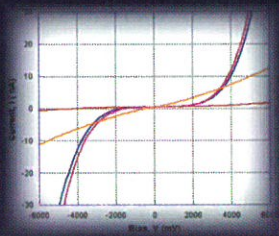
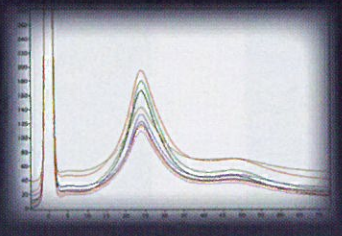


TEM image of the boron nanowire bridging the gap between STM tip and substrate

STEM DF image used for EELS SI



I-V curves acquired from the boron nanowire



EELS SI acquired from boron nanowire

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

In-situ nanocharacterization EELS STEM spectrum image

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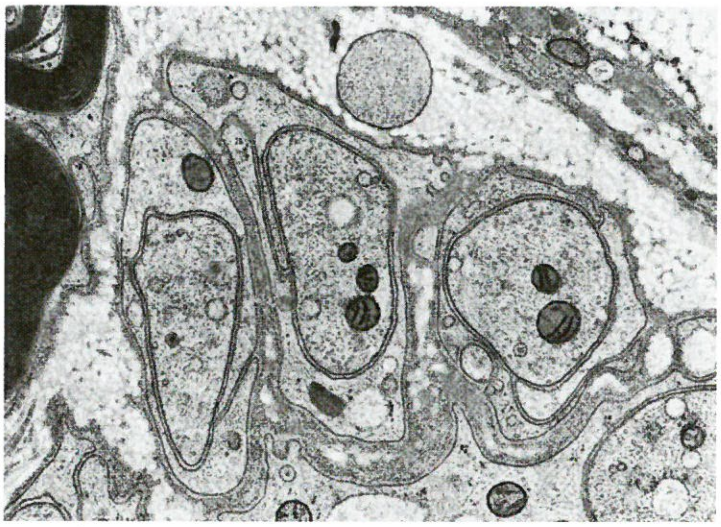
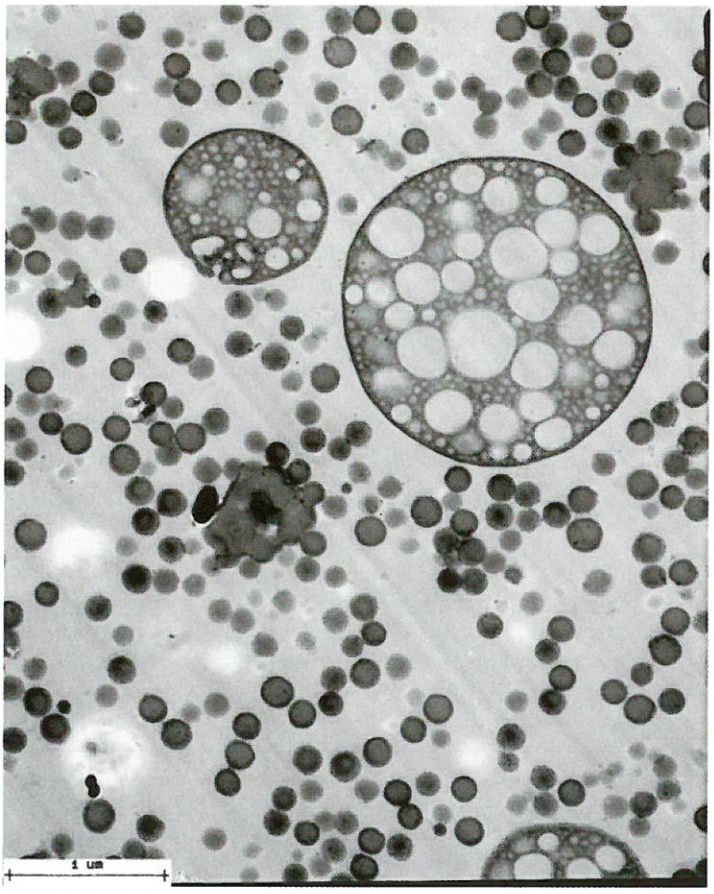


Peripheral nerve (rat), HP frozen, freeze substituted, Epon embedded, cut with the *ultra sonic* knife, section thickness 50nm.



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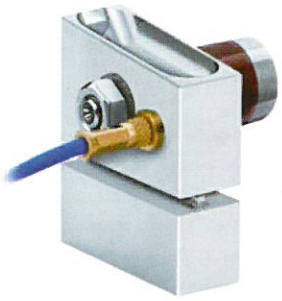

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A SHORT HISTORY OF THE DEVELOPMENT OF ELECTRON MICROSCOPY IN THE GREAT STATE OF TEXAS . . . AS I REMEMBER IT

DONALD DUNCAN

University of Texas Medical Branch at Galveston

TEXAS SOCIETY FOR ELECTRON MICROSCOPY

NEWSLETTER

Vol. 3 No. 2

Spring 1972

Supplement 1

TITLE: A SHORT HISTORY OF DEVELOPMENT OF ELECTRON MICROSCOPY IN THE GREAT STATE OF TEXAS

(As I remember it)
—Donald Duncan

The following is an account of what I know, heard and remember about early electron microscopes in Texas.

Either in 1941 or early in 1942 I proposed and recommended that the Medical Branch acquire an electron microscope. A decade earlier I had good reason to believe that there were nerve fibers too small to be visible with the light microscope. Naturally, I wanted to see them.

In June of 1942 I left UTMB and did not return until February 1, 1946. By that timeit was rumored that there was an electron microscope at the Brooks Army Hospital in San Antonio and there may have been two 'scopes in the possession of and used by industrial firms. It was about 1946, '47 or '48 that there was a microscope in Port Arthur and another in Freeport.

The first University of Texas microscope was purchased in 1947 or 1948. It was acquired by a special legislative appropriation and was assigned to the Electrical Engineering Department on the Austin Campus. The tale I heard was that this was due largely to a young member of the legislature who was also a student at the main campus. World War II produced a number of these.

Upon my return to the Medical Branch I again recommended purchase of an electron microscope. Doctor Chauncey Leake's response to this, after some delay, was that he had consulted with the authorities of the day and they had expressed exceedingly pessimistic opinions on the usefulness of the electron microscope as a biological research tool. They told Doctor Leake that what little could be garnered with this instrument had been harvested already.

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Either in 1941 or early in 1942, I proposed and recommended that the Medical Branch acquire an electron microscope. A decade earlier I had good reason to believe that there were nerve fibers too small to be visible with the light microscope. Naturally, I wanted to see them.

In June of 1942, I left the University of Texas Medical Branch and did not return until February 1, 1946. By that time it was rumored that there was an electron microscope at the Brooks Army Hospital in San Antonio, and there may have been two 'scopes in the possession of and used by industrial firms. It was about 1946, '47 or '48 that there was a microscope in Port Arthur and another in Freeport.

The first University of Texas microscope was purchased in 1947 or 1948. It was acquired by a special legislative appropriation and was assigned to the Electrical Engineering Department on the Austin campus. The tale I heard was that this was due largely to a young member of the legislature who was also a student at the main campus. World War II produced a number of these.

Upon my return to the Medical Branch, I again recommended the purchase of an electron microscope. Doctor Chauncey Leake's response to this, after some delay, was that he had consulted with the authorities of the day and they had expressed exceedingly pessimistic opinions on the usefulness of the electron microscope as a biological research tool. They told Doctor Leake that what little could be garnered with this instrument had been harvested already.

During 1947 and 1948 I sort of fumed and fumbled on this topic. I did propose to the American Optical Company that they make a modification of their microtome by reducing the pitch of the advancing screw so as to permit at least the possibility of sections less than one micron thick. Their response was it couldn't be done despite the fact it had been done long before in connection with the making of diffraction gratings.

In 1948 I learned of the invention of Pease and Baker, a simple device that reduced the unit advance of the Spencer Microtome from 1 μ to 0.1 μ. I acquired one of these wedges as soon as possible

* Donald Duncan was Professor and Chairman of the Department of Anatomy at the University of Texas Medical Branch in Galveston. In addition, he was president of the American Association of Anatomy, a noted authority in the field of neuroanatomy, a pioneer researcher in electron microscopy, and a charter member of the Texas Society of Microscopy, former Texas Society of Electron Microscopy.

AT LEFT – First two pages of Prof. Duncan's original manuscript sent for publication in TSEM Newsletter. The manuscript was produced on a typewritter.

and began to work with it. After much struggle it seemed certain that I had some sections that were considerably less than 1 μ thick. Remember, this was done with a steel knife and no trough to receive the sections. Furthermore, material was doubly embedded in celloidin and paraffin. As to where I learned about and acquired grids to mount them on I have no memory.

With sections in hand, I went to Austin to use the electron microscope. It was in the charge of Mr. Leland Antes, an electrical engineer. Between us, but mostly Antes, we produced the first EM pictures in Texas of biological specimens. After much effort, a little paper on the ultrastructure of peripheral nerves was published in 1950 (1). In the meantime, Francis 0. Schmitt, Fernandez-Moran and F. Sjöstrand had beaten me to the draw.

Antes was succeeded for a brief period by a young lady whose name I have forgotten. After leaving Texas she was for a time the EM technician for Doctor Farquhar and that is the last I know about her. She was followed by Hilton Mollenhauer. Hilton at the time was an electrical engineer. Subsequently, he developed into one of the truly distinguished members among the biologically oriented electron microscopists. Many and many were the days when I was on the road to Austin by 4 a.m. and back in Galveston by 11 p.m. Hilton and I spent many very happy but nearly always frustrating hours together. Each visit was always preceded by weeks of effort here at the Medical Branch. Somehow I did manage to put out another paper, this one on the posterior lobe of the chicken pituitary. Our chief problem was focus. In retrospect, I cannot understand why this was so; but at least 9 pictures out of 10 were hopelessly fuzzy.

One amusing incident occurred on a fine spring day when Hilton and I were working together. Close to noon, a red headed fireball of a young woman burst into the E.M. lab where we were working. (By the way, this was the first air-conditioned facility on the Austin campus). Without introduction or explanation, this lady said, "Are you the people who work with the electron microscope?" We gently allowed that we were. The next blast was .. "I don't want my boss to know that I am here, so I'll not tell you where I come from". More or less glaring at Hilton, she said .. "Can you cut thin sections"?. His answer was no, that is Dr. Duncan's department. So it was my turn, "Where are you from, how do you do it, etc, etc"?. My response was .. "You are from Houston, aren't you"? She wanted to know how I knew that and I said it was fairly obvious. "You won't tell my boss, will you?" "Of course not, but if you will come to Galveston a week from today (Saturday) with some of your blocks I shall try to help you." The story behind this is to the effect that Dr. R. Lee Clark requested funds from the Regents to buy an electron microscope and was turned down. So he went down Fannin on Main Street a few blocks and came back with the money to buy the best available (2). The Medical Branch did not have an electron microscope until Doctor Leake left and the institution was essentially managed by Doctor Blocker. This was carefully disguised as the infamous "Interim Committee", but that is another story. Truman Blocker secured the necessary money for the first 'scope from the Brown-Lupton Foundation. This must have been in 1955. The previous year I received an NIH grant for the development of biological electron microscopy in Texas, a grant that has been approved until 1974.

The initial instrument was the old RCA IML which is still functioning at Lamar University in Beaumont. Charlie Moore of Dallas uncrated it and put it together. He more than anyone I know might have facts and figures on the first ten microscopes in Texas.

Prior to the first one at the Medical Branch, first came the one in the Electrical Engineering Department at Austin. Texas A&M University received one at about the same time but this was put in the hands of a faculty committee and as a result was virtually sterile. Then came the one at M.D. Anderson, and following the Medical Branch, another EML found residence at Southwestern Medical School in Dallas. Not too long after the installation at Southwestern, I was invited to bring specimens and demonstrate its potentialities. Fortunately, I had some very good sections of the pars intermedia of the rat pituitary and took those with me. The Dean, a pathologist by trade, was very interested. Cell boundaries, mitochondria and Golgi apparatus seemed all according to Hoyle. Next we looked at the nucleus and especially the nucleolus. He said, "Do you mean to say that the nucleolus is not a solid round dot?" My response was that the electron microscope says no. He said, "I give up", and left. Subsequently, this microscope was used with ever so great effectiveness by Hilton Mollenhauer.

Probably the third electron microscope in an educational institution came to Rice University under the control of Doctor Milligan. It was a Philips 100. I spent one delightful day with him and the new microscope. Although he was the perfect host and put all else aside for the day, it was obvious without asking that the biologists at Rice or from anywhere else were not to have access to that particular microscope (3). In summary, the first working microscopes in Texas belonged to industry. Now twenty years later the same can be said of the electron probe.

P.S. Our service men were in succession: Charley Moore, Mr. Lubker, Pat Tyner and Bob Shorthose.

EDITOR'S NOTES:

1) Duncan and Antes. Some electron microscope observations on the structure of myelin sheath and axis cylinder in thin sections. Texas Reports on Biology and Medicine, 8: 329-340. 1950.
2) This must have occurred in *1954* or *1955*. The Houston Post reported the installation on December 13, 1955. The microscope was installed for use by Dr. Leon Dmochowski.
3) This electron microscope was installed at Rice University early in the spring of *1951*. Dr. Milligan states: "We ordered the projected electrostatic electron microscope to be made by General Electric in approximately 1943, but none was ever produced and the order later was cancelled.

ACKNOWLEDGEMENT:

Many thanks to Jose A. Mascorro, Tulane University Health Sciences Center, charter member of TSEM, for providing the above article for publication in the Texas Journal of Microscopy for its 40th anniversary.

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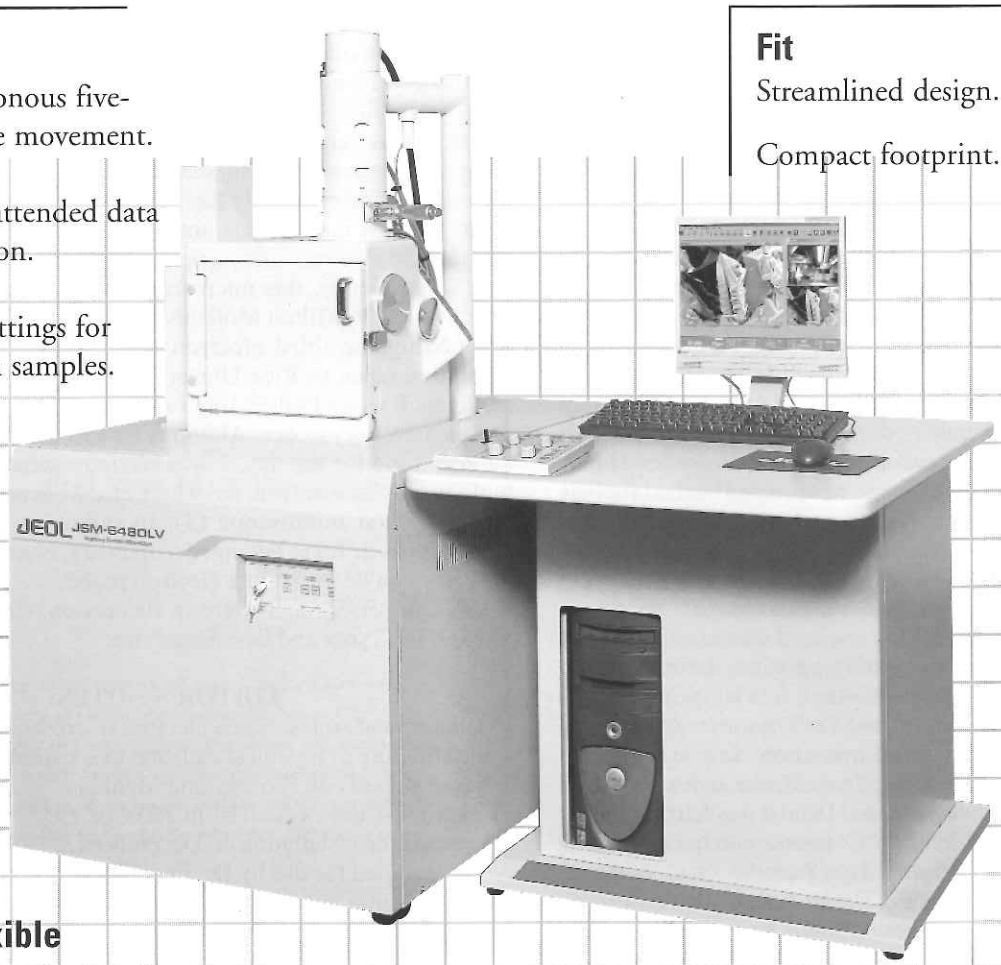

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HOWARD J. ARNOTT

Autobiography-Part One

I was born March 9, 1928, in the Queen of the Angeles Hospital, Los Angeles, California. My parents were of "the working class," they attended high school, but neither went to college, My father, Andrew H. Arnott, still in his early 20's, started as a news photographer; first with newsreels for the *International Newsreel* and later, as a still photographer for *The Los Angles Examiner*, a Hearst Newspaper. While working for the *Examiner*, he made a unique set of photographs of the "Hearst Castle" at San Simeon, California (now the Hearst San Simeon State Historical Monument). For most of his career he worked for the *Los Angeles Times*, where he covered many stories including the building of Hoover Dam, Howard Hughes plane crash, the shooting of Bugsy Siegel, murders, kidnappings and other bizarre episodes so characteristic for Southern California. Sometimes he took me along on assignments; on one occasion I witnessed many stoic Japanese-Americans families boarding a train which took them to internment through WWII. My mother, Evelyn L. Arnott, worked for a department store as a buyer of women's clothes; she often arranged and narrated fashion shows.


About 1932


Easter, 1934

As a child I lived, along with my extended family, in a multi-ethnic neighborhood just east of downtown Los Angeles. From my grand parents home we could easily see the tall pointed Los Angeles City Hall, which was then the tallest building in the city. I remember vegetable peddlers and the ice-man with their horse-driven carts, feeling the Long Beach earthquake, seeing zeppelins, playing "hit-the-can," "hide-and-seek" and other youthful games all on Pomeroy Avenue. My parents often went to local Jewish restaurants where I learned to enjoy *jelly omelets*, *chopped chicken liver sandwiches* and *kosher dill pickles.* I began school at Evergreen Elementary and still have a wooden giraffe that I made in the 3rd grade.

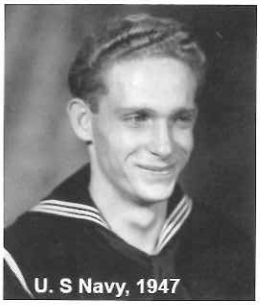
In 1939 we moved to a new 2 bedroom house in a western suburb of LA. With that move I left the multiethnic children of my youth and entered the 6th grade in the 39th Street Elementary School where I met the "upper middle class children" of my second decade. We learned singing, dancing and art in addition to the spelling, history and the various components of "youthful education" of the 1930's. Our new house was located on land that was a former bean field, land that was once part of a famous Los Angeles truck farm; it had marvelous sandy loam soil. The area just north of us remained an open field for many years. It was a place where my friends and I hunted with our BB guns always hoping to kill one of the "great hawks" that lived there. On certain nights the Barnum and Bailey Circus would move onto the field, and in the morning we watched the wonders of the big top coming to life. One afternoon the famous Wrong-Way Corrigan crash-landed in the field and turned his plane upside down, we all (including Corrigan) had a great time!


on Pomeroy Avenue

The area we lived in was subject to flooding causing the city to build a huge underground flood control system. The local storm drain system consisted of two large parallel tunnels, about eight feet tall by six feet wide which extended from our area several miles to Ballona Creek. All through Junior High I watched the system being built. When completed it wasn't long until we realized these "tunnels" were places of *great adventure*; so, first on foot and later on bikes we traveled through them all the way to Ballona Creek. In those days we rode bicycles everywhere.

WORLD WAR II

On a sunny Sunday afternoon in December, 1941, my father ran into the yard to tell our neighbors and I that Pearl Harbor had been bombed. World War II began on that day. My father soon enlisted in the Army Signal Corps and was gone. The next few years were filled with War Bond sales, rubber & scrap metal drives, ration books, blackouts, victory gardens and all manner of efforts to aid the war effort. Because of the strategic importance of Los Angeles, a number of anti-aircraft guns and searchlight units were stationed in our area. When they began firing in the middle of the night it was something to remember. It is not difficult to comprehend the kind of stress which Europe had night after night.


U. S Navy, 1947

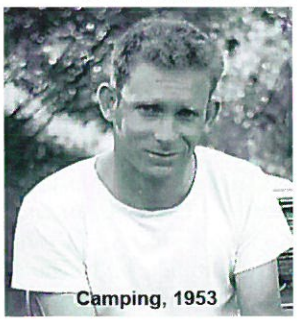
During the war school went on as usual even though there was uncertain urgency about many aspects of life. The government wanted us to complete high school, however, many left to "aid their country and find their fortune" in the service. When I was sixteen I purchased a 1929 Ford roadster for $25.00 and immediately modified it by removing all the fenders and later "updated" it with the addition of a V8 engine; it could run pretty fast. My remembrance of high school is somewhat neutral. I liked Physics, Biology and Radio Shop, endured English and hated Latin. I enjoyed football and soccer and lettered in football. During high school, in the evenings and summers, I worked in a gas station, as delivery boy, a lathe operator and as a roust-a-bout in a commercial bakery. I was working in the bakery on VE Day (May 8, 1945) and continued there until I entered the Navy. When, at last, high school graduation came I became subject to the Draft, which would have meant service in the U.S. Army. I choose to join the U.S. Navy.

U.S. NAVY PHOTO SCHOOL

HJA and Joe Hamilton, 1947

I entered the Navy in May of 1946; taking boot camp in San Diego and later being sent to Air Combat Training School in Florida. It took the coal-burning troop train 6 days to cross from San Diego to Florida but it was my first time to see how big the U.S. is. I went to Air Combat School in Jacksonville, Fl., where we were sorted out for different jobs. I was sent to Photo school at Naval Air Training Base in Pensacola, Fl. to learn to be an aerial photographer. It was an interesting and sometimes exciting six month course. We started with 4x5 Graflex Cameras, worked our way up to Speed Graphics and then went to aerial cameras. We were taught to develop and print our own work and how to make montages of aerial maps. The first aerial work was in the back of SNJ's, a single motor, low wing plane with room for a pilot and a crewman. Photography was done by standing in the rear cockpit and leaning out to the left while the plane circled the target. Later we were taught how to fly map missions where the photographer controls the path that the pilot follows. In the last phase of photo school we learned about movie cameras and making movies. Everything stage, and each photo assignment was graded using the 4.0 system. My best friend in Photo School was Joe Hamilton who later became a TV producer/director and husband of the comedienne Carol Burnett. From photo school I was assigned to a squadron based at the Naval Air Station in San Diego. My squadron had a large photo lab and there I photographed air plane crashes, broken engine parts, basketball games, Admirals in full dress (color) and aerial photos of torpedo practice. I left the Navy with many photographic skills which turned out to be life-long assets.

UNIVERSITY OF SOUTHERN CALIFORNIA


Camping, 1953

After returning home in 1948, I was admitted as a pre-dental student at Univ. of So. Calif. I found many of the pre-dental courses tedious. Luckily, however, I took a botany course with Dr. Thomas Fuller and soon switched my major to botany. As is often the case, a single teacher made all the difference. The botany courses were interesting and well taught. As a junior, I became involved in two interesting research projects: the embryology of *Yucca* (several species) and the sterile culture of embryos extracted from the seeds of *Yucca brevifolia*. In the extracted embryo research I added penicillin in half of the tubes. However, there was no increase in growth when compared to the control. The embryological studies became part of my Masters thesis. Hind sight indicates that experimental embryo culture might have been the better direction for my future research. In 1949 I drove across the country, entered Canada and went fishing at Wedgeport, Nova Scotia. I caught four large blue fin tuna (*Thunnus thynnus)*, the largest 565 lbs. The trip reinforced the great size of the U.S. and the tuna fishing was a once-in-a-life time adventure.

It was just after this trip and mid way through my undergraduate days when I married Jean Cross. I continued to work at the bakery and Jean worked for a bank near the USC campus. The Botany Dept. at USC was small and students and professors got to know each other quite well. Many of my contemporaries went on to Ph.D. work and became successful Botanists in major institutions. After considering the possible positions available to me, I decided to work on a Ph.D.

UNIVERSITY OF CALIFORNIA AT BERKELEY

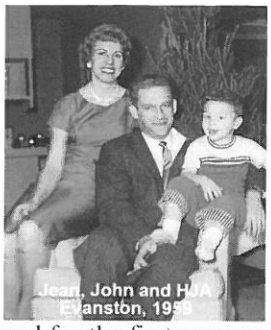

Berkeley, 1956

I was admitted for graduate work at Univ. of California at Berkeley and in 1953 Jean and I began living just north of the campus. At that time the Botany Department had three options: mycology/phycology, structural botany and functional botany. I chose the structural option and decided to work with Adriance S. Foster. The structural option required a certain set of courses, a reading understanding of French and German, oral examinations, an independent research project and dissertation. The course work, orals, research and dissertation were relatively easy for me but the languages, especially German were major stumbling blocks. I shared an office with Marion Cave and learned a great deal about plant embryology from her. Working together we found that *Paeonia* has a free nuclear stage in embryo development thus settling a long standing argument in the literature. In my second semester I began working in the University Herbarium. Under the supervision of Annetta Carter I printed labels, sorted and filed herbarium specimens and in general learned how a herbarium functions.


Berkeley, 1958

Tea Time in the Herbarium presented a special opportunity to meet an amazing group of botanical literati who came, not just from Berkeley, but from all over the world. At that time the Botany Department was one of the best in the world and so its visitors were truly impressive. I remember meeting Linus Pauling, Katerine Esau and Irving Bailey at the pre-seminar coffee sessions. In 1955 I began as a teaching assistant in General Botany working under Bill Jensen. I n 1956 I became Adriance Foster's teaching assistant in Plant Morphology and Plant Anatomy. My research on *Yucca* was independent of Foster; however, after I discovered anastomoses in the leaves of *Ginkgo biloba,* we shared a common interest in open dichotomous venation. We worked together on several projects including the open dichotomous venation in the leaves of *Kingdonia,* an angiosperm. By the end of my time at Berkeley, Adriance Foster and I had become good friends and we often ate lunch at the *True Blue Cafeteria* in downtown Berkeley. In fact, it was on one of our trips to the cafeteria that I collected the first *Ginkgo* leaf with an anastomosis. My "training" at Berkeley was certainly adequate, but with hind sight more course work in plant physiology, chemistry and genetics would have been beneficial. My dissertation, 600 pages in three volumes, was entitled "The Morphology and Anatomy of *Yucca.*" In 1957 our son John was born at Alta Bates Hospital in Berkeley. The next summer we left Berkeley with mixed emotions.

NORTHWESTERN UNIVERSITY


Jean, John and HJA
Evanston, 1959

My graduation in the summer of 1958 was bypassed as the three of us were already in route to my first academic job as an Assistant Professor in the Dept. of Biology at Northwestern University. Northwestern is located in the Chicago suburb of Evanston, at that time it was a lake side community of the wealthy. The Northwestern students were from wealthy to rich families. Housing for Assistant Professors was not abundant, and for the first year we lived in a one bedroom efficiency in the Northwestern Apartments. That year we also learned to live with a climate very different from that of California. The Northwestern Biology Department had been recently formed by the fusion of formerly *separate and competitive* departments of Botany and Zoology and an uneasy truce existed between the parties subject to the fusion.

Northwestern operated on the quarter system with teaching in the summer optional. I was assigned to teach General Biology, Plant Morphology and Plant Anatomy in successive quarters. The latter, were given in small well equipped classes and functioned very well. The General Biology course, however, was large with 600 to 800 students and not as effective. Students were taught from the stage of a large auditorium. I developed lectures for that class, was successful in giving them and helped write a Manual for the accompanying laboratory. My venation research went well and with the assistance of Shirley Tucker and Frances Fletcher we made some interesting discoveries in *Ranunculus* and *Ginkgo*. In 1962 I published a 164 page monograph on "The Seed, Germination, and Seedling of *Yucca*." Katherine Esau used some of my illustrations on the cover of her book "Vascular Differentiation in Plants." In 1962 NSF began supporting my research on *Yucca* anatomy and I became interested in applying electron microscopy to those studies. By then the paper on the corn root by Whaley and Molenhauer had been published and it was clear that electron microscopy would provide great insight into the structure and function of plant cells. While at Northwestern I graduated several masters' students and three excellent doctoral students, Elizabeth Gantt, Frances Fletcher and Harry T. (Jack) Horner. Two of our daughters, Catherine and Susan, were born in Evanston. In those days Northwestern football team suffered constant defeat but the Marching Band was really great!

UNIVERSITY OF TEXAS AT AUSTIN

In 1964 our next adventure led the five of us to the University of Texas where the football team won and the Marching Band was not only great but also great big. I accepted an NIH post doctorial position in the Cell Research Institute (CRI) with W. Gordon Whaley. Dr. Whaley was both directors of the CRI and Graduate Dean at U.T. He had a very large NIH Training Grant to educate individuals in electron microscopy.

Whaley was personally interested in the Golgi apparatus and worked closely with Hilton Mollenhauer and Marianne Dauwalder in trying to understand the development and function of this unique organelle. While Whaley had broad interests, his bias and background leaned toward plants and so the CRI was closely associated with the Botany Department; where Whaley had previously served as Chairman. Although I had already learned to "operate" an electron microscope the CRI gave me one-on-one instruction in every aspect of fixation, embedding, microtomy, light and transmission electron microscopy. Each year Whaley organized a lecture series that brought the best people in cell biology to the UT campus. Help and expertise were available at every hand; in addition to me, many others benefited from this enlightened environment. Early in our stay in Austin our fourth child, Virginia Anne was born.

The CRI was closely related to the Botany Department through both history and joint appointments. I soon came to know many of the botanists. In 1965 I joined that department as an Associate Professor with a half time appointment in the CRI. At Berkeley I had become interested in calcium oxalate crystals in *Yucca* roots. During my last year at Northwestern I did some preliminary electron microscopy on crystals cells. Almost immediately on my arrival in Texas I continued to study calcium oxalate crystal cells, by 1965 I had solved the long standing controversy as to the origin of calcium oxalate in plants. The crystals clearly originate in the vacuole.

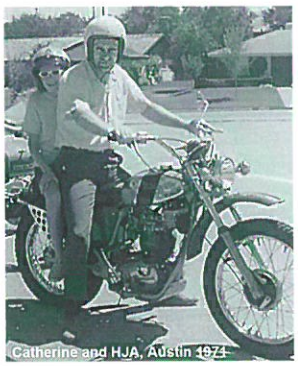
When I came to UT Malcolm Brown was finishing his work with Harold Bold. We became friends and often went to lunch at the Faculty Cafeteria just west of our building. On August 1, 1966 just after noon we left the Botany Building and began our lunch as normal. After we were seated at a window that overlooked the "The Drag" we began to notice that some odd things were happening. Two students had fallen on the sidewalk at the far side of the street. Others (students?) were" running around" in a strange way. It was about then when we heard the gunfire. Charles Whitman was firing from the Tower. Of course at the time we didn't know his name but it was clear that something frightening was occurring on the campus. Very quickly I saw "civilians" with deer rifles firing (back) at the tower, puffs of "smoke" were coming from their bullets but Whitman was still firing. Later we learned that our walk to the cafeteria had taken us directly in Whitman view, apparently he was firing in a different direction as we walked by. That day Whitman killed 14 people and wounded 31 more.

THREE ENGLISHMEN

The first of the three Englishmen entered my academic life because of our common interest in minerals. F. G. E. Pautard was a visitor in the CRI when he became interested in my work on "plant mineralization." In fact, the term "plant mineralization" may have been coined by him (or me). At Leeds University Fred Pautard worked on mineralization in bone and baleen in the Asbury Biophysics Laboratory, so a natural affinity developed between us (also he was a good fisherman). With Whaley and Pautard's encouragement, I delivered a paper, "Studies of calcification in plants." at the Third European Symposium on Calcified Tissues in Davos. The audience was by and large traditional bone and teeth scientists and they were resistant to the thought that crystals develop inside of cells. However, since that time many other workers have demonstrated the veracity of this point of view. Realizing that the crystal structure of calcium oxalate monohydrate had not been worked out, Pautard and I isolated large calcium oxalate crystals from the leaves of *Yucca rupicola*, a local native plant, and did single crystal x-ray diffraction. This allowed us to work out the crystal structure of CaOX-H2O and we published it in *Nature*. We began working on a review paper on Plant Mineralization in 1966-67 and it was published in 1970.


Fred Pautard and HJA
Snowing in Davos, 1965

The second Englishman that I worked with in the CRI was Kenneth M. Smith. Smith was a Professor at Cambridge and a major figure in the study of viruses. He approached me because of my interest in crystals, and introduced me to insect viruses, many of which were associated with protein crystals. He did the virology and I did the electron microscopy...we made a good team! Our insect virology papers showed the way in which viruses became incorporated into protective protein crystals. They also showed that the protein crystals could have different morphologies. We exam-


Catherine and HJA, Austin 1971

ined several plant viruses during that time, including the original discovery of a sunflower virus and its reproduction. The sunflower virus was isolated from plants growing near the Texas Capitol Building that Smith walked by on his way to the lab.

The third Englishman that I worked with while at the CRI was J. A. Colin Nicol. He came to the University of Texas Port Aransas Laboratory from Plymouth Laboratory of the Marine Biological Association. In actuality, Colin was a Canadian. Again, we got together when he learned of my interest in "biological crystals." As with Smith, Nicol fixed the animals and I did the electron microscopy. We worked on the Reflecting spheres in the eyes of weakfishes; the ultrastructure of a retinal tapetum lucidum in the eyes of a teleost; Tapeta lucida in the eyes of seatrout; Reflection of ratfish skin *(Hydrolagus colliei)*; the tapetum lucidum in the eyes of gars; the tapetum lucidum in the eyes of goatsuckers(birds) and other projects which involved quinine crystals in reflecting systems of animals. Of the "three Englishmen" Colin Nicol was by far the most driven and difficult to keep up with.

In addition to the three Englishmen, there were many students and other collaborators that I interacted with at Texas. Most of the research falls into the realm of cell biology. Examples are: Harriet Smith–the Axostyle structure in the termite protozoon *Pyrsonympha vertens* and also Analysis of microtubule structure in *Euglena granulate*; Diane Teigler–Crystal development in the Malpighian tubules of *Bombyx mori*; Judith Morgan–On the Endoplasmic reticulum in trichome glandular cells; Milton Rodgers–Nuclear behavior in *Coprinus*; Genie Eilert–crystal cells in the root of *Yucca Torreyi*; Zeke Rivera–Calcium oxalate in *Opuntia*; Eleanor Cox–The theca of the marine dinoflagellate, *Ensiculifera loeblichii* sp. Nov; Melvin Watson–Ultrastructural morphology of *Microthamnion zoospores*; Sue Adams–The centrioles of *Oedogonium*; Pat Walne–The comparative ultrastructure and possible function of eyespots: *Euglena granulata* and *Chlamydomonas*; Lee Ansell–Chromoplast ultrastructure in the developing fruit of the globeberry *Ibervillia lindheimeri*; Sam Rosso–Plastid ultrastructure in tomato leaves infected with tobacco mosaic virus; Joe Harris, Effects of senescence on chloroplasts of the tobacco leaf; Max Summers–Ultrastructural studies on inclusion formation and virus occlusion in nuclear polyhedrosis and granulosis virus-infected cells of *Trichoplusia ni.*; Malcolm Brown–Structure and function of the algal pyrenoid. Other collaborators were: Hugo Steinfink, Susan Fullilove, Clinton Kawanishi, Nancy Maciolek, C.W. Querfeld, Meredith Blackwell, Larry Thurston, Jim Mills, Donald Stoltz, Mike Bucek, and Marianne Dauwalder.


HJA and W. G Whaley, 1965

Strangely enough, I never cooperated on a publication with Gordon Whaley even though we were both together in the CRI for eight years. In my first years I built several displays and he supervised the art work, not the content. He had definite ideas about how things were to look. For example, for one display the background paper printed a specific color and the ink mixed specifically to match that background. Getting the paper "right" took three trips to the printer while the ink required two more trips. After several years at UT Austin I began to think about going into administration of some kind. Positions for chair were commonly open in departments throughout the country and I began to apply for them. At one time I had three administration offers at the same time—this can easily go to your head. I also interviewed with John Silber, Dean of Arts and Sciences at UT, in an effort to learn something about administration, particularly about something I could do. Unfortunately Silber was fired and my efforts went for naught.

In Part Two I'll write about some of the problems and frustrations I confronted in my 18 years of administrative life.

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Technical Tips

Combined Electron and X-Ray Induced Microbeam XRF in the SEM

Brian J. Cross, *CrossRoads Scientific,*
Kenny C. Witherspoon, *IXRF Systems, Inc.*

Energy-Dispersive X-Ray Spectroscopy (ED-XRS or EDS) is a powerful and easy-to-use technique for the elemental analysis of a wide variety of materials. Most commonly, this technique is called X-Ray Fluorescence (XRF), which classically uses x-ray photon sources to excite the sample. A Scanning Electron Microscope (SEM), of course, uses electrons as the excitation source for microbeam x-ray spectroscopy together with sample imaging using characteristic x rays and/or secondary electrons. These two XRS techniques are used independently, although often the same sample is analysed by both, to provide complementary information.

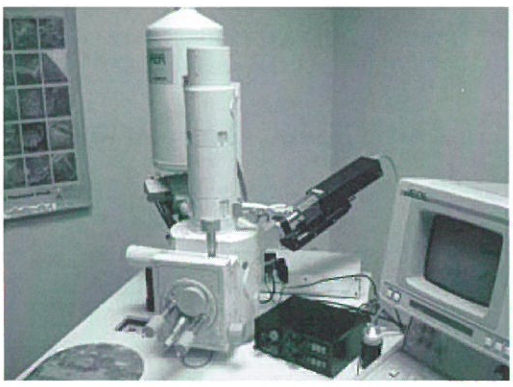
The advantages of both techniques have been reviewed several times [e.g. 1, 2], SEM-EDS being more suited to imaging and microbeam quantitative compositional analysis and maps, and XRF more suited to accurate quantitative analysis, especially for trace elements, while analyzing a much larger area. One weak area of routine laboratory XRF is for low-atomic-number elements, where the excitation efficiencies for low-energy x rays are very poor. In these cases, SEM-EDS analysis can often be better. Conversely, with an x-ray source, there is no need to coat the sample as charging is virtually nonexistent when using x-ray photon excitation.

One can take advantage of the fact that most SEM-EDS microanalysis systems already have a high-quality EDS detector, with an ultrathin window that allows the analysis of critical light elements. Therefore, to enable good-quality XRF within an SEM, one only needs a suitable x-ray source to have both XRF and electron-beam x-ray spectroscopy within the same chamber, *in vacuo* or in air, as appropriate for the sample.

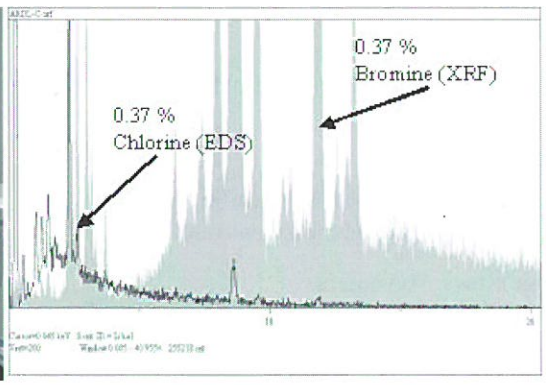
Several attempts have been made to add XRF to SEMs, using the standard EDS detectors already in place [3, 4]. Most of these used the electron beam to create the fluorescing x rays with a thin transmission-target foil placed between the beam and the sample. The main problem with this approach was the low incident x-ray flux onto the sample, especially if the analysis area (x-ray beam) is restricted. The advantages of XRF (e.g., improved sensitivities and peak-to-background ratios) were then lost because of the low count rates achieved.

This problem can be solved by attaching a separate x-ray source onto the SEM, with flux outputs orders of magnitude higher than those produced by low-current SEM beams in transmission foils. By restricting the sample analysis area with apertures or active focusing optics, one can still achieve count rates in these small areas that are typical of standalone XRF spectrometers, with all the advantages of the XRF technique [5, 6].

Examples of mounting and comparative spectra:



μX SEM Tube using a Noran Voyager detector.



μX SEM Tube spectra with trace Chlorine and Bromine

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[4] N.Gao, T.Bievenue, I.Radley, J.Nicolino, K.Witherspoon and B.J.Cross, paper presented at M&M Conference, San Antonio, TX (2003).

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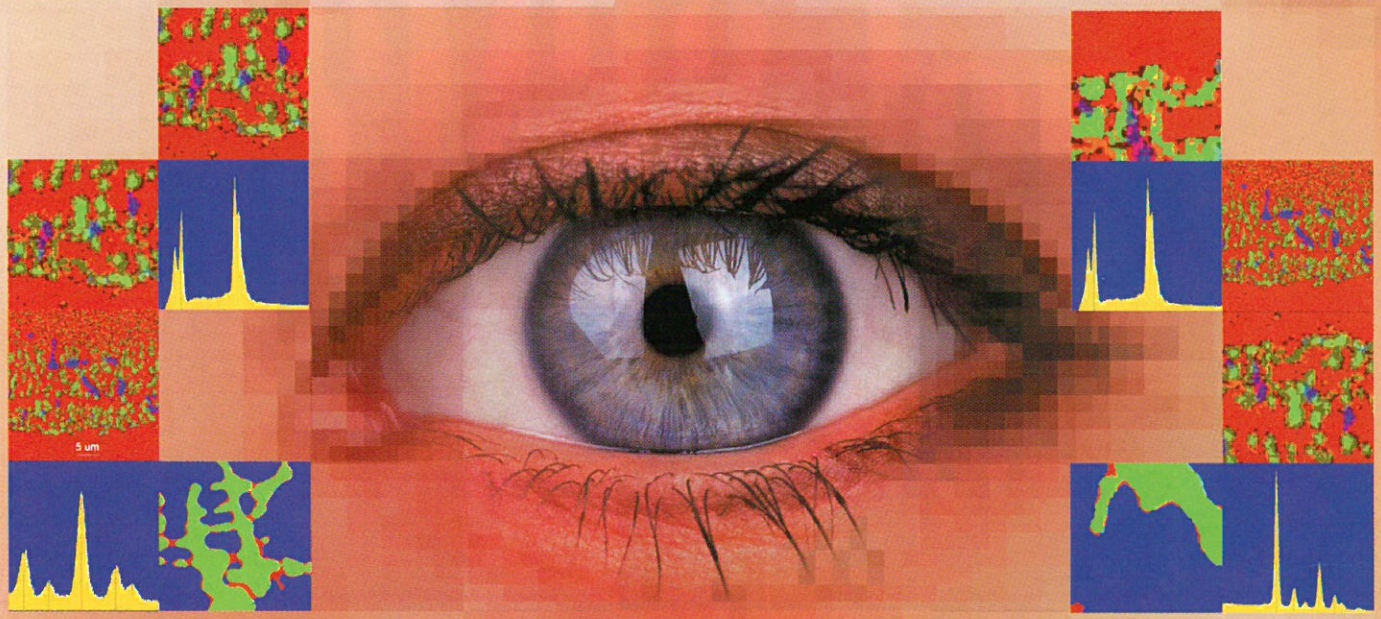

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