

Proceedings of the Southeastern Microscopy Society

Embassy Suites Golf Resort &

Conference Center

Greenville, SC

May 22-24, 2013

Annual Meeting

of the

Southeastern Microscopy Society

Volume 33

ISSN 0149-7887

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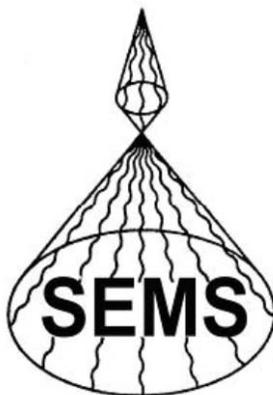
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Acknowledgements

As an affiliate of MSA and MAS we benefit by support for MSA and MAS invited speakers and meeting expenses.

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

Corporate Sponsors and Exhibitors for the meeting as of this printing:

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Dear SEMS Members,

From Cocoa Beach, FL in 2012 we journey to Greenville, South Carolina to meet, to learn, to visit and to inspire one another with our research and our dedication to microscopy. Entwined with our daily routine is the art and science of microscopy; very few analytical techniques depend so heavily on the beautiful images that can be produced with the microscope. When I present microscopy to young people, teachers and to my customers, I always mention that the great thing about being a scientist that uses a quiver of microscopes to investigate a problem is that I get to see things through the microscope that very few people (and sometimes no one) has ever seen before!

The theme of this year's meeting is "Back to Basics". The idea is to present our work and ideas with an emphasis on the proper use of the techniques that inspired the work and led to the results. I hope in this way we can energize our younger members and recapture a bit of the wonder and excitement that occurs when using the microscope to investigate and discover new things.

The core group of the SEMS executive council has once again come together to provide a variety of presentations at a wonderful location. Two workshops on Wednesday will highlight electron microscopy and light microscopy at two different locations on the Clemson campus. Our corporate sponsors once again have committed their time and resources to support our organization.

Following the workshops is the evening poster session and Corporate Sponsor Mixer. This will be a great time to see some great research displayed, get reacquainted with old friends and of course make new ones, and revisit the vendor's exhibits. Be sure to take this opportunity to tell the vendors thanks for all their contributions to our meeting.

Our next two meeting days will be filled with scientific presentations given by a host of internationally recognized invited speakers, a number of our own members and of course those participating in the Ruska student competitions. This mix of presentations shows the strength and diversity of the science of microscopy.

I want to thank each of you for attending and participating in SEMS 2013. I am excited about this year's meeting venue and hope to see each and everyone one of you there. Enjoy the meeting!

Rich Brown, SEMS President 2013

SEMS 2013 PROGRAM

WEDNESDAY AFTERNOON, MAY 22

REGISTRATION - 9AM TO 5 PM

GRAND BALLROOM -DORA

1pm – 5pm Commercial Exhibits

GRAND BALLROOM -DORA

12:00-1:30 Executive Council Mtg and Lunch

ASHVILLE

WORKSHOPS

Transportation by 1:30 pm from the Embassy Suites Hotel- Meet at the SEMS registration desk prior to travel to two campus locations at Clemson University.

Leica Imaging Suite: Sample techniques for creating high resolution images: tips and tricks for great confocal and super resolution sample preparation" - at the **Clemson Light Imaging Facility**.

Tour sponsored by Hitachi and Workshop sponsored by Protochips: A Semiconductor-Based Heating and Electrical Biasing Platform for High-Resolution in situ Electron Microscopy at the **Advanced Materials Research Laboratory, Clemson**.

6:00PM – 8:00PM CORPORATE MIXER

GRAND BALLROOM -DORA

THURSDAY MORNING, MAY 23

Registration – 9am to 5pm

GRAND BALLROOM -DORA

9:10am Opening Remarks – *Rich Brown, President*

9:20 Mantle Metasomatism as Revealed by WDS and EDS X-Ray Maps of Mantle Xenoliths from Southeastern China

Donggao Zhao, University of Texas at Austin, Austin, TX

9:40 Electron Microscopy Sample Preparation and Experiments in a Capsule

Steven L. Goodman, Microscopy Innovations, LLC, Marshfield, WI

10:00-10:20 BREAK (PLEASE VISIT EXHIBITORS) GRAND BALLROOM -DORA

RUSKA Competition

Moderator – Russ Goddard

10:20 Structural and Ultrastructure Analysis of Decellularized Sheep Vaginal Tissues

S. Patnaik, B. Brazile, B. Weed, A. Lawrence, D. Christiansen, P. Ryan, J. Liao
Mississippi State University, Mississippi State, MS.

Contributed Session

10:40 Electron Diffraction Analysis of Mollusk Shells in the SEM

Scott Sitzman, Oxford Instruments America Inc., Pleasanton, CA

11:00 Analytical TEM for Soft and Hybrid Nanomaterials

Jihua Chen, Oak Ridge National Laboratory, Oak Ridge, TN

11:20 – 1:00 LUNCH

SEMS 2013 PROGRAM

THURSDAY AFTERNOON, MAY 23

PRESENTATIONS COLUMBIA/CHARLESTON Moderator: Cynthia Goldsmith

1:00 **INVITED SPEAKER - The Intelligence of the Living Cell**
Brian J. Ford

2:00 **Structure/Function Modifications of Human Nasal Epithelium Associated
with Lifestyle Exposure to Tobacco Smoke**
Johnny L. Carson, The University of North Carolina at Chapel Hill, Chapel Hill,
NC

2:20 **Cryo-EM Structure of the Actin-Tropomyosin Filament**
Duncan Sousa, Florida State University, Gainesville, FL

2:40 **Electron Microscopy & Spectroscopy Studies of YSZ, Ni-YSZ, Ni-alloy, Pure
and doped CeO₂ and LaSrMnO₃ materials useful in Solid Oxide Fuel Cell**
Laxmikant Saraf, Clemson University, Clemson SC

3:00 – 4:00 **BREAK (VISIT EXHIBITORS)** GRAND BALLROOM -DORA

4:00 **INVITED SPEAKER - Basic Digital Imaging and Digital Image Formats**
Jay Jerome, Vanderbilt University, Nashville, TN

6:00-7:00 **SOCIAL** GRAND BALLROOM -DORA

7:00-9:00 **BANQUET** PALMETTO CLUB

SPECIAL LECTURE

BRIAN J. FORD – “THE FIRST MICROSCOPES: WHAT COULD THEY TRULY REVEAL?”

SEMS 2013 PROGRAM

FRIDAY MORNING, MAY 24

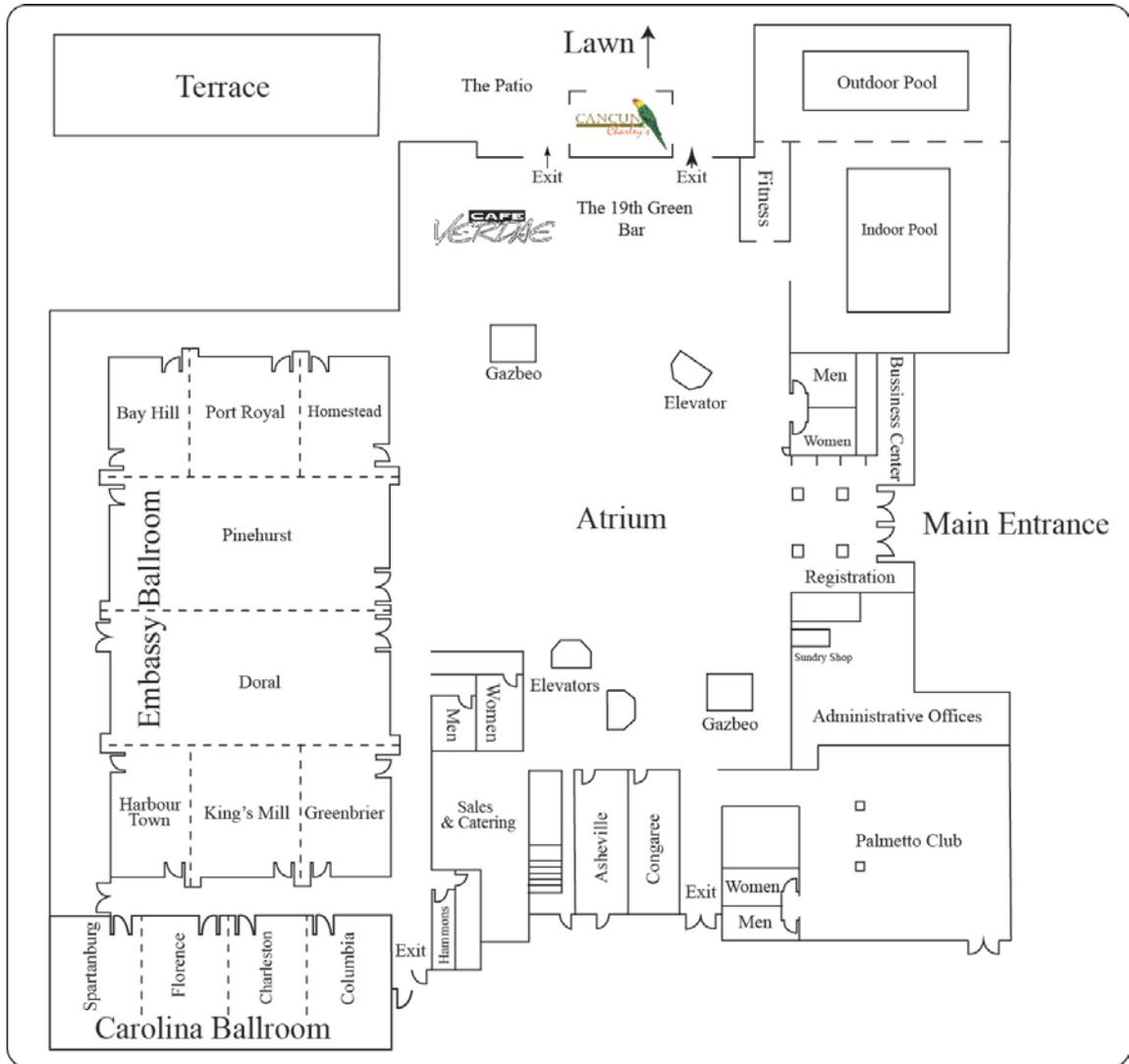
9:00-10:30AM **BUSINESS BREAKFAST** **LOCATION: PALMETTO ROOM**

PRESENTATIONS COLUMBIA/CHARLESTON **Moderator:** -- Jay Jerome

10:30am **Extending Synchrotron X-ray Microscopy to the Laboratory:
Applications for Materials and Life Sciences in 3- and 4-Dimensions**
A.P. Merkle, Xradia Inc., Pleasanton, CA

10:50am **Atom Probe Specimen Preparation using the FIB/SEM**
Richard Martens, Central Analytical Facility, Univ. of Alabama, Tuscaloosa, Al

11:10AM **CLOSING REMARKS:** --, PRESIDENT-ELECT JAY JEROME



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

Columbia/Charleston
 Ballroom - Doral
 Columbia/Charleston
 Asheville
 Ballroom - Doral
 Ballroom - Doral
 Ballroom - Doral
 Palmetto Club
 Palmetto Club
 Ballroom- Doral

Structure/Function Modifications of Human Nasal Epithelium Associated with Lifestyle Exposure to Tobacco Smoke

Johnny L. Carson

Department of Pediatrics and Center for Environmental Medicine, Asthma, and Lung Biology, The University of North Carolina at Chapel Hill, Chapel Hill, NC

The mammalian conducting airways represent a primary interface of interaction of the ambient air with a vital mucosal surface. The airway epithelial surface is a highly organized consortium of cells that mediates secretion, ciliary function, transepithelial permeability, and immune function. At the same time, this surface is highly vulnerable to structural and physiologic modification in ways that may lead to adverse health effects as a function of exposure to environmental contaminants present in the inspired air. Tobacco smoke contains thousands of potentially injurious chemicals and a subset of these is known to cause cancer. This study examined both structural and functional modification of human nasal epithelium associated with lifestyle exposure to tobacco smoke.

This study was reviewed and approved by an institutional review board (IRB) and all subjects gave informed consent prior to participation. Non-smoking and smoking subjects self-identified themselves as generally healthy and without any diagnosis of respiratory disease. Tobacco smoke exposure was documented by urine cotinine assay. Subjects also were assayed for nasal nitric oxide (NO) concentration and were subjected to a non-invasive biopsy of the nasal mucosa lining the inferior turbinates. Epithelial biopsies were assayed for ciliary beat frequency (CBF) and processed for examination by transmission electron microscopy or transitioned to air-liquid interface culture.

Although previous studies of *in vitro* experimental exposure of human ciliated epithelium to cigarette smoke condensate demonstrated marked but reversible suppression of CBF, assessments of CBF documented statistically significant increases in CBF among smokers and non-smokers exposed by lifestyle to environmental tobacco smoke. Assessments of NO in these subjects indicated that nasal NO, a known regulator of ciliary beat frequency, also was elevated among smokers. Ultrastructural evaluation of nasal epithelium from smokers typically revealed normal ciliary membrane organization, a finding consistent with normal or accelerated CBF. However, smoker biopsies routinely demonstrated a noteworthy leukocytic infiltrate and fragmentation of tight junctional complexes findings consistent with previous experimental studies of human air pollutant exposure.

Acceleration of CBF among individuals exposed by lifestyle to tobacco smoke is likely an NO-driven mechanism. Moreover, the fragmentation of junctional complexes suggests that the tobacco smoke milieu coupled with migration of leukocytic cells through the epithelium may create subtle structure/function modifications that may be indicative of early pathologic changes consistent with the emergence of adverse health effects.

This study was supported by a Clinical Innovator Award to the author from the Flight Attendant Medical Research Institute.

Analytical TEM for Soft and Hybrid Nanomaterials

Jihua Chen

Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge, TN

Soft and hybrid nanomaterials represent an important class of cutting-edge materials for future generation technologies including healthcare, energy storage and conversion. Soft materials and their related hybrid systems often suffer from high electron beam sensitivity and low contrast in transmission electron microscopy (TEM) study due to their weak binding forces and low atomic numbers. To tackle these issues, we pursue low-dose, energy filtered TEM which is apply to synthetic polymers, block or branched copolymers, organic crystals, biological and bio-inspired materials, nanocomposites, and other hybrid systems. In this talk, I will focus on our efforts in (1) organic photovoltaic cells (OPVs) and (2) solid polymer electrolytes (SPEs). In each topic, low-dose electron diffraction and energy filtered TEM experiments are performed and we use these results to correlate fabrication methods, device performance and nanomorphology. For OPVs, vertical and planar phase separation of donor and acceptors are investigated with low eV plasma peak and three-window based elemental mapping of carbon and sulfur. For SPEs, quantitative fluorine and oxygen maps are used to study nanoscale salt/ polymer distribution.

POSTER

Nanomorphology- and Polymorphism- Dependent Charge Transport in Solution Crystallized Organic Semiconductor Systems Examined by Low-Dose, Energy Filtered TEM

Jihua Chen

Center for Nanophase Materials Sciences, Oak Ridge National Laboratory

The Center for Nanophase Materials Science (CNMS) recently established imaging capabilities for soft and hybrid nanomaterials with a newly acquired Zeiss Libra 120. This enabled low dose, energy filtered imaging of block and branched copolymers, organic semiconductors and blends, organic solar cell systems, surfactant covered nanoparticles, biological and biomimetic structures, hybrid nanomaterial interfaces and assemblies. Here we use two studies of high performance, solution processed organic semiconductor systems as examples to showcase some possible important applications of soft material, analytical TEM.

In the first example, the effect of solvent choice on the thin film polymorphism of 5,11 bis (triethylsilylethynyl) anthradithiophene (TES ADT) is examined with low-dose electron diffraction and molecular simulation. A new thin-film polymorph of TES ADT was identified which has a gamma angle of 90 degree and a unit cell that is 9 times larger than the most common triclinic TES ADT polymorph. The newly identified thin-film polymorph yields a hole mobility up to 25 times higher than that of the most common thin film polymorph. In the second example, 6,13-bis (triisopropylsilylethynyl) pentacene (TIPS Pentacene)/ conjugated and nonconjugated polymer blends are compared as a function of polymer choice. Energy filtered TEM and selected area electron diffraction was used to study the solution-based crystallization and assembly. A conventional wisdom is that the crystallization of highly crystalline small molecule dominates the self-assembly process of the TIPS pentacene/polymer blends. Here we show that the intermolecular interactions of TIPS pentacene and different polymers tune the vertical and lateral phase separation, leading to novel and potentially useful micro- and nano-structures.

POSTER

A Comparison of Two Techniques for Observing Hair Cuticles

Glenn M. Cohen

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

The cuticle, the outer surface of hair, is best viewed as an air mount, i.e., without histological processing or the use of mounting media and cover slips, because the refractive indices of mounting media (1.5 to 1.55) are similar to those of hair (1.543 to 1.554) and obscure the surface features of hairs. Crocker (*Microscope* 46:169-173, 1998) developed a simple and rapid method for examining the cuticle. He affixed hair to microscope slides with transparent two-sided tape. The technique works best for lightly colored hairs with narrow medullary cores. However, darkly pigmented hairs and hairs with large medullary cores obscured the overlying cuticular patterns. Alternatively, Evans (*J. Forensic. Sci. Soc.* 4:217-218, 1964) affixed hairs to a front surface mirror and observed the cuticular patterns with incident (epi) illumination. In the present study, aluminum foil (shiny side up) substituted for a front surface mirror. The foil was affixed to the slide with two-sided tape. Then, a polarizing filter was inserted in the light path of the epi illuminator to reduce glare and harsh contrasts between the crown and sides of the hair. The present study confirmed and extended the strengths and weaknesses of the two aforementioned techniques. Because these two techniques are non-destructive, hairs can be removed from the tape after observation and immediately processed using routine histological procedures. (Supported in part by a Faculty Development grant from Troy University.)

INVITED SPEAKER

The Intelligence of the Living Cell

Brian Ford

Where does intelligence originate? Back in the 1970s, Brian introduced the idea that microbes showed the roots of intelligent and sentient behavior. In his book *MICROBE POWER* which in 1976 was widely enjoyed in America and other countries, Brian argued that single cells show remarkable abilities which we did not understand.

Over the following decades he has observed single cells performing remarkable acts that reveal their ingenuity. Today Brian will show us algae that can take decisions and single-celled organisms that build homes for themselves and which can tell when tiny components are the wrong way up. This unforgettable presentation will ensure you never look at microbes in the same way again—and it also looks afresh at the way in which human brain cells behave.

By analyzing the traces that neurons emit, Brian shows how we can eavesdrop on the private language of brain cells. He will demonstrate recordings of the modulations hidden within the 40 Hz signals known as 'neuron spike' recordings and offer us all a revolutionary new way of understanding the brain.

This extensively illustrated presentation reveals microbes from every walk of life, and sets them in juxtaposition with accounts regularly broadcast on television. We will never look at living cells in the same way again.

Microscope Imaging for Comparative Analyses of Mammalian Gametes

JM Feugang¹, W Monroe², C I-Wei², A Lawrence², S Patnaik³, R Marcec⁴, CJ Langhorne⁴, RC Youngblood¹, CD McDaniel⁵, RM Hopper⁶, ST Willard⁴, PL Ryan^{1,6}
Departments of ¹Animal and Dairy Sciences, ³Agricultural and Biological Engineering, and ⁴Biochemistry, Molecular Biology, Entomology, and Plant Pathology, ⁵Poultry Science; ⁶Pathobiology and Population Medicine; ²Institute for Imaging and Analytical technologies; Mississippi State University, Mississippi State, Mississippi

Understanding the complexities of gamete biology through *in vitro* analyses is paramount for the success of assisted reproductive technologies, and gamete morphology and quality are important candidates for such evaluation. There are many ways of looking at gametes and capturing their morphological properties; nonetheless, the preparation steps for their visualization remain important decision-makers for the choice of appropriate investigative techniques. Here, we used different microscope imaging approaches to picture gametes of various species for comparative analyses.

Immature cumulus-oocyte complexes (COCs) were aspirated from follicles of post-mortem pig ovaries and matured *in vitro*. Spermatozoa were obtained from fresh semen of boar, stallion, rooster, frog, and salamander. Both COCs (mature and immature) and pig spermatozoa were fixed (4% paraformaldehyde) for immunoprotein detection using FITC-conjugated secondary antibody, followed by their visualization with a confocal laser scanning microscope (CSLM, Zeiss). Spermatozoa of all abovementioned males were prepared for scanning electron microscopy analysis (SEM, JEOL-JSM) using the standard protocol, while other sample subsets were fixed with 4% paraformaldehyde, spread on microscope slides, and air-dried for atomic force microscopy analysis (AFM, Dimension Icon AFM with ScanAsyst).

The CSLM provided high resolution images showing immunofluorescence detection of relaxin protein and its receptors RXFP1 and RXFP2 in pig oocytes and spermatozoa.

Visualization of fixed-spermatozoa on microscope slides indicated possible morphological and topographical analyses that could not be assessed with the CSLM.

The SEM approach provided ultrastructure images with great resolution (6,000X to 12,000X) that allowed basic interpretation of sperm morphology and ultrastructure. The AFM technique revealed images of spermatozoa that were scanned at the nanoscale level, in their “native” structure due to the preliminary fixation with paraformaldehyde.

Interestingly, AFM offered the possibility for qualitative and quantitative analyses of sperm morphology and topography that would permit inter-species comparisons.

In conclusion, the application of traditional microscopic approaches to study mammalian gametes is still essential, and AFM appears as a remarkable tool for gamete analyses at low cost and a faster technique than the SEM. Inter-species analyses using AFM in our study will undoubtedly contribute to a better understanding of adaptive characteristics of spermatozoa to their environments. Supported by USDA-ARS Grant#58-6402-3-0120

Electron Microscopy Sample Preparation and Experiments in a Capsule

Steven L. Goodman

Microscopy Innovations, LLC, 213 Air Park Road, Suite 101, Marshfield, WI 54449-8626

Microscope specimen preparation and TEM grid handling processes have not fundamentally changed in decades. Most processes still require extensive manual handling of one specimen or one grid at a time, while most processing consumes significantly more reagent than necessary.

This presentation will describe the mPrep™ System, a new platform for preparing specimens and for performing “experiments in a capsule.” The System enables flexible and efficient processing, handling, and archiving of TEM and SEM specimens, and grids. The System is capsule-based with each specimen or grid placed in its own labeled mPrep capsule at the time of initial preparation, where it is generally only removed from the capsule to place on the microscope stage. Continuous labeling provides facile integration into lab information management systems to meet GLP, CLIA and other regulatory requirements. Capsules also function like pipette tips, thereby leveraging the capability of inexpensive lab pipetters and other biology labware, to deliver precise reagent volumes to process one or many dozens of specimens or grids in parallel, with the same or different reagents. This makes complex processing such as immuno-labeling easy and reproducible. Additionally, mPrep capsules may be used like microtiter wells for experiments that would heretofore be done in 96-well plates, Petri dishes or similar vessels for cell and tissue culture or other studies. This enables complex cell culture and other fluid processing studies to be done in one vessel from beginning to end, including microscopy, thus enabling “experiments in a capsule.”

POSTER

A Novel Sample Preparation Method That Enables Nucleic Acid Analysis from Ultrathin Sections

Vincent P. Klink¹, Giselle Thibaudeau,² Ronald Altig¹, and Amanda Lawrence²

¹Department of Biological Sciences, Mississippi State University, Mississippi State, MS 39762, USA, ²Institute for Imaging & Analytical Technologies, Mississippi State University, Mississippi State, MS 39762, USA

The ability to isolate and perform nucleic acid analyses of individual cells is critical to studying the development of various cell types and structures. We present a novel biological sample preparation method developed for laser capture microdissection-assisted nucleic-acid analysis of ultrathin cell/tissue sections. We used cells of the mitotic bed of the tadpole teeth of *Lithobates sphenoccephalus* (Southern Leopard Frog). Cells from the mitotic beds at the base of the developing tooth series were isolated and embedded in the methacrylate resin, Technovit®9100®. Intact cells of the mitotic beds were thin sectioned and examined by bright-field and transmission electron microscopy. The cytological and ultrastructural anatomy of the immature and progressively more mature tooth primordia appeared well preserved and intact. A developmental series of tooth primordia were isolated by laser capture microdissection (LCM). Processing of these cells for RNA showed that intact RNA could be isolated. The study demonstrates that Technovit® 9100® can be used as an embedding medium for extremely small tissues and from individual cells, a prerequisite step to LCM and nucleic-acid analyses. A relatively small amount of sample material was needed for the analysis, which makes this technique ideal for cell-specific analyses when the desired cells are limited in quantity.

POSTER

Integrated Microscopy in the Study of Fern Root Development

Guichuan Hou

Department of Biology and Dewel Microscopy Facility, Appalachian State University
Boone, NC28608-2027

One of my research goals is to establish a comprehensive understanding of the root developmental mechanisms in the fern. I have been using homosporous fern *Ceratopteris richardii* as a model system because this species has a track record of successful stories in exploring many different biological questions. Among several approaches utilized in my research, integrated microscopy plays a pivotal role in the investigation of fern root development. Time-lapse optical microscopy has been used to study root growth kinetics. Conventional histology is useful both in tissue pattern visualization and cell lineage analysis in the fixed roots. Laser scanning confocal microscopy is instrumental in investigating the functions of microtubules in cell division and root structural pattern formation. It will also serve as an essential tool for studying gene functions, such as hormone auxin related genes in the root development. Both scanning and transmission electron microscopies have been used in my research on the fern root development. The electron tomography provides much more information of the subcellular structures in the fern root meristem. The purpose of this presentation is to discuss the past, current, and future applications of integrated microscopy in the study of plant root biology.

INVITED SPEAKER

Basic Digital Imaging and Digital Image Formats

W. Gray (Jay) Jerome

Department of Pathology, Microbiology and Immunology and Department of Cancer Biology,
Vanderbilt University, Nashville, TN

Most microscopy now relies on digital imaging to capture and store microscopic information. To fully utilize the capabilities of today's modern microscopes, a microscopist must understand the basics of digital imaging and digital image storage formats. Digital imaging devices have become remarkably easy to use but this masks the many ways in which incorrectly using digital imaging can destroy or distort your data. The field of "scientific" digital imaging is only a small subset of digital imaging. There are lots of things you can do in digital imaging that you should not do in scientific imaging because it perverts the integrity of your data; the image. Unfortunately, with modern digital imaging it is far too easy to inadvertently alter the image without even knowing that you have done so. In this talk, I will review the basics of a digital image and discuss how to match the microscope parameters and image capture parameters in order to maximize image fidelity. We will also discuss post image processing and how these can affect the image data. Finally, the basics of image formats are critical to maintaining the veracity of your data, yet how these formats affect your data are not always understood by microscopists. For this reason, I will include a discussion of the appropriate uses of these formats. Your image is your data. The key to good digital microscopy is understanding that small errors in basic "scientific" digital imaging can lead to accumulation of artefactual errors in your data.

POSTER

A Virtual Microscope for Use in Online and Onsite Biology Labs

T. Kawakami¹, G.M. Cohen², and J Zhong¹

¹Department of Computer Science, Troy University, Troy, AL 36082 and ²Department of Biological and Environmental Sciences, Troy University, Troy, AL 36082

Distance learning has expanded and redefined the educational environment by offering access to almost every discipline independently of time and location. However, conceptual subjects are more successfully presented than those requiring equipment and/or manual skills. For this reason, virtual microscopes must provide equivalent function to simulate their laboratory counterparts. In the present study, we improved and extended three features of our earlier version of the virtual microscope so that it more closely corresponds to the functions of student microscopes. First, we updated the background technology for controlling 3D rotation to provide users with interactive use of the virtual microscope. Second, we added a blurring algorithm on downloaded images to simulate focusing, thereby allowing the focusing (blurring and deblurring) of the image. Third, we added labels with pointers to identify specific structural details of the specimen, along with a short explanation of their cellular functions. The labeling features can also be used for assignments and testing. In short, we have developed a virtual microscope that closely corresponds to the features and operations of student microscopes. (Funded in part by a Troy University Faculty Development grant to G.M. Cohen.)

POSTER

Specialized Setae and Spongiform Lobes in *Strumigenys* Ants (Hymenoptera: Formicidae: Dacetini)

Joe A. MacGown and Richard L. Brown

Mississippi Entomological Museum, Mississippi State University Box 9775, Mississippi State, MS 39762

Scales and squamiform setae are descriptive terms for non-homologous modifications of unicellular setae. Although "scales" are best known in Lepidoptera, flattened setae have evolved independently throughout other Hexapoda, including the Collembola, Apterygota, Homoptera, Psocoptera, Phthiraptera, Coleoptera, Diptera, and Hymenoptera. Scales in Lepidoptera are involved in a variety of functions, but functions of scales within other insect orders are largely unknown. In Hymenoptera scales are present in *Strumigenys* Smith (Formicidae: Dacetini), but the functions of these scales in these ants are unknown. *Strumigenys* is a monophyletic genus of dacetine ants that includes over 900 species worldwide. Forty-eight described species of *Strumigenys* have been reported from the US, but this genus is most speciose in the Southeast where 43 species have been reported. In the US, members of the genus *Strumigenys* can easily be distinguished from other genera by their minute size; 4-6 segmented antennae; elongate, snapping mandibles; unique and often "bizarre" pilosity, and presence of "spongiform lobes" beneath the petiole and postpetiole. The objectives of this research included the following questions. 1) What is the fine structure of the scales and "bizarre pilosity" and how does it vary between species groups of *Strumigenys*? 2) Is the distinctness of *Strumigenys* supported by characters of the body pilosity? 3) What is the fine structure of "spongiform tissue?" Answers to these questions also can contribute to answering the general question of why flattened setae have evolved so many times among insects.

Atom Probe Specimen Preparation using the FIB/SEM

Richard Martens, Central Analytical Facility, Univ. of Alabama, Tuscaloosa, Al

The Atom Probe microscope (AP) is a unique analytical tool capable of three dimensional atomic scale analysis of materials. The technique requires a needle-shaped tip with a radius of curvature of ~100 nm so that the electric field applied by the AP is sufficiently high enough for field evaporation of atoms from the material's surface. To fabricate these needles, electropolishing was historically used. Consequently, metallic samples were needed and capturing a region of interest, such as a grain boundary, was daunting. With the advent of the focused ion beam, (FIB), site specific atom probe specimen needles can be fabricated from a variety of new materials, and site specific areas can be selected for analysis.

New instrumentation in both AP and the FIB/SEM have allowed a wide range of materials to be prepared for analysis, including insulators, ceramics and even geological samples [1]. The FIB/SEM has allowed for site specific areas of materials to be selected for analysis, such as grain boundaries and site specific device analysis of integrated circuits (IC) [2]. The FIB lift-out technique is currently the most common method for preparing specimens for AP analysis [3].

This presentation will discuss the key instrument advances in atom probe and the procedures of preparing atom probe specimens using the FIB/SEM procedure, with particular emphasis towards capturing regions of interest, such as grain boundaries. Gallium ion implantation, sequential milling parameters and lift out and mounting procedures will also be discussed.

[1] *Microsc. Microanal.* 6 vol. 13 (2007) 407-518.

[2] Michael Miller et al., *Microsc. Microanal.* 6 vol. 13 (2007) 428-436.

[3] D. Lawrence et al., *Microsc. Microanal.* 12 (Supp 2) (2006) 1742.

Extending Synchrotron X-ray Microscopy to the Laboratory: Applications for Materials and Life Sciences in 3- and 4-Dimensions

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3D x-ray microscopy has emerged as a powerful imaging technique that obtains information from a range of materials under a variety of conditions and environments. Recently, laboratory-based x-ray sources have been coupled with high resolution x-ray focusing and detection optics from synchrotron-based systems to acquire tomographic datasets with resolution down to 50 nm [1]. This represents an improvement of at least one order of magnitude in true spatial resolution over the limits of conventional laboratory computed tomography (CT) techniques. This talk will explore both the details of the optics involved in such a laboratory-based x-ray microscopes but also several important applications examples of how x-ray tomography has been used as a complement to electron and optical microscopy investigations.

Observing the evolution of microstructure on the identical region of a single sample can rapidly benefit materials modeling techniques, by avoiding the requirement to extrapolate based on statistical samplings from a large number of like specimens. This is largely a unique capacity of x-ray tomography and several examples of in situ and '4D' experiments will be presented, including crack propagation in ceramics, porosity and permeability characterization, deformation of polymer foams under load and the evolution of defects in battery anode materials in Lithium ion batteries [2].

Soft materials, ranging from polymers to biological tissue, consistently pose challenges in generating contrast by several techniques, x-ray absorption included. We demonstrate the application of both phase propagation and Zernike phase contrast techniques on such materials, including polymer electrolyte fuel cells [3] and superconducting materials. Finally, the workflow employing 3D & 4D x-ray microscopy as a complementary step prior to complementary high-resolution 2D and 3D techniques will be discussed.

References

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Structural and Ultrastructure Analysis of Decellularized Sheep Vaginal Tissues

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2. Animal and Dairy Sciences, Mississippi State University, Mississippi State, MS.

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Congenital female genital defects such as cloacal malformations and Mayer–von Rokitansky–Kuster–Hauser (MRKH) syndrome can result in a vagina that is stenotic or short, causing sexual pain and diminished quality of life in the patient. Treatments include vaginoplasties which use autologous skin grafts and sections of intestine. Tissue engineered materials have been proposed as an alternative which has regenerative potential, while not requiring the harvesting of autologous tissues. We thus investigate the potential of decellularized sheep vaginal wall tissue as a potential scaffold for tissue augment and regeneration. It is well known that decellularization protocols have a profound effect on the ultrastructure, biomechanical behavior, and performance of the acellular scaffold product. In this study, we thoroughly characterized the ultrastructural and biomechanical properties of the acellular vaginal wall tissues generated by different decellularization methods.

Sheep vaginal wall tissues were obtained from a commercial abattoir. Specimens were dissected to 20 x 20 mm squares and subjected to one of three different decellularization protocols. The decellularization solutions consist of a stock solution (0.2% EDTA, RNase, DNase and phenylmethanesulfonyl fluoride (PMSF)) with one of the following decellularizing agents: (1) 0.1% sodium dodecyl sulfate (SDS), (2) 0.5% Trypsin, or (3) 1.0% Triton X-100. Specimens were treated with the decellularization agents for either 48 or 96 hours, and stored in PBS for further analyses after thorough PBS rinsing. Tissues were then subjected to biaxial mechanical testing using a custom biaxial system. For ultrastructural analysis, specimens were subjected to series of alcohol gradients, osmium tetroxide fixation and chemically dried overnight in Hexamethyldisilazane (HDMS) solution. Samples were loaded onto aluminum stubs using carbon paste and sputter coated with platinum (30um). Samples were then viewed using JEOL JSM-6500F Field Emission Scanning Electron Microscope (SEM) with secondary electron detectors (SEI), and the voltage was set to 10-12 keV.

SEM results show an alteration in tissue ultrastructure following decellularization. Biaxial mechanical results show important changes in tissue mechanical response following decellularization. Overall, Trypsin treatment appears to be more disruptive than other methods. SDS and Triton treated specimens appeared to be better preserved than Trypsin treated specimens. For native vaginal wall tissue, circumferential direction exhibits stiffer stress-strain behavior when compared to longitudinal direction; however, we notice that this trend is reversed in all the decellularized tissues, which warrants future study for better understanding this mechanical behavior change, and how this will affect the future applications.

Cryo-EM Structure of the Actin-Tropomyosin Filament

Duncan Sousa, Florida State University, Gainesville, FL

Tropomyosin is a key factor in the molecular mechanisms that regulate the binding of myosin motors to actin filaments in most eukaryotic cells. This regulation is achieved by the azimuthal repositioning of tropomyosin along the actin:tropomyosin:troponin thin filament to block or expose binding sites on actin. In striated muscle, including involuntary cardiac muscle, tropomyosin regulates muscle contraction by coupling Ca^{2+} binding by troponin with myosin binding to the thin filament. In smooth muscle, the Ca^{2+} -dependent switch is the posttranslational modification of myosin. Depending on the activation state of troponin and the binding state of myosin, tropomyosin can occupy the blocked, closed, or open position on actin. Using native cryogenic 3DEM, we have directly resolved and visualized cardiac and gizzard muscle tropomyosin on filamentous actin to 12-20 Å resolution. In both structures, tropomyosin occupies the open position even without troponin or myosin present. Further, the actin remains unperturbed, showing no large-scale conformational changes upon tropomyosin binding.

POSTER

Open Access High Throughput TEM at the BSIR

Duncan Sousa, Florida State University, Gainesville, FL

The FSU Biological Science Imaging Resource, BSIR, is an advanced electron microscopy facility open to the user community. We offer affordable, state of the art, automated TEM imaging using FEI's Titan Krios equipped with 4k x 4k CCD and energy filter with 2k x 2k CCD operated by staff with decades of EM experience. Automated data collection is facilitated using Legion and Appion. Remote users coordinate data collection with BSIR staff operating the Titan. Through the Appion system, users can view, in real time via a web browser, data collected and direct BSIR staff during the data acquisition initialization process. Cryo-grids can be sent to the facility for imaging using a standard dry shipper. We currently offer automated single particle, random conical tilt, and tomography data collection using either the Gatan 4k x 4k UltraScan CCD or the Gatan GIF Tridiem energy filter and 2k x 2k CCD. Subject to imaging conditions and specimen quality, a typical day of data collection will result in at least ~2000 single particle images or ~30 tomogram tilt series. Utilizing the BSIR can save a scientist months of tedious data collection and does not require millions of dollars in TEM infrastructure to obtain the very best data.

Electron Microscopy & Spectroscopy Studies of YSZ, Ni-YSZ, Ni-alloy, Pure and Doped CeO₂ and LaSrMnO₃ Materials Useful in Solid Oxide Fuel Cells

Laxmikant Saraf

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In solid oxide fuel cells (SOFC), electricity is electrochemically generated using oxygen ion mobility from oxidizing fuel at high temperature. The materials used in SOFC can be divided into four categories; anode, cathode, electrolyte and interconnects/sealants. Anode can be metal, metal oxide or pure oxide as long as it satisfies the appropriate balance of pure electronic or mixed electronic/ ionic conductivity. From the last ten years, we have been involved in studying pure and doped ceria/zirconia as SOFC anode, Ni-YSZ as anode for direct hydrocarbon fuel feed SOFC, YSZ, ceria micro/nanostructures as SOFC electrolyte, Sr:LaMnO₃ as SOFC cathode and Ni-alloys for SOFC interconnects and seals. The electron microscopy and spectroscopy studies collectively allowed us to better understand the structural design challenges associated with SOFC materials. Surface microstructure analysis of YSZ, Ni-YSZ and Ni-alloy using electron backscatter diffraction (EBSD) resulted in grain orientation and porosity information. Using electron microscopy and spectroscopy studies, we have observed that mobility of Ni in the hydrogen reduced Ni-YSZ exceeds beyond several microns. The electron microscopy studies helped us to design specific ceria/zirconia interfaces to promote ionic conduction. X-ray spectroscopy studies on Sr:LaMnO₃ cathodes revealed a severe change in oxygen environment along with reduced Mn²⁺ presence near the surface. Strong Cr grain boundary segregation effects were also observed in Ni-alloy. In this presentation, we will discuss specific electron microscopy and spectroscopy studies and relate their relationship with chemical and transport properties to get a complete picture and overall understanding about materials challenges to improve SOFC efficiency. This presentation will address role of electron microscopy and spectroscopy to solve the challenges in all of these materials discussed above.

Electron Diffraction Analysis of Mollusk Shells in the SEM

Scott Sitzman

Oxford Instruments America Inc., Pleasanton, CA

Since mollusk shells are crystalline, unique insights into their structure and growth process are accessible via Electron BackScatter Diffraction (EBSD), a well established SEM-based technique in materials science and geology. EBSD collects and analyzes diffraction patterns “reflected” off of well-polished surfaces held at high tilt with respect to an electron beam, to obtain crystallographic phase and orientation information from small interaction volumes (<50nm, depending on material and e-beam conditions). Automatic data collection over a grid of points allows reconstruction of microstructurally representative maps, including for crystallographic orientation, grain size, grain shape, phase distribution, and grain boundary location & character. Applied to the study of shells in two brachiopod species, the technique reveals that grains lie in a related crystallographic orientation across three distinct shell structures. In the study of an oyster shell, two morphologically distinct structures are shown by EBSD to consist of different phases of calcium carbonate (calcite and aragonite), and while all grains in a calcite region lie within 14° of a single orientation, the grains comprising the aragonite region are confined by one crystallographic direction but otherwise lie in a broad range of orientations. Interestingly, the crystallographic grain structure of the aragonite region only partially corresponds to the outward stacked-plate morphology seen in topographic SEM imaging.

POSTER

Synthesis and Characterization of Multiphase Nanocomposites using Stimuli-Responsive Polymers and Magnetic Nanoparticles

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Adhesion, friction, lubrication, wettability, and biocompatibility are surface properties of materials which can be altered by grafting polymers or self-assembled monolayers on the substrate surface. In particular, Stimuli-responsive polymers (SRPs), “smart materials”, which are sensitive to changes in pH, temperature, ionic strength, UV-radiation and many other conditions, can be grafted on the surface of nanoparticles to provide extraordinary responses under different environments. One of the most widely SRPs studied to-date is poly (N-isopropylacrylamide) (PNIPAM). PNIPAM is well known to have a lower critical solution temperature (LCST) at 32 °C. SRPs with pH-responsive capabilities include weak polyelectrolyte brushes such as Poly(methacrylic acid) (PMAA) and Poly(amino (meth)acrylates) polymers which have been previously studied and are known to also provide biocidal and antimicrobial properties. A hybrid inorganic-organic composite system can then be produced using magnetic nanoparticles and stimuli-responsive polymers. This hybrid composite system will combine the capabilities of easy nanoparticle manipulation and responsive behavior in a single material. In this work, the synthesis of several multiphase (co)polymer grafted iron oxide nanostructures is reported.

Mantle Metasomatism as Revealed by WDS and EDS X-Ray Maps of Mantle Xenoliths from Southeastern China

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Studies of mantle-derived xenoliths are important for understanding the composition of the subcontinental lithospheric mantle (SCLM) and mantle evolution and processes. Mantle xenoliths have been found in many locations in China from kimberlites and basalts. The widespread eruption of xenolith-bearing basalts in southeastern China makes it possible to study SCLM and to evaluate its heterogeneity and the processes that produced it. The SCLM of the Southeastern China is dominated by peridotite/lherzolite. The mantle xenoliths studied are spinel peridotite from the Cenozoic Fangshan basaltic rocks, Nanjing, Jiangsu Province, which was erupted during Miocene time (K-Ar ages of 9 Ma). The spinel peridotite xenoliths are mainly composed of olivine, orthopyroxene, clinopyroxene and spinel with some interstitial phases.

Electron probe microanalysis (EPMA) has been utilized to quantitatively characterize the chemical compositions of the well-developed mantle minerals. For example, the olivine was found to be zoned with the rime rich in Fe, an indicator of metasomatism in the SCLM in southeastern China. The EPMA quantitative analysis generally requires a phase with size larger than at least 1 μm . With many fine-grained interstitial phases in the mantle xenoliths, quantitative EPMA may not be a suitable technique to characterize these phases. Distribution of volatile components, such as Na and K, may be missed if only EPMA quantitative analysis is used as EPMA quantitative analysis are usually acquired on coarse-grained phases. WDS and EDS X-ray mappings are useful complementary techniques to EPMA quantitative analysis for studying elemental distribution in mantle xenoliths, especially when mineral phases are small and exist interstitially or around the rim of a mineral crystal. The WDS and EDS X-ray maps of the mantle xenoliths indicate that some spinels are significantly zoned or show symplectitic rim and that fine-grained feldspar occurs as an interstitial phase. The zonation, symplectite and interstitial phases are evidences for infiltration of alkali-rich fluids (Na, K, Fe, etc.) into the mantle xenoliths. With applications of new EDS techniques, e.g., large EDS detector, quantitative mapping and EDS spectrum imaging, EDS X-ray mapping may be able to quickly acquire high quality element maps for minor even trace elements.

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