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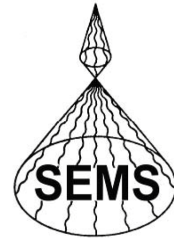

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Our **Corporate Members and Exhibitors** are an important part of our organization and make it possible for SEMS to have outstanding meetings and to publish the SEMS Proceedings. We thank them for their excellent service over the years and look forward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

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Dear SEMS Members and Guests,

Welcome to SEMS 2012 and Cocoa Beach, FL. I attended my first meeting of this organization in 1974 at Chapel Hill, N. C. and our acronym was SEEMS. The extra "E" stood for our affiliation with electron microscopes. Much has changed since 1974; but the quality of the meetings and the opportunities to meet fellow microscopists and improve our professional skills have continued. I hope that this meeting gives you an opportunity to greet old and make new friends.

The success of this and all SEMS meetings is due to the organization and hard work of all the elected and appointed officers as well as many other members. **John Donlon** has been our Local Arrangements Chairman and **John Shields** and **Robert Simmons** have chaired the Program Committee. **Cynthia Goldsmith** (secretary) and **Karen Kelley** (treasurer) have done their usual outstanding jobs in keeping things rolling. **Kim Backer-Kelley** and **Russ Goddard** will be seeing to the Ruska Competition. Please thank our corporate members for all the support that they have given and continue to give to SEMS.

Our program committee has brought us an outstanding group of invited speakers. We welcome MSA President, **Janet Woodward**, back home and welcome **John Henry Scott**, from NIST, and **Philip Howard**, from NASA to SEMS. One of our most important traditions is the Ruska Student Competition which recognizes the best use of microscopy to solve a research problem. I encourage you to attend the Ruska session and to encourage these young people. Over the years I have enjoyed and learned many useful techniques from many of our contributed papers and posters; our corporate members have been additional sources for keeping us current with the technology.

SEMS is one of the most active local affiliates of MSA and is represented throughout the leadership of MSA. A number of SEMS members have been recognized as MSA Fellows: **Ray Hart, Jay Jerome, Sara Miller, Robert Simmons and Janet Woodward. Bob Price** is the latest member of SEMS to be named an MSA Fellow. Congratulations, Bob.

We will celebrate the retirement of **Charles Humphrey** from professional life after many years of loyal service to SEMS and the microscopy community. Unofficially, Charles has probably presented more papers at SEMS meetings than anyone else. We will miss you Charles; but, once you are a SEMS member, you will always be a SEMS member.

We were saddened this past June by the death of long time SEMS member and University of Georgia mycology professor, **Gene Michaels**. He provided many years of leadership and managed the SEMS investments for many years. The SEMS Roth-Michaels Teaching Award was named in honor of Ivan Roth and Gene Michaels. More importantly, he mentored many students and was a good friend of many SEMS members. He will be missed by SEMS and other organizations.

Thank you for attending and participating in SEMS 2012 and it has been my pleasure to serve as your president for 2012. I look forward to greeting you and hope to see you at future SEMS meetings.

E. Ann Ellis
SEMS President 2012

SEMS 2012 PROGRAM

WEDNESDAY, MAY 16

REGISTRATION - 8 AM TO 5 PM

11:00am **Workshop**: Digital Holographic Imaging – 4-Dimensional Capture of Live Biological Material – Dr. Yves Emery, Lyncee Tec — SAWGRASS

1pm – 5pm **Commercial Exhibits** — DUNES & SEA OATS

12:00-1:30 **Executive Council Mtg and Lunch** — ATLANTIS

CORPORATE TALKS SAWGRASS MODERATOR: *JOHN DONLON*

1:30pm -1:45 Patrick Camus, Thermo Scientific, Madison WI
What Do You Want to Publish with an EDS Map?

1:45 – 2:00 Ben Tordoff, Carl Zeiss Microscopy, Cambridge, England
Correlative Light and Electron Microscopy - Bridging the Micro and Nano Worlds

2:00 – 2:15 Richard Brown, MVA, Atlanta GA
Use of a "Poor Man's Hot Stage" to Solve a Unique Indoor Dust Problem

2:15 – 2:30 Lisa Chan, EDAX, Inc., Mahwah, NJ
Electron Backscattered Diffraction Characterization of the Crossed Lamellar Structure in a Molluskan Shell

2:30-2:45 Danielle Elswick, Carl Zeiss Microscopy, Peabody MA
Helium Ion Microscopy – Advancements from 0.35 nm Imaging to Sub 10 nm Nanopatterning

2:45-3:00 Cory Czarnik, Gatan, Inc.
Advancements in Imaging Technology and Characterization for Transmission Electron Microscopy

3:00-3:15 **COFFEE BREAK** **SEA OATS**

6:00PM-8:00PM **POSTER SESSION** DUNES

6:00PM – 8:00PM **CORPORATE MIXER** SEA OATS

THURSDAY MORNING, MAY 17

Registration – 9am to 5pm

9:00am Opening Remarks – *Ann Ellis, President*

RUSKA PRESENTATIONS: SAWGRASS **Moderator:** *Kim Backer-Kelley*

9:15 – 9:30am David Lovett, University of Florida, Gainesville FL
Modulation of Nuclear Shape by Substrate Rigidity

9:30 -9:45am J. Rocco de Gregory, Mississippi State University, Starkville MS
An Examination of Dental Microwear from Two Bronze and Iron Age Sites from East Lokris, Greece

9:45 BREAK (VISIT EXHIBITORS) SEA OATS

10:30 – 11:30am ***MSA INVITED*** Janet Woodward, Buckman USA
Real World Microscopy: Problem Solving in the Paper Industry

11:30-1145am Amelia Dempere, University of Florida, Gainesville FL
Characterization and potential applications of lignin-based nanotubes and nanowires

11:45 – 12:00 John Shields, University of Georgia, Athens GA
Taphonomy of Flood Plain Sedges in the Olduvai Gorge, Tanzania

12:00 – 1:30 LUNCH

THURSDAY AFTERNOON, MAY 17

PRESENTATIONS SAWGRASS Moderator: *E. ANN ELLIS*

1:30 –1:45pm Cynthia Goldsmith, CDC Atlanta GA
Convergence of Two Techniques for the Identification of Previously Unrecognized Viruses

1:45-2:00pm Doria Bowers, University of North Florida, Jacksonville, FL
Alphavirus Hurdles in Mosquitoes; Epithelia and Muscles

2:00-2:30 Charles Humphrey, CDC Atlanta GA
45 Years of Electron Microscopy and Infectious Diseases, 37 Years working with Negative Stain Processing: What Did I Learn?

2:30 – 3:00 Russ Goddard, Valdosta State University, Valdosta, Georgia
Anatomical Comparison of Pecan, Walnut, Pistachio, and Almond Nut Shell Anatomy with Reference to their use in Commercial Products

3:00 – 3:30 **BREAK (*VISIT EXHIBITORS*)** SEA OATS

3:30-3:45 Lingjia Li, Tescan USA, Cranberry Township, PA
Latest Developments in Focused Ion Beam - Scanning Electron Microscope (FIB-SEM) for Advanced Materials Characterization and Microanalysis

3:45-4:00 E. Ann Ellis, Texas A&M, College Station TX
Vapor Staining and Specimen Preparation: A New Look at the Use of Osmium, Ruthenium and Acrolein

4:00-5:00 ***INVITED*** Phil Howard, NASA
Discovery: Under the Microscope at Kennedy Space Center

5:30-7:00 **SOCIAL** **SEA OATS**

7:00-9:00 **BANQUET** **HORIZONS ROOM**

SEMS 2012 PROGRAM

FRIDAY MORNING, MAY 19

8:00-9:30AM	**BUSINESS BREAKFAST**	**LOCATION: HORIZONS**
PRESENTATIONS	SAWGRASS	**Moderator:** *Richard Brown*

9:45-10:45 ***MAS INVITED*** John Henry Scott, NIST
How the Sausage is Made: A Microanalyst's Time in the White House

10:45AM *CLOSING REMARKS:* RICHARD BROWN, PRESIDENT-ELECT

11:15 AM TRIP TO NASA

POSTER

Post-Embryonic Development of the Compound Eye of the Bed Bug, *Cimex lectularius*, (Hemiptera: Cimicidae)

Gerald T. Baker[1], Amanda Lawrence[2], Richard Kuklinski[2], Jerome Goddard[1]

1. Dept. of BCH-EPP, Box 9775, MSU, Mississippi State University, MS 39762
2. Inst. For Imaging & Analytical Technol., Box 6020, MSU, Mississippi State University, MS 39762

The ommatidia that make-up the compound eyes of the five nymphal instars and the adults of the bed bug, *Cimex lectularius*, are round to oval in shape and convex. The number of ommatidia that are in the compound eyes of the various stage are statistically significant, but no significance between the males and females. The ommatidia found on the first three nymphal instars are similar is shape and diameter, ranging from 24.35µm to 27.50µm. Nymphs four and five and the adults have a much great range in the diameter of the ommatidia, from 20.15µm to 30.35µm. By using least-square analysis, a linear relationship exists between the number of ommatidia and the width of the pronotum.

POSTER

Sensilla on the Apex of the Labial and Maxillary Palps of the Workers *of Solenopsis invicta, S. richteri, Nylanderia pubens* (Hymenoptera:Formicidae)

Gerald T. Baker[1], Amanda Lawrence[2], Richard Kuklinski[2], Richard L. Brown[1]

1. Dept. of BCH-EPP, Box 9775, MSU, Mississippi State, MS 39762
2. Inst. for Imaging&Analytical Technol., Box 6020, MSU, Mississippi State, MS 39762

The single segment maxillary palp of the major and minor workers of *Solenopis invicta* and *S. richteri* has a single, apical, porous trichoid sensillum, whereas the maxillary palps of *Nylanderia pubens* has three porous trichoid sensilla on the apex of the fifth papal segment. The apex of the labial palp of the *Solenopsis* species workers has three porous trichoid sensilla, two long sensilla and one short. A short conical peg-like, porous sensillum is also found on the apex of the last papal segment of both types of workers. The palp apex of the *N. pubens* also has three straight and porous trichoid sensilla but the porous peg-like sensillum is not at the apex as in the *Solenopsis* species on the side of the apical papal segment about half way down the segment.

What Do You Want to Publish with an EDS Map?

Patrick Camus
Thermo Scientific, Madison WI

Elemental x-ray mapping provides the spatial distribution of the elements in a sample. However, individual maps do not take into account the affects of peak overlap, sum peaks, escape peaks or matrix correction. This can lead to presenting data that does not represent the correct composition or distribution of elements in the sample.
Quantitative elemental mapping provides an improved interpretation of the sample, but also requires expert knowledge of all of the elements present in the material. These quantitative elemental map results, although more succinct, may not provide the real answer of the phase distribution within the material.
Using a spectral imaging data set as a foundation, a set of statistical analysis routines can be used to unearth hidden and unexpected answers and provide a clearer picture of the phase distribution within your sample. Multivariate statistical analysis (MSA) provides the same interpretable results for analysts of all experience levels and provides the correct phase distribution within the sample. Modern microanalysis systems can provide this level of analysis during the acquisition for increased lab efficiency and report generation.

Alphavirus Hurdles in Mosquitoes; Epithelia and Muscles

Doria F. Bowers
Department of Biology, University of North Florida, Jacksonville, FL 32224

After imbibing a viremic bloodmeal, virus particles are deposited in the midgut of the female mosquito. Infectious virus must attach and enter at the apical aspect of the gut epithelium, replicate in the intracellular arena and then bud via the basolateral aspect of the gut to be released free in the hemolymph. Using Sindbis virus, an arthropod-borne-virus (arbovirus), and adult Aedine mosquitoes we explored tissue-level interactions of a virus in its insect host. Muscles surrounding the gut and ovary, both important “organs of transmission”, responded differentially to Sindbis virus. Temporal and spatial differences were observed using immunofluorescent and TEM, while changes in the diameter of the midgut epithelium were observed using bright field microscopy. From the virus point-of-view; the structural integration of these “organs of transmission” offer both hurdles and insights into the “links in the chain of virus transmission”.

Use of a “Poor Man’s Hot Stage” to Solve a Unique Indoor Dust Problem

Richard S. Brown
MVA Scientific Consultants, 3300 Breckinridge Blvd., Suite 400, Duluth, GA 30096, rbrown@mvainc.com

Sometimes you have to re-create the problem in order to solve the problem. Dust was appearing in the spotlight scaffolding of a major television studio. Workers became concerned about the dust when it would reappear after cleaning. The studio had undergone a complete renovation after a fire and water damage ruined the walls and flooring. Knowing this, the usual concerns about dust, mold and fungus began to circulate throughout the work force. Using a combination of microscopical techniques we were able to identify the dust. The source of the dust remained unclear until we reproduced the dust using a simple home-made hot stage, a polarized light microscope and a lot of patience.

This procedure highlights the problem solving skills employed by the research microscopist to solve problems that occur at the lower limits of ordinary vision. A combination of polarized light microscopy (PLM), Fourier transform infrared microspectroscopy (FTIR) and classic hot stage techniques were used to determine the source of the “dust”….that really wasn’t dust after all!

Electron Backscattered Diffraction Characterization of the Crossed Lamellar Structure In a Molluskan Shell

Lisa H. Chan[1] and Alberto Perez-Huerta[2]

[1]EDAX, Inc., Mahwah, NJ
[2]University of Alabama, Department of Geological Sciences, Tuscaloosa, AL

Electron back-scattered diffraction (EBSD) has been applied for the characterization of formation mechanisms of the complex aragonite cross-lamellar present in many mollusk taxa. A shell sample that survived the 1982-83 El Niño was analyzed to determine crystallographic changes in biomineral structures associated to the maximum seawater temperature anomaly linked to the event. This temperature anomaly is reflected in the shell by the development of a scar and microstructural changes in the first and second order lamellae. Preliminary results indicate that the first order lamellae is composed of less than 0.5 µm size crystallites and that the *c-axis* of aragonite crystals is perpendicular to alignment of lamellae. Furthermore, EBSD data indicate that there are no crystallographic changes across the shell scar related to microstructural modifications triggered by a rise in seawater temperature.

POSTER

A Versatile Virtual Microscope for Biology Labs

T. Kawakami[1], G.M. Cohen[2], and J. Zhong[1]. [1]Department of Computer Science and [2]Department of Biological and Environmental Sciences, Troy University, Troy 36082

Distance learning is playing an increasingly important role in higher education. Natural science courses, however, are burdened by the difficulties of developing virtual labs that emulate their onsite counterparts. For example, several publishers offer virtual microscopes for use in online biology labs. However, these virtual microscopes vary widely in ease of use, adaptability, and effectiveness in emulating classroom microscopes. In response, we have developed a virtual microscope that combines some of the best features of currently available virtual microscopes and allows the user to: a) rotate the microscope 360° for viewing it from every angle; b) select different magnifications by rotating the lens turret; c) focus and control the light intensity; d) identify the parts of the microscope; and e) create slide collections for pedagogical flexibility in the same or different labs.

(Supported by a Troy University Faculty Development Grant to G. Cohen.)

Vapor Staining and Specimen Preparation: A New Look at the Use of Osmium, Ruthenium and Acrolein

E. Ann Ellis and Michael W. Pendleton

Microscopy And Imaging Center, Texas A&M University, College Station, TX 77843

The use of osmium tetroxide vapor fixation dates back to 1884 when it was used for studying spermatogenesis in arthropods. Osmium vapor was used by Keith Porter, Albert Claude and Ernest Fullam to prepare tissue culture cells for transmission electron microscopy (TEM) in 1945. Since then osmium vapor has been used primarily to post fix specimens for TEM. Studies with preparation of insects for TEM and scanning electron microscopy (SEM) have proven this treatment to be very useful. Ruthenium vapor staining is used in preparation of polymers for both TEM and SEM. Ruthenium tetroxide is very unstable; however, ruthenium tetroxide can be prepared and used immediately by mixing 20 mg of ruthenium chloride with 1 ml of 10% sodium hypochlorite. Acrolein vapors can be used for anhydrous specimen preparation for both TEM and SEM and are useful when elemental analysis is needed. All the vapor treatment steps can be set up in a properly functioning fume hood by placing the vapor source in a bottle cap and the specimens on a piece of glass under a deep petri dish or beaker. The reactions can be expedited by placing a flask of hot water on top of the chamber. Timing for vapor treatment must be determined empirically and varies from a few minutes to several hours. Specimens treated with osmium or ruthenium vapors should be examined in the SEM within a few hours or days or stored in a dessicator since the deposited metals oxidize in moist air.

Characterization and Potential Applications of Lignin-Based Nanotubes and Nanowires.

Luisa A. Dempere[1, 2], Wilfred Vermerris[3, 4], and Hector M. Caicedo[5]
1 Major Analytical Instrumentation Center, University of Florida, Gainesville, FL 32606, USA.
2 Department of Materials Science and Engineering, University of Florida, Gainesville, FL 32606, USA.
3 Genetics Institute, University of Florida, Gainesville, FL 32610-3610, USA.
4 Agronomy Department, University of Florida, Gainesville, FL 32610-3610, USA.
[5] Pasteuria Bioscience, Inc. Alachua, FL 32615, USA.

Lignin-based nanotubes can be synthesized using a sacrificial template of commercially available alumina membranes. Lignin is a polymer (a complex cross-linked phenolic macromolecule) found in the plants cell wall that is typically obtained as a waste product from paper mills. It is also one of the waste products of biorefineries that process lingo-cellulosic biomass into fuels and chemicals.

Lignin can be linked to the inner walls of activated alumina membranes through covalent bonding, and its thickness can be adjusted by using phenolic monomers displaying different reactivity. Thus, the thickness of the polymer layer deposited within the pores of the sacrificial template membrane can be changed, which has resulted in the synthesis of nanotubes with a wall thickness of approximately 15 nm or nanowires with diameters of 200 nm [1].

The growing interest in the use of carbon nanotubes (CNTs) in medical applications has encountered limitations based on some of the aspect ratios of the cylindrical buckminster-fullerene structure-based carbon nanotubes that have been used in the evaluation of the CNTs toxicology [2]. When the use of CNTs is considered for medical applications as drug delivery vehicles for therapeutic agents, critical factors such as chemical inertness, and surface characteristics are of concern.

Lignin-based nanotubes are flexible and can be bio-functionalized easily and specifically, which allows a wide range of potential uses and applications. In addition, their intrinsic optical properties, which depend on the their chemical composition as they are functionalized, are ideal for applications in which the nanotubes need to be easily localized and imaged in living cells.

References:

[1] Hector M. Caicedo, Luisa A. Dempere, and Wilfred Vermerris. *Template-mediated synthesis and bio-functionalization of flexible lignin-based nanotubes and nanowires.* Nanotechnology 23 (2012) (12pp).

[2] Kostas Kostarelos. *The long and short of carbon nanotube toxicity.* Nature Biotechnology 26, (2008) (774pp).

POSTER

Cis-Golgi Cisternae of Plants and Algae Serve as Sites of Cisternal Assembly but are Biosynthetically Inactive

Bryon S. Donohoe[12#], Byung-Ho Kang[134#], Mathias Gerl[5], Zachary R. Gergely[1], Colleen M. McMichael[6], Sebastian Y. Bednarek[6], and L. Andrew Staehelin[1]
[1]Department of Molecular Cellular and Developmental Biology, University of Colorado-Boulder, Boulder, CO 80309 [2]National Renewable Energy Laboratory, Golden, CO 80309 [3]Department of Microbiology and Cell Science & Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL 32611 [4]integrative Bioscience and Biotechnology Pohang University of Science and Technology [5]Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden [6]Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706
# These two authors contributed equally to this work.

The cisternal progression/maturation model of Golgi trafficking predicts that *cis*-Golgi cisternae are formed *de novo* from ER-derived COPII vesicles on the *cis*-side of the Golgi. Here we describe structural and functional intermediates of the *cis* cisterna assembly process in high-pressure frozen algae (*Sherffelia dubia*, *Chlamydomonas reinhartii*) and plants (*Arabidopsis thaliana*, venus flytrap) as determined by electron microscopy, electron tomography and immuno-electron microscopy techniques. Our findings are as follows: (1) The *cis*-most (C1) Golgi cisternae are generated *de novo* in Arabidopsis within the Atp115-containing domain of the Golgi scaffold from *cis* cisterna initials produced by the fusion of 3-5 COPII vesicles in contact with a C2 *cis* cisterna. (2) COPII vesicles fuel the growth of the initials, which have to merge into a coherent C1 cisterna before they can nucleate *cis* cisterna initials. (3) When a C1 cisterna nucleates its first initial it becomes a C2 cisterna. (4) C2-Cn *cis* cisternae grow through COPII vesicle fusion. (5) Native BiP is recycled from *cis* cisternae to the ER via COPIa-type vesicles that bud from all *cis* cisternae. (6) *Cis* cisternae are biosynthetically inactive, but C2 cisternae mediate the self-assembly of scale protein complexes. (7) In plants, native mannosidase I localizes exclusively to medial Golgi cisternae. (8) Biochemical activation of *cis* cisternae coincides with their conversion to medial cisternae via COPIb-type recycling of medial cisterna enzymes. These results demonstrate that the assembly and functional maturation of *cis*-Golgi cisternae occurs in a sequential manner, and that the different assembly steps are both temporarily and spatially separated.

Anatomical Comparison of Pecan, Walnut, Pistachio, and Almond Nut Shell Anatomy with Reference to their use in Commercial Products

Russell H. Goddard[1] and John Nizio[2]
[1]Department of Biology, Valdosta State University, Valdosta, Georgia 31698
[2]Southeastern Reduction Company, Division of South Georgia Pecan Company, Inc., Valdosta, Georgia 31601

Over 50 million pounds of pecans are shelled in southern Georgia every year. The nut shell by-product is then ground to a flour and used in a variety of commercial products. The purpose of the present study was to determine anatomically, the composition of the nut shell, and to compare differences in shells of pecans, walnuts, pistachios, and almonds whose shell by-products are also used in commercial products. Nearly 100 percent of the composition of the nut shell was determined to be sclerenchyma tissue, composed of sclereids, or stone, cells. Some fruit walls also consisted of fibers interspersed with sclereids (e.g. almonds). There were differences in cell size and secondary wall thickness in different nut shells whose function may need to be considered in the commercial products for which these by-products are used.

Convergence of Two Techniques for the Identification of Previously Unrecognized Viruses

Cynthia S. Goldsmith, Thomas G. Ksiazek, Pierre E. Rollin, James A. Comer, William L. Nicholson, Teresa C. T. Peret, Dean D. Erdman, William J. Bellini, Michael D. Bowen, Bobbie R. Erickson, Stuart T. Nichol, Julu Bhatnagar, Laura K. McMullan, Christopher D. Paddock, and Sherif R. Zaki.

Centers for Disease Control and Prevention (CDC), Atlanta, GA 30333.

Virus isolation and electron microscopy in combination are powerful tools in the identification of viral illnesses of undetermined cause. The technique of isolating viruses in cell culture allows for the amplification of virus particles to levels where they can be detected by electron microscopy (EM). If the viral isolate cannot be recognized by other detection methods (ELISA, PCR, IFA, etc.), then EM is an ideal method because it can morphologically classify the isolate to a specific virus family. Once the family is known, molecular and immunologic techniques can be employed to determine which virus genus/species has been isolated. In this presentation, there will be several examples given where viruses were isolated by cell culture and identified by EM: SARS coronavirus; Nipah virus, a paramyxovirus; lymphocytic choriomeningitis virus, an arenavirus; Cache Valley virus, a bunyavirus; West Nile virus, a flavivirus; and Heartland virus, a bunnyavirus.

POSTER

Ultrastructural Studies on the Development of Lipid Bodies in Response to Nitrogen Starvation in Biofuel Algae *Auxenochlorella protothecoides*

Elton Goncalves, and Bala Rathinasabapathi
Horticultural Sciences Department, Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32611-0690

The development of clean and renewable sources of fuel is of vital importance for modern society, as petroleum-derived fuels become increasingly scarce over the decades. Among the cleanest and thoroughly renewable sources of biofuels are the oleaginous microalgae, which can accumulate up to 80% of their dry weight as triacylglycerol (TAG) under certain conditions. It has been well known that green algae form lipid bodies [LBs] in response to nitrogen [N] starvation. However, the kinetics of development of lipid bodies and their ultrastructural details in commercially-relevant algae are not well understood. We investigated N-starvation induced lipid accumulation in oleaginous microalgae, *Auxenochlorella protothecoides* UTEX29. We first demonstrated increased TAG accumulation up to 5-fold under mixotrophic growth and N starvation for 48h, by staining the cells with Nile Red, a vital fluorescent dye for intracellular lipids. Transmission electron microscopic observations of cells starved for N for various lengths of time indicated that LBs began developing within three hours following transfer to a N-deplete medium, LBs appeared in the cytoplasm, not in the plastids, and plastids contained pyrenoids and starch granules which tended to decrease upon N-starvation. Many, but not all of the N-starved cells developed trilamellar cell wall indicative of entering into a resting or spore-like stage. This is the first study to date to show the ultrastructure of this biofuel algal species including the determination of early stages of LB formation. The information obtained here will be valuable for choosing the appropriate developmental stage for functional genomics studies to identify genes important for the formation of lipid bodies.

INVITED

Discovery: Under the Microscope at Kennedy Space Center

Philip M. Howard, Kennedy Space Center, Material Science Division, NE-L6
Kennedy Space Center, FL 32899

The National Aeronautics & Space Administration (NASA) is known for discovery, exploration and advancement of knowledge. Since the days of Leeuwenhoek, microscopy has been at the forefront of discovery and knowledge. No truer is that statement than today at Kennedy Space Center, where microscopy plays a major role in unknown contamination identification and is an integral part of failure analysis. Space exploration involves flight hardware undergoing rigorous “visually clean” inspections at every step of processing. The unknown contaminants that are discovered on these inspections can directly impact the mission. What I hope to share with my fellow microscopist is some of the excitement of microscopy and how its discoveries have contributed to successful space flight.

Latest Developments in Focused Ion Beam - Scanning Electron Microscope (FIB-SEM) for Advanced Materials Characterization and Microanalysis

Lingjia Li[1] and Jiri Dluhos[2]
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The Focused Ion Beam – Scanning Electron Microscope (FIB-SEM) system has become an important tool for understanding and manipulating the structure of materials at micro- and nano-scale. The FIB has the unique capability of precisely sculpting, patterning and fabricating structures, while the SEM offers high resolution imaging. This technique has made possible a broad range of applications, from micromachining to TEM sample preparation to 3D visualization of materials. The capabilities of a FIB-SEM workstation can be future expanded with the integration of a variety of analytical techniques, making it a multi-functional nanotechnology tool.

In order to meet the demand of high speed cutting and milling, a new FIB-SEM workstation using a xenon plasma ion beam has been developed recently. This instrument can deliver a beam current of more than 2 microamps, removing materials 50 times faster than traditional FIBs, while still maintaining excellent milling precision at low beam currents and high resolution imaging with SEM. It is ideal for large-scale structure characterization and failure analysis.

RUSKA

An Examination of Dental Microwear from Two Bronze and Iron Age Sites from East Lokris, Greece

J. Rocco de Gregory, Mississippi State University

This research investigates diachronic diet change at two sites during the Bronze Age (BA) and Iron Age (IA) in East Lokris, Greece. The examination of microscopic dental surface features, created by the chewing of food and known as dental microwear analysis (DMA), has shown promise in addressing questions concerning subsistence change among extinct and extant species. The Greek BA and IA are some of the most studied periods in western history, yet little DMA research focusing on this period has been published. The sites of Mitrou and Tragana Agia Triada were used because together they span the transition from BA to IA. Mitrou, which was excavated between 2004 and 2009, is a small island in the bay of Atalanti. Survey and excavation on the island has shown that the occupation extend from the Neolithic period to the Classic Period. Excavations have recovered 76 burials ranging in age from the Early Helladic to the Protogeometric periods. Tragana Agia Triada consists of nine Late Helladic Mycenaean chamber tombs located three kilometers southwest of Mitrou. Due to the geographic and temporal proximity of the sites, it is believed that they are associated. Samples were imaged using a scanning electron microscope, and then quantified using *Microware 4.0*, software developed for DMA. Changes in dental microwear feature frequencies and orientations may indicate dietary shifts. Results from the microwear analysis do suggest that diet was changing during the BA/IA transition. Further research is needed to determine if the cause is due to changes in dietary preference or cooking technologies.

45 Years of Electron Microscopy and Infectious Diseases, 37 Years working with Negative Stain Processing: What Did I Learn?

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Every career has a beginning and an end. I cannot predict the future, but a transition for me is near…to what I do not know. I anticipate and hope for an adjusted pace to the daily laboratory routine of 49 plus years but I doubt I will ever lose my interest for microscopy or bench-level/field science. Microscopy has been more of an avocation than a job. The first-hands on experience I had was with a vintage 1954 RCA EMU 2 C electron microscope (EM) managed by Dr. Clifton Fain in the Ceramics Engineering Department at Clemson University. Within the first week of working with the microscope, despite its limitations, I was hooked. I did not know then that various EMs would become my mistresses for 45 plus years...a year more than my marriage. Thirty-seven of those years, I have attempted negative stain electron microscopy (NSEM) with varied success.

Understanding of thin-section EM, including immune electron microscopy was nearing maturity in 1967 when I began my graduate studies. Negative Stain EM (NSEM) surprisingly was still somewhat a black art and if one follows the Microscopy Society of America listserver, for many, still is today. Upon my arrival at CDC in 1983, I learned that answers to my basic questions about how to prepare **consistently** high quality NSEM grids were unsatisfactorily answered. My particle adsorption questions included such things as which side of the grid should one put the specimen, filmed side or opposite? Should the specimen incubate on the grid or should the grid be placed on the specimen? What is the optimal drop-size and adsorption time? Is any advantage gained by adsorbing the specimen onto a substrate prior to applying a film or filmed grid? Does specimen ultracentrifugation have merit? Can films with different qualities other than formvar, collodion, or carbon be developed? What are the different properties of various negative stains? Can better negative stains be developed? Can quality of NSEM stained grids be maintained; and for how long?

Today, in this presentation, I will share some of what I have learned about the above and other NSEM associated questions.

RUSKA

Modulation of Nuclear Shape by Substrate Rigidity

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Dept. Chemical Engineering, University of Florida, Gainesville, FL

The nucleus is mechanically coupled to the three cytoskeletal elements in the cell via linkages maintained by the LINC complex (for Linker of Nucleoskeleton to Cytoskeleton). It has been shown that mechanical forces from the extracellular matrix (ECM) can be transmitted through the cytoskeleton to the nuclear surface. We asked if substrate rigidity can control nuclear forces. By use of z-stack imaging on a confocal microscope, we found that the nucleus in NIH 3T3 fibroblasts undergoes significant changes in shape as the substrate rigidity is varied. On soft substrates (1 kPa), the nucleus appears rounded in its vertical cross-section, while on stiff substrates (396 kPa), the nucleus becomes flattened. Over-expression of dominant negative Klarsicht ANC-1 Syne Homology (KASH) domains, which disrupt the LINC complex, caused cell rounding and eliminated the sensitivity of nuclear shape to substrate rigidity; myosin inhibition had similar effects. KASH over-expression altered the rigidity dependence of cell motility and cell spreading.
Taken together, our results suggest that nuclear forces are modulated by substrate rigidity, and that a mechanically integrated nucleus-cytoskeleton is required for rigidity sensing. These results are significant because they suggest that substrate rigidity can potentially direct nuclear function and hence cell function.

MSA INVITED

Real World Microscopy: Problem Solving in the Paper Industry

Janet H. Woodward, Buckman USA

In the paper industry, paper breaks and the production of off-quality paper must be addressed quickly. The most basic microscopic tools and techniques often provide rapid and reliable solutions to these problems. Tools include the stereomicroscope and bright field light microscope. Basic techniques include a variety of staining methods: simple and differential staining of deposits, staining for fiber differentiation, and staining for starch. Cross sections of paper or board can be made with a razor blade for microscopic examination. All of these techniques can be done on-site. Other problems, such as coating defects or poor ink receptivity, require tools and techniques that are not available at a mill site. Conventional SEM, ESEM, BSE, EDX, and FTIR are routinely employed to address these and other “real world” problems in the paper industry.

POSTER

Stimulated Emission Depletion (STED) Microscopy and Pacific Orange Dye Optimization for H9c2 COX-1 Imaging via Indirect Immunocytochemistry

John Merriman
Biomedical Engineering, University of South Carolina – Columbia, SC

The fundamental barrier of traditional microscopy has always been the Abbe limit. Diffraction has served to limit image production and microscopic investigation on the sub-cellular level, greatly hindering microbiology and other forms of study. Stimulated Emission Depletion microscopy is one of many new frontiers of microscopy that has recently broken through this diffraction barrier. By utilizing multiple competing sources of light, STED has produced high-resolution, nanoscale images of both biological and non-biological samples, greatly adding to the wealth of knowledge in multiple disciplines. Despite these contributions, several obstacles remain for STED technology. These include pricing and availability of laser usage, as well as inherit qualities of component materials, such as photobleaching of standard fluorophores. Current research aims to create smaller, cheaper STED models capable of using improved dyes that withstand photobleaching.

The purpose of this study is to describe a simple method for standard biological imaging using a novel system software program. Pacific Orange dye was selected for imaging and tested against the STED system: compatibility and photobleaching tests yielded testing parameters for imaging. H9c2 rat embryonic myocardium heart cell samples were passaged and stained via indirect immunofluorescence: COX-1 proteins of the mitochondrial inner-membranes were targeted by primary mouse, anti-COX-1 IgG antibodies, which in turn were targeted by Pacific Orange conjugated secondary F(ab')2 fragment goat, anti-mouse IgG antibodies. Cell samples were identified via CCD camera and then imaged on the STED through the use of a novel the LabWindows/CVI computer program used to minimize testing time and photobleaching while collecting data for dye emissions under only the excitation beam was well as both the excitation beam and the STED beam. Collected data was then processed using Matlab to generate photon emission intensity plots of the cells. While improvement in the images was recognizable through the use of the added STED beam during testing, a large step size used during sample testing movement as well as other sources of error including bandpass filter selection may have prevented full realization of sub-confocal resolution levels. Overall, the experiment described in this report is the basis for an improved technique in biological imaging over standard confocal microscopy

How the Sausage is Made:
A Microanalyst's Time in the White House

John Henry J. Scott
National Institute of Standards and Technology

From late 2007 until 2009 I served as Senior Policy Analyst in the Office of Science and Technology Policy (OSTP) in the White House, a non-political position that gave me a very different view of the federal science and technology enterprise than the one I developed as a microanalyst working at NIST. As part of the George W. Bush and Obama administrations, my portfolio covered the DOE Office of Science, NASA's Science Mission Directorate, and the Mathematical and Physical Sciences at NSF.

During this talk I will describe the basics of the federal science budget process and how the White House helps to coordinate the efforts of many agencies with science and technology roles. Because of the emphasis on government transparency, much of this process is open to public inspection almost in real time -- if you know where to look. In addition to explaining the relevance of this process to the practicing scientist, engineer, or technologist, I will also explain how to learn more and discuss the many opportunities for scientists to participate in the process of informing policy decisions in your field. To lighten things up, I will include anecdotes, photos, and some fun facts about life inside the White House complex, including the exciting times surrounding a transition from one administration to the next.

Taphonomy of Flood Plain Sedges in the Olduvai Gorge, Tanzania

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The western basin, at the drier eastern edge of the Serengeti Plain, may have supported resident hominids, at least during wetter time periods. A better understanding of the seasonal patterns of early Pliestocene animals and plants in this region would help support known evidence of hominid activity discovered in the area. The condition of extant sedge fragments found in similar environmental conditions and exposed to animal pressures would help provide more information to supplement what is thought to be hominid activity in the region.

Cyperus fastigiatus is found in a seasonal wetland of the Seekoeivlei Nature Reserve, South Africa. Sedges are used as a wild plant food for foragers in the region. During the process of animals feeding and moving, these sedges are damaged and eventually buried in mud. The Seekoeleivei site was used because, as a nature reserve, it is protected from domesticated cattle and sheep and exhibits ecological characteristics thought to exist during the early Pleistocene in the Olduvai region.

Because of the resistance of the culm to degradation, fossilized pieces of sedge have been found in the Olduvai region in Tanzania. These fossils exhibit deformations and indications of early Pleistocene animal activity. The taphonomy of fossil sedges was evaluated using the extant species to determine damage and burial patterns. Examples of insect and animal deformations are shown in extant sedge and correlated with fossil sedge fragments to infer pre-burial deformation and damage. Types of inferred taphonomy include snap-broken edges and crimping from trampling, morphologies consistent with drying and varying stages of decomposition, insect damage, and root damage.

POSTER

Micromorphological Characters with Evolutionary Significance in *Scaphosepalum* (Orchidaceae)

Lorena Endara[1,2], Karen Kelley[3] & Norris Williams[1,2]

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2. Department of Biology, University of Florida, Carr-Bartram Hall, P.O. Box 118525, Gainesville, Florida, 32611
3. Electron Microscopy and Bioimaging, Interdisciplinary Center for Biotechnology Research (ICBR) University of Florida, P.O.Box 110700, Gainesville, Florida, 32611

Scaphosepalum is a genus of Neotropical orchids that encompasses 49 species. It belongs to the subtribe Pleurothallidinae that consists of ca. 4000 species and includes the smallest orchid known to science. *Scaphosepalum* is recognized from other pleurothallid orchid genera by osmophores located on the apex of the flower sepals, although this character is observed in its vestigial form in some species of the sister groups. The molecular phylogeny of *Scaphosepalum* consistently supports clades of species with extremely different morphotypes. Morphometric studies using macromorphological characters failed to find shared derived characters (synapomorphies) to support some clades and for this reason, micro-morphological characters were studied using fresh pickled floral material, critical point drying and Scanning Electron Microscopy (SEM). This method contributed greatly to find synapomorphies that were not found using light microscopy, more prominently unicellular hairs in bracts, and different types of hairs in the osmophores and lips that not only have an evolutionary significance that will be reflected in the classification system of this group, but also are directly related to the pollination system of this plants that attract flies. At a higher evolutionary level, the development of the stigma, the rostellum and the level of outgrowth of the column apex found with SEM support the generic circumscription of *Scaphosepalum* and the sister genera.

Correlative Light and Electron Microscopy - Bridging the Micro and Nano Worlds

Dr. Ben Tordoff
Carl Zeiss Microscopy, Cambridge, England

In the past, light and electron microscopy were two successful, yet often independently used technologies. Recently, more and more researchers want to work on the promising combination of these tools to gain new insights into the functionality and the associated ultra-structure of biological specimen. This is the field of “Correlative Microscopy”. An essential prerequisite is the precise retrieval of one and the same region of interest within the sample. As a leading supplier of light as well as electron microscopes, With “Shuttle & Find” Carl Zeiss offers a unique and easy-to-use interface bringing together the realms of wide-field light and scanning electron microscopy. This tool paves the way for making correlative light and electron microscopy part of everyday scientific research.

Shuttle & Find is used in different scientific areas like neurobiology and cancer research as well as in material sciences. In neurobiology typical applications are the investigation of the neuronal network. Proteins of interest are labelled with fluorescence markers. The fluorescence image is related to the ultra-structural image taken by an electron microscope. Functional information from the fluorescence light microscope is combined with ultra-structural information from the electron microscope. In addition to the ultra-structural information analytical information is available using an electron microscope. For example, different kinds of particles, are localized in the light microscope and after retrieving these particles in the electron microscope image, their composition can be analyzed using an EDX detector.

Helium Ion Microscopy – Advancements from 0.35 nm Imaging to Sub 10 nm Nanopatterning

Danielle Elswick
Carl Zeiss NTS, Peabody, MA 01960

The helium ion microscope (HIM) takes advantage of an atomically sharp source to emit a beam of focused He ions so the microscopist today can go beyond imaging resolutions achieved in the Scanning Electron Microscope (SEM). Imaging with ions rather than electrons offers many advantages including the ability to image uncoated non conducting samples at high resolution without damage. Additionally, helium ions can be used to sputter material for nanolithography and nanopatterning applications where sub 10 nm structures are desired.

A gallery of helium ion microscopy results will be presented to showcase the capbility and performance of this novel microscope. The HIM has proven invaluable at characterizing uncoated biological samples as well as other soft materials. Features sizes and material removal via conventional Ga FIB systems is now surpassed using HIM. The HIM-FIB has touched a wide array of applications that range from nanomachining pores for single molecule detection to patterning devices in graphene.

Advancements in Imaging Technology and Characterization for Transmission Electron Microscopy

Cory M. Czarnik
Gatan Inc. 5794 W. Las Positas Blvd. Pleasanton CA

TEM imaging options continue to rapidly evolve as increasing pixel count, higher frame rate, lower noise factor, and higher collection efficiency cameras are successively introduced based on the latest sensor and image transfer technologies. As the myriad of options expands, it becomes increasingly important to understand the inherent advantages and limitations of each technology, as well as the fundamental metrics for characterizing TEM cameras in order to identify the best camera for a given application on a cost / performance scale.

This talk will compare and contrast some of the fundamentals of TEM camera technologies available today including transfer mechanisms of lens-coupling vs. fiber-coupling vs. direct detection (DD) systems. Additional key details will contrast charge-coupled device (CCD), complementary metal-oxide-semiconductor (CMOS), and DD sensor performance and target applications. Finally, it will describe metrics and methods that can be used to compare performance between cameras including detective quantum efficiency (DQE) and modulation transfer function (MTF), as well as other critical aspects of camera performance.

RUSKA AWARD WINNERS

YEAR	RECIPIENT	INSTITUTION
BIOLOGICAL SCIENCES		
1972	Danny Akin	Univ. of Georgia
1973	John Wolosewick	Univ. of Georgia
1974	Murray Bakst	Univ. of Georgia
1975	William Henk	Univ. of Georgia
1976	Durland Fish	Univ. of Florida
1978	Dwayne Findley	N.C. State University
1979	Glen Watkins	N.C. State University
1979	John Weldon	Univ. of Georgia
1980	Michael Dresser	Duke University
1982	Mark Rigler	Univ. of Georgia
1982	Chris Sunderman	Univ. of Georgia
1983	Patricia Jansma	Univ. of Georgia
1985	Mark Brown	Univ. of Georgia
1986	Judy King	E. Tenn State Univ.
1986	Peter Smith	Clemson University
1987	Robert Roberson	Univ. of Georgia
1988	Rajendra Chaubal	Univ. of Georgia
1989	Josephine Taylor	Univ. of Georgia
1990	Chi-Guang Wu	Univ. of Florida
1991	Karen Snetselaar	Univ. of Georgia
1992	Yun-Tao Ma	Clemson University
1992	Theresa Singer	Univ. of Georgia
1993	Julia Kerrigan	Univ. of Georgia
1994	John Shields	Univ. of Georgia
1994	Meral Keskintepe	Univ. of Georgia
1995	Katalin Enkerli	Univ. of Georgia
1996	Rhonda C. Vann	MS State University
1998	Timothy Wakefield	Auburn University
1999	Wendy Riggs	Univ. of Georgia
2000	Gail J. Celio	Univ. of Georgia
2001	Joanne Maki	Univ. of Georgia
2002	Rocio Rivera	Univ. of Florida
2003	Patrick Brown	Univ. of Georgia
2003	Heather Evans	Univ. of S.C. Med.
2005	Janet R. Donaldson	MS State University
2006	Sangmi Lee	MS State University
2007	Jennifer Seltzer	MS State University
2008	Katherine Mills-Lujan	Univ. of Georgia
2009	Shanna Hanes	Auburn University
2010	Kirthi Yadagiri	Clemson University
2011	Maria Mazzillo Mays	Auburn University
PHYSICAL SCIENCES		
1981	Michael Short	West Georgia College
1989	Graham Piper	Clemson University
1992	Kerry Robinson	Clemson University
1997	K. J. Aryana	MS State University
2007	Tao Wu	Georgia Tech

Distinguished Scientists

Jerome Paulin	1984
Ben Spurlock	1985
Ivan Roth	1986
Gene Michaels	1987
Sara Miller	1991
Raymond Hart	1993
James Hubbard	1995
Charles Humphrey	1996
Johnny L. Carson	2000
W. Gray (Jay) Jerome III	2000
Charles W. Mims	2001
Danny Akin	2002
Robert Price	2003
E. Ann Ellis	2008
Glenn Cohen	2010
Robert Simmons	2011

Distinguished Corporate Members

Harvey Merrill	1989
Charles Sutlive	1989
Ted Wilmarth	1989
Ray Gundersdorff	1997
Charles and Betty Sutlive	2000
John Bonnici	2002
Doug Griffith	2007
Robert Hirche	2008
Ron Snow	2009
Al Coritz	2011

Roth-Michaels Teaching Award

James Sheetz	2005
Charles Mims	2006

PRESIDENTS/CHAIRPERSONS

1972-73 Walter Humphreys
1973-75 Jim Hubbard
1975-76 Edward DeLamater
1976-77 Eleanor Smithwick
1977-78 Gene Michaels
1978-79 Edith McRae
1979-80 Jerome Paulin
1980-81 Ken Muse
1981-82 Mary Beth Thomas
1982-83 Jack Munnell
1983-84 Sara Miller
1984-86 Ray Hart
1986-87 Glenn Cohen
1987-88 Gerry Carner
1988-89 Danny Akin
1989-90 Johnny Carson
1990-91 Janet Woodward
1991-92 Charles Mims
1992-93 Charles Humphrey
1993-94 Sandra Silvers
1994-95 JoAn Hudson
1995-96 Jay Jerome
1996-97 Mark Farmer
1997-98 Robert Simmons
1998-99 Robert Price
1999-2000 Buddy Stephens
2000-01 Jim Sheetz
2001-02 Glenn Cohen
2002-03 Charles Mims
2003-04 Greg Erdos
2004-05 John Shields
2005-06 Judy King
2006-07 Johnny Carson
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2008-09 Giselle Thibeadeau
2009-10 Robert Price
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