Workshop on Advanced Topics in EM Structure Determination:
Where do we go from here?

November 9-14, 2014.

National Resource for Automated Molecular Microscopy
The Scripps Research Institute

[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 limitations and possible solutions to the problems, 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.]

All talks will take place in the Hazen Auditorium unless otherwise specified in the Location information.

Sunday November 9 **Theme: Setting the Goals**

4:00 pm ***Registration***

4:45 pm **Welcome** **Bridget Carragher. Clint Potter, Ron Milligan**

Session Chair: Ron Milligan

[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 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. Since the last workshop we have seen major advances in image acquisition resulting in (almost?) atomic level structure determination of a number of specimens. Some of the specimens were well behaved whereas others required considerable biochemical characterization and modification before they were suitable for high resolution imaging and analysis. What limited the resolution in the first place with these "difficult" specimens? Was there a strategy for making them better or was it hit and miss? From this work have we learned any general principles that might be applied to other "difficult" specimens? Were there any special approaches involved in imaging or data processing? What are the prospects for a general high resolution methodology for, e.g. single particles? What are the current limitations? Perhaps illustrate these points with great successes and embarrassing failures (without offending people). Do the problems get bigger as we approach atomic 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 for Cellular Structure Determination** **John Briggs**
[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 sub-nanometer structures; is this the limit or can the method be pushed down to near-atomic resolution? Are there theoretical limitations to what can be achieved? Perhaps illustrate these points with great successes and embarrassing failures (without offending people). 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 November 10 Theme: Specimen preparation

8:00 am Breakfast

Session Chair: David DeRosier

9:00 am Challenges remaining for specimen preparation Tom Walz
[Here we hope to expand on specimen preparation issues introduced in the previous day's talks. 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? Given that many small and heterogeneous samples may only be suitable for examination in negative stain or at low resolution, how to we make sure that the general scientific community (and ours too!) understands that not everything is getting to atomic resolution!]

10:15 am Coffee Break

10:45 am New substrates Lori Passmore
[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 pm Spotiton/Typhon Clint Potter
[Will new approaches to specimen preparation improve quality and throughput? Can we produce thin layers reproducibly? What are the limitations?]

12:00 Lunch

1:00 pm Single cell imaging Henning Stahlberg
[What can be learned about the single whole cell using EM? Where are we now? What are the challenges going forward?]

1:30 pm FIB milling Elizabeth Villa
[What is the advantage of in situ cellular EM? Where are we now? What are the

challenges going forward? What are the practical issues involved in FIB milling? Is it ready for prime time? Is it time consuming? How much skill is required? Will any lab be able to do it?]

2:00 pm Group photo

2:30 pm Break

3:00 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 go on all week and we will have sign up sheets for some of the small group demonstrations that will occur multiple times to ensure that everyone gets a chance to attend the ones they are interested in.]

3:00-3:30pm New NIH announcement for support of regional consortia for cryoEM data collection

Paula Flicker, NIH

Location: Hazen Theory 3rd floor seminar room
[A new announcement for grants to support Regional Consortia for High Resolution Cryoelectron Microscopy has been published by NIGMS (National Institute of General Medical Sciences). See: **http://grants.nih.gov/grants/guide/rfa-files/RFA-GM-16-001.html** *The purpose of this funding opportunity announcement (FOA) is to provide regional access to state-of-the-art data collection capabilities to cryoelectron microscopy (cryoEM) laboratories. Dr. Flicker will discuss the FOA and answer questions]*

3:00 pm Processing Movies from Direct Electron Detection Cameras:

Ben Bammes, Direct Electron

Location: Hazen Theory 1st floor seminar room
[This presentation will include some information about DE products but the focus of the presentation will be on general methods for "movie-processing" that could be used by anyone with a direct detector.]

3:00-3:30 pm Remote Talos Operation of User Interface, EPU, and Tomo 4.0

Sacha De Carlo, FEI Co.

Location: Hazen Suite Control Room
[Hands on demonstration for 6 participants.]

3:00-3:30 pm Talos Technical Overview & What is New

Wim Voorhout, FEI Co.

Location: **Hazen Suite Talos Room**
[Hands on demonstration for 6 participants.]

3:00-3:30 pm Gatan K2 Summit

Chris Booth, Gatan.

Location: **Hazen Suite T3 Room**
[Hands on demonstration for 6 participants.]

3:00-4:00 pm FEI Breakout Session

Matthijn Vos, FEI Co.

Location: **Hazen Auditorium**
[The Manufacturers View on: How do you treat a FEG well; How to set parallel illumination; How to use a Direct electron detector in integration mode or counting mode;

How to check your microscope without re-aligning it; Titan vs. Tecnai, what is the difference, what to do and what not to do.]

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3:30-4:00 pm Direct Detection and High Speed Imaging: What's New and What's Next.
Chris Booth, Gatan.
Location: **Hazen Theory dining room.**
[A presentation to highlight some of the new things that are going on with Gatan and the K2 for people who already have hands on experience with the K2.]

4:00 pm Panel Discussion: David DeRosier (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 (moles) in the audience to get the discussion going.]

5:00 pm Poster Session
[Posters will be displayed in two sessions (probably separated alphabetically). The poster session is one of the most interactive and valuable aspects of the meeting. To maximize the space available for the poster session we plan to display the posters on the walls of the microscopy suite building and they will need to be attached to the wall using very discrete Velcro sticky back coins on the back of the poster.]

6:30 pm Dinner

7:30 pm Structural studies of an AAA+ ATPase by cryo electron microscopy
Minglei Zhao
[This talk will highlight the biology while also drawing attention to the technical advances that made it possible.]

Tuesday November 11 Theme: Image acquisition

8:00 am Breakfast

Session Chair: Bob Glaeser

[A significant part of the discussion today will focus on Direct detectors. DDs appear to be the main reason for improvements in EM structure determination that have been achieved over the past 2 years. Three DDs are available: FEI's Falcon, Gatan's K2, and Direct Electron's DE. We encourage people to share their experiences using these various instruments. What are the particular advantages / disadvantages / limitations associated with each instrument? How is each one best used? Are there issues pairing particular detectors with particular microscopes?]

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 pm Even higher resolution? Holger Stark
[Improving alignment and correcting optical aberrations may well be the future of reducing resolution limiting factors.]

11:15 pm Even better cameras? Greg McMullan
[What are the current pros and cons of the current generation of direct detectors. What does the future hold for improvements in detectors.]

12:00 pm Lunch

1:00 pm Direct Detectors Forum: David Agard (Discussion Leader)
Panelists: Yifan Cheng, Wah Chiu, Carsten Sachse, Sjors Scheres; accompanied by extensive discussion.
[Overview by David on the physics of direct detectors: data acquisition mode (counting vs. integrating mode); MTF, DQE, noise, readout, advantages and drawbacks etc. Panelists will then discuss topics including: How to select the best microscope parameters (mag, dose rate, frame numbers) for a targeted resolution and why for the chosen camera? How to pre-process the frames prior to the image processing pipeline (frame by frame; running average; particle by particle; damage compensation etc.? What works best and what does not work? How to assess the data quality after frame pre-processing and averaging? What are the users' ergonomics in using the direct detectors in terms of data acquisition speed; user interface; automation option and company support? Biological examples that go to atomic resolution for single particle and/or medium resolution for tomography. How do we figure out what are the hurdles to not getting better resolution for a given specimen? How do we handle the anticipated massive data with these cameras ? What are our wish lists for the next generation of cameras?]

2:30 Coffee break

3:00 – 3:30 Phase plates for single particles Rado Danev
[Design of the optimal phase plate. Their use (necessity?) for certain types of single particle work. Do they work routinely? Are they easy to use? Are they expensive to buy and/or maintain? Are there limits on the resolution that can be attained when using phase plate?]

3:30 – 4:00 Phase plates for tomography Wah Chiu
[What are the advantages of using phase plates for recording tomographic data? Do they improve resolution and / or SNR. Are there limits on the resolution that can be attained? Should we all get one? How much money and effort is required?]

4:00 – 5:00 Panel Discussion: Bob Glaeser (Chair)
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5:00 pm Manufacturers Exhibit
[Several manufacturer's have agreed to provide one-on-one or small group demonstrations using the microscopes and cameras in the Hazen Suite during the exhibit hours. To arrange a demonstration please contact the manufacturer representatives attending the meeting or stop by the exhibit and talk to them directly.]

6:30 pm Dinner

7:30 pm Three-dimensional structure of human gamma-secretase
Xiaochen Bai
[This talk will highlight the biology while also drawing attention to the technical advances that made it possible.]

Wednesday November 12 Theme: Processing

8:00 am Breakfast

Session Chair: Joachim Frank

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 two, the presenters are encouraged to discuss this and make a plan.]

10:00 am Coffee Break

10:30 am New challenges for processing heterogeneity 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 Software and processing challenges 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. Are we there yet? Should any lab be able to do this without being embedded in the community for years? Do we need centralized resources? Can a standard lab gather the required resources? Discussion of new classification algorithms both for separating heterogeneous populations and for more accurate orientation determination. Inter-software comparisons as a validation strategy.]

12:00 pm *Panel on mathematical and computational issues in cryo-EM reconstruction.* Chair: Leslie Greengard
[A variety of open problems in cryo-EM reconstruction will be discussed, from algorithm development to forward modeling of the physical processes involved in data collection. Major issues include reconstruction from heterogeneous particles, the incorporation of prior information, the development of bases in which reconstruction can be viewed as sparse, new approaches to 3D variance estimation, and rigorous approaches to quality control. We will also consider the impact of new experimental procedures and new mathematical formulations of the inverse problem.]

12:45 pm Energy landscape analysis Joachim Frank
[A new method of analyzing the energy landscape of an active molecular machine.]

1:00 pm Lunch To Go

Afternoon free to explore San Diego!

Thursday November 13 Theme: Validation and Next steps.

8:00 am *Breakfast*

Session Chair: Eva Nogales

9:00 am Validation Peter Rosenthal
[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?]

10:15 am Coffee Break

10:45 am New approaches to validation Pawel Penczek
[A comprehensive approach to validation].

11:30 am Chasing the chain Frank DiMaio
[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 resutls?]

12:00 Lunch

1:00 pm Near-atomic resolution cryoEM: how far can we go?

David Veesler/Melody Campbell

[Direct detectors have revolutionized the cryoEM field by enabling routine determination of protein structures at near-atomic resolution. What resolution can be achieved by single-particle EM with a given combination of microscope and camera? Is cryoEM competing with x-ray crystallography?]

1:30 pm Panel Discussion: Where do we go from here?

Gabriel Lander (Chair)

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2:30 pm Discussion Group: Model building, fitting & validation using high-resolution cryo-EM maps.

Doreen Matthies, NIH

Location: Hazen Hazen Theory 3rd floor seminar room

[A discussion group to review a selection of methods used to fit or build atomic models into EM-maps of various resolutions, and discuss how these structural models have been validated. The challenge is to make sure that these atomic models are chemically correct and do not include, for example, side-chain clashes, while incorporating the maximum amount of information from the experimental maps in an efficient manner. How do we accomplish that? Current approaches for rigid-body fitting rely on software packages like UCSF Chimera, while flexible fitting may be carried out with programs such as COOT and Phenix, while COOT also allows manual model building and fitting. Alternative approaches include tools originally developed for de novo structure prediction, such as ROSETTA, or for molecular simulation, such as NAMD or CHARMM. The stereochemistry and quality of the resulting models might be assessed with methods such as MolProbity, or in the case of membrane proteins, ProQM. How do we choose the right method? What are the pros and cons? What do we need to be aware of? Most EM-maps do not show the same resolution along the entire molecule. How should we model regions of lower resolution? Densities for glutamates and aspartates seem to be weaker throughout, owing to radiation damage; how should we model these? Can we trust the fits blindly or do we need further validation? What structure validation tools should be used? How can we make our atomic models as good as possible? The goal of this discussion group is to have the participants share their experiences and preferred approaches.]

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5:00 pm Buses leave for dinner

6:00 pm ***Conference dinner***
George's at the Cove
1250 Prospect St, San Diego, CA 92037
(858) 454-4244

Friday November 14 **Theme: Putting theory into practice**

8:00 am ***Breakfast***

[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. We are still considering the topics to be covered so let us know if you have suggestions. The format of the morning will be a series of short talks by experts in the field interspersed with lots of discussion.]

9:00 am **How to choose the optimal microscope/camera combinations.**
[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?]
10 minutes each
John Rubenstein, Henning Stahlberg.

9:45 am **The essentials of a cryoEM lab.**
[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?]
Justin Kollman, Melanie Ohi, Yifan Cheng.

10:30 am ***Coffee Break***

11:00 am **Computational infrastructure.**
[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?]
Steve Ludtke, Daniel Southworth, Holger Stark.

11:45 am **Wrapup**

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