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Southeastern Microscopy
Society**



55th Annual Meeting

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Historian

W. Gray Jerome, III
U-2206
Vanderbilt University
Medical Center
1161 21st Avenue, South
Nashville, TN 37232
615.322.5530
Jay.Jerome@vanderbilt.edu

Endowment

Charles D. Humphrey
Somewhere in the Mountains
Specs1A@hotmail.com

Proceedings Editor

John Shields
Georgia Electron Microscopy
151 Barrow Hall
115 D.W. Brooks Drive
University of Georgia
Athens, GA 30602
jpshield@uga.edu

Photographer

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

Corporate Liaison

Rick Hirche
502 East Oak Hill Ave
Knoxville, TN 37917
865-603-4523
fredhrick@gmail.com

Web Site Contact

Karen Kelley
University of Florida
ICBR
Electron Microscopy Core Lab
PO Box 110700
Gainesville, FL 32611
vau@ufl.edu

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Acknowledgements

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PROTOCHIPS

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Welcome From the President

Dear SEMS members,

It is with great pleasure that I welcome you to the 55th Annual Southeastern Microscopy Society Conference in Chattanooga, Tennessee. *New York Times* named Chattanooga one of the "Top 45 Places to go" in the World, and it is no wonder. Chattanooga is one of America's most breathtaking cities. Along with beautiful Chattanooga, we have an outstanding conference program awaiting your arrival. The conference program will offer an opportunity to explore Chattanooga, presentations from invited speakers, the Ruska Award competition, commercial exhibits, social opportunities for mingling with fellow attendees, and so much more!

On Wednesday, May 16, registration opens at 8:00 a.m. We will start conference events with an outing to Ruby Falls the nation's tallest and deepest underground waterfall. The commercial exhibits will be open all afternoon, followed by the always enjoyable Corporate Mixer and poster exhibits. We have a fantastic program of presentations on Thursday and Friday morning. Once again, we have an exciting group of Ruska competitors and a fantastic line-up of invited speakers.

This year, we are pleased to welcome our MAS-sponsored speaker, **Larry Allard**, from the **Oak Ridge National Laboratory** (Oak Ridge, TN), and our MSA-sponsored speaker, **Amelia Dempere**, from the **University of Florida**. Also, our Ann Ellis Speaker will be **Caroline Miller**, from **Indiana University–Purdue University Indianapolis**. On Thursday evening, our Annual Banquet will feature a wonderful dinner catered by Ruth's Chris Steakhouse and an opportunity to be "entertained." The society's business breakfast will be held on Friday at 9:00 a.m., followed by the last group of presentations before officially closing the meeting at noon.

Looking back on my years of membership in SEMS, I reflect on the memories and the importance of this great organization. I think about the many faces, the vendor socials, and all of the mysterious images! Connecting with SEMS on a regular basis allowed me to educate myself on new products and trends and position myself to serve my facility users better. I am happy to be listed as a regular SEMS member because of the connections we share and the offerings of our regional microscopy society. If this is your first SEMS meeting, please know that we are all very excited that you are with us! We hope that you will consider joining us and becoming more involved in SEMS as we work together to continue to host our annual meetings with more opportunities for professional growth and development for the microscopy community.

One of our most valuable assets as a society is our people and I would be remised if I did not take the time to thank the hardworking executive council, the 2019 Local Arrangements Committee (Chair, Cynthia Goldsmith) and Program Committee members (Chair, Paul Eason) for their commitment to making our time together in Chattanooga exceptional!

Finally, to all of you...our loyal SEMS members and corporate sponsors...thank you for your continued support of this wonderful organization!

Here's to another FANTASTIC meeting!

Brandon M. Walker, SEMS President 2019

SEMS 2019 PROGRAM

WEDNESDAY, MAY 15

REGISTRATION – 8am to 4:30pm

PRE-FUNCTION HALLWAY

Outing to Ruby Falls

1:45pm from Hotel

Executive Council Meeting: 11:30am

RUTH'S CHRIS RESTAURANT (HOTEL)

6:00pm – 8:00pm

CORPORATE MIXER

SALON E-G

POSTERS AND EXHIBITS

THURSDAY MORNING, MAY 16

Registration – 8:30am to 5pm

PF HALLWAY

SESSION 1

RUSKA COMPETITION

MODERATOR: *BRANDON WALKER* *SALON D*

9:00 am – 9:15 am Cell Viability Assessment and Structural Analysis of Filamentous Fungal Biofilms Using Confocal Laser Scanning Microscopy and COMSTAT program A. Shailaja, J.L. Kerrigan- Department of Plant and Environmental Sciences, Clemson University, Clemson, SC

9:15 am – 9:30 am Exosome and extracellular vesicle isolation and biomarker detection using Hydrophobic Interaction Chromatography (HIC) and poly(ethylene terephthalate (PET) Capillary-Channeled Polymer (C-CP) fiber solid phase Tyler Slonecki¹, Terri F. Bruce¹, Sisi Huang², , Lei Wang², Kaylan Kelsey², Rhonda Powell¹, Kenneth Marcus², ¹ Clemson Light Imaging Facility and ² Department of Chemistry, Clemson University, Clemson, SC 29634

9:30 am – 10:00 am BREAK (PLEASE VISIT EXHIBITORS) *SALON E-G*

SESSION 2

MODERATOR: *PAUL EASON*

SALON D

10:30 am – 11:30 am MAS Sponsored Speaker: Latest Experimental Capabilities in *In Situ* Heating and Gas-Reaction Studies in Aberration-Corrected Electron Microscopy, Dr. Larry Allard

11:30 am - 11:45 am Applications of CMOS-Based Imaging Sensors for High-Speed EBSD Mapping, Roger Kerstin

11:45 am - 12:00 pm Leica Microsystems New THUNDER Imagers, Chris Murphy

12:00 pm – 1:30pm LUNCH (*ON YOUR OWN*)

THURSDAY AFTERNOON, MAY 16

- SESSION 5** **MODERATOR: ---** *RICK HIRCHE* *SALON D*
- 1:30 pm - 2:30 pm** **MSA Sponsored Speaker - Combining Tomography and Surface Reconstruction in 3D Characterization.** Luisa A. Dempere, Ph.D.
- 2:30 pm - 3:00 pm** *Break*
- 3:00 pm – 3:30 pm** **Life-Threatening Illnesses Associated with Organ Transplantation** Cynthia S. Goldsmith
- 3:30 pm – 4:00 pm** **Improved Method for Negative Staining of High Density Lipoprotein (HDL) Detects More Surface Detail without Modifying Particle Morphology** Rachel C. Hart¹
- 4:00 pm – 4:50 pm** **Ann Ellis Lecture - Capturing Nature's CAD File: Photogrammetry in an SEM,** E.L. Principe, Ph.D.
- 4:50 pm** **GENERAL SESSION CLOSING REMARKS:** *ERIC FORMO*
- 6:00 pm - 7:00 pm** **SOCIAL** *SALONS E-G*
- 7:00 pm – 9:00 pm** **BANQUET & AWARDS** *SALONS A-C*

FRIDAY MORNING, MAY 17

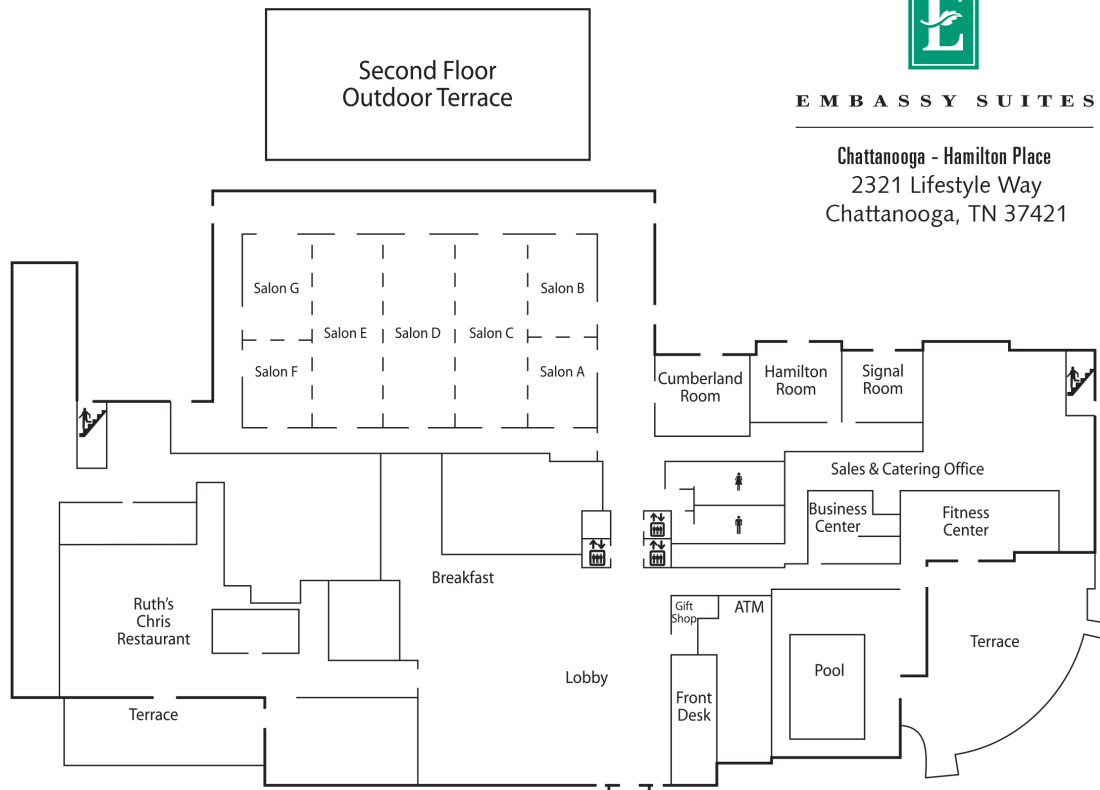
9:00am-10:30am **BUSINESS BREAKFAST** *SALONS A-C*

NO PRESENTATIONS ON FRIDAY



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Pre-Function Hallway

Registration and Poster Session

Salons E-G

Exhibitors, Corporate Mixer, Breaks, and Pre-Banquet Social

Salon D

Presentations

Salons A-C

Banquet and Business Breakfast

RUSKA AWARD WINNERS

1972	Danny Akin Univ. of Georgia		Clemson Univ.	2005	Janet Donaldson MS State Univ.
1973	John Wolosewick Univ. of Georgia	1990	Chi-Guang Wu Univ. of Florida	2006	Sangmi Lee MS State Univ.
1974	Murray Bakst Univ. of Georgia	1991	Karen Snetselaar Univ. of Georgia	2007	Jennifer Seltzer MS State Univ.
1975	William Henk Univ. of Georgia	1992	Yun-Tao Ma Clemson Univ.	2007	Tao Wu Georgia Tech
1976	Durland Fish Univ. of Florida	1992	Theresa Singer Univ. of Georgia	2008	Katherine Mills-Lujan Univ. of Georgia
1978	Dwayne Findley N.C. State Univ.	1992	Kerry Robinson Clemson Univ.	2009	Shanna Hanes Auburn Univ.
1979	Glen Watkins N.C. State Univ.	1993	Julia Kerrigan Univ. of Georgia	2010	Kirthi Yadagiri Clemson Univ.
1979	John Weldon Univ. of Georgia	1994	John Shields Univ. of Georgia	2011	Maria Mazillo-May Auburn Univ.
1980	Michael Dresser Duke University	1994	Meral Keskin-tepe Univ. of Georgia	2012	David Lovett Univ. of Florida
1981	Michael Short W. Georgia Coll.	1995	Katalin Enkerli Univ. of Georgia	2013	S. Patnaik MS State Univ.
1982	Mark Rigler Univ. of Georgia	1996	Rhonda C. Vann MS State Univ.	2014	Katherine Dye Univ. of Georgia
1982	Chris Sunderman Univ. of Georgia	1997	K.J. Aryana MS State Univ.	2015	Zulong Ke Georgia Tech
1983	Patricia Jansma Univ. of Georgia	1998	Timothy Wakefield Auburn Univ.	2015	A.A. Trofimov Clemson Univ.
1985	Mark Brown Univ. of Georgia	1999	Wendy Riggs Univ. of Georgia	2016	Wren Gregory Clemson Univ.
1986	Judy King E. Tenn State Univ.	2000	Gail J. Celio Univ. of Georgia	2016	James Kizziah Univ. Alabama, Birmingham
1986	Peter Smith Clemson Univ.	2001	Joanne Maki Univ. of Georgia	2017	Roshini Ramachandran Univ. of Georgia
1987	Robert Roberson Univ. of Georgia	2002	Rocio Rivera Univ. of Florida	2018	Christopher Isley Univ. South Carolina
1988	Rajendra Chaubal Univ. of Georgia	2003	Patrick Brown Univ. of Georgia		
1989	Josephine Taylor Univ. of Georgia	2003	Heather Evans Univ. of S.C. Medical College		
1989	Graham Piper				

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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 Jerome III	2000
Charles W. Mims	2001
Danny Aiken	2002
Robert Price	2003
E. Ann Ellis	2009
Glenn Cohen	2010
Robert Simmons	2011

DISTINGUISHED CORPORATE

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

ROTH-MICHAELS TEACHING AWARD

James Sheetz	2005
Charles Mims	2006

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1966-67	William Callahan	1997-98	Robert Simmons
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1968-69	Ivan Roth	1999-2000	Buddy Stephens
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1973-75	Jim Hubbard	2004-05	John Shields
1975-76	Edward DeLamater	2005-06	Judy King
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1977-78	Gene Michaels	2007-08	Robert Simmons
1978-79	Edith McRae	2008-09	Giselle Thibeadeau
1979-80	Jerome Paulin	2009-10	Robert Price
1980-81	Ken Muse	2010-11	Michael Miller
1981-82	Mary Beth Thomas	2011-12	E. Ann Ellis
1982-83	Jack Munnell	2012-13	Richard Brown
1983-84	Sara Miller	2013-14	W.Gray (Jay) Jerome
1984-86	Ray Hart	2014-15	Russ Goddard
1986-87	Glenn Cohen	2015-16	Mary Ard
1987-88	Gerry Carner	2016-17	Amelia Dempere
1988-89	Danny Akin	2017-18	Terri Bruce
1989-90	Johnny Carson	2018-19	Brandon Walker
1990-91	Janet Woodward		
1991-92	Charles Mims		
1992-93	Charles Humphrey		
1993-94	Sandra Silvers		
1994-95	JoAn Hudson		

POSTER

Structure of an Insecticidal Toxin and Engineering for Clinical Applications

Cole L. Martin¹, Todd J. Green², Lawrence J. DeLucas³, Christopher D. Radka⁴ and Stephen G. Aller¹

Departments of Pharmacology¹ and Microbiology², University of Alabama at Birmingham; The Aerospace Corporation³ and St. Jude's Children's Hospital⁴.

We have solved the structure of the insecticidal toxin, XptA2, at 3.2 Å resolution utilizing both cryo-EM and x-ray crystallography. XptA2 contains cell surface targeting IgG-like domains constructed around a pentameric toxin delivery channel and transmembrane piercing domains. A flexible linker domain in a similar tripartite toxin ortholog was previously proposed to be the driving force for conformational change into the pore forming state after binding to cell surface targets and internalization. Conversely, our structures reveal a high degree of disorder and a lack of strain on the linker region in the pre-pore state suggesting a low amount of potential energy. We therefore challenge the idea that the linker represents a spring initiating the transition from the pre-pore state into the pore state. We first tested the hypothesis that a continuous linker is not needed for XptA2 to fold and assemble into the pre-pore pentamer. XptA2 was expressed as two halves in which the linker was truncated by a stop codon introduced into “frag1”, and translation of “frag2” was initiated by an artificially introduced Methionine start codon. Two-fragment (“2-frag”) XptA2 was able to form a mature pre-pore pentamer by negative stain electron microscopy. Experiments to convert “2-frag” to the pore state are in progress. In parallel work, we have explored the idea of engineering XptA2 for clinical use. Importantly, wild type XptA2 is non-toxic to mice and is cleared in a manner that is similar to other control non-toxic proteins.

Small animal imaging studies were supported by grant P30CA013148, as part of the UAB Comprehensive Cancer Center's Preclinical Imaging Shared Facility.

RUSKA

Cell Viability Assessment and Structural Analysis of Filamentous Fungal Biofilms Using Confocal Laser Scanning Microscopy and COMSTAT program

A. Shailaja, J.L. Kerrigan

Department of Plant and Environmental Sciences, Clemson University, Clemson, SC.

Biofilms are ubiquitous and of great concern because of their persistence in industrial, medical, and household environments. They are resilient structures, composed of aggregates of microorganisms that are adhered to a surface and enclosed in extracellular polymeric substances. We have been investigating filamentous fungal biofilms that are representative of those in the built environment, *Aspergillus niger* is being studied because it is ubiquitous and a model organism. The objectives of this research are to determine the best method to measure cell viability and to quantify biofilm features. One method utilizes the classical cell stain FUN-1 that exhibits orange-red fluorescent intravacuolar structures in metabolically active cells, while dead cells exhibit green-yellow fluorescence. The second method uses a combination of SYTO9 and Propidium iodide (PI). SYTO9 is a green fluorescent stain with a capacity to penetrate the active cell walls, and PI, is a red fluorescent stain that can penetrate the damaged cell membrane. Confocal microscopy and the computer program COMSTAT are being used to visualize fluorescent labelled cells and quantitating biofilms structures. The combination of nucleic acid stains SYTO9 and PI is more reliable for imaging and live-dead cells differentiation. The center portion of the biofilm contained more live cells when compared to the edge portion. Also, the edge portion contained conidiogenous cells and conidiophores. The viability of the edge portion was not homogenous, much of the biofilm cell was viable, but a minority of red fluorescent nonviable cells was also noted. Once the statistical analysis is completed, this protocol will be employed to test the efficacy of different anti-fungal biofilm agents.

This work is supported by Clemson Light Imaging Facility, Clemson University, SC

RUSKA

Exosome and Extracellular Vesicle Isolation and Biomarker Detection using Hydrophobic Interaction Chromatography (HIC) and Poly(ethylene terephthalate (PET) Capillary-Channeled Polymer (C-CP) Fiber Solid Phase

Tyler Slonecki¹, Terri F. Bruce¹, Sisi Huang², , Lei Wang², Kaylan Kelsey², Rhonda Powell¹, Kenneth Marcus²

¹ Clemson Light Imaging Facility and ² Department of Chemistry, Clemson University, Clemson, SC 29634

Exosomes are a subtype of extracellular vesicles approximately 30-150 nm in diameter that serve a variety of functions within cell communication and, ultimately, in normal and abnormal physiological processes. Manipulation and engineering of exosomes may result in medical applications in fields ranging from disease diagnostics, drug delivery, immunotherapy, regenerative medicine, and cancer therapeutics. Consequently, isolation and separation of intact and stable exosomes is critical for downstream analysis and future applications. Although exosome research is driving incredible innovation in laboratory settings, a consistent, quick, and cost effective exosome isolation method is lacking for clinical translation. This research demonstrates that exosomes and extracellular vesicles can be captured using Hydrophobic Interaction Chromatography (HIC) combined with poly(ethylene terephthalate (PET) Capillary Channeled Polymer (C-CP) fiber solid phase. Furthermore, this technique, with modification, offers a promising exosome isolation method that can offer realistic solutions in a clinical setting. In addition to chromatographic analysis of vesicles from cell culture media, urine, plasma, and cervical mucus, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and fluorescent microscopy have been used to verify the presence of exosomes and extracellular vesicles on the PET C-CP fibers within HIC conditions. In addition to exosome isolation, PET C-CP fibers in combination with protein marker antibodies are being investigated as a viable medium for biomarker detection for ovarian cancer (OC).

Acknowledgements: Dr. Terri Bruce, Rhonda Powell, Dr. Ken Marcus, Sisi Huang, Lei Wang, Kaylan Kelsey. George Wetzal and Donald Mulwee at the Clemson Electron Microscopy Facility for help with SEM and TEM imaging

Funding: The Gibson Foundation The Eppley Foundation for Scientific Research.

MAS Sponsored Speaker:

Latest Experimental Capabilities in *In Situ* Heating and Gas-Reaction Studies in Aberration-Corrected Electron Microscopy

Dr. Larry Allard - Oak Ridge National Laboratory

There has been a marked increase in the past decade in the development of new technologies that permit a variety of *in situ* experiments on materials to be conducted in electron microscopy. Among the many methods (electrical measurements, mechanical property measurements, reactions in liquids, etc.) are the newest capabilities for heating samples and doing elevated temperature gas reactions, using microfabricated heating elements (so-called “MEMS-based devices,” or “E-chips”) as the base platform for both capabilities. We have collaborated with Protochips Co. (Raleigh, NC) since 2006 in the development first of holders for heating in vacuum, and then of holders that incorporate the E-chip heaters in a closed-cell configuration to allow gas reactions at pressures up to a full atmosphere to be conducted *in situ*. A primary issue with the use of E-chip heaters is that specimens have to be electron transparent; powder samples are ideal since they can be deposited easily on the E-chip. Bulk samples must first be thinned appropriately; focused-ion-beam milling is a natural method to make thin foil samples, but Ga-FIB instruments always leave residual Ga on the sample. To avoid Ga, a bad contaminant for aluminum, we have recently explored the use of Xe-plasma FIB methods to cut and affix thin lamellae of Al alloys onto E-chips, with outstanding success...examples of the utility of Xe-PFIB methods to prepare samples for *in situ* heating to study precipitation processes in Al alloys will be given. In the area of gas-cell technology, our latest effort has been to develop the methods and protocols to introduce water vapor into gaseous reactions, at percentages higher than possible at room temperature and atmospheric pressure (about 2%, or ~17 Torr partial pressure). This has involved incorporation of a residual gas analyzer (RGA) into our gas-cell system. The RGA permits not only water vapor in the gas through the cell to be confirmed, but also the total composition of any reacting gas to be quantified, and the ability to confirm that, for reduction reactions, no significant oxygen species are present, as e.g. from an air leak. Examples of these new gas-cell *in situ* capabilities will also be given.

MSA Sponsored Speaker

Combining Tomography and Surface Reconstruction in 3D Characterization.

Luisa A. Dempere¹ , Edward L. Principe² , Gary Scheiffle¹ , Ana C. Bohórquez¹

The combination of multi-scale methods of microscopy and tomography has been proven to provide a comprehensive methodology for 3D characterization of materials. This methodology has been found to be particularly useful for samples with surface features and structures that are critical for the understanding of function, properties or behavior of the component/sample analyzed. Data from the characterization of a Green June Beetle (*Cotinis Nitida*) will be presented as an example of the implementation of this methodology. This scarab exhibits structural color due to interferometric reflections in multilayers starting at the specimen's surface and continuing below it. This example shows how this integrated approach can be used on 3D characterization of samples in which surface characteristics are a critical in complementing the evaluation of volumetric structures.

[1] Research Service Centers, Herbert Wertheim College of Engineering, University of Florida

[2] PanoScientific, Inc.

Ann Ellis Lecture

Capturing Nature's CAD File: Photogrammetry in an SEM

E.L. Principe, Ph.D. - PanoScientific, LLC

We will describe a form of photogrammetry performed in a scanning electron microscope (SEM) to create detailed quantitative micro and nano-scale 3D surface reconstructions of objects. Reconstruction of an arbitrary object can be accomplished by acquiring a series of 2D images while rotating and/or tilting the object on the microscope stage. Sample preparation and imaging conditions are critical to the quality of the reconstruction and ease of data processing. Data processing is similar to macro photogrammetry processing of objects and landscapes. The image capture sequence may be automated, the degree and ease of which depends upon accessibility to programmable stage and image acquisition control on the SEM. We will outline the workflow and provide examples of the method and discuss applications. We will show example data from automated acquisition to control the SEM imaging system and stage to acquire an image set to produce a 3D model. We will demonstrate combining this surface method with complementary x-ray micro CT and FIB-SEM nano tomography volumetric techniques for multi-modal analysis applied to a study of structural color. Captured digital data files may be 3D printed or visualized in a 3D PDF file. But these files may also be imported into 3D physics-based modeling software to analyze mechanical material response, heat transfer, electromagnetic properties, or any other attribute associated with the external surface of the 3D object.

Life-Threatening Illnesses Associated with Organ Transplantation

Cynthia S. Goldsmith

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

Organ and tissue transplantations have become relatively common surgical procedures, and on rare occasions, life-threatening illnesses have occurred in multiple recipients of organs transplanted from a common donor. CDC has been involved in the investigation of several clusters of fatal disease that were seen in organ transplant recipients. Tissue-based diagnostic examination, including histopathology and immunohistochemistry, electron microscopy, viral isolation, and PCR, allowed for the diagnosis of an etiologic agent in these transplant-associated cases. This presentation will cover infectious agents associated with organ transplantation, namely, West Nile virus, rabies virus, lymphocytic choriomeningitis virus, and microsporidia. The immunocompromised status of the organ recipients can contribute to the fulminant infections and profound consequences that followed.

Leica Microsystems New THUNDER Imagers

Chris Murphy

Leica Microsystems, Buffalo Grove, IL

Leica Microsystems has continued its innovation with its new line of Thunder Imagers. These new systems utilize an opto-digital advancement called “Computational Clearing.” This new capability enables users to utilize the speed and sensitivity inherent in widefield microscopes, while minimizing the haze from out of focus light that can limit researcher’s ability to visualize thicker specimen. This talk can offer some insights into how the technology works, as well as as what systems are available with this capability.

Improved Method for Negative Staining of High Density Lipoprotein (HDL) Detects More Surface Detail without Modifying Particle Morphology

Rachel C. Hart¹, John T. Melchior², Scott E. Street², Jamie Morris², Shimpi Bedi², W. Sean Davidson², and W. Gray Jerome¹.

¹Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN, ²Department of Pathology and Laboratory Medicine, University of Cincinnati, OH

Negative stain transmission electron microscopy (TEM) has been used extensively for imaging and characterizing high density lipoproteins (HDL). While traditional negative staining methods have provided useful information, one limitation is the level of detail that can be visualized on the particle surface, particularly the resolution of proteins such as apolipoprotein A-I (APOA1). To overcome this limitation in our current studies, we have implemented a uranyl formate (UF) negative staining procedure for visualizing as well as quantifying diameter sizes in HDL preparations. Starting with the innovative approaches of Rames et al. [1] and by incorporating approaches from Ohi et al. [2], through trial and error we developed a UF procedure that provides more detail on the HDL surface (Fig. 1). Importantly, particle diameter size quantitation analyses using the UF procedure have yielded measurements consistent with our previous staining procedure, suggesting that the new procedure does not significantly alter particle morphology. The additional surface detail now allows us to explore 3-D reconstruction of HDL particles by single particle analysis.

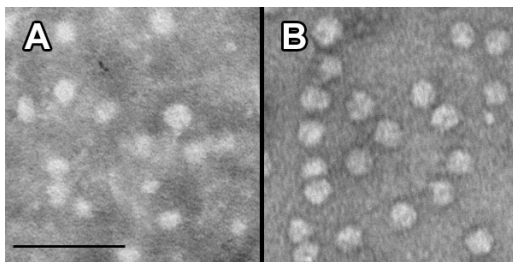


Fig. 1. Micrographs of plasma HDL particles, taken on a Philips/Tecnai T12 100 keV TEM at 150,000x magnification. Scale bar represents 50 nm. **A)** Particles stained with 2% phosphotungstic acid, pH 6.0, using our previous PTA staining procedure. **B)** Particles stained with 0.7% uranyl formate (UF), pH ~4.5, using our new UF staining procedure.

Fig. 1. Micrographs of plasma HDL particles, taken on a Philips/Tecnai T12 100 keV TEM at 150,000x magnification. Scale bar represents 50 nm. **A)** Particles stained with 2% phosphotungstic acid, pH 6.0, using our previous PTA staining procedure. **B)** Particles stained with 0.7% uranyl formate (UF), pH

TEM imaging was performed in part through the use of the Vanderbilt Cell Imaging Shared Resource (supported by NIH grants CA68485, DK20593, DK58404, DK59637 and EY08126). This project was supported by NHLBI grant P01 HL116263.

References:

- [1] Rames M, Yu Y, Ren G. Optimized negative staining: a high-throughput protocol for examining small and asymmetric protein structure by electron microscopy. *J Vis Exp*. 2014;(90):e51087. Published 2014 Aug 15. doi:10.3791/51087
- [2] Ohi M, Li Y, Cheng Y, Walz T. Negative Staining and Image Classification - Powerful Tools in Modern Electron Microscopy. *Biol Proced Online*. 2004;6:23-34.

Applications of CMOS-Based Imaging Sensors for High-Speed EBSD Mapping

Roger Kerstin
EDAX/Ametek, Mahwah, NJ

Complementary Metal-Oxide-Semiconductor (CMOS) based imaging sensors have recently been used for the collection of Electron Backscatter Diffraction (EBSD) patterns in an SEM for orientation imaging applications. The advantage of a CMOS sensor, compared to a traditional Charge Coupled Device (CCD) sensor, is the fast frames rates that are achievable while providing a larger pixel resolution image. In this work, an EDAX Velocity Super CMOS detector was used to index at speeds of 4,500 patterns per second at 120 x 120 pixel resolution, compared to 1,400 patterns per second at 30 x 30 pixel resolution for a CCD-based detector. These speeds can be obtained at comparable SEM beam currents, with equivalent or better quality. Results from different materials including steel, nickel, aluminum, and titanium alloys will be shown to demonstrate the indexing accuracy and precision that can be achieved at these fast acquisition rates.

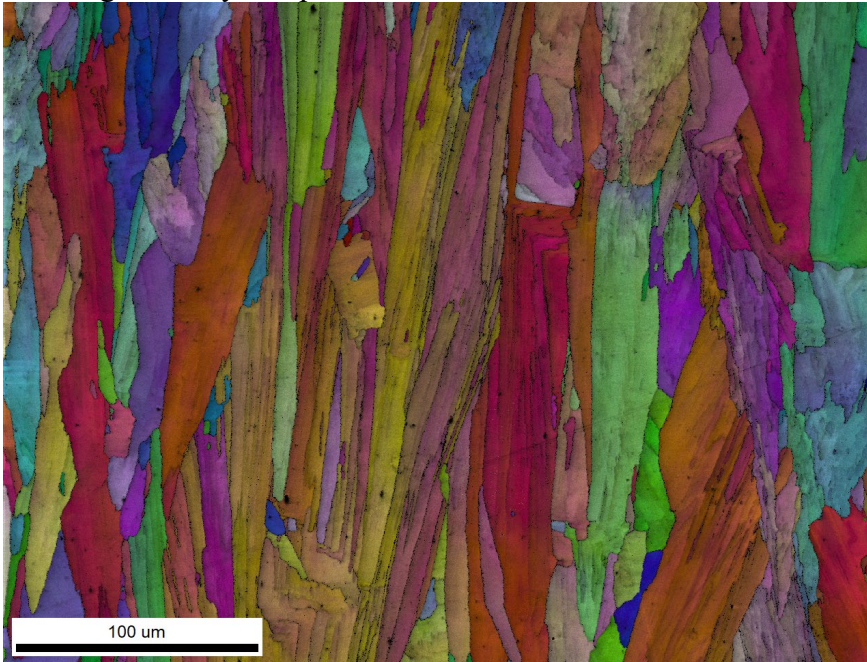


Figure 1-EBSD Image quality and inverse pole figure map collected at 4,500 indexed points per second with CMOS-based EBSD detector

