Workshop on Advanced Topics in EM Structure Determination: Challenges and Opportunities. October 29 - November 3, 2017.

National Resource for Automated Molecular Microscopy
Simons Electron Microscopy Center
The New York Structural Biology Center

[We encourage presenters to address the questions posed below. We ask presenters not to give their usual seminar; we would prefer a broader context for discussion of the issues. Presentations need not and should not be limited to the lecturer's own work. Summarizing the current state of the art (with examples), followed by identification of present challenges and possible opportunities to address issues, is a good overall plan for most lectures. We do not expect presenters to have all the answers and we encourage plenty of questions and discussion both during and after the talks.]

Sunday October 29 **Theme: Setting the Scene**

Session Chair: Clint Potter

4:00 pm Registration opens

4:45 pm Welcome **Bridget Carragher, Clint Potter**

Session Chair: Clint Potter

[These two introductory talks serve to set the scene and introduce the topics for more in depth discussion in the following days.]

5:00 pm Challenges and Opportunities for Molecular Structure Determination
Yifan Cheng

[This introductory talk will describe the big picture: what have we achieved and what do we still need to do. Near atomic resolution single particle structure are now almost routine for well behaved samples. What are the remaining challenges and limitations. Why does this not work for every sample? Why is the resolution still limited for most samples? Are there any general principles that might be applied to "difficult" specimens? Are there well-established best practices for imaging and data processing? How often do these need to be modified? When should we use energy filters, phase plates, Cs correctors, super resolution? What are the challenges in looking at intermediate states? Where should we focus our efforts in the immediate future? What should we pay attention to and what should we NOT do?]

6:00 pm Challenges and Opportunities for Cellular Structure Determination
Elizabeth Villa

[This introductory talk will describe the big picture: what have we achieved and what do we still need to do. How important is it to visualize complexes in their biological environment? What classes of biological questions are best addressed this way? What are the problems associated with imaging large cellular complexes in situ? How do we locate and identify what we are interested in? How important is correlative LM-EM? Has this approach reached a stage of development where it is easily and generally

applicable? What are the challenges associated with recording high-resolution information from cells? What are the general strategies currently in use? What is the role of FIB milling and other advanced specimen preparation methods? What technologies do we still need? Is tomography coupled with sub tomogram averaging the best way to extract the highest resolution information? This approach has recently yielded near atomic resolution structures; could this become routine? Are there theoretical limitations to what can be achieved? What is the current state of the art and the future prospects? What should we pay attention to and what should we NOT do?]

7:00 pm Opening reception

Monday October 30 Theme: Specimen Preparation

8:00 am Breakfast

Session Chair: Tom Walz

9:00 am Challenges remaining for specimen preparation
Bob Glaeser

[Most specimens are still not ready for atomic resolution. What are the specific and general problems? What can be done about them? Which approaches have been tried in the past? How successful have they been? Which approaches look like the most promising for the future?]

10:15 am Coffee Break

10:45 am New approaches to substrates and specimen preparation.
Chris Russo

[Are there treatments that can be applied to thin carbon that are advantageous? Should we be using carbon at all? Are there new substrates that offer advantages over the traditional thin carbon? Can we envision improving on these further using surface treatments?]

11:20 am Spotiton and nanowire grids Bridget Carragher

[Do new approaches to specimen preparation improve quality and throughput? Can we produce thin layers reproducibly? What are the limitations? What else can be done?]

12:00 Lunch

1:00 pm Microfluidic Sample Preparation:
Opportunities, Challenges and Visual Proteomics Thomas Braun

[Challenges faced by miniaturised specimen preparation for TEM, review of current developments, and demonstration of the opportunities resulting from microfluidic preparation methods. In particular, microfluidic single cell lysis and EM grid preparation for 'visual proteomics' will be presented.]

1:30 pm CryoET as a method to understand the air-water interface
Alex Noble

[Where are most particles located in the ice relative to the air-water interface? Can we directly see particle interactions at the air-water interface? What might be happening to particles at the air-water interface? Are the two air-water interfaces different? Do surfactants reduce particles' affinities for the air-water interface? Can grids be plunged faster than the adsorption time for a particle to stick to the air-water interface?]

2:00 pm Time resolved cryoEM Jack Fu
[How can time-resolved cryo-EM help you in your research? What are the obstacles to success?]

2:30 pm Coffee Break

3:00 pm Panel Discussion: Tom Walz (Chair)
[For these panel discussions, all participants are encouraged to submit questions before or during the meeting that will then be discussed in an open forum. Questions are preferably related to topics on which presentations have already been made and should expand on or clarify the lectures of the day. The Chair is encouraged to plant some seeds in the audience to get the discussion going.]

4:00 pm Demos / Vendor breakout sessions / User breakout sessions
[These parallel sessions will involve demonstrations of microscope and cameras, specimen preparation equipment, major software applications, meetings of users groups. Some of these sessions will repeat on Thursday and we will have sign up sheets for some of the small group demonstrations that will occur multiple times to enable as many people as possible to attend the ones they are interested in.]

Improving throughput for cryo-Electron microscopy
Chris Booth, Gatan Inc.
Location: TBD
[In this presentation I will try to address how Gatan is trying to remove bottlenecks for cryo-EM. This presentation will not be so focused on K3, but rather talk about how K3 and software integration are trying to address some of the important issues related to throughput and data management during single particle data collection.]

Toward commercialization of Spotiton
Melanie Adams and Russell King, TTP Labtech
Location: TBD
[Representatives of TTP Labtech will be hosting a breakout session to provide updates on the progress and timelines for a commercial Spotiton device. Participants will be invited to provide input on current challenges and priorities for sample preparation in cryoEM along with wishlist items for Spotiton]

Spotiton demonstration Venkat Dandey and Hui Wei, NRAMM/NYSBC
Location: NRAMM Lab at NYSBC
[The Spotiton vitrification device and nanowire grids will be demonstrated]

DE 20 demonstration Michael Spillman and Benjamin Bammes, Direct Electron
Location: F20 room at NYSBC
[The use of the DE20 direct electron detector will be demonstrated, starting with a short discussion of the camera and the results that have been obtained with it.]

User Group Meeting Mark Herzik
Location: TBD
[A session for all interested parties to discuss microscope and cameras features, equipment for cryo-EM single particle sample preparation and major software applications.]

Titan Krios Overview John Spear, Thermo Fisher
Location: NYSBC Microscopy Suite
[Overview of the Titan Krios microscope.]

Tour of CUNY Advanced Science Research Center **Amedee des Georges**
Location: ASRC

5:00 pm **Poster Session**
[We encourage all participants to present a poster. The poster session is one of the most interactive and valuable aspects of the meeting. Refreshments will be served during the poster session.]

6:15 pm ***Dinner***

7:30 pm **High throughput cryo-ET: Visualizing molecular machines in action**
Jun Liu
[This talk will highlight the biology while also drawing attention to the technical advances that made it possible.]

Tuesday October 31 **Theme: Image acquisition**

8:00 am ***Breakfast***

Session Chair: Scott Stagg

9:00 am **Optimizing image acquisition** **John Rubinstein**
[How to get the most out of your microscope and your direct detector. Details of the methods, formulae, math and common sense needed for optimal data collection.]

10:15 am ***Coffee Break***

10:45 am **Phase plates** **Rado Danev**
[Are these the key to high resolution of small and heterogeneous particles? Can we make them easier to use? What are the remaining issues with using them? Will there be new progress in the near future for these devices?]

11:30 am **Next generation cameras.** **Scott Stagg (DE64)**
Paul Mooney (K3)
TBA

[What does the future hold for improvements in detectors.]

1:00 pm ***Boxed Lunch To Go, Happy Halloween!***

Wednesday November 1 **Theme: Processing**

8:00 am ***Breakfast***

Session Chair: John Rubinstein

9:00 am Introduction and new approaches Sjors Scheres

[A comprehensive overview of the major advances that have taken place in the last few years that have enabled 3D maps to achieve "atomic" resolution. Topic to be covered include: 3D reconstruction, image restoration techniques, how to deal with heterogeneous populations. What are the hot topics in processing? What are the major mathematical approaches and available software? What are the success stories and the failures? Where are the greatest challenges right now and how are we approaching these? Do we need completely new algorithms or just incremental improvements on the current ones? Mistakes to avoid! Some of these topics will be shared between this talk and the next three, the presenters are encouraged to discuss this and make a plan.]

10:00 am Coffee Break

10:30 am New challenges Niko Grigorieff

[It seems that one of the greatest challenges now will be sorting out heterogeneity in 3D. How do we detect heterogeneity and make sure it does not lead us to the incorrect result? What are the signs that it is present in a dataset? How to distinguish conformational vs. compositional variability? What are the prospects for really getting to atomic resolution for a small and heterogeneous particle? Under what circumstances can we hope to be able to do this? Are there some samples that will never be amenable to high resolution reconstruction?]

11:15 am Deep learning methods Steve Ludtke

[Discussion on what we still need to do in terms of providing the computational tools needed by the community to do 3D EM in a routine and reliable manner. Discussion of new algorithms both for separating heterogeneous populations and for more accurate orientation determination.]

12:00 Lunch

1:00 pm Bayesian methods in cryo-EM Marcus Brubaker

[This discussion will focus on the role of Bayesian methods in cryo-EM data processing and the resulting algorithms. Specific topics include: What, exactly, are Bayesian methods? Where have they been used (or not) in cryo-EM? Why are they particularly well suited (or not) for data processing in cryo-EM? What can Bayesian methods tell us about non-Bayesian approaches?]

1:30 pm Software tools to deploy and manage cryo-EM jobs in the cloud Michael Cianfrocco

[Is the cloud useful for cryo-EM? What types of resources are at the disposal of users? What are the appropriate workflows and benchmark comparisons? How does the cost compare to local infrastructure? When should someone use the cloud?]

2:00 pm Panel Discussion Chair: John Rubinstein

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3:00 pm Coffee Break

3:30 pm Manufacturers Exhibit @ NYSBC SEMC Conference room

[Please be sure to visit the vendors that have helped to sponsor this workshop and engage them in discussions about the future needs of our community.]

4:45 pm Poster session
[We encourage all participants to have a poster. The poster session is one of the most interactive and valuable aspects of the meeting. Refreshments will be served during the poster session.]

6:00 pm Dinner

7:30 pm **CFTR, the odd ABC transporter responsible for cystic fibrosis** **Jue Chen**
[This talk will highlight the biology while also drawing attention to the technical advances that made it possible.]

Thursday November 2 Theme: Challenges and Opportunities

8:00 am *Breakfast*

Session Chair: Bridget Carragher

9:00 am Challenges and Opportunities: SP Wah Chiu
[Validation methods have become much better established over the last couple of years. What are the methods that are being used? In what resolution realms are they useful? Do we need more tools? How do we avoid mistakes? Is validation at very high resolution easier than at intermediate resolutions? What about highly heterogeneous datasets?]

10:00 am Coffee Break

10:30 am Challenges and Opportunities: CryoET Julia Mahamid
[What is next for these challenging methods? How hard will it be to do accurate 3D localization for site-specific preparations with cryo-FIB and navigation of tomography data acquisition? Will super resolution cryoLM become a reality? Will high-pressure freezing and FIB lift-out become routine for bulk specimens? How will we solve the segmentation problem? Will deep learning methods help with this or are they over hyped?]

11:15 am Fitting challenges David Veesler
[Methods for fitting backbone tracing into moderate resolution structures. What are the problems, how are they approached, what are the solutions. How do we validate the methods and the results?]

12:00 Lunch

1:00 pm Molecular machines in action Thomas Marlovits
[Some tricks on how to modulate sample orientation on the grid, some results with affinity grids, how to do elegant biochemistry (sample prep) for analyzing machines in action.]

1:30 pm Poster talks (4x10+5) Chair: Dmitry Lyumkis
[Four posters will be selected for short 10 minute talks with an additional 5 minutes for questions.]

2:30 pm Demos / Vendor breakout sessions / User breakout sessions
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Indispensable solutions for Cryo-Electron Microscopy
Yakushevska, Alevtyna, Thermo Fisher
Location: TBA

Titan Krios Overview John Spier, Thermo Fisher
Location: NYSBC Microscopy Suite
[Overview of the Titan Krios microscope.]

Using Amazon Web Services for cryo-EM data analysis Michael Cianfrocco
[Participants will be guided through the nuts and bolts of Amazon Web Services, ranging from specific questions on instance types to running RELION jobs to setting up a new user account. Recently developed cryoem-cloud-tools software package that integrate into the RELION2 GUI, will be demonstrated; these allow users to directly submit jobs to AWS from a local computer.]

3:45 pm Coffee Break

4:15 Group Photo

4:30 pm Poster session
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6:00 pm Conference dinner

Friday November 3 **Theme: Putting theory into practice**

8:00 am Breakfast

Session Chair: Michael Cianfrocco

[This session is targeted at relative newcomers to the field and thus we do not expect all attendees to stay for this, only those with a particular interest in the topics to be covered. We plan to spend the morning on presentations that are focused on practical aspects of doing cryoEM. What does it take to set up a lab? What are the pitfalls of specimen preparation? What instruments should you buy, beg borrow, or steal? What kind of computational resources are needed? What strategies are there available to cope with the onslaught of data resulting from the new generation of detectors.]

9:00 am How to choose the optimal microscope/camera combinations. ***Anchi Cheng***

[What do you need? How do you validate your instrument performance? How many people does each microscope serve? How do you schedule time to optimize the instrument usage and performance?]
Anchi Cheng

9:25 Exploring the Size and Resolution Limits of Single Particle Cryo-electron Microscopy at 200 keV. ***Mark Herzik***
[The Krios is the "go-to" microscope for high resolution. Why is this? Is access to a Krios necessary for a lab to compete in the cryo-EM field? Can conventional cryo-EM be used to solve small structures? How can you optimally use a 200keV scope and what are the limitations?]

9:45 am **The essentials of a cryoEM lab.** ***David Veesler/Joaquin Ortega***
[What do you need? What can you borrow? How do you validate your equipment? How do you service your equipment? How do you assess a new specimen?]

10:30 am Coffee Break

11:00 am Computational infrastructure. **Steve Ludtke/Ed Eng**
[Computation has become a major bottleneck. Data storage (and backup/archive) are major issues. What do you need? What do you buy? Are so called supercomputer centers of value? What about cloud computing? What software do you need up and running? How do you support the hardware and software? How do you validate the software?]

11:45 am Wrapup

12:00 *Lunches To Go and End of Workshop*

October 16, 2017

This course would not be possible without the generous support of the following sponsors: