Southern California Society
for
Microscopy & Microanalysis

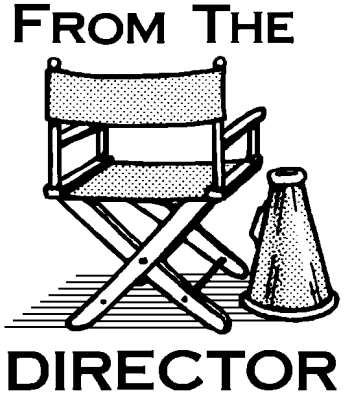
Full-Day Symposium

Saturday
March 30, 2013

FOWLER MUSEUM
at UCLA

Microscopy Society of America Invited Speaker

Daniela Nicastro
Brandeis University



Your SCSMM Board has been working hard to bring you an excellent program for the upcoming March 30th Spring Symposium to be held at Fowler Museum at UCLA. We are focusing our symposium on Cryo-Electron Microscopy, which has truly changed the way life sciences objects are analyzed. At the same time we didn't want to overlook the traditional materials science and physics aspects of microscopy and microanalysis, so there will be presentations representing those disciplines as well. We are pleased that geographically, this year's symposium represents all of Southern California: Santa Barbara to San Diego.

As our Microscopy Society of America Invited Speaker we are honored to have Prof. Daniela Nicastro from Brandeis University. She will have an exciting and inspiring talk "Deconstructing Cilia and Flagella Function with Cryo-Electron Tomography and Structural Proteomics". Along with Prof. Nicastro, our invited speakers Prof. Hong Zhou (CNSI, UCLA), Prof. Timothy S. Baker (UCSD), Dr. Arne Moeller (Scripps), Dr. Martin Pilhofer (Caltech) and Dr. Jinwoo Hwang (UCSB) will guarantee that this year's symposium will be our best.

As has become a tradition, our spring symposium includes a competition for graduate students. The best platform presentation and the best poster presentation not only come with cash awards to support travel to M&M-2013, the M&M program committee has also offered a place in the program for these two winning papers as late breaking posters, free registration and the invitation to a mixer with other M&M winners.

Our board is also evolving. At a brief business meeting at the Symposium, we will present a new board in 2013/2014. The proposed board membership is identified elsewhere in the newsletter and we hope you will support our new team. And I would like to acknowledge the many years of hard work made by our past President, John Porter.

Finally, I would like to acknowledge and thank all of our members including, corporate, flyer advertisers, and meeting sponsors. Spring Symposium is being supported by UCLA's Department of Materials Science and Engineering. As of now our **Gold** Level sponsors are: Carl Zeiss Microscopy LLC, Minus K Technology Inc., Microscopy Innovations LLC, Electron Microscopy Sciences; **Silver**: JEOL USA Inc., Hitachi High Technology, FEI Company, Tescan Inc., Gatan Inc., IXRF Systems, and the **Bronze**: EDAX Inc. and Ted Pella. These significant sponsorships make our meetings what they are.

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Sergey V. Prikhodko

President, SCSMM

SCSMM 2013 Spring Symposium Schedule

08:00 – 9:00	**Registration**
09:00 – 09:10	Welcome address
09:10 – 9:55	Deconstructing Cilia and Flagella Function with Cryo-Electron Tomography and Structural Proteomics *Daniela Nicastro, Brandeis University, MA*
9:55 – 10:25	Imaging Bacterial Cells in a Life-like State, in Three Dimensions and to Molecular Resolution *Martin Pilhofer, Caltech*
10:25-10:40	Vendor Talk Minus K Technologies
10:40 – 11:00	**Coffee Break**
11:00 – 11:30	Atomic Structure of Viral Membrane Proteins by Cryo EM: Dengue Virus Maturation and Fusion *Hong Zhou, CNSI, University California at Los Angeles*
11:30 – 12:00	Virus Structure in 3D at the Microscope in About a Minute or Two *Timothy Baker, UCSD*
12:00 – 12:30	Organization of the Influenza Virus Replication Machinery *Arne Moeller, Scripps*
12:30 – 13:15	**Lunch Break**
13:15 – 13:30	Business Meeting
13:30 – 14:45	Student Presentations: *R. Remenyi (UCLA)* *Yu Chen(UCLA)* *A. Khalajhedayatia (UCI)* *L.Salari Sharif (UCI)* *Chun Zhu (UCLA)*
14:45 – 15:15	Nano-scale Characterization of Materials Using Quantitative Electron Diffraction in Scanning Transmission Electron Microscopy *Jinwoo Hwang, University California at Santa Barbara*
15:15-15:30	Vendor Talk Microscopy Innovations
15:30 – 16:00	**Coffee Break and Poster Session**
16:00 – 16:15	Vendor Talk Carl Zeiss
16:16 – 16:30	Vendor Talk Electron Microscopy Sciences
16:30 – 17:00	Student Awards Presentations and Closing Statement

Directions: Get driving directions and the campus map from the site http://www.ucla.edu/maps-directions-parking. Easy self-service, **Pay-By-Space parking** is available near the Museum by following these directions: Enter UCLA campus **Lot 4** from **Sunset Blvd. at Westwood Plaza** and turn left into the Pay-By-Space area of the lot. Pay-By-Space parking is always available for Fowler visitors, regardless of whether the lot 4 parking kiosk is staffed! Automated pay stations in Pay-By-Space accept $1 and $5 bills and credit cards. The parking fee starts at $1 for 20 minutes and is a maximum of $11 for the whole day. The Fowler is visible to your left when you ascend from the elevator or stairs.

FOWLER MUSEUM
at UCLA
405 Hilgard Avenue.
Los Angeles, CA 90095

Registration & RSVP

Advanced reservation is required.
The event is limited to 100 participants.

Due to the generous support of our corporate members, registration for this meeting is included in the membership dues

Respond no later than 5 p.m. Friday, March 22nd, 2013

Please contact
Mark Armitage
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Regular annual membership for the 2012-2013 term is $25 and $10 for students.
For further details visit SCSMM web site
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Southern California Society for Microscopy & Microanalysis

Membership Application 2012 - 2013

About SCSMM

The **SOUTHERN CALIFORNIA SOCIETY FOR MICROSCOPY & MICROANALYSIS** is dedicated to increasing interest and information in all areas of microscopy and microanalysis, including, but not limited to: transmission electron, scanning electron and electron microprobe, ion probe, microbeam analysis, optical and confocal microscopies, and microspectroscopies. You are invited to join, or renew your membership in the society.

The Society generally meets two times per year at various locations throughout the greater Los Angeles area. The program usually begins with a Social Hour followed by Dinner, then a brief Business Meeting and finally the Scientific Program which consists of one or two presentations in the biological and physical sciences selected to be of sufficient breadth and interest to appeal to the entire membership.

Among our current members are students (graduate and undergraduate), post-docs, college and university professors and research assistants, laboratory directors, vendors of electron microscopes, microanalysis and/or related equipment, laboratory technicians, technologists, assistants, and many others. Their professional work spans the full range of the biological, medical and physical sciences.

In order that we may have precise records, please complete all of the information included on this application, including both your work and home addresses. You may indicate at which address you wish to receive SCSMM mailings. Fax numbers and e-mail address will be used to notify you of last minute changes in scheduled events. This information will be used only for SCSMM business. **The published list of members will include only your work address, phone number, and/or e-mail address and will only be made available to members and meeting sponsors of SCSMM. You may request that your name not be included in the published list.** If your company or laboratory has a web site, we would like to publish this in a directory of services available to Southern California microscopists.

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Southern California Society for Microscopy & Microanalysis

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ABSTRACTS

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Deconstructing Cilia and Flagella Function with Cryo-Electron Tomography and Structural Proteomics

Daniela Nicastro

Brandeis University

Cilia and flagella are conserved and ubiquitous eukaryotic organelles with important biological roles in motility and sensation. Defects in ciliary motility and assembly are linked to severe human diseases. Ciliary beating is driven by thousands of dynein motors that are attached to the microtubule core of cilia, called the axoneme. Despite the importance of cilia and flagella, much remains to be learned about their assembly, detailed structure, regulatory systems and mechanisms that control dynein's activity. Our comparative approach integrates high resolution imaging by cryo-electron tomography (cryo-ET), with genetics and biochemical methods to deconstruct the structural proteome and function of cilia. Cryo-ET combined with subtomogram averaging is a powerful technique for imaging complex biological structures in their native state and in an unperturbed cellular environment.

We have now characterized the cilia of 3 species in WT and more than 20 mutants, dissecting the structures of 8 major complexes at 3-4 nm resolution. This allowed us to discover novel structures (e.g., _ENREF_2Microtubule Inner Proteins), radial spoke heterogeneity and doublet-specific-features. We have solve long-standing mysteries, e.g., by demonstrating that the elusive nexin-link, which is thought to transform dynein-driven microtubule sliding into ciliary bending, is part of the dynein-regulatory-complex (N-DRC). We localized other regulatory complexes (e.g., the CaM- and spoke-associated-complex) and reveal the subunit organization of major assemblies (e.g., of the N-DRC, I1-dynein, and Central Pair Complex). Our long-term goal is to generate a comprehensive molecular blueprint of all major ciliary complexes to gain a better understanding of the inner workings of these intriguing nano-machines.

Imaging bacterial cells in a life-like state, in three dimensions and to molecular resolution

Martin Pilhofer

Caltech

Electron cryotomography (ECT) facilitated groundbreaking insights in Bacterial Cell Biology. One showcase is the bacterial cytoskeleton, which had been overlooked by conventional EM for decades. ECT's ability to reveal the 3D structure of a unique object to molecular resolution and in a life-like state, elucidated surprisingly diverse superstructures of the bacterial cytoskeleton. Understanding the *in vivo* superstructure is the key to understanding function

and evolution of bacterial cytoskeletal elements.For instance, despite a wealth of available biochemical and genetic data on the type 6 secretion (T6S) system, all proposed mechanistic hypotheses turned out to be wrong; however, the mechanism involving a spring-loaded dagger superstructure was immediately clear by combining structural information from ECT with insights on dynamics from live cell fluorescence light microscopy. Insights into the evolution of microtubules came from the finding that bacterial tubulins assemble into five-protofilament bacterial microtubules. Very recently, for the first time we investigated bacteria inside their eukaryotic host by ECT. We uncovered development and morphology of chlamydial inclusions, as well as their interactions with host cell organelles.

Atomic Structure of Viral Membrane Proteins by Cryo EM: Dengue Virus Maturation and Fusion

Hong Zhou

University of California, Los Angeles

Regulated by pH, membrane proteins E and M function during dengue virus maturation and membrane fusion study is presented. Our atomic model of the whole virion derived from cryo electron microscopy at 3.5-Å resolution reveals that in the mature virus at neutral extracellular pH, the N-terminal 20-amino-acid segment of M (involving three pH-sensing histidines) latches and thereby prevents spring-loaded E fusion protein from prematurely exposing its fusion peptide. This M latch is fastened at an earlier stage, during maturation at acid pH in the trans-Golgi network. At a later stage, to initiate infection in response to acid pH in the late endosome, M releases the latch and exposes the fusion peptide. Thus, M serves as a multistep chaperone of E to control the conformational changes accompanying maturation and infection. These pH-sensitive interactions could serve as targets for drug discovery.

Virus structure in 3D at the microscope in about a minute or two

Timothy S. Baker

University of California, San Diego

The development of modern transmission electron cryo-microscopy and computer-based three-dimensional (3D) image reconstruction techniques has revolutionized how the structures of complex, biomacromolecular machines are studied. These and other correlative methods provide a pathway to understand how these machines function at the molecular level. However, this process is quite demanding and can be quite time consuming, and requires the successful completion of a large number of non-trivial steps. These include: specimen isolation, purification, and vitrification; low-dose TEM imaging; 3D

structure determination; and visualization and interpretation of the final structure(s). Much attention has been devoted in recent years to simplifying and automating the imaging and structure determination steps, and this automation has led to dramatic improvements in the resolutions that can be achieved in cryo-reconstruction work. It is now almost "routine" to achieve sub-nanometer resolution results with a wide variety of single-particle specimens. Concomitant with the dramatic improvements that have been achieved with automation to date, the time frame needed to go from "specimen" to "structure" has been drastically contracted from several man-years/months just a decade or so back to weeks/days or hours in highly favorable instances. Such technological breakthroughs now make it feasible to perform 3D reconstructions of symmetric, single particles (e.g. icosahedral viruses) at low-resolution (~20-25 Å) in a matter of minutes and sometimes even faster. Ultimately, the cryo-microscopist would like "instant" 3D feedback *while* the microscopy is performed, as this would, at a minimum, provide important data about the quality of the specimen as well as instrument performance, and such information would help dictate whether extensive imaging is warranted. Results obtained with different samples of icosahedral viruses will be highlighted to illustrate how close we have come to the goal of performing real time 3D at the microscope. Of course, numerous challenges remain and these will also be discussed.

Organization of the Influenza Virus Replication Machinery

Arne Moeller,[1]* Robert N. Kirchdoerfer,[2]* Clinton S. Potter,[1] Bridget Carragher,[1]† Ian A. Wilson[2,3]†

[1] National Resource for Automated Molecular Microscopy, Department of Cell Biology, The Scripps Research Institute, La Jolla, CA 92037, USA. [2]Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, USA. [3] The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037, USA.

Influenza virus ribonucleoprotein complexes (RNPs) are central to the viral life cycle and in adaptation to new host species. RNPs are composed of the viral genome, viral polymerase, and many copies of the viral nucleoprotein. In vitro cell expression of all RNP protein components with four of the eight influenza virus gene segments enabled structural determination of native influenza virus RNPs by means of cryogenic electron microscopy (cryo-EM). The cryo-EM structure reveals the architecture and organization of the native RNP, defining the attributes of its largely helical structure and how polymerase interacts with nucleoprotein and the viral genome. Observations of branched-RNP structures in negative-stain electron microscopy and their putative identification as replication intermediates suggest a mechanism for viral replication by a second polymerase on the RNP template.

*These authors contributed equally to this work.

Nano-scale characterization of materials using quantitative electron diffraction in scanning transmission electron microscopy

Jinwoo Hwang

University of California, Santa Barbara

The atomic scale structural analysis of inorganic materials in scanning transmission electron microscopy (STEM) has been mostly based on Z-contrast high angle annular dark field (HAADF) imaging. In many cases, HAADF imaging provides intuitive understanding of the atomic structure and composition, due to the high dependence of column intensity on the atomic number. One of the limitations of HAADF imaging comes from the difficulty in characterizing structures containing lighter elements, although the recent development of aberration corrected STEM has somewhat improved the situation. We use position averaged convergent beam electron diffraction (PACBED) as an alternative way to examine the atomic structure in nanomaterials. PACBED involves spatially averaging STEM CBED patterns over one or a few unit cells. It is sensitive to lighter atoms, and to the displacement of atoms as small as a few picometers. It also uses the same probe configuration as HAADF imaging, so both PACBED and HAADF images can be acquired simultaneously. By comparing the experimental PACBED to frozen phonon simulations, we have quantified the effect of strain on the NiO_6 octahedral tilts in $LaNiO_3$ perovskite thin films.

Fluctuation electron microscopy (FEM) is another variant of electron diffraction in STEM. FEM uses nano-sized parallel probes to examine nanometer scale medium range atomic order (MRO) in glassy materials. FEM measures the structural fluctuation caused by the MRO by calculating the normalized variance amongst many nanodiffraction patterns. Using a hybrid reverse Monte Carlo simulation which incorporates the FEM data, we discovered crystal-like MRO and aligned icosahedral MRO in the nanoscale structure of Zr-based bulk metallic glass. Both of these MRO had not been captured in the models that only incorporate conventional diffraction data.

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