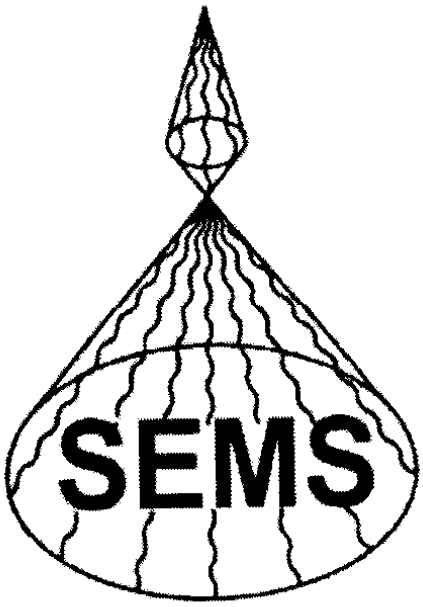
Proceedings of the Southeastern Microscopy Society

2016 Annual Meeting



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Pensacola Beach, FL

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SOUTHEASTERN MICROSCOPY SOCIETY

2016

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ACKNOWLEDGEMENTS

The Southeastern Microscopy Society is a local affiliate of **The Microscopy Society of America (MSA)** an**d The Microbeam Analysis Society (MAS).** The Proceedings are published for members and friends of the Southeastern Microscopy Society (SEMS).

As an affiliate of **MSA** and **MAS**, SEMS benefits by support from these organizations for invited speakers and meeting expenses.

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.

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Southeastern Microscopy Society

Dear SEMS Members,

I sincerely hope you are able to attend our annual meeting this year in Pensacola Beach in warm, sunny Florida. If you brought your families to spend some extra time along the sandy beaches, I hope they find their time quite relaxing.

This year's meeting begins Wednesday morning with a tour of McSwain Engineering, Inc. Corporate Exhibits will round out the afternoon as we come together for the Corporate Mixer and Poster Session in the evening. Thursday looks to be filled with some exciting presentations from students coming to show off their research in the Ruska Competition. We also have some Invited Speakers and contributed papers to round out a full day of microscopy. We will all gather again at our annual SEMS Banquet for some good food, Ruska winner presentation, and a special program for all. Friday morning's Business Breakfast will begin our last half day of presentations, which will include our remaining Invited Speaker.

Hats off to Amanda Lawrence and those on the Local Arrangements Committee who assisted her in getting us back to the beach for an exciting meeting venue. Our program was put together by our Program Chairs, Amelia Dempere and John Shields. The Proceedings was assembled by our own Ann Ellis. Thank you all for your hard work!

I want to personally thank another group that makes this meeting a tremendous success, and they are our Vendors and Corporate Members. Without their support and commitment to SEMS, we would not have the type of meetings we have had in the past. Thank you, Vendors, for your support and your commitment to the society. Please make sure to let them know by stopping by their booths how much we appreciate them here this meeting.

On a more personal note, my two favorite parts of the annual meeting are making new acquaintances and seeing the people I have come to know over the years of being a member of SEMS. You are my colleagues, and I hold a special place in my heart for all of you. Some of you have become my mentors while others have been my encouragers; and there are those of you whom have become my friends for life. Those of you who were not able to come to this meeting, I will definitely miss you. For those of us who are here, let us embrace all who have come, and enjoy another awesome meeting!

Sincerely,

Mary Ard

Mary Ard, SEMS President

SEMS 2016 PROGRAM

WEDNESDAY MORNING, MAY 18

REGISTRATION: 8:00 AM TO 4:30 PM **Foyer 2-3**

WORKSHOP: Tour of the McSwain Labs, Pensacola, FL
9:30 AM-1:30 PM

WEDNESDAY AFTERNOON

1:00-5:00 PM	Commercial Exhibits	Ballroom B
4:00-6:00 PM	**Executive Council Meeting**	**Board Room**
6:00-8:00 PM	Corporate Mixer and Poster Session	Ballroom B

THURSDAY MORNING, MAY 19

REGISTRATION: 8:30 AM-5:00 PM Foyer 2-3

PRESENTATIONS: Ballroom A

9:00 AM Opening Remarks and Welcome Mary Ard, SEMS President

9:10 AM **Invited Speaker: Paul D. Eason**
Department of Mechanical Engineering
University of North Florida
Jacksonville, FL

Creating a Multi-User University Center from Scratch

RUSKA STUDENT COMPETITION Moderator: Terri Bruce

10:00 AM **Swati Kumari**[1], J. Gabriel Monroe[2], Huiyu Wang[2], Rangana Wijayapala[1], Erick S. Vasquez[4], Keisha B. Walters[1], Matthew J. Berg[3], and Scott M. Thompson[2]
[1]Dave C. Swalm School of Chemical Engineering, [2]Dept. of Mechanical Engineering and [3]Dept. of Physics and Astronomy, Mississippi State University; [4]Chemical & Materials Engineering Department, University of Dayton

Structure-Property Relationships of Ferrofluids Used as Working Fluids in Oscillating Heat Pipes for Energy Harvesting

10:15 AM **M.Testa** and B. Kirkland, Department of Geoscience, Mississippi State University, Starkville, MS 39759

Attempting to Identify Spheroidal Structures Found at the Base of Aragonitic Botryoids

THURSDAY MORNING, MAY 19

10:30 AM-11:00 AM **BREAK:** Please visit the vendors and view the posters. Ballroom B

RUSKA STUDENT COMPETITION Cont.

11:00 AM **Xuefeng Zhang**[1], Jilei Zhang[1] and I-Wei Chu[2]
Department of Sustainable Bioproducts[1], Institute of Image & Analytical Technologies[2], Mississippi State University, Mississippi State, MS 39762-9820

Synthesis and Characterization of Carbon-based Nanomaterials from Kraft Lignin

11:15 AM **Wren Gregory**, Achyut J. Raghavendra, and Ramakrishna Podila
Laboratory of Nano-biophysics, Clemson University, Clemson, SC 29634.

Influence of Protein Corona on *in-situ* Aggregation Dynamics of Metal and Metal-oxide Nanostructures

11.30 AM **Achyut J. Raghavendra**, Anthony S. Childress, Jingyi Zhu, Apparao Rao, Sriparna Bhattacharya, and Ramakrishna Podila
Clemson Nanomaterials Center, Clemson University, Clemson, SC 29634

Defects in the graphene flatland: Microscopy, Spectroscopy, and

11:45 AM **James Kizziah** and Terje Dokland
Department of Microbiology, University of Alabama at Birmingham, 845 19th St S, BBRB311, Birmingham, AL 35294

Cryo-EM structure of the *Staphylococcus aureus* Bacteriophage 80α Baseplate

12.00 PM-1:00 PM **LUNCH**

THURSDAY AFTERNOON, MAY 19

PRESENTATIONS: **BALLROOM A**

Moderator: **Cynthia Goldsmith**

1:30 PM **Invited Speaker: Michael S. Kesler**
Materials Science and Engineering
University of Florida
Gainesville, FL

Multi-Scale Characterization Methods Facilitating Alloy Design

2:15 PM **Glenn M. Cohen**, Mona Patel, Cathy Huang, and Abena Prempeh Adaboh
Department of Biological and Environmental Sciences, Troy University, Troy, AL 36082

The Structural Basis of Banding Patterns in Shafts of Human Hair

THURSDAY AFTERNOON, MAY 19

2:30 PM **J. Neville**[1], K. Cannon[1], C. Bartlamet[1], G. Pierce[1], S. Crow[1] and R. Simmons[2].
[1]Applied and Environmental Microbiology and [2]Bioimaging Core Facility,
Georgia State University, Atlanta GA

Delayed Plant Senesce Through Novel ACC-Deaminase Activity in Induced *Rhodococcus rhodochrous* DAP 96253

2:45 PM-3:30 PM **BREAK** Please visit the vendors and view the posters.

3:30 PM **Invited Speaker: Deborah F. Kelly**[1] and Madeline Dukes[2]
Virginia Tech Carilion Research Institute, Virginia Tech, Roanoke, VA 24016
and [2]Protochips Inc., Morrisville, NC 27560

Improving Our View of Nanobiology Through Real-time Imaging and Quantification

4:15 PM **Russell H. Goddard** and Robert Land
Valdosta State University, Department of Biology, Valdosta, GA 31698-0015

Morphogenesis of Venus Flytrap Leaves during Development

4:30 PM Will Harris
Carl Zeiss X-ray Microscopy, Inc., Pleasanton, CA USA

Exploring in 3-D and 4-D with Nondestructive X-ray Microscopy

6:00 PM-7:00 PM SOCIAL PERGOLA (Covered Poolside Deck)
[In Case of Rain: Location will be the foyer of the banquet rooms]

7:00 PM-9:00 PM BANQUET : Awards and Entertainment BALLROOM C

FRIDAY MORNING MAY 20

9:00 AM-10:30 AM SEMS Business Breakfast BALLROOM C

PRESENTATIONS: **BALLROOM A** Moderator: Amelia Dempere

10:30 AM **Invited Speaker:** Edward Princepe
Tescan-USA

Three Dimensional structural and Chemical Imaging: A Discussion of Panoscopic Methods

FRIDAY MORNING MAY 20

11:00 AM **E. Ann Ellis**[1], Gregory A. Johnson[2], Hansoo Kim[3] and J. P. Pellois[4]
[1]Consultant in Biological Electron Microscopy, Thomasville, GA, [2]Merck, Philadelphia, PA, [3]Microscopy & Imaging Center, Texas A&M University, and [4]Department of Biophysics and Biochemistry, Texas A&M University, College Station, TX

Photo-Oxidation of DAB and Detection of Antimicrobial Peptide Conjugates by STEM-EDS

11:15 AM **Gabriella Kiss**
FEI Co, Hillsboro, OR 97124

3-Dimensional Characterization of Resin Embedded Biological Samples Using SBF-SEM and MED-SEM

11:30 AM **Roger Kerstin**
EDAX

Advancements in Material Analysis and Characterization for the SEM

11:45 AM **Jerome Parent**
Lyncee Tec.

DHM for MEMS Meaqsurement and 4-D Topography

12:00 PM **Amelia Dempere**
Materials Science and Engineering, University of Florida
Gainesville, FL

Combining Electron Microscopy and X-ray Microscopy for Materials Analysis

12:15 PM **CLOSING REMARKS**: Amelia Dempere, SEMS President 2017

RUSKA CANDIDATE

Structure-property Relationships Used as Working Fluids in Oscillating Heat Pipes for Energy Harvesting

Swati Kumari[1], J. Gabriel Monroe[2], Huiyu Wang[2], Rangana Wijayapala[1], Erick S. Vasquez[4], Keisha B. Walters[1], Matthew J. Berg[3], Scott M. Thompson[2]
[1] Dave C. Swalm School of Chemical Engineering, Mississippi State University
[2] Dept. of Mechanical Engineering, Mississippi State University
[3] Dept. of Physics and Astronomy, Mississippi State University
[4] Chemical & Materials Engineering Department, University of Dayton

Ferrofluids were synthesized via co-precipitation of nanoscale ferrous oxide and cobalt oxide particles, then surface functionalized to achieve stable suspensions while maintaining effective nanoparticle magnetization. Both as-synthesized (control) and surface-modified nanoparticles were used as working s in an oscillating heat pipe (OHP) for the transport of thermal energy and thermal energy conversion to electrical energy. In this novel process, pulsating or oscillating movement of ferrofluids through an in-line solenoid between two magnets was utilized to generate electric power, demonstrating the concept of ferrofluidic induction. The ferrofluid is oscillating due to an axial thermal gradient and associated phase change. To avoid nanoparticle aggregation in the ferrofluid during long usage periods and thermal cycling, a number of different ligands, including citric acid, were examined as a surface modifiers to maintain the nanoparticles in solution, prevent agglomeration, and maximize the voltage signal from the solenoid. Characterization of the ferrofluid samples was performed using ATR-FTIR spectroscopy, dynamic light scattering, X-ray diffraction, transmission electron microscopy, and atomic/magnetic force microscopy. Cobalt ferrite nanoparticles surface-modified with a variety of different small molecule ligands (e.g., citric acid) demonstrated good magnetic strengths and generated voltages close to those the neat ferrofluids (without surface modification), while maintaining dispersion. When the bias magnets were in place (i.e., harvesting configuration), the OHP's effective thermal conductivity averaged 11% lower than without the bias magnets. The maximum effective thermal conductivity was 12.9 kW/m·K for the non-harvesting ferrofluid test at 470 W input power.

RUSKA CANDIDATE

Attempting to Identify Spheroidal Structures Found at the Base of Aragonitic Botryoids

M.Testa and B. Kirkland, Department of Geoscience, Mississippi State University, Starkville, MS 39759

The association between dendritic structures, microbes, and precipitation of aragonite has been a debated topic among scientists for the past 30 years. The objective of this project is to test the hypothesis that spherical to subspherical structures found in samples deposited during icehouse sea conditions were originally organic in origin. Samples were collected from the Permian Reef, Capitan Formation and Lighthouse Reef Bluehole. Samples were studied in thin section, scanning electron microscopy (SEM) and Energy-dispersive X-ray spectroscopy. The samples showed similar patterns of micritic, microbial, dendritic shrub structures that seem to transition into aragonite botryoids. At the core of the dendritic structures are vast amounts of spherical to subspherical assemblies that may be interpreted as organic structures. These spheroidal structures may be serving as an organic substrate for the aragonite and calcite crystals. Precipitation experiments were conducted to grow calcite and aragonite crystals along with organic molecules in order to replicate the structures found from the sample sites. The organic structures were very similar to what was found at the three sample sites. Even though the organic structures are similar, it is very circumstantial and further research is required to determine if the spheroidal structures are organic and if they promote the growth of aragonitic botryoids.

This work was supported by the National Science Foundation under Grant No. DGE-0947419.

RUSKA CANDIDATE

Synthesis and Characterization of Carbon-based Nanomaterials from Kraft Lignin

Xuefeng Zhang[1], Jilei Zhang[1] and I-Wei Chu[2]
Department of Sustainable Bioproducts[1], Institute of Image & Analytical Technologies[2], Mississippi State University, Mississippi State, MS 39762-9820

Renewable carbon source, wood pulping byproducts lignin was carbonized at 1000 ℃ for the preparation of carbon based nanomaterials with the aid of iron based catalyst. The multilayer graphene (MLG) and multilayer graphene encapsulated iron nanoparticles (MLGEINs) were observed in the products by scanning electron microscopy (SEM) and high-resolution transmission electron microscopy (HRTEM). The formation of carbon based nanomaterials probably related to iron catalytic graphitization of lignin at 1000 ℃. This study indicates the wood pulping byproducts lignin can be used as a carbon source for the production of carbon-based nanomaterials and provides a new research and utilization for lignin

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This work was supported by the USDA Forest Service through Grant no. 11-JV-11111124-129. The authors would thank Domtar Inc. for provided lignin materials, and Rooban Venkatesh K G Thirumalai from Institute of Image & Analytical Technologies, Mississippi State University for the help in the experiments.

RUSKA CANDIDATE

Influence of Protein Corona on *in-situ* Aggregation Dynamics of Metal and Metal-oxide Nanostructures

Wren Gregory, Achyut J. Raghavendra, and Ramakrishna Podila
Laboratory of Nano-biophysics, Clemson University, Clemson, SC 29634.

A comprehensive understanding of interactions between metal nanoparticles and biological media is imperative to elucidate the mechanisms of nanotoxicity and design benign nanomaterials for biomedical applications. Many nanostructures are inherently hydrophobic and thereby tend to aggregate in biological milieu. The inevitable adsorption of proteins on nanoparticle (or the so-called protein corona) surface is expected to alter the aggregation dynamics. Our previous research suggests that: i) shifts in the hyperspectra of nanoparticles can be used to determine the average aggregate size and ii) the spectral profile depends upon the local dielectric environment, which enables differentiation of intra vs. extracellular nanoparticles. Building on these experiments, we are using darkfield hyperspectral imaging (through a CytoViva microscope**)** to study the influence of protein corona on the *in situ* aggregation dynamics of positive and negatively charged Ag and Au nanoparticles**.** This talk will present experimental and theoretical insights into nanoparticle aggregation dynamics and associated spectral changes along with our preliminary results on uptake of nanoparticles by RAW 264.7 macrophage cells.

RUSKA CANDIDATE

Defects in the Graphene Flatland: Microscopy, Spectroscopy, and Applications

Achyut J. Raghavendra, Anthony S. Childress, Jingyi Zhu, Apparao Rao, Sriparna Bhattacharya, and Ramakrishna Podila
Clemson Nanomaterials Center, Clemson University, Clemson, SC 29634

Defects are often wrongly perceived as material performance limiters. Indeed, it is not defects, but the lack of ability to controllably create and manipulate those defects that constrains the realization of new optical, electronic, or thermal properties or functionalities. For instance, doping graphene with nitrogen can result in many interesting configurations that are critical for energy storage, catalysis, and electronics. The rich and intriguing chemistry between C and N atoms manifests at the nanoscale in many forms such as pentagons, hexagons, and octagons with excellent Moire patterns. This talk will present microscopic and spectroscopic characterization of defects in graphene and show how defect configurations could be controlled to achieve desirable properties.

RUSKA CANDIDATE

Cryo-EM Structure of the *Staphylococcus aureus* Bacteriophage 80αBaseplate

James Kizziah and Terje Dokland
Department of Microbiology, University of Alabama at Birmingham, 845 19th St S, BBRB311, Birmingham, AL 35294

Staphylococcus aureus bacteriophage 80α is a siphovirus featuring an icosahedral capsid, a long non-contractile tail, and a baseplate that is used for host cell adhesion, recognition, and DNA ejection. 80α and other phages are an important mode of gene transfer among *S. aureus* because bacteria of this species are not typically transformable and seldom conjugate. Phages can carry genes encoding antibiotic resistance and virulence factors and are involved in the mobilization of *S. aureus* pathogenicity islands (SaPIs). It is therefore important to fully understand how this transfer is mediated. The 80α baseplate, despite its central role in determining host range and facilitating infection, has yet to be characterized. Cryo-EM images of tails with attached baseplates were collected using a mutant incapable of forming capsids. A three-dimensional reconstruction of the baseplates at 17.5 Å was generated using the EMAN and EMAN2 software suites with the application of six-fold symmetry. Several structural features including tail rings, core components of the baseplate, tail fibers, and putative host receptor binding proteins are evident. This study is important to understand the host range of 80α and how it mediates transduction of host genes. With this knowledge, it may become possible to modify the baseplate to adapt 80α for medically significant antibacterial purposes.

The authors would like to thank Dr. Gail Christie for providing the ST247 80α mutant strain and Dr. Altaira Dearborn for collecting the raw data. This research was funded by the NIH grant R01 AI083255.

The Structural Basis of Banding Patterns in Shafts of Human Hair

Glenn M. Cohen, Mona Patel, Cathy Huang, and Abena Prempeh Adaboh

Department of Biological and Environmental Sciences, Troy University, Troy, AL 36082

The hair shaft consists of three regions that are readily visible by routine microscopic methods: 1) the outer cuticle, 2) the cortex, and 3) the centrally located medulla. Despite extensive basic and clinical research on the cortical organization, structural questions remain. For example, the structural basis of bands and banding patterns is unsettled. In the present study, we primarily analyzed shafts of scalp hair. To improve visualization of internal structures, we bleached the hairs to remove pigments. Some hairs were also stained and/or split. We determined that bands consist of repeating barrel-shaped units of fibers that dovetail into each other; interconnecting fibrils attach adjacent bands to each other. Bands are first visible in the upper end of the hair follicle (keratogenous zone), continue along the length of the hair shaft, and end in the hair tip. In straight hair, these barrel-shaped units fill the cortical rim and display a semblance of radial symmetry. However, in hair ranging from curly to kinky, these units shift asymmetrically to one side of the cortex and form looping spirals of varying pitches along the hair shaft. Some hairs display multiple banding patterns along their lengths. In short, we identified the structural details of bands and banding patterns in hairs.

This research was supported in part by a Faculty Development Grant from Troy University, Troy AL 36082.

Delayed Plant Senesce through Novel ACC-deaminase Activity in Induced *Rhodococcus rhodochrous* DAP 96253

Neville, J.[1], K. Cannon[1], C. Bartlamet[1], G. Pierce[1], S. Crow[1] and R. Simmons[2].
[1]Applied and Environmental Microbiology, Georgia State University, Atlanta GA.
[2] Bioimaging Core Facility, Georgia State University, Atlanta GA.

Plant Growth Promoting Bacteria (PGPB) have been an area of interest for the agricultural industry for some time. The direct application of PGPB have been shown to have beneficial effects upon botanical systems from root elongation to the delay of senesce. PGPB colonize the rhizosphere where they initiate plant growth processes by synthesizing auxin that stimulates plant growth and tissue differentiation. In return, the plant produces 1-aminocyclopropane-carbocylic acid (ACC) which the bacteria can use as a nitrogen source. The catabolism of ACC by an ACC-deaminase enzyme has the beneficial side effect of inhibiting the production of ethylene, a signaling molecule that stimulates senesce.

Rhodococcus rhodochrous DAP 96253, a soil dwelling bacteria, has been shown to delay fruit ripening as well as inhibit the growth of select fungi. Based upon these known interactions with eukaryotic systems, *R. rhodochrous* was investigated for its potential to delay the senesce of cut carnations. *R. rhodochrous* was shown to grow in a liquid media and on solid media where ACC was the only nitrogen source indicating the presence of an active ACC-deaminase enzyme. Examination of the *R. rhodochrous* genome did not indicate the presence of an ACC deaminase homologous to any published ACC deaminase gene thereby indicating the presence of a unique enzyme structure and the possibility of unique activity. *R. rhodochrous* ACC-deaminase was tested for activity and the results showed higher activity levels than those of the *Pseudomonas fluorescence*, a well characterized PGPB that has an active ACC-deaminase enzyme

Induced *R. rhodochrous* cells, produced as a 30L fed batch fermentation, were added to a minimal media in which the cut carnations were placed. The carnations exposed to *R. rhodochrous* showed a significant delay in senesce and petal drop compared to unexposed controls. *R. rhodochrous* adhesion was examined using scanning electron microscopy showing an extremely high level of adherence of *R. rhodochrous* to the vascular system of the carnations. Additionally, *R. rhodochrous* that adhered to plant tissue was analyzed using a Baclite live/dead staining kit and imaged using a scanning laser confocal microscope. These images showed a very large viable population of *R. rhodochrous* adhered to the vasculature of the carnations at the end of their *In vitro* life span. These results indicate that *R. rhodochrous* can extend the life of cut flowers compared to traditional cut flower maintenance practices.

Improving Our View of Nanobiology Through Real-time Imaging and Quantification

Deborah F. Kelly[1] and Madeline Dukes[2]
[1]Virginia Tech Carilion Research Institute, Virginia Tech, Roanoke, VA 24016
[2]Protochips Inc., Morrisville, NC 27560

Understanding the properties of molecular machines is a common goal of biologists and engineers. Technical barriers in high-resolution imaging limit our knowledge of dynamic events at the nanoscale level. Transmission electron microscopy (TEM) permits us to peer into the world of cells and molecules. However, functional machines must be fixed in order to enter the ultrahigh vacuum system of a TEM. This task is typically accomplished by freezing specimens at high velocity in a thin layer of vitreous ice. Although ice preserves the structural features of biological assemblies, it also arrests them, making it difficult to understand dynamic mechanisms. Recent advances in the development of materials such as graphene and silicon nitride provide new opportunities for TEM imaging in real-time. Here we demonstrate our efforts to exploit these new materials and to create environmental chambers that permit us to perform experiments *in situ* or "inside" the TEM column using Liquid Cell technology. Using the Poseidon specimen holder (Protochips, Inc.), we can now view biological machinery in a native liquid environment with nanometer resolution. This new imaging modality allows us to visualize dynamic mechanisms in a completely new way. We are currently employing Liquid Cell TEM to improve our view of molecular events involving viral and cellular processes for biomedical applications.

This work is supported by NIH/NIAID [R21AI113402, R01AI116815].

This talk is sponsored by Protochips, Inc.

Morphogenesis of Venus Flytrap Leaves during Development

Russell H. Goddard and Robert Land
Valdosta State University, Department of Biology, Valdosta, GA 31698-0015

Development of multicellular structures requires coordinated growth and differentiation of all cell types composing the tissues of an organ. The Venus flytrap (*Dionaea muscipula*) leaf is a highly complex structure adapted for photosynthesis and for insect prey capture. Additionally, this complex leaf has four different identifiable trichomes that differentiate into hairs each with a unique function. In order to understand the relationship of trichome function to its structure this study was undertaken to examine the morphogenesis of the Venus flytrap leaf using scanning electron microscopy. Leaf primordia are borne singly from the shoot apical meristem and within a few cell divisions; epidermal cell differentiation allows identification of leaf trichome initials that eventually develop into the stellate hairs populating the entire mature leaf except within the trap itself. The leaf primordium undergoes further early differentiation such that a nascent blade can be distinguished. Following this stage rapid and extensive elongation of the petiole occurs with no apparent further growth of the nascent blade. Epidermal cell differentiation produces large numbers of stellate hair initials that quickly develop to cover the surface of the petiole on both adaxial and abaxial sides. The petiole continues to elongate and flatten in to the blade-like portion of the Venus flytrap responsible for most of its photosynthesis. Only after most of this expansion does the nascent blade start to develop into the trap of the leaf. During development of the blade, cells expand along the margins of the midrib to form the flattened, open lamina of the trap. Differential cell growth in the continued development of the blade produces the marginal teeth of the blade. During this development, the differentiation of three different multicellular trichomes on the adaxial surface of the blade is described. Each appears to undergo a similar progression of early cell division with differences in the total number of cells and elongation of cells observed for each of the different trichomes. The developmental questions created from understanding the structure and function of different trichomes are discussed.

Exploring in 3-D and 4-D with Nondestructive X-ray Microscopy

Will Harris, PhD
Carl Zeiss X-ray Microscopy, Inc.,
Pleasanton, CA USA
william.harris@zeiss.com

Analogous to well-known computed tomography or microCT, X-ray microscopy images a specimen, without physical sectioning, to generate a complete 3D view of the object. Uniquely, X-ray microscopes (XRM) incorporate a number of X-ray optical elements that have driven resolution and contrast to levels previously unachievable by conventional CT designs. More specifically, XRM adapts advanced detectors that have been developed at synchrotron facilities over the past decades, coupled with high-energy polychromatic laboratory X-ray sources. Furthermore, continued ongoing developments in synchrotron techniques have increasingly been adapted to laboratory instruments, for example extending the familiar absorption contrast imaging to include phase contrast and diffraction contrast modalities. These advances have broadened the application space of the technique, covering a range of disciplines from materials to life sciences and geosciences, and even including time evolution, so-called 4D, imaging studies.

This talk will draw on several examples to provide an overview of the latest advances in the XRM technique and applications. Discussion will also cover the connections with other familiar microscopy methods, notably SEM and FIB-SEM, to bridge imaging modalities and length scales with correlative workflows.

Photo-oxidation of DAB and Detection of Antimicrobial Peptide Conjugates by STEM-EDS

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Diaminobenzidine (DAB) has been used with osmium tetroxide since it was introduced by Seligman *et al.* in 1968. It was used extensively in horseradish peroxidase leakage studies for a number of years; however, DAB is no longer used very much in cytochemical localization studies with transmission electron microscopy (TEM). Localization of numerous proteins and peptides has been shifted to immunocytochemical studies; however, small proteins and short peptides do not lend themselves as readily to the production of specific antibodies. Photo-activation of DAB with various dyes such as eosin has proved useful in the absence of appropriate antibodies and as alternatives in localization procedures.

Cultures of *Staphylococcus aureus* were treated with the photosensitizer-antimicrobial peptide conjugate eosin Y-$(KLAKLAK)_{2;}$ illuminated under a halogen lamp (525-550 nm) for 2 minutes and then fixed with 1% (vol/vol) acrolein in cacodylate buffer, pH 7.4 for 20 minutes. Samples were washed with buffer and then treated with buffer plus 0.1 M glycine to remove any unbound aldehydes. Some samples were then treated with DAB (1mg/ml) in cacodylate buffer while other samples were controls. Samples were then illuminated for 15 min; the DAB solution was removed and the samples were washed twice with buffer before post fixation in 1% OsO_4 foe 30 minutes at room temperature. All samples were then washed with deionized water and dehydrated in a methanol series (10%-100%); infiltrated overnight and embedded in a Quetol modified Spurr formulation. For conventional TEM, sections were cut at 80-100 nm and examined in a JEOL 1200 EX TEM at 100 kV. For STEM-EDS, sections were cut at 200-250 nm, stabilized with a 10 nm layer of carbon and then elemental analysis was performed in a FEI TECHNAI F20 field emission with a Schottky field emission gun, a high angular dark field (HAADF) detector and Oxford EDS with an ultrathin window detector.

Eosin Y contains four atoms of bromine per molecule and this provides a marker of sites of eosin-$(KLAKLAK)_2$. Photo-oxidized sites of DAB result in areas of increased osmium localization. EDS studies demonstrated that Os peaks and BR co-localized providing a precise cytochemical localization of the binding of the antimicrobial peptide conjugate eosin-$(KLAKLAK)_{2.}$ Localization was confined to the cell wall and plasmalemma and adjacent cytoplasm of all species which were investigated, both gram positive and gram negative bacteria. There was increasing density of the DAB reaction product with increasing light exposure.

This work was part of Gregory Johnson's dissertation research at Texas A&M University. The work was funded by a Robert A. Welch Foundation Grant and the Norman Hackman Advanced Research Program.

Johnson G. A. *et al.* 2014. Photoinduced membrane damage of *E.* coli and *S.* aureus by the photosensitizer-antimicrobial peptide conjugate eosin-$(KLAKKLAK)_2$. PLOS One 9(3):1-14.

3-Dimensional Characterization of Resin Embedded Biological Samples Using SBF-SEM and MED-SEM

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3-dimensional characterization of resin embedded tissue samples in SEM or DualBeam FIB-SEM has demonstrated results equaling traditional 120 keV S/TEM tomography. With a continued interest in larger and larger tissue volumes, there has been a considerable advancement in SEM-based methods for imaging and 3-dimensional reconstruction in the past several years. Serial Block-Face SEM (SBF-SEM) involves combination of imaging and in-situ sectioning of resin embedded tissue within the SEM, allowing for automated imaging and subsequent reconstruction of volumes of tissue. To mitigate charge in what is generally a non-conductive specimen, low energy electrons or the use of low vacuum is employed. In 2014, FEI introduced and launched Teneo VolumeScope (VS), a SEM with integrated SBF and multi-energy deconvolution (MED-SEM). MED-SEM is a non-destructive technique that allows high-resolution imaging and reconstruction of the top layers of a sample. With multi-energy deconvolution one can overcome the traditional resolution limits set by mechanical slicing. After cutting a thin layer of the blockface using a diamond knife, freshly exposed tissue is imaged several times using various accelerating voltages. These images are subsequently used for deconvolving the information into several virtual subsurface layers. This cycle of physical and virtual sectioning offers isotropic datasets with excellent z-resolution and can be fully integrated and automated. This method will be presented as technique for isotropic 3-dimensional characterization of resin embedded tissue specimens.

POSTER

Sensilla on the Mandibles of the Red Imported Fire Ant, *Solenopsis invicta*, the Argentine Ant, *Linepithema humile* and the Crazy Ant, *Nylanderia fulva* (Hymenoptera:Formicidae)

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Three distinct types of cuticular sensilla are found on the mandibular surface of the red imported fire ant, (RIFA), the Argentine ant, (AA) and the crazy ant (CA). The first type is a trichoid sensillum that is a seta-like structure with a distinct socket at the base. The trichoid sensillum is the most numerous of the three types and is situated on the outer and inner surfaces of the mandibles. A small dome shaped sensillum is found at the base of the mandible where it articulates with the head and at the apex near the cusps. Both of these sensilla do not stain with silver nitrate which indicates a lack of porosity. The third type of sensillum is a short cuticular peg found in a shallow pit and has a distinct pore at the tip. The peg-sensillum is located on the external surface of the mandible near the cusps. It stains intensely with silver nitrate which indicates the porosity of the sensillum. The first two types of sensilla are typical mechanoreceptors that provide information on the movements of the mandibles during various activities such as feeding and registering stress that occurs on the mandibles while feeding and other behaviors. The peg-like sensillum is a contact chemoreceptor that is probably monitoring chemicals from possible food sources.

This research was supported by the National Institute of Food and Agriculture and the USDA-ARS Area wide Management of Imported Fire Ant Project (Richard L. Brown, Principal Investigator)

POSTER

MICROSCOPY FACILITY AT APPALACHIAN STATE UNIVERSITY

Guichuan Hou
Department of Biology and Dewel Microscopy Facility, Appalachian State University, Boone, NC 28608-2027

The Dewel Microscopy Facility (DMF) in the College of Arts and Sciences at Appalachian State University (ASU) serves as the core, multi-user microscopy facility for all departments at ASU and communities in the region. The DMF provides a wide variety of services in light and electron microscopy and image analysis. The DMF houses a FEI Quanta 200 environmental scanning electron microscope, a JEM-1400 transmission electron microscope, and ancillary equipment for specimen preparation. There are also other light microscopes, including a Zeiss LSM 510 laser scanning confocal microscope. To maximize contribution of the facility to education, the DMF currently offers BIO4564/5564 Microscopy and BIO3500/5500 Independent Study of Microscopy for undergraduate and graduate students, which are designed to meet students' needs on both theory and sample preparation for light and electron microscopy. Other courses, such as Cell Biology, also have laboratory components using the microscopy facility. Another important mission of the facility is to support faculty and student research at ASU. Scholarships are fostered through collaborative research and instruction among all invested departments in the College and on campus. Meanwhile, the DMF regularly participates in other undergraduate educational programs and works to enhance education of high school students, including STEM and the NC Science Festival. More information about the DMF can be found at: http://casmifa.appstate.edu/. We welcome collaborations and new ideas from individuals and other institutions.

Prof. Dr. Ing. Ernst Ruska
Max-Eyth-Straße 20, 1000 Berlin 33
Tel. 8 32 87 70

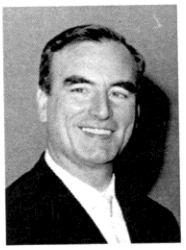
Berlin-Dahlem, den 28 April, 1987

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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