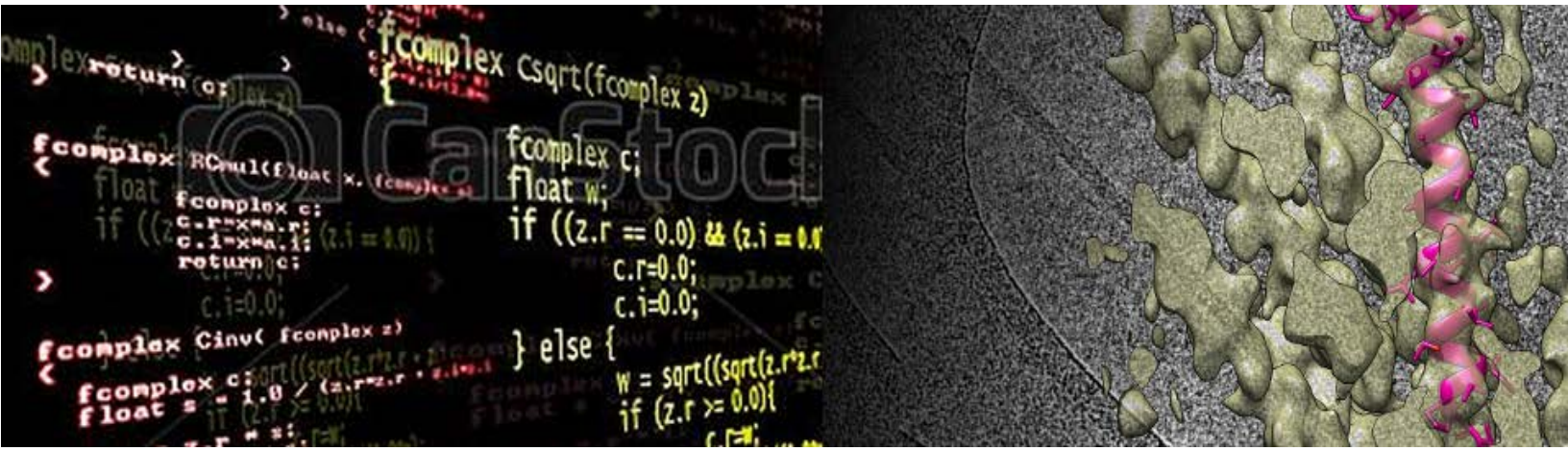
3RD INTERNATIONAL SYMPOSIUM ON CRYO-EM 3D IMAGE ANALYSIS



March 21–24, 2018
Granlibakken Conference Center & Lodge
Lake Tahoe, CA USA

Program Schedule
Abstracts
Registered Participants

3rd International Symposium on Cryo-3D Image Analysis
March 21–24, 2018

Welcome to the third biennial International Symposium on Cryo-3D Image Analysis at Granlibakken, Lake Tahoe. The goal of this meeting is to focus on the computational methods used for analysis of CryoEM and CryoET targeting challenging biological problems. This analysis may range from high resolution 3-D reconstructions, to 2-D and 3-D flexibility/variability analysis of complex systems to analysis of complex cellular environments. While biological problems serve as a driving force, the focus of this specific symposium is on the methods underlying these exciting biological results.

Thank you for joining us in what we are sure will be a profitable meeting.

Dorit Hanein, Chair
Sanford Burnham Prebys
Medical Discovery Institute

Steve Ludtke, Co-Chair
Baylor College of Medicine

Organizing Committee

Wah Chiu
Stanford University

Masahide Kikkawa
University of Tokyo

Henning Stahlberg
University of Basel

Edward H. Egelman
University of Virginia
Medical School

Fei Sun
Chinese Academy
of Sciences

Pawel A. Penczek
University of Texas-Houston
Medical School

Niels Volkmann
Sanford Burnham Prebys
Medical Discovery Institute

Meeting Coordinator

Cassidy Zeiser
Sanford Burnham Prebys

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7:30–7:40 PM	Welcome (Mountain Lake) **Dorit Hanein**, Program Chair Professor, Bioinformatics and Systems Biology, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, USA Introduction, **Wah Chiu**, Session Chair Photon Science Directorate, Professor, Bioengineering, Microbiology & Immunology, Stanford University, Stanford, CA, USA
7:40–8:40 PM	**Wolfgang Baumeister**, **Keynote Speaker** Professor, Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Martinsried, GERMANY
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THURSDAY, MARCH 22ND

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EM Co-Director, Simons Electron Microscopy Center,
New York Structural Biology Center, New York, USA

8:15–8:40 AM ***FOCUS - The Interface Between Data Collection and Data Processing in Cryo-EM***
Henning Stahlberg
Professor, Department for Biosystems Science and Engineering,
Biozentrum, University of Basel, Basel, SWITZERLAND

8:40–9:05 AM ***Frame Alignment and CTF Fitting in IMOD and SerialEM***
David Mastronarde
Professor, Molecular, Cellular and Developmental Biology,
University of Colorado, Boulder, CO, USA

9:05–9:30 AM ***High-resolution Reconstructions and Local-directional Resolution***
Carlos Oscar Sanchez Sorzano
Professor, Biocomputing Unit,
National Center for Biotechnology-CSIC, Madrid, SPAIN

9:30–9:55 AM ***Progress Towards Collecting Tilt-series in Seconds Rather than Minutes with a New "High Precision" Stage***
Grant Jensen
Professor of Biophysics and Biology; Investigator,
Howard Hughes Medical Institute, Division of Biology
and Biological Engineering, Caltech, Pasadena, CA, USA

9:55–10:15 AM Coffee/Tea Break

Session II: Image Correction (Mountain Lake)

10:15–10:30 AM Introduction, **David Derosier**, Session Chair
Professor Emeritus of Life Sciences, Member,
US National Academy of Sciences, Department of Biology,
Brandeis University, Waltham, MA, USA

10:30–10:55 AM ***S2stigmator: A Closed-form Solution for Single-pass Correction of TEM Image Astigmatism***
Wen Jiang
Professor of Biological Sciences, Director of Purdue Cryo-EM Facility, Markey Center for Structural Biology,
Department of Biological Sciences, Purdue University,
West Lafayette, IN, USA

10:55–11:20 AM ***Processing Within the cisTEM Software Package***
Tim Grant
Research Specialist, Howard Hughes Medical Institute,
Janelia Research Campus, Ashburn, VA, USA

11:20–11:45 AM ***CTF Fitting and Performance Checks with the Volta Phase Plate***
Radostin Danev
Professor, Department of Molecular Structural Biology,
Max Planck Institute of Biochemistry, Martinsried, GERMANY

11:45–12:10 PM ***CTF-correction in High-resolution Subtomogram Averaging***
Florian Schur
Assistant Professor, Institute of Science and Technology Austria (IST Austria), Klosterneuburg, AUSTRIA

12:15–1:15 PM Lunch (Granhall)

Poster Session

4:30–6:00 Poster Session **(**Bay Room)

6:00–7:30 Dinner (Granhall)

Session III: Reconstruction Methods (Mountain Lake)

7:30–7:45 PM Introduction, **Pawel Penczek**, Session Chair
Professor of Biochemistry & Molecular Biology
and Director of Structural Biology Imaging Center,
The University of Texas-Houston Medical School, Houston, USA

7:45–8:10 PM ***Algorithms for Accelerated Near-atomic Resolution Single- and Multi-particle 3D Reconstruction***

Hans Elmlund
Senior Research Fellow, Biochemistry and Molecular Biology,
Monash Biomedicine Discovery Institute, Monash University,
Melbourne, AUSTRALIA

8:10–8:35 PM ***Recent Progress of Missing-wedge Compensated Electron Tomography Reconstruction Program ICON***

Fei Sun
Professor, National Laboratory of Biomacromolecules,
IBP, Core Facility for Protein Research, Institute of Biophysics,
Chinese Academy of Sciences, Beijing, CHINA

8:35–9:00 PM ***Kam's Method for Single Particle Reconstruction***

Amit Singer
Professor, Mathematics and Program in Applied
and Computational Mathematics Princeton University,
Princeton, NJ, USA

9:00–11:00 PM Social/Cash bar (Cedar House)

FRIDAY, MARCH 23RD

7:00–8:00 AM Breakfast (Granhall)

Session IV: Deep Learning/CNN Methods (Mountain Lake)

8:00–8:15 AM Introduction, **Niels Volkmann**, Session Chair
Professor, Bioinformatics and Systems Biology,
Sanford Burnham Prebys Medical Discovery Institute,
La Jolla, USA

8:15–8:40 AM ***A Machine Learning Method for Resolving Heterogeneity in CryoEM Single Particle Reconstruction***
Muyuan Chen
Postdoctoral Fellow, Verna and Marrs McLean
Department of Biochemistry and Molecular Biology,
Baylor College of Medicine,
Houston, TX, USA

8:40–9:05 AM ***Deep Learning Based Structural Pattern Mining in Cellular Electron Cryo Tomograms — Several Exploratory Studies***
Min Xu
Assistant Research Professor,
Computational Biology Department, School of Computer Science,
Carnegie Mellon University, Pittsburgh, USA

9:05–9:30 AM ***Advanced Computational Methods in Detection of Secondary Structures from Medium-resolution Density Maps***
Jing He
Professor, Department of Computer Science,
Old Dominion University, Norfolk, VA, USA

9:30–9:45 AM Group Photo

9:45–10:00 AM Coffee/Tea Break

Session V: Computing Hardware Roundtable (Mountain Lake)

10:00–11:00 AM **Steven Ludtke**, Moderator
Professor, Department of Biochemistry and Molecular Biology,
Baylor College of Medicine, Houston, USA

Roundtable Discussants:

Michael A. Cianfrocco
Research Assistant Professor,
Department of Biological Chemistry, Life Sciences Institute
University of Michigan, Ann Arbor, MI, USA

Pawel Penczek
Professor and Co-Director, Structural Biology Imaging Center,
Department of Biochemistry and Molecular Biology,
University of Texas – Houston Medical School, Houston, TX, USA

David Mastronarde
Professor, Molecular, Cellular and Developmental Biology,
University of Colorado, Boulder, CO, USA

Session VI: Improving Maps Roundtable (Mountain Lake)

11:00–12:00 PM **Edward H. Egelman**, Moderator
Harrison Distinguished Professor, Department of Biochemistry
and Molecular Genetics, University of Virginia Medical School,
Charlottesville, VA, USA

Roundtable Discussants:

Paul D. Adams
Division Director, Molecular Biophysics
and Integrated Bioimaging, Structural Biology,
Lawrence Berkeley National Laboratory, Berkeley, CA, USA

Bruno Klaholz
Center for Integrative Biology, Department of Integrated
Structural Biology, Institute of Genetics and Molecular and
Cellular Biology, University of Strasbourg, Strasbourg, FRANCE

Xiao-chen Bai
Assistant Professor and CEMF Co-Director,
Department of Biophysics and Cell Biology,
University of Texas Southwestern Medical Center,
Dallas, TX, USA

12:00–1:00 PM Lunch (Granhall)

Poster Session

4:30–6:00 PM Poster Session (Bay Room)

6:00–7:30 PM Dinner (Granhall)

Session VI: Selected Poster Presentations (Mountain Lake)

7:30–8:50 PM **Masahide Kikkawa**, Chair
Professor of Cell Biology and Anatomy,
Graduate School of Medicine, The University of Tokyo, JAPAN

Selection Committee members:

David DeRosier
Professor Emeritus of Life Sciences, Member,
US National Academy of Sciences, Department of Biology,
Brandeis University, Waltham, MA, USA

Michael Rossmann
Hanley Distinguished Professor of Biological Sciences;
National Academy of Science, Department of Biological Sciences,
Purdue University, West Lafayette, IN, USA

Timothy Baker
Professor, Chemistry, Biochemistry and Molecular Biology,
University of California, San Diego, La Jolla, CA, USA

Kenneth Taylor
Professor, Department of Biological Sciences,
Florida State University, Tallahassee, FL, USA

Selected Poster Presentations:

Data-driven Dose Weighting for High-Resolution Single-Particle Cryo-EM

Alberto Bartesaghi
Biophysics Section, Laboratory of Cell Biology,
Center for Cancer Research, National Cancer Institute,
National Institutes of Health, Bethesda, MD, USA

Multiple Spooled Conformations of a Viral dsRNA Genome Revealed by Cryo-Electron Microscopy

Serban Ilca
Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK

SPHIRE-crYOLO: Accurate and well centered particle picking

Thorsten Wagner
Department of Structural Biochemistry, Max Planck Institute of Molecular Physiology, Dortmund, GERMANY

The Cryo-EM Structure of V-type ATPasd V_O Proton Channel in Lipid Nanodisc

Soung-Hun Roh
Department of Bioengineering and Bio-X Center,
Stanford University, Stanford, CA, USA

8:50–9:00 PM Closing Remarks, **Steve Ludtke**, Program Co-Chair

9:00–11:00 PM Cash Bar/Social Time (Cedar House)

SATURDAY, MARCH 24TH

7:00–9:00 AM Breakfast (Granhall)

Departures

Speaker Abstracts

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Henning Stahlberg, Professor, Department for Biosystems Science and Engineering, Biozentrum, University of Basel, Basel, SWITZERLAND

Frame Alignment and CTF Fitting in IMOD and SerialEM
David N. Mastronarde, Professor, Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO, USA

High-resolution Reconstructions and Local-directional Resolution
Carlos Oscar Sanchez Sorzano, Professor, Biocomputing Unit, National Center for Biotechnology-CSIC, Madrid, SPAIN

Progress Towards Collecting Tilt-series in Second Rather than Minutes with a New "High Precision" Stage
Grant Jensen, Professor of Biophysics and Biology; Investigator, Howard Hughes Medical Institute, Division of Biology and Biological Engineering, Caltech, Pasadena, CA, USA

S2stigmator: A Closed-form Solution for Single-pass Correction of TEM Image Astigmatism
Wen Jiang, Professor of Biological Sciences; Director of Purdue Cryo-EM Facility, Markey Center for Structural Biology, Department of Biological Sciences, Purdue University, West Lafayette, IN, USA

Processing Within the cisTEM Software Package
Tim Grant, Research Specialist, Howard Hughes Medical Institute Janelia Research Campus, Ashburn, VA, USA

CTF Fitting and Performance Checks with the Volta Phase Plate
Radostin Danev, Professor, Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Martinsried, GERMANY

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Florian Schur, Assistant Professor, Institute of Science and Technology Austria (IST Austria), Klosterneuburg, AUSTRIA

Algorithms for Accelerated Near-atomic Resolution Single- and Multi-particle 3D Reconstruction
Hans Elmlund, Senior Research Fellow, Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University, Melbourne, AUSTRALIA

Recent Progress of Missing-wedge Compensated Electron Tomography Reconstruction Program ICON
Fei Sun, Professor, National Laboratory of Biomacromolecules, IBP, Core Facility for Protein Research, Institute of Biophysics, Chinese Academy of Sciences, Beijing, CHINA

Kam's Method for Single Particle Reconstruction
Amit Singer, Professor, Mathematics and Program in Applied and Computational Mathematics Princeton University, Princeton, NJ, USA

A Machine Learning Method for Resolving Heterogeneity in CryoEM Single Particle Reconstruction
Muyuan Chen, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX, USA

Deep Learning Based Structural Pattern Mining in Cellular Electron Cryo Tomograms — Several Exploratory Studies
Min Xu, Assistant Research Professor, Computational Biology Department, School of Computer Science, Carnegie Mellon University, Pittsburgh, USA

Advanced Computational Methods in Detection of Secondary Structures from Medium-resolution Density Maps
Jing He, Professor, Department of Computer Science, Old Dominion University, Norfolk, VA, USA

FOCUS - The Interface Between Data Collection and Data Processing in cryo-EM

Henning Stahlberg

C-CINA, Biozentrum, University of Basel, SWITZERLAND
henning.stahlberg@unibas.ch

FOCUS is a software package that interfaces cryo-transmission electron microscopy (cryo-EM) data collection with computer image processing, using own and third-party software packages. FOCUS creates a user-friendly environment to import and manage data recorded by direct electron detectors and perform elemental image processing tasks in a high-throughput manner while new data is being acquired at the microscope. It provides the functionality required to remotely monitor the progress of data collection and data processing, which is essential now that automation in cryo-EM allows a steady flow of images of single particles, two-dimensional crystals, or electron tomography data to be recorded in overnight sessions. FOCUS computes several parameters to judge the quality of a data collection session, such as distribution of CTF resolutions, or amounts of image drift, or iciness of the samples. It also can for single particle projects perform an initial 2D class average calculation, or for 2D crystal projects compute 2D and 3D reconstructions, while the data are being recorded. The online quality monitoring and the rapid detection of any errors that may occur during data collection greatly increases the productivity of recording sessions at the electron microscope.

Frame Alignment and CTF Fitting in IMOD and SerialEM

David N. Mastronarde

Department of MCD Biology, University of Colorado, Boulder, CO, USA

I will describe two synergistic developments in the IMOD image processing package and SerialEM data acquisition program. The first is alignment of movie frames from direct detectors. A frame alignment module was first developed within IMOD and incorporated into the program Alignframes. The main alignment strategy involves cross-correlating all pairs of frames within a set of frames and solving for the best shifts among that set. Alignframes is particularly well suited for aligning tilt series taken with SerialEM; it can produce a tilt series output file in the correct order and with essential metadata preserved. The same frame alignment module has been incorporated into SerialEM, first for alignment of frames from K2 cameras, and more recently for alignment of frames from Falcon 2 and 3 cameras, and from Direct Electron cameras run from a new version of the DE server. Alignment during acquisition is particularly valuable for tilt series, where a shift alignment is adequate. The second development is the adaptation of the Ctffind4 program of Rohou and Grigorieff into an IMOD library. This library is used in SerialEM to provide accurate estimates of defocus and astigmatism from images with Thon rings. New routines for astigmatism correction and coma-free alignment are based on direct measurement of astigmatism; these are more reliable than previously implemented routines using beam-tilt induced image displacements. In IMOD, the ctffind library is being incorporated into the CTF determination program, Ctfplotter. This program combine spectra from different Z heights in a tilted image with scaling to align and reinforce the CTF zeros; it can also combine spectra from multiple tilt angles when necessary. This capability, extended to 2D spectra, allows astigmatism to be determined more accurately from multiple adjacent tilt angles than from a single image, while defocus can still be determined for single images.

High-resolution Reconstructions and Local-directional Resolution

C. O. S. Sorzano[1*], J. Vargas[2], J.L. Vilas[1], A. Jiménez[1], J. M. Carazo[1]

[1]*Biocomputing Unit, Centro Nacional de Biotecnología-CSIC, C/ Darwin 3, 28049, Cantoblanco (Madrid), SPAIN*

[2]*Departament of Anatomy and Cell Biology, McGill University, 3640 Rue University, Montréal, QC H3A 0C7, CANADA*

*Correspondence to coss@cnb.csic.es

We will present a new reconstruction protocol called highres for reconstructing homogeneous populations of particles. The algorithm is similar to an statistical version projection matching with some important modifications: (1) the weights and the angular assignment is performed considering not only the value of the cross-correlation between experimental images and volume reprojections but also their significance in the distribution of cross-correlations of the dataset; (2) the CTF amplitude is corrected before entering 3D reconstruction using a Wiener filter; (3) there is an extensive signal detection step in which features not significantly larger than noise are dampened. We show that this approach significantly increases the resolution of the reconstructed volumes.

We will also present an algorithm to perform a directional and local resolution analysis of the reconstructed volumes. This information may be useful to distinguish structural heterogeneity from angular assignment uncertainty. It may also be exploited in a signal restoration algorithm or the fitting of atomic models.

Progress Towards Collecting Tilt-series in Seconds Rather than Minutes with a New "High Precision" Stage

Grant Jensen

Howard Hughes Medical Institute, Division of Biology and Biological Engineering, Caltech, Pasadena, CA, USA

Given the fact that direct electron detectors can read out tens of frames per second, we thought it might be possible to record quick tilt-series by continuously rolling the sample around an axis while the camera was recording data (rather than tilting the sample incrementally and taking individual images at each tilt angle). In this way we thought we might be able to record tilt-series in tens of seconds instead of tens of minutes. For this to work, the stage would have to be eucentric enough that the image of a target of interest would remain on the camera face throughout the tilt-series without any image or stage shifts being applied. Towards this end we asked ThermoFisher to develop a more stable stage, and we have now received and installed their prototype "high precision stage" on our Titan Krios. In initial tests, this stage appears to be successful: targets move only ~200 nm away from the center during complete tilt-series at a variety of speeds, and the non-eucentricity is remarkably reproducible (and therefore predictable). The rate-limiting step turns out to be the speed at which the camera can count individual electrons. As a result, tilt-series at higher magnifications or lower doses can be recorded more quickly. Resulting tilt-series can have hundreds or even thousands of frames. To align such tilt-series, we have developed protocols to stretch, align, and average groups of closely related frames to enhance the visibility of the gold fiducials. Our initial results make us optimisitic that we will soon be able to record up to several hundreds of tilt-series per day, with further speed-ups possible with faster camera and computer communications. Of course this will be a big boost to tomography applications, but because for a given dose, more information is obtained from a tilt-series than a single projection, fast tilt-series may replace projections in single particle experiments as well.

S^2stigmator: A Closed-form Solution for Single-pass Correction of TEM Image Astigmatism

Rui Yan, Kunpeng Li, and **Wen Jiang**

Markey Center for Structural Biology, Department of Biological Sciences, Purdue University, West Lafayette, IN, USA

Minimization of the astigmatism of the objective lens is a critical daily instrument alignment task essential for high resolution TEM imaging. Fast and sensitive detection of astigmatism is needed to provide real-time feedback and adjust the stigmators to efficiently reduce astigmatism. We have developed a s^2-power spectra based method, *s^2stigmator*, to allow fast and sensitive detection of the astigmatism in TEM live images. Using a fast, closed-form solution to determine the defocus and astigmatism of the images, this method presents the astigmatism parameters in a "radar"-style display to provide real-time feedbacks to guide the adjustment of the objective lens stigmators. Such unique capability allowed us to discover the mapping of the two stigmators to the trajectories of the astigmatism in the "radar" plot and to develop a single-pass tuning strategy for the minimization of the objective lens astigmatism. Using this method, we have investigated how image astigmatism varies with the imaging conditions (e.g. defocus, magnification). We have found that the large change of defocus/magnification between visual correction of astigmatism and subsequent data collection tasks, or during data collection, will inevitably result in undesirable astigmatism in the final images. The dependence of astigmatism on the imaging conditions varies significantly from time to time, so that it cannot be reliably compensated by pre-calibration of the microscope. Based on these findings, we recommend that the same magnification and the median defocus of the intended defocus range for final data collection are used in the objective lens astigmatism correction task during microscope alignment and in the focus mode of the iterative low-dose imaging. It is also desirable to develop a fast, accurate method that can perform dynamic correction of the astigmatism for different intended defocuses during automated imaging. Our findings also suggest that the slope of astigmatism changes caused by varying defocuses can be used as a convenient measurement of objective lens rotation symmetry and potentially an acceptance test of new electron microscopes.

Processing Within the cisTEM Software Package

Tim Grant

HHMI Janelia Research Campus, Ashburn, VA, USA

cisTEM (computational imaging system for transmission electron microscopy) is a newly developed open-source software package for processing single-particle cryo-EM data. It features a graphical user interface that is used to submit jobs, monitor their progress, and display results. It implements a full processing pipeline including movie processing, image defocus determination, automatic particle picking, 2D classification, ab-initio 3D map generation from random parameters, 3D classification, and high-resolution refinement and reconstruction. In this talk, a general overview to cisTEM will be given, and a more detailed description of some of the new methods included will be provided.

CTF Fitting and Performance Checks with the Volta Phase Plate

Radostin Danev

Max Planck Institute of Biochemistry, Martinsried, GERMANY

An ideal Zernike phase plate introduces a constant phase shift of half pi. The Volta phase plate utilizes beam-induces phase shift that is not constant and increases with the accumulated dose. Consequently, the phase shift becomes a free parameter in the contrast transfer function (CTF) and must be determined for each experimental image. Despite being just one additional free parameter, the variable phase shift unpins the CTF curve at the zeroth spatial frequency and thus significantly reduces the robustness of CTF fits. In order to improve the reliability of the fits, one has to specify proper search ranges for the phase shift and the defocus, corresponding to the actual ranges of these variables in the dataset. In addition, the strong low frequency components in phase plate images could bias the CTF fits. To reduce such low frequency bias, it is recommended to increase the low-resolution fit limit from the typical 30 Å to ~20 Å.

Overall, CTF fitting is the most critical step in the data processing workflow of phase plate data. Monitoring of the process and possibly iterative refinement of the parameters and very important. The stability of the measured astigmatism and the proper behavior of the measured phase shift are good indicators of the success of the fits. Plots and histograms of the measured defocuses and phase shifts should be used to determine their actual ranges and iteratively refine the fits. Finally, a histogram of the estimated CTF resolutions is a very good measure of the overall technical quality of the dataset. It also provides a rough guideline for the final resolution of the 3D reconstruction, assuming the sample has no performance limiting issues, such as preferred orientations, low particle numbers, heterogeneity, flexibility, etc.

CTF-correction in High-resolution Subtomogram Averaging

Beata Turonova[1], **Florian K.M. Schur[1,2]**, William Wan[1,3], Robert A. Dick[4], Dustin M. Morado[5], Wim J.H. Hagen[1], Volker M. Vogt[4], John A.G. Briggs[1,5]

[1]Structural and Computational Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, GERMANY

[2]Institute of Science and Technology Austria, Klosterneuburg, AUSTRIA

[3]Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Martinsried, GERMANY

[4]Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA

[5]MRC Laboratory of Molecular Biology, Cambridge, UK

Cryo-electron tomography (cryo-ET) can provide three-dimensional insights into the unperturbed organization of tissues, cells and viruses. In order to study the structure of proteins or macromolecular complexes within reconstructed tomograms at higher detail, subtomogram averaging is a powerful tool, and in optimal cases it can generate protein structures at medium to high resolutions. In order to achieve the highest possible resolution, an accurate correction of the contrast transfer function (CTF) is necessary. For tomography this means not only that the defocus change across the tilted image needs to be corrected, but also that the thickness of the sample needs to be taken into account. Our recent implementation of a three-dimensional CTF-correction approach (NovaCTF) allows a more accurate restoration of high-resolution information in cryo-electron tomography. Using an optimal retroviral sample, we showed that this 3D-CTF-correction approach results in higher-resolution structures derived from subtomogram averaging than via standard 2D-CTF-correction. Currently, our bestresolution has been determined to 3.4 Å. In follow-up experiments using a different retroviral sample, we now consistently see significantly improved reconstructions using NovaCTF, both in terms of resolution and signal restoration. We also show that we can obtain high-resolution structures with significantly smaller datasets than via conventional 2D-CTF-correction. In addition to discussing the background and implementation of 3D-CTF correction in our image processing workflow, and the importance of accurate defocus estimation from low-dose/low-signal cryo-electron micrographs, also current limitations in defocus determination will be discussed.

Algorithms for Accelerated Near-atomic Resolution Single- and Multi-particle 3D Reconstruction

Hans Elmlund

Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University, Melbourne, AUSTRALIA

Cryogenic electron microscopy (cryo-EM) and single-particle analysis enable determination of near-atomic resolution structures of biological molecules. However, large computational requirements limit throughput and rapid testing of new image processing tools. We developed PRIME, an algorithm part of the SIMPLE software suite, for determination of the relative 3D orientations of single-particle projection images. PRIME has primarily found use for generation of initial *ab initio* 3D reconstructions. In my talk, I will describe how the strategy behind PRIME, iterative estimation of per-particle orientation distributions with stochastic hill climbing, achieves near-atomic resolution single-particle 3D reconstruction. I will introduce a number of mathematical techniques for accelerating the convergence rate, leading to an overall speedup of nearly two orders of magnitude. Another focus area of my team is development of multi-particle 3D reconstruction algorithms for capturing conformational and/or compositional states that may co-exist within a single data set. Building on our recent approach for identifying homogeneous subsets of images in 2D (Reboul *et. al* 2017, Protein Science), we have developed a method for estimating the necessary parameters in 3D. If time permits, I will also introduce our stochastic quantization method for rapid multi-particle sorting.

Recent Progress of Missing-wedge Compensated Electron Tomography Reconstruction Program ICON

Fei Sun[1] and Fa Zhang[2]

[1]*Institute of Biophysics, Chinese Academy of Sciences*

[2]*Institute of Computation Technology, Chinese Academy of Sciences*

Cryo-electron tomography (ET) has demonstrated its potential in revealing the high-resolution structure of macromolecules in the cell/tissue, and will become a unique approach for the future *in situ* structural biology. However, the existence of missing information due to the limited angular sampling in ET experiments makes ET reconstruction as the 'ill-posed' problem, which hampers the subsequent sub-tomogram alignment and average. We recently published a work to describe a new algorithm ICON (Iterative Compressed-sensing Optimized NUFFT reconstruction) to restore a portion of non-sampled information and yield a tomogram with a better contrast. Here, I will report our recent studies of the algorithm of ICON, including the change of weights in NUFFT, a new post-processing procedure, GPU implementation, and applications in real experimental data processing. Besides, I will also give a short report of our recent published ET package AuTOM that aims for automatic processing of ET tilt data.

Kam's Method for Single Particle Reconstruction

Amit Singer

Princeton University, Mathematics and Program in Applied and Computational Mathematics, Princeton, NJ, USA

In a seminal paper that was published in 1980, Zvi Kam proposed a radical new approach for 3-D *ab-initio* reconstruction that completely sidesteps the estimation of rotations. Kam's method is based on computing the autocorrelation and higher order correlation functions of the 3-D structure in reciprocal space from the 2-D noisy projection images. Remarkably, these correlation functions uniquely determine the 3-D structure. Unlike maximum likelihood estimation that requires multiple passes over the data and requires an initial model, Kam's method needs just one pass over the data for computing the correlation functions and does not require any initial model. As a result, it can be extremely fast and may even operate in a streaming mode of computation in which data is processed on the fly while being acquired. Despite these clear advantages, Kam's method was not adopted by the EM community for several reasons. One clear disadvantage of Kam's method is that it requires a uniform distribution of viewing directions. Also, accurate estimation of the autocorrelation functions involves relatively sophisticated and modern tools from high dimensional statistics, as well as highly non-trivial numerical implementation. The computation of higher order correlation functions is even more challenging. Finally, Kam's method assumes images are perfectly centered, limiting the resolution that it can achieve.

In this talk, the speaker will discuss ongoing efforts made in his research group to make Kam's method viable, specifically, by extending the original method to non-uniform distributions of viewing directions and non-perfect centering of the images. In addition, we will address the question of how many images are required for reconstruction as a function of the signal to noise ratio (SNR).

A Machine Learning Method for Resolving Heterogeneity in CryoEM Single Particle Reconstruction

Muyuan Chen, James M. Bell, and Steven J. Ludtke

Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX, USA

The function of most protein molecules involves structural flexibility and/or dynamic interactions with other molecules. CryoEM provides direct visualization of individual proteins in different conformational states. While many methods are available for classification of discrete states, characterization of continuous conformational changes without human supervision is still challenging. Here we present a machine learning algorithm to determine the conformational landscape of proteins through a 3-D Gaussian representation mapped onto 2-D images in known orientations. Using a neural-network-like architecture, the method is capable of highlighting flexible regions, resolving compositional/conformational heterogeneity and map particles onto eigen-motion trajectories. We have applied this method to several different biomolecular systems to explore conformational changes at a range of scales.

Deep Learning Based Structural Pattern Mining in Cellular Electron Cryo Tomograms — Several Exploratory Sudies

Min Xu

Computational Biology Department, School of Computer Science, Carnegie Mellon University, Pittsburgh, USA

Cellular Electron CryoTomography (CECT) has enabled 3D visualization of subcellular structures at the submolecular resolution and in the near-native state. However, the systematic structural identification and recovery in CECT are difficult due to the structural complexity and imaging limits. During recent years, deep learning techniques, such as convolutional neural networks (CNN), have emerged as a main-stream technique of a broad range of computer vision tasks. In this talk, I will present several exploratory projects that we have been conducting on using CNN for facilitating structural pattern mining in cellular tomograms. These projects include: (1) large-scale subtomogram coarse supervised classification, structural feature extraction, and semi-supervised subdivision [4]; (2) improving classification accuracy using deeper models [1]; (3) improving classification speed through model compression [2]; (4) autoencoder based unsupervised image pattern discovery and weekly-supervised segmentation [5]; and (5) supervised subtomogram segmentation [3]. Our exploratory studies showed that deep learning is potentially a very powerful tool for systematic structural identification and recovery in cellular tomograms.

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Advanced Computational Methods in Detection of Secondary Structures from Medium-resolution Density Maps

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One of the most distinct characters in density maps at medium resolutions, such as those with 5–10 Å resolutions, is the visibility of protein secondary structures. Although computational methods have been developed, accurate detection of helices and β-strands from cryo-EM density maps is still challenging. In this talk, I will discuss our approaches in secondary structure detection using geometric properties, machine learning, and convolutional neural networks. In order to evaluate the accuracy of secondary structure detection, we have developed AxisComparison, a tool that is integrated in Chimera for quantification of the detected helices and beta-strands. Our recent effort in characterizing atomic structures solved from high-resolution cryo-EM density maps will be discussed.

Poster Abstracts

Data-driven Dose Weighting for High-Resolution Single-Particle Cryo-EM

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Improvements in the DQE of direct electron detectors combined with their ability to operate in movie mode, have created unique opportunities for the development of computational strategies in single-particle cryo-EM capable of routinely achieving near-atomic resolution. By harnessing the time-resolved information contained in movie frames, these techniques have managed to overcome fundamental resolution barriers inherent to imaging biological samples using high energy electrons. Despite these advances, the accuracy of CTF parameter estimation and alignment of movie frames on a per-particle basis is still limited by the combination of poor SNR and the small image area occupied by single particles. In addition, the inability of existing dose compensation strategies to capture the highest resolution data recorded during the earliest part of the exposure remains a major resolution-limiting factor. Here, we report the development of methods to account for local variations in defocus and beam-induced drift, as well as the implementation of a new data-driven dose compensation scheme for efficiently extracting high-resolution information recorded during the exposure. These advances enable determination of a significantly improved cryo-EM density map for inhibitor-bound □-galactosidase where the ordered regions of the protein show clear delineation of the contours for non H-atoms.

APPLE Picker, Automatic Particle Picking for Cryo-EM

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Particle picking is a crucial first step in the computational pipeline of single-particle cryo-electron microscopy (cryo-EM). Selecting particles from the micrographs is difficult especially for small particles with low contrast. As high-resolution reconstruction typically requires hundreds of thousands of particles, manually picking that many particles is often too time-consuming. While semi-automated particle picking is currently a popular approach, it may suffer from introducing manual bias into the selection process. In addition, semi-automated particle picking is still somewhat time-consuming. This paper presents the APPLE picker, a simple and novel approach for fast, accurate, and fully automatic particle picking. While our approach was inspired by template matching, it is completely template free. This approach is evaluated on publicly available datasets containing micrographs of β-galactosidase and keyhole limpet hemo-cyanin projections.

3D Sorting in cryo-EM with Outcome Validation

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Introduction: Sorting of 2D projection data in single particle cryo-EM is a challenging problem. The commonly used *K*-means clustering algorithm (or its version) has well-known deficiencies: its outcome strongly depends on the initialization, i.e., on the template maps provided by the user to start the algorithm. It also depends on the number of groups *K*, a key parameter that has to be provided by the user. When combined with 3D refinement, 3D sorting suffers from prohibitively long time of calculations, which makes validation of the outcome all but impossible. In addition, the difficulties are compounded by the fact that the dataset is inhomogeneous, i.e., the input 2D projection images are collected under different imaging conditions (varied defocus, ice thickness, and so on), and their angular distribution tends to be highly uneven, thus SNR of the structure has directional dependence.

Method: The proposed method, EQUIPART, employs a modified *K*-means algorithm to accomplish balanced partition of the data and, to increase computational efficiency and robustness of the outcome, separates group assignment from 3D refinement. EQUIPART proceeds by testing reproducibility of multiple randomly restarted clusterings, which is accomplished by evaluating two-way agreements of group assignment. Monte Carlo simulations provide assessment of the agreement level under the assumption that assignments were random and only groups whose reproducibility raises above random level are accepted. This methodology is used to provide validation of the ultimate result. In addition, EQUIPART adjusts the number of groups by repetitively applying the entire clustering procedure to projection images that could not be reproducibly assigned to groups in a given pass through the data.

Results: We demonstrate efficacy of the method in the application to the previously published 80S ribosomal dataset, in which case EQUIPART was able to determine well-defined subgroups in a set thought to be homogeneous. We also show a case of hemocynin complex where visibly convincing large-scale confirmation changes were discarded as irreproducible using the Monte Carlo-based validation.

Multiple Spooled Conformations of a Viral dsRNA Genome Revealed by Cryo-Electron Microscopy

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Bacteriophage ϕ6 of the *Cystoviridae* family is a prototypic dsRNA virus that packages its three-segmented genome in ssRNA form into preassembled procapsids. Genome replication and the addition of a second protein layer leads to the formation of the nucleocapsid. Both the procapsid and nucleocapsid have been structurally characterized previously, however, the organization of the dsRNA has remained elusive due to the symmetry mismatch with the icosahedral shells. Here we developed a structure refinement approach utilizing multimodal priors in a maximum likelihood framework to reconstruct the nucleocapsid genome from single particle cryo-electron microscopy data. The structure we obtained shows that the dsRNA adopts a spooled arrangement, resembling the canonical models for viral dsDNA genomes much more closely than the nonspooled dsRNA structures previously determined. Upon further analysis, two major conformations were discerned, each representing an average of multiple locally distinct sub-conformations. In both averaged structures, capsid proximal regions were resolved to sub-nanometer resolutions, whereas the remainder of the genome appeared considerably less ordered. To tackle the increased flexibility of the inner RNA layers, we sequentially subtracted the contribution of each layer to facilitate the local asymmetric refinement of the following one. The attained resolution degraded progressively, yet remained sufficiently high to distinguish individual strands and to unambiguously fit dsRNA models. This enabled tracing approximately 75% of the whole genome and analysing the different liquid crystalline geometries exhibited by such tightly packed nucleic acid.

Volta Phase Plate Data Collection Facilitates Image Processing and cryo-EM Structure Determination

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Single particle cryo electron microscopy (cryo-EM) is showing unprecedented developments in structural biology regarding instrumentation, image reconstruction and analysis, which increasingly allows resolving large macromolecular structures to near atomic resolution, with a strong exponential increase since the introduction of direct electron detectors ~2012/13 (reviewed in Orlov *et al.*, 2017). Following the first high-resolution cryo-EM structure of the human ribosome (Khatter *et al., Nature* 2015) and of a first ligand complex (Myasnikov *et al.,* 2016) we now determined its structure to a resolution level allowing for the first time to visualize over 130 individual rRNA modifications explaining their structural and functional roles (Natchiar *et al.*, 2017). This high-resolution work involved sorting of different structural states through advanced image processing tools such as maximum likelihood based 3D sorting (Scheres, *Methods in Enzymology* 2010) and 3D sampling & classification (Simonetti *et al.*, *Nature* 2008; Klaholz 2015), local/focused classifications (Klaholz *et al.*, 2004) and refinements (von Loeffelholz *et al.*, 2017).

Here we will present our latest work on the processing of phase plate data (von Loeffelholz *et al.*, 2018). Volta phase plates (VPP) introduce a phase shift in the contrast transfer and drastically increase the contrast of the recorded low-dose cryo-EM images while preserving high frequency information. This comparative study addresses the behaviour of different data sets during image processing and quantifies important parameters during structure refinement. Automated VPP data collection was done from the same human ribosome sample either as a conventional defocus range dataset or with a Volta phase plate close to focus (cfVPP) or with a small defocus (dfVPP). The analysis of image processing parameters shows that dfVPP data behave more robustly during cryo-EM structure refinement because particle alignments, Euler angle assignments and 2D & 3D classifications behave more stably and converge faster. In particular, less particle images are required to reach the same resolution in the 3D reconstructions. Finally, we find that defocus range data collection is also applicable to VPP. This study shows that data processing and cryo-EM map interpretation, including atomic model refinement, are facilitated significantly by performing VPP cryo-EM, which will have an important impact on structural biology (von Loeffelholz *et al.*, 2018).

Furthermore, we are involved in a series of developments that facilitate processing and data interpretation. An important aspect is atomic model building and refinement into high-resolution cryo-EM maps which allows deriving atomic models with correct geometric parameters for which we developed a protocol based on least squares and

maximum likelihood target functions using tools at the interface of crystallography and cryo-EM (Natchiar *et al.*, 2017) and a novel quality assessment of these atomic models using global validation metrics, including methods and tools for the evaluation of EM-derived atomic models (Afonine *et al.*, *Acta Cryst D,* 2018*, under revision*). Moreover, to extend multi-scale integration towards correlative imaging techniques we are developing new tools for super-resolution fluorescence microscopy (PALM, STORM, GSDIM etc.): SharpViSu, an interactive open-source software that allows performing drift correction and FRC-based resolution estimation (Andronov *et al.*, 2016), and ClusterViSu for image segmentation based on Voronoi tessellation of the individual fluorescence events in 2D (Andronov *et al.*, 2016) and most recently in 3D (Andronov *et al.*, 2018), based on similar concepts as our recent weighted back-projection 3D reconstruction algorithm (Orlov *et al.*, *submitted*). Together, these developments allow bridging various scales and resolution levels, from the atomic to the cellular level, to address the function of large nucleoprotein complexes in an integrated manner including also structure-sequence analysis with our IBiSS database (Beinsteiner *et al.*, 2015).

Towards Automatic Screening for Cryo-EM Autogrid Selection

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Having the best grid quality is crucial to obtain micrographs suitable for high resolution Cryo Electron Microscopy. Sample homogeneity, vitrification process and grid transfer have a profound effect on the final images acquired and the 3D reconstruction quality. Users typically prepare multiple grids and select the best one for acquisition by screening them for useable areas. Currently, both Autogrid transfer from the Autoloader into the microscope column and Autogrid “Atlas” creation by combining multiple low-magnification images are steps performed automatically by the microscope, but requiring operator supervision. In addition, the assessment of the Autogrid quality (holey film integrity, ice thickness and contaminations) is a tedious and time consuming task performed manually by the operator. Here we propose a method to automate this screening task and assist the user to select the most suitable Autogrids.

Autogrids of interest are pre-preselected by the operator and then transferred successively, their respective Atlases being acquired automatically. The grid squares displayed on the Atlases are detected automatically and categorized based on image analysis and unsupervised machine learning, where squares with a similar appearance will be grouped into the same category. The outcome of the automated analysis is displayed using colored annotations in an interface, where the user can review and confirm which squares from which Autogrids are the most suitable to acquire a high resolution dataset.

The availability of this feature on microscopes like the new Glacios used for screening campaign of various samples, will save precious time on the high-end Krios that is typically dedicated to acquire the high resolution dataset required to obtain near atomic resolution structures.

PyFilamentPicker: Semi-automated Flament Tracer for cryo-EM Structure Determination

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Cryo-EM structure determination of filamentous specimens includes particle picking, classification of the images, and iterative refinement of 3D structures. Although the latter two steps are common to “single” particle analysis, the first step: particle picking from filamentous specimens is not optimized and most of existing software tools require considerable effort and their sensitivity and specificity are insufficient. Here, we developed a semi-automated reference-free filament tracing tool: PyFilamentPicker. This tool can precisely trace a filament only by clicking a part of it. It adopts a correlation-based method to trace a filament, using the image of the filament at the initial clicked position as a reference image. We tested PyFilamentPicker using various kinds of filaments, such as microtubule, actin, and tobacco mosaic virus (TMV) and showed that its tracing capabilities even from a low signal-to-ratio images recorded on films. It enables users to intuitively, accurately, and quickly prepare datasets for three-dimensional reconstruction for other software.

Hyper-molecule Representation of the Continuum of Molecular Conformations in cryo-EM

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We introduce “hyper-molecules,” which simultaneously capture the entire continuum of conformations of a molecule in a single higher dimensional “hyper-object.” We discuss how prior knowledge about the molecule is encoded in such hyper-molecules, and introduce a Bayesian framework for recovering the continuum of conformations from cryo-EM images.

A Novel Filamentous Virus Infects Hyperthermophilic Acidophiles

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Sulfolobus filamentous virus (SFV) was isolated from very hot, highly acidic springs in Beppu, Japan. It is membrane enveloped, and contains a 37 kbp double stranded DNA genome which encodes for two major capsid proteins. A BLAST search using either of these proteins fails to recognize any sequence similarity with any other protein in the database, suggesting that archaeal viral proteins are grossly undersampled in existing databases. Further, there is no apparent sequence similarity between these two proteins. We have generated a cryo-EM reconstruction at better than 3.7 Å resolution, and show that there are 17.14 asymmetric units per 46 Å turn of the solenoid. The asymmetric unit contains the two proteins, which form a pseudo-symmetrical heterodimer, wrapping around 12 bp of DNA. In SIRV2 (DiMaio et al., Science, 2015) identical capsid proteins form symmetrical homodimers, while in AFV1 (Kasson et al., eLife, 2017) two different capsid proteins form a pseudo-symmetrical heterodimer. Surprisingly, the pseudosymmetrical heterodimer in SFV is more similar to the homodimer in SIRV2 than it is to the heterodimer in AFV1. As with AFV1 and SIRV2, where we showed that the DNA is found in A-form, it is in A-form in SFV although the supercoiling is at a much larger radius. This is consistent with our previous prediction that DNA will be maintained in A-form by both bacterial spores and viruses that infect extremophiles. As with AFV1, the membrane enveloping in SFV is anomalously thin. These studies provide new insights into how both proteins and DNA can be stable in the most aggressive environments.

The Cryo-EM Structure of V-type ATPase V_O Proton Channel in Lipid Nanodisc

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The mechanism of transmembrane proton translocation in rotary motor ATPases is poorly understood. Here we report the 3.5 Å resolution cryoEM reconstruction of the lipidnanodiscreconstituted V_O proton channel of the yeast vacuolar H^+-ATPase (V-ATPase), captured in a physiologically relevant, autoinhibited state. The resulting atomic model provides unprecedented detail for the residues at the interface of the proteolipid ring and the transmembrane portion of the *a* subunit that constitute the proton pathway, and together with previous mutagenesis studies, we propose the chemical basis of transmembrane proton transport. Moreover, we discovered that the C-terminus of the assembly factor Voa1 is an integral component of mature V_O. Voa1's C-terminal transmembrane α helix is bound inside the proteolipid ring where it contributes to the stability of the complex. Our structure rationalizes possible mechanisms by which mutations in human V_O can result in disease phenotypes, thus providing new avenues for therapeutic interventions.

The Structure of Zika Virus

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The structures of the immature Zika virus, the mature Zika virus and the mature Zika virus complexed with a neutralizing antibody have been determined by cryo electron microscopy to 3.8Å, 9Å and 6.2Å resolution, respectively. The biggest structural difference between Zika virus and dengue virus (another flavi virus) occurs at a glycosylated Asn residue that is probably a site where the virus can attach to a cellular receptor. The immature Zika virus structure shows vestiges of a viral capsid inside the viral membrane, not seen in other flavi viruses. Asymmetric reconstructions of Zika and Kunjin viruses shows that the core is placed asymmetrically placed inside the glycoprotein shell suggesting that there is a special vertex produced by budding and needed for RNA packaging. A number of neutralizing antibodies are being studied when in complex with the virus. One of these neutralizes by cross-linking the surface glycoproteins. Because the three independent Fab attachment sites are closer together than the diameter of the Fab molecules, not all sites can be occupied simultaneously. This reduces the concentration of the antibody required for neutralization, thus increasing the antibody's potency.

A Single-particle EM Approach for Differential Visual Proteomics

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We introduce a method for differential visual proteomics. The method consists of (i) an instrument for single cell lysis [1] and for blotting-free sample preparation for electron microscopy (EM) [2-4] as well as (ii) a novel image-processing pipeline for data analysis.

There is a strong link between protein functions and cellular phenotypes. Proteomics give important conclusions about how different molecular pathways correlate to variations in the cellular traits. Traditionally, mass spectrometry (MS) is used for quantifying the protein expression levels and for identifying links between protein abundances and cellular traits. However, MS is only partially suitable for single-cell analysis and provides limited information about the structures of the involved proteins. Visual proteomics refers to the qualitative and quantitative description of the cellular proteome by EM and the recognition of proteins and protein complexes according to their structure and shape. Cryo-electron tomography (cryo-ET) provides a protein map at the single-cell level, it is however restricted to thin cell sections, often suffers from limited resolution and is not suitable for high-throughput analysis.

Here we present a method where single-particle EM is used for obtaining highresolution images of the cell-proteome. Initially, individual cells are lysed by electroporation. The cell lysate (~3nL) is subsequently conditioned, prepared in a lossless manner and imaged by negative stain EM or cryo-EM. Image-analysis software is employed for detecting and classifying the protein particles in the collected EM images. A subsequent image processing and quantitative analysis of the detected proteins can reveal differences in the proteome structure of different cells, *i.e.* the proteome of healthy and unhealthy cells.

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Improving 3D reconstructions of macromolecular conformations

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Cryo-electron microscopy using single particle analysis requires the computational averaging of thousands of projection images captured from identical macromolecules. However, macromolecules usually present some degree of heterogeneity showing different conformations. Reconstruction of these conformations is essential to infer the macromolecular function and understand how it works. Computational approaches are then required to classify heterogeneous single particle images into homogeneous sets at different conformations. Nonetheless, sometimes the attainable resolution of reconstructions obtained from these smaller homogeneous sets is compromised because of reduced number of particles or lack of images at certain macromolecular orientations. In these situations, the unique solution to improve map resolution is returning to the electron microscope and collect more data. In this work, we present an approach to overcome this limitation for heterogeneous datasets. Our method is based on moving particles between different conformations using an optical flow approach. Particles are then merged in a unique conformation obtaining reconstructions with improved resolution, contrast and signal-to-noise ratio.

SPHIRE-crYOLO: Accurate and well centered particle picking

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Manual single particle selection is a tedious and time consuming process. Therefore several automatic particle picking procedures have been developed, however, most of them still fail with challenging datasets. Here, we present an automatic particle picking procedure called crYOLO, which is based on the well-known deep learning object detection system “You Only Look Once” (YOLO). We used three cryo-EM datasets from the EMPIAR databank to test crYOLO: After training crYOLO with less than 1,500 particles per dataset, the program selected the particles with high precision and accuracy. In addition, also unevenly shaped particles are well centered during selection. Using standard NVIDIA GPUs particle selection takes less than a second per image. crYOLO is part of the SPHIRE software package and freely available under http://sphire.mpg.de/.

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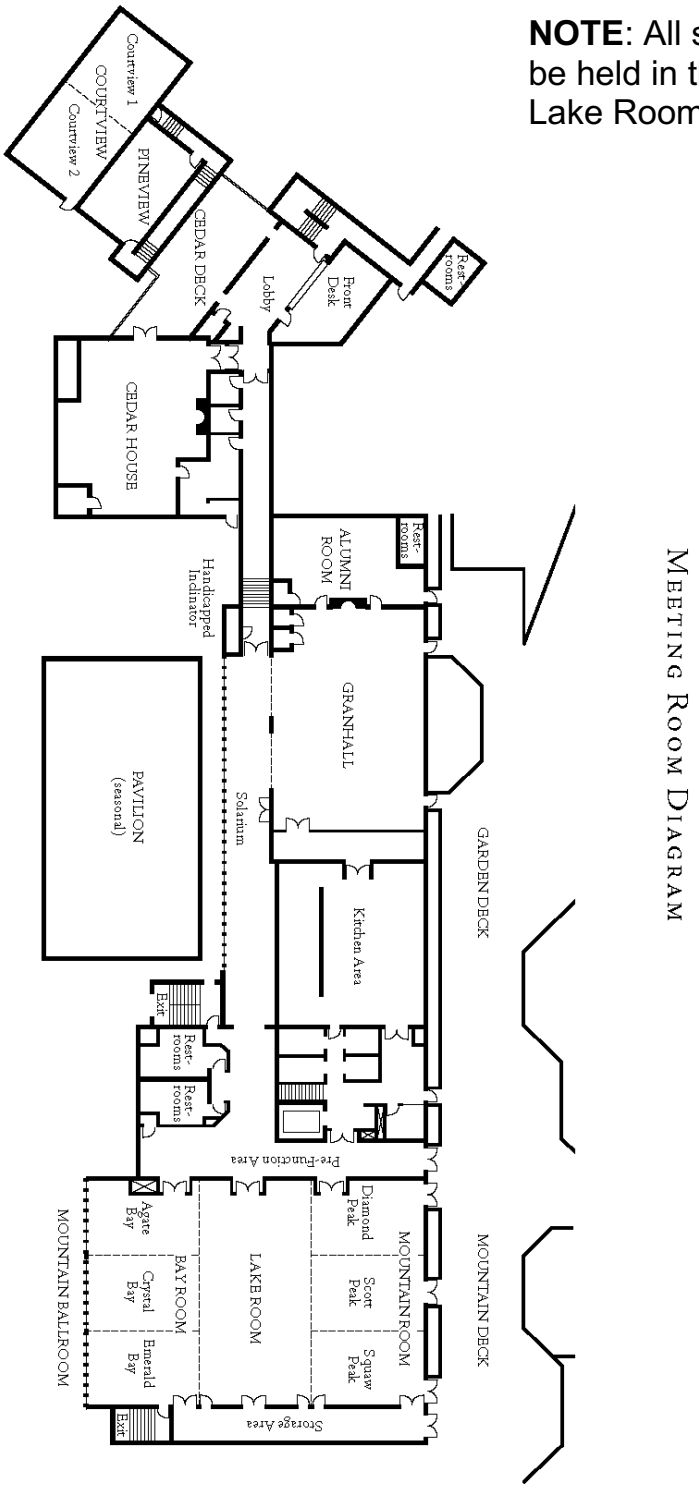
Meeting Room Diagram

NOTE: All sessions will be held in the Mountain-Lake Room



MEETING ROOM DIAGRAM

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