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May 20-22, 2015

Annual Meeting
of the
Southeastern Microscopy Society

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Please Bring These Proceedings to the Meeting!

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EXECUTIVE COUNCIL

President

Russell H. Goddard
Department of Biology
Valdosta State University
Valdosta, GA 31698
229-249-2642
rgoddard@valdosta.edu

Secretary

Cynthia Goldsmith
1600 Clifton Rd.
CDC Mailstop G32
Atlanta, GA 30333
404.639.3306
cgoldsmith@cdc.gov

Member-at-Large

Terri Bruce
132 Long Hall
Clemson University
Clemson SC 29634
terri@clemson.edu

Past-President

W. Gray (Jay) Jerome III
U-2206 MCN
Vanderbilt University Medical Center
1161 21st Avenue, South
Nashville, TN 37232-2561
615-322-5530
Jay.jerome@vanderbilt.edu

President-Elect

Mary Ard
Electron Microscopy Lab
Department of Pathology
College of Veterinary Medicine
University of Georgia
Athens, GA 30602
706-542-5537
maryard@uga.edu

Treasurer

Karen Kelley
University of Florida
ICBR Electron Microscopy BioImaging
Lab.
P.O. Box 110700
Gainesville, FL 32611
352.392.1184
klk@biotech.ufl.edu

Member-at-Large

Amanda Lawrence
PO Box 9775
Institute for Imaging & Analytical
Technologies
Mississippi State University
Mississippi State, MS 39762
662-325-7998
alawrence@i2at.msstate.edu

APPOINTED OFFICERS

Historian

W. Gray Jerome, III
U-2206 MCN
Vanderbilt University Medical Center
1161 21st Avenue, South
Nashville, TN 37232-2561
615-322-5530
Jay.jerome@vanderbilt.edu

Endowment

Charles D. Humphrey
336-461-3602
Specs1a@hotmail.com

Proceedings Editor

E. Ann Ellis
PO Box 6124
97 White Blossom Trail
Thomasville, GA 31758
eann.ellisem@gmail.com

Photographer

Dayton Cash
Electron Microscope Facility
Clemson University
91 Technology Drive
Anderson, SC 29625
864.656.2465
ecash@clemson.edu

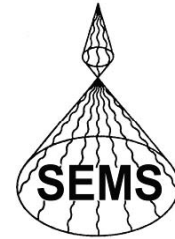
Corporate Co-Liaison

Rich Fiore
Tescan USA, Southeastern Sales
Cranberry TWP, PA 16066
919-928-6777
rich@tescan-usa.com

Web Site Contact

Karen Kelley
University of Florida
ICBR Electron Microscopy BioImaging
Lab.
P.O. Box 110700
Gainesville, FL 32611
352.392.1184
klk@biotech.ufl.edu

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Acknowledgements

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Our **Corporate Members and Exhibitors** are an important part of our organization and make it possible for SEMS to have outstanding meetings and to publish the SEMS Proceedings. We thank them for their excellent service over the years and look forward to a bright and productive future.

Corporate/Institution Members and Exhibitors for the meeting as of this printing:

| | | | |
|--|---|--|---|
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Southeastern Microscopy Society

Dear Fellow SEMS Members,

It has been an honor to serve SEMS over the past year as president and I am pleased to welcome you to the 51st meeting of the Southeastern Microscopy Society! Although last year's 50th anniversary was very special, this year's meeting holds promise of providing the membership with exceptional opportunities to explore new research and technology through our meeting activities, and to reacquaint ourselves with old friends or foster new friendships. I look forward to visiting with you at this year's meeting. Robert Simmons and Cynthia Goldsmith on our Local Arrangements Committee, together with John Shields and Heather Evans-Anderson (Program Chairs), have put together an outstanding meeting program for us. The meeting has commercial exhibits well-represented by all of our corporate members, workshops, and technical talks. As always, I want to express my sincere thanks to the corporate vendors who support our society and make our meetings more vibrant with their exhibits. Workshops start on Wednesday morning this year with Leica and JEOL presenting a cryo-TEM preparation and imaging workshop at the Emory Imaging Center and Henry Oliver of Leica demonstrating cryo-sample preparation at the Emory location also. There will be a tour and workshop on ionic liquids by Georgia Tech and Hitachi at the Georgia Tech NanoFacility and finally a Forensics Workshop is offered by Tescan USA and MVA Scientific Consultants at the MVA facility. Our Corporate Mixer is scheduled for Wednesday evening again held among our corporate exhibits. SEMS meetings depend on our corporate sponsors and this is a great time for everyone to visit and catch up on the latest technology offered by them. Be sure to take this opportunity to tell the vendors thanks for all their contributions to our meeting and the society.

In between meeting activities and over the lunch hour, you will be free to discover Decatur. Downtown Decatur offers a number of restaurants, eclectic shops, and historical sites. Additionally, there are many activities in the Atlanta area that you may consider before and after the meeting. For further information on what to see and do while in Decatur, consider visiting the Decatur Visitors Center at 113 Clairemont Ave.

Finally, I must acknowledge and thank all the hard work done by the executive council for their work over the last year and for preparing for this meeting. Cynthia Goldsmith and Karen Kelley tirelessly contribute to the Society every year; Terri Bruce and Amanda Lawrence have prepared our Ruska session; Ann Ellis and John Shields have been working hard on our Proceedings; Mary Ard prepared this year's ballot with the goal of improving member participation in the society; and Jay (Jerome) has continued assisting the society and has been an excellent role model to which I could set some goals for the society. Finally, thank you to our appointed officers who support many other functions of SEMS.

Thank you everyone for attending and participating in SEMS 2015. Enjoy the meeting!

Russ Goddard, SEMS President 2015

SEMS 2015 PROGRAM

WEDNESDAY MORNING, MAY 20

REGISTRATION – 8:00 AM TO 4:30 PM

ROTUNDA

WORKSHOP:

Leica Microsystems and JEOL at Emory Imaging Center: Cryo TEM Preparation and Imaging

Van to begin transport by 8 am from the Rotunda

WEDNESDAY AFTERNOON

1pm – 5pm Commercial Exhibits

DECATUR B

TOUR AND WORKSHOP: Georgia Tech and Hitachi at Georgia Tech NanoFacility
Ionic Liquid Workshop – *Lunch is provided.*

Transportation at noon from the Rotunda

WORKSHOP: MVA and Tescan: Particle Characterization at MVA Scientific
Consultants

Lunch is provided

Transportation at noon from the Rotunda

6:30PM – 8:00PM

CORPORATE MIXER AND POSTER SESSION

DECATUR B

THURSDAY MORNING, MAY 21

REGISTRATION – 8:30 AM TO 5:00 PM

ROTUNDA

7:30 – 9:00 am *Executive Council Meeting*

MARY GAY D

9:00am **Opening Remarks – Russ Goddard, SEMS President**

PRESENTATIONS:

SWANTON AMPHITHEATRE

9:10 [MAS INVITED SPEAKER] *Performing High Accuracy, High Precision Electron-excited X-ray Microanalysis with SEM/SDD-EDS and NIST DTSA-II, Even When Severe Peak Overlap Occurs (and So Can You)!*

Dale E. Newbury, NIST Fellow, NIST, Gaithersburg, MD

RUSKA Competition

Moderator: *Amanda Lawrence*

9:55 *Densification and Grain Growth of Lu₃Al₅O₁₂:Pr Sintered Ceramics*

A.A. Trofimov, Clemson University, Clemson SC

10:10 *Human Respiratory Syncytial Virus Assembly and Budding Visualized by Cryo-Electron Tomography*

Zunlong Ke, Georgia Institute of Technology, Atlanta GA

10:25-10:45

BREAK

DECATUR B

10:50 *Biomechanical Characterizations of Scar ECM during the Acute to Chronic Stages of Myocardial Infarction*

Bryn Brazile, Mississippi State University, Mississippi State MS

11:05 *Analysis of Plant Responses to Titanium Dioxide (TiO₂) Nanoparticles*

Kaydee Smith, University of South Carolina, Columbia SC

11:20 *Characterization of the Ability of Yeast Probiotics and Paraprobiotics to Directly Interact with Gram Positive and Gram Negative Bacteria*

Gabe Posadas, Mississippi State University, Mississippi State MS

11:35 *Interactions of the BHV-1 Immediate Early Protein bICP0 with the Host Cell Splicing Factors hnRNP A/B and SFPQ*

Katie Foster, Mississippi State University, Mississippi State MS

11:50 *Microscopic Investigation of Plant Leaf Surface Characteristics and Foliar Uptake*

Frank Gibson Bethea Jr., Clemson University, Clemson SC

12:05 – 1:30

LUNCH

SEMS 2015 PROGRAM

THURSDAY AFTERNOON, MAY 20

PRESENTATIONS SWANTON AMPITHEATRE Moderator: *Russell Goddard*

1:30pm *Ebola Virus Disease*

Cynthia S. Goldsmith, Infectious Diseases Pathology Branch, Centers for Disease Control and Prevention (CDC), Atlanta, GA

1:45 *Quantification of the Morphological Transition in Cadmium Selenide Nanocrystals as a Function of Reaction Temperature*

Albert D. Dukes III, Lander University, Greenwood, SC

2:00 *Cryo-TEM: A New Era for 3D Structural Analysis of Protein Complexes*

Jeff Lengyel, FEI Inc, Hillsboro OR

2:15 **[INVITED]** *Superresolution Fluorescence Microscopy Goes Deep*

Peter Kner, College of Engineering, University of Georgia, Athens, GA

3:00 – 3:45

BREAK (VISIT EXHIBITORS & POSTERS)

DECATUR B

3:45 *Innovative 120kV TEM/STEM for Nanotechnology and Nanomedicine*

Barbara Armbruster, Hitachi High Technologies America, Inc., Pleasanton, CA

4:00 *Scanning electron microscopy cathodoluminescence of quartz: principles, techniques and applications in geosciences*

Donggao Zhao, University of Texas at Austin, Austin TX

4:15 *Secondary Electron and Transmitted Electron Imaging of Nanocrystals*

Jane Howe, Hitachi High-Technologies Canada Inc, Toronto, Canada

4:30 *Petrography for the Built Environment*

Steven Stokowski, TEC Services, Inc., Lawrenceville, GA

4:45 *Molecular Sieves Synthesis from Lignin Nanotubes Synthesis Waste*

Luisa A. Dempere, University of Florida, Gainesville, FL

6:00-7:00

SOCIAL AND POSTERS

ROTUNDA

7:00-9:00

BANQUET

MARY GAY

SPECIAL PRESENTATION:

Microscopy into Art: Adaptive Interpretation

R.B. Simmons, Imaging Core Facility, Georgia State University, Atlanta, GA

SEMS 2015 PROGRAM

FRIDAY MORNING, MAY 22

9:00-10:30AM **BUSINESS BREAKFAST** **LOCATION: MARY GAY C**

PRESENTATIONS **LOCATION SWANTON AMPHITHEATRE** **Moderator: Mary Ard**

10:30am **[MSA INVITED]** *FIB-based Techniques for Specimen Preparation, Materials Characterization, and Prototyping*

Lucille A. Giannuzzi, L.A. Giannuzzi & Associates LLC, Fort Myers, FL

11:10 *What's Below the Surface? Uncovering Silicon in Plant Tissue*

T. Nylese, EDAX, Mahwah, NJ

11:25 *Use of Dow Corning Z-6040 to Improve Adhesion of Embedding Media to Specimens*

E. Ann Ellis, Consultant in Biological Electron Microscopy

11:40 *EDS Large Area Mapping of Non-Flat Surfaces to Find Particles*

Warren J. MoberlyChan, Oxford Instruments America, Inc.

NOON **CLOSING REMARKS: MARY ARD, PRESIDENT-ELECT**

POSTERS

DECATUR B

Development of Dionaea muscipula (Venus Flytrap) Leaves and Leaf Trichomes

Russell H. Goddard, Valdosta State University, Valdosta, GA

Fine Structure of Allogromia sp. ZG, an Easily Cultured Foraminiferan from Long Key, Florida (USA)

Susan T. Goldstein, University of Georgia, Athens GA

Ultra Low Voltage Electron Microscopy for the Enhancement of Energy-Filtered BSE image

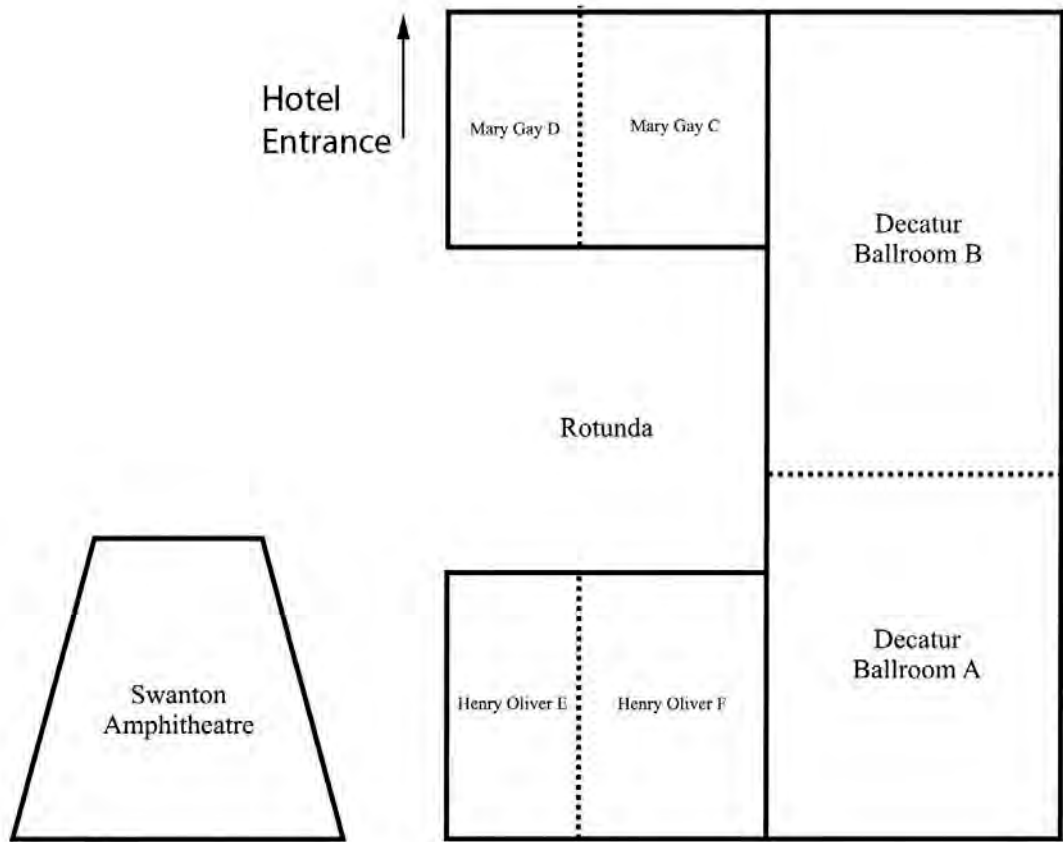
Y. Hashimoto, Hitachi High Technologies America, Clarksburg, MD

Economic Development and the Central Analytical Facility at the University of Alabama: Marketing and Promoting Core Research Facilities

R.L. Martens, University of Alabama, Tuscaloosa, AL

Application of Ionic Liquid on Various Samples for SEM Sample Preparation

A. Muto, Hitachi High Technologies America, Clarksburg, MD



Presentations
 Exhibitors
 Registration
 Poster Sessions
 Corporate Mixer
 Wednesday Night Social
 Banquet
 Business Breakfast
 Breaks
 Executive Council Meeting

Swanton Amphitheatre
 Decatur B
 Rotunda
 Decatur B
 Decatur B
 Decatur B
 Mary Gay
 Mary Gay C
 Decatur B
 Mary Gay D

MAS INVITED SPEAKER

Performing High Accuracy, High Precision Electron-excited X-ray Microanalysis with SEM/SDD-EDS and NIST DTSA-II, Even When Severe Peak Overlap Occurs (and So Can You)!

Dale E. Newbury, NIST Fellow
NIST, Gaithersburg, MD 20899

Silicon drift detector energy dispersive spectrometry (SDD/EDS) features high throughput, enabling the collection of high count spectra (1M to 10 M counts or more) within modest collection times (10 s to 100 s). Equally important, SDD-EDS operates with extremely stable peak shape (resolution) and peak position (calibration) across the entire input-output range. Stable peak shape and position are vital to the critical first step in quantitative analysis: the accurate determination of characteristic x-ray peak intensities by multiple linear least squares (MLLS) fitting of reference peaks to the spectrum of the unknown. With these measured peak intensities from the unknown and appropriate standard(s), the classic Castaing k-ratio protocol [$k = I(\text{unknown})/I(\text{standard})$] with calculated matrix (inter-element) corrections can then be performed to yield elemental concentrations. Remarkably accurate quantitative x-ray microanalysis can be achieved with this procedure, as embedded in NIST DTSA-II [1], even in the case of severe peak interferences, e.g., S – K and Pb-M; S – K and Mo-L; SrL and W M, N-K and Ti-L; Ti-K and Ba-L, etc., and even when the interfering constituents have large concentration ratios, e.g., Ba/Ti = 25:1. With this level of analytical performance despite severe interferences, SDD-EDS can replace wavelength dispersive spectrometry (WDS) for microanalysis of major (concentration $C > 0.1$ mass fraction), minor ($0.01 \leq C \leq 0.1$), and trace ($0.0005 \leq C < 0.01$) constituents with lower electron dose to achieve the same measurement precision as WDS [2].

[1] NIST DTSA-II is available free at:

<http://www.cstl.nist.gov/div837/837.02/epq/dtsa2/index.html>

[2] Newbury, D. E. and Ritchie, N. W. M., “Review: Performing Elemental Microanalysis with High Accuracy and High Precision by Scanning Electron Microscopy/Silicon Drift Detector Energy Dispersive X-ray Spectrometry (SEM/SDD-EDS)”, *J. Materials Science* 50 (2015) 493-518, electronic version:

<http://www.springerlink.com/openurl.asp?genre=article&id=doi:10.1007/s10853-014-8685-2>

Densification and Grain Growth of $\text{Lu}_3\text{Al}_5\text{O}_{12}:\text{Pr}$ Sintered Ceramics

A.A. Trofimov¹, M.R. Marchewka¹, and L.G. Jacobsohn^{1,2}

¹Department of Materials Science and Engineering, Clemson University, Clemson, SC 29634

²Center for Optical Materials Science and Engineering Technologies – COMSET, Clemson University, Anderson, SC 29625

$(\text{Lu}_{1-x}\text{Pr}_x)_3\text{Al}_5\text{O}_{12}$ (LuAG:Pr) is a fast dense scintillator that has attracted the attention of the scientific community. The fabrication of this scintillator as a transparent ceramic is a challenging problem that involves sintering at high temperatures. In this work, the densification and grain growth of LuAG:Pr ceramics is investigated. Ceramic samples were prepared from nanopowders obtained by co-precipitation and calcination, followed by sintering in air for 20 hrs. Samples were doped with Pr at 0.18 at.%, as determined by SEM/EDX. Sintering was carried out at a single temperature, from 1400 to 1700 °C, or using a two-step approach that has been suggested to decouple densification and grain growth. In this approach, once the temperature reaches the highest desired temperature value, it is immediately decreased to a lower value where it remains for 20 hrs. Temperature combinations from 1500/1400 to 1800/1700 °C were used. Density measurements were carried out according to the Archimedes method, with values ranging from 57 to 97 % of the density of LuAG single crystals (6.73 g/cm³), depending on the sintering conditions. Grain size was determined by means of SEM measurements using the lineal intercept method according to ASTM standard E112-96, with average sizes ranging from 0.4 to 1.9 μm. Sintering effects on the light emission from the Pr³⁺ ions were evaluated by means of photoluminescence measurements.

Acknowledgements: This material is based upon work supported by the National Science Foundation under Grant No. 1207080.

Human Respiratory Syncytial Virus Assembly and Budding Visualized by Cryo-Electron Tomography

Zunlong Ke¹, Rebecca S. Dillard², Cheri M. Hampton², Joshua D. Strauss², Kristen M. Lamb², Eric Alonas³, Hong Yi⁴, Barney S. Graham⁵, Philip J. Santangelo³, and Elizabeth R. Wright^{2,4,*}

1, School of Biology, Georgia Institute of Technology, Atlanta, GA 30332

2, Division of Pediatric Infectious Diseases, Emory University School of Medicine, Children's Healthcare of Atlanta, Atlanta, GA 30322

3, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30332

4, Robert P. Apkarian Integrated Electron Microscopy Core, Emory University, Atlanta, GA 30322

5, Vaccine Research Center, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD 20892

Human respiratory syncytial virus (hRSV) is an enveloped RNA virus that belongs to *Paramyxoviridae* family. HRSV infection may lead to the development of bronchiolitis and pneumonia; and in severe cases in immuno-compromised children and adults, death. Due to its pleomorphic nature, the three-dimensional structure of hRSV is not well understood. In order to characterize the molecular mechanisms underlying hRSV assembly and viral and cellular protein dynamics, we use cryo-electron tomography (cryo-ET) to examine hRSV infected cells.

Our cryo-ET studies have captured stages of hRSV assembly including initiation at the plasma membrane, viral filament elongation, and scission in lung-derived cell types. We are able to resolve the relative locations of the viral structural proteins during assembly, including the surface glycoproteins, the matrix protein, and the ribonucleoprotein (RNP) complex. We have observed two arrangements of the surface glycoprotein: ordered rows and a hexagonal lattice. The matrix protein also forms oligomers at sites of assembly and filament elongation. In addition, we have resolved early assembly events at the plasma membrane using cryo-immunogold labeling specific for the fusion (F) glycoprotein. Cryo-ET combined with native labeling will aid in determining the order of viral protein recruitment to sites of assembly. We frequently resolve cellular factors, such as cytoskeletal networks and ribosomes, near and within the assembling viral filaments. Future experiments will determine the biological significance of ordered glycoprotein arrangements and the role of matrix protein oligomers in the assembly process. We propose that the fusion glycoproteins initiate hRSV assembly and matrix protein oligomers are required to facilitate elongation by recruiting the RNP complex and driving subsequent filament elongation. We anticipate that our proposed model for hRSV will contribute to structure-directed anti-viral drug and vaccine design, and potentially other paramyxoviruses.

We would like to thank the Robert P. Apkarian Integrated Electron Microscopy Core, Emory University for microscopy services and support. This work was supported in part by Emory University, Children's Healthcare of Atlanta, and the Georgia Research Alliance to E.R.W.; the Center for AIDS Research at Emory University (P30 AI050409); public health service grant AI101775 to E.R.W. from the NIH/NIAID; NSF grant 0923395 to E.R.W.; and public health service grant GM094198 to P.J.S. from the NIH/NIGMS.

Biomechanical Characterizations of Scar ECM during the Acute to Chronic Stages of Myocardial Infarction

Bryn Brazile¹, J. Ryan Butler², Sourav S. Patnaik¹, Yanyi Xu³, Amanda Lawrence⁴, Andrew Claude², Raj Prabhu¹, Lakiesha Williams¹, Jianjun Guan³, and Jun Liao¹

¹Department of Biological Engineering, Mississippi State University, Mississippi State, MS.

²Department of Clinical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.

³Department of Materials Science and Engineering, Ohio State University, Columbus, OH.

⁴Institute for Imaging & Analytical Technologies, Mississippi State University, Mississippi State, MS.

Myocardial infarction (MI) affects more than 8 million Americans, causing massive heart cell death and heart function decrease. After MI, the infarcted tissue experiences a dynamic and time dependent process, i.e., the necrotic phase, the fibrotic phase, and the remodeling phase; each phase exhibits unique structural and mechanical properties. However, the biomechanical knowledge on the extracellular matrix (ECM) of scar tissue, mainly consisting of collagen fibers, is currently lacking. In this study, we took a novel approach to obtain scar ECM by applying a decellularization procedure to MI scar tissues created by permanent left coronary artery ligation (PLCAL) in a rat model. The time-dependent biomechanical and structural properties of scar ECM were then examined by thorough characterizations utilizing light microscopy of tissue histology as well as biaxial tensile biomechanical testing. Infarcted rat heart tissues were generated by a standardized PLCAL procedure. The infarcted hearts were harvested 15 min, 1, 2, 4, and 12 weeks after the PLCAL procedure (N = 6 per group), to capture scar tissue development. Decellularized native heart tissues were used as the control. To obtain scar ECM, the infarcted hearts were subjected to 0.1% sodium dodecyl sulfate (SDS) solution at room temperature for 3 weeks. A biaxial mechanical testing system was used to characterize the scar ECM under physiologically-relevant loading conditions. The biaxial data showed that the scar ECM preserved an overall anisotropy response observed in rat ventricle tissues and rat scar tissues, i.e., the longitudinal direction was more extensible than the circumferential direction. Scar ECM 15 minutes, 1 week, 2 weeks, and 4 weeks post infarction showed a stiffening biaxial behavior along with the time in both directions. The decrease of extensibility along longitudinal direction was more noticeable than the circumferential direction, which resulted in a decrease in degree of anisotropy. Scar ECM 12 weeks post infarction maintained a longitudinal extensibility similar to the 4-week scar ECM, while experiencing an increased extensibility in the circumferential direction. The time evolving biomechanical properties of scar ECM implied the alterations of collagen fiber network from the acute to chronic stages of MI, which was verified by the histology of the scar ECM. These biaxial mechanical properties are a direct function of the microstructural properties or its ECM. Understanding the structure-property relationship of the scar ECM will provide insights into the disease progression, as well as the biomechanical environment of the scar tissues. Future studies will involve more post-infarction time points, as well as quantification of the fiber network in the scar ECM.

This work is supported by AHA 13GRNT17150041.

Analysis of Plant Responses to Titanium Dioxide (TiO₂) Nanoparticles

Kaydee Smith^{1,2}, Kajal Ghoshroy³, and Soumitra Ghoshroy^{1,2}

¹Electron Microscopy Center, University of South Carolina, Columbia, SC 29208 USA

²Department of Biological Sciences, University of South Carolina, Columbia, SC 29208 USA

³Division of Science, Mathematics, and Engineering, University of South Carolina, Sumter, SC 29150 USA

As a result of a rise in the amount of nanoparticles used in consumer products, nanomaterials are expected to appear in the environment over time. The presence of these nanomaterials raises concerns for both wildlife and plant life. This study aims to analyze the effect of one nanomaterial (Titanium Dioxide, TiO₂) on the physiology and structure of the cells and organelles of two plant species [jalapeno (*Capsicum annuum*) and corn (*Zea mays*)]. Each plant species was exposed to varying concentrations of TiO₂ and analyzed using Scanning Electron Microscopy, Light Microscopy, and Transmission Electron Microscopy techniques. High concentrations of TiO₂ revealed structural damage to corn and jalapeno root cells, as well as intercellular changes. Root surface structure damage in both plant species appears to be similar, but TEM analysis suggested that TiO₂ might have varying effects on corn and jalapeno plant cells. This study highlights the importance of implementing safe procedures for the disposal of nanomaterials, as they have the potential to cause damage to economically important plants.

Characterization of the Ability of Yeast Probiotics and Paraprobiotics to Directly Interact with Gram Positive and Gram Negative Bacteria

Gabe Posadas¹, Jeffery A. Carroll², Jimmie Corley³, Amanda Lawrence⁴, and Janet R. Donaldson¹

¹Department of Biological Sciences, Mississippi State University, Mississippi State, MS 39762

²Livestock Issues and Research Unit, USDA-ARS, Lubbock, TX 79403

³Lesaffre Feed Additives, Milwaukee, WI 53214

⁴Institute for Imaging and Analytical Technologies, Mississippi State University, Mississippi State, MS 39762

Probiotics are widely utilized in the livestock industry to improve health and overall productivity. Despite the extensive amount of research performed on examining the mechanisms by which probiotics confer positive effects upon hosts, their use is still highly debated, and they are undoubtedly under characterized. The hypothesis for this study was that variations exist in the binding potential of probiotics to Gram negative and Gram positive bacteria. To test this hypothesis, the binding capability of five different types of live yeast probiotics or yeast paraprobiotics to two Gram negative bacteria (*Salmonella typhimurium* and *Escherichia coli* O157:H7) and two Gram positive bacteria (*Listeria monocytogenes*, *Clostridium perfringens*) was analyzed by both qualitative and quantitative assays using scanning electron microscopy and filtrations assays, respectively. SEM was used to visually assess surface-to-surface interactions between the yeast products and the bacteria. In preparation for viewing under SEM, the yeasts were incubated on coverslips then co-cultured with bacteria (1:100 yeast:bacteria ratio). To assess each probiotic's propensity towards adhesion, extensive washings of the coverslips were done. This removal of unbound bacteria determined that any bacteria remaining resulted from direct interactions between the pathogens and the products, inferring antagonistic behavior of adhesion displayed by the probiotics. Though the Gram negative bacteria did not demonstrate significant differences in binding to any of the products, Gram positive bacteria exhibited significant preference in binding to one of the probiotics and one of the paraprobiotics. Membrane filtration adhesion assays were also performed in order to test this mechanism. This assay includes similar yeast-bacteria co-incubation sample preparation to the SEM adhesion assay, but the sample is filtered using 3.0 μm membrane filters which are large enough to trap yeasts but small enough to allow unadhered bacteria to flow through. The filtrate was then plated and compared to a bacteria-only control filtrate. While *L. monocytogenes* exhibited significant binding to all samples ($p < 0.05$), *E. coli* O157:H7 and *S. typhimurium* demonstrated specificity in binding to particular products. Together, these data suggest that mechanisms of action of the yeast-based probiotics demonstrate strain specificity to particular pathogens. Further research is warranted to determine how these variations in binding potential influence the activity of these products *in vivo*.

Interactions of the BHV-1 Immediate Early Protein bICP0 with the Host Cell Splicing Factors hnRNP A/B and SFPQ

Katie Foster, Mississippi State University and Dr. Florencia Meyer, Mississippi State University

Bovine herpesvirus 1 is one of the major contributing factors in bovine respiratory disease. Once established within the host, BHV-1 causes a lifelong latent infection that can be reactivated under conditions of stress. Increased host susceptibility to secondary bacterial infections leading to pneumonia, and abortions, are symptoms of active infection and decrease production in both the dairy and beef industries. The BHV-1 immediate early protein, bICP0, is necessary for establishing an efficient infection in the host cells. Earlier work has shown that bICP0 interacts with different host cell proteins in infected cells. Western blot analysis showed that the cellular splicing factors hnRNP A/B and SFPQ experienced upregulation after infection. hnRNP A/B influences pre-mRNA processing and other aspects of mRNA metabolism and transport, while SFPQ is required for the assembly of the spliceosome. Using confocal microscopy, we aim to investigate the cellular localization and distribution of these cellular splicing factors at early and late times post infection, as they relate to bICP0 in the infected host cell. Differential cell lysis of infected cells followed by western blot will complement the microscopy work to finely tune the timing and movement of these proteins. Further investigation will determine if viral transcripts are interacting with these cellular splicing factors as well. We expect to learn whether or not these host cell proteins are involved with viral transcription within infected cells with an overall objective of discovering the mechanisms exploited by the virus to establish an efficient infection.

Microscopic Investigation of Plant Leaf Surface Characteristics and Foliar Uptake

Frank Gibson Bethea Jr., Haibo Liu and Terri Bruce
College of Agriculture, Forestry & Life Sciences, Clemson University, Clemson SC

Foliar applications of fertilizers and pesticides are a popular practice in the turfgrass industry. There is currently a lack of knowledge of how foliar applications interact with the leaf surface and the variables that are responsible for the hydrophobicity of the leaf or how the product enters into the plant. Understanding the mechanisms involved in uptake of the chemicals is pivotal to increasing uptake and reducing overall losses. Various grass species were studied to determine the leaf characteristics that influence hydrophobicity and uptake of foliar applied solutions.

Cuticle morphology was studied using scanning electron microscopy and cuticle roughness was analyzed using fractal analysis. Cuticle composition was studied by cuticle extraction and analyzed with gas chromatography. Leaf width and surface morphology was studied using optical profiler to analyze leaf topography. Results indicate cuticle morphology is directly dependent upon chemical composition of the cuticle and leaf morphology and topography is dependent upon species. Hydrophobicity of leaf surface was tested using a static water droplet and analyzing the contact angle of a droplet on each surface. Correlation analysis revealed total cuticle wax correlates significantly with hydrophobicity, but the relationship is not as significant with leaf roughness. Confocal microscopy revealed that uptake of a solution penetrates through stomata on the leaf surface, and stomatal size and density could be a major factor for foliar absorption. Future aims are to build a predictive model based on leaf characteristics to aid in product formulation and adjust management techniques to improve overall uptake of solutions.

Ebola Virus Disease

Cynthia S. Goldsmith

Infectious Diseases Pathology Branch, Centers for Disease Control and Prevention (CDC), Atlanta, GA 30333.

This past year has seen the largest Ebola virus outbreak on record, centered in West Africa in Guinea, Liberia, and Sierra Leone. At the time of this writing, within these three countries, there have been 23,014 cases, with 14,379 cases being laboratory-confirmed. There have been 9840 deaths, although the number may be higher due to under-reporting. Organizations from several countries have been working to defeat this disease, by treating the sick patients, by providing infrastructure, and by tracing and monitoring contacts of the patients.

Tissue specimens were collected for electron microscopy in a previous Ebola virus outbreak in 1995. Overwhelming viremia was present, as evidenced by the presence of numerous Ebola virus particles in the blood vessels. In the liver, a large number of viral particles, both normal and aberrant, were present in the sinusoids. Large filamentous inclusions, indicative of viral replication, were observed in hepatocytes, and infected cells were particularly concentrated close to the portal tract. In the lung, mature Ebola virus particles were observed within the alveolar space and in the interstitium, and viral inclusions were seen in alveolar macrophages. In the dermis of the skin, mature particles were observed in the connective tissue matrix, and inclusions were present in endothelial cells.

The presence of mature virus particles in the dermis of the skin and in the alveolar space of the lung may have important implications in terms of person-to-person transmission. The involvement of endothelial cells may explain the increased vascular permeability and shock seen in patients. Electron microscopic studies should further our understanding of the pathology and pathogenesis of this devastating disease.

Quantification of the Morphological Transition in Cadmium Selenide Nanocrystals as a Function of Reaction Temperature

Albert D. Dukes III, Michael Tanner Cameron, and Jordan A. Rogerson, Lander University, Greenwood, SC 29649

Controlling the morphology of semiconductor nanocrystals has typically relied on controlling the concentration and type of surface ligands utilized during synthesis. We demonstrate the temperature is also a significant factor in determining the final morphology of CdSe nanocrystals. Morphology was characterized by imaging the nanocrystals utilizing scanning transmission electron microscopy. In this work we quantify how the morphology changes as the reaction temperature is modulated. When the reaction temperature is above 280°C a dot morphology is predominant. However, when the reaction temperature is lowered, the nanocrystals exhibit a branched morphology, with a bi-pod structure being the most abundant.

This work was supported by the Lander Foundation

Cryo-TEM: A New Era for 3D Structural Analysis of Protein Complexes

Jeff Lengyel, FEI, Inc. Hillsboro OR

A new frontier exists in unraveling interactive biological and biochemical processes and pathways at the macromolecular level. Of critical importance is the three-dimensional visualization of macromolecular structures and molecular machines in their native functional state. Three techniques play a major role in orchestrating this. Nuclear magnetic resonance (NMR) has the capability to study specific protein domains or fragments and their functional role in protein folding and dynamics and in ligand binding whereas X-Ray crystallography (XRD) allows visualizing high-resolution but more static 3D structures of apo and liganded proteins, mainly in a monomeric or dimeric state after crystallization. To unravel more physiologically relevant situations however, it is essential to visualize multimeric complexes in their tertiary and quaternary state and their interaction with other complexes. By performing typical cryo-TEM applications like single particle analysis or tomography, this can be achieved. In this so-called translational methodology, cryo-TEM thus provides complementary information to NMR and XRD that can be crucial for drug discovery, e.g. in terms of a better understanding of the mechanism of action inferred from the EM structure of the physiologically relevant complex.

Latest developments in the cryo-TEM workflow have brought the 3 major structural biology technologies closer together. Now, finally, a continuum has been reached on all important aspects with regards to resolution and macromolecular scales which allows for the full integration and synergy of the combination of these technologies. These latest developments have enhanced the utility of structural biology in not just answering fundamental questions, but actually contribute to curing diseases and improving health.

INVITED

Superresolution Fluorescence Microscopy Goes Deep

Peter Kner

College of Engineering, University of Georgia, Athens, GA 30602

Fluorescence microscopy is an indispensable tool in the biological sciences which allows individual proteins to be imaged in living cells. Unfortunately, the resolution of a conventional widefield or confocal microscope is limited by diffraction to roughly 250nm. Recently, several novel techniques have been developed which bring the resolution of fluorescence microscopy down to between 100nm and 20nm. These techniques, superresolution microscopy, were recognized by the 2014 Nobel Prize in chemistry. Superresolution techniques have so far been limited to thin tissue culture samples of only a few microns thickness. When imaging into thicker biological samples, optical aberrations and scattering degrade the resolution and contrast, limiting the ability of fluorescence microscopy to achieve even the conventional resolution of 250nm. By modifying the phase of the fluorescence emission before it reaches the camera, adaptive optics and wavefront shaping can correct for optical aberrations and scattering, restoring the resolution. Adaptive Optics has been combined with confocal and multiphoton fluorescence microscopy to improve diffraction limited imaging in thick tissue, but the resolution is still limited by diffraction. We have combined Adaptive Optics (AO) with both Structured Illumination Microscopy (SIM) and Stochastic Optical Reconstruction Microscopy (STORM) to achieve sub-diffraction resolution through over 30 μ m of tissue [1,2]. The combination of AO with superresolution microscopy requires different considerations than the use of AO with confocal and multiphoton microscopy. In this talk, I will give an overview of superresolution fluorescence microscopy and adaptive optics, and discuss our recent work on combining these two important techniques.

This work was supported by the National Science Foundation through grants MCB1052672 and DBI1350654.

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Innovative 120kV TEM/STEM for Nanotechnology and Nanomedicine

Barbara L. Armbruster

Hitachi High Technologies America, Inc., Nanotechnology Systems Division,
5960 Inglewood Drive, Pleasanton, CA 94588

The performance of advanced nanotechnology-based therapeutics, which promises to significantly improve the treatment outcomes for oncological diseases, is closely related to their composition, morphology and crystal structure. At the nanoscale, high resolution TEM provides an essential foundation for the next phase of cancer nanomedicine in the clinic, the rational design of nanomaterial-drug combinations [1]. Many nanomaterials developed for biomedical applications are sensitive to electron beam irradiation; consequently TEM users want to observe and analyze crystal structures of their samples with minimum electron dose. The selection of the electron beam energy that is ideal for a given nanoparticle composition is a desirable option for reducing irradiation damage. The flexibility of a 120kV microscope to easily provide an acceleration voltage range from 40 to 120kV is excellent for many nanoparticle-based therapeutics including carbon as well as noble metals. This is a significant change from earlier requirements for working at higher voltages: for nanotechnology-based therapeutics, contrast and flexibility in the selection of high voltage outweigh the more expensive and less flexible 200kV microscopes.

The Hitachi HT7700 is a visibly redesigned nanoscience-oriented 40-120kV transmission electron microscope [2]. The microscope was planned from the start with a fully digital imaging design concept. Optional brightfield/darkfield STEM detectors facilitate x-ray mapping, support z-contrast imaging and the imaging of thick specimens with minimal chromatic aberration in brightfield STEM mode [3]. In addition to the standard objective lenses, Hitachi offers the novel objective lens “EXALENS” [4, 5] for 2Å resolution with on-axis illumination at 120kV with a spherical aberration coefficient of 1.76 mm and a chromatic aberration coefficient of 1.73 mm. The EXALENS maximum specimen tilt angle is +/- 30 degrees. The included electron source is a single crystal LaB₆ cathode but a standard tungsten filament is also available. An energy dispersive X-ray (EDX) analyzer with a silicon drift detector (SDD) can be added to expand the analytical capabilities of the TEM/STEM. A typical solid angle of the SDD is 0.237 sr., which is a factor of 1.7 times greater than the previous model. Point/line analysis and elemental mapping are available by means of the STEM option.

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Scanning Electron Microscopy Cathodoluminescence of Quartz: Principles, Techniques and Applications in Geosciences

Donggao Zhao, Stefanie N. Frelinger, Matthew D. Ledvina, J. Richard Kyle
Department of Geological Sciences, Jackson School of Geosciences, University of Texas
at Austin, Austin, TX 78712

Scanning electron microscopy cathodoluminescence (SEM-CL) technique has been a prevalent research technique in geosciences, especially for quartz, zircon and diamond. Principles, instrumentation, and sample preparation techniques of SEM-CL are reviewed in this study. Quartz from specific geological environments, such as porphyry and epithermal systems, reveals unique SEM-CL textures, which can be used to constrain the evolution of ore-bearing systems when complemented by fluid inclusion, hyperspectral mapping, and trace element studies. Experimental conditions for quartz SEM-CL imaging, such as accelerating voltage, beam spot size, working distance, vacuum conditions, and image acquisition, were examined using an XL 30 ESEM and the attached Gatan PanaCL detector. The optimized experimental conditions for high quality panochromatic SEM-CL images of quartz are as follows: 15 kV accelerating voltage, relative beam spot size 6 (approximately 500 nm in diameter), and photomultiplier tube (PMT) voltage HT -570 V to -580 V at high vacuum mode for carbon-coated samples or -550 V to -560 V at low vacuum mode for non-conductive samples. Working distances (WD) vary depending on the position of the retractable CL detector and the so-called “hot spot” of CL signal from the uniform epoxy or other materials on the sample. A CL hot spot corresponds to the focus point of the CL mirror that can be found by adjusting WD to maximize CL signal from epoxy. The sample surface should be as close as possible to the CL detector. A reduced WD increases the CL intensity. But a 1 mm clearance between the detector and the sample surface is recommended to prevent the detector from possible damage by the sample. Several minutes of beam exposure prior to image acquisition at 320 second scan speeds at 50X-1500X magnifications is recommended to generate the greatest CL emission. Monochromatic CL imaging requires three scans over the same area using red, green, and blue optical filters that can be merged to produce a “true color” image. The red and green filters require stronger PMT voltages to produce sufficient CL emissions. The most common application of quartz SEM-CL in ore geology is to reveal the relative timing of mineral precipitation, mineral dissolution, and inherited structural features. Understanding of temporal relations among these events makes it possible to select specific generations of quartz within a vein for further studies. SEM-CL imaging of vein quartz is explored through case studies of the Red Hills Porphyry Cu-Mo Deposit, Texas, USA, and the Ertsberg-Grasberg Cu-Au District, Papua, Indonesia.

Simultaneous Atomic-Resolution Secondary Electron and Transmitted Electron Imaging of Nanocrystals

Jane Howe¹, Xue Wang², Madeline Vara², David Hoyle¹, Tom Schamp³, and Younan Xia²

¹. Hitachi High-Technologies Canada Inc, Toronto, Canada.

². The School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, USA.

³. Hitachi High-Technologies America Inc, Gaithersburg, USA.

Our ability to image surface and bulk features of nanomaterials plays an important role in the field of nano-scaled materials research. Secondary electron microscopy (SEM) has been widely used for characterization of a broad range of materials. Conventional SEM operates at an accelerating voltage ranging from a few hundred volts to 30 kV, commonly using secondary electrons (SE) for imaging. SE imaging at high voltages up to 300 kV can also be obtained in a scanning transmission electron microscope (STEM) equipped with an SE detector. The Hitachi HD-2700A is a Cs-corrected STEM with an SE detector operating at 80-200 kV. In this paper, we will discuss the simultaneous atomic-resolution SE and bright-field and dark-field transmission electron imaging of Pd@Pt core-shell nanocubes and other nanomaterials. Incident electrons of 200 kV have a penetration depth of over 200 nm, thus providing both the surface and bulk information of these nano crystals from SE and TE signals. We will also discuss the use UV light and ozone to remove the hydrocarbon from the surfaces of these specimens for achieving optimal resolution. This new cleaning technique can be applied to both bulk SEM and TEM specimens.

Petrography for the Built Environment

Stokowski, Steven J., TEC Services, Inc., 234 Buford Drive, Lawrenceville, GA 30043
sstokowski@tecservices.com

Microscopic analyses and geologic understanding are essential for the correct diagnosis when building materials deteriorate, as these three case histories illustrate. The first case history is of the 1930's Fore River Bridge between Quincy and Weymouth, Massachusetts. This bascule drawbridge is a choke point for ship access to the historic Quincy shipyard and for commuter access to a portion of the Boston metropolitan area. The Depression-era concrete was originally of surprisingly good quality. Deterioration of the mass concrete bridge elements was, however, severe after 64 years. Alkali silica reactivity (ASR) in the concrete bascule piers formed silica gel in the concrete that causes expansion of the concrete that caused the steel drawbridge mechanism to bind and jam when the bridge was raised to the upright position, resulting in long commuter delays. ASR caused the bascule bridge structure to be removed at the cost of \$6 million; it is now replaced by a temporary vertical-lift galvanized steel structure (\$60 million+ in one press account). The new bridge under construction may cost \$244 million. The second case history is of the deteriorated brownstone loggia at the Oakes Ames Memorial Hall, North Easton, MA. This building is one of many designed and constructed by H. H. Richardson, a famous 1800's architect who designed many durable multi-hued brownstone and granite buildings that are part of the New England heritage. However, flat stone panels, arches, and decoratively carved columns in the loggia of this building began to deteriorate 100 years after construction. The cause of the deterioration was the formation of the sodium-sulfate salts, mirabolite and thenardite, consequent to the weathering of pyrite in the brownstone. In addition, calcium chloride deicing salt, a hygroscopic material also identified in the stone, probably increased the number of cycles of destructive hydration volume changes of the sodium sulfate. The recommended corrective action included polticing, as opposed to the proposed infrared heaters, which would have accelerated the deterioration due to the sodium sulfate salts. The final case history is of a total-replacement failure of a large memorial to WWII veterans in the Rhode Island Veterans Cemetery, Exeter, RI. This memorial, which consisted of large "black granite" panels carved with the names of the veterans from The Greatest Generation, was constructed with community-raised funds, was designed as a focal point of the cemetery, and was to be a showpiece for the contractor who diligently and expeditiously constructed the memorial through one of the worst summer droughts in RI history. Unfortunately, the onset of rain resulted in staining, cracking, and widespread deterioration (36 of 58) of the stone panels. The cause of the deterioration was expansion of the water-absorptive, smectite-group clay saponite, the hydrothermal alteration product of small amounts of orthopyroxenes in the black anorthosite panels. These were selected as an alternate source by the landscape architect after political sanctions prevented importation of the specified original stone, another "black granite." Contractor delisting and multiple lawsuits ensued, but eventually the contractor was vindicated.

Molecular Sieves Synthesis from Lignin Nanotubes Synthesis Waste

William E. Kerstein¹, Luisa A. Dempere^{1,2} and W. Vermerris^{3,4}

¹Department of Materials Science and Engineering ²Major Analytical Instrumentation Center ³Department of Microbiology and Cell Science ⁴Genetics Institute, University of Florida, Gainesville, FL 32611

The synthesis of molecular sieves in a hydrothermal solution procedure modified with lignin and other impurities was undertaken to provide a pathway for utilizing lignin nanotube synthesis waste. The experiments were designed to examine the synthesis parameters. Results seem to indicate that this could be a possible pathway for a cradle to cradle approach in the scale up of the synthesis of lignin nanotubes using sacrificial alumina templates. The intended molecular sieve (AIPO-5), and an interfering material Berlinite (a compact aluminophosphate), were created by these experiments according to the characterization with X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM).

The generation of molecular sieves is a serendipitous finding that creates a secondary waste-to-product synergy beneficial to biofuels projects that produce lignin as a byproduct from cellulosic ethanol. Besides desiccation of ethanol and the usage as a molecular sieve for organic species refinement, AIPO-5 has been used to catalyze processes such as the Fischer-Tropsch hydrocarbon synthesis, and syngas production. There are numerous aluminophosphate molecular sieves that could be synthesized, in addition to the possibility of adding silicon or metals to get other molecular sieve families that could utilize the heterogeneous composition of this particular waste solution.

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Microscopy into Art: Adaptive Interpretation

R.B. Simmons

Imaging Core Facility, Georgia State University, Atlanta, GA, 30303.

Beautiful images captured through microscopes are often considered works of art in themselves. Artists have been taking these images as inspiration for creative adventures using media other than photography to convey the complex and often breathtaking sights previously seen by only a select few. In the hands of artist such as Salvador Dali, Fuco Verdura and Luke Jerram the (mostly) unseen world comes alive through the use of gemstones, glass, paper, paint and other media bringing microscopic worlds to light. The ever-present influence of microscopy on the art world is enlightening, inspiring and often quite surprising.

MSA INVITED SPEAKER

FIB-Based Techniques for Specimen Preparation, Materials Characterization, and Prototyping

Lucille A. Giannuzzi, Ph.D., FAVS, FMSA

L.A. Giannuzzi & Associates LLC, Fort Myers, FL 33913

Lucille@LAGiannuzzi.com

Focused ion beam (FIB) based instruments are now routinely used for specimen preparation for a range of analytical instruments. Dual platform instruments (a FIB column and a scanning electron microscope (SEM) column on the same platform) can be used for unattended and automated site specific cross-sectioning or TEM specimen preparation. The SEM can be used for end-pointing any FIB technique via live or intermittent imaging. In addition, the synergistic use of the FIB with the SEM allows for automated acquisition of serial slices for subsequent 3D reconstruction and tomography of microstructure (via SEM imaging), crystallography (via EBSD) and/or elemental composition (via EDS). Automated and advanced digital patterning capabilities can be used to either remove or deposit material lithography or micro- and nano-prototyping. Advances in different ion sources enable FIB to be used from nano- to macro- scale lengths. Examples of the methods and techniques mentioned above will be presented from a variety of materials and advances in specimen preparation lift out methods will also be discussed.

What's Below the Surface? Uncovering Silicon in Plant Tissue

T. Nylese and R. Kerstin

EDAX, A Division of Ametek Materials Analysis Division 91 McKee Dr. Mahwah, NJ 07430

The fields of SEM and EDS imaging and microanalysis follow many traditional operating conditions to conduct certain types of analyses. For example, biological materials often require a conductive coating or low beam current and voltage operating conditions to prevent damage to the cell materials. Variable pressure SEMs have created better capabilities for analysis of delicate materials while minimizing sample damage and have resulted in greater characterization of biological materials. A combination of low vacuum conditions and higher beam energy permits x-ray collection from materials that are located below the surface of biological tissue. Varying the beam energy to increasing energies and collecting x-ray maps from serially deeper areas lends an understanding to the depth, character and concentration of these materials that are located within soft tissues.

In this work, silicon, which has been determined to play an important role in several types of plant species, was identified present in a sample of saw tooth sedge grass, *Cladium masriscus*, when using above 5 kV electron beam energy. Initial work at 5 kV and lower failed to reveal any of the suspected silicon, so the revelation at 10 kV highlighted that it was indeed present, yet below the surface tissue. Full investigation of several areas of the plant shows the various locations of the silicon bodies, including the remarkably shaped dumbbell morphology and the “glassy” thorns. Subsequent work was done at incrementally increasing beam kV to show the progressively deeper presence and intensity of this unusual, yet useful element in the plant structure.

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Use of Dow Corning Z-6040 to Improve Adhesion of Embedding Media to Specimens

E. Ann Ellis

Consultant in Biological Electron Microscopy

Some specimens are difficult to handle in that the specimen releases from the embedding medium at the junction of the specimen surface and the embedding medium. The following technique has been used to embed and section specimens of zeolite particles, thin films and leaf sections with thick, waxy epicuticular coatings. Treatment of the specimen surfaces with Dow Corning Z-6040, a silane, has been used to solve the problem of adhesion of the epoxy resin to the specimen.

Zeolite particles, ground to a fine suspension, were prepared in a 1% (wt/vol) of absolute or 100% ethanol or methanol and aliquoted into 0.5ml in microcentrifuge tubes. To this suspension, 0.5 ml of 0.1% (wt/vol) in absolute alcohol was added to each tube. The tubes were capped and inverted several times to mix the solutions. Tubes were spun in a microfuge for 10 minutes. A small, visible pellet appears at the tip of the tube. Carefully decant the alcohol from the tube and then use a filter paper point and carefully touch the point to the alcohol remaining in the tube. Do not touch the paper point to the pellet. Working in a properly functioning fume hood, add a drop of propylene oxide (PO) to each tube. The PO will diffuse through the pellet; allow the PO to volatilize off the samples and carefully remove any excess with a paper point being careful not to touch the pellet. Prepare a fresh batch of LX-112 (Ladd Research) or Eponate 12 (Ted Pella Inc.) with Araldite 502 with a hard formulation [anhydride:epoxide of 1.0]. [LX-112 2.3 g; Araldite 502 1.49 g; DDSA 6.13 g; BDMA 0.25 ml]. Carefully add the well mixed resin to the tubes to a level of approximately 0.5 ml. Cap the tubes and then place the tubes in a rack to remain upright; polymerize overnight. After polymerization, carefully cut the Eppendorf tube off the polymerized block. **Do not** trim a block face; the conical tip is the block face. Set the ultramicrotome to cut sections of 80-100 nm and carefully approach the block face as you would in sectioning cell cultures *en face*. Be sure to keep the water level correct with a mirror-like surface. The first 10-20 sections will have the highest concentration of particles and as the block face increases in size, the particles will appear more toward the outside of the sections. Pick up the sections on an appropriate sized (200 mesh) cleaned grid and allow the sections to dry down. Grids are now ready to be examined in the transmission electron microscope at an accelerating voltage of 100-200 kV.

Thin films on polymer substrates are carbon coated to help identify the top surface of the sections. Add 100 μ l of Dow Corning Z-6040 to 10 grams of epoxy resin and then flat embed the thin film coated polymer in flat peel-away molds. Polymerize overnight and then cut appropriate sized blocks from the large block.

With plant materials with thick epicuticular layers of wax, it is necessary to preserve the epicuticular wax with 0.1% (wt/vol) malachite green in the primary fixative. After osmication and dehydration in a methanol series, leaf sections were infiltrated and embedded in a Quetol 651 modified Spurr formulation (Ellis, E. A. 2006, *Microscopy Today*) which contained 100 μ l of Dow Corning Z-6040 in 10 grams of complete resin. Specimens were embedded in the same resin including Z-6040. Results were improved preservation of the epicuticular layer and the sections stayed intact during sectioning.

EDS Large Area Mapping of Non-Flat Surfaces to Find Particles

Warren J. MoberlyChan and Craig Theberge

Oxford Instruments America, Inc. NanoAnalysis Applications, Concord, MA 01742

With the development of Silicon-Drift-Detectors and fast count rates, EDS mapping requires only seconds. Coupled with fast computers automatically controlling microscope stage motion, Large Area Mapping (LAM) can investigate several centimeters in minutes. When applied to surfaces that are neither flat nor dense, EDS mapping may find particles that are not readily visible in SEM images. Furthermore, EDS mapping of large areas (i.e. 1-5x magnifications!) can ensure application of microscopy to statistically meaningful regions of our samples.

Traditionally, EDS is often applied to surfaces that are both polished flat and dense; however, many SEM investigations require us to study neither flat nor dense materials (e.g. particles in a porous matrix, particles on a filter paper, contamination on a biological material). Since the EDS detector is at an angle, surface roughness causes shadowing in an EDS map, often limiting its interpretation. However, with high counts rates, and especially excellent analysis of low energy x-rays, EDS mapping of all elements simultaneously enables the microscopist to both interpret surface roughness as well as automatically find chemically distinct features that are not readily discerned by SEM imaging.

Traditionally, Microanalysis is the application of sequential *Look* (SEM imaging), *Analyze* (EDS spot mode spectra of a few areas), and *Map* (which historically took hours often without revealing anything new; i.e. So why map?) Also a statistical concern is that we focus our use of the SEM to go to higher and higher magnifications, seeing the roughness of the bark on a tree while ignoring the forest. With all the autofocus and power of SEM computers, we now often can increase the magnification to >10,000x in seconds, making crucial our decision on what we image, or not. (And when we *analyze* after we *look*, that decision is more crucial.) But fast EDS Mapping means we can *Map* before we *Look*; a fast computer means we can *Analyze* the map while we acquire, before we *Look*. Performing *Large Area Maps* reverses our approach to Microanalysis, before we increase the magnification in the SEM, to ensure we focus on the right roughness on the right bark on the right tree in our forest.

POSTER

Development of *Dionaea muscipula* (Venus Flytrap) Leaves and Leaf Trichomes

Russell H. Goddard and Robert Land

Valdosta State University, Department of Biology, Valdosta, GA 31698-0015

Trichomes provide a useful system to study cellular differentiation and development. The Venus flytrap is a carnivorous plant that has highly developed leaves that contain four distinct populations of trichomes. Leaf primordia from apical shoots from type II tissue culture explants were dissected, observed with scanning electron microscopy, and the developmental progression of leaf development was noted with respect to the development and differentiation of leaf trichomes. Earliest leaf primordia emerging from the apical meristem bore large single cell trichome initials that give rise to stellate trichomes (touch receptors) distributed over much of the mature leaf. The differentiation of a blade (lamina) is apparent early but this remains undeveloped during extensive elongation of the petiole and only starts to grow and differentiate after the petiole reaches nearly 1 cm in length or more. The development of stellate trichomes on the petiole and abaxial side of the blade occurs throughout leaf development while the development of the three trichomes restricted to the adaxial side of the blade occurs later. Digestive gland and trigger hair development occur during early blade differentiation, while nectar glands are borne in the axes of marginal teeth and migrate due to cell expansion into the margin area of the adaxial side of the blade late in development. Similarities in cell division patterns as well as differences are noted to show how early development of the different trichomes appears homologous, but with later differences that lead to specific structures resulting in different functions for each trichome.

POSTER

Fine Structure of *Allogromia* sp. ZG, an Easily Cultured Foraminiferan from Long Key, Florida (USA)

Susan T. Goldstein¹, Deniz Z. Altin Ballero², and Elizabeth A. Richardson³,
¹Department of Geology, University of Georgia, Athens, GA 30602, ²Georgia Perimeter College, Covington, GA 30014, ³Department of Plant Biology, University of Georgia, Athens, GA 30602

Organic-walled allogromiids are common members of foraminiferal associations in reef and back-reef settings of the Florida Keys, and many live in cryptic or otherwise protected microhabitats associated with macroalgae and seagrasses. A new species of *Allogromia* was isolated from the alga *Dasycladus vermicularis* (Scopoli) collected from prop-roots of the red mangrove in Zane Grey Creek, Long Key, Florida. Materials brushed from the surfaces of the alga were maintained at 18 - 20° C in incubators with illumination on a 12-hr cycle. This allogromiid, present in the initial collections, reproduced among the algal washings over several months. Specimens were picked individually and placed in clean, artificial seawater and then transferred through new, clean wells over several days, facilitating the removal associated microbiota and ingested food organisms. Individuals were then placed in cultures with the mat-forming cyanobacterium, *Spirulina major* Kützing. Individuals soon extruded extensive reticulopodial nets and burrowed into the mats. These foraminifera opened large areas in these mats and ultimately reproduced. These cultures have been subcultured numerous times and maintained since 2010. This foraminiferan can also be reared on the coccolithophore *Isochrysis galbana*. This species, referred to here as “*Allogromia* sp., ZG”, genetically matches sequences of undescribed and uncharacterized allogromiids deposited in GenBank from Cyprus and Jamaica. *Allogromia* sp. ZG is also distinct from *A. laticollaris* Arnold, and from environmental DNA sequences of morphologically unknown allogromiids, as well as from several undescribed allogromiids known in the literature by nicknames (e.g., “squatter,” “rubble dome,” “twinkle”). Collectively, these allogromiids belong to Clade M of the monothalamid foraminifera. This species therefore appears to be broadly distributed and is fairly easy to isolate and rear in culture. Individuals typically have a bright orange cytoplasm, high flexibility, a variable number of apertures, and can assume a wide range of shapes. TEM (using high-pressure freezing followed by freeze substitution) shows that the test has a complex fine structure that varies in thickness. It consists of two layers: a thick inner layer with a “herringbone” fine structure that microscopically resembles elastin, and a thinner outer electron-dense mesh-like layer that occurs in patches. The number of nuclei varies per individual, and the non-reproductive nucleus is morphologically similar to that of many other basal-clade foraminifera. In culture, this species reproduces asexually by budding and multiple fission, and sexually and by releasing numerous flagellated gametes into the surrounding medium. This life cycle differs significantly from that of *Allogromia laticollaris*.

This work is part of a broader project on the biodiversity of basal-clade foraminifera and was supported by NSF grant DEB0445181.

POSTER

Ultra Low Voltage Electron Microscopy for the Enhancement of Energy-Filtered BSE image

Y. Hashimoto¹, A. Muto¹, T. Walters², E. Woods², and D. C. Joy^{3,4}

¹Nano-Technology Systems Div., Hitachi High Technologies America, Clarksburg, MD 20871

²Institute for Electronics and Nanotechnology, Georgia Institute of Technology, Atlanta, GA 30332, ³Material Science and Engineering, University of Tennessee, Knoxville, TN 37831

⁴Center for Nanophase Materials Science, Oak Ridge National Laboratory, Oak Ridge, TN 37831

SEM images are conventionally pictured at the accelerating voltage range from 10 to 30 kV. Lower accelerating voltage is useful to improve image contrasts and a unique technique, the deceleration method is applied at a landing voltage (V_i) below 1 kV. Morphological and compositional information at specimen surface is important in the field of material science and engineering, because it strongly affects material properties. SEM imaging at ultra low voltage (ULV) is a suitable method for obtaining topmost surface information and reducing specimen damage. In this study, we report an imaging method using energy filtered BSE signal at ULV and its application.

Figure 1 shows a schematic of the signal detection system of SU8200 series FE-SEM using deceleration mode. To control the signal detected by the top detector, an energy filter called “top filter” is used. The top filter allows only electrons with higher energy than filtering voltage (V_f) to be detected. Figure 2 shows the BSE images of the carbon nanotube (CNT) / polytetrafluoroethylene (PTFE) composite film at 0.3 kV. In image (a) taken by the upper detector, CNT and PTFE can be identified by their topographic features but the contrast between the materials is not obvious. In image (b) taken by the top detector with the top filter, the contrast between CNT and PTFE is clearly observed. In image (c) taken at higher magnification, intertwined CNT of 10 to 50 nm in diameter, and dispersion state of CNT at PTFE surface can be seen. In order to better understand the different BSE signal intensity between CNT and PTFE, we investigated BSE signal behavior at ULV with fundamental experiments and electron simulation techniques. We will disclose more details in the session.

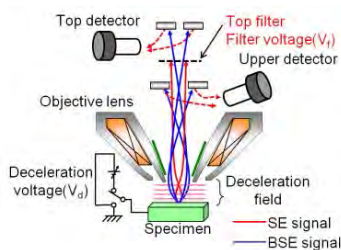


Fig. 1 Schematic of the signal detection system of SU8200 series FE-SEM using deceleration mode

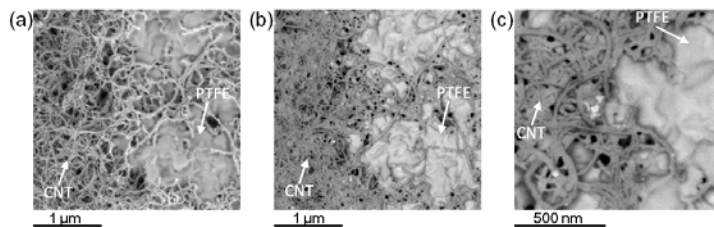


Fig. 2 BSE images of the CNT / PTFE composite film at ULV, taken by (a) the upper detector, (b)(c) the top detector with the top filter. (V_i :0.3 kV, V_d :0.5 kV, V_f :0.7 kV)

Specimen courtesy : Prof. Yoshiyuki SHOW of Tokai University

POSTER

Economic Development and the Central Analytical Facility at the University of Alabama: Marketing and Promoting Core Research Facilities

R.L. Martens¹, J.R. Goodwin¹, R.A. Holler¹, A.S. Brasher¹, M. Robinson¹, J.R. Foster¹, D.E. Nilkes², G.B. Thompson³, C.A. Pinkert⁴

¹ Central Analytical Facility, University of Alabama, Tuscaloosa, AL 35487

² Department of Chemistry, University of Alabama, Tuscaloosa, AL 35487

³ Department of Metallurgical & Materials Engineering, University of Alabama, Tuscaloosa, AL 35487

⁴ Office for Research and Economic Development, University of Alabama, Tuscaloosa, AL 35487

Managing and maintaining a core research facility in academia can be challenging. Normal day to day operations and activities take priority at any core facility: education of students for research experience and instrument maintenance. While the success of core facilities is largely driven by faculty, many would not exist without the support of administration. Having as many “key” faculty and “power users” whose research depends on the instrumentation, and who have a strong track record of publications and proposal submissions/awards can be enough to ensure a core facilities’ success. However, there are many additional activities, educational outreach opportunities and “economic development” efforts that core research facilities should take advantage of for continued success, support, marketing and public relations. Promoting the facilities/capabilities and attracting industrial users can be crucial for survival. Collaborations between faculty, departments, other institutions and industry are also very important.

The Central Analytical Facility (CAF) [1] has a mission of service in support of the teaching, research and service for the University of Alabama (UA) [2]. The CAF has emerged as a première microanalytical and microstructural characterization facility within the Southeast region. Its primary focus is to assist on and off campus academic research institutions with their characterization needs. Being a “hands-on” facility and training students to operate the equipment develops a critical skill set attractive to future employers. It also encourages graduates in the high-tech and research fields/jobs to return to the facility to conduct company research. The CAF enables research that leads to patents, licensing, and other intellectual property (IP) which supports departments such as the “Office for Technical Transfer” [3]. Once research IP is established, faculty who have won Small Business Innovation Research awards (SBIR) and can grow their business in the Alabama Innovation and Mentoring of Entrepreneurs (AIME) small business incubator [4]. This connection between research, intellectual property, and assisting a business start-up are strategic “Economic Developments” a core research facility can actively facilitate. Organizing workshops and symposiums, either directly with vendors or within a specific research community, increases visibility and reputation of facilities. Having an established core research facility also helps attract new research faculty.

[1] www.caf.ua.edu

[2] www.ua.edu

[3] www.ott.ua.edu

[4] www.aime.ua.edu

POSTER

Application of Ionic Liquid on Various Samples for SEM Sample Preparation

Y. Hashimoto¹, L. Blubaugh¹, T. Walters², E. Woods² and A. Muto¹

¹ Nano-Technology Systems Div., Hitachi High Technologies America, Clarksburg, MD 20871

² Institute for Electronics and Nanotechnology, Georgia Institute of Technology, Atlanta, GA 30332

Ionic liquids have unique features. Some of them can be non-volatile and highly conductive at the same time. IL1000 is an ionic liquid that is highly attractive for use in electron microscopy. It is stable under vacuum, conductive, hydrophobic and hyperosmotic, while also being inert and chemically safe. Furthermore, it has the unique capability to maintain wet samples in a “hydrated” status under vacuum conditions. [1][2].

Current applications for IL1000 include primarily biological samples, which traditionally require difficult and time consuming preparation before imaging by electron microscope; IL1000 serves as a quick and easy alternative to conventional preparation methods [4]. Although many convincing results have already been obtained from biological samples prepared with IL1000, the ideal preparation parameters such as ionic liquid concentration and treatment time seem to be somewhat sample dependent.

In this study, we report the results of several biological samples prepared using IL1000 with varying parameters. Additionally, several non-biological samples, including polymers and Aerogels with nano-porous structures were treated with IL1000 and showed promising result. Figure 1 shows an example of non-conductive Aerogel fractures. Aerogel fractures (a) are mixed with 5% IL1000 (b), treated for 1 hour, and dropped on Al foil(c). Excess liquid was removed using filter paper (d) then the sample was observed by SU8230 FE-SEM with high vacuum condition without coating (e). Fine porous structure was clearly confirmed with no charge-up artifacts. Furthermore, we were able to image the sample under high vacuum without degradation of image quality over time. We believe that IL1000 provides an extremely easy and reliable sample preparation solution for imaging of non-conductive or wet samples in a high resolution SEM.



Fig. 1 Aerogel sample preparation and SEM image taken by SU8230 FE-SEM

(a) Raw sample in plastic bag

(b) Preparation step : Dropping mixture of aerogel and ILs on SEM Stub

(c) Preparation step : Removing excessed liquid by filter paper

(d) SEM picture taken by SU8230 FE-SEM (Landing voltage: 300V, Magnification :x150k)

Specimen courtesy: Antonia Antoniou of Georgia Institute of Technology

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Dear recipient of the Ruska Award 1987,

When I learnt a couple of years ago that the Southeast Electron Microscopy Society donated a student achievement award, I was very pleased, because I know how important it can be to a young scientist to find early recognition for his work. I congratulate you on receiving the Ruska Award, and I hope that it will be possible for you to continue in this most interesting field of work.

With kind greetings,

Ernst Ruska



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