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Corporate Member Representative: ROBERT D. MEYER Meyer Instruments, Inc. 1304 Langham Creek Dr. Suite 235 Houston, Texas 77]84 (713) 579-0342

Student Representative: TAMMY HANCOCK Dept. of Biology

Univ. of Texas at Arlington P.O. Box 19498 Arlington, Texas 76019 (214) 274-1020

TSEM Journal Editor: RONALD W. DAVIS Department of Medical Anatomy Texas A&M University College Station, Texas 77843 (409) 845-7904

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TEXAS SOCIETY FOR ELECTRON MICROSCOPY, INC. JOURNAL VOLUME 21, NUMBER 1, 1990 ISSN 0196-5662

Ronald W. Davis, Editor Department of Medical Anatomy, Texas A&M Univ., College, Station, TX 77843

Texas Society for Electron Microscopy, Inc.

"For the purpose of dissemination of research with the electron microscope."

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#### ON THE COVER

Glochidia (pl.), glochid (sing.), are the small hair-like thorns found at the base of the large spines on the cactus *Opuntia*. They are particularly troublesome because they do not appear to be very threatening, or at least not like the large thorns that they surround. They are, however, very sharp and have backward pointing barbs that allow them to move forward and prevent them from coming out. They are very irritating, hard to see, and almost impossible to grasp, thus making it extremely difficult to remove them from skin. I have been told that the best way to remove glochidia is to scrape the outer layer of skin where they are embedded with the edge of a knife. This removes the outer dead skin and the glochidia too.

Ronald W. Davis, Medical Anatomy Department, Texas A&M University, College Station, Texas 77843-1114. (Magnification approximately 300X.)



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#### Electron Microscopy Sciences

# President's Message

Although the numbers were small, the quality was immense at the Arlington meeting. Variety was the nature of the day. We began with the President of the Oklahoma Society telling us all about the nastiest and ugliest creature known to man. That was followed by octopus parasites and the sexual activities of dragonflies. We saw airplanes, bicycles, teeth filling, alligator glands, glue, Australian plants, Guatemalan fighting plants, brick paved sewer systems of silk worms, unusual cilia and, as amazing as it may seem, the electron microsopy of a drop of water! Then, of course, what would a TSEM meeting be without Louis Bragg's seed coats that had been branded with a horseshoe!

I was especially impressed and excited by the discussions of the environmental electron microscope that allows for the viewing of wet samples and the tunneling microscope that allows for the viewing of atoms. These, along with ultracryomicrotomy and thick section high voltage microscope, that we have concentrated on in previous meetings, are certainly the future of electron microscopy. They should open up many new avenues of research.

The upcoming Kerrville meeting draws to a close my year as President of TSEM, Inc., and I wish to thank all the members of the Executive Council for their patience and assistance during the year. They did all the work of running the Society while I sat back and smiled. I also wish to thank all the members who presented papers and posters at the meetings, some of whom rescued the prospects of a

paperless meeting at the last minute.

One note of a discouraging nature that I must address is that of dwindling revenue. As can be seen in the enclosed (pp. 7, 9) and previous Treasurer's Reports, our assets are steadily decreasing. This is due primarily to meeting expenses. It is essentially impossible to "break even" on meetings when so few members attend. Even an increase in registration fees, which are already high, would be of little effect when you consider that we rarely have more than 75 members at each meeting. I can only see two possible solutions. We can increase membership dues by five or ten dollars a year (with 600 members, we would gaint \$3-6,000 in revenue) or we can go to one meeting a year, which would decrease expenses and possibly draw a larger group since many of our members cannot attend both meetings.

This topic will be discussed in the coming days, and I am sure that the executive council members will welcome your suggestions.

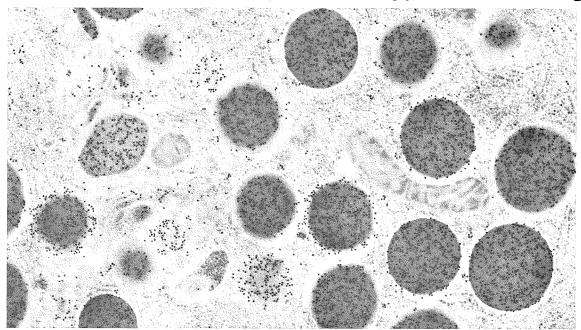
I would encourage everyone to continue vigorous support of the next president by continued submission of quality abstracts. I look forward to learning about the great variety of plants, animals and "things" that the members of TSEM view with their scopes.

H. Wayne Sampson President, TSEM, Inc.

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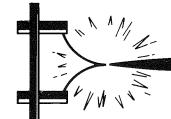
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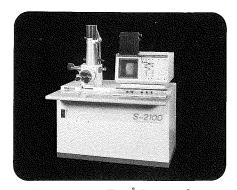
#### TREASURER'S REPORT

For Period Ending 27 February, 1990

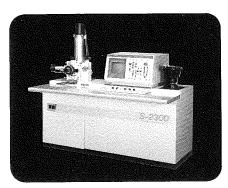
ASSETS ON 1 JANUARY, 1990:	
Certificate of Deposit No. 177576	
Certificate of Deposit No. 10-0003893	
Checking Account No. 01600041996	\$7,360.41
RECEIPTS:	
Arlington Meeting / Exhibitor Fees	
Dues	
Journal Ad Revenue 20:2625.00	
Journal Ad Revenue 20:1	
Checking Account Interest	\$ 890.04
EXPENSES:	
General Mailouts	
Checking Account Service Charge	\$ 617.04
ASSETS AS OF FEBRUARY 27, 1990:	
Certificate of Deposit No. 177576	
Certificate of Deposit No. 10-0003897(**)	
Checking Account No. 01600041996	\$7,633.41



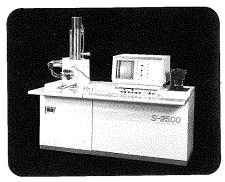
# FOCUS ON HITACHI'S WIDE RANGE OF INNOVATIVE SEM'S

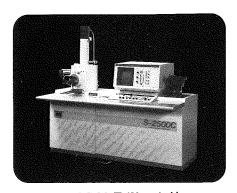


Model S-2100 SEM: ☐ 60 Å Guaranteed
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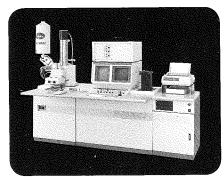
Model S-2300 SEM:  $\square$  Unique new objective lens design guarantees 45 Å with tungsten source  $\square$  Accelerating voltage range from 500V to 25kV in 35 steps  $\square$  Large specimen chamber accommodates 6" specimens  $\square$  Fully automatic computerized astigmatism correction



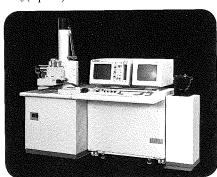


**Model S-2500C SEM:**  $\square$  60° conical lens allows high resolution on large samples at high tilts.  $\square$  High take-off angle for EDX and WDX at 12 mm working distance  $\square$  Mouse-driven computer stage option  $\square$  40Å guaranteed resolution with W, 30Å with LaB<sub>6</sub> (Option)

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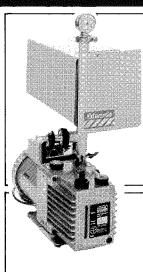


#### TREASURER'S REPORT CONTINUED

#### TREASURER'S REPORT For Period Ending 31 December, 1989

ASSETS ON 1 JANUARY, 1989:	
Certificate of Deposit No. 15-7199995	
Certificate of Deposit No. 177576	
Certificate of Deposit No. 11-8829764	<b>4.1.</b> 222 22
Checking Account No. 015210-01	\$11,380.89
RECEIPTS: Austin Meeting / Registration & Exhibitor Fees \$ 2,427.00	
Stereology Workshop	
Arlington Meeting / Registration & Exhibitor Fees	
Individual and Corporate Dues	
Journal Ad Revenue 19:1	
Journal Ad Revenue 20:1	
Journal Ad Revenue 20:2	
Checking Account Interest	
Certificate of Deposit Interest	
Donations	\$13,211.96
200000000000000000000000000000000000000	φ10,211.00
EXPENSES:	
Austin Meeting Announcement/Program	
Printing and Mailout	
Austin Meetin Expenses	
Arrington Meeting Amoutcement Manout	
and Program Printing	
Kerrville Meeting Announcement/Program	
Printing and Mailout	
Kerrville Meeting Expenses	
Journal Printing & Postage	
EMSA Symposia	ė.
Student Travel / Competition Award	
Treasurer's Expenses	
Reimbursement(+)	
Checking Account Service Charge	
Data Base Computer Program	\$17,232.44
	. ,
ASSETS AS OF 31 DECEMBER, 1989:	
Certificate of Deposit No. 177576	
Certificate of Deposit No. 10-0003897(*)	
Checking Account No. 01600041996(*)	\$ 7,360.41
(*) E l. CD #44 0000504	
(*) Formerly CD #11-8829764	
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(+) Reimbursement of Austin Registration Overpayment	

# Buyers Guide to EM specimen preparation



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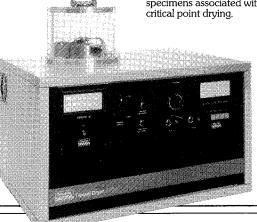


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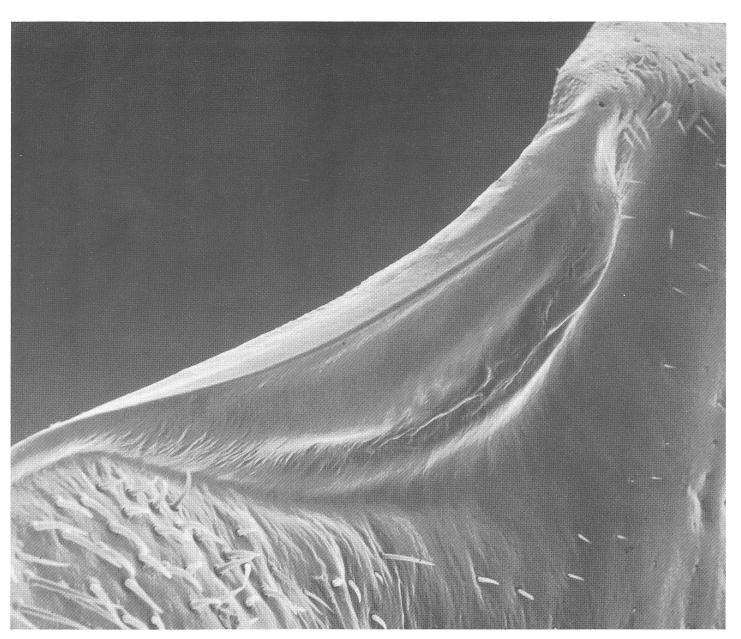
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# What Is It?

#### **Answer from Back Cover**

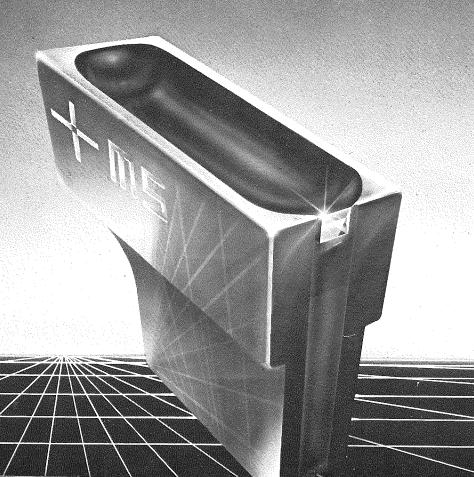
The "What Is It" picture illustrates what is called the "file" on the underside of the wing of a male cricket. The file is part of the stridulatory organ, which is used to produce sound by rubbing one body part against another. Sound is produced when the wings are raised slightly above the body and moved back and forth so that the scraper (illustrated above), which is on the edge of the opposite wing, rubs against the file.

The "songs" of the various species differ mostly in the pulse rate and the way the pulses are grouped. The tympanae (eardrums) are relatively insensitive to changes in pitch, but are quite sensitive to changes in rhythm.

If you want to see the stridulatory organ yourself, you will have to be able to tell the boys from the girls. The male, which is the only one that has this feature, has two spines at the rear of the abdomen, the female has three. The middle spine on the female is the ovipositor.

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# TSEM By-laws

#### ARTICLE I - NAME

The name of the Corporation shall be the Texas Society for Electron Microscopy, Inc.

#### ARTICLE II - PURPOSE

This Corporation, henceforth referred to as the Society, is organized exclusively as a scientific and educational organization. The purpose of this Society shall be: (a) to increase and disseminate knowledge concerning the biological and physical applications of electron microscopy and related instrumentation, and (b) to promote free exchange of ideas and information among electron microscopists and interested participants. Not withstanding any other provision of these articles, this Society shall not, except to an insubstantial degree, engage in any activities, or exercise any powers that are not in furtherance of the purposes of this Society. No substantial part of the activities of the Society shall be the carrying on of propaganda, or otherwise attempting to influence legislation; and the Society shall not participate in, or intervene in (including the publishing or distribution of statements) and political campaign on behalf of any candidate for public office.

#### ARTICLE III - MEMBERSHIP

Membership in the Society shall be open to individuals who share the stated purpose of the Society. The Society shall consist of regular members, student members, corporate members, and honorary members.

An applicant, other than a corporate organization, having an interest in electron microscopy, may be considered for regular membership. An applicant enrolled in an undergraduate or graduate academic program will be considered for student membership. Students wishing to become more involved in the Society may elect to apply for regular membership. Any applying commercial organization having an interest in electron microscopy shall be considered for corporate membership. A corporate membership shall entitle that corporation to designate one representative who shall receive membership benefits as a regular member. Other representatives of the same organization may apply for regular membership to receive Society privileges. Honorary membership shall be restricted to: (a) distinguished scientists who are not members of the Society, but who have made significant contributions to this Society, (b) to Society members for extended and outstanding service to this Society, or (c) members who have completed a term as President of the Society.

Application for regular, student, and corporate membership shall be made to the Secretary who, with the approval of the Executive Council, shall report same at the next business meeting of the Society. A two-thirds vote of the regular members present shall elect applicants to membership.

Nominations for honorary membership may be made by any member of the Society. Nominations shall be made in writing to any member of the Executive Council and must be accompanied by written evidence of the nominee's eligibility. The member of the Executive Council shall present the nomination for consideration at the next meeting of the Executive Council. The Executive Council

shall act upon the nomination within one year of its presentation and shall notify the nominator of the final action taken on the nomination.

Only members shall have the right to vote and to serve on committees. The right to hold elective office is restricted to regular members. Corporate members may exhibit at the Society's meetings (additional exhibition charges may be levied by the Executive Council). An honorary member shall be exempt from dues and shall be entitled to all privileges or regular membership. All members shall receive Society mailouts.

Membership dues for regular, student, and corporate members will be set by the Executive Council. Changes in dues shall be made by the Executive Council and notification of such shall be made by announcement at the fall meeting immediately prior to the year they go into effect. Dues shall become payable on January 1 of each year. Members whose dues remain unpaid by the spring meeting will be dropped from membership.

#### **ARTICLE IV - OFFICERS**(A) Elected Officers

The elected officers of the Society shall be President, President-Elect, Immediate Past President, Secretary, Treasurer, Program Chairman, and Program Chairman-Elect. The President-Elect shall serve one year as such, the following year as President, and the following year as Immediate Past President. The Secretary shall be elected in even-numbered years and serve for a two year term. The Treasurer shall be elected in odd-numbered years and serve for a two year term. The Program Chairman-Elect shall serve one year as such, followed by one year as Program Chairman. The installation of incoming officers shall be at the spring meeting. All officers shall arrange for the orderly and timely transition of their offices within 30 days after the installation of officers. However, all officers shall continue until relieved by their successors. The duties of the officers shall be:

- (1) PRESIDENT: shall preside at all business meetings of the Society and at meetings of the Executive Council. The President, or his designee may represent the Society at the annual meeting of the Electron Microscopy Society of America. The President shall conduct the business of the Society between Executive Council meetings.
- (2) PRESIDENT-ELECT: shall assist the President, and substitute for him in his absence, and perform such duties as assigned by the President.
- (3) IMMEDIATE PAST PRESIDENT: shall assist the President and the Executive Council, and shall keep those statistics of the Society as deemed necessary by the Executive Council.
- (4) SECRETARY: shall maintain the records of the Society, other and financial, and distribute announcements to the membership.
- (5) TREASURER: shall be custodian of the Society funds and shall account for them in accordance with accepted business practice. The Treasurer shall be bonded, and the cost of such shall be borne by the Society. The Treasurer shall have his records examined annually by an internal audit committee chosen by the Executive Council at the fall

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Telephone: 916-243-2200 • 1-800-237-3526 (U.S.A.) • 1-800-637-3526 (Calif.) • 1-800-243-7765 (Canada) meeting. A written report of the internal audit shall be presented to the Executive Council at the spring meeting.

- (6) PROGRAM CHAIRMAN: shall be responsible for organizing the various scientific activities of the Society with the advice of the President. The Program Chairman shall not commit any funds of the Society unless authorized by the Executive Council or as authorized by the President and Treasurer.
- (7) PROGRAM CHAIRMAN-ELECT: shall assist the Program Chairman and substitute for him in his absence and, additionally, extend the planning of programs into his own term of office as Program Chairman.

#### (B) Appointed Officers

The appointed officers of the Society shall be the Journal Editor, the Student Representative, and the Corporate Representative, who shall be appointed by the Executive Council.

- (1) JOURNAL EDITOR: shall publish a Journal twice a year promoting the purpose of the Society, unless otherwise ordered by the Executive Council. The term of appointment shall be for two years and may be renewed.
- (2) STUDENT REPRESENTATIVE: shall represent the student membership of the Society on the Executive Council. The term of appointment shall be for one year during which he is a student member in good standing.
- (3) CORPORATE REPRESENTATIVE: shall represent the corporate membership of the Society on the Executive Council. The term of appointment shall be for one year.

Additionally, the officers of the Society shall perform the duties prescribed by the bylaws and, as appropriate, by the parliamentary authority adopted by the Society. No part of the net earnings of the Society shall incur to the benefit of, or be distributed to, its members, trustees, officers, or other private persons, except that the Society shall be authorize and empowered to pay reasonable compensation for services rendered and to make payments and distributions in furtherance of the purposes set forth in Article Two hereof.

#### ARTICLE V - MEETINGS

There shall be two scientific meetings per year: fall and spring, unless otherwise ordered by the Executive Council. Exact times and places of these meetings shall be designated by the Executive Council. A business meeting will be held at each scientific meeting of the Society. Parliamentary procedures to be followed in the business meeting shall be those specified in the current edition of Robert's Rules of Order Newly Revised. Ten percent of the regular members, or 35 members, whichever is smaller, shall constitute a quorum at a business meeting. The Secretary shall determine if a quorum exist and inform the President at the meeting, prior to actions requiring a vote. The presence or lack of a quorum shall be noted in the minutes.

#### ARTICLE VI- EXECUTIVE COUNCIL

The Executive Council shall be responsible for the scientific and administrative obligations of the Society. It shall determine policies for the good of the Society in accordance with these bylaws; it shall plan scientific and business meetings; it shall authorize the expenditure of Society funds; and it shall conduct other duties as required for the benefit of the Society. The Executive Council shall meet prior to the business meeting at each scientific meeting of the Society. Special meetings of the Executive Council can be called by the President, and shall be called upon the written request of three elected members of the Executive Council.

At each fall meeting, the Executive Council shall appoint a Student Representative and a Corporate Representative, who shall represent the student and corporate membership respectively, the following year as voting members. The Executive Council shall also appoint Local Arrangements Chairman for each of the various meetings and in so doing shall duly consider the recommendations of the Program Chairman and the President. Local Arrangements Chairmen are ad-hoc, nonvoting members of the Executive Council.

Any member of the Society may attend the regular meeting of the Executive Council upon prior approval of the President or presiding officer.

The elected and appointed officers shall constitute the Executive Council. The President and three other elected officers, or the President-Elect and three other elected officers, shall constitute a quorum.

#### ARTICLE VII - FISCAL YEAR

The fiscal year of the Society shall run from January 1 to December 31 of each calendar year.

#### ARTICLE VIII - COMMITTEES

Standing or special committees shall be appointed by the President as directed by these bylaws, or as the Society, or the Executive Council, shall from time to time deem necessary to carry on the work of the Society. The President may appoint advisory committees at any time without prior consultation with the Executive Council. The President shall be an ex-officio member of committees except the Nominating Committee.

#### ARTICLE IX - ELECTIONS AND INTERIM VACANCIES

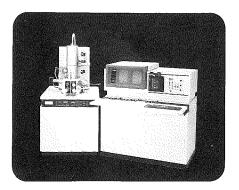
At the spring meeting each year the Executive Council shall appoint three regular members to serve on the Nominating Committee with the newly elected President-Elect and the Secretary. The Secretary shall serve as chairman of the Nominating Committee. The Nominating Committee shall nominate two candidates for each officer position becoming vacant that year. In preparing the slate of nominees, due consideration shall be given to the geographical area and fields of interest represented by the membership of the Society and to the nominee's previous participation in the Society's affairs. The Nominating Committee shall also ascertain the willingness of each nominee to serve if elected. The report of the Nominating Committee shall be announced to the Executive Council at the fall meeting of the Executive Council and then to the membership with the first announcement and call for abstracts for the spring meeting.

Additional nominations may be initiated by the membership by a petition to the Secretary, signed by a minimum of ten members. Such petitions must be received by the Secretary by eleven weeks prior to the spring meeting.

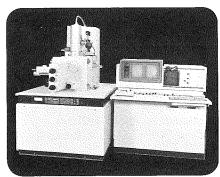
Ballots shall be mailed to members at least seven weeks prior to the spring meeting, and completed ballots shall be accepted by the President until fourteen days prior to the meeting of the Executive Council during the spring meeting. The Secretary and President shall independently count the ballots prior to the Executive Council Meeting, announce the results at the Executive Council Meeting, and at the spring business meeting, and in the next general mailout to the membership. Any member may examine the ballots at the spring business meeting.

The candidate receiving the largest number of votes shall be the winner. In the event of a tie vote, the Executive

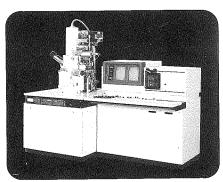
# LOOK INTO HITACHI'S BROAD LINE OF FIELD EMISSION SEM'S



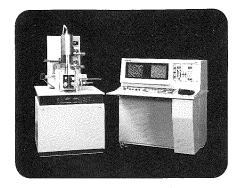
Model S-800 Field Emission Research SEM:  $\square$  20 Å Guaranteed  $\square$  Accelerating Voltage Range (1 kV  $\sim$  30 kV)  $\square$  Maintenance-free, long life, cold cathode emitter



Model S-806 Field Emission Wafer Inspection SEM:  $\square$  60Å Guaranteed at 25 kV  $\square$  200Å Guaranteed at 1 kV (150 Å Attainable)  $\square$  3″ to 6″ Wafer Capability (8″ capability option)  $\square$  Fully Programmable Stage  $\square$  Dry Vacuum System  $\square$  Conical Lens Option (allows 15 mm W.D. at 60° tilt)

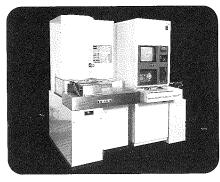


Model S-900 Field Emission Ultra High
Resolution SEM: □ 7Å Guaranteed at 30 kV
□ Ultra High Magnification (150 × ~800,000 ×)
□ Remote Control Side Entry Goniometer
Stage □ Anti Contamination Device □ Air
Lock Specimen Exchange □ Dry Vacuum
System

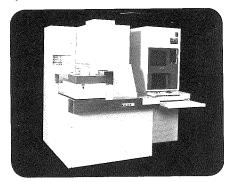


Model S-4000 SEM: ☐ Built-in real time frame averaging ☐ High brightness F.E. gun with 15Å guaranteed resolution ☐ Built-in beam blanker for low specimen dosage ☐ 3″ specimen airlock ☐ Color imaging (Option) ☐ CPU-controlled instrument parameters

every application and budget.



Model S-6000 Field Emission CD Measurement SEM:  $\Box$  150 Å Guaranteed at  $1\,\mathrm{kV}$   $\Box$  Measurement Precision  $\pm 1\%$  (or  $\pm 0.02~\mu\mathrm{m}$ )  $\Box$  3" to 6" Wafer Capability  $\Box$  Auto Wafer Loading  $\Box$  Fully Programmable Stage  $\Box$  Real Time Imaging



Model S-7000 Field Emission Wafer Inspection and CD Measurement SEM:  $\square$  150Å Guaranteed at 1 kV  $\square$  4" to 8" Wafer Capability  $\square$  Auto Wafer Loading  $\square$  5-axis Position Correction Computer-controlled Eucentric Stage  $\square$  Conical Lens 60° Tilt at 15 mm W.D.  $\square$  Real Time Imaging  $\square$  Measurement Precision  $\pm 1\%$  (or  $\pm 0.02~\mu m$ )

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Council shall decide the winner. The ballots may be examined by the Executive Council at the spring meeting.

A two-thirds vote of the entire membership of the Executive Council shall remove any officer or appointee derelict in their duties. The Executive Council shall accept resignations in good faith.

An interim vacancy in the presidency shall be filled by advancement of the President-Elect, who will go on to serve this anticipated terms as President and Immediate Past President. In the event there is no President-Elect to advance, the Executive Council shall elect one of its members as acting President to serve until the completion of the next regular election. An interim vacancy in the office of Program Chairman shall be filled by the Program Chairman-Elect, who will go on the serve his anticipated term as Program Chairman. If there is no Program Chairman-Elect to advance, the Executive Council shall appoint a Program Chairman to serve until the completion of the next regular election. Interim vacancies in the officers of Secretary or Treasurer shall be filled by appointment by the Executive Council until completion of the next regular election. Interim vacancies in the officers of Journal Editor, Student Representative, or Corporate Representative shall be filled by an appointment made by the Executive Council.

#### ARTICLE X - DISSOLUTION

Upon the dissolution of the Society, the Executive Council shall, after paying or making provision for the payment of all the liabilities of the Society, dispose of all the assets of the Society exclusively for the purposes of the Society in such manner, or to the Electron Microscopy Society of America. Any such assets, not so disposed, shall be disposed of by the Court of Common Pleas of the county in which the principal office of the Society is then located, exclusively for such purposes, or to such organizations, as said court shall determine, which are organized and operated exclusively for such purposes.

#### ARTICLE XI- INDEMNIFICATION BY THE SOCIETY

The Society shall indemnify each member of the Executive Council, director, officer, person who is serving or has served at its request as a director, officer, or employee of another corporation, against expenses, in connection with the defense of any pending or threatened action, suit, proceeding, criminal or civil, to which he is or may be made a party by reason of being or having been such a member of the Executive Council, director, officer, or employee, provided that a determination is made:

(A) That he was not and has not been adjudicated to have been negligent or guilty of misconduct in the performance of his duty to the Society of which he is or was a member of the Executive Council, director, officer or employee;

(B) That he acted in good faith in what he reasonably believed to be in the best interest of the Society; and

(C) That, in any matter the subject of criminal action, suit or proceeding, he had no reasonable cause to believe that his conduct was unlawful.

The determination as to the foregoing matters with respect to each action, suit or proceeding shall be made:

(i) By a majority of the Executive Council of the Society acting at a meeting at which a quorum consisting of officers who are not parties to or threatened with such action, such officers vote; or

(ii) By independent legal counsel in written opinion, if such quorum cannot be obtained to vote on such indemnification, or even if obtainable, the officers qualified to vote so direct.

The termination of any action, suit or proceeding upon a plea of nolo contendere or its legal equivalent, shall not, of itself, create a presumption that any member of the Executive Council, director, officer or employee did not act in good faith in what he reasonably believed to be the best interest of the Society or had reasonable cause to believe that his conduct was unlawful. Expenses incurred by any person in defending any action, suit or proceeding may be paid by the Society in advance of the final disposition of such action, suit or proceeding as authorized by the Executive Council in the specific case upon receipt of an undertaking by or on behalf of such person to repay such amount unless it shall ultimately be determined that he is entitled to be indemnified by the Society. The indemnification provided in this Article shall not be deemed exclusive of any rights to which those seeking indemnification may be entitled under any regulation, bylaw, agreement, insurance policy purchased by the Society, vote of the members or otherwise, or of any other indemnification which may be granted to any person who has ceased to be a member of the Executive Council, director, officer or employee of the Society, and shall insure to the benefit of the heirs, executors, successors and administrators of such a person.

#### ARTICLE XII - AMENDMENTS AND PERIODIC REVIEW

Amendments to these bylaws may be initiated by individual members of the Executive Council, or by petition to the Secretary, signed by ten regular members of the Society. Amendments must be approved by a two-thirds majority of the Executive Council. The Secretary shall then be promptly submitted by mail to the membership for approval, with statements of support and/or opposition by the Executive Council. The ballots shall be accepted by the Executive Council for one month after the date of mailing. The Executive Council shall count the ballots; the amendment(s) shall be ratified if it received a favorable two-thirds majority of the votes cast. Any member may, if he so desires, be present at the counting of the ballots.

These bylaws shall be reviewed for amendment at regular intervals, not to exceed three years, by a committee of voting members of the Executive Council appointed by the President. The date of the latest review and/or amendment shall be stated in the last paragraph.

These bylaws were last reviewed and/or amended by vote of the Executive Council on 12 October, 1989.



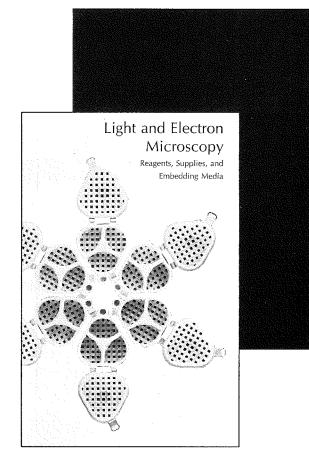
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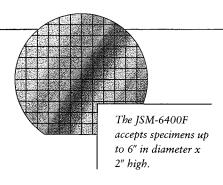
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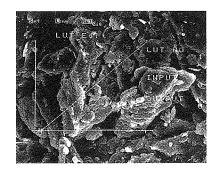
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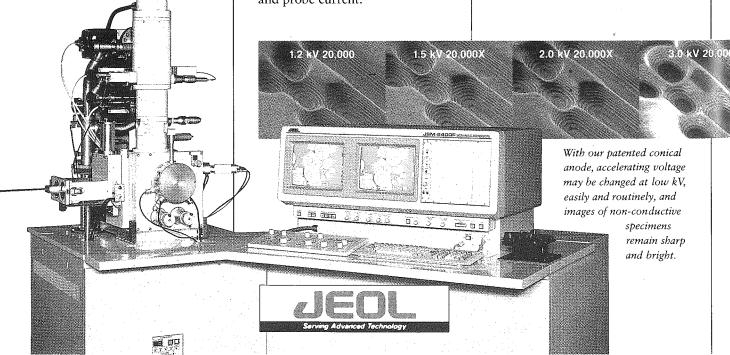
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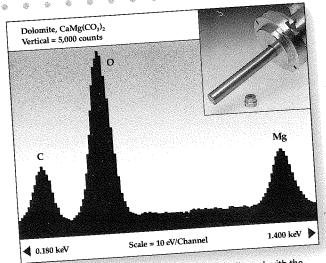
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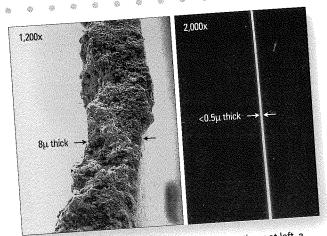
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#### **EDITORIAL POLICY**

#### LETTERS TO THE EDITOR

Letters to the editor are printed as they are received in the order of their arrival. These letters reflect the opinion of the individual TSEM member and do not necessarily reflect the opinions of the editor or the society. The content of the letters should be concerned with the philosophical or operational aspects of the TSEM, the Journal and its contents, academic or national policies as they apply to TSEM and/or its members and electron microscopy in general. Editorial privilege may be evoked to insure that the LETTERS SECTION will neither be used as a political forum nor violate the memberships' trust.

#### **ELECTRON MICROGRAPHS AND COVER PHOTOS**

Micrographs submitted for cover photos should be marked as such. The choice of photographs will be made by the editor. Photograph receipt and/or dispensation will not be acknowledged. Photographs will not be returned. Electron micrographs to be used for cover photos and text fillers are welcome and should be selected with some attention to aesthetic appeal as well as excellence both in technique and in scientific information content.

#### REGIONAL NEWS

News items should be submitted through the regional editor in your area and made to conform to the standard format used by the regional news section. Regional contributions should be sent to the Regional News Editor. Editorial privilege may be executed for the sake of brevity or to preserve the philisophical nature of the TSEM Journal.

The JOB OPPORTUNITIES section will be comprised of a "Jobs Available" and a "Jobs Wanted" sub-section.

Anonymity of individuals listing in the Jobs Wanted or Jobs Available sub-sections may be maintained by correspondence routed through the Regional News Editor's office.

#### TECHNICAL SECTION

The Technical Section will publish TECHNIQUES PAPERS, HELPFUL HINTS, and JOB OPPORTUNITIES. The TECHNICAL PAPERS will describe new or improved methods for existing techniques and give examples of the results obtained with methods. The format of the Technique Papers will be the same as that used for regular research reports. HELPFUL HINTS will be in the form of a brief report with an accompanying illustration, if required for clarity. Helpful Hints should embody techniques which will improve or expedite processes and/or procedures used in EM.

#### **PUBLICATION PRIVILEGES**

The right to publish in the TSEMJ is restricted to TSEM members or to those whose membership is pending. A membership application form can usually be found in each issue of the TSEMJ. Membership dues are as follows: student \$2.00; regular members \$10.00; Corporate members \$75.00. Individuals who belong to TSEM by virtue of a corporate membership are invited to participate in Journal submissions as are our regular or student members. However, papers of a commercial nature, either stated or implied, will not be accepted for publication as a Research Report or Techniques Paper. Such papers may be acceptable as advertising copy.

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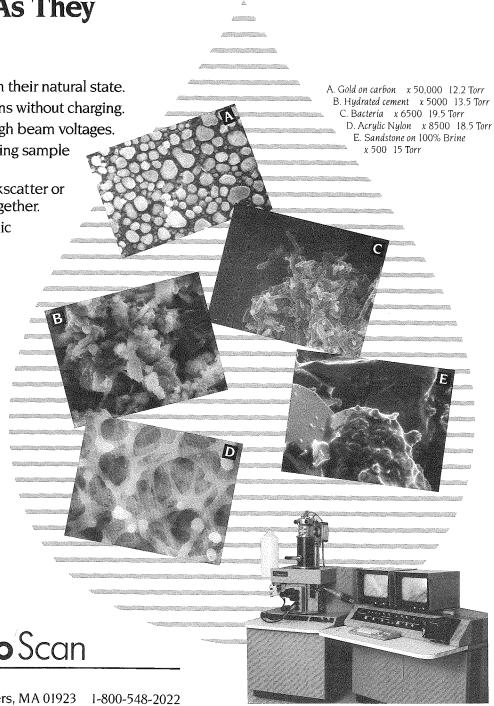
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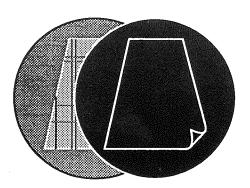
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## **Abstracts**

#### **BIOLOGICAL SCIENCES**

#### PLATFORM PRESENTATION — WINTER 1989

WHOLE CELLS VIEWED WITH ELECTRON MICROSCOPY AT 200KV AND 100KV. D.J. Ohlmer, C.L. Seidel, H.A. Steuckrath, and M.A. Goldstein, Dept. of Medicine, Baylor College of Medicine, Houston. TX 77030

Houston, TX 77030.

The whole cell mount technique has been used predominantly with high voltage electron microscopy. However, in cultured cells all but regions around the nucleus can be viewed successfully with intermediate and even conventional voltages. We have devised an easy and practical method for examining adjacent cultured cells with whole cell TEM, SEM, and LM in conjunction with immunocytochemistry. Nickel grids are sandwiched between a floating (2x4cm) formvar film and a sheet (1x2cm) of thermonox plastic. The floating sandwich is removed with a Parafilm strip, dried, and sterilized by UV. Cells are cultured on the formvar surface by standard proce-Some samples are then aldehyde fixed; others are permeabilized, then fixed. All samples are post fixed in OsO4, dehydrated in ETOH to 100% and critical point dried. Grids can be stabilized with a thin coat of carbon and viewed in the EM. Whole cell TEM processing can take as little as two hours. We have examined the cytoskeletal structure of vascular smooth muscle cells using a combination of immuno-fluorescent probes and immuno gold labeling. TEM and SEM of whole cells were compared to LM and to conventional thin sectioned, embedded, adjacent cells. The combined techniques provide the sensitivity of immunofluorescence and the resolution of E.M. localization on a single population.

A COMPARISON OF THE PARADERMAL AND TRANSVERSE VIEWS OF DEVELOPING PROSOPIS GLANDULOSA SEED COATS. Rebecca S. Westover and Louis H. Bragg. Department of Biology, University of Texas at Arlington. Arlington. Tx. 76019

University of Texas at Arlington, Arlington, Tx. 76019.

Prosopis glandulosa (mesquite) is a representative member of the woody legumes within the subfamily Mimosoideae. A pleurogram and a cracked "tile" surface with distinct microsurface patterns are characteristic of the Prosopis seed coat. These surface characters have been studied for their taxonomical uses, but little is known about the developmental sequence in which these characters are formed. Earlier observations revealed the stages of development of the surface features of the seed coat. Transverse sections of the mature seed reveal distinct epidermal and hypodermal layers composed of various cell types. The developmental sequence of these different cell types will be determined.

ECOLOGICAL ANATOMY OF NUPHAR LUTEUM LEAVES. PAULA S. WILLIAMSON, EDWARD L. SCHNEIDER, AND DAVID E. LEMKE. Department of Biology, Southwest Texas State University, San Marcos 17, 78666.

San Marcos, TX 78666.

Plants of Nuphar luteum (L.) Sibth. & Sm. growing in the San Marcos River, Hays Co., Texas, have been observed to produce emergent, floating, and submerged leaves on the same individual. While many aquatic macrophytes produce both floating and submerged leaves, it is somewhat unusual for an individual plant to also produce emergent leaves. Structural differences occur among these leaf types. Emergent and floating leaves are leathery in texture, entire margined, and possess a thick cuticle covering the upper surface, stomata scattered throughout the upper surface, palisade and spongy parenchyma, collenchyma associated with the veins, astrosclereids, and air canals. Submerged leaves are extremely thin, with a texture similar to lettuce leaves, lack stomata, and possess an undifferentiated mesophyll. Compared with emergent and floating leaves, submerged leaves exhibit less lignification of the tracheary elements and reduced supportive tissue.

ULTRASTRUCTURAL & IMMUNOFLUORESCENT FINDINGS IN SURAL NERVE BIOPSIES, S.C. Bauserman, M.D. S&W Clinic, TAMU College of Medicine, Temple, Tx.

One of the most common clinical indications for peripheral nerve biopsy is a suspicion that the patient may have inflammatory or immune-mediated peripheral neuropathy. These particular forms of the disease often respond to steroid or other immunosuppressive therapies. In recent years we have been performing immunofluorescent stains for immunoglobulins and complement (C3) in addition to the routine use of electron microscopy and teased-fiber preparations.

Our studies have revealed a high degree of correlation between steroid-responsive neuropathies and evidence of demyelinating neuropathy with prominent segmental demyelination (predominantly Schwann cell injury). In addition, we have seen selective deposition of IgM at the perineurial membrane to correlate with responsive- ness. We speculate that the blood-nerve barrier at the perineurium has allowed the deposition of this particular immunoglobulin as an additional manifestation of this disimmune disease process. We intend to continue the study and periodically assess this observation in a larger aggregate of case material. Some of the morphologic and graphic findings to date are demonstrated in this paper. Technical aspects are not unusually demanding, and the use of immuno-electron microscopy may be indicated in the future.

CHONDROCYTE DIFFERENTIATION IN AN <u>IN VITRO</u> SYSTEM: AN ULTRASTRUCTURAL COMPARISON. J. T. Ellard, M.A. Machado, W. A. Horton. Department of Pediatrics, Division of Medical Genetics, University of Texas Medical School, Houston, Texas, 77225.

The study and evaluation of various human chondrodysplasias has been enhanced by a new cell culture system. Both normal and affected chondrocytes are obtained from human costochondral cartilage and grown in monolayer to allow for cell dedifferentiation and amplification in number. Following resuspension and culturing on agarose, the chondrocytes redifferentiate forming aggregates grossly resembling cartilage tissue. The cell morphology and matrical components of the cultured "chondroids" appear strikingly similar to human chondrocytes and matrix observed in intact tissue. Cells are spherical containing centrally-located nuclei, numerous lipid droplets and transport vesicles, and abundant rough endoplasmic reticulum and golgi complexes. When stained with ruthenuium hexamine trichloride, the matrix reveals numerous proteoglycan granules in close association with collagen fibrils. Chondrocytes from two of the human chondrodystrophies, Hypochondrogenesis and Achondrogenesis IA were also cultured. Cellular and matrical similarities between the chondroids and original tissue were observed. This system provides an accurate model of human chondrocyte differentiation which will allow for further morphological and biochemical studies of the human chondrodysplasias.

CRYSTALS IN THE VEGETATIVE AND REPRODUCTIVE PARTS OF <u>OXALIS</u>
<u>DILLENII</u> JACQ. Louis H. Bragg and Khalil G. Ghanem. <u>Department</u>
of <u>Biology</u>, The University of Texas at Arlington, Arlington,

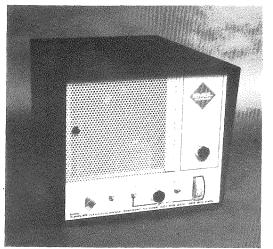
Both light and electron microscopy were used to determine the presence or absence of crystals in the leaves, stems, and roots as well as the flowers, fruit, and seeds of Oxalis dillenii. Crystals were abundant in the leaves and seed coats. Elemental analysis suggests the crystals in the leaves are different in their chemistry compared to the crystals in the seed coats.



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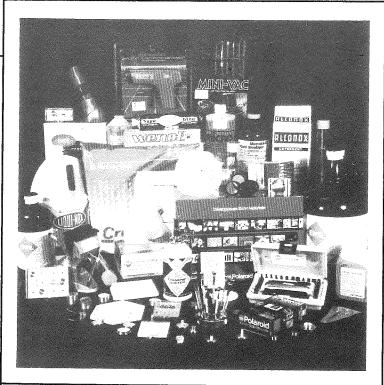
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TISSUE RESPONSE TO HARD TISSUE REPLACEMENT IN RATS.
A.D. Pearsall, R. Spears, K. Sarubbi, Department of Anatomy,
Baylor College of Dentistry, Dallas TX, 75246.

Hard Tissue Replacement (HTR) is a alloplastic bone
substitute that combines a polymethylmethacrylate core with a
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capsule. Unfortunately, such studies provide only isolated
views of the bone-polymer interface and little information in
regard to the fine structural features. Accordingly, the aim regard to the fine structural features. Accordingly, the aim of this study was to examine the <u>in vivo</u> interfacial reactions between bone and HTR using scanning electron microscopy (SEM).

Adult male Sprague-Dawley rats were anesthetized, a hole (1 x 2.0 mm) was drilled in the calvarium, the HTR inserted and the wound closed by sutures. Fifty-six days later, the animals were anesthetized, exsanguinated, and the tissues prepared for examination by SEM. Implantation in excavated bone tissue resulted in the ingrowth of calcified tissue both between and within the polymer. This was confirmed by x-ray energy dispersive analysis (XEDA) which indicated that calcium was concentrated in this region. Immediately adjacent to the HTR, however, the collagenous matrix formed an extensive sheet (ca. 2 µm thick) which tended to parallel the contour of the polymer. The appearance of the fibers was more as a continuum rather than as separate bundles but the latter was also observed. Interestingly, XEDA suggested that this region showed no sign of mineralization. These results suggest that the bone-HTR interface is more complex than originally surmised. Additional studies will be necessary to elucidate the attachment mechanism of this synthetic bone polymer. (Supported by NIH Traineeship DE07188)

ULTRASTRUCTURAL EXAMINATION OF CILIA: A COMPARISON OF TWO TECHNIQUES FOR SPECIMEN COLLECTION. L.D. GRAY, D. SUEZ\* and D.E. BRADY, Dept. Cell Biology and Environmental Sciences, \*Dept. Allergy and Immunology, The University of Texas Health Center at Tyler, P.O. Box 2003, Tyler, TX, 75710.

Primary ciliary dyskinesia (The Immotile Cilia Syndrome) is a group of genetic defects afflicting the ciliary and sperm flagellar axonemes. Pediatric patients in particular who present with chronic respiratory infections of both upper and lower airways with no apparent underlying cause (such as immune deficiency) may be suspected of having this syndrome. Ideally, the respiratory cilia of these patients should be examined by electron microscopy in order to confirm or rule out this diagnosis. Traditionally, a nasal or bronchial biopsy is required to obtain the necessary specimens and typical forceps biopsies include some discomfort and risk to the patient. Additionally, mechanical damage and denudation of cilia by chronic mechanical damage and denduation of citia by chronic infections can render biopsies of small areas unusable. have recently tried a technique utilizing a disposable cytology brush to lightly brush the nasal epithelium in order to collect ciliated cells. The procedure is rapid, painless to the patient, less expensive, and samples a large area. No anesthetic is necessary and there is virtually no risk involved. This technique has, on a small sample size, yielded far more cilia with better quality than the forceps biopsies we have examined in the past Although other similar procedures have been published in the literature, we have used methods described in personal communication with Dr. Bjorn Afzelius of the University of Stockholm, Sweden. Examples from both biopsies and brushings will be shown.

A COMPARISON OF LEAF TISSUES FROM JUNIPERUS VIRGINIANA L. AND J.  $\frac{ASHEI}{Biology}$ , The University of Texas at Arlington, Arlington,

TX, 76019.

Populations of Juniperus virginiana and J. ashei were examined by both light and electron microscopical techniques.
J. virginiana (eastern red cedar) is the more widespread of the two species and has a more eastward distribution. J. ashei (mountain cedar) inhabits the drier regions to the west. The two species overlap in their ranges throughout central Texas. Epidermal and subepidermal tissues were examined for possible structural differences associated with their distribution patterns.

THE STRUCTURE OF HEMOLYMPH AND INTRAERYTHROCYTIC STAGES OF BABESIA BOVIS. R.E. Droleskey<sup>1</sup>, P.J. Holman<sup>2</sup>, D. Cruz<sup>2</sup>, J.R. DeLoach<sup>1</sup>, & G.G. Wagner<sup>2</sup>. <sup>1</sup>Veterinary Toxicology & Entomology Research Laboratory, USDA-ARS, Rt. 5 Box 810, College Station, Texas, 77840 and <sup>2</sup>Dept. of Veterinary Microbiology and Parasitology, Texas A&M University, College Station, Texas, 77843.

Boophilus microplus ticks were fed to repletion on Babesia bovis parasitized cattle. engorged females were maintained at 28°C, 90% relative humidity for oviposition. Three days after repletion hemolymph was obtained by removing part of one leg and hemolymph drops collected into PBS. The hemolymph was fixed in a glutaraldehyde fixative and prepared for both transmission (TEM) and scanning electron microscopic (SEM) Additionally, erythrocytes from B. bovis examination. infected cattle and from in vitro cultures were fixed for TEM and SEM examination. Vermicules in the hemolymph were pear-shaped, 10 - 15µm long, with a depression near their anterior polar end. Merozoites, vacuoles, and some cellular debris were observed within both <u>in vivo</u> and <u>in vitro</u> <u>B</u>. <u>bovis</u> infected erythrocytes. <u>In vivo</u> and <u>in vitro</u> infected erythrocytes had projections on their erythrocyte membranes which did not appear to differ between the samples although the shape of in vivo infected erythrocytes differed from their in vitro counterparts.

AN SEM AND X-RAY MICROANALYSIS STUDY OF SPORANGIAL COMPONENTS IN TWO SPECIES OF PHYSARUM. H. J. ARNOTT AND H. W. KELLER. Department of Biology, University of Texas at Arlington, Arlington, TX 76019-0498.

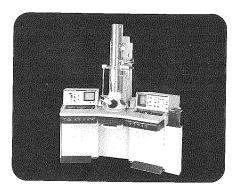
Five major components that make up the brightly colored sporangia of Physarum pulcherimum and P. roseum have been examined by SEM and energy dispersive x-ray analysis. The stalk, peridial granules, peridium, capillitial nodes and spores were examined from several specimens from each of several collections of both species. SEM observations several collections of both species. SEM observations revealed that the peridial granules were smooth in <u>P. roseum</u> but markedly roughened in <u>P. pulcherrimum</u>; small flattened "spines" were found on the latter species. The sporangial stalk in <u>P. roseum</u> is longitudinally wrinkled, but the large number of granules found in the stalk of <u>P. pulcherrimum</u> is absent. Capillitial nodes are common in the latter species but relatively rare in the sporangium of <u>P. roseum</u>. The distribution of several mineral elements is different in the five sporangial components we studied, and there are five sporangial components we studied, and there are differences between the two species. For example, barium and manganese were found in the peridium of <u>P. roseum</u> but not in the peridium of <u>P. pulcherrimum</u>. Calcium, manganese and sulfur were found in the peridial granules of <u>P. roseum</u> but not in the other species.

ULTRASTRUCTURAL ASPECTS OF <u>CLADOSPORIUM</u> <u>CARYIGENUM</u> INFECTION OF PECAN LEAVES. A. E. Rushing and A. J. Latham, Department of Biology, Baylor University, Waco, TX 76798 and Alabama Agricultural Experiment Station, Auburn University, AL 36849. Infection of pecan leaves by the fungus

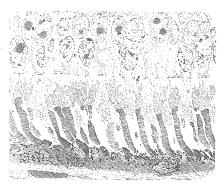
Cladosporium caryigenum is by direct penetration of the host cuticle. Hyphae then extend subcuticularly over both the upper and lower leaf epidermis; they were not observed growing directly on the leaf surface. Hyphae reach the interior of the leaf by extending intrusively through the middle lamella region of adjacent epidermal cells. All hyphal growth is intercellular. Conidiophores are produced only from subcuticular hyphal cell. Production of conidiophores is preceded by the melanization of the walls of those fungal cells that will subsequently be involved in conidiophore formation. Conidiophore extension is accomplished by the rupture of the plant cuticle and the outward expansion of the fungal cell. There is an accumulation of vesicles near the point of rupture suggesting enzymatic involvement, possibly in conjunction with mechanical force. A collar, formed by the ruptured cuticle, surrounds the base of the conidiophore. Clusters of concentric bodies are found in conidiogenous cells but not in vegetative hyphae.



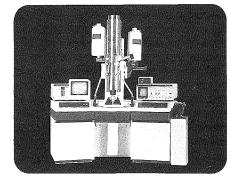
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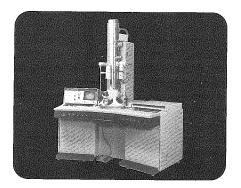
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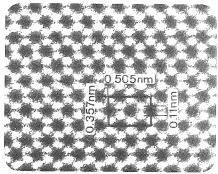


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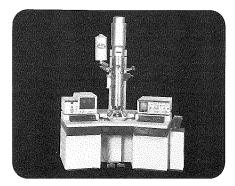
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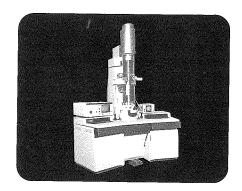
Specimen: Diamond (110) taken with the H-9000



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A QUANTITATIVE STUDY OF CALCIUM OXALATE CRYSTALS ON THE HYPHAE OF LITTER FUNGI. V.L.BLACKMON and H.J. ARNOTT, Department of Biology, University of Texas at Arlington, Arlington, TX 76019-0498.

This work is part of an overall attempt to understand the relationship between the structure of calcium oxalate and its effect on the structure and function of fungi. In this paper we attempt to provide a quantitative picture of the distribution of calcium oxalate crystals on the hyphae of certain litter fungi. Using litter samples collected under pine and juniper trees from several states, we have examined the fungi using both LM and SEM. Using image processing, we have developed quantitative methods to measure the longitudinal distribution of calcium oxalate along hyphae; this variation includes both the morphology of the crystals and the actual volume of crystalline material associated with component hyphae. Previous studies merely have reported the calcium oxalate without providing any indication of the amount associated with various types of hyphal morphology; for example, see Graustein et al., Science (1977) 198:1252-1254 and Arnott and Fryar, Scanning Electron Microsc. (1984) IV:1745-1750. As reported in other cases, the calcium oxalate crystals seem to be entirely weddellite, calcium oxalate dihydrate.

THE PRESERVATION OF PLANT SAMPLES USING MICROWAVE STIMULATED HEATING IN THE PRESENCE OF GLUTARALDEHYDE. D.J. Ohlmer, Dept. Biology, Texas Southern University, Houston, Texas 77004.

It has been found that the heating of plant samples by microwave irridation in the presence of glutaraldehyde promotes fixation. By nature, plant tissue is very difficult to preserve. The microwave heating method overcomes the difficulties presented by dense cell walls, low protein content, high pH gradients, hydrolytic enzyme rich vacuoles and waxy coatings. It is well-documented that heating minimizes cellular autolysis. Conventional heating methods result in significant temperature gradients with little control of final sample temperature. With microwave heating, the temperature is evenly distributed and easier to control. This method allows fixation of larger samples while still promoting quality preservation. The microwave technique uses a conventional microwave oven ordinarily found in most homes. Domestic microwave ovens usually have an energy frequency of 2.45 GHz and are in the 400 to 800 watt range making them ideal for the rapid heating of samples. Average heating time is between 10 and 30 sec. when using 15mls. of fixative in Acknowledgment: Dr. G. Howze, Biology Dept., T.S.U.

ULTRASTRUCTURAL LOCALIZATION OF ANTIBODIES TO PECTIN AND ACYL-MANNOSIDASE IN SOYBEAN CELLS AND PROTOPLASTS.
R.D. Record, L.R. Griffing, G.P. Kaushal, and A.D. Elbein.
Texas A&M University, College Station and University of
Texas Health Science Center, San Antonio

Membrane-mediated trafficking of pectin and arylmannosidase in soybean (<u>Glycine max</u> (L.) Merr.) suspension cultures was shown using immunocytochemistry. JIM 5, a monoclonal antibody to polygalacturonic acid, a major component of pectin molecules, was previously shown to label the primary cell wall of recently divided cells and the middle lamella of older, non-dividing cells in pea nodules (VandenBosch et al., 1989 EMBO J 8, 335-342). This anti-polygalacturonide was used in this study to demonstrate the intracellular localization of pectins in soybean suspension culture cells and protoplasts. Pectin localization gives insite into the route taken by secreted polysaccharides. In contrast, aryl-mannosidase may be used to demonstrate the route taken by vacuole-targeted molecules. Homogenization and fractionation revealed a peak of aryl-mannosidase activity in the vacuole fraction. Immunocytochemistry was done using both monoclonal and polyclonal antibodies to aryl-mannoside from mungbean.

INTRACELLULAR LIPID PRESERVATION BY MALACHITE GREEN-GLUTARALDEHYDE FIXATION OF INTERVERTEBRAL DISKS FROM PROGRESSIVE ANKYLOSIS MICE. H.W. SAMPSON, Dept. Anatomy, College of Medicine, Texas A&M University, College Station, TX 77843.

Intervertebral disk chondrocytes from progressive ankylosis mice prepared by conventional techniques for electron microscopy contain large empty intracellular vacuoles. In order to determine the nature of these vacuoles, tissue from normal and ankylosing mice were fixed for 18 hours in 3% glutaraldehyde buffered with 0.067 M sodium cacodylate either with or without 0.1% Malachite Green. The fixed tissue was washed briefly in buffer and postfixed for 8 hours in 2% osmium tetroxide. This fixative has been demonstrated to preserve phospholipids, cerebrasides, cholesterol, fatty acids, fatty aldehydes and lipoproteins, possibly by forming a bond between the lipid and osmium.

Intervertebral disks, prepared in this manner revealed lipid droplets in most chondrocytes from all animals, but ankylosing animals contained massive accumulations of lipid.

Biol. Reprod. 10:565-577 (1974) J. Chromatog. 88:425-427 (1974) J. Chromatog. 105:195-196 (1975) Stain Tech. 53:29-35 (1978) J. Micro. 154:83-92 (1989)

LM AND TEM ANALYSIS OF BUFO VALLICEPS INTEGUMENTARY GLANDS. S. Dominey, M. Johnson, J. Turner, and T. Hoage. Biology Program, Sam Houston State University, Huntsville, TX 77341.

Current literature does not clearly identify the integumentary gland distribution nor the secretory states of the various sized glands within toad integument. Comparative data consists of Bufo americanus integument studies completed in 1909. The current study evaluates distribution and morphology of Bufo valliceps abdominal and pelvic patch glands. Young (Î to 12 months) and old (12 and up months) toads were collected and maintained in an earth filled terrarium under standard conditions. Tissues were collected from pithed animals, fixed in 3% gluteraldehyde (in iso-osmotic phosphate buffer, pH-7.3) and prepared for both light and transmission electron microscopy by standard methods. Tissue sections were photographed and glandular characteristics evaluated by morphometric analysis. Glands ranged in size from 0.35 to 3.02 mm (Mean = 1.21±0.29 mm) with an average distribution of 3.8±1.1 glands/0.0026 cm². Secretion products consist of large mucous granules in small glands to mucous bound protein fractions in large glands. Ultrastructural changes associated with secretory activity were observed.

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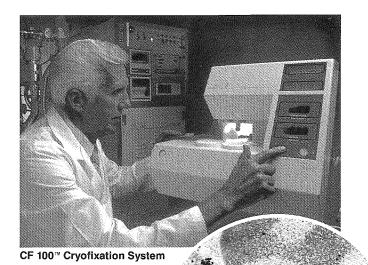
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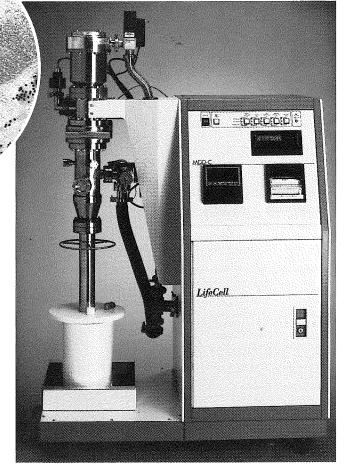
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Philips Electronic Instruments Company A Division of North American Philips Corporation 85 McKee Drive Mahwah, NJ 07430 Tel. (201) 529-3800 ULTRASTRUCTURAL, 1H NMR SPECTROSCOPIC AND BIOCHEMICAL CORRELATES OF TRIGLYCERIDE ACCUMULATION IN RAT STRIATED MUSCLES. W.B. VANWINKLE<sup>1</sup> and P.E. WOLKOWICZ<sup>2</sup>, <sup>1</sup>Cryobiology Research Center, UTHSC-Houston TX 77381 and <sup>2</sup>Division of Cardiovascular Disease, Univ. of Alabama at Birmingham, AL 35294.

Electron microscopy of cardiac cells bordering a central zone in myocardial infarcts often reveals increased numbers of lipid (triglyceride) droplets possibly due to alterations in lipid metabolism. Nuclear magnetic resonance (NMR) spectroscopy of ischemic tissue has shown a concomitant increase in a signal possibly attributable to increased triglyceride. As part of a study to clarify the origin of the NMR spectra and to assess the diagnostic potential of detection of areas of ischemia by NMR, we fed rats a diet enriched in rapeseed oil, the main fatty acyl component being erucic acid (22:1) which, while metabolized by liver is not readily oxidized in heart. After two days, hearts were removed and perfused ( $\pm$  acetate substrate) in, and  $1\mathrm{H}$ spectra acquired from, a Bruker 360 Hz spectrometer. Control, rapeseed oil-fed and substrate free perfused hearts were examined by EM followed by morphometric analysis for determination of of organellar % volume. Increases in specific lipid NMR spectra correlated well with a 10-fold increase in intracellular lipid droplet volume (as well as droplet diameter increase). These findings agreed with data derived from triglyceride biochemical assay and chromatographic determinations. Substrate-free perfusion resulted in a return to control levels judged by these techniques. In rapesee oil-fed rats, EM examination of diaphragm revealed that slow twitch, oxidative (SO), but not fast twitch, oxidative-glycolytic (FOG), fibers had increased lipid droplets. In contrast, SO fibers in leg muscles did not show an increase in lipid droplet volume. These findings not only show the efficacy of NMR detection of ischemic zones but suggest that muscle activity and metabolism may regulate lipid utilization.

CRYOFIXTION OF <u>VICIA FABA</u> ROOT HAIRS. D. J. SHERRIER AND K.A. VANDENBOSCH, Dept. of Biology, Texas A&M University, College Station TX 77843

Root hairs are the root cell type suceptible to initial infection by Rhizobium. A successful infection results in the familiar nitrogen-fixation symbiosis between legumes and bacteria. Tip-directed secretion of glycoproteins and cell wall constituents in root hairs may provide substrates necessary for а successful infection. Unfortunately, exocytic vesicles and cytoskeletal elements are not well preserved by conventional fixation for TEM examination. Freeze fixation and freeze substitution provide a means of preserving these transient events and structures. We plunge-froze sections of three day old roots in liquid propane at -176C. The fixed roots were then transferred to acetone with 1% OsO<sub>4</sub> at -90C where the tissue substituted for three days. The resulting tissue was characterized by well defined vesicular and membranes, smooth membrane, and some cytoskeletal preservation.

SOCIAL ENVIRONMENT AND ATHEROSCLEROSIS IN LABORATORY RATS. Michelle Martin, J.L. Humphreys, and F.R. Wilson II, Department of Biology, Baylor University, Waco, TX 76798.

Socialization has been implicated as a factor slowing the rate of development of atherosclerotic damage in rabbits and monkeys. To test this idea in laboratory rats, 12 rats were housed in individual cages and fed a two percent cholesterol diet for 39 days. The animals were divided into a control group that received normal laboratory maintenance and an experimental group that received additional social stimulation from the experimenters for approximately 10 minutes twice each day during the 39 day period. At the end of the experimental period, aortas were removed and examined for evidence of atherosclerotic damage using scanning electron microscopy. Aortas from animals in the experimental group showed less evidence of intimal damage from atherosclerosis than those from the control group. In the control group 54.2% of intercostal arteries were occluded with atherosclerotic plaques at their junctions with the aorta as opposed to only 25.5% in the experimental group. These results suggest that even minimal amounts of socialization (with the experimenters) may have a significant effect on the rate of development of atherosclerosis in rats.

AN ULTRASTRUCTURAL AND MORPHOLOGICAL COMPARISON OF IN VITRO PROPAGATED CORD BLOOD STEM CELLS USING THREE DIFFERENT COLONY STIMULATING FACTORS. G.Krannig, B.Frenck, S.Vadhan-Raj, S.Buescher. Cryobiol. Res. Cntr., Dept. of Pediatr., Dept. of Clin. Imm., & Biol. Res., M.D.Anderson Cancer Cntr., U.T.H.S.C., Houston, Tx.

Myelopoietic stem cell division and differentiation are dependent on a continuous supply of highly specific growth factors. Using two different, in vitro, growth conditions, myelopoietic progenitor cells purified from human umbilical cord blood were stimulated to proliferate into two different cell populations. Cells continuously exposed to IL-3 plus GM-CSF yielded eosinophilic granulocytes. By 2 Wks., the majority of cells displayed dilated endoplasmic reticulum (ER) and large interconnected vesicles containing flocculent material. At 3 wks., the vesicles were heterogenous and contained aggregates of dense particulate material which contained antigenic peroxidase. By 4 wks., nuclear segmentation and large densely cored granules were visible which stained positively with soybean agglutinin (SBA), a lectin marker for eosinophils. Cells which were briefly exposed to IL-3 for 2 hrs., washed and grown continuously in G-CSF alone, differentiated into neutrophilic granulocytes. At 2 wks., some of these cells contained rough ER and elongated dense granules. Others showed large segregated vesicles containing flocculent material with occasional small condensations. Heterogenous granules and cleft nuclei were seen by 3 wks. By 4 wks., nuclear segmentation and granule heterogeneity were obvious. Occasional SBA (+) granules were seen and antigenic peroxidase was demonstrated at 2,3, and 4 wks., using immuno-labeling with colloidal gold. Based upon previous descriptions of other authors, the ultrastructural characteristics of these cells grown in vitro are similar to characteristics of cells grown in vivo.

ULITRASTRUCTURAL PRESERVATION OF SOY BEAN PROTOPLASTS BY FREEZE SUBSTITUTION AND MOLECULAR DISTILLATION DRYING. L.R. GRIFFING AND G.R. ALIAGA, Dept. Biology, Texas A&M University, College Station TX 77843.

Plant cells are difficult to rapidly freeze without artifacts due to the high level of hydration within vacuoles with standard cryofixation methods. In order to maintain antiquenicity and cellular location of water soluble molecules the Life Cell Process, which differs from conventional freeze substitution through the use of molecular distillation of water, osmium or aldehyde vapor fixation, and direct impregnation with resin was used in this study to examine the ultrastructure of soybean suspension culture cells and protoplasts. The cells or protoplasts are quick frozen with a metal mirror cryofixation system, transferred to the Molecular Distillation Dryer, followed by vapor phase fixation. show for the first time that the ultrastructure of protoplasts as well as of suspension culture cells can be effectively maintained through this process. Through a combination of fixation parameters we show that: 1) protoplasts can be well fixed in that portion which actually comes into contact with the metal mirror freezing surface, while the same cell will show freezing damage further away; 2) a longer resin infiltration time was required for plant cells than typically required for animal cells; 3) labile plasma membrane receptors are preserved. The Life Cell Process allowed the identification of membrane receptors, and has great promise for the identification of water soluble or membrane-soluble plant growth regulators or alkaloids through immunocytochemistry.

A COMPARISON OF <u>EN BLOC</u> STAINING METHODS USING PANCREATIC ACINAR CELLS. J.L. Humphreys and A.E. Rushing, Department of Biology, Baylor University, Waco, TX 76798.

To evaluate the quality of stains used en bloc, samples of pancreatic tissue from a normal laboratory rat, fixed by standard glutaraldehyde and osmium tetroxide methods, were stained en bloc using seven staining combinations. Sections from each sample were examined for contrast and preservation quality without post-staining. Each sample was photographed and compared to a sample that was not stained en bloc but was post-stained with uranyl acetate and lead citrate. Both phosphotungstic acid and tannic acid yielded good contrast when used alone, but when combined with uranyl acetate yielded contrast similar to that obtained with standard post-staining methods. These results suggest that some en bloc staining methods, even without further post-staining, may yield results as good as those obtained with standard post-staining techniques.



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#### PLATFORM PRESENTATION — WINTER 1989

TEM STUDIES OF YBa $_2$ Cu $_3$ O $_{7-8}$  PREPARED FROM LOW-TEMPERATURE PRECURSORS. D. C. DUFNER, R. A. MOHAN RAM\*, and A. CLEARFIELD\*, Electron Microscopy Center, Department of Chemistry, Texas Electron Microscopy Center, \*Department of A&M University, College Station, TX 77843.

Solid state reactions are the most commonly employed processes for the preparation of high-T<sub>c</sub> superconducting ceramic oxides. However, there has been considerable effort expended to prepare the YBa<sub>2</sub>Cu<sub>3</sub>O<sub>7-S</sub> (1-2-3) superconductor by low-temperature methods in order to obtain a more homogeneous oxide mixture resulting in products with superior superconducting properties. In this work, we illustrate how TEM can be used to characterize the microstructure of these materials as a

Our laboratory has been preparing low-temperature precursors by sol-gel and other methods. These methods were employed in the preparation of the 1-2-3 superconductors. The precursors were heated at various temperatures in the 750-930 C range for 12-60 hours in flowing O<sub>2</sub>. The resulting powders were found to be superconducting at 93.2K. TEM characterization was carried out on the Philips EM400T operating at 120kV.

TEM was employed to study the difference in the micro-

structure of ceramic oxides and those prepared by our method. Unit cell dimensions of  $\underline{a}=0.3821$ nm,  $\underline{b}=0.3889$ nm, and  $\underline{c}=1.168$ nm were confirmed by x-ray and electron diffraction. Domain structures, ranging from 15-50nm in width, were observed in all samples. However, observations of these twin domains were less frequent in crystals heated for shorter periods of time. Thus, nearly "domain-free" particles of 1-2-3 superconductors could be obtained by heating the precipitates at higher temperatures for short periods of time.

#### TEM-Scale Shock-Wave Damage in Silicates And The Cretaceous-Tertiary Boundary Event

Alan R. Huffman, Neville L. Carter, and Andreas K. Kronenberg, Center For Tectonophysics, Texas A&M University, College Station, TX. 77843-3113

The discovery of iridium enrichment and shocked quartz grains at the Cretaceous-Tertiary Boundary (KTB) have been used as primary evidence that a large meteorite struck the earth 66 million years ago. Since Alvarez et al (1980) proposed the bolide theory, a significant body of literature has been written on this mass extinction event. Recently, Officer et al (1987) proposed that volcanism could be equally viable as a cause of the KTB extinctions. The observation of shock damage in quartz and feldspar from various explosive volcanic centers has led to the proposal that not all shocked silicates are impact-generated.

Detailed optical and transmission electron microscopy of naturallyand artificially-shocked quartz and feldspar reveals a wide range of deformation microstructures indicative of brittle and plastic deformation and shock-induced phase transformation in silicates under shock loading. The sharp contrast between optical microstructures and TEM-scale submicrostructures indicates that the optical scale deformation under shock loading. The TEM-scale structures also provide information on how the optical-scale microstructures develop by coalescence of TEM-scale features. Natural shock microstructures from the KTB show differences in TEM features due to 66 million years of post-shock alteration.

Alvarez et al, 1980, Science, 208: 1095-1108 Officer et al, 1987, Nature, 326: 143-149

#### Grain-Boundary Dynamics During Annealing of Dunite

Alan R. Huffman, Center For Tectonophysics, Texas A&M University, College Station, TX. 77843-3113.

Grain-boundary dynamics at the SEM scale are described for Balsam Gap Dunite annealed in a sealed vacuum for 10 hours at temperatures to 900°C. The grain-boundary assemblage chlorite-lizardite-taletremolite breaks down to form olivine and clinopyroxene. Grain-boundary disruption and intergranular fracturing accompany sample dehydration. EDAX analysis suggests that significant ion mobility occurs during dewatering.

The presence of fine-grained material at grain boundaries is expected to have an appreciable effect on the mechanical response of specimens deformed in laboratory experiments. Diffusion-controlled grain-boundary processes may dominate to strains required for direct olivine intergranular contacts; internal dislocation creep should control the strain rate thereafter. In addition, the clinopyroxene formed during treatment, if in sufficient quantities, may also affect the mechanical response of the material due to homologous-temperature effects. It is concluded that the role of pre-treatment, and grain-boundary phenomena must be evaluated natural deformations

#### MICROSTRUCTURAL FEATURES OF A SUPERPLASTICALLY DEFORMED AL-LI ALLOY (2090 0E-16)

Rajasekaran Balasubramanian, Malur N. Srinivasan, and Ramon E. Goforth Department of Mechanical Engineering Texas A&M University College Station, TX 77843-3123

An investigation was carried out to study the superplastic behavior of 2090 (OE-16) Al-Li alloy at different combinations of temperature, strain rate and specimen thickness. The microstructure of each deformed specimen was examined in detail using optical and transmission microscopy. The results indicate that both static and dynamic grain growth occur in this alloy. Examination of transmission electron micrographs indicate that grain growth and dynamic recrystallization act competitively during the superplastic deformation of this alloy.

LOCATION OF DESIGN MARGINALITIES ON THE MC68010

LOCATION OF DESIGN MARGINALITIES ON THE MC68010 MICROPROCESSOR USING ELECTRON BEAM MICROPROBING.

J.A. LANGE, W. VINEYARD, and J. HAMILTON, Motorola Inc., Austin, Texas 78735-8598.

The USLI microprocessor design life cycle is scheduled to include a shrinking of the design geometries and chip size. This accomplishes two things, one being an economy of scale brought about by allowing more chips to be manufactured on a given size wafer. The other is an increase in on-chip clock speed and processor throughput, the to the lowered cumulative resistance of signal paths due to the lowered cumulative resistance of signal paths and, to a lesser extent, the path length of the signals. Computer simulations allow for most of the necessary circuit parameter adjustments to be made before first silicon is processed. However, due to the millions of factors inherent in a multi-hundred thousand transistor design, some probe time should be expected to fully optimize a design for speed. The complexity of these modern designs are forcing the use of Electron Beam Microprobing (EBM), and will continue even more so with the increasing use of more vertical integration manufacturing methods. We describe a case history of design optimization using  $\underline{\mathsf{EBM}}$  as a means of introducing a discussion of stimulus-EBM system considerations.

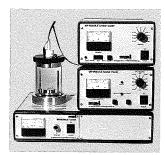




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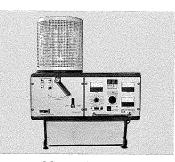
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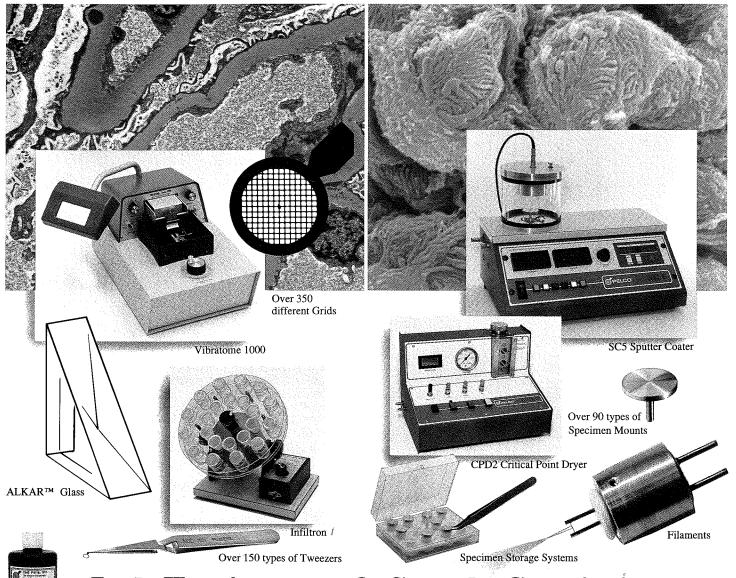


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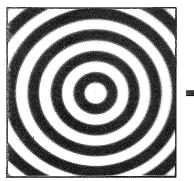
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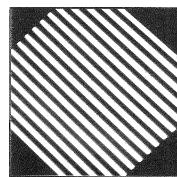
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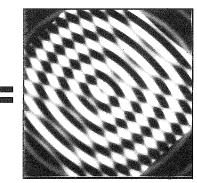
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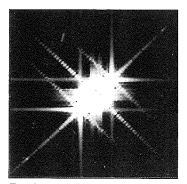


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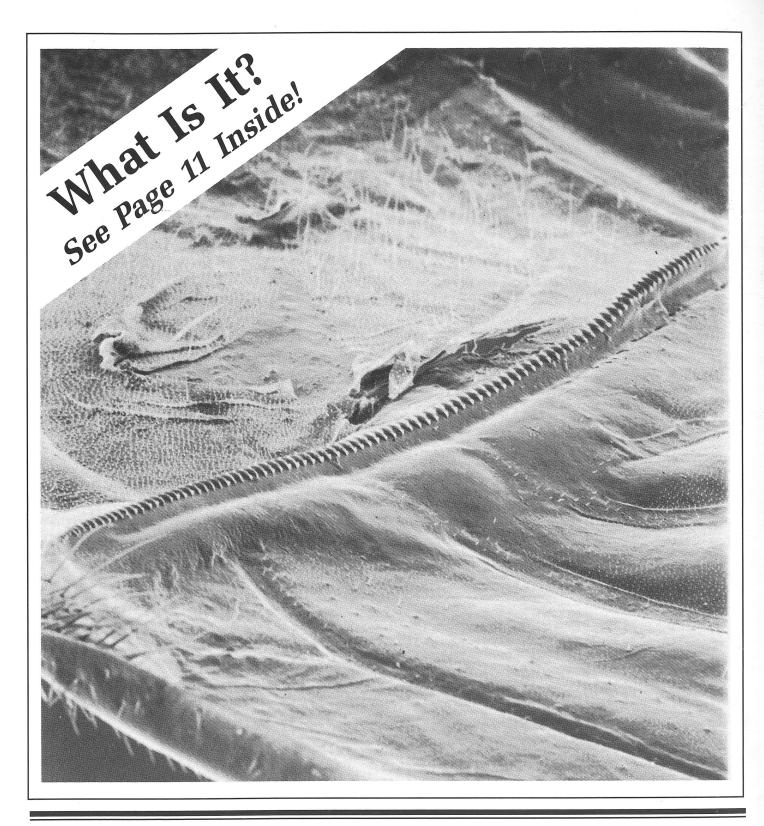
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