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Please Bring These Proceedings to the Meeting!

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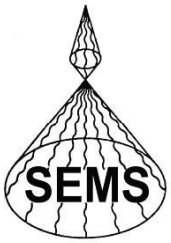

Acknowledgements

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

Our **Corporate Members and Exhibitors** are an important part of our organization and make it possible for SEMS to have outstanding meetings and to publish our newsletter, the BEAM and 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,

This year's meeting in the historic city of Charleston, South Carolina, promises to be one of our best ever. To get the meeting started Local Arrangements Chair **Bryan Majkrzak** will be hosting a low country boil on Sunday night which will provide great food, drink and fun for all that are able to attend. For those that survive the night of festivities at the low country boil we have an excellent list of pre-meeting workshops on Monday morning followed by more than two days of presentations from a number of internationally recognized invited speakers, many of our southeast regional favorites speaking about their research, and of course the Ruska student competition which this year has a list of eleven participants. The Program Committee (**Giselle Thibaudeau, Amanda Lawrence,** and **Bill Monroe**) has done an outstanding job of putting together a program filled with state-of-the-art science and microscopy.

We will also have one of our best turnouts ever for the exhibits and I hope everyone will be able to attend the Exhibitor's Mixer on Monday night. Please remember to thank the Exhibitors for attending and contributing to the success of our meeting. Without their contribution it would be impossible to hold a successful meeting.

In addition to our excellent meeting venue at the Francis Marion Hotel, Charleston offers many opportunities to enjoy the food and history of the Low Country of South Carolina. While we certainly hope that all of you attend all of the talks and visit the exhibits, you should also try to find time to visit the Market area, take a walk around the Battery, or enjoy a carriage ride around the city. Many of us are also planning a post meeting trip to Fort Sumter on Wednesday afternoon and the more of our friends that can be with us for the boat ride to the Fort the better.

Thanks for attending and participating in SEMS 2010. SEMS is one of the most active Microscopy Society of America Affiliate Societies and your participation here helps us to continue to lead the way. Take care and I hope that after a successful meeting all of you have a safe trip home.

Bob Price, ***President 2010***

SEMS 2010 PROGRAM

SUNDAY, MAY 23

4:00 PM – 9:00 PM **LOW COUNTRY BOIL SOCIAL**

SHUTTLE TO SOCIAL FROM LOBBY OF FRANCIS MARION HOTEL.
*SHUTTLE WILL BEGIN AT **3:30 PM** AND CONTINUE EVERY **20 MINUTES***

MONDAY MORNING, MAY 24

PREMEETING EVENTS

Workshop 1: Practical Digital Imaging for Microscopists
When: Monday May, 24 - 8:30 am – 11:30 am
Where: Laurens Room, Francis Marion Hotel

Workshop 2: Confocal Laser Scanning Microscopy
When: Monday May, 24 - 8:30 am – 11:30 am
Where: MUSIC Basic Science Building 6th floor Imaging Suite

Tour: Robert Bosch Corporation
When: Monday May 24 - 8:30 - 10:30
Where: Bosch Corporate facility
Shuttle van will leave the Francis Marion at 8:30 am and will return by 10:30 am.

9:45 – 10:15 AM **BREAK** *(FOOD AND COFFEE)*

10:00 - 11:00 Discussion on Core Facility Support during Difficult Economic Times
Carolina "B"

11:00 - 12:30 Executive Council Meeting and Lunch
Bridgeview Suite Francis Marion

SEMS 2010 PROGRAM

MONDAY AFTERNOON, MAY 24

1:00 Opening Remarks – *Bob Price, President*

Presentations: Carolina "B" Moderator: Bob Price

1:05 **[INVITED]** ***Microanalysis and the FBI's Amerithrax Investigation of the 2001 Anthrax Attacks***
Paul G. Kotula and Joseph R. Michael, Sandia National Laboratories

1:50 ***Quality Assurance Testing of Recombinantly Expressed Human Papilloma Virus Capsids by Negative Stain Electron Microscopy***
Charles Humphrey, Caitlyn Kryston, Gitika Panicker, Maureen Metcalfe, Elizabeth Unger Centers for Disease Control and Prevention (CDC)

2:10 ***Ultrastructural and Histopathological Studies of 2009 Pandemic Influenza A (H1N1) Virus***
Cynthia S. Goldsmith, Maureen G. Metcalfe, Wun-Ju Shieh, Dianna M. Blau, Dominique C. Rollin, Xiyan Xu, and Sherif R. Zaki.Centers for Disease Control and Prevention (CDC)

2:30 **[INVITED]** ***Intravital Visualization of Liver Function***
John J. Lemasters, Venkat K. Ramshesh, and Zhi Zhong, Medical University of South Carolina

3:00-3:20 BREAK (*VISIT EXHIBITORS)* CAROLINA "A"/ PREFUNCTION "A"

3:20 **[INVITED]** ***Myocardial Regeneration in* Ciona Intestinalis**
Heather J. Evans Anderson, Winthrop University

3:50 **[INVITED]** ***A Meeting of Microscopy and Biomechanics***
Susan M. Lessner and Michael A. Sutton, University of South Carolina

4:20 ***Study of Liver Function and Injury after Hepatic Ischemia/Reperfusion and Liver Transplantation using Intravital Confocal/Multiphoton Imaging***
Zhi Zhong, Tom P. Theruvath, Venkat K. Remshesh, Hasibur Rehman, and John J. Lemasters Medical University of South Carolina

4:40 ***Functional Morphology and Seed Anatomy of the Invasive Weed, Benghal Dayflower (*Commelina Benghalensis*): Implications for Dispersal by Mourning Doves***
Russell H. Goddard[1], Theodore M. Webster[2], Richard Carter[1], and Timothy Grey[3] Valdosta State University[1], USDA, ARS[2], and University of Georgia[3]

5:00 ***A Simplified Manual Dissection Method for Isolating BETCs from Developing Maize Kernels***
Yuquing Xiong, University of Florida

5:30-6:30 POSTER SESSION AND CORPORATE MIXER CAROLINA "A"

TUESDAY MORNING, MAY 25

Coffee/Water Provided at 8:00am

RUSKA COMPETITION CAROLINA "B" MODERATOR: *RICHARD BROWN*

8:15 ***Development of a Shack-Hartmann Wavefront Sensor for Measuring the Optical Aberrations of Biological Samples***
Ben Thomas and Peter Kner, University of Georgia

8:30 ***Electromechanical Characterization of Living Cells in Electrolyte Solutions using Line Mode Band Excitation Piezoresponse Force Microscopy***
Gary L. Thompson[1], Vladimir V. Reukov[1], Maxim P. Nikiforov[2], Senli Guo[2], Stephen Jesse[2], Sergei V. Kalinin[2], Alexey A. Vertegel[1] , [1]Clemson University, [2]Oak Ridge National Laboratory

8:45 ***Fluid Flow Forces Regulate Embryonic Atrioventricular (AV) Valve Development via Rho A/Rho Kinase Dependent Pathway.***
Hong Tan [1], Delphine Dean [2], Stefanie Biechler [1], Lorain Junor [1], Michael Yost [1], Richard L. Goodwin [1] , University of South Carolina [1], Clemson University [2]

9:00 ***The Role of Processing Body Formation in the Regulation of mRNA Decay During Colorectal Tumorigenesis***
Fernando F. Blanco and Dan A. Dixon, University of South Carolina

9:15 ***The Role of Lis1 in Dynein-mediated Organelle Transport in Mature Neurons***
Jai Pandey[1], Mariano T. Mesngon[2], Sachin Hebbar[1], Deanna Smith[1] , [1]University of South Carolina, [2]Aberdeen Proving Ground, Aberdeen, MD

9:30 ***Movement of Activator of G-Protein Signaling 3 within the Aggresome Pathway***
Ali Vural, Sukru Sadik Oner, Ningfei An, Joe B. Blumer, Stephen M. Lanier. Medical University of South Carolina

9:45 ***The Role of Multipotent Epicardial Cells in the Formation of Cardiac Valves and Septa.***
Andrea Roberts, L Junor, JD Potts, MJ Yost and RL Goodwin, University of South Carolina Medical School

10:00-10:30 **BREAK (*VISIT EXHIBITORS*)** CAROLINA "A"/PREFUNCTION "A"

RUSKA COMPETITION CONTINUED

10:30 ***Mucilage Secretion in Different* Symbiodinium *Strains***
Maria Mazzillo and Stephen C. Kempf, Auburn University

10:45 ***Examination of Alterations in the Membrane Integrity of Virulent and Avirulent Strains of* Listeria Monocytogenes *During an in vitro Cold-smoking Process by Transmission Electron Microscopy***
Joseph R. Pittman and Janet R. Donaldson, Mississippi State University

11:00 ***A Histological and Immunohistochemical Investigation of Neural Ontogeny in the Larval Oyster* Crassostrea virginica *(Bivalvia)***
Ivey R. Ellis and Stephen C. Kempf, Auburn University

11:15 ***Modulating Chemosensitivity of Tumor Stroma to Enhance Anti-tumor Drug Response***
Grishma Acharya, Maria Marjorette O. Peña , University of South Carolina

11:30 ***Ultrastructure, Life Cycle, and Pathology of* Labyrinthula terrestris*, Causal Agent of Rapid Blight on Turfgrass***
K. K. Yadagiri and J. L. Kerrigan, Clemson University

11:45-1:00 **LUNCH**

TUESDAY AFTERNOON, MAY 25

PRESENTATIONS CAROLINA "B" MODERATOR: GISELLE THIBAUDEAU

1:00 **[INVITED]** ***Scanning Microscopy for the 21st Century***
David C Joy, University of Tennessee and Oak Ridge National Laboratory

1:50 ***Direct Measurement of Live Cell Volume with a Conventional Microscope***
Michael A. Model[1], Jennifer L. Reese[2], and Daniel C. Focht[2], Kent State University[1] and Bioptechs Inc. [2]

2:10 ***High Dynamic Range (HDR) Imaging with Reflected Light Microscopy***
Rich Brown, MVA Scientific Consultants

2:30 ***Comprehensive Phase Analysis of Energy Dispersive Spectroscopic Data With Live Time Investigation***
Craig Theberge, EDAX, Inc.

2:50-3:20 **BREAK (*VISIT EXHIBITORS*)** CAROLINA "A"/PREFUNCTION "A"

3:20 ***New Tools for Automated Megamontaging and Serial Section TEM***
B.L. Armbruster, J. Brink and T. Isabell, JEOL USA

3:40 ***Super-Resolution Micrograph Reconstruction by Nonlocal-Means Applied to HAADF-STEM***
Peter Binev[1], Francisco Blanco-Silva[1], Douglas Blom[1], Wolfgang Dahmen[2], Robert Sharpley[1] and Thomas Vogt[1]
University of South Carolina[1] and RWTH-Aachen University, Germany[2]

4:00 **[INVITED]** ***Odyssey of a Chemical Microscopist***
Elaine F. Schumacher[1] and John Gustav Delly[2] , [1]McCrone Associates, Inc. and [2] College of Microscopy

4:45 ***Microscopy Education for the 21ST Century***
E. Ann Ellis, Texas A & M University

5:00-5:30 ***Follow-up Round Table Discussion on Microscopy Education***

6:00-7:00 **SOCIAL** CAROLINA "A"/ PREFUNCTION "A"

7:00-9:00 **BANQUET** CAROLINA "B"

SEMS 2010 PROGRAM

WEDNESDAY MORNING, MAY 26

7:30-9:00 **BUSINESS BREAKFAST** **CALHOUN ROOM**

PRESENTATIONS CAROLINA "B" MODERATOR: MIKE MILLER

9:00 **[INVITED]** ***Instrumentation, Metrology and Standards, Three Keys to the Nanotech Kingdom***
Michael T. Postek, National Institute of Standards and Technology

9:50 ***Ultrafast Electron Microscopy – Adding Time Resolution***
Alan W. Nicholls, W. Andreas Schroeder and Joel A. Berger, University of Illinois at Chicago

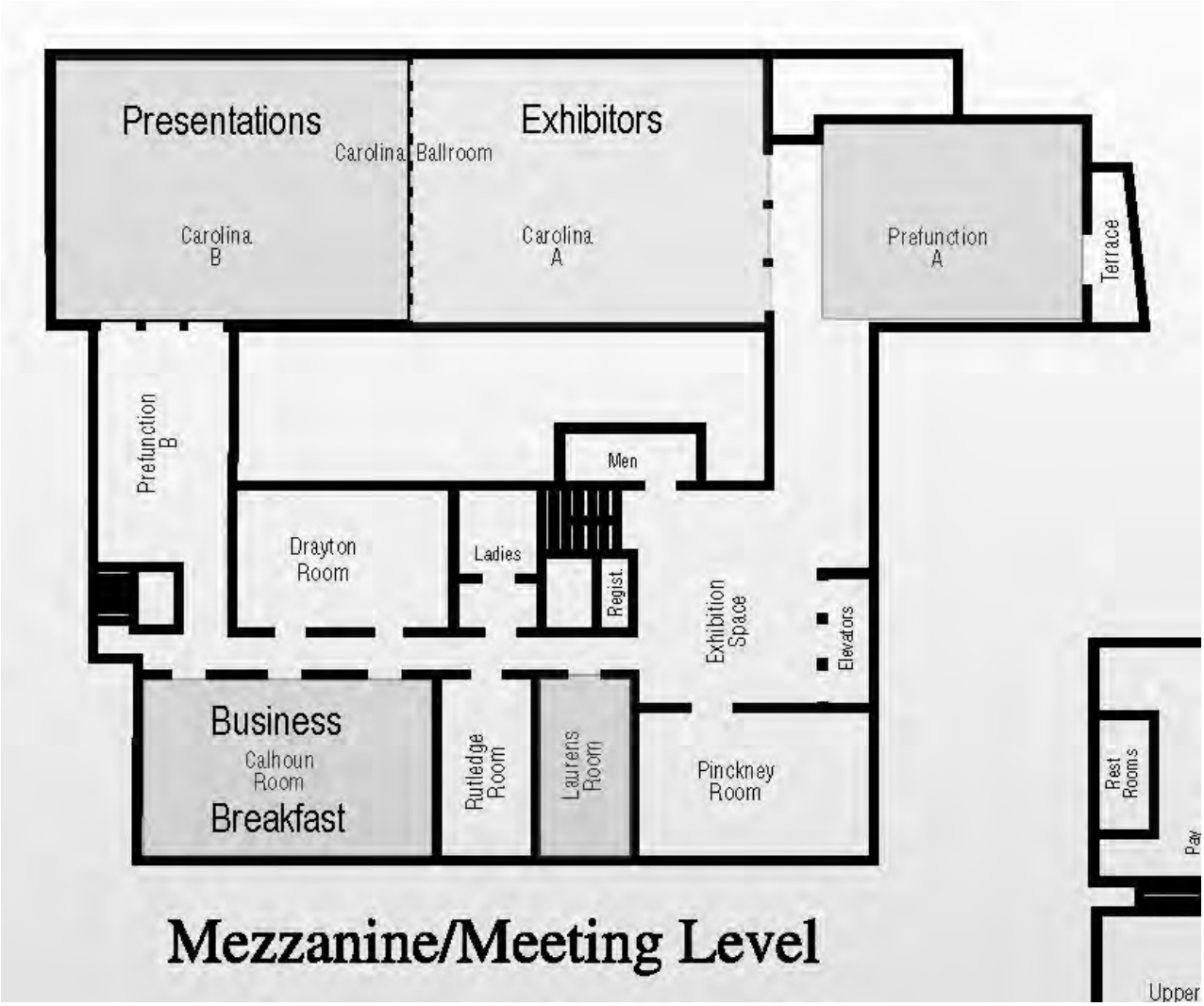
10:10-10:30 **BREAK**

10:30 ***Combining High Resolution Optical and Scanning Probe Microscopy with WITec Instruments***
Jianyong Yang, WITec

10:50 **[INVITED]** ***Liquid Scanning Transmission Electron Microscopy of Biological Specimens***
Niels de Jonge, Oak Ridge National Laboratory

11:10 **[INVITED]** ***Imaging the Molecular Mechanisms of Glucose-Stimulated Insulin Secretion***
David W. Piston, Vanderbilt University

12:00 **CLOSING REMARKS**: MICHAEL MILLER, PRESIDENT-ELECT



Presentations	Carolina “B”
Exhibitors	Carolina “A”
Executive Council Meeting	Bridgeview Suite
Poster Sessions	Carolina “A”
Corporate Mixer	Carolina “A”
Tuesday Night Social	Carolina “A”/Prefunction “A”
Banquet	Carolina “B”
Business Breakfast	Calhoun Room
Breaks	Carolina “A”/Prefunction “A”

RUSKA

Modulating Chemosensitivity of Tumor Stroma to Enhance Anti-tumor Drug Response

Grishma Acharya[1] , Maria Marjorette O. Peña[1,2]
[1]Dept. of Biological Sciences, [2]Center for Colon Cancer Research **,** University of South Carolina, Columbia, South Carolina, 29201

Inhibitors of Thymidylate Synthase enzyme (TS) have been in use for many years in the clinical management of a variety of cancers. TS catalyzes the reductive methylation of dUMP to dTMP. In the absence of exogenous thymidine, this reaction is the only source of dTMP for DNA synthesis in actively dividing cells, thus making TS an important target of anti-neoplastic drugs. However the use of TS inhibitors is severely limited by their lack of specificity for cancer cells, resulting in hematopoietic and gastro-intestinal toxicity. While traditionally anti-neoplastic drugs were developed to target cancer cells directly, in recent years increasing attention has been focused on the normal non-neoplastic cells around the tumor which together with the extra cellular matrix, make up the tumor microenvironment or stroma. These cells create a conducive environment for tumor progression and proliferation by secreting pro-tumorigenic molecules and hence to reduce the probability of relapse, it is important to target anti-neoplastic drugs towards these cells along with cancer cells. Hematopoietic or bone marrow derived cells make up a vast majority of tumor stromal cells since they get recruited to the primary tumor and sites of metastasis. We hypothesize that these infiltrating hematopoietic derived cells are direct targets of anti-cancer drugs and can dictate the tumor response to therapy. We use $APC^{min/+}$ mouse that are predisposed to spontaneous development of tumors of the small intestine and colon for our study. We have devised a protocol to replace their bone marrow with the GFP labeled bone marrow from a donor C57BL/6-UB1-GFP mouse and generate a chimeric $APC^{min/+}$mouse. Confocal microscopy imaging of the intestines and other organs of these mice verifies the engraftment of GFP labeled bone marrow derived cells in the recipient $APC^{min/+}$mouse. Gene expression signatures of the stromal cells within tumors are modulated by the tumor microenvironment and are known to be different from signatures in the stromal cells of normal tissues. The promoters of such tumor stromal specific genes can thus be utilized to drive the expression of TS-siRNA specifically to the tumor stroma to down-regulate TS levels in tumor stromal cells while protecting the normal tissues from drug induced toxicity, enhancing the TS inhibitors' efficacy. Confocal imaging of intestinal tumor sections from the transplanted mice proves that one such protein, namely Osteopontin, is upregulated specifically in the intestinal tumor stromal cells and absent in the stromal cells of normal tissues. Also, Osteopontin has been found to colocalize with the GFP labeled tumor infiltrating bone marrow cells by confocal imaging. We aim to create a retroviral construct wherein Osteopontin promoter drives the expression of TS si-RNA to the intestinal tumor stroma of $APC^{min/+}$mouse. Bone marrow of the donor C57BL/6-UB1-GFP mouse can be transduced with this construct and transplanted into a recipient $APC^{min/}$mouse so as to chemosensitize the recipient to TS inhibitor drug therapy.

INVITED

Myocardial Regeneration in *Ciona Intestinalis*

Heather J. Evans Anderson
Department of Biology, Winthrop University, Rock Hill, SC 29733

Ciona intestinalis is an invertebrate animal model system that provides many advantages in the study of heart development due to the relatively simple heart design, reduced genetic redundancy, and the possession of numerous genes homologous to those found in vertebrates. Cardiac myocytes in *Ciona* are reported to have the ability to regenerate new cells in order to replace old or damaged cells throughout the lifespan of the adult *Ciona*, an ability which does not occur in postnatal mammals. However, regeneration of cardiac myocytes in *Ciona* has not been verified using modern techniques. Whole-mount and section immunohistochemistry using proliferation markers phosphohistone H3 (PHH3) and BrdU in addition to sarcomeric muscle marker MF20 was performed to identify regions of cardiac myocyte proliferation and to determine the baseline rate of proliferation in the *Ciona*. Preliminary data show distinct regions of proliferation in the differentiated cardiac myocytes of the myocardium. Proliferating cardiac myocytes were found between the outer layer of pericardial connective tissue and inner layer of myocardium. This data suggests that a maturation gradient exists among cardiac myocytes within the *Ciona* myocardium, which is likely to contribute to regeneration. These studies will provide the foundation for further studies into the molecular differences between regenerative cardiac myocytes in *Ciona* and non-regenerative cardiac myocytes in mammals.
The project described was supported by NIH Grant Number P20 RR-16461 from the National Center for Research Resources for support of the program entitled "South Carolina IDeA Networks of Biomedical Research Excellence" (SC-INBRE).

New Tools for Automated Megamontaging and Serial Section TEM

B.L. Armbruster, J. Brink and T. Isabell
JEOL USA, Inc. Peabody, MA 01960

Traditional serial section TEM is a powerful technique that has been poorly adapted to the demands of the high-throughput lab and internet-based collaborations. Through the efforts of several groups, new tools overcome all computational barriers, providing highly standardized collaborative environments for mapping and screening the neural circuitry, or connectome, of the eye and brain.

A complete suite of software tools and strategies that utilize existing ultrastructural resources has been developed by researchers at the University of Colorado at Boulder and the Moran Eye Center at the University of Utah. By providing tools to automatically tile 1000 images into large two-dimensional mosaics, to precisely register serial mosaics into three-dimensional volumes and to browse gigabyte image sets and terabyte volumes, molecular level maps of the retina are being posted on the web to encourage other labs to tackle similarly challenging questions. A 0.25mm diameter serial-section tissue column spanning the inner nuclear, inner plexiform and ganglion cell layers was imaged at a resolution of 2.18 nm/pixel, yielding over 350,000 image tiles in a 16.5 terabyte volume captured automatically over 5 months at 3000 images/day.

To further distinguish neuronal classes, the Moran Eye Center has developed TEM-compatible molecular probes and classification software, allowing researchers to tag every cell with a molecular signature that is used to create more descriptive "color" TEM images. The tools used to build this interactive model are revolutionizing neuroanatomy by creating models of healthy and diseased tissues, and providing new methods to understand traumatic brain injuries, epilepsy and neurodegenerative diseases.

Super-Resolution Micrograph Reconstruction by Nonlocal-Means Applied to HAADF-STEM

Peter Binev[1], Francisco Blanco-Silva[1], Douglas Blom[4], Wolfgang Dahmen[5], Robert Sharpley[1] and Thomas Vogt[2,3]
Departments of Mathematics[1], Chemistry[2], Biochemistry[3], and Electron Microscopy Center[4], University of South Carolina, Columbia, SC, and Institut für Geometrie und Praktische Mathematik[5], RWTH-Aachen University, Germany.

We outline a new systematic approach to extracting high-resolution information from HAADF–STEM images which will be beneficial to the characterization of beam sensitive materials. The idea is to treat several, possibly many low electron dose images with specially adapted digital image processing concepts at a minimum allowable spatial resolution. Our goal is to keep the overall cumulative electron dose as low as possible while still staying close to an acceptable level of physical resolution. We shall present the main conceptual imaging concepts and restoration methods that we believe are suitable for carrying out such a program and, in particular, allow one to correct special acquisition artifacts which result in blurring, aliasing, rastering distortions and noise.

RUSKA

The Role of Processing Body Formation in the Regulation of mRNA Decay During Colorectal Tumorigenesis

Fernando F. Blanco and Dan A. Dixon
University of South Carolina, Dept. of Biological Sciences and Cancer Research Center, Columbia, SC 29208.

Colon cancer is mediated through various genetic alterations that promote the initiation and progression of tumorigenesis. As a consequence of these defects, overexpression of many oncogenic factors promoting enhanced cell growth and inflammation are observed. In the normal intestinal epithelium, the mRNAs of oncogenic factors contain AU-rich mRNA elements (AREs) that serve to target mRNAs for rapid decay and regulate their expression. To this extent, mRNA decay has been shown to occur in discrete cytoplasmic foci called processing bodies (P-bodies). The mRNA decay factor tristetraprolin (TTP) binds and delivers ARE-containing mRNAs to P-bodies. Here, we show that treatment of intestinal epithelial cells with transforming growth factor-b (TGF-b̩ promotes TTP expression via a SMAD-mediated mechanism. The functional consequence of TTP induction results in a 2-fold decrease in ARE-mRNA expression and increased numbers of P-bodies. Immunofluorescence analysis and 3D-image co-localization studies revealed that TGF-b treatment promotes increased numbers of TTP-containing P-bodies. These findings indicate that induction of post-transcriptional regulation and P-body assembly is a novel growth-inhibitory feature of TGF-b in normal cells. On the other hand, transformed intestinal epithelial cells bearing activating mutations in the RAS proto-oncogene are refractory to TGF-b-dependent growth inhibition. To this extent, treatment of these cells with TGF-b did not promote TTP expression, leading to a 3- to 4-fold enrichment in ARE-containing genes. Immunofluorescence analysis of Ras-transformed intestinal epithelial cells revealed a significant 2-fold decrease in P-body numbers as compared to non-transformed controls. Consistent with this, confocal imaging of normal colon and tumor tissue revealed a significant loss of TTP expression and P-body formation in tumors. Taken together, these results demonstrate that loss of the mRNA decay factor TTP occurs during colon tumorigenesis, thereby compromising P-body formation and ARE-mRNA decay, thus resulting in stabilization of various cancer-associated mRNAs.

High Dynamic Range (HDR) Imaging with Reflected Light Microscopy

Rich Brown
MVA Scientific Consultants, Atlanta GA

Some of the most disappointing images I have taken as a microscopist involve reflected light and the stereomicroscope. Usually, I have a variety of particles with variable colors, transparency, shape, size, luster and focal depths all in the same field of view. While there are many ways to overcome each problem using diffuse light (different backgrounds, exposures and patience), I usually opt for getting a decent amount of light and a series of exposures; when in doubt, bracket.

Exposure bracketing in photomicrography is not new. Exposure bracketing is how photomicrography is performed and in every exposure there are dark particles that look good, white particles that look good; rarely (at least in my images) is there an exposure where all the different range of colors in the field of view look good!

Digital imaging offers hope for the frustrated! Using high dynamic range (HDR) imaging techniques and software, all of my bracketed images can be combined into a single image that represents the entire range of exposures taken. Examples of the images produced, software that is available and some basic considerations will be enough to get you started and experimenting with this new digital photography technique.

POSTER

The Sanguine Future of Microscope Slides in Traditional Undergraduate Histology Laboratories

Glenn M. Cohen
Department of Biological and Environmental Sciences, Troy University, Troy, AL 36082

Microscope slides have served as the mainstay of histology courses for generations of students. Although virtual slides have begun to erode the dominance of glass microscope slides in professional schools, we do not foresee their replacement in undergraduate histology courses for several reasons. First, most slide collections offer a wider variety of tissue sections than those available from virtual slide vendors. Second, slide collections can readily add slides that have been prepared with specialized stains to reveal cell types, organelles, connective tissue, etc. Third, students commonly use consumer digital cameras in histology labs to capture images of sections by photographing through the eyepieces of standard lab microscopes. Fourth, access to virtual slides collections are expensive and often exceed the replacement costs of traditional slide collections. Fifth, off-campus access to the virtual slide collections creates security issues with university computer systems. Although some professional schools can bear the financial costs of balancing access and security, undergraduate departments do not have these financial resources. In short, although microscope slides and virtual slides will increasingly blend together in many pedagogical settings, virtual slides will not replace microscopes slides in undergraduate histology courses in the intermediate future. (Supported in part by a Troy University Faculty Development Grant.)

INVITED

Liquid Scanning Transmission Electron Microscopy of Biological Specimens

Niels de Jonge
Molecular Physiology and Biophysics, Vanderbilt University Medical Center, 2215 Garland Ave, Nashville TN 37232-0615. Materials Science and Technology Division, Oak Ridge National Laboratory, 1 Bethel Valley Rd., Oak Ridge, TN 37831-6064.

Liquid scanning transmission electron microscope (STEM) is a novel technique for imaging whole cells in liquid with nanometer spatial resolution on individually labeled proteins. The cells in liquid are placed in a micro-fluidic system with electron-transparent windows of silicon nitride. This system is placed in the vacuum chamber of the STEM. Imaging occurs by scanning the focused electron beam over the specimen and collecting transmitted electrons. Due to the sensitivity of the contrast mechanism of STEM on the atomic number, it is possible to detect nanoparticle-labels of heavy materials, such as gold, inside a micrometers-thick layer of water. Images were obtained of gold-tagged epidermal growth factor (EGF) molecules bound to EGF receptors on fibroblast cells. This talk will discuss the functioning of the micro-fluidic system, the basics of image formation, and the imaging of biological specimen. Possible application of this system in other areas of science will also be discussed, e.g., the imaging of specimen in gaseous environments and the imaging of nanoparticles in liquid with TEM.

References

1. de Jonge, N., Peckys, D.B., Kremers, G.J. & Piston, D.W., "Electron microscopy of whole cells in liquid with nanometer resolution," *Proc. Natl. Acad. Sci*. 106, 2159-2164 (2009).
2. Peckys, D.B., Veith, G.M., Joy, C.S., Piston, D.W., and de Jonge, N., "Nanoscale imaging of wet biological specimens with scanning transmission electron microscopy," *PLoS* One 4, e8214-1-7 (2009).

Microscopy Education for the 21ST Century

E. Ann Ellis
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Education of current and future technologists is concern for all electron microscopy laboratories. In the early days of electron microscopy most of the technical staff in biological laboratories was trained in the laboratory and on the job by the principal investigator. Many of these people were histotechnology technicians who went through the process in cookbook fashion. In the 1960's courses in electron microscopy specimen preparation were initiated in many departments to train graduate students and some technical staff. A number of institutions started short courses for initial training and some taught summer school courses for people from other schools. In 1970 a two year training program which led to an associates degree with a certificate in electron microscopy was started at San Joaquin Delta Community College in Stockton, CA. This program is still active and a recognized source for well trained electron microscopy technologists. Another two year program is at the Madison Area Technical School, Madison, WI. More recently, a four program for biology majors with an interest in microscopy has been initiated at Central Michigan University, Mt. Pleasant, MI. There are still a number of schools that teach electron microscopy specimen preparation and microscope use to graduate students and other technical staff who require microscopy in their research. The Electron Microscopy Society of America (now the Microscopy Society of America) initiated a certification program in 1978 to establish standards of technical skills for biological transmission electron microscopy technologists. Candidates for certification must have two years of full time experience in an electron microscopy laboratory and pass both written and practical examinations. Initial certification is for one year and technologists then re-certify every five years. Although there are formal programs for electron microscopy technologist education, these programs are limited by their numbers and size. It is still our responsibility to train staff members who have the aptitude and necessary manual dexterity for electron microscopy specimen preparation and microscope use.

RUSKA

A Histological and Immunohistochemical Investigation of Neural Ontogeny in the Larval Oyster *Crassostrea virginica* (Bivalvia)

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Although few investigations have considered neuronal aspects of bivalve larval ontogeny, the central nervous system of bivalve veligers has yet to be described in detail. Additionally, little attention has been paid to the occurrence and function of small cardioactive peptides (SCPs) in larval molluscs. This study provides an in depth examination of both the central and peripheral components of the larval nervous system and the larval SCPnergic innervations in the Eastern oyster, *Crassostrea virginica*. Larvae at the D-hinge, newly eyed and pediveliger stages were analyzed at the light level (histology) by means of serial, 0.5 or 1.0 μm section sets and confocal microscopy (SCPs). Histologically, D-hinge larvae were found to have limited neurogenesis. As development progressed to the newly eyed larval stage, a single apical ganglion and paired cerebro-pleural, pedal, and visceral ganglia were present forming a typical bivalve central nervous system loop. The nervous system of pediveligers was similar to that of newly eyed larvae, but added an additional accessory ganglion on the left and right sides, posterior to each visceral ganglion. Various peripheral innervations were also documented, including nerves extending from the pedal and visceral ganglia in both newly eyed and pediveliger larvae. In addition, results indicate that SCPs are present early in development (D-hinge larvae) and increase towards competency (pediveliger larvae). In newly eyed and pediveligers, SCPs were found in a varying number of neurons within all central ganglia. Furthermore, several peripheral tissues were innervated by SCPergic axons including the velum, foot, esophagus, mantle and various musculatures. Due to the location of SCPs in *C. virginica* larvae, it is likely that these neuropeptides modulate muscle contraction and / or ciliary beating in larval molluscs.

POSTER

3D Imaging of Micron-scale Permeability Pathways and Biofilm Surfaces within Sandstone Pore Networks using CT X-Ray

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CT X-ray was used to image surface features of biofilm and permeability pathways within the micron-scale pore networks of an oil-reservoir sandstone core (depth 4,796.2 ft), Eutaw Formation, Jasper County, Mississippi. To culture bacteria present in the core, samples were kept in anaerobic conditions and immersed for 16 days in low-concentration nutrient solutions, and then reduced to 3 mm diameter using a gentle tap-fracture procedure. CT images were taken using a Phoenix Dual-Tube X-ray CT system at 116 kV and 48 μA for the x-ray beam, achieving a beam spot-size of 5.6 Watts and voxel size of 5.7 cubic microns. Taking 720 images (every half degree), a 3D reconstruction yielded a three-dimensional grayscale data set for attenuation of the x-ray beam, corresponding to the density of the sample in grayscale values 0-255. Setting the baseline density at grayscale=0, corresponding to the density of the air at STP, all voxels of non-zero grayscale value were deleted electronically, leaving only air-density voxels in the 3D image. A binary, negative image then displayed only the porosity of the 3 mm sample, thereby revealing surface textures of the biofilm mass and permeability pathways with a 5.7 cubic micron maximum resolution. The 3D image was easily manipulated for rotation and magnification for a visual tour through the biofilm-coated pore structure.

Functional Morphology and Seed Anatomy of the Invasive Weed, Benghal Dayflower (*Commelina Benghalensis*): Implications for Dispersal by Mourning Doves

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Benghal dayflower is an introduced noxious weed that infests and reduces yields in many agricultural crops. The weed has quickly spread throughout southeastern Georgia (USA) due to its tolerance to many commonly used herbicides, particularly glyphosate. The potential dispersal of this weed by migratory Mourning doves was investigated in this study. Evidence shows that doves feed on Benghal dayflower seeds with some birds containing hundreds of seeds in their guts (crop, proventriculus, and gizzard). Seeds extracted from harvested birds were examined for changes in their external morphology during digestion by scanning electron microscopy (SEM), and their viability by germination. Control seeds were used to ascertain seed tolerance to acid environments and for their normal structure. Benghal dayflower seeds showed reduced but high germination rates (45%) in seeds extracted from dove gizzards, and very high tolerance to a 2 h treatment in 1.0 M HCl for 2 h (82% germination) as compared to controls (92%). Benghal dayflower seeds germinate by rupturing through the micropyle region and lifting the embryotega, a callus-like covering over the micropyle. Even after imbibition, the seed coat does not rupture relating to its strength. The seed coat itself appears structurally reinforced with an intricate underlying cell layer with a complex of lateral cell walls that form a “honeycomb” pattern with little or no intercellular spaces. The outer “honeycomb” patterned layer is subtended by a continuous thick cell wall layer. The lumen of the outer layer of cells contains many inclusions whose chemistry has not yet been determined. The structure of these seeds is discussed with respect to their survival in bird intestinal tracts and with reference to dispersal and the potential establishment of new infestations of Benghal dayflower by doves and other birds.

Ultrastructural and Histopathological Studies of 2009 Pandemic Influenza A (H1N1) Virus

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In April 2009, a novel H1N1 influenza virus was first detected in 2 patients in California, and the same virus was found to be circulating in Mexico. The virus rapidly spread worldwide, and by June, the World Health Organization had declared a global pandemic. The ultrastructural characteristics of influenza viruses have been well documented. In viruses grown in cell culture, the hemagglutinin and neuraminidase glycoproteins form prominent protrusions (spikes) from the viral envelope. Inside the virions are 8 individual nucleocapsids, which contain the RNA of the virus wrapped within a nucleoprotein Influenza virions are known to form upon the apical plasma membrane in tissue culture cells that are polarized. However, in 2009 pandemic influenza (H1N1) virus infection, not only are virions budding at the plasma membrane, but also upon the membranes of the Golgi complex. Dark tubular structures can be found in the nuclei of infected cells, and by using immunogold labeling these tubules were shown to contain the matrix (M) protein. The tubular structures can also be found in autopsy tissue, and in cell cultures infected with other influenza A viruses. Electron microscopy of autopsy lung tissue revealed characteristic extracellular influenza virus particles. Histopathological examination of lungs from autopsy tissues found evidence of diffuse alveolar damage (DAD), including inflammation, edema, and hyaline membranes. Morphological and pathological studies are instrumental in the understanding of this novel virus, and may provide information for treatment and vaccine development

POSTER

Dissecting the Embryonic Root Development in the Fern *Ceratopteris richardii* using Integrated Microscopy

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The purpose of this research is to characterize the developmental anatomy of the embryonic root in the homosporous fern *Ceratopteris richardii*. This species is an emerging model system for studying a range of developmental and genetic problems in the non-flowering plants. The spores were germinated on half-strength basal salt medium in petri dishes. With constant light at 20^{o}C, gametophytes became sexually mature in about three weeks. The developing embryo and emerging first root were studied using a dissecting microscope and a laser scanning confocal microscope (LSCM). The confocal microscopy was able to reveal cellular patterns of an entire embryo without conventional sectioning. Samples of different stages in embryo and root development were also fixed for further histological, scanning electron and transmission electron microscopy investigations. The data indicated that the embryonic root initiation and structural pattern formation each followed a relatively rigid cellular sequence and are reminiscent of developmental patterns previously described for the fifth root in *C. richardii* and for the heterosporous fern *Azolla*. It is hoped that further molecular and genetic investigations of the different root populations in *C. richardii* will advance our understanding of the cell lineage regulation and, among others, the origin of variations in root ontogeny.

Quality Assurance Testing of Recombinantly Expressed Human Papilloma Virus Capsids by Negative Stain Electron Microscopy

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Cervical cancer is highly curable when detected early. However, it remains one of the leading causes of cancer related death in women worldwide. The etiological link between human papillomaviruses (HPV) and cervical cancer has led to efforts to improve cervical cancer screening and to developing vaccines against HPV infection. Recombinant virus-like particle (VLP) based subunit vaccines have been released that target either HPV16, 18, 6, and 11 or only HPV16 and 18. HPV16 and 18 are associated with the majority of cervical intraepithelial neoplasias (CIN). HPV6 and HPV11 are less associated with CIN but highly associated with anogenital warts. Unlike similarly structured polyomaviruses, HPV cannot be grown in conventional cell culture. This problem complicates specific antigen production for vaccine development, and immunodiagnostic studies. Fortunately, the genomes of the major pathogenic HPV types are well characterized. The availability of these genotypes facilitates recombinant protein expression methods that may be used to produce specific capsid proteins (L1 and/or L2) that self-assemble to form VLPs that are conformationally similar to HPV. The VLPs can be produced in insect cells, yeast, bacteria, or human cells. Once the VLPs are expressed they may be purified by traditional protein purification techniques including gel filtration, gradient centrifugation in sucrose, cesium chloride, or iodixanol (Optiprep®). They are then used for developing vaccines, or immunodiagnostic tests. Prior to their use for test development, we evaluate the VLPs for quality and quantity by using negative stain electron microscopy (EM). EM is used as a monitor during VLP propagation, post purification, and periodically during storage. Some of the VLP preparations provide challenges to EM observation. The challenges include one or both of the following. 1. Visualizing the VLPs within extraneous cellular and VLP associated debris. 2. Visualizing the VLPs despite the effects caused by various chemicals used for preparation and purification. Details regarding observation of the preparations, the difficulties encountered, and the means by which the difficulties were overcome will be described in the presentation.

POSTER

Agricultural Significance of Seed Dispersal by Migratory Doves

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Invasive weeds can reduce crop yields in the agricultural industry. For larger seeds, birds can often serve as vectors in the proliferation and expansion in range of plants. In this study, the gut contents of sixty-four doves collected over four different years (2003 – 2006) were studied. Intact, morphologically similar seeds were separated from each gut collection for each dove and photographed by light microscopy and scanning electron microscopy. Preliminary results indicate that of the doves investigated; twenty-four different types of seeds were identified representing 24 different species of plants. In general, multiple different seeds were found in most doves investigated indicating doves are eating a wide variety of seeds. Preliminary seed identification of the different seed types is progressing and further analysis of the numbers and types of seeds found in each dove will be made with regard to the impact doves may have in spreading invasive weeds in the southeastern United States and the potential harm this can have in an agricultural environment.

INVITED

Scanning Microscopy for the 21st Century

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The Scanning Electron Microscopy (SEM) is the most widely used of all electron microscopes because it offers a powerful combination of performance, versatility, and convenience. However, further enhancements in its capabilities can no longer be anticipated because fundamental constraints - such as the large wavelength of low energy electrons and problems with electron-optical lenses – cannot be eliminated by better engineering or novel design. The Helium Ion Microscope (HIM) - the ORION from Carl Zeiss SMT - combines all the virtues of the SEM - such as a instant access to a wide range of magnifications and the intuitive power of imaging with secondary electrons - with important new benefits and advantages. These include significantly better resolution as a result of the much smaller wavelength of the He+ ions, a much improved depth of field, reduced beam penetration which provides enhanced surface sensitivity, and excellent chemical and crystallographic contrast. Examples of such images will be shown, and the possibilities that exist for microanalysis with ORION will also be discussed

INVITED

Microanalysis and the FBI's Amerithrax Investigation of the 2001 Anthrax Attacks

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The Anthrax attacks of 2001 in the US killed 5, sickened 22 others and caused a significant disruption of mail and other government facilities. Although the attack materials were for the most part recovered (*Bacillus Anthracis*) in powder form in sealed envelopes, the US Federal Bureau of Investigation (FBI) was unprepared to perform the needed forensic analyses on these bio-weapon materials. In particular, it was identified that microanalysis from the micro- to nano-scale was a key missing piece of their capabilities. As a result, Sandia was asked to analyze the materials from the attacks by early 2002 and we reached our general conclusions within a few months. We also analyzed over 200 samples of *B. anthracis* between 2002 and 2008 in an attempt to discern the method of manufacture of the attack materials.

This talk will describe Sandia's involvement in the FBI's investigation and in particular the power of microanalysis in answering several critical questions: Was the *Bacillus Anthracis* intentionally weaponized (i.e., contain an additive to make it disperse predictably) and were the materials from the attacks from the same source? In particular x-ray spectral imaging (in the SEM and STEM) combined with multivariate statistical analysis [1-3] were used to answer these questions. Specimen preparation was both by conventional microtomy and focused ion beam (FIB) sectioning of spore preparations. In addition, significant advances in analytical throughput were achieved by modification of a FE-SEM with an annular Si-drift detector with a solid angle of over 1 steradian. STEM in SEM was then performed with this new hybrid instrument in order to analyze large numbers of spores in a short time.

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INVITED

Intravital Visualization of Liver Function

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The liver is a favorable organ for study by intravital microscopy. After anesthesia and laparotomy, the liver can be gently pulled from the abdomen and placed in view of a microscope objective. Wide field microscopy has long allowed identification of most hepatic cell types and revealed directly sinusoidal movement and margination of blood cells after various stresses. Based on microanatomy, hepatic sublobular regions can also be identified and compared. Stellate cells have a unique autofluorescence signature and fluoresce blue with UV excitation, which bleaches rapidly, whereas hepatocytes display a more robust but dimmer autofluorescence under visible light. Using introduced fluorophores and GFP-expressing transgenic mice, numerous functional parameters may be monitored. By eliminating superimposed out-of-focus fluorescence, confocal and multiphoton fluorescence microscopy effectively increases spatial resolution compared to widefield microscopy.
In multiphoton microscopy, red/infrared excitation allows deeper tissue penetration with less photodamage and more efficient collection of fluorescence in a scattering environment than confocal microscopy. Intravital multiphoton micrographs rival in quality and resolution the corresponding images collected from cell monolayers. Examples will be shown illustrating use of intravital microscopy to document changes of microcirculation, leukocyte adherence, mitochondrial function, and cell viability after various stresses.

INVITED

A Meeting of Microscopy and Biomechanics

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New developments in three-dimensional digital image correlation (3D-DIC) have allowed us to transform a simple fluorescence stereomicroscope into part of a sophisticated computer vision system to analyze biomechanical properties of soft tissues on micrometer length scales under either pressurization or tensile loading. Application of 3D-DIC to soft tissues has required overcoming several technical challenges intrinsic to these materials. First, we have developed new approaches to create a sufficiently high-contrast, random speckle pattern on the specimen surface that will remain intact when the specimen is submerged. Calibration of the stereovision system at reduced length scales also requires custom-manufactured, high-precision standards. Finally, since many biological specimens must be maintained in a physiological aqueous environment to preserve their native material properties, we developed a method to correct for refractive index changes at the liquid interface when imaging submerged objects. We will discuss solutions to these challenges that have enabled us to measure surface deformations and strains during pressurization and/or tensile loading of mouse carotid arteries, combining these measurements with carotid artery mathematical models to estimate anisotropic material properties

POSTER

Free Tubulin and PKA Activation Dynamically Modulate Mitochondrial Membrane Potential in Cancer Cells

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Mitochondrial membrane potential ($\Delta\Psi$) generated by respiration or hydrolysis of ATP depends on respiratory substrates, ATP, ADP and phosphate passing through the voltage-dependent anion channel (VDAC) in the mitochondrial outer membrane. *In vitro*, both tubulin binding and phosphorylation induce VDAC closure. Here, we hypothesized that endogenous free tubulin and cAMP-dependent phosphorylation by protein kinase A (PKA) modulate $\Delta\Psi$. Our AIM was to induce VDAC closure and opening in intact cells by: increasing and decreasing endogenous free tubulin and by promoting and blocking PKA activation. HepG2 human hepatoma cells and A549 lung carcinoma cells were incubated in Hank's solution with 5% CO_2/air, and $\Delta\Psi$ was assessed by confocal microscopy of tetramethylrhodamine methyl ester (TMRM). Free and polymerized tubulin was determined using a Microtubules/Tubulin *In Vivo* Assay Kit (Cytoskeleton). In HepG2 cells, myxothiazol (10 μM), a respiratory inhibitor, caused a slight decrease of TMRM fluorescence, but subsequent addition of oligomycin (10 μg/ml), an ATP synthase inhibitor, collapsed $\Delta\Psi$ almost completely, indicating that inhibition of both respiration and ATP hydrolysis are required to collapse $\Delta\Psi$. Stabilization of microtubules by paclitaxel (10 μM) increased $\Delta\Psi$ by 60%, whereas microtubule disruption by colchicine (10 μM) or nocodazol (10 μM) decreased $\Delta\Psi$ by 60-70%. Paclitaxel pretreatment prevented the effect of colchicine and nocodazol on $\Delta\Psi$. Dibutyryl cAMP (1 mM) decreased $\Delta\Psi$ by 45% whereas H89 (1 μM), a specific inhibitor of PKA, increased $\Delta\Psi$ by 94% and blocked the depolarization induced by dibutyryl cAMP. In A549 cells, nocodozale also depolarized mitochondria and this effect was prevented by pretreatment with paclitaxel. Up and down regulation of $\Delta\Psi$ by tubulin polymerization/depolymerization and PKA-dependent phosphorylation/dephosphorylation are consistent with the hypothesis of VDAC conductance dynamically regulating mitochondrial metabolism.

RUSKA

Mucilage Secretion in Different *Symbiodinium* Strains

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Symbiodinium are brown, unicellular dinoflagellates that reside intracellularly in a variety of invertebrate hosts, including many cnidarians. In this mutualism, the endosymbiotic algae are enclosed in a symbiosome membrane (both host and symbiont-derived) and donate photosynthetically fixed carbon to their host in exchange for nutrients. *Symbiodinium* is a diverse genus of 8 clades with multiple strains in each clade. The specificity of the association between symbiont and host varies with some relationships being highly specific and others of a more general nature. The symbiont secretes mucilage that lies at the interface between host and symbiont (as part of the symbiont contribution to the symbiosome membrane). Cultured *Symbiodinium* from a variety of clades were labeled with one of 2 antibodies to symbiont mucilage (PC3, developed to a clade B alga cultured from *Aiptasia pallida;* BF10, developed to a clade C alga cultured from *Briareum* sp.). The labeling was visualized with a fluorescent marker and examined with a confocal microscope. PC3 antigen was found in cultured *Symbiodinium* from clades A and B but not in clades C and D. BF10 antigen was more specific and was only found in strains closely related to the strain the antibody was created against. These results indicate that the mucilage secretions do vary amongst *Symbiodinium* strains. This could be the location for any molecules involved in specificity since it varies between strains and is present at the host-symbiont interface.

Direct Measurement of Live Cell Volume with a Conventional Microscope

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Cell volume is one of the basic characteristics of a cell and is being extensively studied in relationship to a variety of processes, such as proliferation, apoptosis, fertility or locomotion. At the same time, a simple microscopic technique for its measurement is lacking. The method we describe uses negative transmission contrast rendered to cells by a strongly absorbing dye present in the extracellular medium. Cells are placed in a shallow (0.03 mm) chamber, and a nontoxic and cell-impermeant dye, such as Acid Blue 9 or Patent Blue V, is added to the medium at 5 mg/ml. Transmission images are collected at a wavelength of maximum dye absorption (630 nm). Where the cell body displaces the dye, the thickness of the absorbing layer is reduced; thus, an increase in cell thickness produces brighter images, and vice versa. Since the absorption coefficient of the dye can be measured, the intensity is easily converted into the absolute values for cell thickness and volume. The method is fast, impervious to fluctuations in the light source and has a high signal-to-noise ratio; it can be realized either on a laser scanning or conventional microscope equipped with an appropriate filter. Long-term experiments require a special chamber, and we use a Bioptechs FCS2 perfusion chamber fitted with a thin gasket and an additional port for rapid switching of solutions. We demonstrate the utility of this method for measuring the cell volume response to apoptosis-inducing chemicals staurosporine and ionomycin or to a hypotonic buffer.

Ultrafast Electron Microscopy – Adding Time Resolution

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Ultrafast electron microscopy (UEM) aims to combine the high spatial (sub-nm) resolution of transmission electron microscopy with the high temporal (sub-ps) resolution possible using today's ultrashort pulse lasers. The goal of such an instrument is to be able to directly visualize individual atomic motions, which would have a major impact on our understanding of fundamental ultrafast processes in nature. Key to the development of such a research instrument is a laser-driven photoemission source with high brightness. Initial work at other laboratories has taken existing TEM columns and retrofitted laser-pulsed electron sources. However the long electron column, with electron beam-crossovers, suffers from deleterious space-charge effects that limit the space-time resolution attainable in converted TEMs. The methods employed by these other groups to get around the space-charge effects include using a train of single electron pulses, or using a single pulse with insufficient electrons for imaging but sufficient for diffraction studies. At UIC we are redesigning the gun and pre-specimen column to generate sufficient electrons for single shot imaging. This involves using a nano-patterned photocathode, making the column above the specimen short (~50cm) and also introducing an RF pulse compression cavity that will correct any temporal space-charge pulse broadening at the specimen.

The Role of Lis1 in Dynein-mediated Organelle Transport in Mature Neurons

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Lissencephaly, which means smooth brain in Greek, is a rare brain formation disorder caused by defective neuronal migration during the early weeks of gestation, resulting in a lack of development of brain folds (gyri) and grooves (sulci). Patients exhibit increasingly severe seizures early in life. Children with lissencephaly are severely neurologically impaired and often die within several months of birth. Classical lissencephaly, characterized by agyria/pachygyria and neuronal mispositioning, is caused by Lis1 haploinsufficiency. Many studies have linked Lis1 to mitosis and migration of neurons during embryonic development. Similar defects in humans may be sufficient to cause the seizures observed in these patients. If developmental defects are solely responsible for the seizures then treatment options are very limited. However, Lis1 expression remains high in the adult brain. Lis1 binds to cytoplasmic dynein, a microtubule motor critical for retrograde axonal transport. It has been shown that LIS1 is required for dynein, but the underlying mechanism is poorly understood. . We showed earlier that Lis1 overexpression alters dynein distribution and microtubule organization in non-neuronal cells, and that Lis1 stimulates dynein's ATPase activity in vitro. If Lis1 reduction causes defects in organelle transport, neuron function may be compromised, which in turn could cause seizures. Our studies are designed to determine if Lis1 malfunction in mature neurons can contribute to defects in axonal transport, leading to seizures. We have examined the role of Lis1 in dynein-dependent transport in cultured cells both neuronal and non-neuronal cells using steady state distribution and time-lapse analysis of motile vesicles. We find that Lis1 reduction inhibits dynein-dependent retrograde organelle motility in non-neuronal cells, and reduces the pool of the fast-moving organelles in embryonic cortical neurons. Moreover, we find that expression of a Lis1 point mutant unable to bind dynein does not trigger the same changes induced by expression of wild type Lis1. We are now examining how Lis1 reduction, or expression of the dynein-binding mutant, affects retrograde axon transport in adult rat sensory neurons. If Lis1 regulates transport of Lis1 in mature neurons, then knocking it out after brain development occurs may cause seizures or other types of neuronal defects.

INVITED

Imaging the Molecular Mechanisms of Glucose-Stimulated Insulin Secretion

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The islet of Langerhans is the functional unit responsible for glucose-stimulated insulin secretion (GSIS), and thus plays a key role in blood glucose homeostasis. The importance of the islet is demonstrated by the proven ability of islet transplants to reverse Type I diabetes pathologies in human patients. Over the last 10 years, we have been interested in understanding the multicellular mechanisms of islet function, and their role in the regulation of blood glucose under normal and pathological conditions. In many ways, the islet appears to function as a syncytium, which exhibits synchronous behavior of membrane action potentials, Ca2+ oscillations, and pulsatile insulin secretion across all -cells in the islet. In other ways, the islet works as individual cells, especially in the regulation of gene transcription. Using our unique quantitative optical imaging methods and novel microfluidic devices, the *dynamics* of these molecular mechanisms can be followed quantitatively in living cells within intact islets. These investigations utilize transgenic and tissue-specific knock-out mouse models with demonstrated phenotypes, as well as traditional biochemical and molecular biological approaches.

RUSKA

Examination of Alterations in the Membrane Integrity of Virulent and Avirulent Strains of *Listeria Monocytogenes* During an *in vitro* Cold-smoking Process by Transmission Electron Microscopy

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Listeria monocytogenes is one of the most deadly food-borne pathogens and is responsible for an estimated 2,500 cases of listeriosis and 500 deaths annually in the United States. Individuals most susceptible to *L. monocytogenes* are the elderly, immunocompromised, pregnant women, and neonates. Ready-to-eat foods such as smoked rainbow trout, smoked mussels, and smoked salmon are commonly contaminated with *L. monocytogenes*. *L. monocytogenes* is also a persistent contaminant in plants that produce smoked finfish. It has been proposed that minimal exposure to environmental stresses could trigger a stress response in bacteria, resulting in alterations of the bacterial cell membrane that allow for the organism to become resistant to subsequent stresses. To determine if the minimal processing procedures used in preparing smoked salmon precondition *L. monocytogenes* to be able to survive during downstream processing procedures, we analyzed the cell envelope and growth of virulent and avirulent strains of Listeria following exposure to a mock cold-smoking procedure. The membrane integrity and changes in the thickness of the cell membrane, cell wall, and, cell envelope were evaluated for each strain following exposure to each stress involved in the cold smoking process by transmission electron microscopy. Preliminary results indicate differences exist in the viability of virulent and avirulent strains during the smoking process.

INVITED

Instrumentation, Metrology and Standards, Three Keys to the Nanotech Kingdom

Michael T. Postek
Precision Engineering Division, Manufacturing Engineering Laboratory, National Institute of Standards and Technology

Most researchers do not consider the need for advanced instrumentation, measurements (metrology) and standards as necessary components of the research infrastructure of the U. S. nanotechnology effort. Yet they are critical to its success. The U. S. National Nanotechnology Initiative has invested over $12 billion in research at the nanoscale. This investment needs to demonstrate to the U. S. taxpayer that this money was invested well. In order to recoup this investment, the development of successful manufacturing (nanomanufacturing) is critical. Creation of new jobs and manufacturing productivity reinforces the value of the investments. Nanomanufacturing comes in all shapes and colors, but infrastructural measurements are critical. Size matters, especially for nanotechnology. If you cannot measure it, you cannot manufacture it. Knowing dimensionality with a known uncertainty is primary to understanding the "unique" function of nanomaterials. Whether or not a new material property is being exhibited at 5 nm or 6 nm is a question which needs an accurate answer. Many of the material databases currently under development are incorporating such data from multiple sources. Are these sources accurate in the generation of these data? In order to describe the properties of a nanomaterial, accurate measurement infrastructure is needed and adopted so that these data are valid and useful. Accuracy is needed so decisions being made regarding the suitability of these materials as commercial products, components of commercial products or its effects on the environment are made on a strong ***scientific*** basis and not hype. This presentation will discuss some of these issues and potential solutions.

POSTER

Intravital Multiphoton Imaging of Ethanol-induced Liver Mitochondrial Depolarization

Zhi Zhong[1,3], Venkat K. Ramshesh[3,4], Hasibur Rehman[1,3] and John J. Lemasters[1-4]
Departments of [1]Pharmaceutical Sciences and [2]Biochemistry & Molecular Biology, [3]Center for Cell Death, Injury and Regeneration, and [4]Hollings Cancer Center, Medical University of South Carolina, Charleston, SC

Acute treatment with ethanol increases hepatic oxygen consumption and decreases hepatic ATP levels, suggesting an alteration of mitochondrial function. Accordingly, the goal of this study was to investigate changes of mitochondrial polarization and permeability *in vivo* after acute alcohol treatment using intravital multiphoton microscopy. Mice were treated with a single inebriating oral dose of ethanol (6 g/kg). Mitochondrial polarization, mitochondrial inner membrane permeability and loss of cell viability were assessed by intravital multiphoton microscopy of rhodamine 123 (Rh123), calcein and propidium iodide (PI), respectively, at 1-24 h after ethanol treatment. In saline-treated mice, hepatocytes exhibited punctate green Rh123 fluorescence, documenting the presence of polarized mitochondria. By contrast after ethanol treatment, many hepatocytes did not take up Rh123, indicating mitochondrial depolarization. After ethanol treatment, depolarization occurred in ~15%, ~50% and ~95% of hepatocytes, respectively, at 1, 4 and 6 h and remained >85% at 12 h. Thus, wide-spread, severe mitochondrial dysfunction was occurring. Subsequently, mitochondria repolarized such that only ~15% of hepatocytes contained depolarized mitochondria at 24 h after treatment. Virtually all hepatocytes not taking up Rh123 excluded PI at all time points studied, indicating that loss of cell viability was minimal. Calcein, a 623 Da fluorophore that loads the cytosol and only gains entrance to the mitochondrial matrix space when mitochondrial permeability transition (MPT) pores open, outlined mitochondria as dark voids in hepatocytes of saline-treated mice. These voids remained 6 h after ethanol treatment despite mitochondrial depolarization. Moreover, cyclosporin A and NIM811, specific inhibitors of MPT pores, did not prevent depolarization after ethanol treatment. These findings indicate that ethanol-induced mitochondrial depolarization *in vivo* was not due to opening of MPT pores. This ethanol-induced and MPT-independent mitochondrial dysfunction may contribute to steatosis after acute and hepatotoxicity after chronic ethanol exposure.

Comprehensive Phase Analysis of Energy Dispersive Spectroscopic Data With Live Time Investigation

Craig Theberge
EDAX Inc.

Traditionally, X-ray mapping involved the creation of multiple elemental maps, often one of the goals for the analysis was the interpretation of phases from the individual maps. This interpretation was done after the maps were collected and was strongly dependent on the skill and experience of the operator. Different operators would likely make different interpretations using a manual method where the analyst would be forced to consider three steps for their analysis – data collection, phase analysis, and interpretation. Automating Phase Mapping to generate and visualize phase information while the maps are collected minimizes the dependence on these tasks and operator knowledge and experience. Live phase mapping enables the phase determination to occur while the primary map data is collected and does not require a separate processing step after collection, nor is prior knowledge of the sample chemistry required and no parameters need be defined. Examples of phase maps of complex materials will be presented.

RUSKA

The Role of Multipotent Epicardial Cells in the Formation of Cardiac Valves and Septa.

Andrea Roberts, L Junor, JD Potts, MJ Yost and RL Goodwin
University of South Carolina Medical School. Columbia, SC

Epicardial cells have an emerging role as cardiac stem cells. These cells and their progenitors, proepicardial (PE) cells, have been demonstrated to give rise to all resident cell types in the heart. The contribution of PE-derived cells to the atrioventricular (AV) septum and valves is not well established. However, recently published reports and our own studies indicate that AV valve progenitor cells stimulate PE cells to migrate into this region of the developing heart. Once there, PE cells are reported to stimulate the deposition of fibrous extracellular matrix (ECM) molecules including periostin and collagen. The expression and localization of these ECM proteins is critical to the proper development and function of the heart. Using a unique 3-D culturing modeling, we have found that AV cushion tissues can induce PE cells to undergo an epithelial to mesenchymal transformation (EMT) by 100 fold compared to PE without cushion. These transformed cells were then observed to migrate into the developing valve tissues. Our early studies indicate that members of the TGFb family of proteins, specifically the combination of TGFb1, 2 and 3, are critical regulators of this process. When PE cells were cultured on the tube scaffolds containing TGFb1, 2 and 3 together, PE cells underwent EMT by 9 fold compared to PE without TGFb. Using confocal analysis and real time PCR, we have also observed the expression and localization of ECM molecules in these transformed cells. These observations indicate that the AV cushions release TGFb molecules that attract PE cells, which then express fibrous ECM proteins. Since AV valve and sepal birth defects are amongst the most common of all birth defects, delineating molecular mechanisms of their formation is critical. We further suggest that understanding the mechanisms of valve formation, and specifically the role of these multipotent cells, will benefit the long term goal of developing new therapies for these birth defects and pave the way for the in vitro production of replacement valvular and septal tissues.

INVITED

Odyssey of a Chemical Microscopist

Elaine F. Schumacher[1] and John Gustav Delly[2]
[1]McCrone Associates, Inc. and [2] College of Microscopy 850 Pasquinelli Drive, Westmont, IL 60559-5539

John G. Delly is a world-renowned expert in the use of polarized light microscopy and microchemical testing to characterize materials. This presentation describes the evolution of his professional career, launched by the gift of a Gilbert chemistry set at age six and continuing to the present day, a journey of some seventy years. The emphasis will be on early influences, and the critical role of self-teaching in childhood, when imagination and curiosity drive the process. Acquisition of a series of increasingly complex chemistry and microscopy kits, and progression through the experiments in the high quality manuals that came with them fostered learning about optics, chemical principles, glassblowing and more. The user was encouraged to "explore the world with the microscope"; specialized kits and manuals allowed a child to practice everything from chemical magic to mineralogy, and illustrations provided role models for careers in science, engineering and other technical fields.

We share concerns today about how to attract students to the disciplines of science, mathematics and engineering, and how to teach them effectively. We recognize the value of microscopy as a teaching tool, a way to get students at all levels involved in hands-on science learning. Those of us who have found a career in microscopy to be personally and professionally rewarding (as well as just plain fun!), take tremendous pleasure in sharing our knowledge and enthusiasm with others, whether it's organizing a Saturday K-12 outreach program, teaching a short course, or developing a college level microscopy curriculum. A look back at some early science "toys" will illustrate principles and practices that we can use today to ignite an interest in science learning that, for some students, will last a lifetime.

RUSKA

Fluid Flow Forces Regulate Embryonic Atrioventricular (AV) Valve Development via Rho A/Rho Kinase Dependent Pathway

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Cardiac valve formation can be divided into two phases: Epithelial to Mesenchymal Transformation (EMT) phase and post-EMT phase. Formation of endocardial cushions, the primordia of cardiac valves, involves extracellular matrix (ECM) deposition and an EMT of endothelial cells. After endocardial cushion formation, the mound-like swellings attenuate, elongate and stiffen into fibrous valve tissues during the post-EMT phase. Intensive studies have focused on the EMT phase and this process has been well described. By comparison, little is known about the post-EMT phase. It has been postulated that the increasing fluid flow forces that occur during cardiovascular development play an active role in the morphogenesis of valve tissues. To investigate the effects and the underlying molecular mechanisms of fluid flow forces on valve maturation, we have utilized a novel three-dimensional in vitro culture system. In our model, AV cushion explants from chicken embryos are cultured inside a tubular collagen scaffold, which is connected to a pulsatile pump. Estimated in vivo fluid flows were applied to developing valve tissues. Data from real-time RT-PCR, immunofluorescence stain, Western blot analyses and atomic force microscopy (AFM) found that fluid flow regulates the expression and localization of fibrous ECM proteins (tenascin C, periostin and Type I collagen (col1)), correspondingly enhancing the material properties of developing AV cushions. The Rho A/Rho kinase pathway has long been implicated as an important mediator of mechanical signals in endothelial cells. Our current work supports the importance of the Rho pathway in fibrous valve development. Our data showed that RhoA mRNA level in AV cushion explants increased by 1.6 folds under flow conditions. Further investigation demonstrated that, compared to controls, when treated with a RhoA activator--LPA (lysophosphatidic acid), cushion explants showed increases of Tenascin C and col1 protein expression and exhibit stiffer as well as stronger mechanical properties. *Y-27632 (a* Rho-kinase inhibitor) suppresses Tenascin C and col1 protein expression in cushion explants induced by fluid flow forces, and consequently the tissue is less stiff. Our data indicate that the flow mediated mechanical forces can drive expression and deposition of the fibrous ECM proteins that are critical for proper valve morphogenesis and function.

RUSKA

Development of a Shack-Hartmann Wavefront Sensor for Measuring the Optical Aberrations of Biological Samples

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When imaging with fluorescence microscopy into thick tissue samples, index of refraction differences in the sample will distort the light from the focal plane as it travels through the sample. The distortion of the image will reduce the signal-to-noise ratio and affect the image fidelity. In thick tissue samples, the peak intensity can easily be reduced by more than a factor of 10. This reduction in peak intensity means that weak signals from fluorescently labeled structures can easily be lost in the noise. This distortion can be corrected using adaptive optics technology, the process of measuring the wavefront and then correcting the distortions with a deformable mirror. Adaptive optics has been used with great success in astronomy where Shack-Hartmann Wavefront Sensors are used to measure the optical aberrations caused by the earth's atmosphere, using a bright star as a reference. Here we describe the design of a Shack-Hartmann Wavefront Sensor for the measurement of optical aberrations in biological samples. We discuss the optical design of the sensor, the algorithm used to calculate the wavefront from the raw measurement and the sensitivity of the sensor. We show the results of measurements of the wavefront using fluorescent microspheres as reference "stars".

RUSKA

Electromechanical Characterization of Living Cells in Electrolyte Solutions using Line Mode Band Excitation Piezoresponse Force Microscopy

Gary L. Thompson[1], Vladimir V. Reukov[1], Maxim P. Nikiforov[2], Senli Guo[2], Stephen Jesse[2], Sergei V. Kalinin[2], and Alexey A. Vertegel[1]
[1]Clemson University, Department of Bioengineering, Clemson, SC 29634, [2]Oak Ridge National Laboratory, Oak Ridge, TN 37831

The immediate goal of this project was to establish rapid, nanoscale electromechanical imaging of cells in physiologically-relevant solutions. Extracting local maps of electromechanical response, elasticity and viscoelasticity within cells will give insight into the dynamic functional properties of cells, including regionalized responses to environments, and will allow functional identification of cell types. Band excitation piezoresponse force microscopy (BEPFM) allows for deconvolution of the electromechanical signal from a signal generated by changes in contact resonance-dependent parameters, such as elasticity and topography, by acquiring broadband frequency spectra. Line mode BEPFM is a contact mode, rastered scanning probe technique that provides nanoscale resolution of electromechanical responses at the tip-sample interaction during application of a band of chirplets of ac biases from the conductive tip to the surface of the sample. Bacteria were retained as model biological systems representing simple living cells because they can tolerate a range of temperatures and liquid environments, from deionized water to common cell culture media. In this study, Gram-positive *Micrococcus luteus* (ML, spherical) and Gram-negative *Pseudomonas fluorescens* (PF, rod-shaped) bacteria adsorbed onto poly-L-lysine (PLL)-coated mica were imaged in three different liquids: water, Dulbecco's phosphate buffered saline (DPBS) and Dulbecco's modified Eagle's medium (DMEM) using an Asylum Research MFP-3D atomic force microscope (AFM) customized to perform BEPFM. Contrast between the bacteria and PLL substrate is observed in each liquid, albeit diminished in electrolytes. The physical nature of the multiple peaks within the amplitude versus frequency spectra and the influence of applied bias and indentation are crucial discussion points. The dominant peak of each set of spectra in each liquid on bacteria or PLL was used to plot how the amplitude changes with applied bias. In water, there is a parabolic relationship for all surfaces, whereas in DMEM and DPBS the relationship is usually linear. When combined with response versus indentation data, this suggests that electrical double layer interactions are the main contributors to the electromechanical signal. The results presented here support our previous publications, perhaps explaining the efficacy of functional recognition imaging of bacteria types, and will set up the next round of analyses estimating the relative mechanical properties of ML and PF bacteria, rat aortic smooth muscle cells, and other living systems from BEPFM and force volume data. BEPFM imaging in physiologically-relevant electrolyte solutions is possible with reduced contrast, whereas imaging in water is dominated by additional forces that result in a broadened spectral peak.

RUSKA

Movement of Activator of G-Protein Signaling 3 within the Aggresome Pathway

Ali Vural, Sukru Sadik Oner, Ningfei An, Joe B. Blumer, Stephen M. Lanier.
Medical University of South Carolina, Charleston, SC

AGS3, a receptor independent activator of G-protein signaling, is involved in unexpected functional diversity for G-protein signaling systems. AGS3 has seven tetratricopeptide (TPR) motifs upstream of four G-protein regulatory (GPR) motifs, each of which bind and stabilizes the GDP bound conformation of Giα. The positioning of AGS3 within the cell and the intra-molecular dynamics between different domains of the proteins are likely key determinants of
their ability to influence G-protein signaling. We report that AGS3 enters into the aggresome pathway and that this positioning of the protein is differentially regulated by the AGS3 binding partners Giα and mInsc. Giα rescues AGS3 from the aggresome, whereas mInsc augments the aggresomal distribution of AGS3. The distribution of AGS3 to the aggresome is dependent upon the TPR domain and it is accelerated by disruption of the TPR organizational structure or introduction of a non-synonymous single nucleotide polymorphism. These data present AGS3, Gproteins and mInsc as candidate proteins involved in regulating cellular stress associated with protein processing pathologies.

A Simplified Manual Dissection Method for Isolating BETCs from Developing Maize Kernels

Yuquing Xiong
University of Florida

Endosperm in a developing seed of maize is a major storage tissue for starch and proteins, and is of immense economic significance for its role in food, feed and biofuel production. Basal endosperm transfer cells (BETCs) constitute one of the four cell types in an endosperm with a major role in solute acquisition and transport functions from the mother plant. The BETCs, with their wall-in-growth (WIG) feature that greatly increase plasma membrane area of each cell, are critical for seed development in all cereals. Although several BETC-specific genes are described in maize, there is as yet no global analysis or a catalogue of all genes expressed in the BETCs.

First, we developed a simplified manual dissection method to microdissect BETCs from developing kernels at 12 days after pollination, a stage of one of the most active metabolic phases of seed development. RNA was isolated from microdissected BETCs and used in cDNA synthesis. A single 454 GS-FLX sequencing run on the cDNA generated 5790 reads with an average read length of 218 nucleotides. After cleaning and repeat masking, 4505 reads were used for clustering and assembly and a complement of 2473 unique sequences were generated. Validation of the BETC-specificity of the 454 sequences was done by qPCR and by *in situ* hybridization. Based on high abundance of the previously identified BETC marker genes and absence of the gene transcripts unique to a neighboring cell-type, we suggest that our method has yielded BETC-specific RNAs. Functional annotation and categorization of the 2473 unique sequences showed that the largest proportion of the BETC genes were engaged in functions related to mitochondrial activity, protein metabolism, nucleotide and protein binding activities and defense functions.

A method that does not use laser microdissection was developed for the isolation of the BETCs, which has led us to its first transcriptome analysis in a developing endosperm in maize. Consistent with our recent cellular and ultra-cellular level analyses that identify maize BETCs a model system to study polarized secretion in plant cells, these data show high abundance of transcripts related to mitochondrial activity, stress stimulus, alkaloid biosynthesis related to defense genes and various signaling functions essential for seed development.

RUSKA

Ultrastructure, Life Cycle, and Pathology of *Labyrinthula terrestris*, Causal Agent of Rapid Blight on Turfgrass

K. K. Yadagiri and J. L. Kerrigan
Department of Entomology, Clemson University, Clemson, SC 29634-0315

Labyrinthula terrestris belongs to a group of organisms commonly referred to as marine net slime molds, which have primarily been isolated from aquatic environments. This species causes the disease rapid blight on cool-season turfgrasses. Symptoms include water-soaked lesions and browning of foliage that lead to yellowing and death of the infected turf, causing unsightly spots on golf course greens. Little is known about this terrestrial pathogen; therefore its ultrastructure, life cycle, and pathology are being investigated with light and electron microscopy. Ultrastructural observations reveal that each cell contains a single nucleus, nucleolus, mitochondria, Golgi apparatus, liposomes, and cell surface organelles called bothrosomes that secrete a double membrane, extracellular matrix. Our pathology experiments indicate that *Labyrinthula terrestris* can infect hosts indirectly through stomata, trichome bases, cut ends of grass blades, and wounds, and through direct penetration of the host tissue. Once inside the host, it has been observed in cells of the epidermis, mesophyll, bundle sheath, vascular bundle, and sclerenchyma, and is able to divide and move within host cells and across host cell walls. Outside of the host, cells aggregate into clusters that are able to give rise to new cells when placed on fresh medium, suggesting these aggregates serve as resistant and overwintering structures. Better understanding of the basic biology of *L. terrestris* can be ultimately applied to provide better means of controlling rapid blight disease.

Combining High Resolution Optical and Scanning Probe Microscopy with WITec Instruments

Jianyong Yang
WITec Applications Scientist

High resolution optical and scanning probe microscopy covers a wide range of techniques, many of which are more and more commonly seen in today's research on nanotechnologies. Here we want to give a detailed introduction to the operational principles and instrumental configurations relevant to confocal Raman and Scanning Probe Microscopy including atomic force microscopy (AFM), scanning near-field optical microscopy (SNOM), and Pulsed Force Mode. Particularly, we are presenting a system that integrates all the above mentioned techniques together. As a result, one can exam the same sample area (often of μm or sub-μm size) with multiple approaches. The advantages are obvious, for example, with combined confocal Raman imaging and AFM measurement we are not only getting the high resolution topographic image and mechanical properties (such as adhesion and stiffness at the nm scale) but also getting the Raman image with chemical sensitivity. For confocal Raman imaging, typical research fields include pharmaceutics and cosmetics, materials and polymer sciences, archaeology and geosciences, forensics, coatings, thin films and other areas where a clear identification of the distribution of chemical compounds is a necessity.

Study of Liver Function and Injury After Hepatic Ischemia/Reperfusion and Liver Transplantation using Intravital Confocal/Multiphoton Imaging

Zhi Zhong, Tom P. Theruvath, Venkat K. Remshesh, Hasibur Rehman, and John J. Lemasters
Department of Pharmaceutical & Biomedical Sciences, Medical University of South Carolina, Charleston, SC 29425

Many pathophysiological processes occur after hepatic ischemia/reperfusion (IR), leading to subsequent liver injury. Monitoring these pathophysiological processes in living animals has been difficult. Recent developments in intravital confocal/multiphoton microscopy using introduced fluorophores and fluorescent protein-expressing mice provide novel approaches to visualize pathophysiological processes such as mitochondrial depolarization, onset of the mitochondrial permeability transition (MPT), disturbances of microcirculation, inflammation, and cell death in living animals. Confocal/multiphoton microscopy achieves higher axial resolution compared to widefield microscopy. Red/infrared excitation used in multiphoton microscopy allows deeper tissue penetration with less photodamage and more efficient collection of fluorescence in a scattering environment. We used these novel imaging technologies to document pathophysiological disturbances after hepatic IR. Mitochondrial polarization, cell death, and the MPT were assessed using rhodamine 123 (Rh123), propidium iodide (PI), and calcein, respectively. In sham-operated mice, green Rh123 fluorescence was punctate in virtually all hepatocytes, indicating mitochondrial polarization. Two hours after warm IR, mitochondria depolarized in >70% of cells, but cell death in majority of these hepatocytes had not yet occurred. Calcein is a cytosolic fluorophore that gains entrance to the mitochondrial matrix space when MPT pores open. Calcein outlined mitochondria as dark voids in the hepatocytes of sham-operated mice. Entry of calcein into mitochondria after IR indicated MPT onset *in vivo*. NIM811, a specific inhibitor of the MPT, and ethyl 3,4-dihyroxybenzoate, a prolyl hydroxylase inhibitor that upregulates HIF-1α, prevented MPT onset, mitochondrial depolarization and liver injury after IR. NIM811, minocycline, and ischemic preconditioning also protected against MPT onset and liver graft failure after full-size and small-for-size rat liver transplantation, as again documented by intravital confocal/multiphoton microscopy. After transplantation, blood flow velocity and sinusoidal diameter revealed by intravital microscopy of fluorescein-labeled erythrocytes decreased markedly in eNOS-deficient liver grafts compared to wild-type grafts. Such microcirculatory disturbances were associated with more severe graft injury, indicating that donor eNOS attenuates storage/reperfusion injury partially by improving microcirculation. Together, these examples show that intravital confocal/multiphoton imaging is a powerful new technology to study alteration of liver function after IR.

POSTER

Scanning Electron Microscopy of Cornea at Low Energy and Low Current

Jijin Yang and Doug Wei
Nano Technology Systems Division, Carl Zeiss SMT Inc., Peabody, MA

The cornea is the main component of the outer fibrous shell of the eye and consists mainly of collagen fibrils surrounded by a ground substance containing proteoglycans and other proteins. High resolution SEM imaging of biological samples like cornea is often challenged by surface charging and beam damage. Strategies for dealing with these issues are to use low acceleration voltage and/or low probe current. However, low kV and low probe current introduce other difficulties during imaging. For example, low kV deteriorates resolution and causes faster contamination buildup at high magnification. Signal to noise ratio is another concern when using low probe current. Recent development of Zeiss Merlin FE-SEM, which has excellent signal at whole range of voltage and current that helps overcome the difficulties in imaging at low kV and low current. Here we used Zeiss Merlin FE-SEM to image collagens and ground substance in cornea. The cornea samples were neither coated with conductive layer nor stained. To avoid surface charging, all imaging was done at an energy range below 2 kV, mostly below 0.5 kV, and probe currents were from a couple of pA up to 28 pA. The SEM images of the cornea at high magnification show that the typical collagen fibrils with a characteristic 65 nm D-periodicity or D-banding. Nanostructures of ~5 nm in diameter in the matrix of proteoglycans and other proteins form a 3-D cross-bridge network that cover the corneal collagen fibrils can be seen clearly. The cross-bridges between collagen fibrils have not been imaged directly by SEM before. “Minor” bands on the collagen fibril possibly the “a” and “b” bands that haven’t been resolved by SEM before, can also be seen with Merlin FE-SEM. Similar to the diameter of the collagen fibrils, the cross-bridge width is also not uniform. Compared with TEM and AFM images, the high resolution SEM images show something different. Detailed data of imaging will be reported in this presentation.

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
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	2009

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Charles Sutlive	1989
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Charles and Betty Sutlive	2000
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James Sheetz	2005
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1972-73 Walter Humphreys
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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
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