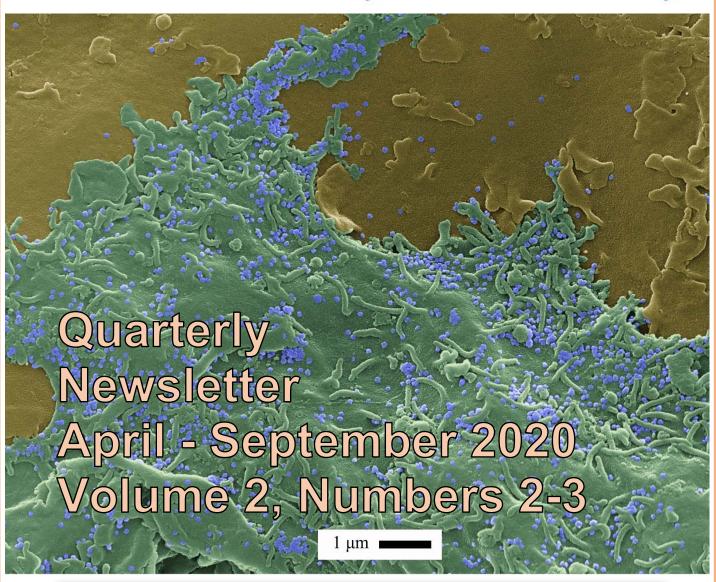


# Chesapeake Microscopy & Microanalysis Society



Published Quarterly by CMMS - The Chesapeake Microscopy and Microanalysis Society

# President's Column



Dear CMMS members and non-member readers. It seems that it has been an extremely long time since the last newsletter and I hope that this double issue will provide you some leisurely microscopy related and non-political reading. As a board, we had many plans for in-person meetings this year, including getting together in Milwaukee at the M&M, our spring meeting with associated seminars, and our Fall Social meeting. Alas, these went the way of other in-person meetings. To ensure the health of our members, we cancelled all in-person meetings, and subsequently had a Summer Speaker Series via online seminars. These were very well presented seminars and there is a synopsis of these in the newsletter. I would like to thank Joe Mowery for initiating these seminars and ensuring they went smoothly. Our featured local microscopist is Dr. Christine Brantner of George Washington University, and our featured laboratory is the Correlative Light and Electron Microscopy Workshop hosted by George Washington University.

As we deal with Covid-19 and all the social interruptions this virus has imposed upon us, we must always remember that the virus can be lethal, and we must be vigilant in the protection of ourselves and others until a successful vaccine is approved, distributed, administered and is effective for the majority of the population. Many of you are involved with researching issues related to the virus. I know of colleagues that have investigated the effectiveness of decontamination of PPE for re-use, investigating the lifecycle of the virus, and those investigating vaccine structure and efficacy.

Looking forward to 2021, we still want to reach out to members of local colleges, universities, government entities and industry to invite any microscopy-minded persons to become more involved with microscopy to strengthen the society. Future issues will continue to spotlight regional microscopists and microscopy related persons.

While we will not be able to meet during this calendar year, I hope to see many of you at the 2021 MSA/MAS meeting in Pittsburgh. Once we are able to gather, many of us will want to meet and socialize to discuss issues that have occurred during the pandemic, and because of the pandemic. Until that time, we will publish as much information as we can about online microscopy resources for training, education and enhancement. As always, please feel free to contact us if you have any suggestions or feedback.

While the year started out wonderfully, and when this particular pandemic has passed, I still hope to meet as many of you as possible and physically shake your hand. In the meantime, I am sending you best wishes for health and happiness.

Robert K. Pope President of CMMS, 2020 November 30, 2020



#### **CMMS 2020 Board Members**

#### **President**

Dr. Robert K. Pope
Senior Principal Investigator,
Bacteriology and Electron Microscopy
Battelle National Biodefense Institute
National Bioforensic Analysis Center



#### **President Elect**

Mr. Joseph Mowery
Biologist
Electron and Confocal Microscopy Unit
USDA Agricultural Research Service



#### **Treasurer**

Dr. Emma Bullock EPMA Laboratory Manager Geophysical Laboratory Carnegie Institution for Science



#### Secretary

Dr. Kedar Narayan Group Leader Center for Molecular Microscopy Frederick National Laboratory



#### **Communications Officer**

Dr. Tagide deCarvalho
Research Assistant Professor
Director, Keith R. Porter Facility
University of Maryland, Baltimore County



#### **Outreach Officer**

Dr. Thomas Lam
Physical Scientist
Smithsonian Museum Conservation Institute



#### Past President, Board Advisor

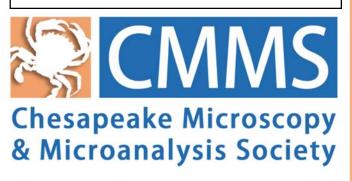
Dr. Ru-Ching Hsia
Associate Professor
Department of Neural Pain Sciences
University of Maryland School of Dentistry



Scanning Electron Micrograph of tissue culture cells infected with Severe Acute Respiratory Syndrome Corona Virus-2 (SARS CoV-2), the virus that causes COVID-19. The Vero cells were infected with one virus particle for every 100 Vero cells present. After two days, the cells were inactivated to preserve structure, harvested and sterility tested to demonstrate that no live virus remained. The Vero cells can be seen in green and tan, with blue-purple virus particles budding out of the heavily infected (green) Vero cell. The virus particles can be seen sticking to the surface of less infected (tan) Vero cells.

This work was funded under Agreement No. HSHQDC-15-C-00064 awarded to Battelle National Biodefense Institute (BNBI) by the Department of Homeland Security (DHS) Science and Technology (S&T) Directorate for the management and operation of the National Biodefense Analysis and Countermeasures Center (NBACC), a Federally Funded Research and Development Center. The views and conclusions contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed or implied, of DHS or the U.S. Government. The DHS does not endorse any products or commercial services mentioned in this presentation. In no event shall the DHS, BNBI or NBACC have any responsibility or liability for any use, misuse, inability to use, or reliance upon the information contained herein. In addition, no warranty of fitness for a particular purpose, merchantability, accuracy or adequacy is provided regarding the contents of this document.

Notice: This photograph has been produced by BNBI under Contract No. HSHQDC-15-C-00064 with the DHS. The US Government retains and the publisher, by accepting the photograph for publication, acknowledges that the USG retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this photograph, or allow others to do so, for USG purposes. Views and conclusions contained herein are those of the authors and should not be interpreted to represent policies, expressed or implied, of the DHS.



## "FOCUS" ON A LOCAL MICROSCOPIST



#### **Christine Brantner**

Christine Brantner is a Senior Scientist at George Washington University Nanofabrication and Imaging Center (GWNIC).

**CMMS:** Would you briefly introduce yourself to the members of the CMMS community?

**CB:** Hi, I'm Chris Brantner, the Senior Research Scientist for Electron Microscopy at the George Washington Nanofabrication and Imaging Center (GWNIC). I have been at GW for 5 years after spending time at NINDS, Janelia, DHS and NHLBI. I am past president of CMMS and I have hosted numerous CMMS events at GW.

**CMMS**: What was your training background, and what led you to become a microscopist?

I did not know what I wanted to do with the rest of my life when I was finished with college. Because of a Research Experience

for Undergraduates program, I knew about the University of Wisconsin at Milwaukee's Great Lakes Research Facility. I applied to go to graduate school to find a path in life. My initial training as a microscopist was at UWM in the Electron Microscope Lab of the Biology Department. My thesis adviser took me to meet the Director as soon as I reported for graduate school. I became the microscopist for several labs during my years as a graduate student so I was introduced to several projects and types of samples to look at with EM. When it came time for graduation, working in a Core or Center on many different instruments was projects and interesting to me than starting my own research lab. I answered an ad for a microscopist from NINDS at NIH and moved half way across the country. I have worked with many great people at several institutions around the DC area since.

**CMMS:** Could you briefly describe the focus of your research, and the type of EM specimens you typically work with?

**CB:** As a part of the GWNIC, I do not have an individual research focus. I have effort on several grants with users and I am part of the "research team" of any user who brings me a sample to look at in the SEM or TEM. I had mostly processed and imaged biological samples before arriving at GW. I have now broadened my repertoire of samples to include materials samples as GW has strong participation imaging from Nanofabrication lab, Chemistry and Physics Departments as well as the Engineering School and the Biomedical Engineering While my background is Department. microbiology, I tell users that if we can figure out how to get it into a microscope, I will try to image anything.

**CMMS:** What types of instrument do you have, and what techniques are performed in your lab?

CB: The full list of the instruments of the GWNIC can be found on our web page (www.nic.gwu.edu). I assist with everything experimental design to preparation to imaging for projects for the SEM, FIBSEM and TEM microscopes. We train or provide as a service the following at the GWNIC: SEM sample prep and basic imaging for biological and materials samples, SEM EDS mapping, SEM VolumeScope 3D, TEM sample prep and basic imaging for biological and materials samples, TEM EDS, TEM hi resolution lattice imaging, Focused Ion Beam SEM for 3D imaging and for TEM lamella preparation as well as large area imaging in the FIBSEM that looks like a TEM image except we are able to image very large samples to retain the 2D context of the high magnification images of structures. We can perform freeze substitution, immunogold labeling, correlative light and electron microscopy, and negative staining. If you find it in the literature, we will see if we can help you to make it happen at GW.

**CMMS:** What do you consider is the high point of your career and the best achievement so far?

CB: I have always been excited to start a new job and the great new experiences that await. The potential for growth, both professionally and personally, that each position has presented to me has led to a rich experience in the Washington DC area. I read The Hot Zone by Richard Preston while I was a graduate student; so one highlight for me over the years has been the time I spent working for the Department of Homeland Security at the Battelle National Biodefense Institute where I was afforded the chance to work in the BSL-4 lab to prepare samples for electron microscopy.

Two fun achievements that I possess are: a production company used several micrographs for a PBS special on microbes and I once held a top secret security clearance with DHS.

**CMMS:** In your opinion, what is the most challenging part of your job?

CB: That is a tough question. It is always a challenge to keep all of the instrumentation running in a Core Facility. And it is a fun challenge to determine the best way to process and image a sample that you have never seen before. I enjoy working in a Core/Center setting where I get to see all of the exciting ideas that the users have on a daily basis.

**CMMS:** What is the most important training that helped you with your career?

**CB:** The first training that I had in graduate school was probably the most important. My mentor in the UWM EM Lab knowledgeable, technology savvy, creative with the maintenance and repair of equipment and patient in teaching. She was involved in the local professional society and introduced me to a whole group of people who were there electronically if you needed to ask questions. My mentor and advisor took me to national meetings, had me present posters and allowed me opportunity to attend workshops. All of these things were great for learning and by their example, I have continued to go to meetings and workshops and to take all interested microscopists, young and old. From this base of learning electron microscopy to produce my thesis. I have added and built on that with the rest of the experiences I have had at all of my jobs, leading to the state of my craft and my knowledge at this moment.

**CMMS:** What are the pros and cons working in industry, academic or federal agency?

**CB:** I have spent most of my career in a government agency. I am sure that the pace is slower than in industry, but usually there were policies and procedures that when followed allowed one to get the work done at some point in time. I am now in an academic setting and this seems to have a very slow pace for action where administration is required. The type of samples and projects that I have seen in academics have been the most varied and creative. In government agencies, many times the money and resources are more stable and reliable than in academics so as to allow research to different continue through economic situations. The pay is higher in industry jobs, but there is a lack of independence that an individual has related to projects because it is all about the company's products and profits.

**CMMS:** Do you have any words of advice for the junior members of our EM community when they choose a career path in industry, academia or government?

**CB:** These things I have mentioned here have probably all been pointed out before, but the trick is knowing yourself well enough to know which environment you will thrive in. When you interview for a position, try to speak with as many different people at as many different levels and departments as you can so that you get a better feeling for what it might be like to work there. It is always possible to do a course correction in your career as circumstances at a place or within yourself change with time. Don't fight it. Go with it. The hot job at the current time may go out of fashion over years. It is no longer the norm for people to stay in a position for most or all of their career.

**CMMS:** Do you have any suggestion for future CMMS events or what CMMS can do

to promote EM techniques or help the career advancement of our members?

**CB:** Members could make themselves available for informational interviews about what it is like to work at \_\_\_\_\_. I am open to having people contact me for informational interviews about what it was like working somewhere.

Put digital tours of our labs on the CMMS website.

Members could self-report publications or upcoming talks to CMMS, and these could be posted on the CMMS website. I am thinking about a digital bulletin board of some sort for members to browse.

**CMMS:** Can you tell us about some of the other activities in which you have been involved?

**CB:** Here at GW, I have been involved in the creation and execution of annual CLEM workshops. (June 2020 was cancelled, so our 4th annual CLEM workshop will be in June 2021. Please see additional article for more information on this week-long, intensive look at all things CLEM.

**CMMS:** What are the strangest EM samples you have ever encountered? What are the most difficult EM specimen you have ever worked with? What are the worst mistakes you have ever made?

Mistakes in the lab that were memorable?

CB: On my first job out of my PhD, I needed to learn how to use a Denton coater (manually valved vacuum chamber). I had never seen one before. I watched and was trained and then set free to use it. Well, on my first solo attempt to coat something, I did not valve it correctly and the boss came running because it was making a terrible

noise "trying to pull a vacuum on the whole room". Oops!

More recently, I set about making some epoxy resin in the lab. I added all of the components, put it on the stir plate and walked away. My lab manager came and asked me what I was doing in the lab. When I indicated that I was making resin, she

disagreed with me and encouraged me to go and look at it. Well, I had a great colored, layered bunch of resin components with a thick crust on the top. No one, including me, knows what happened with that. I use these as extreme examples while I am training users to emphasize certain details that are important in these and other protocols in the lab.

#### **CMMS Contact Information**

Instagram

www.instagram.com/chesapeakemicroscopy

**Twitter** 

www.twitter.com/chesapeakemms

Website

www.chesapeakemicroscopy.org

**Email** 

ChesapeakeMicroscopySociety@gmail.com

#### **Submission Deadline**

Submission deadline for the October – December edition of the newsletter is December 18, 2020. Submit all potential articles, photos and information you would like to share with the local microscopy community to the following address.

ChesapeakeMicroscopySociety@gmail.com.





# Upcoming Microscopy Meetings

FCEM 2021 - Frontiers in Cryo-Electron Microscopy

February 2-5 • Online Conference

http://www.keystonesymposia.org/ks/Online/Events/2021B1/Details.aspx?EventKey=2021B1

FOM2021 - Focus on Microscopy 2021 - Online Conference

March 28-31 • Online Conference

http://focusonmicroscopy.org/

**BIOPHOTONICS CONGRESS: Optics in the Life Sciences** 

Novel Techniques in Microscopy

April 12-15 • Vancouver, British Columbia, Canada

https://www.osa.org/en-us/meetings/osa meetings/osa biophotonics congress/program/

MICROSCOPY, HISTOPATHOLOGY AND ANALYTICS

Apr 24-27 • Fort Lauderdale, Florida, United States

https://www.osa.org/es-

es/meetings/global calendar/events/microscopy histopathology and analytics/

IUMAS-8 — 8TH MEETING OF THE INTERNATIONAL UNION OF MICROBEAM ANALYSIS SOCIETIES May 24-28 • Banff, Alberta, Canada

https://www.microbeamanalysis.eu/events/event/74-iumas-8-8th-meeting-of-the-international-union-of-microbeam-analysis-societies

GORDON RESEARCH SEMINAR — THREE-DIMENSIONAL ELECTRON MICROSCOPY

June 13-18 • Newry, ME, United States

https://www.grc.org/three-dimensional-electron-microscopy-conference/2021/

GORDON RESEARCH SEMINAR — TISSUE MICROSTRUCTURE IMAGING

July 18-23 • Stonehill College, Easton, MA, United States

https://www.grc.org/tissue-microstructure-imaging-conference/2021/

MICROSCOPY AND MICROANALYSIS 2021

August 1-5 • Pittsburgh, PA

https://www.microbeamanalysis.eu/events/event/47-microscopy-microanalysis-2021

## HITACHI Inspire the Next

# Size without Compromise

The SU3900 VP-SEM with an Extra-Large Chamber

Hitachi's SU3900 Variable-Pressure SEM features an extra-large chamber to accommodate a multitude of analytical applications: EDS, EBSD, micro XRF, in situ, heating, cooling, tensile testing, and more. Equipped with 20 ports and a robust stage, the SU3900 can handle it all! Whether your sample is large, small, heavy, magnetic, or non-conductive, the SU3900 is the instrument for your lab.

### Extra-Large Chamber & Heavy-Sample Stage

Superior X/Y range of motion for up to 5 kg sample weight

#### **Ultra-Wide NaviCam FOV**

Covering full observation sample area (200 mm  $\Phi$ )

#### Intelligent Filament Technology

Auto optimization for improved filament performance and lifetime

# Fully Integrated High-Quality EDS Capability

Seamless SEM and EDS operation from a single GUI

For more information: microscopy@hitachi-hta.com





Sample: Pure Titanium Accelerating Voltage: 5 kV Magnication: 800x



Extra-large chamber for max. 300 mm ø, 130 mm tall, and 5 kg sample

Innovation • Synergy • Solutions



Hitachi High Technologies America, Inc.
 www.hitachi-hightech.com/us 800-253-3053 microscopy@hitachi-hta.com

© 2020 Hitachi High Technologies America, Inc. All rights reserved

From Eye to Insight



#### CRYO-EM MICROSCOPY SAMPLE PREPARATION AND WORKFLOW SEMINAR & DEMONSTRATION



#### **NEW! THUNDER IMAGER** EM CRYO CLEM

The new THUNDLR Imager LM Cryo CLLM from Leica utilizes a new, exclusive opto-digital method called Computational Clearing that eliminates the haze of traditional cryp widefield images by algorithmically removing the nut-of-focus data without compromising the raw image data. This results in crystal clear visualization of cryn images without the baze, and provides precise identification of structures-of-interest.

SEMINAR: Tuesday, February 18, 2020 Bodian Room (2-200)/1830 E. Monument St. 1:30 PM

Refreshments provided - Please RSVP!

Presenter: Louise Bertrand Product Performance Manager, Widefield Guest Speaker: Gleb Shtengel, PhD Senior Scientist, Janelia Farm Research Campus

HANDS-ON DEMONSTRATIONS: February 18-21, 2020

Building Rangos/855 N. Wolfe St. Please RSVP to schedule your time! HOSTED BY:

Shigeki Watanabe, PhD Dept. of Cell Biology

Scot Kuo, PhD

SCAN HERE TO RSVP:

For more information or to RSVP, please visit https://www2.leica-microsystems.com/JHU-CLEM,

(443) 257-2941

paula.cranfill@leica-microsystems.com

Paula McGuire, Account Manager - Widefield Mark Kukucka, Account Manager - EM Sample Prep (410) 443-1761

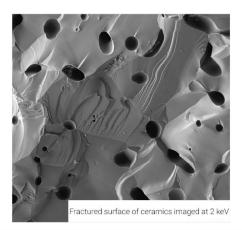
Mark.Kukucka@leica-microsystems.com



## **TESCAN CLARA**

**NEW FIELD-FREE UHR SEM** 

Ultra-High Resolution Scanning Electron Microscope (SEM) takes materials characterization to the next level



#### Find out more at www.tescan.com

Angstrom Scientific, Inc. distributes key products to complement and support electron Microscopy and EM sample prep equipment. Specifically:

#### HITACHI Inspire the Next

#### TM 4000 Table Top SEM

The TM4000 provides a solution for SEM users to easily obtain high-quality data and quickly generates reports for a very efficient workflow.

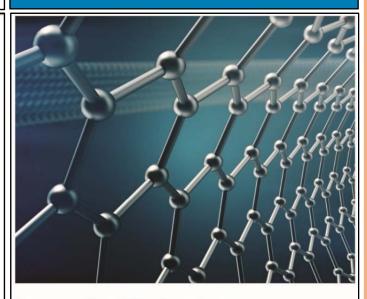


From Eye to Insight

Leica Array Tomography Solution: Artos 3D Consistent, ultrathin serial sections for array tomography in less time with the ARTOS 3D ultramicrotome.

#### LEICA EM CRYO CLEM

One system to serve your workflow needs in Correlative Light and Electron Microscopy.



## Innovative Technology, Global Reach

Make critical decisions with confidence

Find out more at thermofisher.com/EM



2019 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.



Position		
Institute Laboratory	Brief Description	More information
CryoEM Staff Scientist  Howard Hughes Medical Institute Janelia Research Campus  Ashburn, Virginia, United States	Janelia Research Campus is a pioneering research center in Ashburn, Virginia, where scientists pursue fundamental questions in neuroscience and imaging. The Howard Hughes Medical Institute (HHMI) launched Janelia in 2006, establishing an intellectually distinctive environment for scientists to do creative, collaborative, hands-on work. Our integrated teams of biologists, computational scientists, and tool-builders pursue a small number of scientific questions with potential for transformative impact. We share our methods, results, and tools with the scientific community. It is a uniquely innovative and collaborative atmosphere that reflects HHMI's reputation for excellence.	https://hhmi.wd1.myw orkdayjobs.com/en- US/External/job/Janelia -Research- Campus/CryoEM-Staff- Scientist R-186
Electron Microscopy Specialist Embryology Department Carnegie Institution for Science	The most important responsibility of this position is to provide electron microscopy service to all labs who need it. This involves acquisition and fixation of specimens, embedding, cutting and photography of samples as well as instruction on any phase of the process that the lab may choose. Routine upkeep and maintenance of the EM and all other laboratory equipment. It also includes providing histology and cryoscopy services and instruction to all labs.	https://jobs.carnegie science.edu/jobs/ele ctron-microscopy- specialist/
STAFF SCIENTIST MICROSCOPY CORE HEAD  Laboratory of Cellular and Molecular Biology (LCMB), Center for Cancer Research (CCR), NCI, NIH, HHS	The Laboratory of Cellular and Molecular Biology (LCMB), Center for Cancer Research (CCR), NCI, NIH, HHS is looking for a motivated and skilled microscopist to fill the position of Head of its Microscopy Core. The Core provides state-of-the-art equipment, advice and training to approximately 40 researchers from the LCMB groups, although other trained CCR researchers are welcome to use the instruments with approval of the Core Head. The Core places an emphasis on training independent users, but the staff is available to assist in all phases of experiments. This includes experimental design, data acquisition, and data analysis. The facility staff has particular expertise in live cell imaging, Fluorescence Lifetime Imaging (FLIM), and single molecule localization microscopy (SMLM) including both photoactivation localization microscopy (dSTORM). The Core Head will also be expected to participate in meetings of the CCR Microscopy Core Heads and to interact extensively with the other microscopy facilities in CCR. The Core's equipment includes a Leica SP8 LSCM with white light laser and a Falcon FLIM system, a Nikon Spinning Disk, and a Nikon Total internal reflection fluorescence microscopy (TIRF) system.	https://jobs.microscop y.org/job/director-of- the-integrated-imaging- center-iic/54742802/

Position		
Institute	Brief Description	More information
Laboratory	·	
Scientist II, Electron Microscopist  Frederick National Laboratory  National Cancer Institute	Provide technical advice to investigators on sample preparation, and data collection  * Operate TEM microscopes, specifically Titan Krios and Talos Arctica; task will include loading samples, screening, and performing high-resolution data collection for single particle studies as well as cryoelectron tomography  * Manage and use CryoSPARC live software as well as other software related to EM (Relion, IMOD, Amira)  * Manage maintenance for the Titan Krios and the Talos Arctica as well as associated instruments (T20, Talos L120C)  * Train new CMM employees and other users in the use of the Krios, Tecnai T20 and Talos L120C as well as in data collection software and in the sample preparation techniques  * Interact closely with colleagues within the CMM as well as other groups	https://jobs.microscop y.org/jobs/view/scienti st-ii-electron- microscopist- req1413/ba5122c0- 13314507545/
Post-doctoral Fellow Frederick National Laboratory National Cancer Institute	Fully funded postdoctoral research positions are available in the Dr. Kylie Walters' Protein Processing Section within the Structural Biophysics Laboratory at the Center for Cancer Research (CCR), National Cancer Institute (NCI). This research section integrates structural and cellular biology methods to uncover atomic-level mechanistic information on the cellular processes that drive disease and can be harnessed for cancer therapy. These projects will provide the postdoctoral fellow unique opportunities to develop and apply cutting-edge structure-based drug design approaches, including cryoelectron microscopy, x-ray crystallography, and NMR, as well as chemical biology approaches that incorporate degrader warheads. The fellow will join a highly collaborative and growing research environment with new cryoelectron microscopy equipment and state-of-the-art core facilities including, but not limited to, gene editing, next-generation sequencing, mass spectrometry and cell imaging. The candidate will be fully funded by a competitive intramural fellowship.	https://ccr.cancer.gov/careers/post-doctoral-fellow-structural-biology-chemical-biology-structure-based-drug-design-small-molecule-degraders/26281
Post Doc -Cryo- Electron Microscopy of Membrane Proteins  University of Maryland School of Medicine  Baltimore MD	The Hong ( <a href="http://www.medschool.umaryland.edu/hong-lab">http://www.medschool.umaryland.edu/hong-lab</a> ) and Weber ( <a href="https://www.medschool.umaryland.edu/profiles/Weber-David/">https://www.medschool.umaryland.edu/profiles/Weber-David/</a> ) laboratories are recruiting a post-doctoral fellow with an interest in using high-resolution single particle cryo-electron microscopy (cryoEM) to investigate the structural biology of membrane proteins implicated in cancer and infectious diseases. The CBT houses a new 200 keV Thermo Fisher Talos Arctica cryoEM microscope equipped with a Gatan K3 direct electron detector and a second 200 keV Thermo Fisher Glacios cryoEM microscope equipped with a Falcon III direct electron detector. A 120 keV FEI Tecnai T12 TEM equipped with a CCD camera is available for negative stain imaging as are 300 keV systems at the nearby NCI National cryoEM center.	https://umb.taleo.net/careersection/umb_fac_ulty+and+post+docs/jobdetail.ftl?job=157041 &source=Indeed.com



### **Related Links**

Chesapeake Microscopy and Microanalysis Society www.chesapeakemicroscopy.org

Microscopy Society of America www.msa.org

Microanalysis Society www.microbeamanalysis.org

American Microscopical Society www.amicros.org

AASP – The Palynological Society www.palynology.org

Geological Society of America www.geosociety.org



# CMMS

# Chesapeake Microscopy & Microanalysis Society

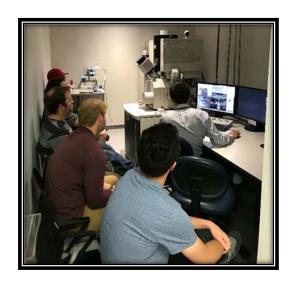
## SPOTLIGHT ON A MICROSCOPY WORKSHOP

Third Annual Correlative Light and Electron Microscopy (CLEM) Workshop With an Emphasis on Biological Samples at George Washington University Nanofabrication and Imaging Center (GWNIC)

The Washington University George Nanofabrication and Imaging Center (GWNIC) hosted the third annual Correlative Light and Electron Microscopy (CLEM) Workshop on June 10-14, 2019. The workshop, attended by 20 participants from various institutions, was a combination of lectures from invited speakers, applications scientists and staff as well as demonstrations and discussions instrumentation and protocols. Vendors were invited to assist with instrument demonstrations as well as to introduce new instrumentation that could be used for CLEM. The workshop was geared towards anyone interested in learning new techniques related to CLEM.



Anastas Popratiloff, GWNIC, at Teneo SEM impressing upon attendees the importance of matching pixel size for overlaying image files for correlative microscopy



Ken Wu, Thermo Fisher, at Helios FIBSEM performing the milling step for the fiducial marker for using the Slice and View software to create a 3D stack of images through a sample

The main theme of the workshop was correlating large-area images of the same sample created with both light and high-resolution electron microscopy. Strategies for sample preparation suitable for both light microscopy (LM) and electron microscopy (EM) were demonstrated and discussed. The GWNIC staff shared their real-world workflows for these experiments. Data collection from modern microscopes with multiple modalities was demonstrated. This was followed by discussions of data management of large data sets.

The first day was a lineup of lectures introducing the topics that would be demonstrated and discussed throughout the workshop. Day two concentrated on LM. Several vendors brought confocal microscopes to the workshop and were given samples prepared by GWNIC staff to simulate the type of preparations that are

routinely imaged for CLEM experiments at GWU. From there, day three began with demonstrations and discussions of sample preparation (vibratome slicing, processing and embedding in resin, ultramicrotomy) after LM imaging to prepare the sample for EM.



Sarah Crowe, Leica Microsystems, at TCS SP8 Multiphoton Confocal discussing the basics of image formation in the microscope

There are many challenges to finding the same feature in LM and EM. Several tips and tricks were shared to make this process easier. Then 2D EM for large-area imaging was demonstrated in the SEM and FIBSEM where images are inverted to look like transmission electron microscope (TEM) images. A workflow for immunogold labeled, large-area resin sections on silicon wafers was shown. The fourth day brought discussions of 3D EM. The Thermo Fisher VolumeScope was demonstrated along with the use of the FIBSEM to create large data sets of structural information. The final day was devoted to image analysis and data handling. These are topics that should not be left out of a correlative workshop as it is necessary to know what to do with data once it is collected from the complex microscopes and complex sample preparations in the CLEM workflow. Three software packages were demonstrated and discussed as they related to use for the overlay of data from several instruments.



James Shaw, from Bitplane, doing a demonstration of the many new aspects of the Imaris software for image analysis

The 4th Annual GWNIC Correlative Light and Electron Microscopy Workshop will, hopefully, be presented in June of 2021 as the COVID-19 pandemic caused the cancelation of it for June 2020.

#### Day one Lecture titles and presenters

Multi- or single modality layered image data sets as a tool for navigation, precise and inclusive data collection **Anastas Popratiloff** 

Using Fluorescent Proteins and Genetically Encoded Sensors **Erik Rodriguez, GWU Chemistry** 

Introduction to Confocal Microscopy <u>Sarah</u> <u>Crowe, Leica</u>

Microscopic Imaging Across Multiple Workflows: Synergy of TEM, SEM and LM <u>Tara Nylese</u>, ThermoFisher

Overlaying 3D CLEM data in the arivis Scalable Image Storage environment **Chris Zugates, Arivis** 

Development and Applications of Fluorescent Proteins (FP) for Correlative Light and Electron Microscopy (CLEM) <u>Gaby Paez</u> <u>Segala, Janelia Research Campus</u>

Zeiss 3D Cryo-Workflows – High-Content, High-Resolution, High-Flexibility <u>Geoff Perumal, Zeiss</u>

# **NEW PRODUCTS**

## PELCO® Secondary Antibody Gold Conjugates for Light & Electron Micrscopy



Precisely engineered to give uniform gold spheres with a narrow size distribution.

We currently offer conjugates with gold particle sizes ranging from 5nm to 40nm.

Also available: FluoroNanogold™ Secondary Antibody Conjugates

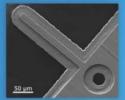
### PELCO® CLEM Silicon Support Grids with Silicon Nitride Membranes



3.0mm major diameter with two parallel 1mm flats for repeatable alignment

# PELCO<sup>®</sup> Indicator FIB Lift-Out TEM Grids, 5 or 8-pin





SEM images of 5-pin Indicator FIB Grid

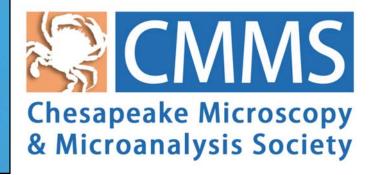
# TED PELLA, INC. Microscopy Products for Science and Industry

www.tedpella.com 800.237.3526

# **Classics Corner**



Robert K. Pope took this photo in 2005 of a
Siemens Elmiskop 102 at the
Histology Center in the old Hospital in
downtown Strasbourg France. The microscope
still worked brilliantly.



## **CORPORATE SPONSORS FOR 2020**

**Platinum Level** 











## **Gold Level**







ONLINE COURSES AND WEBINARS RELATED TO MICROSCOPY & IMAGING					
Optical Microscopy					
Microscopy Series	iBiology	https://www.ibiology.org/online-biology- courses/microscopy-series/			
Microcourses	Harvard Medical School	https://www.youtube.com/c/Microcourses/videos			
MyScope Microscopy Training	Microscopy Australia	https://myscope.training/			
Guidance for Quantitative Confocal Microscopy	Olympus	https://www.olympus- lifescience.com/en/resources/webinars/ocap-guidance- for-quantitative-confocal-microscopy/			
Electron Microscopy					
TEM for materials science	Swiss Federal Institute of Technology Lausanne	https://www.coursera.org/learn/microscopy			
Nanotechnology: A Maker's Course	Duke University, NCSU, UNC	https://www.coursera.org/learn/nanotechnology			
Getting started in cryo-EM	Caltech	https://www.coursera.org/learn/cryo-em			
Imaging SARS-COV-2 safely: Protecting the microscopy community	Francis Crick Institute	https://www.crick.ac.uk/whats-on/webinarimaging-sars- cov-2-safely-protecting-the-microscopy-community			
EDS in the TEM: Fundamentals & Principles	EDAX	https://www.workcast.com/register?cpak=441889406045 7230&elq mid=33818&elq cid=10009956			
	Energy-dispersive X-ray spectro	scopy			
EDS Mapping: Data Collection, Representation, Extraction & Mining	EDAX	https://www.workcast.com/register?elq_mid=25834&elq_cid=10009956&cpak=9199173860181816&elqTrackId=9_b1cfbdb5a9745d0b14762378544fbfb&elq=6adbd887cbd3_4f3e8e340203e2074643&elqaid=25834&elqat=1&elqCam_paignId=16597_			
Art, Science, Microscopy and EDS	Oxford Instruments	https://view6.workcast.net/register?cpak=126758522916 8117&referrer=wastt			
	Image Processing & Analysi	is			
Image Processing and Analysis for Life Scientists	Swiss Federal Institute of Technology Lausanne	https://www.edx.org/course/image-processing-and- analysis-for-life-scientists			
Introduction to Machine Learning	Duke University	https://www.coursera.org/learn/machine-learning-duke			
Image Analysis with Fiji	University of Liverpool	http://pcwww.liv.ac.uk/~cci/reveal_ia/ImageAnalysisWith Fiji.html			
Introductory Python Tutorials for Image Processing	Zeiss/Apeer Micro	https://www.youtube.com/playlist?list=PLHae9ggVvqPgy RQQOtENr6hK0m1UquGaG&mkt_tok=eyJpljoiWmpVMU1 6azVOMIEzTmpBMSIsInQiOil3YWI0dIRJN2EyOWI6NXIPME 5UY1VDUIZ3aFd4eVwvTGdcL09cLzUrQURkVDhWMkU5alh 6MytHMGFVTnU4Vmpra2ZvY3NIMEkxeFZnSmRwTWppb1 wvTk5FZnc9PSJ9			
Image and Video Processing: From Mars to Hollywood with a Stop at the Hospital	Duke University	https://www.coursera.org/learn/image-processing			
Fundamentals of Digital Image & Video Processing	Northwestern University	https://www.coursera.org/learn/digital			
Bioimage Analysis Course	iBiology	https://www.ibiology.org/online-biology- courses/bioimage-analysis-course/			
Beginner's Guide to Colouring EM Images	Microscopy Australia	https://micro.org.au/events/webinar-beginners-guide-to-colouring-em-images/			

# **Quarterly Microscopy Puzzle**

```
CMSPAJCXHLPREGEDBS
DKWAKGVHXREGNQEIA
LACOFNOCETUIPXHSVS
       UMCESHALE
     Ι
            QCAKN
       \mathsf{L} \mathsf{M} \mathsf{M}
  SRAVONTVSP
                  G
               Z 0
     TRGEOE
                  Ε
                    Ι
       OWRC
             R
               N
                Ι
                  Ν
                   A D
  ZCABSOR
                Ι
             Р
               Т
                  0
                   Ν
            Ι
                    Ι
       \mathsf{C} \mathsf{N}
          Υ
             UF
                  Ε
        L
          GBAM
                7
       F
         Н
          B H H
               R
                  Μ
    RFUHCHXIGAN
                     F
  YKGUANCDHDF
    QNUKWEAZGHEK
               QGJ
   SVGNJ
          0 0 L
                   A X V
WYHWUYDHFATXXRZLEE
 CSNELBXTDGNSC
                     ISWL
```

ABSORPTION
ARGON
CHESAPEAKE
CONFOCAL
DISPERSIVE
ELECTRON
EMISSION
ETCHING

GALLIUM
ION
LENS
MICROANALYSIS
MICRON
SECONDARY
SPECTROMETER
WEHNELT

# **Last Quarterly Microscopy Puzzle Answer**

**Spot the Difference for Electron Microscopists** 

Spot the 10 differences in the Carnegie Electron Probe lab!







Chesapeake Microscopy & Microanalysis Society

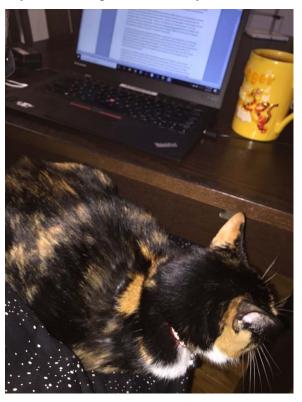
# Probing in the time of COVID-19 by Emma Bullock

The first time I heard about the coronavirus was back in January. I was just about to go on holiday to Mexico, and at the time, the situation didn't seem so bad. As the weeks went on, I think we all began to realize the seriousness of this novel virus. Finally, in March, the Carnegie Institution for Science made the decision to close our campus with the goal of keeping all of the people working there safe. On Friday, March 13th, our instruments were put into standby and we said "au revoir" to our colleagues. I remember commenting at the time that I'd see people in a couple of weeks – I never imagined that it would be months before I would go back, and that we still don't have a definite date for fully reopening!



Me, sporting my homemade mask, in front of my electron probe at the Carnegie Institution for Science on June 8<sup>th</sup>, 2020.

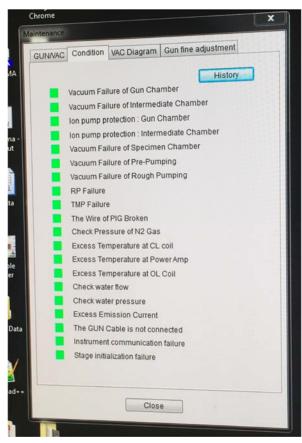
The first few weeks of the lockdown provided an opportunity to catch up with some reading and paperwork – the things I always wanted to do but never seemed to quite have the time for. My cats were delighted that I was home all the time, and got endless treats and snuggles. I really missed my labs though. My instruments were not connected to the internet, so I had no way of checking them remotely.



My "coworker" Bella, helping me to read and write articles...

At the beginning of June, we got word that the campus would be open on a very limited basis and that those folks who wanted to check out the condition of their instruments could seek permission to do so. On Monday, June 8<sup>th</sup>, after almost three months away, I set foot on campus again. It was strange to

see the parking lot empty, and the corridors – where in normal times you're always certain to run into someone on their way to grab coffee – were silent. As for the instruments? Much to my delight, both the SEM and my electron probe were perfectly happy, with green lights across the board. I checked them both out, and they ran just fine. It was like I'd never left!



Green lights across the board – just what I hoped to see!

Over the subsequent weeks, the electron probe lab re-opened on a very limited basis. I would set the instrument up and sit at the back of the room providing assistance when needed. I am lucky that my instrument is in a large room, which provides plenty of space for physical distancing. Masks and gloves were mandatory, and Carnegie also supplied us with disinfectant and hand sanitizer. Our janitorial staff did an amazing job of cleaning regularly too. My users were happy to be

able to collect that last bit of data they needed for presentations and publications, and I was happy to see my instrument being utilized again.

Just last week, we finally managed to get a long-planned upgrade to our electron probe computer installed. We made the switch to the Windows 10 operating system. This means that my electron probe is now back on the network, and I can run it remotely again! I'll still need to go in to change samples, but it will make it much easier to troubleshoot issues or set the probe up without necessarily being on campus. I do miss interacting with my colleagues though – at Carnegie, we have a very active community, and would often get together to discuss what we're working on, or what exciting developments in our field have been published.

How will things look in the future? Well, we're slowly moving towards increasing the number of people on campus, but I think it'll be a while before we're back to normal. My colleagues and I still regularly meet via Zoom, and have seminars and tea-breaks together. Meeting virtually allows us to catch up with colleagues and friends that are now spread out across the globe. Some part of me hopes that we can keep doing this, even when it's safe to meet up in person again. It's been nice to catch up with the reading that I've put off for too long, and I am grateful that I can now run the lab from home, but I am looking forward to interacting with people again. In the meantime, my main hope is that everyone stays safe and healthy, so that in the future we can all get together and have CMMS meetings in person again.

How has your lab been affected by the coronavirus? Are you allowed back on site, or are you working remotely? Share your pictures and stories with us at our website: <a href="https://chesapeakemicroscopy.org/">https://chesapeakemicroscopy.org/</a>, under the "Image Gallery" tab.

# Recap of the CMMS Virtual Summer Speaker Series

## By Joe Mowery

This summer CMMS hosted a virtual speaker series during the lead up to M&M 2020. We were pleased to feature three engaging microscopy talks in July by guest speakers Nestor J. Zaluzec, John Shields and Zechmann. The talks were well attended with 30-50 participants and served as a good opportunity to reconnect with local colleagues during a time when we were all isolated indoors. One advantage of a virtual meeting format is the ability to invite speakers from outside our local region who we don't often get to hear from.



Nestor Zaluzec, Senior Scientist Argonne National Laboratory

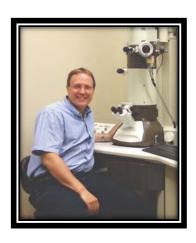
Nestor Zaluzec was the first presenter in the series with a talk on hyperspectral imaging using his high

efficiency detectors in a state of the art analytical electron microscopy. His instruments enable him to capture hundreds of time resolved spectral images from multiple bands across the electromagnetic spectrum to measure the elemental distribution within a time. These sample over advancements are enabling greater opportunities to study beam sensitive soft materials and biological samples, in a field of study previously reserved for hard materials.



John Shields, Director of Georgia Electron Microscopy at the University of Georgia

At CMMS, we always aim to alternate between material science talks and biological talks, so we were pleased to have John Shields present on the challenges in preparing food science samples for microscopy. His talk highlighted the problem solving mindset, and the arsenal of techniques that are required by a microscopist to adapt to widely variable food samples. John emphasized an excellent quote by G. Meeks which states "specimen preparation is still very much of an empirical art based on experience and to a certain extent on intuition".



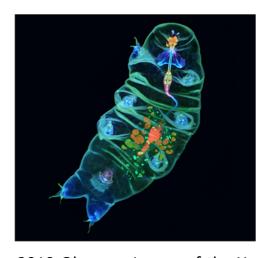
Bernd Zechmann, Director of the Center for Microscopy and Imaging at Baylor University

The third talk was by Bernd Zechmann, who presented on the preparation of plant samples for TEM and SEM. Bernd discussed his use of the automated Leica AMW microwave processor, which increases the rate of diffusion of fixatives and reagents into samples. of diffusion This increased rate drastically speeds up sample processing times. Bernd also described a simple technique using dental putty to create surface replicas of plants

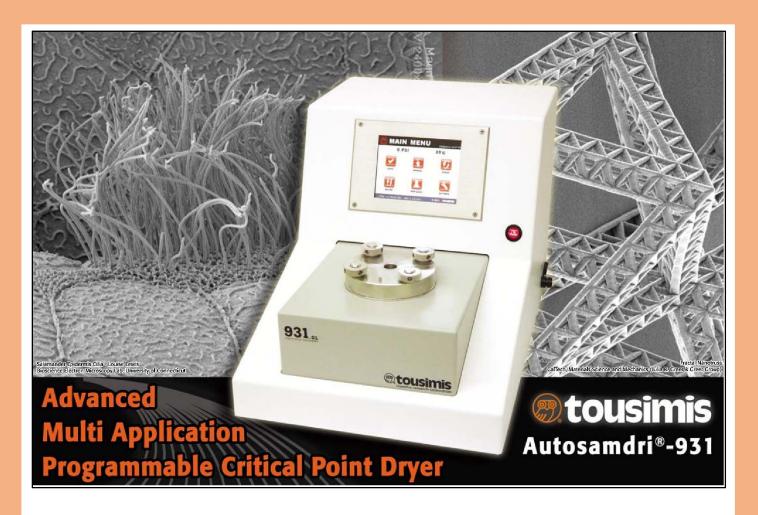
leaves, which can be used for rapid SEM studies, without chemical processing artifacts. Bernd concluded his talk discussing the rising cost of samples preparation equipment and instruments that gradually is diminishing the ability of small labs and universities keep to up with technological advances.

CMMS would like to thank all the speakers, and everyone who attended the speaker series. We would love to receive feedback if you are interested in attending additional virtual talks.

#### **Previous Issue Cover Photo**



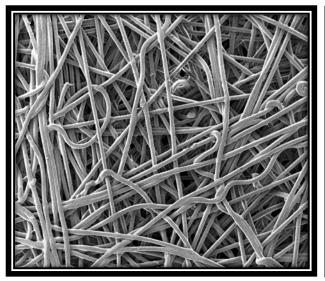
The 2019 Olympus Image of the Year, Americas regional prize was awarded to CMMS board member, Tagide deCarvalho. This colorful tardigrade micrograph was produced via Calcofluor white and Congo red staining and imaged with a 20x objective on a Zeiss LSM 900 confocal microscope

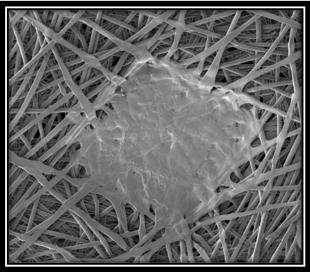


## Are you a Microscopist?

Guess the material from the images below?

Send responses to <a href="mailto:ChesapeakeMicroscopySociety@gmail.com">ChesapeakeMicroscopySociety@gmail.com</a>
All that guess correctly will be listed in the next newsletter.





# **FEATURED PHOTOGRAPH**



The featured photograph in this issue was submitted by Tagide deCarvalho, Director of the Keith R.

Porter Facility, University of Maryland, Baltimore County (UMBC).

The photograph is of a moss rosette exhibiting autofluorescence on a Leica SP5 confocal microscope.

The micrograph was awarded an Image of Distinction in Nikon's Small World 2020 contest.