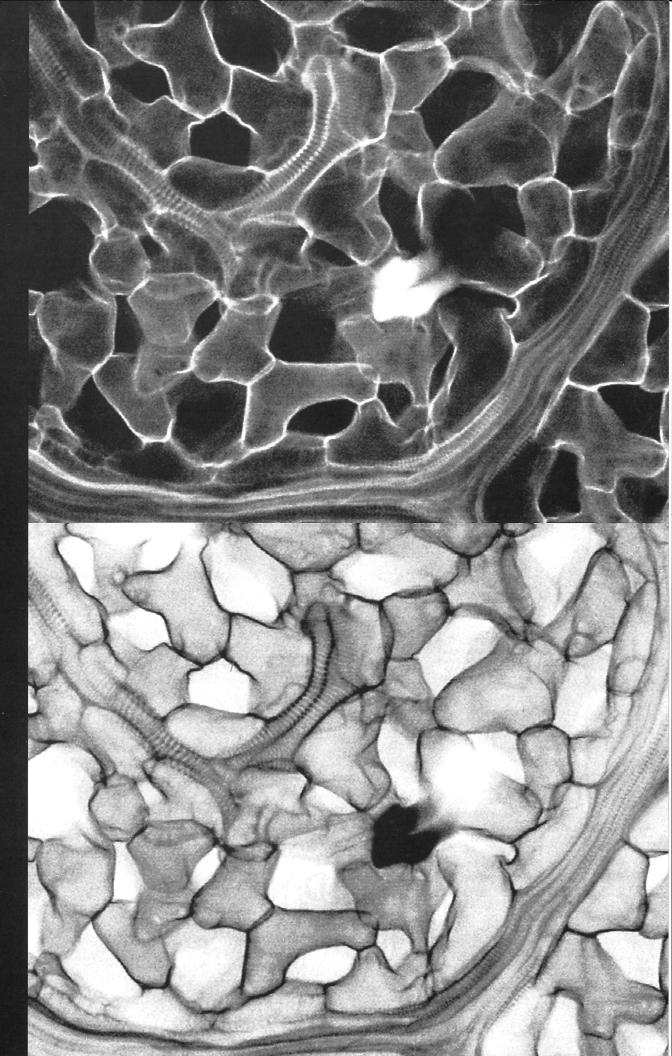
ISM e

T e x a s Journal of Microscopy

Volume 30, Number 1, 1999 ISSN 0196-5662



# WANTED

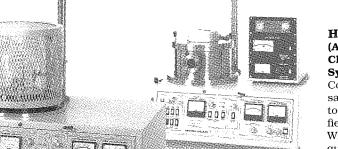
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TEXAS JOURNAL OF MICROSCOPY VOLUME 30, NUMBER 1, 1999 ISSN 0196-5662



David C. Garrett, Editor

Department of Biological Sciences, University of North Texas, Denton, TX 76203

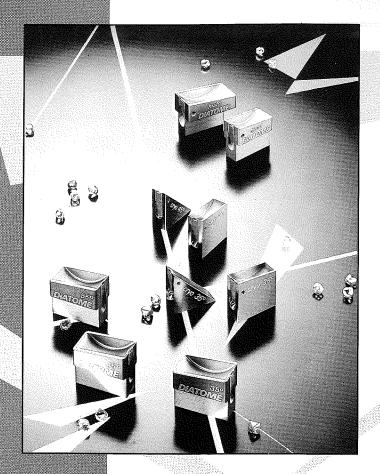
#### Official Journal of the Texas Society for Microscopy

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Answer to "What Is It" from Tex. J. Micros. 29:2
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TSM Application For Membership
What Is It? Back Cover

#### ON THE COVER

The cover presents a laser confocal optical section made in the paradermal plane of cleared leaf of tea (Thea sinensis) made with a Laser Scanning Confocal Microscope. This is optical section number "149," the complete series stored in the computer consisted of 181 optical sections; successive sections passing down through the upper epidermis, the palisade parenchyma and finally through the spongy parenchyma. In this plate, section 149 is rendered as positive in the upper part of the cover, the lower part of the cover, a duplicate of section 149, is rendered as a negative image. Section 149 shows branching veins and the characteristic cells and airspaces of the palisade parenchyma of *Thea*. In the central part of this optical section a sclereid idioblast is seen. This sclereid could also be followed in many other sections in the series. Sclereid idioblasts are emblematic for the mesophyll of *Thea* leaves. This optical section provides good resolution of many leaf features, e.g., note the very fine rendition of the secondary wall thickenings in the tracheids making up the veins. It is also worth mentioning that the positive and negative versions each emphasize different features of the leaf structure. The original leaf preparation, produced in 1955, was made by clearing a tea leaf in NaOH and chloral hydrate; the specimen was then washed, stained with safranin, mounted on a slide in a synthetic resin and coverslipped. Mike Davis and Howard J. Arnott, The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.



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# President's Message

s we approach the annual Spring meeting of our Society, I look back on our fall meeting with mixed feelings. We were very fortunate to attract several noted invited speakers for both our Thursday workshop and our regular sessions. Dr. Robert Burghardt, with the assistance of Dr. Roula Mouneimme and Zeiss, staged a very informative though sparsely attended workshop on fluorescent techniques capable of detecting cellular responses at the micrometer scale. Additionally, Dr. Burghardt gave an invited talk on the application of those techniques in studying calcium signaling in mammalian cells. We were also very fortunately to have Dr. David Piston, a pioneer in the field of multi-photon microscopy, as an invited speaker. He was able to cover applications of twophoton excitation microscopy and green fluorescent proteins in a one-hour talk. These two talks in conjunction with the workshop represented a mini-symposium on some of the cutting edge techniques presently used in the in situ study cellular function. It is a shame that more members did not take advantage of the opportunity to learn about these techniques and applications.

I would like to thank all those who attended that meeting, and especially those that made presentations. I would like to express my sincere appreciation to Dr. Howard Arnott for his effort in making the contributed paper session possible almost single-handedly. Approximately 60% of all the submitted papers at the Bandera meeting were associated with Dr. Arnott's lab. In recent years Dr. Arnott and his students, along with the students of Dr. L. E. Murr from the University of Texas at El Paso, have been some of the prime supporters of our meetings. While I have singled these people out for notation there are many others that have always managed to present a paper once a year. The society values and needs the support of all of these individuals. Frankly, without this kind of support from more of our membership TSM will cease to be the TSM that we have all worked so hard to create.

Our meetings must have a critical mass in terms of attendance and presentations to make them worthwhile.

Putting together two meetings a year along with the accompanying workshops constitutes a tremendous amount of effort. We are dangerously close to not having that critical mass of attendance and presentations that will constitute a viable society. Workshops that are sparely attended, meetings that are not broadly supported, and executive council positions for which candidates must be coerced to run are not indicative of a healthy society. There are a number of reasons for this, many of which have been printed on this page by previous Presidents and me. But the fact remains that TSM belongs to all of us, and if we all do not contribute to our Society, TSM will cease to exist.

I invite all the members to become engaged in a dialogue regarding the future of our Society, a dialogue that began at the fall meeting. We must also try to expand the horizons of TSM by bringing new members with more diverse interests into our Society. One of the hallmarks of our society has been that it was formed around the common interest of electron microscopy and not around any one particular scientific discipline. Researchers from many different scientific fields attended and presented papers at our meetings because they valued the honest exchange of scientific ideas and the development of professional relationships with others who came from a diverse background but whose commonality was the use of microscopy in their research. As we move away from focusing solely on electron microscopy we must try and use the historical basis of our Society as a selling point to entice new members and participants.

There can be no stronger advocate for a society than its members, and I trust that the membership of TSM can rise to the occasion and bring our Society into the next century.

Sincerely, Bob Droleskey President 1998-1999

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Advertiser	Page Located	Advertiser	Page Located
Denton Vaccuum, Inc		M.E. Taylor Engineering, Inc	6
		Micro Star Technologies, Inc	
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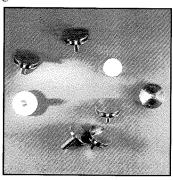
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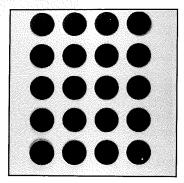
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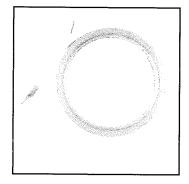
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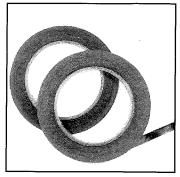
Carbon Conductive Tabs are a double-sided 30µ polycarbonate foil covered on both sides by a 30µ conductive glue.



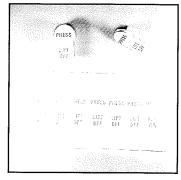
Use this 2-part Conductive Silver Epoxy for adhering microscope samples and solderless connections.



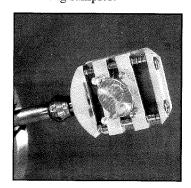
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# Treasurer's Report

#### TEXAS SOCIETY FOR MICROSCOPY TREASURER'S REPORT For Period Ending March 12, 1999

ASSETS AS OF JANUARY 1, 1999:		
Norwest Bank Checking Account No. 110649558		
Bank One Certificate of Deposit No. 1882289323	\$4,079.37	
TOTAL	\$8,931	.27
RECEIPTS:		
Dues\$1,090.00		
Journal 29:2 Advertisement Revenue		
Checking Account Interest		
Interest on Certificate of Deposit No. 1882289323\$53.98 TOTAL RECEIPTS	#2 022 00	
TOTAL RECEIF IS		
EXPENSES:		
Spring Meeting 1999 Waco Texas		
Waco Convention Center Insurance		
Secretary's Account/ Mailout Office expense \$500.00		
TOTAL EXPENSES	\$578.75	
ASSETS AS OF MARCH 12, 1999		
Norwest Checking Account No. 110649558		
NationsBank Checking Account No.005772227833		
(Treasurer-elect Account)\$500.00		
Bank One Certificate of Deposit No. 1882289323\$4,079.37 TOTAL	¢10.275	. 53
101AL	\$10,373	.54
TREASURER'S 1998 YEAR END REP For period beginning Jan. 1, 1998 and ending Dec.	ORT 31, 1998	
ASSETS AS OF JANUARY 1, 1998:		
Norwest Checking Account No. 110649558	\$5,258.91	
Bank One Certificate of Deposit No. 1882289323	\$4,079.37	
TOTAL	\$9,338	.28
RECEIPTS:		
Dues		
Spring Meeting 1998 Lake Texoma, OK		
Meeting Registration/Banquet\$2,110.00		
Workshop\$240.00		
Donations/Grants\$225.00		
Fall Meeting 1998, Bandera TX		
Meeting Registration\$1,345.00		
Workshop		
Donations/Grants		
Journal Advertisement Revenue		
Journal 28:2 \$500.00 Journal 29:1 \$1,850.00		
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Journal 29-2 \$875.00		
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Grants and Donations         \$100.00           Checking Account Interest         \$60.09           Interest on Certificate of Deposit No. 1882289323         \$237.38           TOTAL RECEIPTS           EXPENSES:           Journal Printing           Journal 29:1         \$1,712.94           Journal 29:2         \$1,922.88           Student Travel         \$1,211.52           Student Award         \$150.00           Secretary's Account/ Mailout Office expense         \$1,000.00           Spring 1998 Meeting expenses         \$2,978.22           Fall 1998 Meeting Expenses         \$1,821.83           Spring 1999 Meeting Expenses         \$1,821.83           Spring 1999 Meeting Expenses         \$1,000.00           Legal Fees for name change         \$375.00           Officer Bonding         \$144.59           Past Presidents Plaque         \$61.50	\$11,103.47	
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# **Abstracts**

## BIOLOGICAL SCIENCES PLATFORM PRESENTATION—SPRING 1999

COMPARATIVE STUDY ON SPERMODERM AND ANATOMY OF SEEDS OF SELECTED LEGUMINOUS TAXA FROM LIGHT AND SCANNING ELECTRON MICROSCOPIC OBSERVATIONS. NABARUN GHOSH A. CHATTERJEE and DON W. SMITH I Biology, South Texas Community College, McAllen, CAS, Department of Botany, University of Calcutta, India. Juniversity of North Texas, Denton. TX

The use of the Scanning Electron Microscope as a tool to provide new data in plant systematics and their application to the angiosperm classification date back to the late fifties of our century but until more recently have remained without influence upon the shaping the angiosperm systems. This lack of use of the ultrastructural potential was due to the dearth of available comparative data. A recent paper (Wantanabe et al. 1999) offered the use of ultrastructural features obtained from SEM study to delineate 45 taxa of the family Solanaceae (Watanabe et al. 1999). In this abstract we summarize the detailed micromorphological data on 26 leguminous genera. We used both light microscope and SEM to compare the seed surface patterns as well as anatomical features. This study revealed seed surface ornamentation and cracking pattern that were species specific. We found seed surface patterns were not enough to characterize the seed of a species because sometimes the seed surface study did not provide enough features to compare different species. We cut transverse and longitudinal sections from different regions of seeds; midseed, near the hilum and two distal ends. The anatomical study with SEM on the seed sections revealed the size, shape, and number of tiers and cellular organization of the epidermis, hypodermis, endosperm and internal structural details. The notable anatomical features of the epidermal and endospermic tissues that we obtained in this study on different species of Acacia, Albizia Cassia and Dalbergia can be used in revisions and circumscriptions of Leguminous taxa. 1. Watanabe, Hitoshi, Toshio Ando, Eshio Nishino et al. (1999) Three groups of species in Petunia sensu Jussieu (Solanaceae) inferred from intact seed morphology. American Journal of Botany 86(2): 302-305.1999.

STRUCTURAL CHANGES IN WHITE LEGHORN EGGSHELLS AFTER TREATMENT WITH BUFFER SOLUTIONS FROM pH 6.6 TO pH 7.6. SANDRA L. WESTMORELAND AND HOWARD J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, Texas 76019.

Chicken embryos derive over 80% of the calcium needed for their development from the eggshell. Calcium is mobilized from the mammillary cones, the most internal region of the eggshell, during days 13 to 21 of the incubation period, resulting in visible structural changes (Westmoreland and Arnott, 1998). This process occurs as calcite of the eggshell is dissolved by carbonic acid formed from respiratory carbon dioxide produced by the growing embryo (Tuan, 1987). We propose that selective solubility of the internal region of the eggshell by carbonic acid is necessary to preserve the structural integrity of the shell during incubation of the embryo. If the entire thickness of the shell were equally soluble, the shell could become weakened before the hatching date resulting in shell cracking and death of the embryo. It was the purpose of this experiment to demonstrate what in vitro changes may occur in the various shell regions when subjected to buffered solutions in a limited range of pH, 6.6 to 7.6. This pH range was selected in part due to data collected by Arad, et. al., 1989, who determined that the range of pH in uterine fluid during shell formation in a laying hen is 7.25-7.53. In preparation for these experiments 5.25% sodium hypochlorite was used to remove both the external organic shell cuticle and the internal proteinaceous shell membranes from eggshells of the White Leghorn chicken. In separate trials samples of the shell were then exposed to solutions of pH 6.6, 7.0, and 7.6 buffered with Sorensen's phosphate buffer. They were incubated at 37 degrees Centigrade for 120 minutes. Untreated samples were used as controls. After treatment, samples were rinsed twice in distilled water, air dried, mounted on aluminum stubs, sputter coated with gold and palladium, and examined with scanning electron microscopy to determine if changes occurred in the shell structure.

ULTRASTRUCTURE OF ACCESSORY PENES AND CAUDAL APPENDAGES OF SELECTED SPECIES OF ISCHNURAN DAMSELFLIES (ODONATA: COENAGRIONIDAE). ALICE M. STACEY, HOWARD J. ARNOTT AND JAMES V. ROBINSON. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

Male damselflies have both primary and secondary (accessory) genitalic structures used in copulation. Primary genitalia include caudal appendages located on the tenth segment of their abdomens which serve to grasp the female prior to copulation. Male damselflies have secondary genitalia between the second and third segment of their abdomens which contain the intromittent organ known as the accessory penis. The accessory penes and caudal appendages of nineteen species of ischnuran damselflies were examined using scanning electron microscopy. The penes were comprised of a chitinous stem, a sclerotized body with paired basal spines and a membranous glans with paired flagella. Thirteen variables of the accessory penes were measured for each species using the VitalScan imaging program. The glans portion of the penes was the region with the greatest degree of spination of the flagella among species. Caudal appendages were comprised of three components known as the dorsal tubercle, cerci (dorsal) and paraprocts (ventral). The dorsal tubercle of all species were bifid and minute structures were visible on their surfaces. The cerci and paraprocts were covered with hair-like projections attached directly to the cuticle. All species examined had hairs on the upper portion of the dorsal appendages which did not conform to the typical pattern. This discrepancy may be attributed to differences in the function of the hairs in the insect's sensory system.

AN EXAMINATION OF IRIDESCENCE IN CHELICERAE OF SALTICIDAE (ARANEAE). TIMOTHY L. HENRY. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019-0498.

Chelicerae are the first appendages (jaws) of spiders that function in grasping and injecting venom into prey. The chelicerae of certain jumping spiders (Araneae, Salticidae) have iridescence qualities, specifically those of the genus *Phidippus*. These quick and intelligent arachnids are predatory hunters, noted for their large brains and visual acuity. Iridescence is related to these attributes in courtship and aposematisic behavior. The structures of these iridescent chelicerae appear to be species-typical and could be used as a reliable method of species identification. In this study, iridescent chelicerae of *Phidippus audax* (Hentz), *Phidippus johnsoni* (Peckham and Peckham) and *Phidippus texanus* (Banks) were examined using light microscopy (LM) and scanning electron microscopy (SEM). Cheliceral molts were examined, both externally and internally, using SEM and LM microscopy. Non-iridescent chelicerae of the salticid *Mavia inclemens* (Walckenaer) were compared to those of *Phidippus*.

MICROSCOPY OF THE MUNDANE. II. HOWARD J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

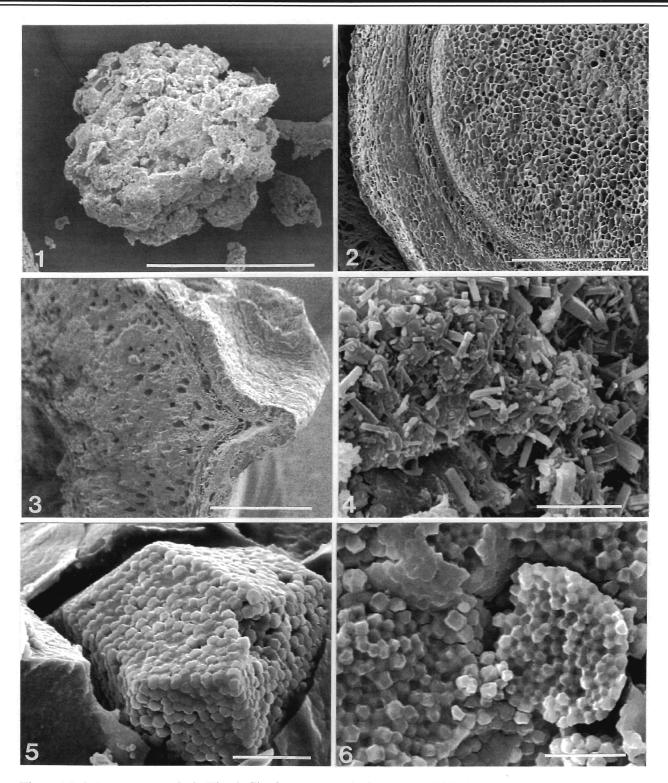
Commonplace things and widgets of life often seem unworthy of much notice or inspection. However, the most mundane items can be of considerable interest when viewed with a light or electron microscope. Leeuwenhoek, who was in fact one of the "first mundane microscopists," proved the value of examining commonplace things of his environment in the 17th century. When viewed with a microscope the structure and morphology of cliché structures (pond water, blood etc.) often provide entertaining and illuminating answers to our most basic questions about the objects around us. For example, what does the surface of a match look like before and after burning? Can the match's structure be associated with the burning events? What interesting things can you find in a coffee pot? In this second tour, we will look at a number of everyday objects using light and electron microscopy. Some have intrinsic interest; others, because of their bizarre structure, may (might) be considered "art," and finally, some just may be fun to cogitate. This tour starts by reexamining a pencil. I missed a lot of chances the first time around. We will look at some pills, bottle covers, wrapping materials and in between, a few items connected with the personal computer will be examined for diagnostic purposes.

A LIGHT AND ELECTRON MICROSCOPIC STUDY OF A "PEPPERCORN MÉLANGE." HOWARD J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington TX, 76019

Commercial black pepper products derived from the fruit of *Piper nigrum* can be purchased in a myriad of different forms, e.g. black peppercorns, coarse, medium and fine ground black pepper (Fig 1), green peppercorns (freeze dried or pickled in brine), white peppercorns and ground white pepper, etc. The product "Peppercorn Mélange" contains green, black and white peppercorns derived from P. nigrum as well as bright red "peppercorns" derived from Schinus terebinthfolius, the Brazilian pepper tree, a species unrelated to Piper. A bottle of the mélange provided material with which to investigate the structure of each of the four components. The green peppercorns of the mélange had undergone considerable internal damage when you compare their structure to that of green peppercorns stored in brine. In all likelihood the structural damage resulted from being freeze dried. Attempts to resuscitate the "freeze dried" green peppercorns from the mélange by soaking the fruits in water were not successful. Only the outer part of the pericarp remained intact and, the fruits internal structure was difficult to image. On the contrary, green peppercorns in brine were easily sectioned with a freezing microtome and their internal structure could be determined without difficulty by either light or electron microscopy (Fig. 2). Likewise, it was possible to examine the other three mélange components using preparations made with the freezing microtome.

The peppercorns (fruits) of P. nigrum are composed of a single seed contained within the pericarp (surrounding fruit tissues). The exterior of the fruit is composed of dense layer of sclereids below a thin epidermis; a second layer of large sclereids is found at the seed/pericarp boundary. In white peppercorns the pericarp has been removed from black peppercorns by processing. The central portion of the peppercorns is composed of a reserve food tissue called the perisperm, a diploid tissue derived from ovule cells, and a small embryo. Perisperm is the basic component of white peppercorns and white pepper, and by volume represents the major part of either green or black peppercorns. The perisperm of green, black and white peppercorns contain many oil idioblasts distributed within a parenchymatous starchy tissue. Depending on the age of individual peppercorns perisperm cells will have only a few or many starch grains. At maturation starch grains are jammed together in the individual cells (Fig. 5.). Often the extremely tight packing causes the shape of the starch grains to become polygonal, in all probability this occurs because of pressure of the drying process during fruit maturation (Fig. 6). Each oil idioblast contains one or more large droplets of pepper oil. The oil is contained in what appears to be "a sac" which is attached to the cell wall. In the immature green peppercorns the oil idioblast may have numerous oil droplets of variable size. Apparently during maturation the small droplets fuse to form the large, usually single, oil droplet. The pepper oil is light to dark brown when viewed by light microscopy. A profusion of small crystals was found within many cells of the pericarp and the seed coat (Fig. 4). These birefringent euhedral crystals often pack individual cells and can be found in green, black and white peppercorns. The crystals may be a form of peperine. In addition to the oil cells the inner (white) portion of the seed contains cells packed with small starch grains. White peppercorns are essentially the same as the inner part of the black peppercorns. The perisperm tissue, which makes up most of the white peppercorn, contains cells packed with intermixed oil idioblasts. The interior of the red "peppercorns," fruits of S. terebinthfolius is entirely different than the black peppercorns. Each fruit contains a single large seed which has a very thick seed coat formed by many elongate sclereids tightly fused together.

<sup>&</sup>lt;sup>1</sup> A Trade Mark of McCormick & Co, Inc., Hunt Valley, Md.



Figures 1-6. *Piper nigrum* fruit. Fig. 1. Single pepper grain from ground black pepper. Bar =  $400 \, \mu m$ . Fig. 2. Cross sectional view of green peppercorn preserved in brine, cryo-sectioned. Bar =  $1000 \, \mu m$ . Fig. 3. Fractured dry black peppercorn showing riged pericarp which large sclereids, persperm on left. Bar =  $1000 \, \mu m$ . Fig. 4. Crystals found in cells of the pericarp in a dry fractured black peppercorn. Bar =  $10 \, \mu m$ . Fig. 5. Starch containing cell from the perisperm of a black peppercorn. The starch grains adhere to each other because of the pressure caused by shrinkage of the perisperm cells during drying. Bar =  $20 \, \mu m$ . Fig. 6. Starch grains from perisperm of black peppercorn showing polygonal shape caused by close packing. Bar =  $10 \, \mu m$ .

THE "Z": IMAGING AND ANALYSIS IN THE LEAST-EXPLORED PLANE OF SECTION. MIKE DAVIS AND HOWARD J. ARNOTT. Department of Biology and the Center for Electron Microscopy, The University of Texas at Arlington, Arlington, Texas, 76019.

Sectioning specimens for microscopic evaluation oftentimes masks the true three-dimensional characteristics of the sample. Volumetric relationships, which are often very useful aspects of the specimen structure, may be overlooked. However, with the advent of Z-plane stepper motors and the laser scanning confocal microscope (LSCM), we can now observe thicker sections microscopically without disturbing the *in situ* relationship and positioning of features in our sample. This holds many advantages over serial sectioning: LSCM sections in the Z plane can be sub-microns in thickness, and these sections can be acquired, compiled, and analyzed by a computer very rapidly.

The true three-dimensional structure of leaves is particularly difficult to reconstruct from a series of sections, and although cleared leaf specimens can help understand leaf structure, traditional micrographs of clearings do not have high quality resolution. This study uses the LSCM to view the structure of various cleared leaves preparations. The technique of "clearing" uses a 5% solution of sodium hydroxide and a saturated solution of chloral hydrate to remove the pigmentation from leaves or other plant parts starting with either living, fixed or pressed specimens (Foster, 1949). After pigment removal the plant components are then washed, stained with aqueous safranin, dehydrated through 100% ETOH, transferred to xylene, mounted on a microscope slide in a medium such as permount and finally a cover slip is added. This technique provides a specimen in which the three-dimensional relationships of the leaf cells are intact and can be viewed with an ordinary light microscope. In this case, however, several mounted tea (Thea sinensis) leaf sections were viewed with the LSCM, and areas with sclereids and other points of interest were acquired through the entire leaf in the Z plane at 0.2um per optical section (Fig. 4). Safranin autofluoresces in the ultraviolet wavelengths, so it was unnecessary to apply any fluorescent staining technique to visualize the entire leaf structure with the confocal microscope. Sequential images were captured using a photomultiplier tube camera (PMT) at various magnifications, and then reconstructed as stacks of images. Image stacks were then processed using Metamorph Imaging Series software (Universal Imaging Co., Trenton NJ). Figure 1 and the front cover show images characteristic for each section of a series of 181 optical sections. The quality of the information in each of these images is quite good.

Using computer analysis of the optical sections volumetric data was obtained on structures of interest. Three-dimensional reconstruction of the entire image stack was also performed to evaluate the spatial organization of these areas of interest. Additionally, wireframe models were generated of objects of interest to better isolate them from the surrounding tissue and to visualize their three-dimensional shape and orientation (Figs. 2, 3). Using the sequential sections the computer can generate "fly-throughs," and plane rotation from reconstructions of the leaf. Fly-throughs, plane rotation and other uses of the data from the stacks of optical sections provides an exceptional means of viewing the internal structure of leaves, not only useful in research but also especially useful in teaching.

Stereographic views and montages were also produced from our areas of interest. These data help reconstruct the actual shape and spatial organization of structures in various leaves, as well as render accurate volumetric measurement data for comparison and analysis.

#### References:

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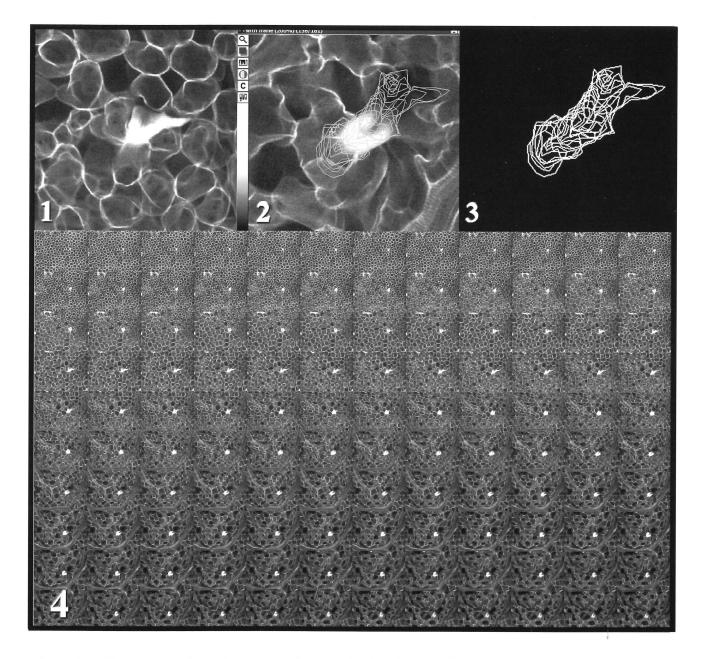


Figure 1. Single plane of a multiplane confocal stack of a sclereid cell.

- Figure 2. Construction of a wireframe around the sclereid in multiple Z planes of section.
- **Figure 3.** Wireframe model of a sclereid cell resulting from the sequential sections shown in the previous figure.

Figure 4. Montage of entire Z series through the sclereid cell.

AN INVESTIGATION INTO THE EXOSKELETAL ULTRASTRUCTURE OF PLUSIOTIS GLORIOSA FOR COMPARISON TO AN UNKNOWN SPECIES. CATHERINE A. BOYLES, HOWARD J. ARNOTT AND CHARLES S. WOLFE. The Department of Biology and The Center for Electron Microscopy, The University of

Texas at Arlington, Arlington, Texas 76019.

Plusiotis gloriosa is a scarab beetle so named for its 'glorious' external markingsmetallic silver stripes running along the length of iridescent green elytra. Its range extends from West Texas and into New Mexico, Arizona and Mexico, where it is found in association with Juniper trees. It is common within its range and is often collected incidentally by entomologists searching for other species. Although slight variations in markings and size exist, overall external morphology remains consistent. However, in about 1% of the P. gloriosa collected in West Texas a "red-type" variation is found. The "red-type" variation is also iridescent being copper/brown in color with similar markings. Basically the structure is the same, the green color is merely replaced with "red." Because P. gloriosa is both well collected and taxonomically well understood, particular interest was paid to a single specimen collected in the Davis Mountains of Texas in 1996 by collector/entomologist David Marqua. Similar in most respects to P. gloriosa, the exoskeleton showed a radically different sculpturing and color. All efforts to taxonomically place this specimen into a known group have failed, and it appears to be a currently undescribed (new) species. Since differences between P. gloriosa and this unknown appear limited to the exoskeleton, our microscopic investigation targets that area. An understanding of the differences separating the more common green type from the rare "red-type" and the radically different unknown depends upon a thorough understanding of the exoskeletal makeup of P. gloriosa. Available literature lacks consistency in description of insect exoskeleton structure within and among insect groups, and is particularly poor for scarab beetles. Even the techniques to make these studies are not well documented. Because of the unique character of the "new species" careful investigation of the common green P. gloriosa is necessary, prior to any invasive investigation of the unknown specimen. Both light and scanning microscopy were utilized to study the green specimens.

A COMPARATIVE STUDY OF THE DIGESTIVE GLANDS OF THE DIONAEA MUSCIPULA ELLIS (VENUS FLYTRAP). TRACY NEFF AND HOWARD J. ARNOTT. The Department of Biology and The Center for Electron Microscopy, The University of Texas at Arlington, Arlington, Texas 76019.

Dionaea muscipula Ellis, more commonly known as the Venus Flytrap, is an insectivorous plant indigenous to the bogs of North and South Carolina. Unfortunately the D. muscipula has become an endangered species due to the encroachment of man onto its native habitat and voracious collecting. Charles Darwin was the first to extensively study the D. muscipula and although he was able to suggest reasonable explanations for the mechanisms of trap closure, however, the mechanisms of digestion remains a mystery. There has been a great deal of study done on the mechanisms of trap closure, but all efforts to find information on the mechanisms of the digestive glands have failed. The D. muscipula demonstrates three distinct phases upon trap closure: (1) Closure - the first step in trapping prey, this stage occurs within one second of trigger hair stimulation; (2) Sealing – this step occurs within thirty minutes post stimulation; (3) Depression - the trap begins to depress on one side of the trap to bring both sides of the trap in closer contact with the prey inside the stimulated trap -- this step occurs within two hours post stimulation. This is the time frame used for this study. The digestive glands of the D. muscipula were examined unstimulated; thirty minutes post stimulation through four hours post stimulation. The time frame of greatest observable change in digestive gland structure was two hours post stimulation. While each stage that was examined showed a clear increase of the turgidity of the digestive gland, at two hours post stimulation "pores" appears on the secretory head cells of the digestive glands. At this time the function of these "pores" is not yet understood, it may be that these "pores" provide an exit for the digestive enzymes to leave the secretory head cells and enter the sealed trap to begin the digestion of the trapped prey. Further study will be required to determine if this is in fact the function of these "pores". Both light and scanning microscopy were utilized to study the digestive glands of the D. muscipula.

CHARACTERIZATION OF A PUTATIVE OXYGEN CHEMORECEPTOR ISOLATED FROM THE GILL OF THE CHANNEL CATFISH, ICTALURUS PUNCTATUS. S.E. MERCER, M.L. BURLESON AND H.J. ARNOTT. Department of Biology and the Center for Electron Microscopy, The University of Texas at Arlington, Arlington, TX 76019.

Virtually nothing is known regarding the mechanisms of oxygen chemoreception in animals other than mammals. Efforts to determine the transduction process using the mammalian carotid body have proven difficult because the sensory organs have evolved numerous highly derived and overlapping neural circuits based on the interaction of multiple neurochemicals. The first gill arch of fish is believed to be the phylogenetic precursor of the mammalian carotid body. Efforts to develop a comparative vertebrate model for electrophysiological study of oxygen-sensing mechanisms have resulted in a dissociation and partial enrichment procedure to isolate neuroepithelial cells from the first gill arch of channel catfish (Ictalurus punctatus). A subgroup of cells with morphological and immunohistochemical similarities to mammalian chemoreceptor cells have been identified as candidate O2 chemoreceptors based on the presence of neuron specific enolase, serotonin and tyrosine hydroxylase. The heterogeneous primary culture environment was examined using scanning electron microscopy. The target cells were spherical bodies with an average diameter of 5-6  $\mu m$  and a convoluted surface. The putative chemoreceptor cells were generally associated with sustentacular or glial cells and were often paired or clustered. Putative chemoreceptors were found to represent a significant percentage of cultured cells (ca. 25%). The culture also contained numerous cells with morphologies characteristic of neurons. Electron microscopy confirmed the viability of the dissociation and enrichment procedure. This cell culture model offers the potential to study the electrophysiology of oxygen-sensing cells in a less derived system with numerous naturally occurring neuronal

### BIOLOGICAL SCIENCES POSTER PRESENTATION—SPRING 1999

THE PTEROSTIGMA OF COENAGRIONID DAMSELFLIES. R. OSBORN, Dept. Biology, University of Texas at Arlington, TX 76019.

The morphology and degree of sexual dimorphism in the pterostigma of three species of coenagrionid damselflies was investigated using SEM. *Ischmura posita* was the most dimorphic in shape, size and color. The male pterostigma of this species was larger than that of the female, and there were differences in the number of chitinous microfibres in the matrix. The hindwing pterostigma of female *Telebasis salva* was lighter in color and larger than that of the male of this species. Differences in color were also found in the female and male pterostigma of *Enallagma basidens*. Although the three species had different types and arrangements of spination, there were no differences between sexes within a species. Sexually dimorphic charactes were mainly size, shape and color. Such larger scale differences can be expected since these differences may be attributable to the fact that the pterostigma functions not only for species-recognition, but also as a mate-recognition signal.

MORPHOLOGICAL STUDIES OF A NOVEL CAPSULE-FORMING BACTERIUM.

PATAMAPORN SUKPLANG, ACHARAWAN THONGMEE, DAVID GARRETT AND G.R. VELA, Dept of Biological Sciences, University of North Texas, Denton, TX 76203.

Scanning and transmission electron microscopy were used to study the morphology of a Gram negative capsule-forming bacterium recently isolated from soils. This bacterium is rod shaped, 1-2  $\mu$ m x 4-6  $\mu$ m, and has an extremely large transparent capsule measuring 15  $\mu$ m x 25  $\mu$ m. Studies were performed on cells grown on Burk's nitrogen free agar, Tryptic Soy Agar and Dialyzed soil agar. Scanning electron micrographs revealed the different amounts of capsule material produced by cells grown on each medium, i.e. cells grown on Burk's Medium were surrounded by more capsular material than those grown on Tryptic Soy Agar and Dialyzed Soil agar. Transmission electron microscopy of cells grown on these media showed that this organism has a multilayered cell wall identical to that of other Gram negative bacteria. Cells grown on Burk's Agar contained intracellular inclusions which became larger as the cultures aged. Bacterial spores were seen with TEM when cells were grown on Dialyzed Soil Agar after two days of incubation while not seen in the other two media even after 7 days. Spore bearing cells also showed rib like ridges not previously observed in other bacteria. These structures were not observed by light or phase contrast microscopy and were observed only by electron microscopy.

#### CHARACTERIZATION OF A TMV COAT PROTEIN MUTANT USING TRANSMISSION ELECTRON MICROSCOPY. Tamarah L.

Adair, Ann E. Rushing, and Christopher M. Kearney. Department of Biology, Baylor University, Waco, TX 76798.

Plant viruses move through plants either by cell-to-cell movement or by long distance (systemic) movement. This project investigates the role of coat protein (cp) on systemic movement and symptom formation using two different genetically modified TMV vectors and the host plant Nicotiana benthamiana. The two vectors differ in their cp sequence: the cp from one TMV vector (30B.KO.GFP) consists of the entire TMV-U5 cp sequence, while the other vector (30B.GFP.Acp) lacks one-third of this cp sequence. 30B.KO.GFP moves systemically through the host plant causing symptoms such as stunting, mosaics, and structural changes in the leaves. At the ultrastructure level, features associated with normal TMV infection have been observed. In contrast, symptoms and systemic movement are delayed or absent in the plants infected with 30B.GFP. Acp. The cp mutation interrupts the coat protein open reading frame, thus a virion coat is not produced. Virions are not observed at the ultrastructure level, but other features, such as the densely staining large filaments characteristic of normal TMV infections, have been found. Replication and cell-to-cell movement of 30B.GFP.∆cp are comparable to 30B.KO.GFP. These results indicate that the coat protein is important in efficient systemic movement and in symptom formation. Future studies will use immunocytochemistry and in situ hybridization to analyze the mechanisms involved in systemic movement in this plant-host system.

#### THE MOSS GENUS TREMATODON IN NORTH AMERICA.

Emily M. Coe and Ann E. Rushing. Bay City High School, Bay City, TX 77414 and Department of Biology, Baylor University, Waco, TX 76798.

The moss genus <u>Trematodon Michx</u> is represented in the North American flora by five species: <u>T. ambiguus</u>, <u>T. boasii</u>, <u>T. brevicollis</u>, <u>T. longicollis</u>, and <u>T. montanus</u>. These species can be distinguished based on a number of prophological for the prop morphological features including the structure and ornamentation patterns of the spores and the peristome teeth. Surrounding the sporophyte capsule, T. longicollis and T. ambiguus have elongated, highly ornamented peristome teeth while the teeth of the other three species are shorter and have less elaborate expressions patterns. The spores of all five species have distinguish proving the proving the spores of all five species have distinguished proving the spores. ornamentation patterns. The spores of all five species have distinct proximal and distal surfaces. The distal surface of <u>T. boasii</u>, <u>T. brevicollis</u>, and <u>T. montanus</u> spores has a warty ornamentation pattern. Spores of <u>T. longicollis</u> and <u>T. ambiguus</u> vary from warty to spinose in distal ornamentation. This variation may be due to the degree of spore maturity. Similarities in peristome structure and ornamentation and in spore ornamentation patterns reveal that T.  $\underline{\text{boasii}}$  and  $\underline{\text{T. montanus}}$ , the two most recently named species, are closely related and also share features in common with  $\underline{\text{T. brevicollis}}$ . The other two species, T. <u>longicollis</u> and T. <u>ambiguus</u>, are distinguished by more well developed peristome teeth and spores. Other sporophyte and gametophyte features support these distinctions.

#### MATERIALS SCIENCES PLATFORM PRESENTATION—SPRING 1999

THE ELECTRONIC STRUCTURE OF ALKALI-DOPED MoS, STUDIED BY ELECTRON SPECTROSCOPY FOR CHEMICAL ANALYSIS (ESCA). Kenneth T. Park, Department of Physics, Baylor University, Waco, TX 76798

Interaction of a MoS<sub>2</sub>(0002) surface and surface-deposited alkali metal is investigated by high resolution Electron Spectroscopy for Chemical Analysis (HRESCA or HRXPS) and a full-potential linearized augmented planewave density functional theory (FLAPW-DFT). The adsorption of initial sub-monolayer amount of alkali metal (Li, K, and Cs) onto the basal plane introduces a new density of states above the valence band maximum (VBM) of  $MoS_2$ : for Li, at ca~0.2~eV above the VBM or 1.2~eV above the VB peak "A" of the MoS<sub>2</sub> and for K and Cs, ca 1.25 eV above the VBM. Angle-resolved VB-XPS and theoretical analysis locates this electron density in the MoS<sub>2</sub> layer, thus transferred from alkali adatoms. The adsorbed Li further gives rise to new, distinct surface components in both the Mo 3d and S 2p core levels, which are shifted toward lower binding energy by 1.13 eV and 0.68 eV respectively. On the other hand, the surface deposited Cs and K only broaden the Mo and S core levels without introducing such distinct low binding energy components. As more Li atoms are deposited onto the surface, the "reduced" components of both Mo and S continue to grow, and near the Li saturation coverage, they account for more than 40 % of the total photoelectron intensities from the substrate. The combination of surface core level shifts and X-ray photoelectron diffraction indicates that the deposited Li are also present below surface whereas most Cs adatoms are in the form of disordered thin overlayer on the  $MoS_2$  basal plane. Theoretical account for observed phenomena using the FLAPW-DFT and the chemical reactivity of alkali-modified MoS2 will be also discussed.

SITE SPECIFIC COPPER DEPOSITION DURING SHORT TERM CORROSION TESTING OF ALUMINUM ALLOY 2024 AIRCRAFT SHEET, H.M. Obispo, E.A. Trillo, R.M. Arrowood, L.E. Murr, and W.W. Fisher, Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, TX 79968.

Pitting corrosion with regards to aircraft materials has been the subject of recent interest due to the need for longer aircraft service times. Aged Al 2024 sheets contain many precipitates, which seem to be preferential sites for pitting corrosion. Al-Cu-Mg containing particles act as anodic sites, while Al-Cu-Mn-Fe containing particles act as cathodes. In addition to the pits, deposits have been found near Al-Cu-Mn-Fe precipitates after being tested in NaCl solutions. These copper deposits may be a consequence of the iron present in some of the particles.

In order to fully understand the role that specific precipitates play in the pitting/deposition process, potentiostatic tests were performed on 2024 Al aircraft sheets at -200 mV vs SCE for 180 seconds in a 3.5% NaCl solution at a temperature of 22 °C. Optical microscopy revealed a severely pitted microstructure after corrosion testing. Scanning electron microscopy (SEM) was used to examine the surface before and after testing. Deposits were clearly visible on the surface after testing and EDS was used to identify them as Cu rich. A Hitachi-H 8000 scanning transmission electron microscope (STEM) was then used to document the as-received microstructure, as well as to identify the constituent particles and particle regimes. Research supported by the future Aerospace Technology (FAST) Center at the University of Texas at El Paso; supported by AFOSR Grant f49620-95-1-0518.

CHARACTERIZATION OF EXFOLIATION CORROSION IN AL 2024 AND AL 2524 ALLOYS, David A. Roberson, Roy Arrowood, and L.E. Murr, Dept. of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX, 79968.

Exfoliation corrosion is a specialized form of grain boundary attack detrimental to wrought aluminum alloys. This form of corrosion is characterized by metal splitting along grain boundaries and lifting away from the bulk material to create a layered structure resembling strudel pastry. Though there is substantial information concerning exfoliation in 7000 series aluminum, not much is known about the mechanisms of the phenomena in 2000 series aluminum. In service the time for exfoliation corrosion to occur ranges from months to years.

The scope of this research is to create a test that produces quick reproducible results that will aid in the definition of the mechanisms behind exfoliation corrosion. By exposing Al 2024 and Al 2524 samples to strongly alkaline solutions, exfoliation corrosion can occur in a matter of days. The samples were immersed in .5M NaOH solution in a sealed container and monitored visually until signs of exfoliation became evident. The type and magnitude of the damage was evaluated via optical, scanning electron and/or transmission electron Microscopy (SEM and/or TEM). The magnitude of damage was then compared between aluminum-clad Al 2024, bare Al 2024, and aluminum-clad Al 2524, a relatively new alloy. This research is conducted through The University of Texas at El Paso FAST Center for Structural Integrity of Aerospace Systems. This effort is sponsored by the Air Force Office of Scientific Research, Air Force Materiel Command, under grant number F49620-95-1-0518.

MICROSTRUCTURAL ANALYSIS OF THE CARIBBEAN STEEL DRUM, E.A. TRILLO, E. FERREYRA T., S. PAPPU, C. KENNEDY, D.P. RUSSELL, AND L.E. MURR, Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, Tx.

The Carribean steel drum is an instrument that has only been invented and developed in the last 50 years. Although it has gained some interest in the musical arena, not much is known about the metallurgical aspects that contribute to its unique sound. This research focuses on steel drum fabrication utilizing light microscopy (LM), transmission electron microscopy (TEM), and Vickers microhardness values to characterize microstructures at various stages of production. The initial sinking of the drum head produces a reduction in thickness of up to 50% at the bottom of the drum head. TEM analysis reveals carbide precipitation and extensive dislocation densities in this area that can exceed 1010 cm<sup>-2</sup>. Comparisons of the steel drum material to 40% cold rolled 316 stainless steel plates reveal that deformation has an important affect on the acoustic signal. The heat treatment that the drum is subjected to during fabrication has been found to involve strain aging which also plays a significant role in the harmonic signal, especially during tuning. Strain aging along with strain hardening produces a requisite elastic-plastic interaction, which allows for multi-harmonic tuning. Elastic-plastic and plastic hardness profiles were compared which highlight the note zone regions. The analysis of the acoustic spectra for specific note zones illustrate their complex, non-linear behavior, and the role that deformation induced defects play in acoustic dispersion and multi-harmonic signal production. Research supported in part by the U.S. Department of Defense, Defense Logistics Agency, Defense National Stockpile Center under Grant DN-009 and also in part by a Shell Oil Company Foundation Grant.

ELECTROCHEMICAL AND MECHANICAL EVALUATION OF TITA ALLOYS FOR METALLIC IMPLANT CONSIDERATION, E.A. TRILLO, C. RAMIREZ, R. VILLA, S.W. STAFFORD, and L.E. MURR, Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, Tx, 79968

For orthopaedic surgery, titanium and its alloys have been the biomaterials of choice due to their excellent corrosion resistance and high strength-to weight ratio. However, current demands require longer implantation times which have raised concerns over possible metal ion release into the body. This research looks at two relatively new TiTa alloys (Ti40Ta and Ti50Ta) as possible alternatives to current Ti materials. Electrochemical and mechanical tests were performed over a range of heat treatments to characterize these materials and to select the best thermomechanical processing. Potentiodynamic scans reveal a passive region on all samples comparable to Ti6Al4V, the current biomaterial standard. Vickers microhardness tests were performed, whose values were converted to tensile strength. The tensile strength values were also as high as the Ti6Al4V samples and in some instances exceeded them. Optical microscopy and transmission electron microscopy revealed a wide variety of microstructures depending on the heat treatment. An  $\alpha$ ,  $\alpha + \beta$ , and martensite phases were clearly present. The variation in density and shape of the a and martensite phases depended on the amount of Ta and the thermal treatment performed. The results show great promise in utilizing these TiTa alloys for orthopaedic use. Research supported in part by the U.S. Department of Defense, Defense Logistics Agency, Defense National Stockpile Center under Grant DN-009 and also in part by a Shell Oil Company Foundation Grant.

AN INVESTIGATION OF THE MECHANICAL PROPERTIES AND MICROSTRUCTURE OF SILVER-COATED Ag4Sn DENTAL ALLOY PARTICLES CONDENSED IN VITRO, Viviana Aguero, J.A. Marquez, and L.E. Murr, Dept. of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX, 79968

Mercury-containing dental amalgam is the most widely used restorative material in the world. When a mercury amalgam sets, it becomes a brittle metal-matrix composite (Hg and Ag-Sn-Cu) with mechanical and physical properties which can withstand the oral environment conditions. If mercury penetrates into the nerve tissue of the tooth, its toxicity may affect the nervous system. Efforts to replace Hg-containing amalgams have thus been made to help eliminate such risks.

This research focuses on obtaining a dental alloy with properties similar to conventional alloys with the use of less mercury or no mercury. Silver-coated Ag4Sn dental alloy particles have been selected as potential replacements. In order to coat the particles they are immersed in a silver cyanide electrolyte over a vibrating cathode plate, which eases adsorption of silver onto the particles. The powders under study are 11%, 16%, 18%, and 25% silver coating and a coppersilver coated powder. The powders have been characterized for differences in morphology, surface features, internal structure, and mechanical properties. The powders were amalgamated with mercury in amounts less than is conventionally used. Increasing the silver content in the particle coatings to 18% strengthened the alloy, but above 25% the alloy weakened dramatically. Copper and tin powders were introduced to remedy this condition. Eta' Cu6Sn5 crystals are expected to precipitate during the amalgamation reaction. The eta' phase is desired because it is known to strengthen dental amalgam by restricting plastic flow. Current research concentrates on the physical and mechanical properties of the powders with the addition of copper and tin to the alloy system. The results obtained will be compared with established properties of conventional dental amalgams to determine its commercial feasibility. Supported in part by a Shell Oil Company Foundation Grant.

EVALUATION OF TITA OXIDE LAYERS VIA ELLIPSOMETRY FOR BIO-MATERIAL CONSIDERATION. C. RAMIREZ, R. VILLA, E. A. TRILLO, S. W. STAFFORD, L. E. MURR, Dept. of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX 79968

Titanium-tantalum alloys, which are being considered for biomedical implants have been shown to respond favorably to various heat treatments by exhibiting excellent mechanical and corrosion properties. The alloys, specifically Ti40Ta and Ti50Ta, were divided into five sets including one sample of each alloy and subjected to different heat treatments. One set was heat-treated at a temperature of 1000 °C for one hour and then furnace cooled. Another was subjected to the same heat treatment but was quenched in water. A third set was heat-treated at a temperature of 400 °C for three hours and quenched in water while a fourth set was heat-treated at 400 °C for ten hours and quenched in water. A fifth set was kept in the asreceived condition for reference. The properties of these alloys are being compared to those of Ti6Al4V, currently the most common titanium alloy in use as a biomedical material. The next step in this research is to analyze the surface of the heat-treated samples using ellipsometry analysis. From this, it will be possible to determine the type of oxide layer present on the samples. The information obtained will be compared with potentiodynamic test scans which were performed using a simulated biological fluid as the electrolyte. The potentiodynamic scans show stable passive layers for both materials under all conditions except for the Ti50Ta heattreated at 1000 °C and quenched. By comparing the structure of the oxide layer to the passive region of the potentiodynamic test scans, a relationship between the oxide layer and the passive region can be found. The determination and study of such a relationship will allow for optimization of corrosion properties during the preparation of these alloys for use in biomedical applications. This research was supported by the U.S. Department of Defense, Defense Logistics Agency, Defense National Stockpile Center under Grant DN-009.

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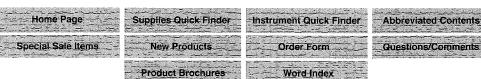
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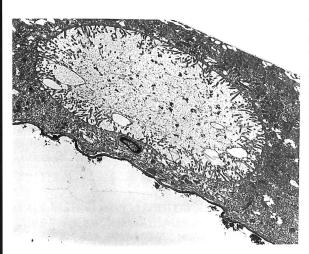
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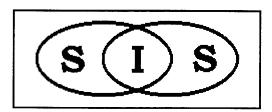
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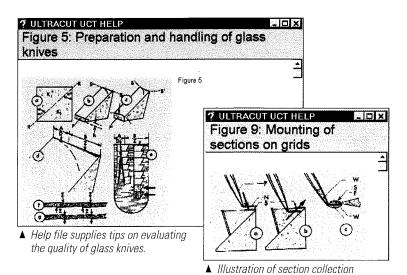
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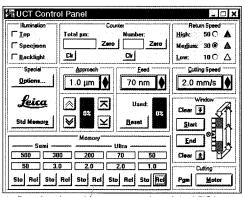
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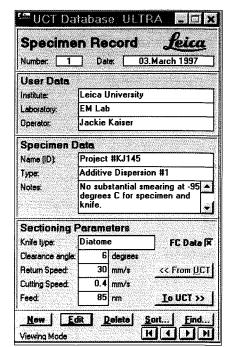
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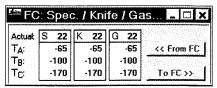
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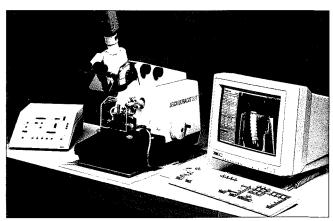
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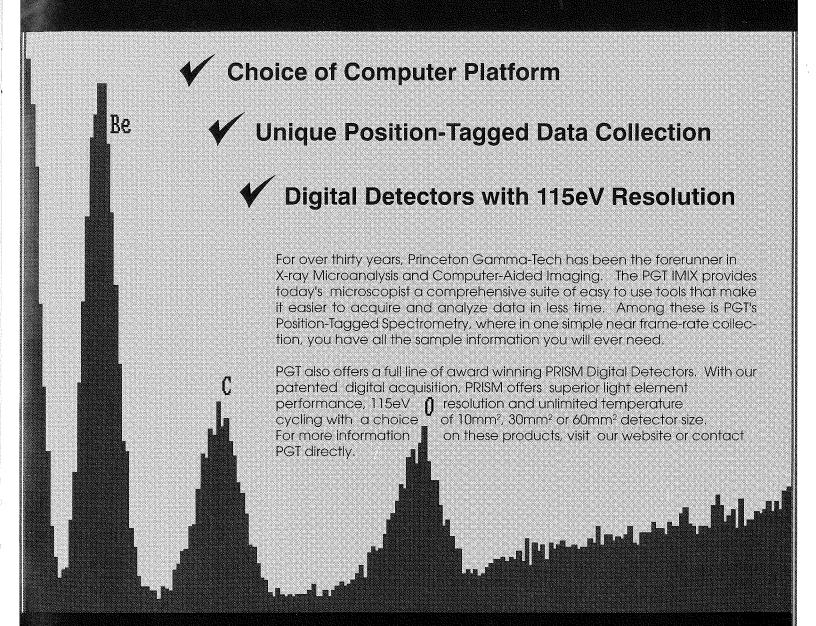
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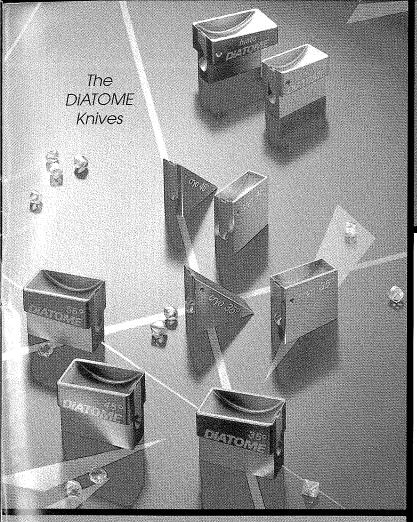
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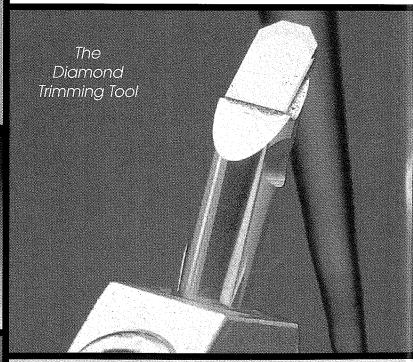
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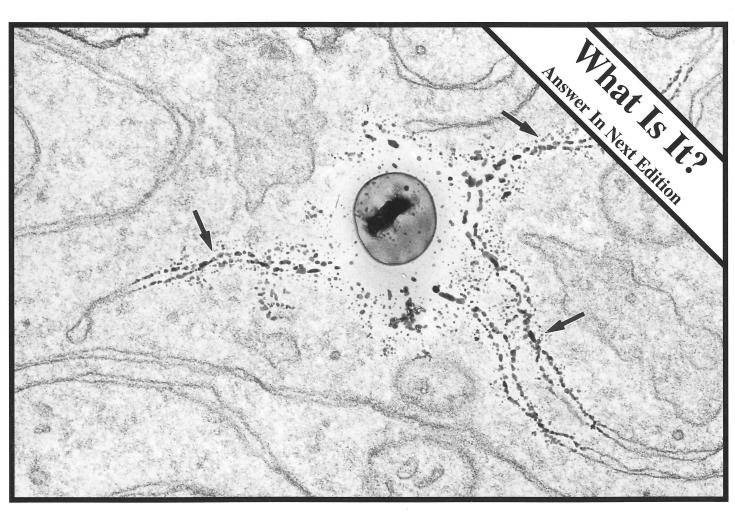
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