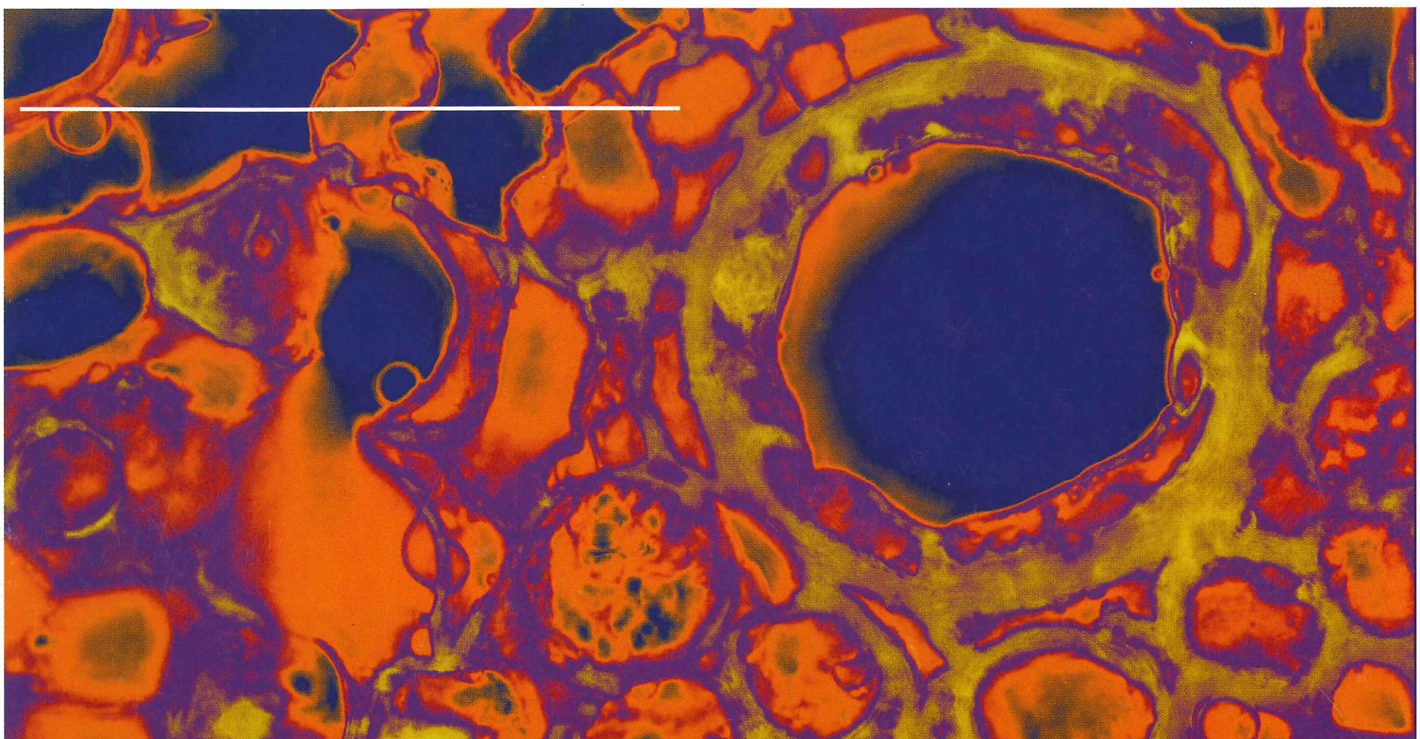
Texas Journal of Microscopy



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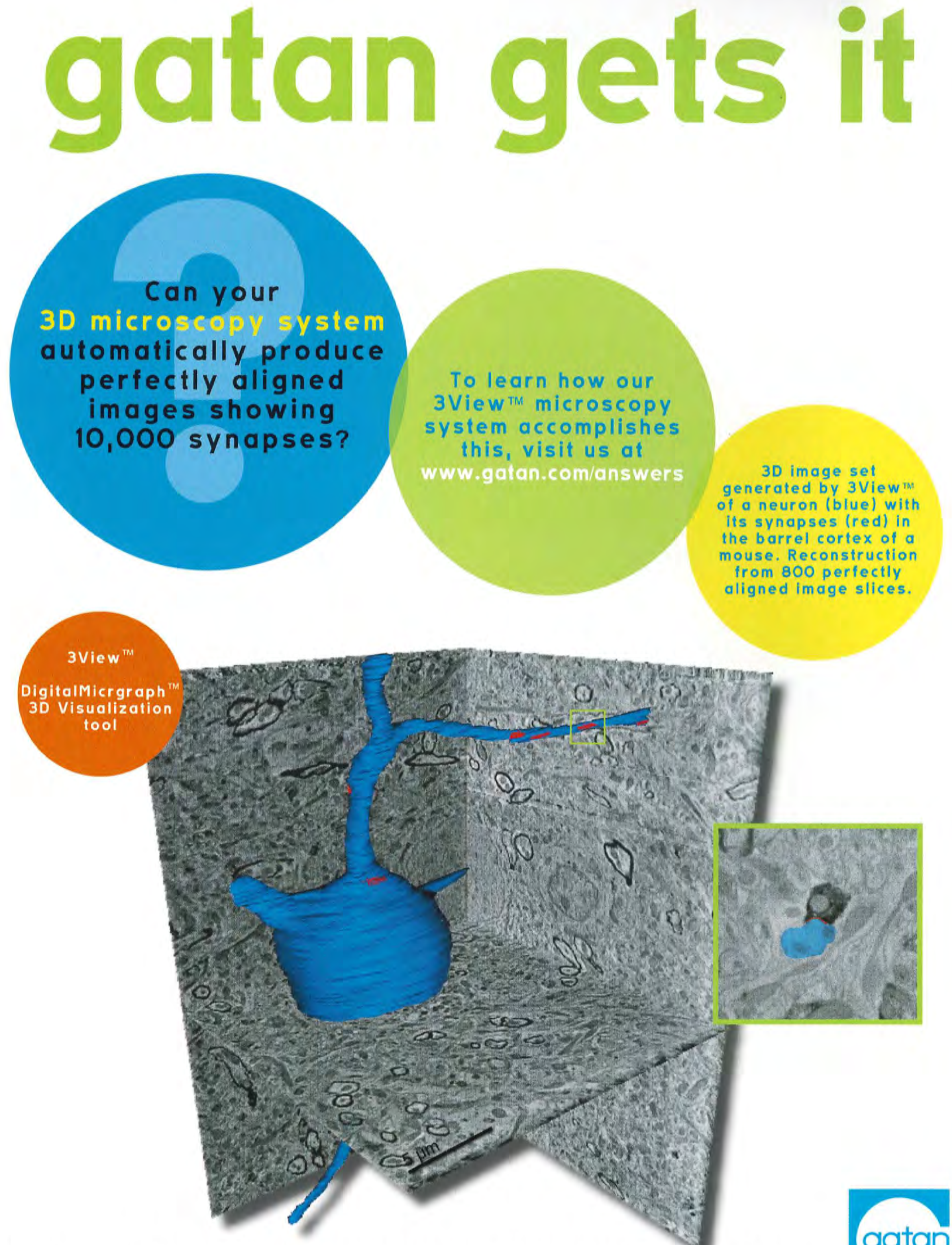

Image showing a neuropil area through a mouse barrel cortex (neuron shown in blue and synapses shown in red) processed for EM and generated using Gatan's 3View™ SBFSEM microscopy system. Eight hundred perfectly aligned image slices of 50 nm were acquired. Gatan's DigitalMicrograph™ 3D Visualization tool was used to show the Z projection of the 800 slices. Manual segmentation using Reconstruct (GNU General Public License version 2) software generated a 3D reconstruction of the neuronal cell including the dendrites and axon. The inset image shows an identified synapse between a dendrite of the reconstructed neuron and the axon labeled for parvalbumine (pre-embedding immunochemistry with biotinilated antibody). Sample courtesy of Dr. Graham Knott, DBCM, University of Lausanne.

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Department of Biology, Texas Woman's University, Denton, TX 76204

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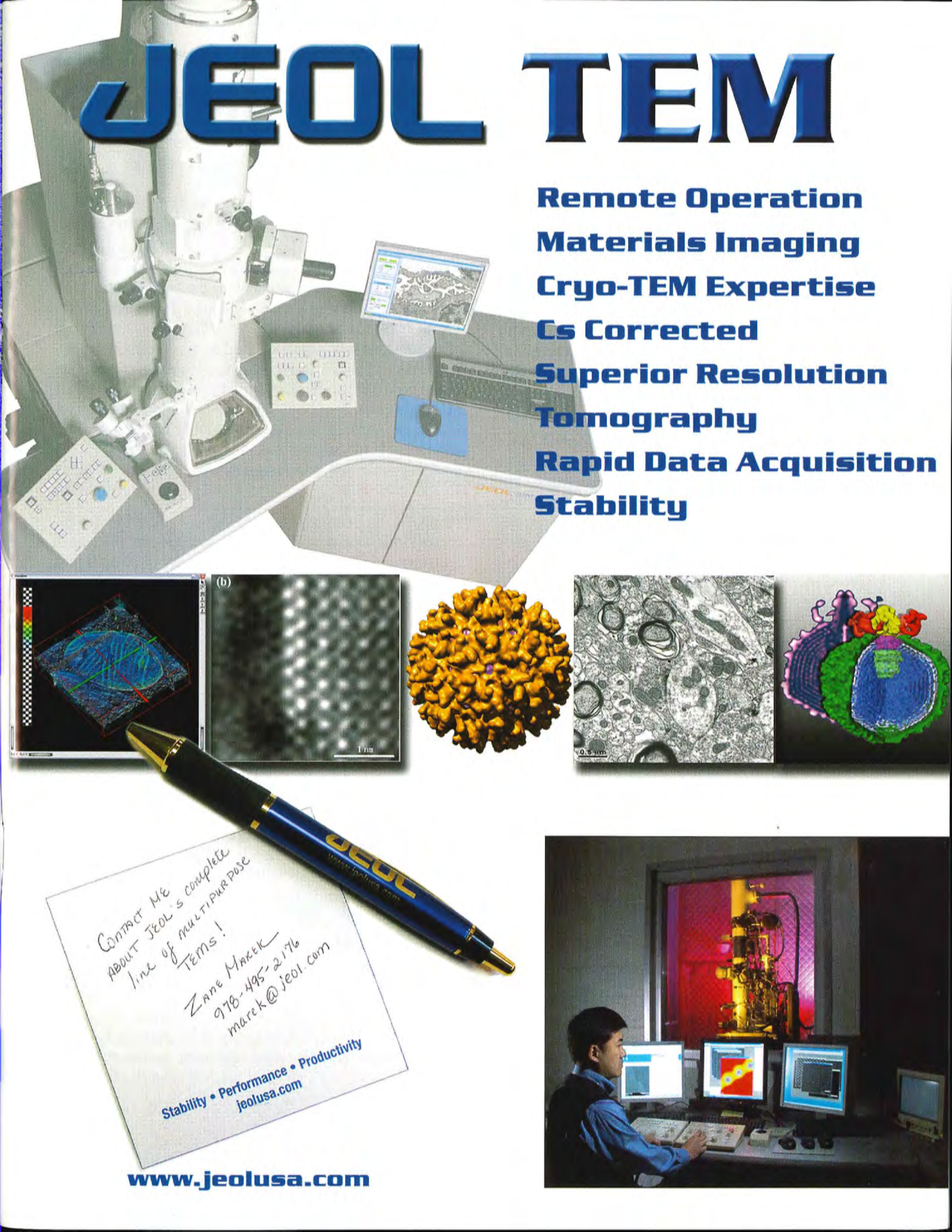
ON THE COVER

Resin duct system in bristlecone pine wood – Bristlecone pines owe much of their long-term stability to resins produced by the numerous resin duct cells present in the wood. The wood piece on the cover originated from a dormant bristlecone pine growing in the White Mountains of California (collected in May 2007 by Christine Hallman using an increment borer and fixed in FAA on site). The image shows a cross section of a resin duct and its accessory cells. Resin ducts are longitudinal tubes surrounded by a series of living secretive (epithelial) and parenchymatic cells. Five excretion cells and approximately 15 smaller parenchyma cells, some of which contain starch grains, surround the duct in the image. The resin duct developed from cambial derivatives at the same time late wood tracheids were produced. Two annual rings are shown, the lower one is the youngest, and the section is oriented as though the bark is under the area we are viewing. In addition to the cell wall layers, one can also see a number of bordered pits in the lowermost tracheids. Sections of the core were made with a freezing microtome and stained with safranin and fast green. Images were taken with a Nikon's Eclipse 80*i* microscope, DXM 1200F digital camera and NIS Elements software. The image on the cover is a gradient map in Photoshop to attain the present colors and hues that help distinguish the wall layers in the early wood tracheids (see the three lowermost rings of cells). Howard J. Arnott, Department of Biology, The University of Texas Arlington, 76019. Bar = 100 μm.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President's Message

First and foremost I want to give my sincere thanks to the tremendous job that Paulo Ferreira has done in the organizing of the spring meeting in Austin. He has done a stellar job. At the writing of this message we can look forward to an excellent workshop on aberration-free TEM, which includes a presentation not only by Larry Allard but the participation of several of our corporate members in both presentations and financial support. In addition to the workshop Paulo has arranged for two plenary presentations. My thanks also go out to our program chairperson, Phoebe Doss, who has spent a great deal of time and energy coordinating the events for the Austin program. She has worked tirelessly on the hotel and facility arrangements and has put together a great program.

I want to acknowledge the contribution of our treasurer, Ann Ellis, and secretary Tina Halupnik to the Society and their help with the planning of the spring meeting. Another key person that deserves a great deal of thanks is Becky Holdford, our web master. The web site has been an invaluable tool for keeping all of us coordinated. And finally, I extend my great appreciation to Camelia Maier for her extremely important work. She has had to coordinate the abstracts for the meeting. Camelia is truly a key player through her service as the editor of our journal. Again, my sincere thanks to all in the executive council.

As many of you know, there is a proposal that we meet only once a year instead of our usual two times per year. There was a survey sent out to get the sense of the society on this question, but the response was low. Less than 1% of the members responded and there was no clear agreement even in these few responses. This brings me to the point of communication among the members. I propose using a list server or other ways of making communication easier among members of our Society. I also propose that the secretary supplies all of the executive committee members with the email addresses of the membership so that any member of the committee can alert the membership when needed. I believe that modernizing the communication between the TSM members would also lead to a greater participation in our meetings.

Finally, I am happy to see that we are beginning to have presentations and attendance from medical and dental schools. I remember that in the early days of our Society participation from the medical community was substantial. Twenty or thirty years ago the main excitement centered on electron microscopy. To be sure, electron microscopy still holds forth as a valuable instrument, especially in the material sciences. But we, as a society, have changed our name from Texas Society for Electron Microscopy to Texas Society for Microscopy. This means that we have evolved to include fluorescence microscopy, confocal microscopy, atomic force microscopy and many other forms of microscopy. We should continue recruiting students, faculty and staff who use any forms of microscopy for presenting at TSM meetings. We need to better organize our meeting programs since interesting programs bring in people. Better and timely communication, increased membership, and fruitful meetings will make our Society stronger in serving Texas and the nation as an important vehicle of scientific information about the latest advances in microscopy.

Ernest F. Couch
2007-2008 President,
Texas Society for Microscopy

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Manuscripts for publication in the Texas Journal of Microscopy are accepted any time. Please, send your work as research articles, review articles, or short communications in biological sciences, materials science and other disciplines employing microscopy techniques to:

Camelia G.-A. Maier, TSM Journal Editor
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DISSECTING THE BACTERIOPHAGE f29 DNA PACKAGING MOTOR

M.C. MORAIS,[1] J.S. KOTI,[3] V.D. BOWMAN,[2] E. REYES-ALDRETE,[1] D.L. ANDERSON,[4] AND M.G. ROSSMANN[2]

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Linear dsDNA viruses, including tailed bacteriophages, adenoviruses, herpesviruses, and poxviruses, first package their genomes into preformed protein shells and later inject their DNA, via elaborate tail structures in the case of bacteriophages, into the cytoplasm of the host cell [1]. The process of genome encapsidation by dsDNA viruses is remarkable considering the entropic, electrostatic, and bending energies of DNA that must be overcome in order to package DNA to near crystalline densities. Indeed, phage DNA packaging motors are among the most powerful biological motors known, capable of generating forces in excess of 57 picoNewtons [2]. Accumulated data from a variety of phage systems indicate that packaging is driven by a molecular motor that converts energy obtained from ATP hydrolysis into the translocation of DNA [3]. The viral ATPases that power genome encapsidation belong to a large family of ATPases whose members are involved in various DNA remodeling tasks including cell division, chromosome segregation, DNA recombination, strand separation, and conjugation [4, 5].

Bacteriophage f29, a tailed dsDNA virus (family *Podoviridae*) that infects *Bacillus subtilis,* has long been used as a model system for studying DNA packaging. An efficient *in vitro* packaging system has been developed for f29 that has been adapted for both robust bulk and single-particle assays [1, 6]. The f29 genome is approximately 19.3 kb, codes for 20 proteins and is covalently linked at both 5 ends to the phage protein gene product 3. Proheads, the first particles assembled during morphogenesis, consist of the head-tail connector protein, scaffolding protein, the major capsid protein, the head fiber protein, and a phage-encoded, 174-base RNA molecule (pRNA). A virus-encoded ATPase drives packaging of the phage genome into the prohead, which subsequently triggers the release of the scaffolding protein. Thus, the f29 DNA packaging motor consists of the prohead, the connector, the pRNA, and an ATPase. Upon completion of packaging, assembly of the lower collar results in release of the pRNA and ATPase. Subsequently, the tail knob and appendages assemble to form the phage tail and complete virus maturation.

In order to understand how the f29 DNA packaging motor operates, it is necessary to know how the component parts are arranged with respect to one another. Here we report a series of cryoEM reconstructions, which unambiguously locate the different components of the packaging motor and determine their molecular envelopes on the virus. CryoEM reconstructions were determined for various particles missing different components of the motor, such as the connector, the ATPase, full-length 174-base pRNA, as well as missing different domains within the pRNA. The resulting reconstructions delineate the molecular boundaries of individual motor components, and suggest a mechanism for translocation of the genomic DNA in which the connector and the ATPase function as valves whose operation is coordinated by the pRNA to ensure unidirectional transport of DNA.

Figure 1. Structure of the f29 DNA packaging motor as determined by cryo-electron microscopy. Two protein components of the motor, the connector and the ATPase, are shown in green and blue, respectively, and an RNA component, the pRNA, is shown in magenta. The atomic structure of a segment of double-stranded DNA, shown as spheres colored by atomic element, was placed in the central channel of the motor to illustrate the path of the DNA during genome packaging. The motor translocates viral DNA into a preformed viral capsid.

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THE STRUCTURE OF WESTERN EQUINE ENCEPHALITIS VIRUS

M.B. SHERMAN AND S.C. WEAVER

University of Texas Medical Branch at Galveston, Galveston, TX 77555, USA

Western equine encephalitis virus (WEEV; family *Togaviridae*, genus *Alphavirus*) is an enveloped RNA virus that is readily transmitted to vertebrate hosts by infected mosquitoes or *via* aerosol. This virus and closely related Venezuelan and Eastern equine encephalitis viruses are among the leading causes of viral encephalitis in humans and horses in the Americas. Human infections result in a range of diseases, from mild flu-like illness to encephalitis, coma and death. Survivors of acute infection often experience severe and permanent neurological damage. No WEE vaccine has been produced so far. The above viruses could also become bioterror agents in the wrong hands.

Alphavirus genus has been extensively studied by EM, cryoEM in particular. However, the Western equine encephalitis virus has never been studied by EM due to its high infectivity and lack of safe, biologically contained cryoEM facilities. This virus is classified as biological safety level 3 (BSL-3) agent requiring biological containment for its growth, purification and subsequent handling. We report here on the first cryoEM imaging attempt with a JEOL 2200FS microscope in a BSL3 containment in the recently built cryoEM facility at UTMB. All specimen preparations were done in a biosafety cabinet in containment by personnel wearing personal protective equipment (respirator with air blower, hood, gown, and gloves). Samples have never left the containment without being destroyed by heat or chemicals. A 3.5 µl WEEV suspension (approximately 10^{10} particles/ml) was applied to C-flat holey films and plunge-frozen in liquid ethane. The images were acquired on a GATAN 895 UltraScan camera at 40,000x using low dose imaging. A number of 5347 images of the WEEV particles were boxed from CCD frames for image analysis and reconstruction. We used PFT programs developed in T. S. Baker's lab. Figure 1 shows a WEEV map at 15Å resolution. The virus contains an ssRNA genome that encodes four nonstructural and three main structural proteins. The mature virus is approximately 70 nm in diameter. The envelope proteins form 80 trimeric spikes that are arranged on the virus surface in a T=4 icosahedral lattice. The capsid protein forms a T=4 icosahedral nucleocapsid, with distinct pentameric and hexameric capsomeres on the outer layer of the nucleocapsid. The envelope and nucleocapsid structures are separated by a lipid bilayer.

There is a high degree of sequence identity between different lineages of alphaviruses ranging from 35% to 80% (Weaver *et al.*, 1997). Old World alphaviruses show very similar envelope and nucleocapsid organization (Fuller, 1987; Paredes *et al.*, 2001). In contrast, it was found that nucleocapsids of VEE virus and the Old World alphaviruses show pronounced differences in capsomere orientation and structure (Paredes *et al.*, 2001). We are currently analysing the similarities and differences between WEEV, VEEV and other alphaviruses. At the same time the map is being refined to higher resolution to reveal detailed organization of WEEV.

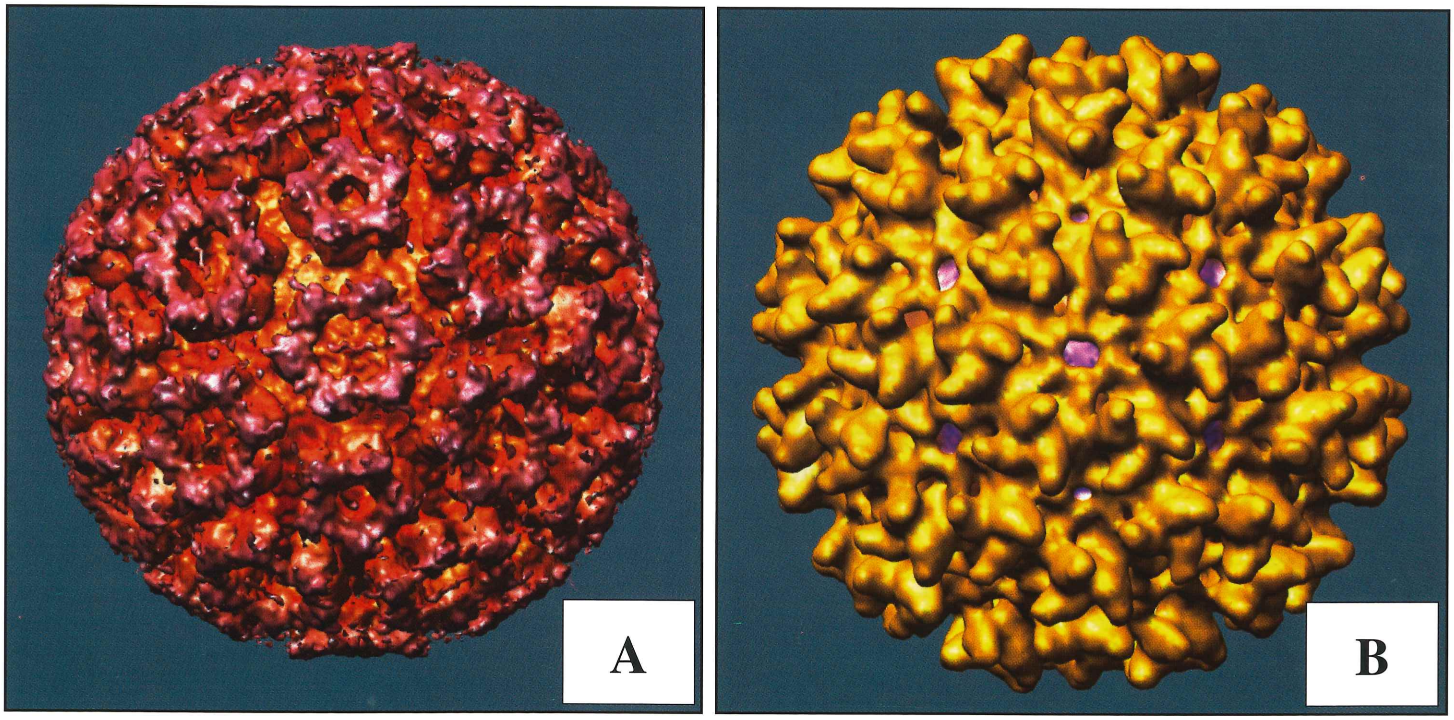

Figure 1. CryoEM 3D map of WEEV at 15Å resolution. The virion is approximately 700Å in diameter (A) with T=4 icosahedral symmetry. The viral envelope has 80 trimeric spikes. Inside the envelope there is a nucleocapsid (B) formed by capsid proteins. Distinct pentameric and hexameric capsomeres form T=4 lattice.

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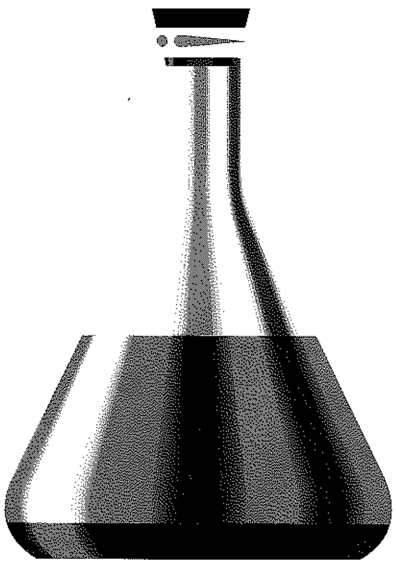

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Abstracts

BIOLOGICAL SCIENCES
Spring 2008

ALPHAVIRUS STRUCTURE REVEALED BY CRYO-EM. ANGEL M PAREDES and DENNIS BROWN, Dept Pathology and Laboratory Medicine, University of Texas Health Science Center Houston and North Carolina State University.

Alphaviruses are an important group of arboviruses (mosquito vectored viruses). Some members of this group like Eastern Equine encephalitis and Western Equine Encephalitis virus cause debilitating encephalopathy type disease in both humans and domesticated animals. Because these viruses are easily grown and purified, they also pose a bioterror threat to the civilized world. They are spherical containing a host derived lipid bilayer sandwiched between two concentric icosahedral protein shells. Virus assembly is an important process in the lifecycle of any virus with the ultimate goal being assembled infectious particles. We explore alphavirus assembly by studying the 3D structure of the final product of this assembly process, the native virus particle. We also study the structures of chemically treated virus that give us insight into assembly. Structure is determined by electron cryomicroscopy and image analysis of frozen hydrated infectious specimen.

DECREASED NADH OXIDASE IN GALACTOSE INDUCED DIABETIC RETINOPATHY IN FEMALE AS COMPARED TO MALE C57BL/6J MICE. E. ANN ELLIS[1], R. L. LITTLETON[1] and MARIA B. GRANT[2], [1]Microscopy and Imaging Center, Texas A&M University, College Station, Texas 77843-2257, and [2]Department of Pharmacology and Therapeutics, College of Medicine, University of Florida, Gainesville, Florida 32610.

Diabetes is a disease of vascular complications with NADN oxidase as the major source of superoxide and oxidative stress. Elevated levels of NADH oxidase have been demonstrated in retinal endothelial cells of animal models of diabetic retinopathy using cerium based cytochemical localization. Cell culture studies have shown that 17ß-estradiol inhibits or reduces NADH oxidase activity. NADH oxidase levels were compared in age-matched female and male C57BL/6J mice with galactose induced diabetic retinopathy.

Diabetes was induced in age-matched female and male C57BL/6J mice by feeding a rodent diet containg 30% galactose (Purina Mills, Richmomd, ID). After 12 months of the galactose diet, mice were euthanized, the eyes were enucleated, fixed and processed for cytochemical localization of NADH oxidase. Semi-quantitation of NADH oxidase localization was based on the percentage of vessels positive for NADH oxidase. Female diabetic mice had lower levels (77.3% ± 11.14%) of NADH oxidase as compared to males (91.4% ± 1.25 %).

Pre-menopausal women are at a lower risk for cardiovascular diseases than postmenopausal women or age-matched men and lack of circulating estrogen puts postmenopausal women at risk for cardiovascular diseases. In diabetes, increased levels of superoxide and peroxynitrite lead to reduced availability of nitric oxide in the vascular endothelium and vascular complications. The data presented in this pilot study supports a role for estrogen in reducing superoxide production and oxidative stress in diabetic pre-menopausal female mice as compared to age-matched diabetic male mice.

FLUORESCENCE MICROSCOPY AS A TOOL TO CHARACTERIZE SOME BIOLOGICAL SPECIMENS. MANDY WHITESIDE[1], NABARUN GHOSH[1], ESTHER VILLANUEUEVA[1], and DON W. SMITH[2], [1]Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, TX 79016 and [2]Department of biology, University of North Texas, Denton, Texas 76201.

The advent of fluorescent labeling plus the plethora of sophisticated light microscopy techniques make studying dynamic processes in living cells commonplace (Stephens and Allen, 2003). It is noninvasive, provides imaging in three dimensions, has high sensitivity down to the single molecule level, and allows the study of molecular- and organelle-specific signals (Weiss, 2000). We studied auto-fluorescing structures of several species of phytoplankton, and pollen from two plant families, *Asteraceae* and *Liliaceae.* We have also studied leaves and petioles from viral infected *Beta vulgaris* L. (sugar beet) fluorescently stained with Fluorol Yellow 088. For all the specimens, wet mounting was the best approach to prepare slides. Chlorophyll and phycoerythrin caused autofluorescence in phytoplankton species under study. Pollen and sugar beet organs were stained to demonstrate that the particular cellular components would fluoresce when excited. Phytoplankton and pollen specimens were observed using an Olympus BX40 Microscope equipped with fluorescein-isothiocyanate (FITC) and tetramethylrhodamine (TRITC) filters, a mercury lamp source, and an Olympus DP70 digital camera with Image Pro 6.0 software. The sugar beet organs were examined with the BX61 Olympus Microscope with FITC and TRITC filters, a UV light source, and a magnifier digital camera. The specimens were viewed and photographed at 40X using bright field, FITC, and TRITC filters. A fluorescent source was used to excite the storage molecules or proteins of the specimens, which exhibited fluorescence. The FITC filter revealed characteristic colpi, sulci and sutures in the pollen grains, green fluorescent proteins (GFP and EGFP), and the TRITC filter revealed red fluorescent proteins (DsRed) in the phytoplankton. The sugar beet leaves and petioles exhibited characteristic fluorescence in the virus-infected tissues that was confirmed by DAS-ELISA Double Sandwich method. This investigation demonstrated that the fluorescent microscopy could be used to characterize and annotate many biological specimens.

RELATIONSHIP BETWEEN PRIMATE RETINAL NERVE FIBER BIREFRINGENCE AND ITS ANATOMICAL CONSTITUENTS. GINGER POCOCK, MIA MARKEY, MAURA BOYLE, LAURA STRONG, KACIE HATTAWAY, and H. GRADY RYLANDER III, University of Texas at Austin.

Glaucoma is an irreversible ocular disease that causes gradual damage to the optic nerve, which carries visual information from the eye to the brain. Thinning of the Retinal Nerve Fiber Layer (RNFL) is a pathological symptom of glaucoma and is the result of degeneration of the neurotubule cytoskeleton structures. Studies suggest the source of the birefringence emanating from the retina could be attributed to the retinal ganglion cell (RGC) axon neurotubules throughout the RNFL. A relationship between

RNFL birefringence and neurotubule density was investigated using polarization-sensitive optical coherence tomography (PS-OCT) and transmission electron microscopy (TEM). PS-OCT was used to make *in vivo* birefringence measurements of the peripapillary RNFL (Rylander *et al.*, 2005). Birefringence measurements are correlated with neurotubule densities and other RGC axon organelles sampled from TEM images in corresponding regions surrounding the optic nerve head. Theoretical estimations of neurotubule density are also calculated using an expression based on an approximation of Wiener's theory for mixed dielectrics (Oldenbourg *et al.*, 1998) and RNFL birefringence measurements. If a significant correlation exists between neurotubule density and RNFL birefringence, PS-OCT could potentially be used as a clinical instrument to detect early stage glaucoma.

MICROSCOPIC STUDIES OF VENUS FLYTRAP LEAVES. DIXIE SUAREZ and CAMELIA MAIER, Department of Biology, Texas Woman's University, Denton, Texas 76204-5799.

Venus flytrap, *Dionaea muscipula* (*Droseraceae*), is a carnivorous plant endemic to the bogs of North Carolina and northern South Carolina, characterized by acidic, nutrient poor soils. The plant acquires nitrogen by capturing insects with its leaves transformed into traps. Scanning electron microscopy was used to examine the traps before and after an insect meal, at different developmental stages. Traps are found at the tip of petioles and require the stimulation of trigger hairs in order to capture insects. They closed with such a force that the insect exoskeleton cracked. The plant then initiates the digestion of the insect prey by expelling enzymes from digestive glands that cover the surface of the trap. The trap surface hosts a microfilm of bacteria, yeast, and fungi that may help with digesting trapped insects. Future study will try to identify if the microbes are beneficial or parasitic to *Dionaea muscipula.*

MINERAL DEPOSITS OF MULBERY SPECIES. SAMUEL OYEBODE and CAMELIA MAIER, Department of Biology, Texas Woman's University, Denton, Texas 76204-5799.

Many organisms are actively depositing minerals, mainly calcium oxalate, silica, and calcium carbonate. The distribution, morphology and chemical composition of these mineral deposits constitute taxonomic characteristics for a number of plant families. The objective of this study was to characterize the mineral deposits in various tissues of *Morus alba* and *M. rubra (Moraceae).* Vegetative and reproductive tissues were sampled from mulberry male and female trees in winter, spring and summer. Tissue preparation and deposit isolation were performed for light and electron microscopy and histochemical studies. All tissues examined contained prismatic calcium oxalate crystals. Leaves showed calcium carbonate and silica cystoliths in specialized cells in the upper epidermis. Druses of calcium oxalate were found along the veins and stem tissues. Only prismatic crystals were observed in buds. Future studies will focus on the mulberry sexual dimorphism involving mineral deposits and the functions of mineral deposits in plants.

PLOIDY ANALYSIS FOR THE DETERMINATION OF *ERYTHRONIUM* SPECIES IN NORTH CENTRAL TEXAS POPULATIONS. ANNIE PETRIE, PALLAVI UPADHYAY, and CAMELIA MAIER, Department of Biology, Texas Woman's University, Denton, Texas 76204-5799.

Erythronium albidum (tetraploid) and *Erythronium mesochoreum* (diploid) *(Liliaceae)* are two plant species native to North Central Texas. The two species have very similar morphological and anatomical characteristics making visual identification difficult. The objective of this study was to determine the species of *Erythronium* in the plant populations in North Central Texas. The best method to identify these species is through ploidy analysis. Root tips were fixed in 0.01% colchicine and the root squash method was employed to observe the chromosomes under a light microscope. Number of chloroplasts in guard cells and length of stomata are reported to be directly proportional with ploidy level. Also, pollen size/diameter is a known indicator of ploidy level. The average pollen diameter for plants collected at two location showed a significant difference, 18.4 and 20.15 um, respectively. No conclusive results were obtained with chromosome chloroplast counts. Future experiments will employed more accurate techniques for determining the ploidy in Erythronium species in North Central Texas, such as fluorescence *in situ* hybridization (FISH) with repetitive DNA (45 S rDNA) and flow cytometry. This work was done in collaboration with The Native Plant Society of Texas, Trinity Fork Chapter.

EXAMINING THE MORPHOLOGY OF DIFFERENT EFFECTS OF ESTROGEN ON ZEBRA FINCH EGGSHELL THICKNESS. H. POURARSALAN, S.L. WESTMORELAND and K. SCHUG, Department of Biology and The Center for Electron Microscopy, University of Texas at Arlington, Arlington, Texas 76019.

Female Zebra finch chicks are exposed to phytoestrogens in their daily consumption of seeds. We hypothesized that 1) estrogen will cause eggshell thinning in Zebra Finch eggs, 2) eggshells from chicks treated with estrogen would show a higher mammillary cone density, and 3) the eggshells from chicks treated with estradiol benzoate would show a higher concentration of magnesium than those from untreated chicks. Jim Millam (University of California at Davis) orally administered estradiol benzoate to experimental female Zebra finch chicks as an equivalent of the normal phytoestrogen consumption in the natural habitat. The control Zebra finch chicks group was treated with canola oil for 7 days. Three samples were removed from each eggshell and digital electron micrographs of the eggs were taken at 500X. Thickness of the eggshells was determined using image analysis software, Image ProPlus. It was found that the treated eggshells were significantly thinner than the control ($p=0.02$). The mammillary cone tips were marked on the images taken using Microsoft Photoshop and tips were counted using Image Pro Plus. The mean mammillary tip value for treated eggshells was 65.97 ($SD=11.2$) while the mean value for control was 63 ($SD=14.6$). Although this difference was not statistically significant ($p=0.228$), it shows a trend: the thicker the eggshell, the lower the mammillary cone density. Eight eggshells from each experimental and control groups were ashed and flame atomic absorption spectroscopy was used to measure the magnesium concentration. No significant difference was found between the experimental and control groups ($p=0.48$). We concluded that treatment with estradiol benzoate causes eggshell thinning in Zebra Finch, but has no effect on the mammillary cone density and magnesium content.

***SARRACENIA ALATA* WOOD: AN ANATOMICAL STUDY.** RACHAEL N. JONES and DENNIS A. GRAVATT, Department of Biology, Stephen F. Austin State University, Nacogdoches, Texas 75962.

Sarracenia alata Wood (pitcher plant or yellow trumpet, *Sarraceniaceae*) is typically found in saturated, acidic, nutrient-deprived soils. The populations used in this study were located in the Angelina National Forest near Boykin Springs Park, Angelina County, Texas. Plant samples were collected from herbaceous seeps embedded within dry sandy uplands. The tubular leaves of *S. alata* are composed of a liquid-filled pitcher with a lip-like peristome, seam-like ala and overlying hood. Internally, leaves are divided into four zones: zone 1 refers to the hood; zone 2 contains the peristome; zone 3 includes the top portion of the pitcher; and zone 4 is the bottom portion of the pitcher. Samples from internal and external portions of all four leaf zones were collected and prepared for SEM. The presence of key identifying features, such as trichomes, stomata, and nectar glands, were noted for each zone and their densities quantified. Trichome morphology and density were found to differ amongst the leaf zones. This morphological study will serve as foundational work for future investigations of *S. alata*.

MATERIAL SCIENCES
SPRING 2008

SELF HEALING NANOPARTICLES. [1]C.E. CARLTON, [2]O. LOURIE, and [1]P.J. FERREIRA, [1]Materials Science and Engineering Program The University of Texas at Austin, Austin, TX, 78712 and [2]Nanofactory Instruments AB, Gothenburg, 412 58, Sweden.

A silver nanoparticle with a diameter of approximately 10 nm was deformed *in-situ* in a transmission electron microscope. Deformation was done to observe the microstructural mechanism for plastic deformation in nanostructured mechanical systems. The results of the experiment are interesting both for their novelty and the insight they grant into other nano-deformation systems, particularly the mechanical testing of nanopillars, a subject containing a lively debate over the deformation mechanism. While dislocation motion is accepted as the main agent of plastic deformation in indented nanopillars, it is unclear were the dislocation come from. This work shows that it is possible to generate dislocations even in the absence Frank-Reed sources. Additionally, the mere presence of dislocations in a 10nm particle is quite surprising.

TEM STUDIES OF SEMICONDUCTOR AND METALLIC NANOPARTICLES GENERATED BY LASER ABLATION OF NANOPARTICLES, IGNACIO F. GALLARDO, KAY HOFFMAN, and JOHN W. KETO, Department of Physics, The University of Texas at Austin, Austin, TX 78712.

Laser Ablation of Microparticles (LAM) is a process of nanoparticle formation in which microparticles in a flowing aerosol are continuously ablated by high power laser pulses (Nichols *et al.*, 2000). For the first time, we have produced CdSe/ZnS core/shell nanoparticles using a double ablation apparatus, designed to undergo a two-step LAM process. This process can be inverted to produce ZnS/CdSe core/shell nanoparticles. The present work focuses on approximately 30 nm diameter heterostructures and used high resolution transmission electron microscopy (HRTEM) to image core and shells. For smaller particles, core shell structures have been detected with energy dispersive spectroscopy (EDS) with 5 nm spot size beam, and fast Fourier transform (FFT) spectra. Differences in the ablation behavior were measured between the two IIB-VIA type semiconductors. To study the effects of the nanoparticles in the second cell under high UV laser pulses, thermodynamic numerical calculations (Kompa *et al.*, 1988) were made and tested for well known Silver nanoparticles. We discuss temperature and size distributions for CdSe and ZnS nanoparticles. A two-laser pulse experiment is designed to monitor nanoparticle size before and after laser interaction. We show HRTEM images of particles before and after surface evaporation. First results show that Ag nanoparticle radius decreases from 3.5 nm to 2.2 nm, keeping a constant standard deviation of 20%. Our theoretical model is in good agreement with the experiments. This study was supported by The Center for Nano and Molecular Science and Technology (CNM), the Robert A. Welch Foundation, the Strategic Partnership for Research In Nanotechnology (SPRING), The Texas Materials Institute (TMI), and CONACYT.

WHAT IS NEEDED FOR COMBINING ABERRATION CORRECTION WITH DIFFERENT APPLICATIONS? JAN RINGNALDA, FEI Company.

With the advent of corrector technology being made available to many laboratories and operators, the tuning and tweaking of electron microscopes has increased significantly in order to get the required performance firstly out of tuning samples, and secondly to get the systems ready and tuned for actually doing work on the samples the systems were purchased for.

While it seems that most of the early systems purchased went through a lengthy characterization stage and 'preliminary results' was an acceptable publication title for work being done with a new system that was not yet fully tweaked and analyzed, this is now no longer an acceptable delay period. A system needs to be installed in a timely fashion, and produce results in chorus with the expectation of the grant awarding body who supplied the funding for the purchase of such a system.

In this presentation the common issues with performance-hampering issues will be highlighted, together with some historical solutions to get these issues taken care of. It should be noted that the microscope systems of today are extremely sensitive measuring devices of external effects such as fields, vibrations and temperature fluctuations, and when simple effects such as thermal expansion are taken into account, it is not trivial to maintain a stability of less than 0.5 nm/min in an image over an acceptable period of time.

Some data from the TEAM instrument will be presented, also a description of the actions taken to really improve the environment into which this system was placed. As the requirements of materials science and biology are getting more demanding, the stability and reliability aspect of these systems with ever increasing complexity will play a more and more important role in the completion of successful experiments.

LOCAL STRAIN MEASUREMENT BY GEOMETRICAL PHASE ANALYSIS IN TEM APPLIED TO STRAIN-ENGINEERED DEVICES. Jayhoon Chung*, Guoda Lian*, Cathy Vartuli*, Sri Rajagopalan** and Lew Rabenberg**, *Texas Instruments, Dallas, Texas 75243, **Texas Materials Institute, University of Texas at Austin, Austin, Texas 78712.

The local strain around the transistor was measured by the newly developed geometric phase analysis (GPA) applied to high-resolution transmission electron microscope (HRTEM) images. The stresses in a Si device can cause device failure. Also, it can be used to improve device performance by strained-engineering techniques. Therefore, the stress measurement in transistor level is a critical tool for failure analysis and process development. However, it is very hard to see the stress quantitatively and two-dimensionally in nanoscale resolution. GPA technique can be easily applied to conventional HRTEM images to reconstruct quantitative and two-dimensional strain map. This work presents the local strain measurement of Si in transistor level by the geometric phase analysis with 10^{-3} order of strain sensitivity and nanometer spatial resolution. GPA was successfully applied to both biaxail and uniaxial stress conditions. The measured strain values are well matched to the values calculated mathematically. This technique can be applied to the analysis of stress-related device failures and process monitoring for the device with SiGe material, such as strained CMOS and Si/SiGe bipolar transistors.

$LIFEPO_4$ NANORODS NETWORKED WITH MULTI-WALLED CARBON NANOTUBES FOR ENERGY STORAGE APPLICATIONS. T. MURALIGANTH, A. VADIVEL MURUGAN, D. FERRER, P. FERREIRA and A. MANTHIRAM, Electrochemical Energy Laboratory and Materials Science and Engineering Program, The University of Texas at Austin, Austin, TX 78712.

$LiFePO_4$ crystallizing in the olivine structure has drawn much attention in recent years as a cathode material for high power lithium ion batteries. This type of batteries is currently very much of interest for hybrid electric vehicles as Fe is inexpensive and environmentally benign and the covalently bonded PO_4 groups offer excellent safety. However, the poor lithium ion conductivity, due to the one-dimensional lithium ion diffusion and its low electronic conductivity have prompted the synthesis of $LiFePO_4$ in controlled nanostructures decorated with conductive species. We present here a novel low cost approach to synthesize highly crystalline $LiFePO_4$ nanorods with controlled particle size at temperatures as low as 300°C within a short time. The $LiFePO_4$ nanorods are characterized by high-resolution TEM, SEM, XRD, and electrochemical measurements. The size of the nanorods increases from 20 nm width and 100 nm length to 45 nm width and 1 μm length as the concentration of the reactants in the reaction medium increases. Subsequent networking of the $LiFePO_4$ nanorods with multi-walled carbon nanotubes (MWCNT) resulted in nanocomposite electrodes that exhibited excellent capacity retention and high power capability in lithium cells. The networking was performed at ambient temperatures without requiring any post annealing treatment in reducing gas atmospheres. The effect of $LiFePO_4$ particle size on the rate capability is presented by investigating $LiFePO_4$ nanorods with various dimensions.

USING ENERGY DISPERSIVE SPECTROMETRY TO COMPARE THE EFFICIENCY OF METAL COATING TECHNIQUES FOR SCANNING ELECTRON MICROSCOPY OF INSECTS. BONNIE B. PENDLETON*, MICHAEL W. PENDLETON**, and AND E. ANN ELLIS**, *Dept. of Agric. Sciences, West Texas A&M Univ., Canyon, TX 79016-0001, **Microscopy & Imaging Center, Texas A&M Univ., College Station, TX 77843-2257.

To obtain secondary electron images of insects using SEM, specimens usually are coated with a conductive material. This study compared the amount of conductive metal covering two maize weevil (*Sitophilus zeamais* Motschulsky) specimens prepared for SEM imaging. The gold/palladium metal covering of one specimen was produced by sputter coating (Echlin, 1978) and the ruthenium metal covering of the other specimen was produced by vapor coating (Ellis and Pendleton, 2007). These two coating techniques were compared using counts of X-ray signals received from analogous locations of both specimens using a Princeton Gamma-Tech energy-dispersive spectrometry (EDS) detector mounted in a JEOL 6400 SEM. The insect to be sputtered was air dried, mounted on a stub, and placed in a Hummer II sputter coater. The gold/palladium coating produced by sputtering (with argon) was estimated to be 30 nm thick. The insect to be vapor coated was also air dried and mounted in the same way but placed in a glass petri dish with a glass lid in a fume hood. Ruthenium tetroxide was added to a vial and placed in the petri dish with the insect. A beaker of hot water on the lid of the petri dish expedited the vapor coating (5 minutes of coating required). Three areas were tested by EDS on each of the dorsal, ventral and lateral sides of both the sputter coated and vapor coated weevils. The resulting EDS plots imply that surfaces not facing the target are not well coated by sputtering and also that surfaces well coated by vapor fixation are unrelated to the position of the insect within the petri dish. Therefore the best procedure for metal coating insects for SEM observation is to use a combination of both sputtering and vapor coating.

OPTIMUM CONDITIONS FOR GEOMETRICAL PHASE ANALYSIS OF HIGH RESOLUTION TEM IMAGES OF STRAINED CRYSTALS. LEW RABENBERG[1], SRI RAJAGOPALAN[1], and JAYHOON CHUNG[2], [1]Texas Materials Institute, University of Texas at Austin, Austin, TX 78712, and [2]Texas Instruments, Dallas, TX 75243

Geometric phase analysis (GPA) is an image processing technique that creates a map of local atomic displacements or strains from high-resolution transmission electron microscope (HRTEM) images. Briefly, an image of the area of interest, along with an unstrained reference region, is subjected to Fourier transformation. Intensity maxima in the resulting power spectrum were individually masked, translated to the center, then inverse-transformed. The resulting phase images can be displayed as local displacements or as strains in any direction of interest within the 2-dimensional image. This talk will describe the basic GPA process and its limitations, including issues associated with specimen thinning, and masking. In particular, specimens exhibiting step-function strain discontinuities will be used to analyze the effects of rapidly varying strains. Image simulations and analytical models are used to define an optimum defocus for GPA.

EDUCATIONAL
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THE USE OF TECHNOLOGY TO PROMOTE ACTIVE LEARNING IN THE SCIENCE CLASSROOM. S.L. WESTMORELAND, The University of Texas at Arlington, Department of Biology and The Center for Electron Microscopy, Arlington, Texas.

In active learning students are at the center of the learning process. The role of the teacher is to define learning objectives and to design appropriate experiences to enable students to achieve these objectives. The emphasis is on active engagement of the students in the learning process through reading, discussion, writing, problem-solving, investigating, and reflecting. No longer is the student a passive observer or receiver in the classroom. Through active learning, students retain information better and develop higher level thinking skills. Students are also more engaged and can use a variety of learning styles. One of the newest "tools for success" in my classes is the use of technology in a wide variety of applications. As a part of my plan for student success through active learning, I have integrated technology into all of my classes in an array of applications. I am currently conducting a controlled study to investigate the effects of using online activities and homework and personal answer devices or "clickers" in the large biology lecture classroom. The hypotheses are that the following changes will occur in the class using technology: high attendance, decreased attrition, increased final exam scores, and change in grade distribution with fewer failures. Student acceptance of the clickers has been excellent with 100% compliance in purchase and registration. We have had numerous successful sessions with increased student focus and engagement. Preliminary results indicate that classes with clickers have a higher success rate and high attendance. Technology promotes active learning while engaging students in a way that relates to "their world." It uses a variety of learning styles. It enables students to learn skills that are vital for success in science careers. It gives students more autonomy, as they work at their own pace through online activities and monitor their own success. It gives them a "voice" in a large classroom. Technology is a vital part of the active learning environment Technology is truly a bridge to student success!

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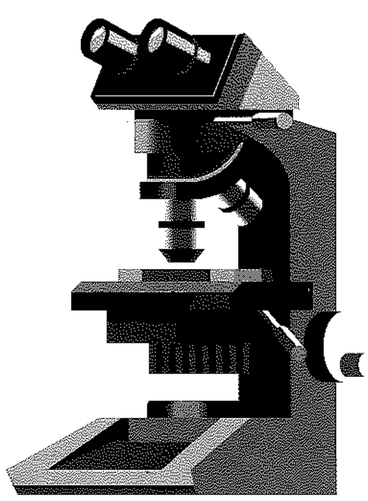

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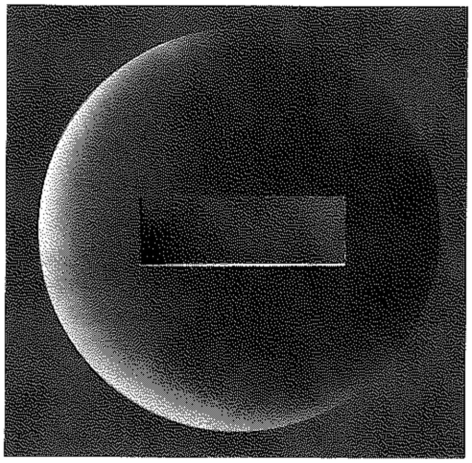

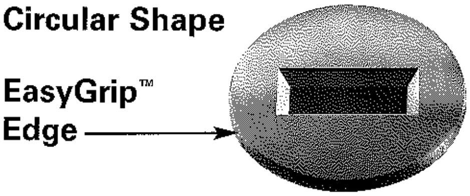

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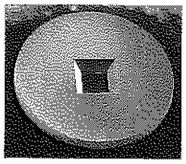

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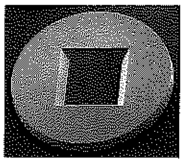

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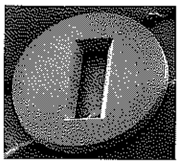

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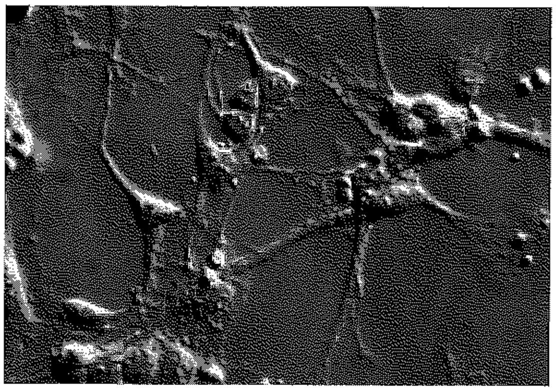

DIC image of hippocampus neurons grown on a silicon nitride substrate by Prof. M. Stowell, et. al., MCDB, CU-Boulder, Colorado

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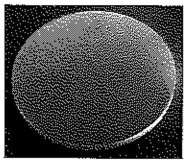

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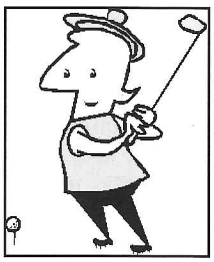
"Wood – The Final Frontier"

Howard J. Arnott

Autobiography-Part Five

Part Five of my autobiography deals with the reawakening of my interest in the structure of wood. Wood is so familiar that most of us do not think about it at all. My "wood reawakening" came about through a series of accidents, some great opportunities and a personal time frame where change could be accommodated. Together these forces rekindled a curiosity that lay dormant for several decades following graduate school. While this change was a continuous and time dependant process, the reader will soon find that the account is not always given in chronological order.

DISCOVERING THE GREAT BASIN BRISTLECONE PINES



Just before the start of the third millennium, my wife Jean and I visited our daughter Virginia's home in Half Moon Bay, California. Of course, while there we were able to visit with her family including three of our grandchildren. It was very amusing and enlightening to watch the many golfers that passed by my daughter's windows; we could see them but they didn't see us. It was really astonishing to see how many of them cheated! When they thought no one was looking, a little push of the ball here, a small kick of it there, a pick up and drop; does it really matter? Earlier in this autobiography series, I made some observations about golfers in Florida. They drank a lot, actually quite a lot, and they talked a lot but only about golf or liquor prices. Maybe (probably), I am naïve, but as far as I know the Floridians didn't cheat. During our stay in Half Moon Bay, Craig Scott, my son-in-law, Alex Scott, my grandson, and I went fishing; you may remember that fishing is one of my passions. Although I was seasick most of the day, I was lucky enough to catch a 30 lb salmon and it was very tasty. Subsequently, Jean and I left the Pacific coast and traveled back toward Texas *via* Yosemite (the first accident—we could have gone back many other ways). After leaving Yosemite *via* its eastern entrance at Tioga Pass, we arrived in the Owens's River Valley where we stayed overnight at the Best Western Creekside Motel in Bishop, California. Prominently displayed in their lobby was a placard on the subject of The Bristlecone Forest in the White Mountains, home of the "oldest living things". Being trained as a botanist, I had heard about bristlecone pines. Obviously, at that time, I didn't know enough about them to understand how interesting they are.

Neither of us had ever seen the bristlecones, *Pinus longaeva* Bailey (*Pinaceae*), and because they were touted as both old and bizarre we decided to have a look. The next day we made the first of many drives to Ancient Bristlecone Pine Forest in the White Mountains of California. From Highway 168 there is a turnoff where the Ancient Bristlecone Scenic Byway leads to The U. S. Forest Service Visitors Station at the Schulman Grove, at an altitude of about 10,000 feet (Fig. 1). The well-designed station house is at the end of a paved road with a large parking area beside it. The trailhead for the Discovery Trail is located at a short hundred meters from the station. We hiked up the Discovery Trail into the Schulman Grove through living bristlecones, some of which are over 4000 years old. Pine Alpha, the first 4000-year old bristlecone that Schulman found, is along this trail and there were plenty of other bizarre twisted trees (Plate 1E). On the trail, we saw both the purple (normal) and green cones of *P. longaeva*, the Great Basin bristlecone pine; we also noted that these bristlecones seem to grow on dolomite only (Plate 1A).

Also located at the station is the trailhead for the Methuselah Walk Trail (a 4-mile hike). Along that trail you can "walk" to the area where the oldest known living tree still survives. It lives among more than 40 trees, 4000 years old and older. The majority of these old trees are found in what is called the Methuselah Grove, only about two miles from the trailhead. There is nothing like the Methuselah Grove anyplace in the world, it is a one of a kind phenomenon. I was amazed to walk among the living and dead trees of all shapes and sizes that grow there; sometimes you have to work your way around or through these old trees (Fig. 2; Plate 1D). Some trees have only one branch still alive that is supported by a narrow band of living bark; these trees are aptly called strip bark trees. Many trees are still standing although they may have died several centuries ago. One of the major ecological features of this grove is the white mineral called dolomite (calcium magnesium carbonate), which makes up the soil. Another environmental feature of this area is a minimal rainfall of 3 to 5 inches, a limited period of growth (six or seven weeks) and a steep slope on which to grow (Plate 1A). All these things combine to make bristlecone pine growth very difficult. Many authorities think that it is the difficult growing conditions that have led to the longevity of these trees (Plate 1, A, C, E). At first, this was a difficult concept for me to accept.

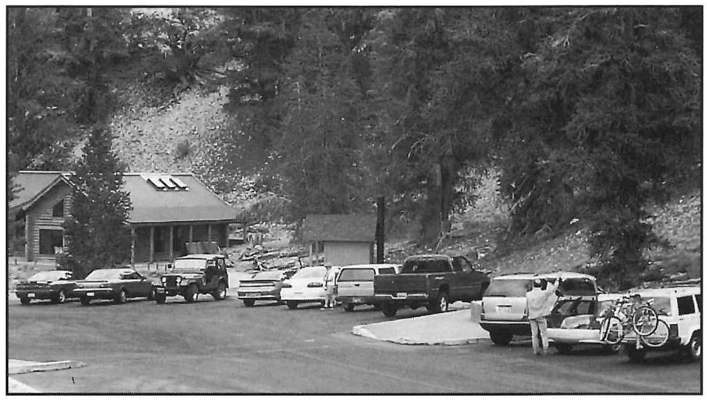


Figure 1. Schulman Grove Visitor Station, Discovery Trail starts behind us. The Methuselah Walk is to the left, and the Mexican Mine Trail to the right.

After we came back from the Methuselah Walk, we continued to drive north on The Ancient Bristlecone Scenic Byway, which becomes a rough dirt road. Twelve miles and four turn offs later you come to the Patriarch Grove at an elevation of about 11,500

Figure 2. Methuselah Walk (trail). Note the steep slope that the trail passes through.

feet (Fig. 3). If you continue on this road it goes to the University of California's Barcroft Station at 12,500 feet. At the Patriarch Grove we saw what many believe to be the largest bristlecone pine, The Patriarch Tree (Fig. 4). At first glance, The Patriarch Tree looks as if many individual trees have fused together, but DNA tests have shown that it is in fact one tree. Anecdotes and legends about this tree brought Edmund Schulman to the White Mountains in the 1950's. In the Patriarch Grove there are many bizarrely shaped bristlecone pines; some look like they are half dead and may have looked like that for centuries. Mixed with them are trees that look like normal pine trees. The great age found in the Methuselah Grove is not duplicated here; the age of the Patriarch Tree is less than 2000 years. We drove to the Patriarch Grove in a normal sedan without incident; far different from the second time we traveled there (see later). This brief examination of these

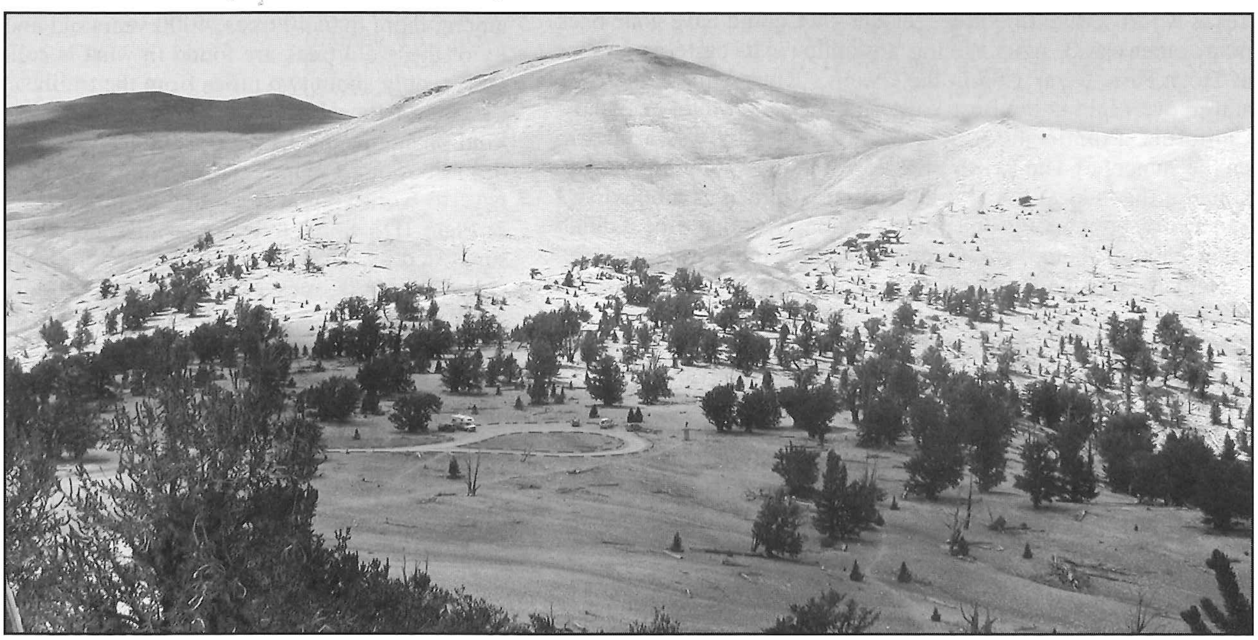


Figure 3. View of the Patriarch Grove and Sheep Mountain. The two frost rings study areas are on both edges of this picture. Sheep Mountain is at the top of the photo, the road to Barcroft station passes between the peak and the uppermost tree line. The loop seen in the center is the parking area for the Patriarch Grove; The Patriarch tree is just to the right of the loop.

Figure 4. The Patriarch Tree is about 1750 years old, has a diameter of approximately 17 feet, and growth at an altitude of 11,500 feet.

amazing trees was another completely unplanned "accident." However, it initiated what is now an ongoing interest in the Great Basin Bristlecone Pines and these trees became a major part of my new frontier. Our enjoyable experiences in the White Mountains made me wonder not only about these amazing trees, but also about Edmund Schulman, for whom the Schulman grove was named.

Back at the station, I noticed a National Geographic article by Schulman; as soon as we got home, I purchased two copies of the March 1958 National Geographic article for $1.50 each at the Half Price Books. That edition contained Schulman's article entitled "Bristlecone pine, oldest known living thing" (Schulman, 1958). In his article, which contains many high quality color plates, he reports finding several living bristlecones over 4000 years old. According to his study, the oldest tree he called Methuselah was at least 4700 years old. These trees were one and a half times older than redwood species, which previously had held the record for being the oldest known trees. The above article was published three months after Schulman's death at 49.

In his article, Schulman tells about traveling to the White Mountains in search of old trees with Dr. Fritz Went. I met Dr.

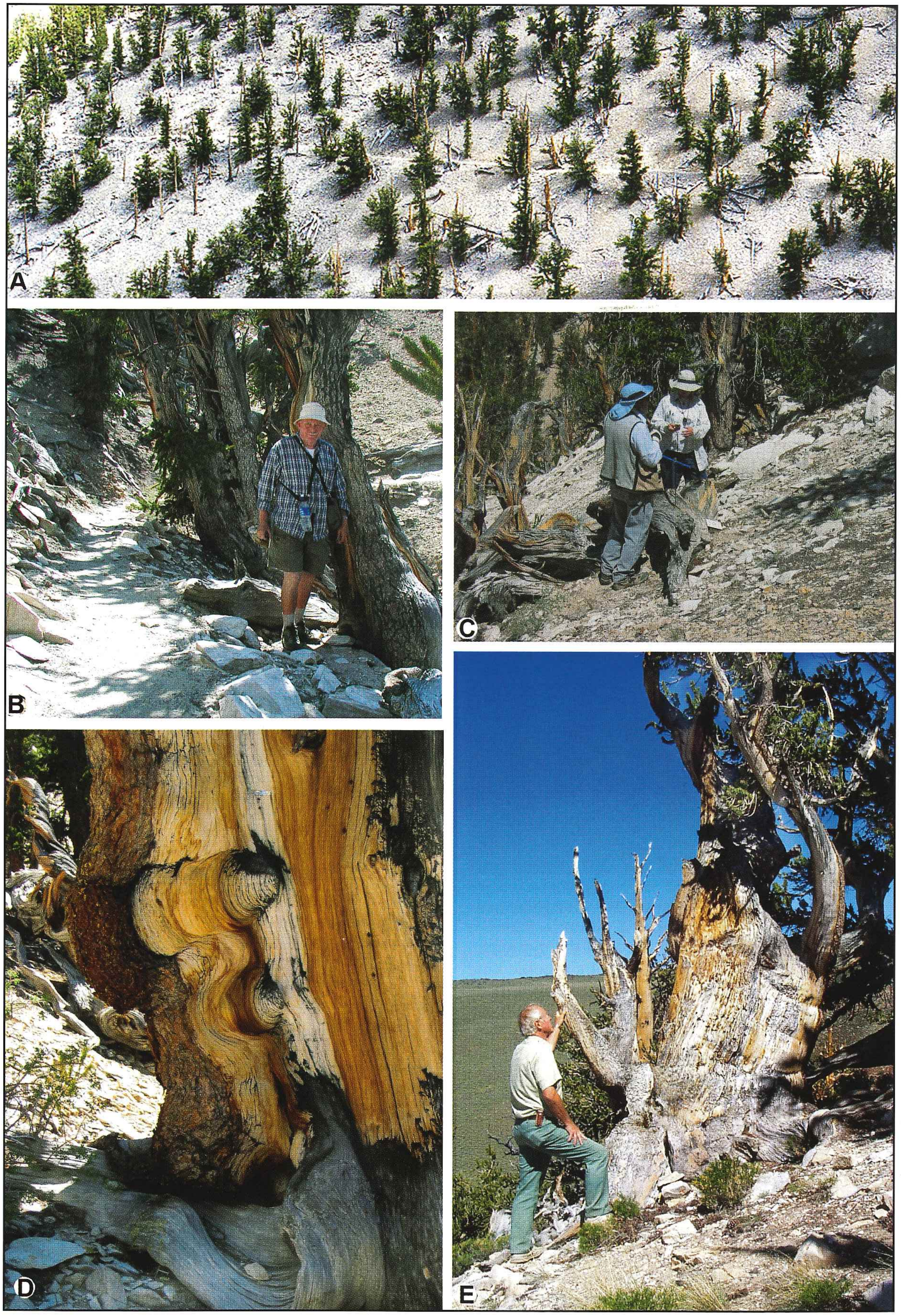


Plate 1. A. Methuselah Grove trail crosses in the middle of photo. Note white dolomite. B. H.J. Arnott resting on the Methuselah Grove trail. C. Rex Adams and Christine Hallman are looking for remnants on a steep slope in the Methuselah Grove. D. Stem of an eroded tree in the Methuselah Grove showing the origin of two branches, whose conical shapes can be seen because of stem erosion. E. George Novinger by a 4000-year plus tree called *Pine Alpha*.

Went on several occasions, both as a graduate student at USC and UC Berkeley, and later as a young professor at Northwestern. Fritz Went was a child prodigy. He discovered the important plant hormone auxin at the age of 23 and he continued as a botanical innovator throughout his life. For me, Went was real, someone I had known and been on field trips with. This connection made Schulman "genuine". Like other adventurers in the West, I could imagine the grand times Schulman and Went had while looking for the "super-old trees". Recently, I found that Schulman had published an earlier account of the 4000-year old trees in a more obscure book entitled "Dendroclimatic Changes in Semiarid America' published by the University of Arizona Press in 1956.

AN ATTEMPT TO FATHOM MY RENEWED INTEREST IN WOOD STRUCTURE

I can not clearly explain my recent attraction to the study of wood; after half of century of investigating all kinds of other things, wood was a surprise to me. I am not sure that one's interests require an intellectual explanation. However, here goes. To me, the appeal of wood studies is multifaceted and deep seated. In some ways, it was brought to life by seeing the bristlecones, by reading Schulman's and others' articles, and by talking to people in the Laboratory of Tree-Ring Research; they all played a role in creating this interest. However, I keep feeling that part of my interest goes back to building clocks. When building wooden clocks or pieces of furniture, you have the power of creativity; you can cut and sand the wood into an object of beauty and utility.

"THE POWER OF CREATIVITY IS A GREATER FORCE THAN MOST OF US WANT TO ADMIT."

I've been interested in clocks for forty years. Early on, I developed a special "love" for the *wooden shelf clocks* designed and made in the 1820's by Eli Terry in Plymouth Hollow, CN (see Part III of my autobiography) (Fig. 5). Like most clocks of the day, Eli Terry's clocks had wooden cases. And because metal was scarce in the 1820's, the early versions of these clocks had movements made of wood as well. In fact, each piece of the movement was made of a specific kind of wood: a gear was made of one wood, pinions from another and the back plates from still another wood. Beside the uniquely designed elements of the pillar and scroll shelf clocks, Eli Terry was also responsible for the concept of interchangeable parts that could be assembled by relatively unskilled workers. Prior to that time, each clock mechanism was made from start to finish by one craftsman.

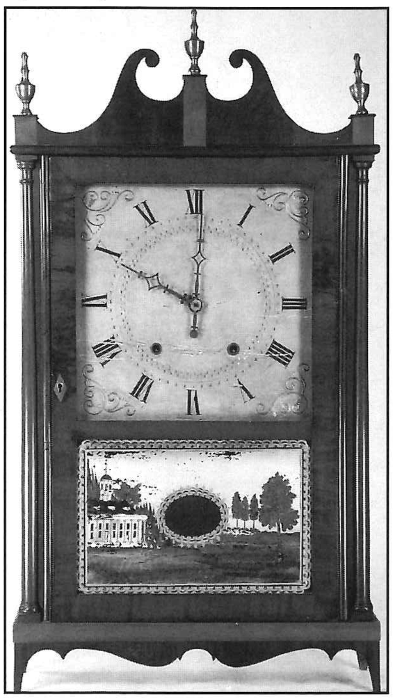


Figure 5. Eli Terry Pillar and Scroll wooden clock.

The concept of interchangeable parts developed by Eli Terry brought us directly to the assembly line. Henry Ford and others brought the assembly line to fruition in America and eventually in the world.

Clocks like the ones made in the first half of the nineteenth century by Eli Terry and his apprentice, Seth Thomas, are often excellent investments. Twenty years ago you could buy an excellent Eli Terry Pillar and Scroll clock for $1,000. Today, a similar clock could sell for $40,000, if you can find one!

On many occasions during my botanical preparation (see part III), the structure and development of secondary xylem, or wood, was introduced. At USC, Dr. Tomas Fuller taught me wood structure in both general botany and plant anatomy classes. Dr. Adriance Foster did it once again at UC, Berkeley. Although both professors made brief allusions to size and age, by tradition they were more interested in presenting the minute details of wood rather than the size and variation, which occurs in the secondary xylem. After all, size and shape were practical problems. Instead of investigating the wood of trees, we studied microscope slides of minute wood samples; even the question of lumber was generally ignored. Ask yourself how many botany students know what knots in wood are and where they originate from? We used microscope slides as a proxy for secondary xylem structure and from those slides we expected students to extrapolate what a tree is. Now, after teaching plant anatomy for over 50 years, this pedagogical approach seems completely backwards. I say this while acknowledging the fact that I had followed almost the same approach in my teaching that Fuller and Foster did when I was a student.

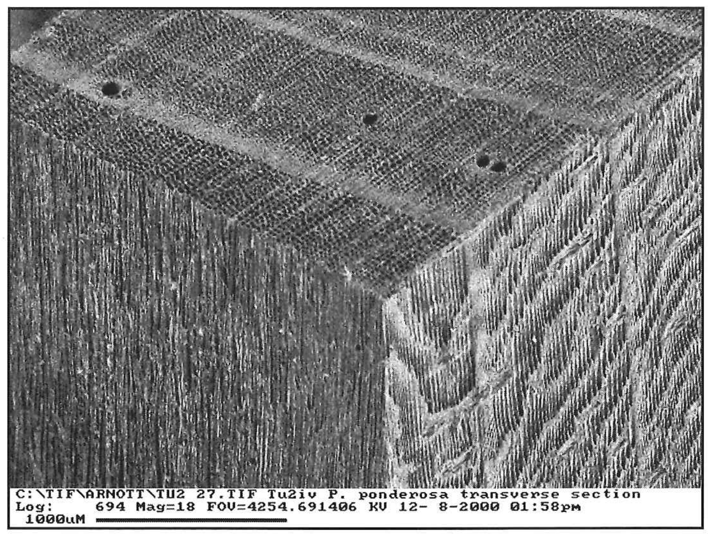


Figure 6. "Cubed" piece of *Pinus ponderosa* showing all three primary planes: radial on the lower right, tangential on the lower left, and transverse (cross) on the upper face.

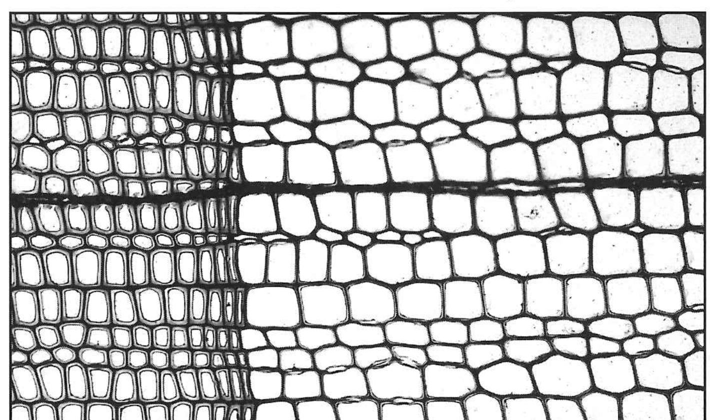


Figure 7. Light micrograph of a cross section of the wood of *Pinus lambertiana*. Note the regularity of the cells.

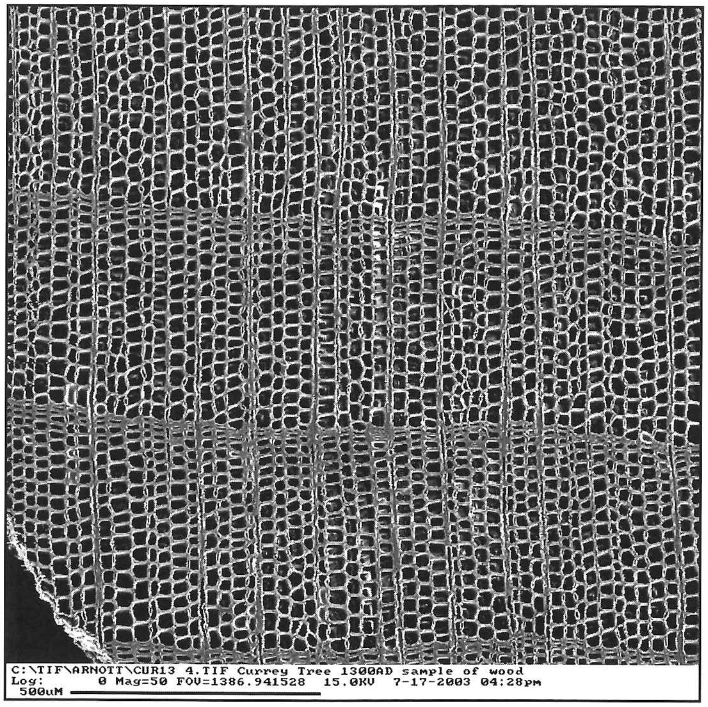


Figure 8. SEM of bristlecone wood. Note the cellular regularity in this piece of wood.

But again, let's come back to why is wood of interest to me. One of the important attractions of wood is its regularity. Wood *is* organized! It is organized into a three dimensional system made up of radial, transverse and tangential coordinates (Fig. 6). The marvelous structure of wood, or secondary xylem, is ultimately derived from one circumferential living cell layer called the vascular cambium. Every cell that makes up wood (or for that matter the bark) derives its very existence from this astonishing lateral meristem. Not only does wood have an organized and easily described structure, but its growth and development are also highly organized (Figs. 7, 8). On top of that, wood is made up of limited number of cell types. Each cell type has a unique and unchanging typological structure and function. This is true even though the trees from which the cells come may be completely distinctive and entirely dissimilar to each other. In that sense, the cell types in wood are not unlike the cell types which make up the organs of our body; your liver cells or your kidney cells are exactly like mine, no matter how different we look, or how old we are, or how differently we think or whether we live in a red or blue state.

Wood from different species varies in its resistance to decomposition. The wood of the bristlecone pine is particularly resistant to decay, due mostly to a high content of resins. However, the arid environment where they live and die is also very important in their resistance. Because of this resistance, we can look at the cells of bristlecone wood that are over 10,000 years in age. In many ways, these old cells are "exactly" like those found in living or recently dead trees. One of the great attributes of wood is that their cells are programmed to die so that they can conduct water to the living parts of the plant. In bristlecones the wood cells die but they don't decompose or decay. This is due to a complicated chemistry of the cell walls that is superimposed on a very strong cellulose base. The permanence of priceless piece of wood furniture is due to the structure and chemical composition of dead cells.

Superimposed on wood's regularity is the ability to vary! Wood varies, but it always varies in a regular way! In a way, it is like "Lego Blocks" which can be put together in many different ways. Annual rings provide still another level of structure in wood. Much of the grain that we value in furniture and floors is due to the structure of annual rings. By simple observation of a cross section of a stem you can see that some rings are wide while others are narrow; occasionally some rings are very wide or very narrow. Dendrochronologists pay special attention to the annual rings of stems because this gives them an account of what happened to their "host" tree, hundreds, or even thousands of years ago. The examination of ring structure can show us how and when temperature, rainfall or other factors affected a particular tree at some time in the past (Glock, 1934). Examination of tree rings can tell us about frost damage that occurred in a specific year, this has become my major interest. In a way, tree rings are like written music. When you can read music, you can produce wonderful sounds. When you can read tree rings, you can understand history, the history of an individual tree or the history of a population of trees.

IN AND OUT OF THE LABORATORY OF TREE-RING RESEARCH (LTRR)

Few of my professional years have been committed to wood studies. Only in the last few years I have been studying "wood." Members of the Texas Society for Microscopy will quickly realize this, since I have used our journal to document some of my "wood" work. When we talk about wood, we are really speaking about trees. Of course secondary xylem is produced in the stems and roots of shrubs and even in some annuals but it is the stems of trees "where wood rules." At the Laboratory of Tree-Ring Research (University of Arizona, Tucson) "wood rules." So it was that I ventured to the LTRR sometime in 1999. Jean and I drove to Tucson to find out what the LTRR was like. Admittedly, it was a bit of a "lark" or a "vacation." On that occasion, I found out some things about the LTRR, but it took several more trips to learn what goes on there. It didn't take me long, however, to find out that the LTRR is a National Treasure, both in terms of people and especially in terms of its collections. There are collections there that can not be made again as the material no longer exists in nature. There are collections there that are one of a kind; the opportunity to collect them again is gone. There are collections there that illuminate the development of dendrochronology from its inception. However, let's start with the beginning of my relationship with the LTRR.

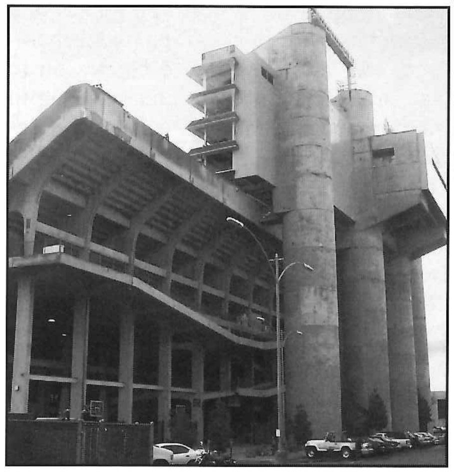


Figure 9. University of Arizona Football Stadium. LTRR is located on the first and second floor under the stands.

We found on our arrival in Tucson that the LTRR is situated in the football stadium; it has been there almost since its establishment, in 1937 (Fig. 9). The construction of the stadium was started in 1928, the birth year of Mickey Mouse and of Howard Arnott. Rooms under the stands provided the space for research and offices of the LTRR. To the public, LTRR is represented by two solid dark blue doors on the street level of the west side of the stadium between gates 15 and 17. On the second floor, but still under the stands, many professors find their office directly across from the hot dog stands that operate full blast during football season. The University band practices in the stadium during football season and the band music can be clearly heard in any part of the tree ring lab. I have never been there on a football day, however, I am sure it is remarkable - probably also deafening!

The LTRR was founded by A. E. Douglass, an astronomer;

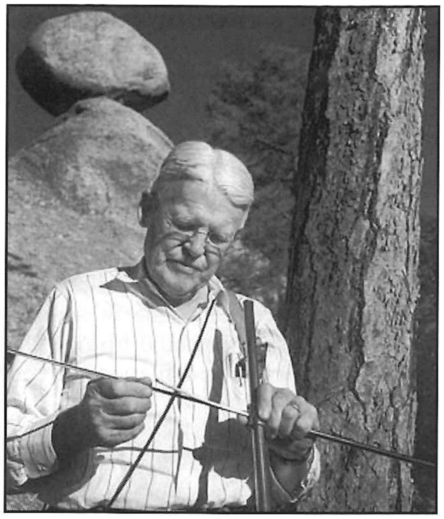


Figure 10. Andrew E. Douglass, founder of the LTRR. Photo courtesy of LTRR.

many consider him the father of dendrochronology (Glock, 1934) (Fig. 10). Because of my previous administrative experience, the organizational plan of the tree ring lab is of interest to me. Although it is a part of The University of Arizona, the LTRR is not organized like a traditional department or college where professors have allegiance to only their own department and/or college. Before he started tree work, A. E. Douglass was an astronomer and he kept his association with astronomy throughout the time he worked with tree rings, even as he became the prime initiator and developer of dendrochronology. His example may have set the *modus operandi* for the LTRR. In that organization, each professor has allegiances to both the LTRR and to one or more other departments such as geography, anthropology, archeology or geology, etc. This divergence carries on to the corresponding graduate programs since students working in the LTRR are from a variety of departments and will receive degrees from those departments. Obviously, members of the LTRR may have very different backgrounds and perhaps the LTRR organizational scheme leads to something akin to "hybrid vigor."

After finding the football stadium, I walked into one of the blue doors with LTRR stenciled on the door. This door led to a large laboratory where 10 or 12 students were working on projects. What happened next is another one of those "accidents" that brought back my interest in wood structure. The lab had a familiar enough look since it had microscopes and other teaching paraphernalia in evidence (Fig. 11). On the walls, there were large sections of stems, framed growth charts and photos, and a blackboard. I remember that the lab seemed cluttered with chairs, boxes of wood (stem) sections, student backpacks and other items. A class was underway in the lab and the students paid no attention to me and went on with their work. Someone, probably Tom Harlan, asked me what I wanted. I answered, "I am interested in wood and whatever you are you doing here." Very soon a general explanation of what was happening was put forward and I was made at to feel at "home." In a very short period of time I was introduced to Thomas Harlan, Rex Adams (Fig. 12), and Christine Hallman (Fig. 13). Each, in his/her way, was a key to understanding both dendrochronology and the LTRR. Not only did they provide access to the Lab on that day, but also later, all three were instrumental in helping me regain access to the Lab. Later that afternoon I learned about frost rings from Christine and the next day I was given a tour of the laboratory by Rex.

On the tour, Rex introduced me to several scientists that were working on tree rings, and I was privileged to see some of the research that was taking place in their facility. I specifically remember the "fire scar studies" led by the director, Tom Swetnam. Later Rex took me on a tour of the "storeroom," a kind of "wood herbarium." I had seen the Wood Herbarium at Kew Gardens in England, with their highly organized and uniform small pieces of wood; it was rather "dry." However, that did not prepare me for the LTRR storeroom. That was a new experience in itself! The storeroom contained fascinating pieces of wood, many of them of historical significance. For example, Rex pointed out the sections of trees that were collected by Edmund Schulman and Fritz Went during their quest for old trees. Two things were immediately obvious about LTRR storeroom: space was used to the maximum and curators were operating at a minimum. Obviously they were trying, but they were simply swamped by the magnitude of the collection; a problem that continues to grow as new material is added. Later I will describe the new opportunities for specimen storage. The nice well cut and ordered specimens of Kew were not what I found in this storeroom. Rather, I found an assortment of large and small pieces of wood that were stuck in every possible cranny from floor to roof. In some areas of the storeroom there were boxes and open shelves that contained wood. In the center there was a long and tortuous path that leads through piles of boxes. Somewhere, near the middle, the path branched in two. Here and there you were surrounded by stacks of color coded boxes. At the middle of the path there was a very old filing cabinet where the pieces of bristlecone wood used to recalibrate the C^{14}

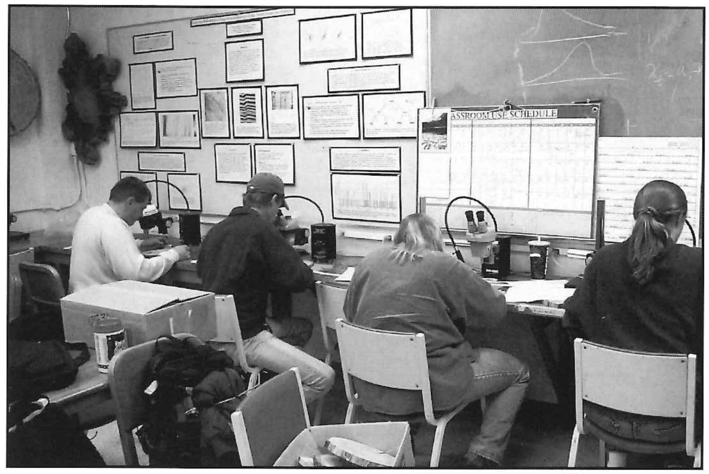


Figure 11. Students working in the old dendrochronology teaching lab. This space has now been converted into graduate students' cubicles and is euphemistically called The Tree Fort.

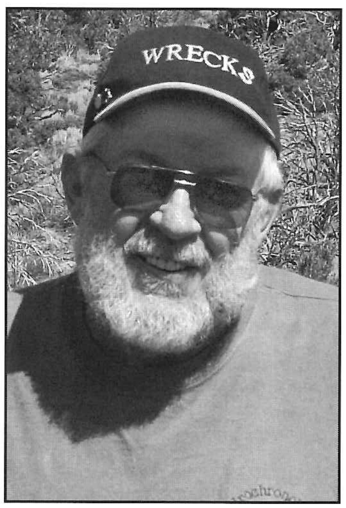


Figure 12. Rex Adams, a living encyclopedia of dendrochronology.

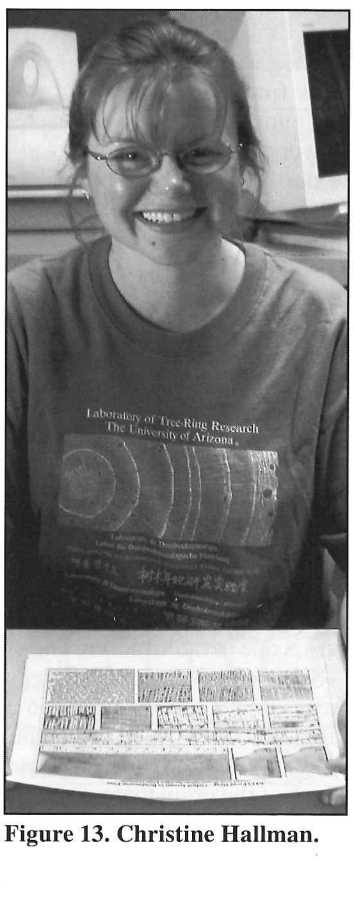


Figure 13. Christine Hallman.

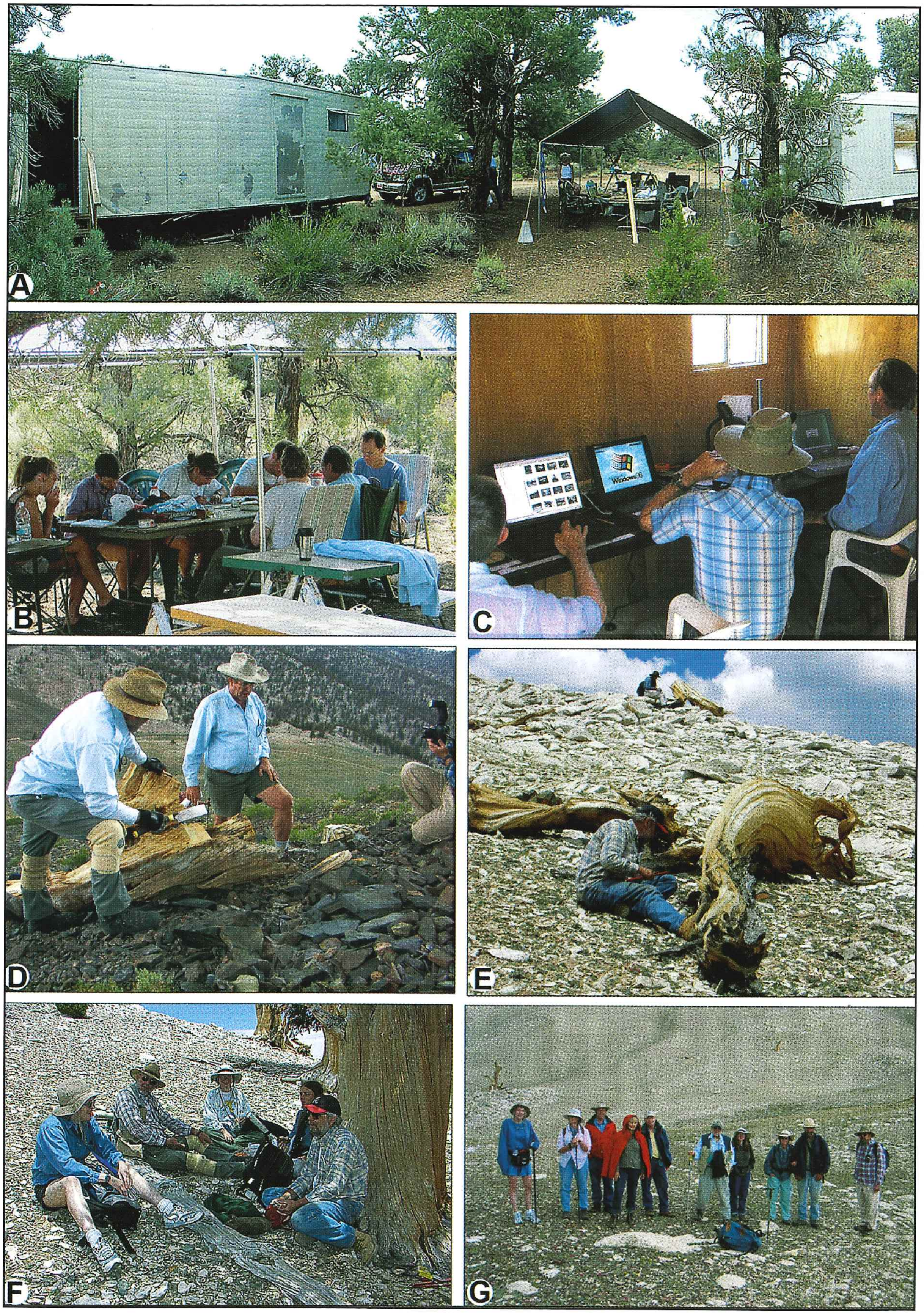


Plate 2. Camp Harlan. A. Trailers make up the center of Camp Harlan. The one on the left contains equipment maps and computers, while the one on the right represents living quarters. The canopy tent between is a resting and working area. B. Students from Swansea University, Wales, taking instructions in dating from Tom Harlan, 2nd on right. C. Equipment trailer inside view showing computers, etc. D. Rex Adams (left) and Tom Harlan working on a remnant piece on Campito Mountain. E. Jim Burns working on remnant wood of bristlecone pine on Sheep Mountain. F. Lunch break; (from left to right), Catherine Arnott-Thornton, Rex Adams, Christine Hallman, Angelika Clemens and Jim Burns. G. Group from Camp Harlan on their way back from the Skeleton Forest.

Figure 14. The new LTRR teaching laboratory and lecture hall located in the Math Annex Building (panoramic photo).

radioactive dating curve were stored. If you continued on this tortuous path, at its very end, one finds a series of 6 foot in diameter pieces of redwood collected by A. E. Douglass as he studied the age of redwoods. The description given above is not a criticism of the LTRR; it is a statement of fact and clearly the *powers that be* are well aware of this problem. If ever a place needed a curator, this is one. Of course, Rex, Tom, and Chris Basin with almost ninety years of service between them, can find things because they helped store them.

I have just described the way the storeroom looked like when I first visited the LTRR. Let's jump forward now and see what has happened since then. The biggest improvement to date is that the basement of the Math Annex building is now occupied by the LTRR. Among other things, there is a large area devoted to *Sequoia* slabs with fire scars; these are on steel frames and off the ground. The shop, with its very large band saw, was moved there and finally a class room/lecture hall was made (Fig. 14). In addition, a series of measuring devices have been collected in the "measuring room". These changes were great but of course have not alleviated the storeroom problem. However, late in 2007 the LTRR received a $9,000,000 grant from Mrs. Emil Haury to build a proper storage facility. Her husband, Dr. Emil Haury, was a distinguished member of the of LTRR. Currently, the plan for an improved storage facility is in the hands of the University of Arizona architects.

Because I expressed interest in the wood of Prometheus, it was great when Rex quickly located the Currey Tree material stacked on shelves in the storeroom. During the afternoon and the next day, I was given a chance to examine these collections by myself. What a thrill it was to look at and feel the wood of a 4800 year old tree. However, one continues to worry, was it really necessary to cut the living tree down? The Prometheus material was sometimes labeled as Currey Tree or WPN-114, but not Prometheus. Many of the pieces had been dated with ink writing or labels glued on wood surfaces. Many pieces were coated with some kind of sealer, perhaps varnish, which to me diminished their value. However, I did not have the background or "chutzpah" to realize that they represented a jig-saw puzzle waiting to be put together. Later Christine Hallman performed a miracle by assembling pieces of the puzzle into the "third slab" of Prometheus (see later). I was able to photograph many of the pieces by putting them on the stadium seats, in broad sunlight. Rex was kind enough to cut off some small pieces of the LTRR Currey Tree material. I immediately used the wood when I returned to UTA. One of the first things that I did was to macerate Prometheus wood and to examine the results with the Scanning Electron Microscope (SEM) (Figs. 15, 16). In one piece of the Currey Tree wood I was lucky enough to find the 1453 late frost ring. I made light and electron micrographs of that frost ring and they have been seen on Christine Hallman's web site for many years.

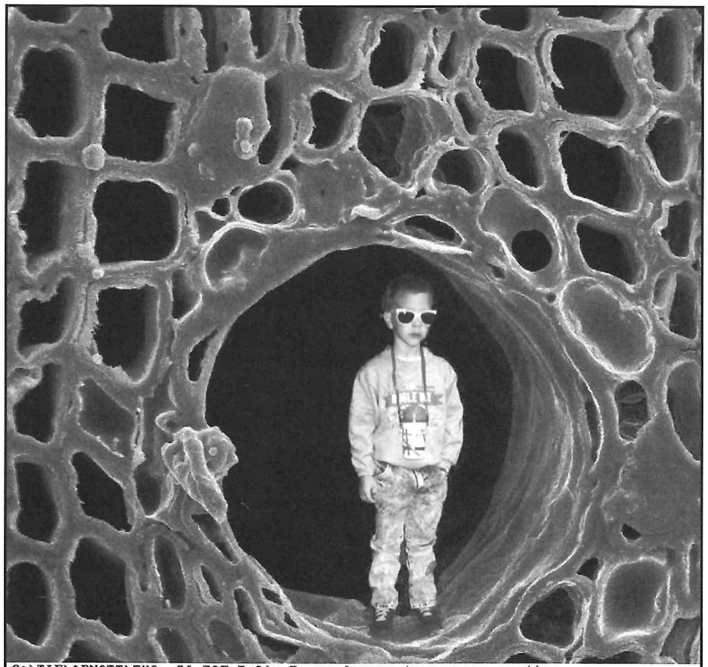


Figure 15. Sometimes you need a scale object. Here, a very young James Arnott is standing inside of a resin duct of *P. longaeva*.

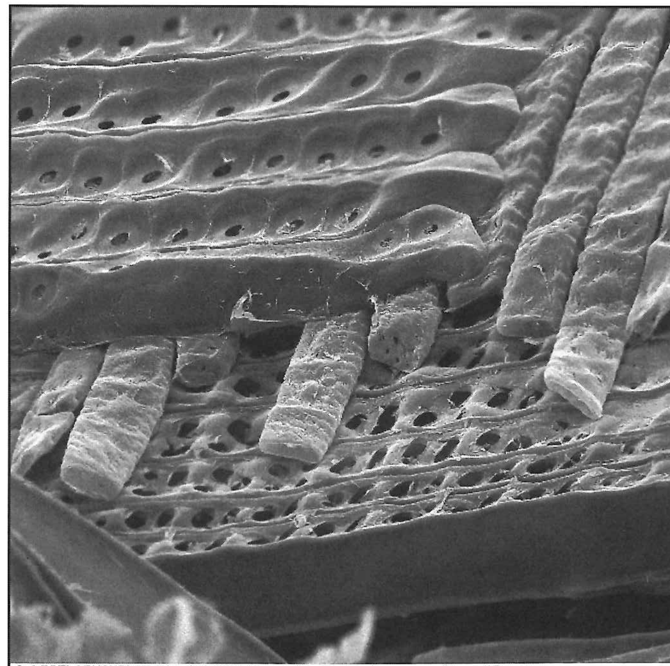


Figure 16. Macerated wood of Prometheus showing the interrelationship between tracheids, horizontal cells with circular bordered pits, and the ray cells (vertical) in this photo.

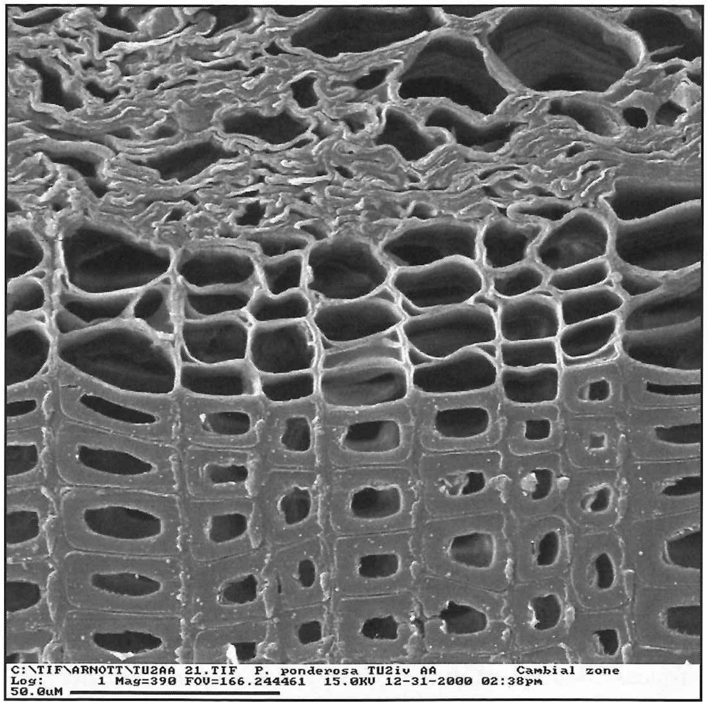


Figure 17. Vascular cambium of *Pinus ponderosa*. Section made from a completely dry "cookie".

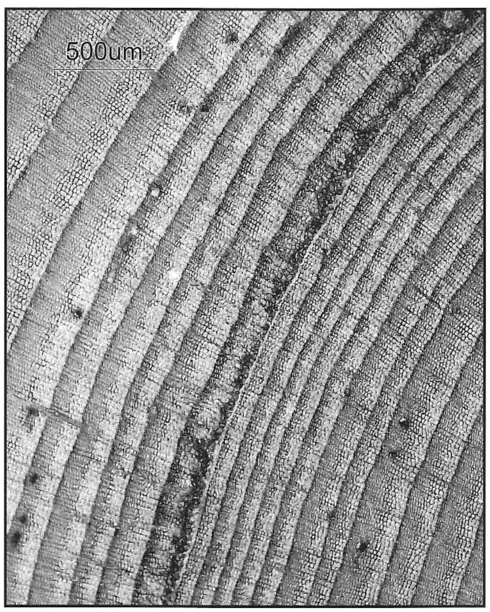


Figure 18. Cross section of bristlecone wood showing a prominent frost ring (ninth annual ring from the left).

I have worked in the LTRR several times; the first time I spent a week in 2002. During that time I got to know Hal Fritts and his postdoc, Debbie Hemming who were working onacambiummodel. The model, as I understood it, was very complicated because it attempted to factor in every element of cambial structure and physiology that they could envision. Debbie Henning challenged me to make some SEM pictures of the cambium from dead ponderosa pine wood (Fig. 17). As I left, she gave me several "cookies". A cookie in this instance is a cross section of a stem. As it turned out, those cookies were extremely useful both for studying the cambium and also for studying frost rings. In fact, those cookies have some of the best frost ring material I have yet to observe. I was also able to connect the 1971 frost ring with temperature data provided by Fritz and thus establish that this ring was due to local freezing conditions and not some far away volcanic eruption induced climatic change. Careful study of the several frost rings showed exactly how the original tissues were crushed and structurally modified during the frost event (Figs. 18, 19).

Because of my interest in frost rings, Dr. Katherine Hirschboeck sponsored my first official 6-week visit to the LTRR. Her interest in frost rings goes back to a now classic paper she published with Valmore LaMarche in which they tied a 1628/27 BC frost ring in

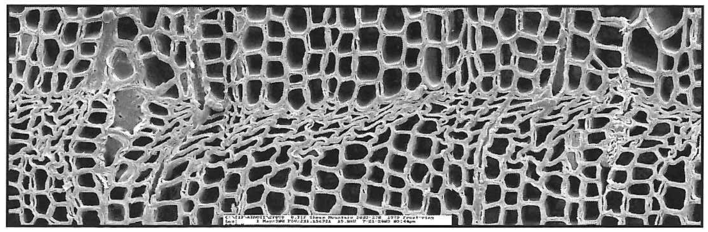


Figure 19. SEM montage of the 1965 late frost ring in a bristlecone pine tree from Sheep Mt.

bristlecone pine with the explosion of Santorini volcano (Santorini or Thera Island) (Hirschboeck, 1980; LaMarche and Hirschboeck, 1984). Their date was accepted by many as the definitive age of the explosion that blew away 80% of the island. The presumption is that an atmospheric aerosol veil developed from the explosion, traveled around the world, and caused a lowering of the temperature and hence the production of frost rings. Hirschboeck is still concerned with frost rings and volcanoes; however, it is clear not every frost ring is born from a volcanic eruption; Glock (1951) generated frost rings using dry ice and a 55-gallon drum. Recently, Schweingruber (2007) and others seem to have cast doubt on the connection between all frost rings and volcanoes. The date for the Santorini explosion still remains controversial.

During this visit, I was housed in a second story office shared with Dr. Bryant Bannister, retired director of the LTRR. Naturally, I got to know him and still talk to him whenever I visit the lab. He seems to remember everything that has happened to the LTRR and is willing to share his knowledge. He also has a great sense of humor, unlike others that I have known in their retirement. During those 6 weeks, Dr. Hirschboeck was very busy with some out-of-the-ordinary teaching duties and, as a result, we had only brief discussions during my stay. In fact, although unofficial, Rex Adams was my real mentor. That was a providential break because I learned a great deal of basic dendrochronology from him as he was more than willing to teach me, both about the past and current history of LTRR. Rex was great at finding materials with frost rings and through him I "harvested" a significant amount of wood that now resides at UTA. It was then that I learned that the LTRR has many correspondence and research files from people like Douglass, Schulman, and Ferguson. They are interesting reading and certainly grist for new volumes about them.

Each fall, during the dendrochronology course, the students take a field trip to learn how to perform the major tasks of dendrochronology in the field. Rex (Fig. 12) asked me if I would like to go along and, of course, I accepted. Field trips are one of the very best ways to learn about any kind of subject. This field trip went up through northern Arizona and into New Mexico; it involved two overnight stays, one at a house and the second camping out in tents. The tent I borrowed from Rex was interesting in that it was an "instant tent." One minute you had a coiled up flat round object and the next second it opened up into a full tent - an instant tent! However, I still remember the camp-out night as one of the most miserable that I have ever spent. This was mainly due to the rocky ground; a camp site which no pseudo-mattress drop cloth could disguise, so that even a pebble only 1/2 inch in diameter could be a terror in the night. Moving the tent, which might seem an alternative, simply took you to a new set of rocks. Secondly it was miserable because the tent "dripped or rained" on me. Some "technical detail" in tent construction seems to cause this; but clearly motels are the way to go.

Aside from that wretched night, the field trip was very enjoyable and informative. We hiked and toured El Morro National Monument (www.nps.gov/elmo/index.htm, which is a site with several prehistoric dwellings, only a small part of which has been investigated. El Morro is an astonishing place to visit and it

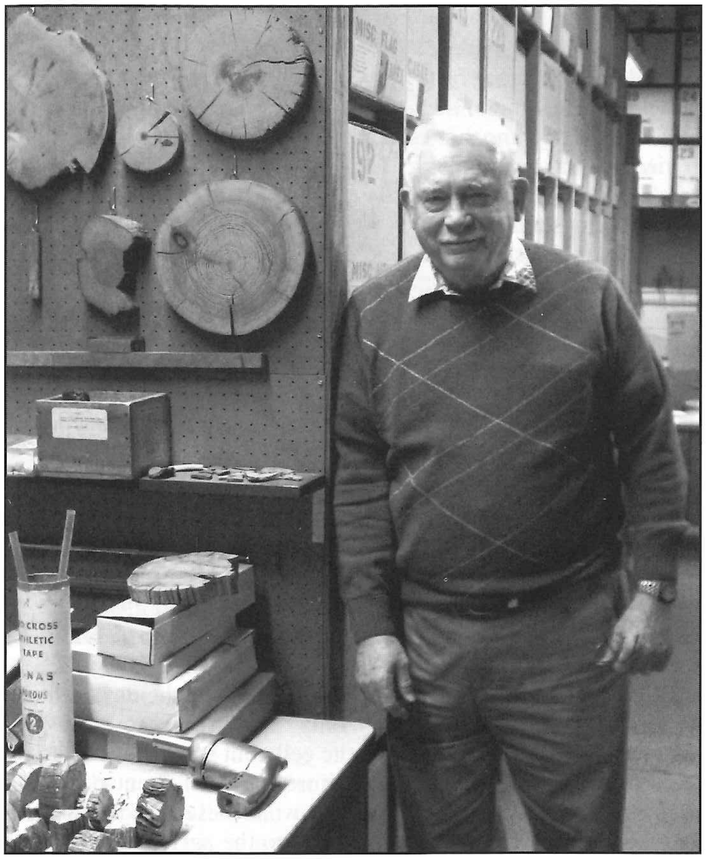


Figure 20. HJA in the archeological collections of the LTRR. Note storage boxes on shelves. Photo by Dick Warren.

was very enjoyable. We spent some time at El Malpais National Monument where we saw the Ice Caves and after a good walk over a very jagged lava flow we saw the oldest Douglass fir in the southern US. Incidentally, a fall on that lava meant serious injury. Later we drove into a mixed forest in the Zuni Mountains where the students practiced using increment borers and GPS units. All in all, the field trip was a great success. Oh yes! I forgot that we stopped at Show Low, AZ, where the piece of charcoal (HH 39) that "bridged the gap" for A.E. Douglass was found (Douglass, 1929). At Show Low we heard an informal lecture by Dr. Jeffrey Dean, Professor of Dendrochronology, about the importance of bridging the chronology gap in *P. ponderosa* studies. Once done, Douglass could immediately date many prehistoric southwestern sites. Currently the spot where the charcoal was found is covered by concrete.

I gave two seminars to the LTRR group during that stay. "Frost rings" and "What ever happened to the world's oldest tree, Prometheus?" (Plate 3). The first talk gave information about the formation of frost rings and was based on the studies I did on *Pinus ponderosa* from Mt. Lemon, AZ. Basically the work was on the same cookies that had been given to me by Dr. Debbie Hemming. I later found out that the cookies were "borrowed" from Ed Wright and were a part of his dissertation material. Subsequently, Hal Fritts took me to see where the trees grew before harvest. All three trees had been cut within 1 inch of the ground. In the Prometheus paper I informed them about the way in which "my" slab came in to my possession and what we were doing with it. I also showed them pictures of the Prometheus site and the importance of the section from the "V" cut; most of this is covered in detail later. Since Christine had, by then, put together the LTRR's slab, I was able to compare and contrast the two slabs as well as deal with how the latter slab got to the tree ring lab. I also compared it to the third slab that is in the Bristlecone Convention Center in Ely, NV.

Near the end of this visit, I began looking at frost rings in cores taken from timbers of Spruce Tree House in Mesa Verde National Park. This was interesting in that many of the cores were dated in terms of the starting date (beginning of the secondary xylem formation) and the cutting date (the year in which the Mesa Verde people actually cut the tree). With those dates in hand, it is easy to exactly date frost rings to the year. Some of the cores taken in the early years are 1 inch in diameter while more modern cores are either 1/2 or 3/8 of an inch in diameter. Rex was very helpful in this research since many of the cores had to be cut and sanded or re-sanded. Unless the cores are carefully sanded frost rings are often impossible to see. It was not difficult to extrapolate that archeological cores and cookies would be a ready source of frost rings. This *cache* of frost rings that interested me; it was like a gold mine of frost rings. It was only later that the timing of the frost rings became interesting; the chronology of each frost ring is probably much more important than the nature of the frost ring. Unfortunately, before I could do much I had to return to UTA, however, some of the cores went with me so that they could be studied by light and electron microscopy.

By the end of my stay, Christine had put together the LTTR's slab of Prometheus and it was just a mater of time until she dated it. During the last few days, I used the measuring machine to measure the widths of the individual annual rings; this was done for most of the main piece section and for the piece section containing the pith. Chris Basin helped me "cross date" the two pieces using COFECHA and we came out with 4901 as the total number of annual rings. This is 57 more rings than Currey's count and 39 more than Greybill's count of 4862. Recently, Tina Halupnik cross dated the two pieces and came up with a count of 4842 annual rings. The later count is essentially the same as Currey's count reported in the journal Ecology (Currey, 1965). Tina Halupnik used a complicated method of photography to measure the rings very accurately and the Greg Lazear and Tom Harlan's cross dating program to make her count.

My second visit to LTRR was on a Sabbatical in the spring of 2007. My visit was sponsored by Dr. Jeffrey Dean, the Associate Director of the LTRR and the person in charge of the Archeology Collections. It was from those collections that I obtained the Mesa Verde cores mentioned above. Although he was busy we still had regular meetings every Monday morning. In those meetings we went over whatever findings I had made during the previous week and decided where the next set of cores would come from. The archeology collection has well over 400,000 specimens and I was grateful that Jeff took care of finding the various specimens. In general, the specimens are contained in boxes that give

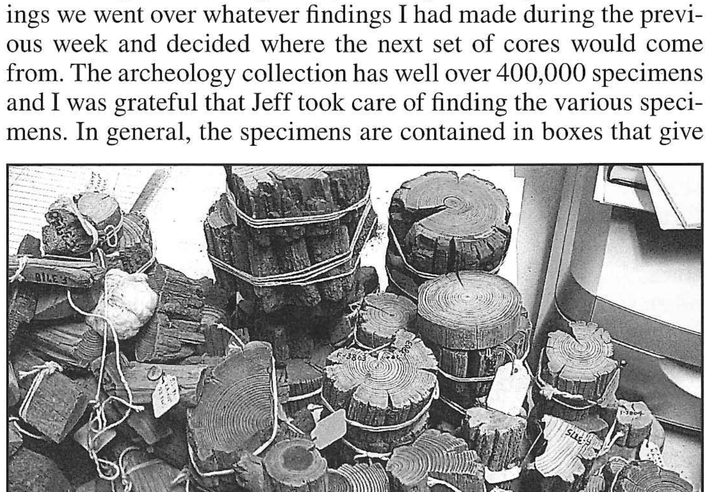


Figure 21. Some of the "cookies" from Wupatki Pueblo.

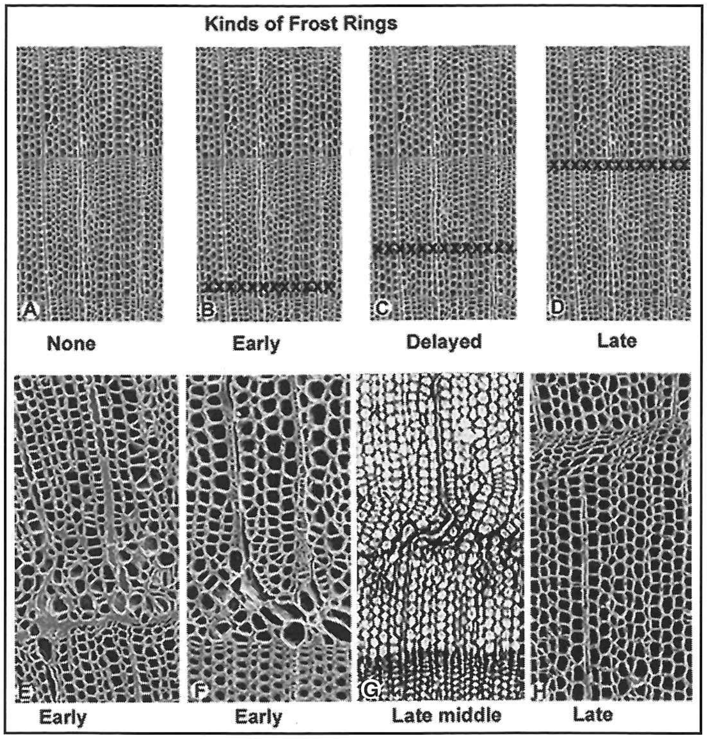


Figure 22. Types of Frost rings are illustrated. When an annual ring has frost damage in the ring it is referred to as a frost ring. For example if the 1925 annual ring has frost damage then we call it a 1925 frost ring. A-D. Are four electron microscopic examples of the same annual ring which exemplify different types of frost rings. The X's mark the part of the annual ring in which the frost damage would be found. In the delayed frost ring case the X's are in the middle of the annual ring, in the early frost ring case the damage is in the oldest part of the annual ring and in the late frost ring type the damaged cells are in the youngest part of the annual ring. E. and F. are electron microscopic examples of early frost rings, the dense grey areas represent crushed tracheids. In E there are only a few late wood cells marking the boundary of the annual ring, whereas in F several rows of late wood cells are seen. G. Shows a light micrograph of a delayed frost ring where several rows of tracheids have been produced prior to the freezing episode. H. Shows a normal late frost ring in which the main "damage" is an alteration in the direction of the tracheid files.

the appropriate content data on the outside. The individual boxes are about 14 inches square and contain 1.6 cubic feet of space. With careful packing many cookies can be stored in 1.6 cubic feet. One box might have cores and cookies from Long House and another would have cores and cookies from Spring House. Some sites were represented by a number of these boxes, for example there are 26 boxes of material from Pueblo Bonito or a total of over 41.6 cubic feet of stored materials. There are probably more since, in some cases, the Pueblo Bonito material is combined with other Chaco Canyon material. By this time, you should have a vision of the vast amounts of materials that are contained in this collection (Figs. 20, 21).

During the five months that I worked on the Mesa Verde materials, I studied cores from the following sites: Spruce Tree House, Spring House, Square Tower House, Balcony House, Long House and Cliff Palace. Of course, there are many more sites in Mesa Verde. Some say the number may be as high as 4000 different ruins. Some sites are very small, perhaps consisting of only one room or a simple storage area. The six houses cited above represent a very good sample of the larger ruins in Mesa Verde. You may ask what the major findings of this research are. The first finding is that there are many frost rings in the cores of Mesa Verde timbers and, by extrapolation many frost rings should be present in the cores taken from other southwestern ruins. As I indicated above, prehistoric sites are a potential gold mine of frost rings. A second finding is that frost rings have a greater occurrence in certain years than others. Because my study was on a random sample of cores, this means that certain years are more important in the environmental and ecological conditions of Mesa Verde that others. The most "frequent" years observed in cores and representing frost rings are AD 1179, 1189, 1219, 1229, and 1248.

The frost rings in Mesa Verde are over 98% early frost rings (see explanation in Fig. 22). This is entirely different from what we found on cores from Sheep Mt. trees. On Sheep Mt., an individual year will always have either early or late frost rings (see later). There is a relatively simple explanation for this early frost ring phenomenon. The explanation concerns the time in which the trees of the Mesa Verde area are actively growing. Douglas fir and pinyon pine grow in the early part of the year and their growth is completed by early summer. Hence they are never growing later in the year where they could suffer from freezing temperatures and form late frost rings. The growth period of *Juniperus* has not been critically established. However, the predominance of early frost rings indicates that these species are finished growing long before the subzero weather returns. Another interesting finding is the relatively common occurrence of "delayed" frost rings. These are frost rings, which have a series of normal tracheids laid down before the frost event. Generally, only three to eight rows are produced before freezing damages the cells but sometimes up to 30 rows of tracheids may develop before the frost event occurs. It is of course interesting to speculate what these periods of cold, as demonstrated by frost rings, meant to the agriculture of these early peoples. Perhaps both cold and drought have conspired to make their agricultural experience difficult. It is quite possible that cold played its role in causing the abandonment of the cliff dwellings at Mesa Verde in the late 1200's.

In order to accomplish the Mesa Verde frost ring study, I "imported" my microscope (Nikon 80i), camera, computer and other equipment from Arlington. The computer was loaded with three image processing pieces of software and was an absolute necessity to accomplish this research. I also worked out a method to photograph the wood's surface without sectioning it. Both Mike Johnson and Mike Davis of Nikon were very helpful in working out a satisfactory technique. The process of sanding the surface of the wood is an essential part of the preparation technique. I need to emphasize that without proper wood sanding you can not see frost rings. With ordinary belt sanders you have to go through a series of stages in sanding. If the surface is reasonable you can start using 220 grit, then you go to 320 grit, and follow that by a 400 or 500 grit belt. Even then some fine-tuning of the surface may be necessary to get the proper finish; in the later case 600

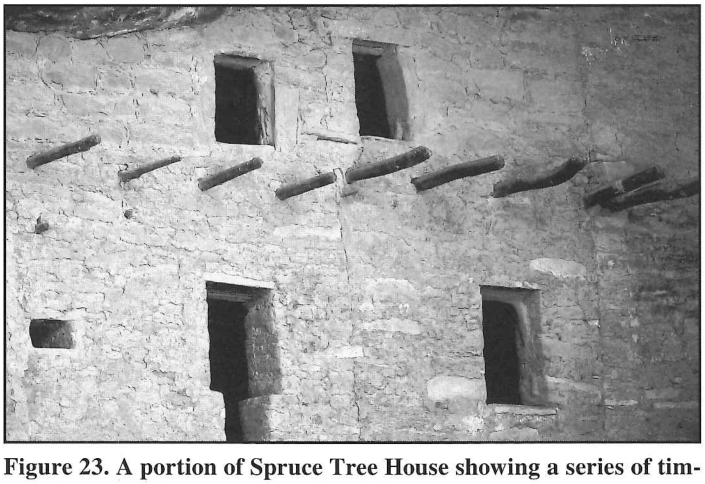


Figure 23. A portion of Spruce Tree House showing a series of timbers from which cores were extracted.

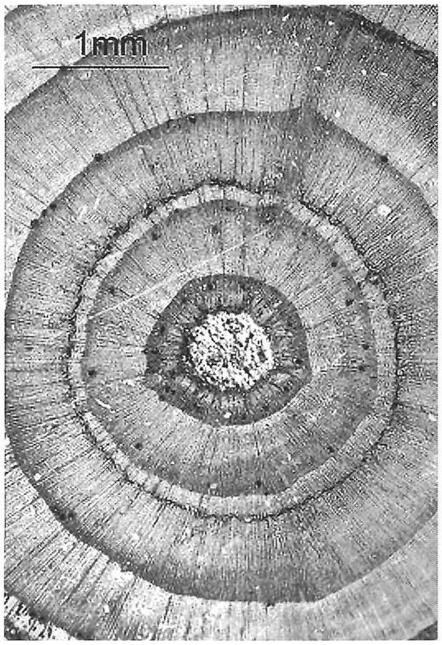


Figure 24. Cross section of a frost ring in a timber from Spring House, Mesa Verde, Colorado.

grit polishing cloth is recommended.

In a session of Tree Ring Talks (seminar), I summarized the findings for Mesa Verde (Figs. 23, 24). First I showed that frost rings occurred in the timbers of the six cliff houses studied. That being said, there were differences in the relative numbers of frost rings found in each house. Secondly I found that frost rings were more common in juniper wood. Five sixths of the cores that had frost rings were juniper. In part this was due to a larger number of juniper timbers but it was also due to the fact that multiple frost rings were found in juniper wood. The amount of juniper found in each cliff dwelling varied substantially. In all I had found over 1000 frost rings in cores that I studied. This is not a substitute for a proper publication so I am not trying to provide all the facts in a detailed manner.

In the spring of 2008, UTA awarded me another sabbatical to continue the frost ring studies at the LTRR. Jeff Dean served as my sponsor and he responded to any and all of my questions and needs. The main purpose of this second investigation period was to extend the frost ring studies done in Mesa Verde to other sites with the objective of developing a frost ring chronology for the southwest US region. I studied the following sites (listed in alphabetical order with the number of frost rings given in parenthesis): Betatkin (50), Broken Flute Cave (44), Casa Chiquita (4), Chetro Ketl (72), du Pont Cave (4), Kiet Siel (54), Kin Kietso (20), Mummy Cave (64), Mummy Cave 2 (11), Obelisk Cave (11), Prayer Rock Cave (3), Pueblo Bonito (271), Pueblo del Arroyo (50), Sliding Rock Ruins (2), Tabeguache Cave (18), Tse-Ya-Tso (15), Una Vida (4), Walpi Pueblo (446), White House (24), and Wupatki Pubelo (117).

Currently, the frost ring chronology extends from 261 B.C. to 1922 A.D. The oldest frost rings (261 B.C.) were found in specimens from Tabeguache Cave, a basket maker II site, in Montrose County, South Western Colorado. The latest (1922 A.D.) was found in Walpi Pueblo, a Hopi site that is still occupied on First Mesa in North Eastern Arizona. The total number of dated frost rings found in Southwestern archeological sites in the latter study is 1284 which if added to the 813 previously found in Mesa Verde makes a grand total of 2097 dated frost rings.

Throughout the periods that I have been at the LTRR, Rex Adams has been the one that I could count on for help. Both Tom Harlan and Christine Hallman were always helpful when asked, but retirement (for Tom) and many responsibilities of her graduate program (for Christine) made communication more difficult. During summers, I continued to cement relationships with all three at the White Mountains camp. In the next section I will explain some of the activities that occur in that camp.

CAMP HARLAN – A BRISTLECONE HERITAGE SITE

For years Tom Harlan has organized a field camp in the White Mountains. Anywhere from 10 to 30 individuals may be present on days in July and August; all are working on bristlecone research (Plate 2, A-G). The surprising part of this camp was that the vast majority of individuals are donating their services; many seriously feel that this is their summer vacation. It is a real tribute to Tom and Annita Harlan that people return year after year to help with the various bristlecone research projects that are centered in the camp. I can make no claim as to understanding the array of activities that are done using the camp as a base. However, I know the main force that drives Tom Harlan; it is the hope of extending the bristlecone chronology back to the ice age, back to a time perhaps 11 to 15 thousand years before the present. It seems quite possible that this will happen; it also seems clear that there is only a "small" gap (missing link) between the present chronology and much older wood already in the LTRR collection. Everyone hopes for Tom's sake, that the gap will soon be closed. Currently the bristlecone chronology is over 8800 years long. The Henry Michaels' piece (a large piece of remnant wood from the Methuselah Grove) in the LTRR storeroom has over 3000 rings in it. When that remnant can be integrated into the bristlecone chronology it will extend back 11,800 years.

The individuals that reside in Camp Harlan come from all walks of life; Tom Harlan ("a ranch kid") was born in Harper, Texas, Annita Harlan is from Van Horn, Texas (Fig. 25). Their visitors have a wide spectrum of vocations and avocations. They are extremely talented and finally they are amazingly friendly. Other than Tom as the "leader," there is little perceptible in terms of lines of authority. Perhaps both Rex Adams and Annita Harlan serve in the capacity of "lieutenants;" if so, they do it with great modesty and circumspection. While Tom and Annita live in a trailer, most, including Rex, camp out in tents. Meals are made on cook stoves, a latrine and portable potties are provided by the Forest Service, water is available and shower facilities are sometime available. I remember that at one edge of the camp there was a rock. Everyone used that rock to check his or her GPS units; it was a simple but useful routine. The altitude at the camp is about 7500 feet, which gives people who will be working at higher elevations in the mountains an opportunity to acclimate. Catherine and I usually stayed at a motel in Bishop so we never knew what went on in camp at night. I often speculate that their activities may include sitting around a campfire telling ghost stories or jokes; Rex has an unlimited supply of the latter. Here's one of Rex' jokes, for example: A termite walks into a bar and asks "Is the bar tender here?" Of course, some individuals may just collapse after a day of strenuous hiking and backbreaking inspection of tree after tree. Somehow, I always thought that we missed something with our city slicker ways.

Figure 25. Annita and Tom Harlan, summer 2003.

When Catherine and I first met this group in July of 1999, my intentions were simply to learn about bristlecones trees, which had definitely caught my fancy. I also wanted to see dendrochronologists in action in the mountains of California. At that time, my shift toward the study of wood was still in its infancy and other than frost rings I had no special interests. With many years of university administration under my belt, it was amazing to see how people worked without any obvious lines of authority. In universities of my experience almost everyone worked for themselves; it was only with real pressure that they cooperated and often their cooperation was faked. At Camp Harlan people work like "ants." The ant analogy is not meant as a putdown but simply as an observation. When you watch ants in movies or if you have the talent for observing them in nature, there are no apparent lines of authority but still a great deal of work gets done. So it was in Camp Harlan (Plate 2).

Collecting remnant wood was the assignment of most people in camp (Plate 1, C; Plate 2, D-F). There is, of course, a substantial routine associated with such activity. When a piece of remnant wood is found, it is tagged, photographed *in situ*, and its location (GPS data) is collected, whole small pieces were returned to camp. Larger pieces are either cored or representative samples are cut from them, or both (Plate 2D). Finally all the data is logged into Tom's bristlecone data base. Efforts on remnant wood collecting are concentrated in certain areas deemed to be regions of importance. In the world of remnant wood collection, the collectors' motto is "the older the better." Sheep and Campito Mountains, Sliver Canyon and Methuselah Trail are investigated over and over. Recently, in the latter, Annita Harlan found a very old piece of remnant wood by following a rabbit, the piece is now called the "bunny" or "bunrab piece". When Tom examined the specimen, it turned out to be over 8800 years old and is currently the oldest piece of bristlecone wood to be cross-dated. Based on extrapolations of current data, new areas are mapped for exploration each year. After cutting and sanding a new specimen, skeleton plots were immediately prepared for many samples. The beginning and ending dates are determined by cross dating, either by hand or using the computer cross dating program that was developed by Greg Lazear. For cross dating, the bristlecone chronology, developed for the White Mountains during the years since Schulman first explored the area, is used. Some camp members are engaged in re-sampling remnant wood, especially wood that does not have GPS data. Another important assignment is to find the location of trees that were photographed in the past. Often, the exact site where the photograph was taken was only vaguely described. Small teams of two or three people cover the landscape trying to incorporate GPS data with old and new photos. Others are engaged in finding tagged specimens and taking GPS and photographic data; occasionally some are retagging trees where the tags are lost or worn out. As this is done, new photographs and GPS data also are accumulated. Recently some individuals have distributed and downloaded "I buttons" which record temperature during the year.

The camp site has recently moved a short distance and some permanent quarters are now available. Prior to the recent upgrade, the camp was organized around two trailers, donated by the Forest Service. One trailer provided working space, electricity, and security for equipment such as microscopes, etc (Plate 2, A). The second trailer served as Tom and Annita's "home away from home." One or more large canopy tents were set up between the trailers; they provided work space, a place to eat and relax and finally a place to escape from the sun. When not in the field, Tom provides "campers" with help in wood preparation, hand and computer cross dating instructions, and general information as needed. One year, Tom had a group from Swansea University in Wales; he taught them dating techniques as a class (Plate 2, B). I would be remiss if I did not mention the fact that Tom loves to tell stories. His stories are about the good old days, about search and rescue teams he was on, about the characters in bristlecone research or just about people and places he knows.

I was never engaged directly in remnant wood collection, retagging, or photo reconnaissance, but I observed others as they went about their work. Catherine and I followed a group up the north flank of Campito Mt. to a large almost entire tree remnant log, which had been found the year before. Using hand saws, Tom Harlan, Rex Adams, and Christine Hallman cut substantial pieces from this downed log (Plate 2, D). The tree TRL 2001-682 turned out to be more that 7200 years old. The oldest frost ring found in bristlecones is from TRL1979-139 from the Methuselah Grove and dated -6413 or 8422 years ago. I examined this frost ring using both light and electron microscopy. Newspaper reporters and photographers often come to Tom's camp in search of interesting stories. In the episode described above, both a photographer and

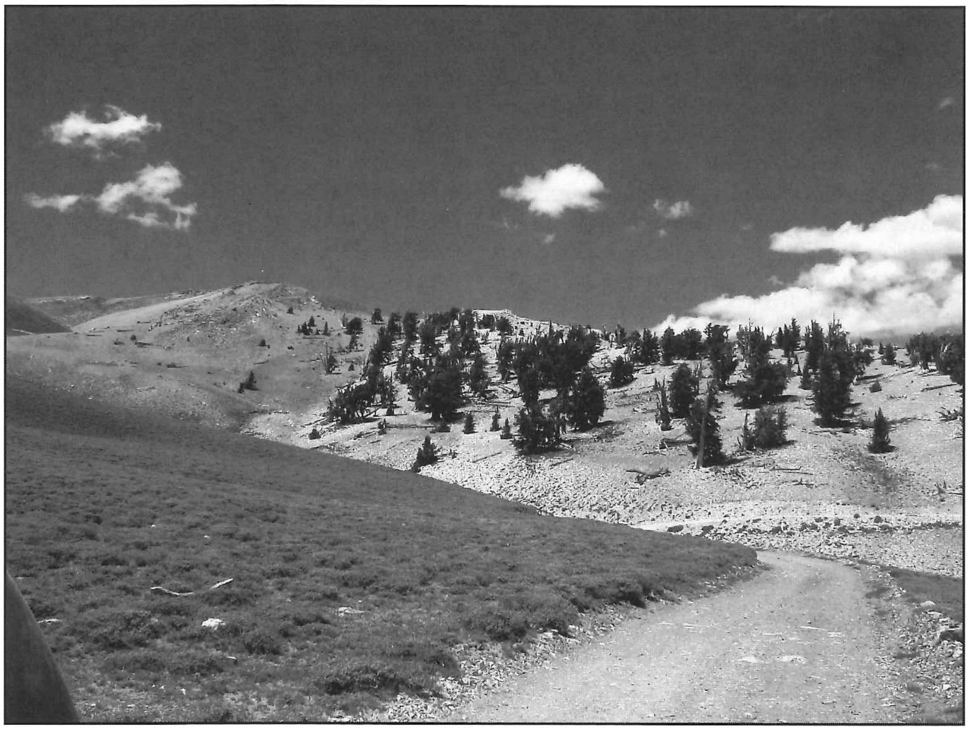


Figure 26. Sheep Mountain viewed from the west. Many of the trees used by Christine Hallman and HJA in our frost ring study are from this group of trees.

a reporter from the Los Angeles Times were on the mountain asking questions and taking pictures. This resulted in an article by Lee Romney published in the Los Angeles Times on Sept. 13, 2002. Al Seib was the photographer with Lee Romney; it was exceptional to talk to him about the L.A. Times' photo department since my father had worked there for over 30 years. While in college, I often took football and Rose Parade pictures for the LA Times Mirror, a branch of the Times.

To illustrate remnant wood collecting I have a photo of Rex Adams and Christine Hallman gathering a sample from the Methuselah grove (Plate 1, C). A second photograph shows Jim Burns taking a core from a large remnant sample just off the ridge of Sheep Mt; in the background Angelika Clemens is doing the same (Plate 2, E). Although this work may seem trivial to some, it is really the heart and sole of Tom's program.

Over time, we started several projects on Sheep Mt. including looking at remnant wood frost rings. Catherine and I spent a good deal of time there (Fig. 26). The most important project was done with Christine Hallman; it was a study of frost rings in young bristlecone trees. In this venture we collected two sets of cores from each tree, one with headings of north and south and another with headings of east and west; usually the second was taken about an inch higher. With these cores we can construct virtual cross sections of the stem of individual trees without actually cutting them down. We collected strongly in two regions, one on the upper western flank of Sheep Mt. and a second just east of the Patriarch Tree (Fig. 3). One thing we hoped to find from these studies was any directionality in the position of the frost rings in the specimens as an indication of localized effect of environmental factors. The results of that study are still pending. However, one impressive result was the finding that the frost rings generated in a particular year were either early or late frost rings. In other words, a specific year could be characterized as an "early frost ring year" or a "late frost ring year." Frost rings from 1954 and 1992 are all early frost rings, while those from 1965 and 1978 are all late frost rings. In 1987 most frost-damaged cells were produced in the middle of the annual ring.

While we were in the mountains, I tended to be an observer or a "giver of advice." Catherine on the other hand almost always pitched in and helped with what ever the "chores" were going on (Fig. 27). In the Sheep Mt. project with Christine, she often helped in the coring by being the "backstop," that is, she worked on the opposite side of the person who was doing the coring. In the Sheep Mt. frost ring study it was important to get the entire core including the bark on both sides of the tree, hence the need for a backstop. Catherine helped with photography and tagging trees for our study. Besides Rex, Christine and Catherine we had many generous people helping with the coring; Rebecca Franklin, Alex McCord, John King, Angelika Clemens, Richard Franklin, and Adelia Barber are some of those who helped.

On a few occasions, we witnessed to the fury of afternoon storms in the high mountains. Once, when Jean and I were coming back from the Patriarch Grove, it started to rain about two miles from the Schulman Station. By the time we drove by the Station it was a fully blown torrential rainstorm. As we started down the paved road leading to Highway 168, the intensity of the rain increased and then it began to hail. By the time we got to the Vista Point, the water and hail were cascading horizontally off the upper shoulder of the road; it was difficult to see. Conditions like that do not generate good feelings when you are going down on a winding mountain road. Finally we came to a camp ground and pulled under some trees to wait out the storm. That day, I learned that when there is rain on the upper part of a mountain, there will be trouble down below; the water will come flying down ravines, carrying mud, rocks, and trees with it. *The steeper the mountains the faster the water flows*; of course this is not a unique observation. When it finally cleared and we could see again there were many others who had opted to stay in the campground. Very soon, we heard that there was a washout about 300-400 yards down the road. We drove down to see the "washout" which was actually a large pile of mud, trees and branches spread out across the road. Most people opted to stay in the camp. We watched as a military type jeep in front of us went through the debris without too much trouble. We were in a 4-wheel drive vehicle so I decided to try it. Luckily, we made it through the debris and eventually made it to Highway 168. However, just downhill from the bristlecone turnoff there is a section of highway that becomes very narrow, actually one lane, with high cliffs on either side of the road. When we got there, about a foot of water was flowing very fast through the narrow area looking like a river. I went through the flowing water without any trouble. Just below, the authorities had closed the road to upcoming traffic. A half a mile later there was no water at all. I have often wondered where it all went. Somewhat shaken, Jean and I went back to the motel in Bishop. Later that evening, the TV showed that the roads we were on had been washed out, the pavement just washed away (Fig. 28). Several people had to be rescued from cars trapped by the high water. Highway 168 was closed for five days and people left on

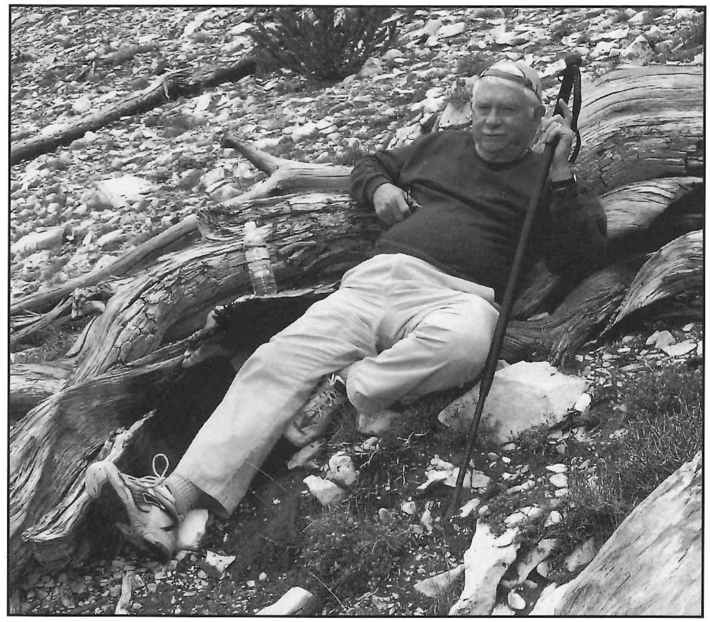


Figure 27. HJA "working" in the bristlecone pine forest, 11,500 feet in altitude. Photo by David Garrett.

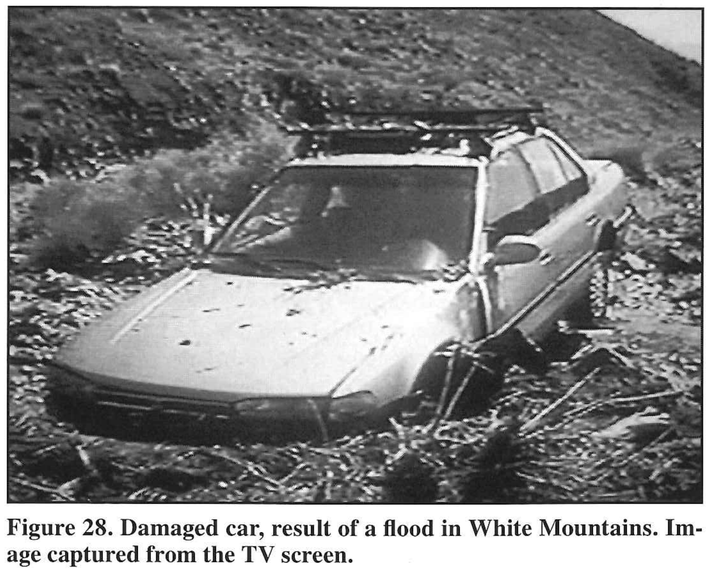


Figure 28. Damaged car, result of a flood in White Mountains. Image captured from the TV screen.

the mountain just had to wait it out. We were lucky to have made it out and not to have been caught by the water. The next day the newspaper reviewed the problems that the storm had caused. Later, we went on to Nevada looking for more bristlecones. In this situation, it is of interest to remember how quickly the weather changes, from perfectly clear to deadly weather in only a few minutes.

On several occasions, we left the mountains because of approaching storms with lightning and thunder. A group of about 25 people (including Catherine and I) were hiking to a spot in the Skeleton Forest where a memorial service for Frank Haussman was to take place (Plate 2, G). A year before, while collecting remnant wood Frank died of a heart attack. As we got to the edge of the Skeleton Forest the sky was clear. However, as we rested there for a few minutes it began to get dark and we soon saw lightning and heard thunder in the distance. Over a short time the thunder was getting louder. At that point, the leaders decided to head back for the cars, which were about a mile and half away. By the time the storm reached us, most of the people, including Catherine, were already back to their cars. However, I had been slow and was still about 300 yards from the cars when it started to hail. Luckily, the hail remained small even though it was getting colder and the storm's future was unknown. When I reached Rex's truck, I was quite relieved. As the hail continued Rex headed the pickup back toward the Schulman Grove. By the time we got past Campito Mt. the storm was over. When we stopped to assess the situation we found that the pickup bed was full of hail, Catherine and Rex made snow (hail) balls and I took pictures. On another occasion, Catherine was pelleted by hail stones as we came down Campito Mt. As we were walking to the car she said, "they sting."

PROMETHEUS *"THE MARTYRED ONE"*

For an introduction to bristlecones there is no better place to begin than Leonard Miller's web site "The Ancient Bristlecone Pine" (http://sonic.net/bristlecone/). It was there that I learned about living bristlecone trees named "Buddha," Socrates" and "Prometheus," trees growing in what is now The Great Basin National Park in Nevada. One tree, Prometheus, was cut by the U.S. Forest Service and Donald R. Currey in 1964; when analyzed, Currey reported that the tree had 4844 annual rings (Currey, 1965). Before cutting it was the *oldest living tree*! This is the tree that has been called "The Martyred One", "Prometheus," "WPN-114" and the "Currey Tree" by various authors. The following citations will give you a substantial background regarding the problems that arose from the cutting of this tree: Currey (1965), Cohen (1998), Lanner (2007), and Lambert (1991). For the purposes of this narration I have gathered most of the Prometheus story in one section (Plate 3, and other figures).

The ranger at the Schulman Grove Station told me about a large stand of Great Basin bristlecone pines some 70 miles east of Ely on the border between Nevada and Utah in Great Basin National Park. We also discussed the very old specimen that had been cut down (*Prometheus*) and that it was approximately 4800 years old. The ranger believed that the stump of this tree was just off the trail that led from the parking lot (at 10,000 feet) to the rock glacier in the cirque at the foot of Wheeler Peak. "It would be easy to find." The latter statement is absolutely true if in fact you know where an object is. It is "just off the trail" if you consider a half mile over treacherous rocks and a steep climb to the top of a lateral moraine followed by travel through a dense stand of bristlecones to be just off the trail. In retrospect, my information about this stump, as we will see, was pretty darn slim, to say the least.

Naturally, the ranger's information presented a "great opportunity," and since we needed to cross Nevada on our way home to Texas, Jean and I were soon on the way to G.B.N.P. In the area of the Great Basin National Park there is not much to choose from in terms of motels. We stayed at the Border Inn, a motel which my wife, and later my daughter Catherine, dubbed "the motel from hell." It is located on the border between Nevada and Utah on Highway 50; you could eat and gamble in Nevada and sleep or buy gas in Utah. In general it was also a gamble whether the air conditioning, electricity or plumbing worked or not; in July-August the temperature is a real concern. This trip occurred in 1999 just before the Y2K.

From the park headquarters it is an easy drive up to the Wheeler Peak Campground located at about 10,000 feet; on the ride you gain about 4000 feet. The trailhead for the Bristlecone and Glacier Trail leads up from the parking lot. About 2.5 miles up the trail there is an interpretive area with signed specimens, etc. As you first begin going up the trail there is a mixed forest with bristlecones and other gymnosperms. Later, the trail runs along the edge of a glacial moraine and, finally, it is on the moraine. Flashback: in Bishop, CA, we priced walking sticks at a sporting goods store and found that they were high dollar items. In lieu of "official" walking sticks, I purchased two mop handles, one blue and one yellow. I sharpened them and, on the day in question, we used them on our hike up the trail. While our walking sticks were utilitarian, they were also something of a novelty to the other hikers who quickly thought of us as proletarians and as hiking amateurs. From my perspective we were high level innovators. As we hiked up the trail, the path narrowed and there was a sharp drop off on one side of the trail. Jean decided to wait for me at a prominence, which was both safe and big enough for hikers to get by. Later, Catherine and I called this "Mom's Point" and was a place where we rested. I continued on up the trail far enough to see the old bristlecones in the signed area. As I traveled back toward where Jean was waiting, I was surprised to meet several people who recognized me as the husband of "my wife who was just down the trail" or "you must belong to the lady sitting on the rock down below." Obviously mop handles are attention grabbers. However, on that day *Prometheus* was not seen.

The next day, Jean remained in the parking lot reading. I traveled up the trail determined to find *Prometheus*. This time, I ventured out on the moraine hoping that this vantage would allow me to find the stump of Prometheus since it was "just off the trail." At this level, the moraine was several hundred feet wide composed of very large pieces of rock from "refrigerator to room size." While there were great views of Wheeler Peak, the Wheeler Cirque, the Wheeler glacier and some large isolated trees, there were no stumps to be found. At the time, I had no real idea what the stump looked like. However, I thought, "It would become clear when I saw it." (On occasion, I have a disadvantage of being *too* optimistic). While walking on the moraine, I learned that many of the rocks, which were just dropped on the moraine by the melting of the glacier, are balanced in such a way that my weight would tip them in one way or another. I found this out the *hard* way! Stepping on a medium size bolder it tipped and caused me to fall. I landed upside down on my head. In the process I cut my head, leg and tore my jeans. After I regained my composure, I realized that my very best pair of sunglasses was now forever lost in the crevices between the moraine boulders; luckily my camera was strapped to me. I quickly got off the moraine and I wasn't sure that I would ever be willing to travel on it again. Jean and I left G.B.N.P. with only a salt shaker (bearing the G.B.N.P logo) and a couple of T-shirts.

During 2000, I had some correspondence with the Science Officer at the Great Basin National Park regarding the possibility of collecting litter from around the Prometheus stump. He gave me permission to collect litter, and also the name of a ranger that had recently visited the stump site. I wrote the ranger and she kindly gave me directions to the site. From the time of my accident onward, when doing field work, I always was accompanied by my daughter, Catherine Arnott-Thornton. The companion idea was a very wise move "dictated" by Jean; by that time, I was in my

Figure 29. Wheeler Peak (upper right) and the Prometheus site. Prometheus grew on the lateral moraine seen at the left center.

Figure 30. The Prometheus Site. The main part of the plant lies in a slight depression near the center of this picture. The stump is obscured by shadows but is just to the right of the main stem.

Figure 31. Prometheus stump. Catherine Arnott-Thornton and HJA, on the occasion of first sighting of the Prometheus area.

early 70's, and although I was in good health, it was clear that accidents do happen! I am forever indebted to Catherine for taking her time to "watch" over me and to Jean for being adamant about her decision.

In 2000, after working in the White Mountains at Camp Harlan, Catherine and I traveled on to Nevada and attempted to follow the directions given by a ranger who had been to the stump. The ranger's plan called for crossing the moraine where it reached its narrowest point (50 to 100 m) some distance up the trail to an altitude almost equal to the surface of the lateral moraine. From there, we were to reach the bench on which WPN-114 grew by "walking" on scree at the side of the valley, thus climbing "horizontally" along the canyon wall to the moraine bench. After considerable exertion, we reached the bench, a level place on the lateral moraine. Although it is only a few hundred feet higher than the moraine, the climb was difficult. The "floor" of the bench on top of the lateral moraine has a splendid grove of bristlecone trees growing on it; however, the surface is composed of small boulders from about 6 inches to 2 feet in diameter. Walking or even standing on the bench was not easy. Only rarely did we find any soil or a flat place to stand (Plate 3, A, D; Fig. 30). We searched for several hours but were not able to find what was left of Prometheus (Fig. 30). We did make several collections of litter, which I carried back to the lab. The trip down the rocky moraine wall was almost as difficult as going up. When we got to the bottom, we still had to cross the dreaded moraine proper, which at that point was several hundred yards wide. I was not exactly sure how to cross it, but I never felt that we were lost. By that time, it was getting dark and threatening to rain, not a happy situation. Once back on the trail and then finally back at the "motel from hell," I was sure that we (I) would never do that again.

However, that night, or the next morning, I decided to see if Catherine was willing to try again; we had come to see *Prometheus* and we should try again. I also thought that there was an easier way to get to the lateral moraine - by going in a straight line across the moraine, leaving from the interpretive area and climbing directly up the wall of the lateral moraine. The next day, we followed my plan. As we crossed the main moraine, we set up piles of stones to guide us back and with care we crossed it without incident, tipping rocks and all (Fig. 29). As we climbed the wall of scree we looked for areas with moss and dirt as it was easier to get footing in these small zones. When we got to the top of the lateral moraine we crossed the bench to the opposite side, which was slightly higher than the edge we came over. The bench is populated with both mature and juvenile bristlecones. When we reached the opposite side of the bench, we found that the mountain went up at a strong angle and that its surface was composed of scree. The point where the mountain inclined from the bench marked the extent of bristlecones; the trees extend only to where the scree starts upward at a sharp angle. Moving left (toward Utah) along that line of demarcation, we started looking for the stump; we had some reference photographs that helped during this phase of our search. A half hour later Catherine yelled that she found the stump and I soon had my first look at *Prometheus.* It was great to find the stump along with the additional remains of the tree, but it was also discouraging to realize that this was the oldest living tree known to man, and it had been sawed down while still alive (Fig. 31, Plate 3, B). Looking at the main stem it had been eroded by constant bombardment from sand and ice crystals from the upside of the bench.

The site, where the remains of *Prometheus* lie, is at the edge of a slight depression only a short distance from the major upturn of scree (Fig. 30) (Currey, 1965). Currey's claim that a major landslide could cover Prometheus and the other trees of the area seems quite justified. The major portion of the stem (bole) lays in this slight depression a couple of meters from the stump (Fig. 30, Plate 3, A). The bole is more than a meter in diameter and is quite impressive. One can imagine how impressive it must have been in life. The bole has a large "V" cut in its top. Many other pieces are spread on the ground around the bole and the stump (Plate 3, A; Fig. 30). No pieces that look like the formerly living part of the tree could be identified. There are a number of other "large" trees around the site and many other "large" trees in the vicinity, which were not necessarily old trees. We did not have time to categorize them. I photographed everything I could and then we were forced by time to leave. Incidentally, all three times we went to the Prometheus site, we had a radio telephone borrowed from Park Service with us and, as usual, Catherine loaded us up with bottles of water.

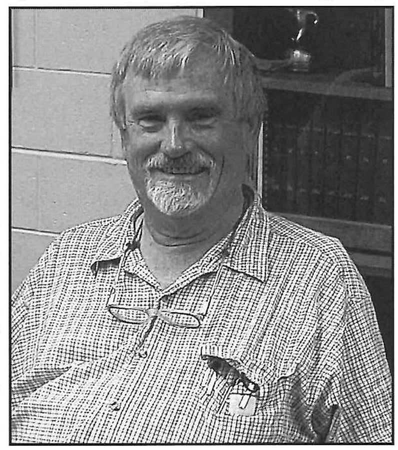


Figure 32. Donald Currey, 2003.

Near by the Prometheus site there is an interesting specimen that is likely to be quite old; I called it the "Five Sisters" (Plate 3, D). It is located a few meters from the stump in the general direction of Wheeler Peak and almost at the scree line. The tree consists of several large stems, which have developed in a "piggy-back" style. Schulman illustrated this kind of growth in his 1953 National Geographic

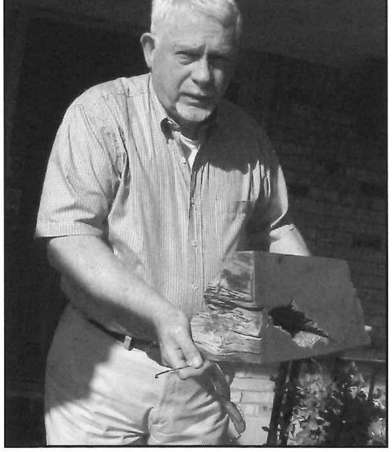


Figure 33. HJA with the "V" section of Prometheus (the 'pith' portion). Photo by Jean Arnott.

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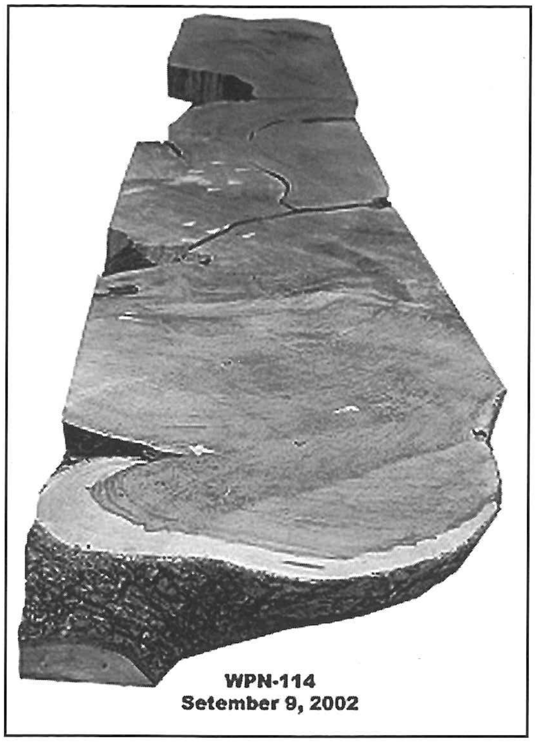

Figure 34. Prometheus slab at UTA.

article. It appears that the tree continuously formed a series of "branches" on the sheltered or downwind side; eventually these branches developed into major stems and all of them are in a row. The oldest and largest stem has been severely eroded down past the pith, a situation that happened to Prometheus. I was not able to locate any increment bore holes in this specimen and it may never have been sampled. I think it could rival the oldest bristlecones in terms of age. Another tree, only a few meters from the stump, appears to be as big in diameter as Prometheus. Many trees in that area are bigger in diameter than Methuselah. On this occasion, we also found a large tree with a horizontal "V" section removed from the non-living side (Plate 3, A). Recently, this tree has been dubbed Buddy and was cross dated with Prometheus by Tina Halupnik. However, it is important to remember that large diameter is not necessarily correlated with old age.

On this occasion, I collected litter found around and lying on top of the Prometheus stump. A lot of interesting micrographs resulted from that litter. As in other gymnosperm litter, there were many samples with calcium oxalate crystals (Arnott, 1995, 2006). While there, I did quite a bit of photography, some of which has been used in various talks I have given. Because this time we knew what to expect, going down was somewhat less arduous. The piles of rocks were excellent guides in crossing the moraine. As we crossed, we destroyed each pile so as not to confuse others. Catherine and I made one more trip to the WPN-114 site but I will leave that for later.

Figure 35. Catherine Arnott-Thornton interviewing Mr. Harvie L. Tibbs, a former Forest Service Ranger at his home in Ely, Nevada.

Since we were in Utah, I decided to attempt a visit with Dr. Donald R. Currey who was a faculty member in Geography at the University of Utah, Salt Lake City (Fig. 32). Catherine and I arrived at the Utah campus and with some luck we were able to contact and meet with Currey. We met him in a conference room where I told him something about my work and showed him electron and light micrographs of *Prometheus* wood obtained at the LTRR. We also showed him recent pictures of the *Prometheus* or WPN-114 site taken two days earlier. Incidentally I asked Currey what WPN-114 means. He told me that it simply meant White Pine Number 114. My expectation in talking to Currey was only that he might have some small pieces of WPN 114 wood that I could use in my research. Our interview was very friendly and Currey did not seem resistant to talking about the world's oldest living tree. As a scientist, I can not criticize the cutting of WPN 114, however, after the fact, one still wonders whether there might have been another way!

In time, I asked Currey if he had any *Prometheus* wood he would be willing to give me. Much to my surprise and continued delight to this day, he decided to give me his personal slab that he had kept since the cutting in 1964 (see later). After he agreed in principle to give us the wood, he made several phone calls to an associate who actually had the wood stored in his basement. The associate agreed to meet us at his house and we followed Currey's car to the meeting place some distance from the campus. From his basement, his friend brought out a large ammunition case that contained the carefully packed Prometheus slab. He also produced the pith section of *Prometheus*, a piece that came from the "V" cut in the bole mentioned earlier. The latter was something that I did not know existed and finally explained the reason for the "V" cut. As an additional surprise he also brought out the "V" section cut from the second tree on the bench of the moraine mentioned earlier (Fig. 33). They also gave me several bristlecone cores from Utah. We briefly unpacked the wood and looked at it in amazement. Quickly, Catherine and I repacked it and placed the ammo case and other pieces in the car. We thanked Currey again and promptly left Salt Lake City. I hurried from that meeting knowing all the time that people have second thoughts. Currey might have thought again about giving up such a precious specimen. In hindsight, I believe he had made up his mind and it was completely unnecessary for me to worry. Donald Curry died four years later. I believe he sensed that I would use the material in an appropriate scientific manner. The slab was returned to the University of Texas Arlington (Fig. 34).

Some of the citations given at the beginning of this section describe the cutting of Prometheus. The US Forest Service used chain saws to originally fell the tree in 1964. Then cut sections were carefully carried down the mountain and surfaced. You may wonder how many complete slabs were cut from the *Prometheus* stump. I believe the correct number is three. In 2003, Catherine and I interviewed Mr. Harvie L. Tibbs, a former forest ranger at his home in Ely, Nevada (Fig. 35). He was one of a group of Forrest Service workers who helped carry the parts of *Prometheus* from the mountain. He was along when one of his colleagues had a heart attack and died as they carried the wood down the mountain. He told us that he helped prepare slabs of the Currey material in the Ely shop of the Nevada Northern Railroad. His recollection was that three slabs were made from the original material. I know where all three slabs are in 2008. One is present at UTA in my possession, another is on display at the Bristlecone Convention Center in Ely, Nevada, and the third original slab is in the collection of the LTRR in Tucson, AZ. Before its display at the convention center the second slab was exhibited in the lobby of the Hotel

Nevada in Ely. That slab is mounted in a glass case and it is very difficult to photograph. It is displayed with prominent historical dates located on the surface. For many years the third slab was lost in the LTRR storeroom until Christine Hallman reassembled it.

The exact manner in which the third slab reached its final destination is not clear. It was only in 2005 that Christine Hallman was able to find all the pieces in the LTRR collection (Plate 3, F). Before Christine, I had been through "all" the "Currey Tree" collection at the LTRR; however, I was not able to piece them together into a slab. Some people believe the third slab passed through the hands of the U.S. Forest Service and was given to Dr. Wesley Ferguson (a faculty member of the LTRR), who then took it back to the LTRR sometime in the 70's. At some point the third slab is believed to have been on display at the LTRR; that may have been the reason why the surface was varnished. One additional slab exists in the display area of the Great Basin National Park; it is of a different shape and was apparently made from the bole at a much later date. I have seen additional pieces (not complete slabs), that are in private hands and I have heard rumors that some pieces are located at the Forrest Genetics Laboratory in Placerville, California.

When the *Prometheus* slab was brought to my lab at UT Arlington in August of 2000, the first thing we did was to photograph and scan it. Very quickly we had a photographic record of the slab. The dimensions of the slab when put together are 82 inches (2.08 m) in length and 11.6 inches (29.4 cm) at the widest point and 2.75 inches (7 cm) thick (Fig. 34; Plate 3, E). The 1964 annual ring is at the youngest end with the bark still in place over most of the curved face that represented the living part of the stem. The other end of the slab ends with ring 4568. Obviously the slab had the marks Currey made on its surface while preparing his ring count; Currey reported that the total number of annual rings was 4844 (Currey, 1965).

In order to scan the slab, because of its width, we purchased a "long bed" (14 x 8.5 inch) HP scanner. Using that scanner upside down we set up a straight edge board along one side of the slab. We used that board to control the axis of the scanner as it was moved along. Martha Gracey and I were able to scan the entire slab at 900dpi. A series of 11 scans were made and they were fitted together using Photoshop. As the individual scans were already large files, when put together they made an even larger file. However, after reducing the size of this file we were able to print "life size" images of the entire slab. These prints demonstrated the rings clearly, but it was not possible to see the tracheids that made up the wood of *Prometheus*. I first showed these scans at Camp Harlan and later at the LTTR during a seminar I gave.

It is not clear to me how Currey obtained the figure of 4844 annual rings. The main slab contains only 4568 rings. There are about 900 rings in the V section (containing the pith), which overlaps with the main piece. Somehow, Donald Currey must have integrated the annual rings from main and pith sections. Perhaps the two were cross dated but no evidence for that exists. Likewise, it is not clear how at a later time, Don Graybill of the LTRR came up with the number 4862, again perhaps he had access to the pith and he cross dated them to establish the final count. Graybill had access to various chronologies and he probably used them in this dating; however, without the V section you do not know where the beginning annual ring lies.

By examination of the wood in the main portion of the slab, I was again able to find the 1453 frost ring. This is a common frost ring in many Great Basin bristlecones (Hallman, personal communication). The presence of this frost ring provides evidence that Prometheus was subject to some of the same weather elements as many other trees in the arid southwest. In the "pith section" I found another frost ring in the 30th ring from the pith. Using Graybill's count this means that the frost ring occurred 4874 years ago or in 2866 B.C.

Catherine and I made our third trip to the Prometheus site in the following year. In the time between the second and third trips I had been trying to find an easier way to reach the moraine bench. I worked out what seemed to be a good trail, which took us by Brown Lake and then up on the bench. On our final climb, we started out on the new trail and made it to Brown Lake without trouble. Brown Lake is a small alpine lake with bristlecones all around it. From there, we were going to climb directly up to the north end of the bench; however, this trail turned out to be impossible for us to climb because of a very steep rocky zone that we couldn't pass. This route took some three or more hours. Even though we started early, we had lost a good deal of time. We then went to plan "B," and followed the "trail" we had used before. That is we crossed over the moraine then climbed directly up the side to the lateral moraine. It was no easier than before with the exception of the fact that we knew where we were going.

I brought full scale printouts to the site so we could compare them directly with the stump and with the "V" cut in the bole. The printouts matched exactly except for the fact that the printout had to be turned upside down to fit (Plate 3, C). That meant that it was the lower surface of the slab that was finished in the Curry specimen Of course we have pictures showing that and the con-

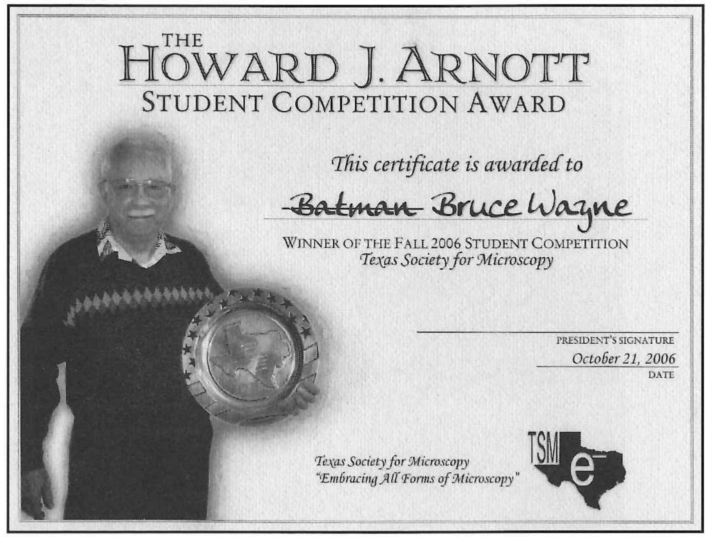

Figure 36. Student Competition Award Certificate designed by Amanda Halupnik.

Figure 37. Mike Johnson at the University of Arizona, spring 2007.

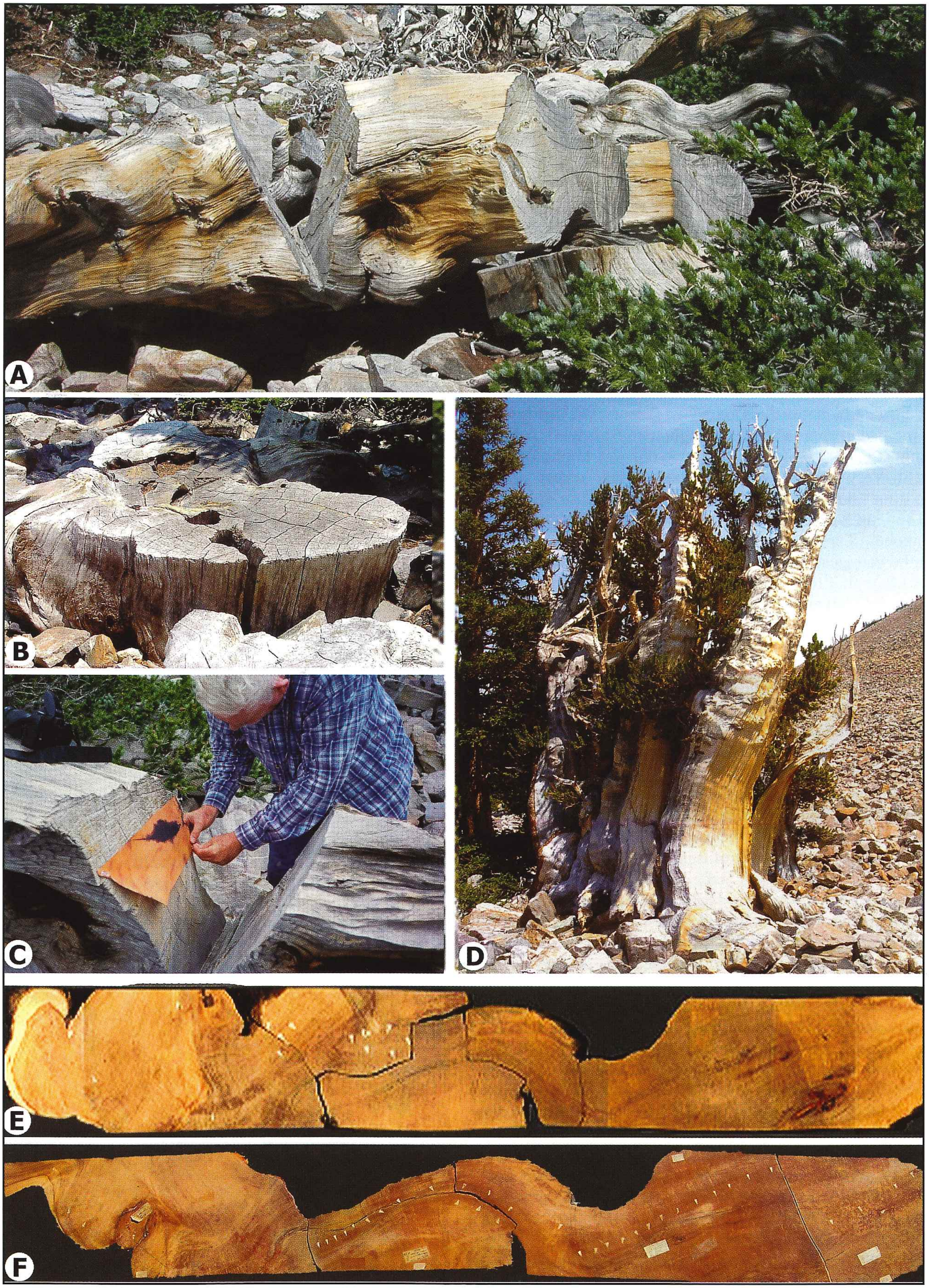

Plate 3. A. Prometheus site with large "V" cut in the stem and lack of bark on the stem surface. B. Stump of Prometheus showing irregularities and cracks. C. HJA applying a copy of the "V" piece to its place of origin. The fit is exact. D. The Five Sisters Tree. Note the erosion on the lead branch, the steep ascent of the mountain in the background, and the very rocky "soil." E. Photo of the Currey slab at UTA with sapwood and bark on the left end. F. The LTRR slab with a slightly different profile at the left end. Yellow flags are 100 annual rings apart.

nection of the pith piece with the V cut. Nothing had changed during the year since we first found the site. However, on that climb we learned what mountain (altitude) sickness is like. During that afternoon, however, the trip down from the bench and across the moraine was without problems. Once again we had piled up rocks to guide us back across the moraine and back to the trail. I sincerely doubt that I could make the climb now.

THE TEXAS SOCIETY OF MICROSCOPY

I have always enjoyed my relationship with the Texas Society of Microscopy. The first meeting that I attended was at Rice University sometime in the 1960's and I remember that we met in a large sloping auditorium and believe Lee Rudee was the organizer of that meeting. Since that time the society has met on many University campuses, University of Texas, Austin being the latest. On a few occasions we had "joint" meetings with other state societies and I always thought they brought added value to the participants. We also met at Bandera where the opportunity for recreation around a camp fire was also an addition. Perhaps the most important thing is that we kept meeting and let's hope that will continue.

I appreciate the opportunity to get some of my thoughts out in the open. If my sometimes candid comments have offended anyone, 'c'est la vie, however, I apologize. I am grateful for the many things TSM has done for me; special plaque, special meeting with my former students, but most of all naming the student awards after me (Fig. 36). I must first thank Dr. Ann E. Rushing who suggested that I write this autobiography, probably it is not exactly what she expected, but she has been a good sport about it. Of course, I thank Dr. Camelia Maier for her encouragement and excellent editorial work on the several parts. In addition many other people helped with various aspects of manuscript preparation. I thank my wife, Jean Arnott; my daughters Catherine Arnott-Thornton and Susan Garrett, Martha Gracey, Rex Adams, Sandra Westmoreland, Tina Halupnik and others. Many helped directly with the research and also deserve my wholehearted thanks; Martha Gracey, Catherine Arnott-Thornton, Christine Hallman, Rex Adams, Tom Harlan, Jeff Dean, Dick Warren, Kelsey Pendley, Tina Halupnik and others. Finally, I want to thank Mike Johnson, of the Nikon Co, for constantly making sure my microscope and software functioned properly. However, flying to Tucson to check my microscope is far beyond what anyone could expect, but such actions are typical of Mike (Fig. 37).

That's 30.

ADDENDUM (MAY 13, 2008)

Several individuals, including the editor, have asked me. "What does "That's 30" mean?" "That's 30" is a newspaper term, somewhat archaic now, that marks the end of a story. I learned the term from my father, Andrew Hugh Arnott, who was a newspaper photographer for over 40 years. Inserting this here is a way of acknowledging my father's role in creating my interest in photography. Even as a kid, he "shoved" a speed graphic camera in my hands and started my photography lessons. Incidentally, I am currently working on an article entitled "Three generations of photographers;" I will describe and compare the photographic careers of my great grandmother (Nellie Merritt Alderman), my father and I.

Speaking of photographs, except where noted, all the photographs in this article were taken by me.

Since finishing this manuscript, my 80th birthday occurred and that day emphasized to me that change is catching up with me, I can feel it in many ways. In my early life I had at least 15 (perhaps more) living blood relatives. Now, not counting our children I have only one living blood relative, my aunt, May Leonie Donnelly Myers Mabe, she is 95. (Note: My Aunt May passed away May 16, 2008 in Houston, Texas). This realization caused me to consider the future (and also the past). As most will agree, the future is all about change. The ability to deal with change is a prime characteristic of *Homo sapiens.* Individual adaptation to change allows each of us to survive and sometimes to live a "happy" life. It is interesting to reflect on the lives of the ancestral Puebloans that lived in Mesa Verde. What caused them to abandon their magnificent cliff dwellings in the last part of the 13th century? The ***"wood dictionary"*** helps explain some of the conditions (drought, frost, etc.) that occurred at the time and may have caused them to leave, but we can still ask the question, "Why couldn't they adapt?".

In the light of this essay, Part Five, it is interesting that wood (dendrochronology) is playing a very important role in the current controversy about "global warming." Tree ring studies are a basic part of the data being used to show that the rate of tree growth has increased in the last 150 years. Many think that the basic cause of global warming is the input of CO_2 into the atmosphere caused by the burning of fossil fuels. Others seem to think that burning wood should cause no need for worry because wood is a "renewable resource." You only have to visit New Hampshire in the winter to see the pollution that comes from burning wood. In my opinion, burning wood is not green.

Here is another way of signing off. In the immortal words of Woody Woodpecker, "That's all folks!"

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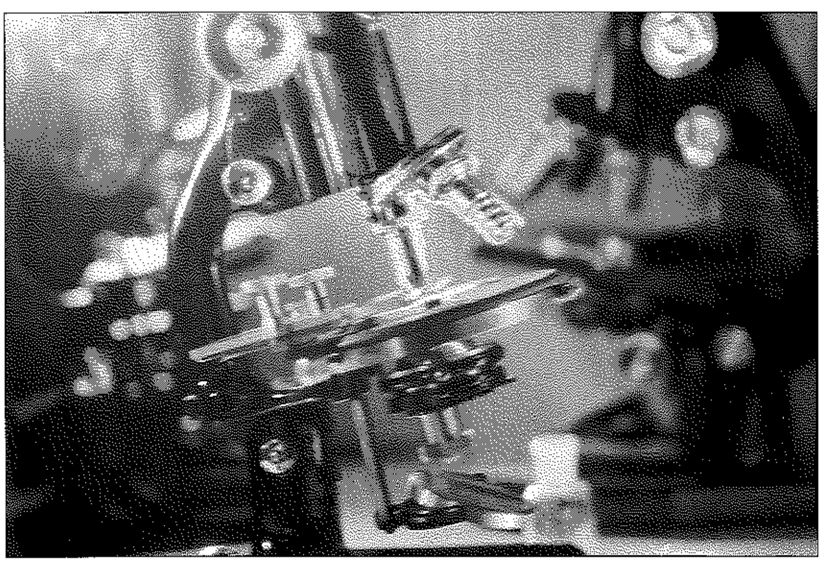
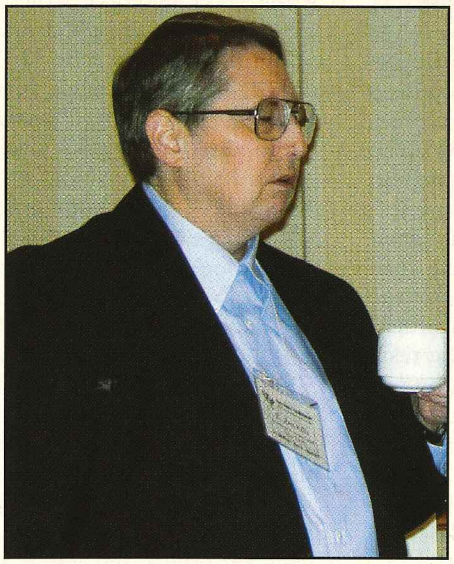


TSM Member Honored with Distinguished Scientist Award

E. Ann Ellis, TSM Treasurer was recently honored with the Distinguished Scientist Award at the Southeastern Microscopy Society (SEMS). As described on the SEMS' web page, this is an award for "members of long standing who exemplify personal and intellectual integrity, perennial scholarship, contributions to the field of electron microscopy, excellence in teaching and service to the SOCIETY above and beyond the call of duty". The Distinguished Scientist Award is not given on a regular basis, but at times when qualified SEMS members are identified and nominated by the general membership.



E. Ann Ellis, Senior Research Associate at the Microscopy and Imaging Center at Texas A&M University (TAMU), has 30 plus years experience in biological electron microscopy including plants, insects and other animal models of disease processes. Ann also has a "solid background in correlative microscopy" (TAMU web site). She is a specialist in employing combined methods of cytochemical localization and immunocytochemistry and loves to take the challenge of working with hard-to-prepare specimens. One of her strengths is to develop and adapt methods for the preparation and analysis of such specimens. In dealing with difficult specimens, Ann's favorite saying is "If we don't have a method, we develop one" (TAMU web site).

Ann is a member of the MSA Certification Board and past chair of the board. At TAMU she is also involved in training students through microscopy by teaching Fundamentals of Transmission Electron Microscopy. Her scholarship is impressive and diverse. Apart from her numerous presentations at different meetings, Ann published research articles in the *Journal of Materials Science, Investigative Ophthalmology and Visual Science, Biomacromolecules, Analytical Biochemistry, Microscopy and Microanalysis, Antioxidants & Redox Signaling, Experimental Eye Research, Proceedings of the National Academy of Sciences of the United States of America*, and *Texas Journal of Microscopy*, among other peer-reviewed scientific journals. One of her latest publications (2008) as co-author is 'Protein screening using cold microwave technology' in Analytical Biochemistry (Vol. 375). Ann also published the chapter "Poststaining Grids for Transmission Electron Microscopy" in the book series Methods in Molecular Biology: Electron Microscopy, Second Edition Methods and Protocols (John Kuo, ed.) in 2007. Her publications are cited by Ted Pella, Inc., for example, as references for their products. Here is one example: *Ellis, E Ann, Solutions to the Problem of Substitution of ERL 4221 for Vinyl Cyclohexene Dioxide in Spurr Low Viscosity Embedding Formulations, Microscopy Today*, V 14, No 4, July 2006 (www.tedpella.com/technote_html/18300-4221%20TN.pdf and www.tedpella.com/chemical_html/chem2.htm).

The TSM is very proud of E. Ann Ellis' achievements.

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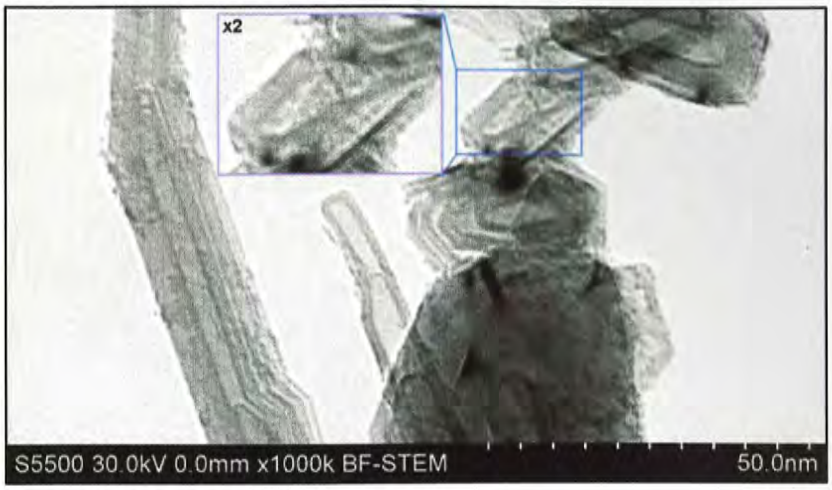

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