2ND INTERNATIONAL SYMPOSIUM ON CRYO-EM 3D IMAGE ANALYSIS

March 30 – April 2, 2016
Granlibakken Conference Center & Lodge
Lake Tahoe, CA USA

Program Schedule
Abstracts
List of Participants

2nd International Symposium on Cryo-3D Image Analysis

March 30-April 2, 2016

Welcome to the second 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. We would encourage speakers and the audience to focus their presentations and discussions on technical aspects of the analysis. As in our previous meeting, posters will be displayed for the entire event, and several will be selected during the poster sessions to give short-talks on the final day of the meeting. Lecture sessions will leave plenty of time for discussions, and we encourage the speakers to keep well within their time limits to permit this. Thank you for joining us in what we are sure will be a profitable meeting.

http://blake.bcm.edu/tahoe2016

Dorit Hanein, Chair
SBP

Steven J. Ludtke, Co-Chair
Baylor College of Medicine

Organizing Committee

Wah Chiu
Baylor College of Medicine

Edward H. Egelman
University of Virginia Medical School

Ben Hankamer
University of Queensland

Masahide Kikkawa
University of Tokyo

David N. Mastronarde
University of Colorado

Pawel A. Penczek
University of Texas-Houston Medical School

Henning Stahlberg
University of Basel

Fei Sun
Chinese Academy of Sciences

Niels Volkmann
Sanford Burnham Prebys

Meeting Coordinator

Alicia Uncangco
Sanford Burnham Prebys Medical Discovery Institute

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Notes:

Program

WEDNESDAY, MARCH 30TH

4:00-6:00	Meeting Registration / Check-in
5:00-6:00	Reception (Granhall)
6:00-7:30	Dinner (Granhall)
7:30-7:40	Welcome, **Dorit Hanein**, Program Chair Professor of Bioinformatics and Systems Biology, Sanford Burnham Prebys, La Jolla, USA Introduction, **Wah Chiu**, Session Chair Distinguished Professor, Director National Center for Macromolecular Imaging at the Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, USA
7:40-8:40	**Joachim Frank**, **Keynote Speaker** Investigator, Howard Hughes Medical Institute and Professor, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, USA
8:40-11:00	Cash bar (Granhall)

THURSDAY, MARCH 31ST

7:00-8:00	Breakfast (Granhall)

Session I (morning), Handling Compositional and Conformational Variability
(Mountain-Lake)

8:00-8:05	Introduction, **Niels Volkmann,** Session chair Professor of Bioinformatics and Systems Biology, Sanford Burnham Prebys, La Jolla, USA
8:05-8:30	**Friedrich Förster***, Platform Speaker Group Leader, Department of Structural Biology, Max-Planck Institute for Biochemistry, Martinsried, GERMANY

*Speakers from outside the U.S.

8:30-8:55 **Pawel Penczek**, Platform Speaker
Professor of Biochemistry & Molecular Biology and Director of Structural Biology Imaging Center, The University of Texas-Houston Medical School, Houston, USA

8:55-9:20 **Peter C. Doerschuk**, Platform Speaker
Professor, Meinig School of Biomedical Engineering, Cornell University, Ithaka, USA

9:20-10:00 Coffee/Tea Break and **Group Photo**

Session II, High Resolution Subtomogram Averaging and Related Techniques
(Mountain-Lake)

10:20-10:25 Introduction, **David N. Mastronarde**, Session Chair
Professor and Co-director of Boulder Laboratory for 3D EM of Cells, Department of MCD Biology, University of Colorado, Boulder, USA

10:25-10:50 **Zhiguo Shang**, Platform Speaker
Associate Professor, Departments of Cell Biology and Biophysics, UT Southwestern Medical Center at Dallas, USA

10:50-11:15 **Fei Sun***, Platform Speaker
Professor of Structural Biology and Leader & Chief Scientist of Center for Biological Imaging, Institute of Biophysics, Chinese Academy of Sciences, CHINA

11:15-11:30 **Jesus G. Galaz-Montoya,** Platform Speaker
Postdoctoral, Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, USA

11:30-12:00 **Ben Hankamer,** In Memoriam of **Peter van der Heide**

12:00-1:00 Lunch (Granhall)

Poster Session I (Afternoon)

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

6:00-7:30 Dinner (Granhall)

*Speakers from outside the U.S.

Session III (Evening), High Resolution Single Particle Analysis
(Mountain-Lake)

7:30-7:35 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

7:35-8:00 **Wen Jiang,** Platform Speaker
Associate Professor of Markey Center for Structural Biology, Department of Biological Sciences, Purdue University, West Lafayette, USA

8:00-8:25 **Kai Zhang***, Platform Speaker
Postdoctoral, MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, Cambridge, UK

8:25-8:50 **Rui Zhang**, Platform Speaker
Research Specialist, Howard Hughes Medical Institute, University of California Berkeley, Berkeley, USA

8:50-11:00 Cash bar (Cedar House)

FRIDAY, APRIL 1ST

7:00-8:00 Breakfast (Granhall)

Session IV (Morning), Resolution Assessment, Model and Map Validation
(Mountain-Lake)

8:00-8:05 Introduction, **Henning Stahlberg***, Session Chair
Professor of C-CINA, Biozentrum, University of Basel, Basel, SWITZERLAND

8:05-8:30 **Javier Vargas***, Platform Speaker
Instruct Image Processing Center, Spanish National Center for Biotechnology CNB-CSIC, Madrid, SPAIN

8:30-8:55 **Edward H. Egelman**, Platform Speaker
Professor of Biochemistry and Molecular Genetics, University of Virginia Medical School, Charlottesville, USA

*Speakers from outside the U.S.

8:55-9:20 **Nikolaus Grigorieff**, Platform Speaker
Investigator, Howard Hughes Medical Institute, Professor, Janelia Research Campus, Loudoun County, USA

9:20-9:45 **Niels Volkmann,** Platform Speaker
Professor of Bioinformatics and Systems Biology, Sanford Burnham Prebys, La Jolla, USA

9:45-10:30 Coffee/Tea Break

Session V, Data Storage Roundtable
(Mountain-Lake)

10:30-12:00 **Steven Ludtke**, Moderator
Professor, Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, USA

Grant Jensen, Roundtable Discussant
Professor, Department of Biology, California Institute of Technology, Pasadena, USA

David N. Mastronarde, Roundtable Discussant
Associate Professor and Co-director of Boulder Laboratory for 3D EM of Cells, Department of MCD Biology, University of Colorado, Boulder, USA

Roberto Marabini*, Roundtable Discussant
Escuela Superior Politécnica Superior, Universidad Autónoma de Madrid, Madrid, SPAIN

Ardan Patwardhan*, Roundtable Discussant
Senior Scientific Officer, Protein Data Bank in Europe, European Molecular Biology Laboratory, European Bioinformatics Institute, Cambridge, UK

12:00-1:00 Lunch (Granhall)

1:30-3:30 Satellite Meeting: EMDataBank map challenge.

Wah Chiu, Overview EMDataBank Challenges and Map Challenge Goals

Cathy Lawson, Status of Map Challenge Submissions, Data Access

Ardan Patwardhan, Current Validation Services for EM Maps

Open discussion: criteria for map submissions

Poster Session II (Afternoon) – location

4:30-6:00 Poster Session II (Bay Room)

6:00-7:30 Dinner (Granhall)

Session VI (Evening), Seven Selected Poster Talks

7:30-8:50 **Edward H. Egelman**, Session Chair
Professor of Biochemistry and Molecular Genetics, University of Virginia Medical School, Charlottesville, USA

Christopher Aylett, Selected Short Talk
Postdoc, Biology, Institute of Molecular Biology and Biophysics

Timothy Grant, Selected Short Talk
Postdoc, HHMI / Janelia Research Campus

Slavica Jonic, Selected Short Talk
Faculty, IMPMC, Sorbonne Universités French National Centre for Scientific Research - CNRS

Matthew Johnson, Selected Short Talk
Postdoc, Institute of Molecular Biophysics, Florida State University

Robert Louder, Selected Short Talk
Student, Biophysics, UC Berkeley

Vidya Prasad, Selected Short Talk
Postdoc, Biological Sciences, Purdue University

William Wan, Selected Short Talk
Postdoc, Structural and Computational Biology Unit, European Molecular Biology Laboratory

Masahide Kikkawa*, Selection Committee
Professor of Cell Biology and Anatomy, Graduate School of Medicine, The University of Tokyo, JAPAN

Ben Hankamer*, Selection Committee
Professor, Institute of Molecular Bioscience
The University of Queensland, AUSTRALIA

Fei Sun*, Selection Committee
Professor of Structural Biology and Leader & Chief Scientist of Center for Biological Imaging, Institute of Biophysics, Chinese Academy of Sciences, CHINA

David DeRosier, Selection Committee
Professor of Biology, Emeritus, Brandeis University, Waltham, USA

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

9:00-12:00 Cash Bar/Social Time (Cedar House)

SATURDAY, APRIL 2ND

7:00-9:00 Breakfast (Granhall)

Departures

Speaker Abstracts

Cryo-Electron Microscopy: the Sky is the Limit
Joachim Frank, Investigator, Howard Hughes Medical Institute and Professor, Department of Biochemistry and Molecular Biophysics, Columbia University, USA

Classifying and correlating structural heterogeneity in situ and in vitro
Friedrich Förster, Max-Planck Institut fuer Biochemie, D-82152 Martinsried, Germany

Identification of Reproducible 3-D Structures in Heterogeneous Cryo-EM Data Sets
Pawel Penczek, Professor of Biochemistry & Molecular Biology and Director of Structural Biology Imaging Center, The University of Texas-Houston Medical School, Houston, USA

Characterizing discrete and continuous 3-D particle heterogeneity from cryo EM images
Peter Doerschuk, Faculty, Biomedical and Electrical and Computer Engineering, Cornell University, Ithaca, USA

Tomography-Guided 3D Reconstruction of Subcellular Structures (TYGRESS) improves the resolution of cellular imaging
Zhiguo Shang, Associate Professor, Departments of Cell Biology and Biophysics, UT Southwestern Medical Center at Dallas, USA

Electron tomography reconstruction with restoration of missing information
Fei Sun, Professor of Structural Biology and Leader & Chief Scientist of Center for Biological Imaging, Institute of Biophysics, Chinese Academy of Sciences, CHINA

CTF Correction and Alignment Algorithms for Single Particle Cryo Electron Tomography in EMAN2
Jesus Galaz-Montoya, Postdoctoral, Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, USA

Sub-3 Angstrom Cryo-EM 3-D Reconstruction of Viruses: Affinity Grids and Image Corrections
Wen Jiang, Associate Professor of Markey Center for Structural Biology, Department of Biological Sciences, Purdue University, West Lafayette, USA

High Resolution Cryo-EM at High Speed and Low Cost
Kai Zhang, Postdoctoral, MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, Cambridge, UK

A new protocol to accurately determine microtubule lattice seam location
Rui Zhang, Research Specialist, Howard Hughes Medical Institute, University of California Berkeley, Berkeley, USA

Soft alignment validation in cryoelectron microscopy
Javier Vargas, Instruct Image Processing Center, Spanish National Center for Biotechnology CNB-CSIC, Madrid, SPAIN

Validation of Helical Structures: Lies, Damned Lies and Resolution Statistics
Edward H. Egelman, Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, USA

Optimizing Image Contrast for Cryo-EM
Nikolaus Grigorieff, Investigator, Howard Hughes Medical Institute, Professor, Janelia Research Campus, Loudoun County, USA

Validation of low-resolution EM models using statistics-based tools
Niels Volkmann Professor of Bioinformatics and Systems Biology, Sanford Burnham Prebys, La Jolla, USA

Cryo-Electron Microscopy: the Sky is the Limit

Joachim Frank

Howard Hughes Medical Institute and Professor, Department of Biochemistry and Molecular Biophysics, Columbia University, USA

Molecular structure research by cryo-EM and single-particle reconstruction has gotten very far by now, after several decades during which the mathematical concepts and computational approaches were developed. The resolution barrier posed by suboptimal recording media, about 5Å for asymmetric particles, has recently been overcome, and structures in the 2-3Å range are no longer exceptions. It is interesting to think of this situation as a starting point, not an end. I will take this talk as an opportunity to sketch out future developments, at the risk of being proven wrong in the span of a few years.

Classifying and correlating structural heterogeneity *in situ* and *in vitro*

Friedrich Förster

Max-Planck Institut fuer Biochemie, D-82152 Martinsried, Germany

In order to fulfill their cellular function macromolecular complexes adopt different conformational states and assemble transiently with cofactors. Cryo-electron tomography is uniquely suited to capture three-dimensional (3-D) snapshots of complexes in their native environment, ultimately the cell, whereas cryo-EM single particle analysis provides higher resolution structural insights into well-defined, purified components of the cellular machinery. Combined use of these approaches can provide a mechanistic understanding of cellular processes. Both approaches rely on statistical analysis of many images of the same type of molecule (sub-tomogram and single particle analysis, respectively) that require simultaneous alignment of the data to the same coordinate system and classification according to their underlying structural differences. Here, we use the 26S proteasome as an example to illustrate the integration of cellular cryo tomography and single particle analysis for a structural description of regulated intracellular protein degradation. Simultaneous subtomogram alignment and classification by 'autofocused classification in 3D' (AC3D) provides low-resolution structures of different 26S proteasome states in intact neurons as well as their relative abundances. Focused classification of single particle data reveals the different conformations of the 26S proteasome in vitro as well as the relative changes of the conformational equilibrium upon cofactor binding and the precise cofactor binding mode. Together, the results show how deubiquitylating enzymes modulate the conformational space of the 26S proteasome and regulate proteasomal degradation.

Identification of Reproducible 3-D Structures in Heterogeneous Cryo-EM Data Sets

Zhong Huang, **Pawel A. Penczek**

Department of Biochemistry & Molecular Biology, The University of Texas at Houston

Available methodologies for sorting out 3-D heterogeneity given 2-D cryo-EM projection data set typically adopt a version of multireference alignment, which is based on K-means clustering method. However, K-means has numerous drawbacks: it converges to local a minimum and solution depends on the initial assignments to groups and will change if the initial assignment changes. It is also unclear how to determine the appropriate number of groups, smaller groups tend to be absorbed into larger ones and the outcome validation methodologies are lacking. Here we present a sorting protocol that is based on the concept of reproducibility of the result, and thus provides a measure of validation. To overcome shortcoming of the basic K-means clustering algorithm we developed a 3D version of an equal group size K-means (EQK-means). We first apply it to the data set multiple times with random initializations and then we employ pair-wise comparisons of the resulting group assignments to identify reproducible subsets. Pair-wise solution that has the highest reproducibility rate is used as a seed for subsequent straightforward K-means clustering. We demonstrate efficacy of the method using simulated data and we also applied it to a ribosome test data set previously analyzed by others. We show that the method allows us to identify additional, well-defined functional states of the ribosome.

Characterizing discrete and continuous 3-D particle heterogeneity from cryo EM images

Peter C. Doerschuk

Biomedical and Electrical and Computer Engineering, Cornell University, Ithaca, USA

By representing the electron scattering intensity of a particle by a Fourier series, describing the Fourier coefficients as random variables, and estimating the mean vector and covariance matrix of the random variables from cryo EM images by maximum likelihood estimation, both discrete and continuous heterogeneity of the particle can be characterized. Application of these tools, in collaboration with Professor John E. Johnson (TSRI), to study the time course of maturation in the RNA T=4 icosahedral insect virus Nudaurelia Capensis Omega (NwV) and the role of the viral protease in the maturation of the dsDNA T=7l bacteriophage Hong Kong 97 (HK97) will be described.

Tomography-Guided 3D Reconstruction of Subcellular Structures (TYGRESS) improves the resolution of cellular imaging

Zhiguo Shang, Xiaofeng Fu, Kangkang Song, Nikolaus Grigorieff, and Daniela Nicastro

Associate Professor, Departments of Cell Biology and Biophysics, UT Southwestern Medical Center at Dallas, USA

Cryo-electron tomography (cryo-ET) is a powerful tool to visualize the 3D structure of macromolecules, organelles and cells. But the resolution of cryo-ET reconstructions is limited by several factors: including radiation sensitivity of biological samples and structural damage cause by accumulated electron dose, sample thickness, low contrast and signal-to-noise ratio (SNR), which makes correction of the contrast transfer function (CTF) challenging. These limitations ultimately prevent cryo-ET from achieving resolutions that are typical for cryo-electron microscopy (cryo-EM) reconstructions of single particles. Here, we developed a new hybrid-approach called "TomographY-Guided 3D REconstruction of Subcellular Structures" (TYGRESS). Essentially, this algorithm combines the advantages of both single-particle cryo-EM and cryo-ET/subtomogram averaging, i.e., (1) per region of interest, e.g. macromolecular complexes in situ, one single-particle-type image with relative good SNR and contrast is recorded and ultimately used for the "high-resolution" 3D reconstruction, while (2) a subsequent traditional cryo-ET tilt series is acquired and reconstructed for the same region to guide particle picking and image alignment, steps that usually prohibit single-particle cryo-EM reconstruction of complex cellular specimen. We tested TYGRESS on rapidly frozen, intact axonemes (>200 nm diameter) and obtained a 3D map of the averaged 96 nm axonemal repeat unit at 22 Å (0.143 criterion), which clearly exceeds the resolutions previously attained by traditional cryo-ET of axonemes (30-40 Å). Because TYGRESS reconstructions are not limited by radiation damage, it will benefit from the same factors as traditional single particle reconstructions, such as increased particle numbers and use of direct electron detectors. TYGRESS is applicable to the same samples that are suitable for cryo-ET and subtomogram averaging, while providing 3D reconstructions with superior resolution.

Electron tomography reconstruction with restoration of missing information

Fei Sun

Professor of Structural Biology and Leader & Chief Scientist of Center for Biological Imaging, Institute of Biophysics, Chinese Academy of Sciences, CHINA Institute of Biophysics, Chinese Academy of Sciences

Cryo-electron tomography (ET) plays an important role in revealing biological structures, ranging from macromolecule scale to subcellular scale. After acquiring series of pictures with different angular assignment, one performs an inverse radon transform and gets the 3D structure of biological specimen. However, since high angle tilt image cannot be acquired with respect to certain physical limits, ET reconstruction suffers from a well-known bias called 'missing wedge', which makes ET reconstruction as a kind of 'ill-posed' problem.

Many computation techniques have been proposed to find the solution for the underdetermined sampling pattern in ET. One main category of the reconstruction schemes takes no prior information into consideration, like weighted back projection (WBP), ART/SIRT, SART, INFR (Iterative Non-uniform Fourier-transformation Reconstruction) and etc. Their common goal is to ensure the data consistence within the sampled Fourier domain, while making no intentionally modification to the 'missing wedge' area.

Here we describe two new reconstruction algorithms by utilizing prior information of biological specimen, FIRT (filtered iterative reconstruction technique) and ICON (Iterative Compressed-sensing Optimized NUFFT reconstruction). Using simulated data, we proved that FIRT and ICON can generate a better reconstruction by observing the reduced ray artifacts and significant improved correlation with ground truth. For experimental data, using the 90-degree projection and the projection-omitting strategy, we proved that both algorithms could efficiently recover missing information at the non-sampled angular region. The recovered information provided significant benefits for 3D volume alignments during sub-tomogram averaging process.

CTF Correction and Alignment Algorithms for Single Particle Cryo Electron Tomography in EMAN2

Galaz-Montoya JG[1], Flanagan J[1], Schmid MF[1], Ludtke SJ[2]

[1]National Center for Macromolecular Imaging, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030, USA; [2]National Center for Macromolecular Imaging, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030,
USA. Electronic address: sludtke@bcm.edu.

Single particle cryo-electron tomography (cryoSPT) extracts features from cryo-electron tomograms, followed by 3D classification, alignment and averaging to generate improved 3D density maps of such features. Robust methods to correct for the contrast transfer function (CTF) of the electron microscope are necessary for cryoSPT to reach its resolution potential. Many factors can make CTF correction for cryoSPT challenging, such as lack of eucentricity of the specimen stage, inherent low dose per image, specimen charging, beam-induced specimen motions, and defocus gradients resulting both from specimen tilting and from unpredictable ice thickness variations. We have implemented CTF correction algorithms and tools in EMAN2 without making any assumptions common in the field; namely, that the defocus at the center of an image is the same across all images of a tiltseries, that the particles all lie at the same Z-height in the embedding ice, and/or that the specimen grid and carbon support are flat. We also introduce speed and accuracy improvements and a higher degree of automation to the subtomogram averaging algorithms available in EMAN2.

Sub-3 Angstrom Cryo-EM 3-D Reconstruction of Viruses: Affinity Grids and Image Corrections

Wen Jiang

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

Single particle cryo-EM has been used to determine many structures of viruses and protein complexes to 3-5 Å resolutions and a few cases at sub-3 Å resolutions in recent years. To allow routine achievement of sub-3 Å resolution 3-D reconstructions, we have been developing methods to improve all relevant steps for cryo-EM projects from sample preparation to computing algorithms. Here we will report two technique advances that allowed us to obtain several virus structures at ~2.5 Å resolutions. The first is computational determination and correction of elliptical magnifications that distort the images. Our method is formulated as a generalized 2-D alignment problem that is performed during image refinement instead of relying on pre-calibrations using poly-crystalline samples. This formulation discovers the correction parameters from the data and automatically adaptive to potential variations of the distortions. The second advance is optimization of antibody-based affinity grid sample preparation method to capture low-yield/low concentration particles on grid substrate and at the same time without introducing excessive background noises. We showed that affinity grid method is capable of achieving near-atomic resolution 3-D reconstruction.

High Resolution Cryo-EM at High Speed and Low Cost

Kai Zhang

MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, Cambridge, UK

The recent revolution of cryo-electron microscopy (cryo-EM) allows the determination of macro-biomolecules to near-atomic resolution. In spite of its great improvement, cryo-EM is still far from the limit that physics allows. Better classification, higher resolution and faster speed have always been important targets for further improvement. Computational resource has been one big issue in structural determination by cryo-EM, especially for dataset with huge box size or severely heterogeneous conformations. The use of GPU and deep optimization of the algorithm will significantly reduce the cost of computation. On the other hand, due to the many times faster speed, lots of challenging parameters and statistics in cryo-EM data processing could be extensively investigated and optimized. Several newly developed fast GPU programs have reduced parts of the computation cost to almost nothing. The much faster speed could help to interactively optimize parameters in real-time and in turn improve the robustness of algorithm itself. Several practical datasets will be discussed to show the philosophy: speed is far beyond saving time.

A new protocol to accurately determine microtubule lattice seam location

Zhang Rui, Nogales E.

Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA; Howard Hughes Medical Institute, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA.

Microtubules (MTs) are cylindrical polymers of αβ-tubulin that display pseudo-helical symmetry due to the presence of a lattice seam of heterologous lateral contacts. The structural similarity between α- and β-tubulin makes it difficult to computationally distinguish them in the noisy cryo-EM images. We have developed a new data processing protocol that relies on the signals at tubulin dimeric repeat to accurately determine αβ-tubulin register and seam location for each MT segment. Our strategy can deal with difficult situations, where the decoration protein is relatively small (such as EB proteins) or the decoration is sparse. Our protocol even works well for un-decorated MTs. Using this protocol, combined with movie data processing, we were able to determine the cryo-EM structures of MT at 3.5 Å resolution in different nucleotide states, and with different decoration proteins. Microtubules (MTs) are cylindrical polymers of αβ-tubulin that display pseudo-helical symmetry due to the presence of a lattice seam of heterologous lateral contacts. The structural similarity between α- and β-tubulin makes it difficult to computationally distinguish them in the noisy cryo-EM images. We have developed a new data processing protocol that relies on the signals at tubulin dimeric repeat to accurately determine αβ-tubulin register and seam location for each MT segment. Our strategy can deal with difficult situations, where the decoration protein is relatively small (such as EB proteins) or the decoration is sparse. Our protocol even works well for un-decorated MTs. Using this protocol, combined with movie data processing, we were able to determine the cryo-EM structures of MT at 3.5 Å resolution in different nucleotide states, and with different decoration proteins.

Soft alignment validation in cryoelectron microscopy

Javier Vargas

Instruct Image Processing Center, Spanish National Center for Biotechnology CNB-CSIC, Madrid, SPAIN

Electron Microscopy is reaching new capabilities thanks to the combined effect of new technologies and new image processing methods. However, the reconstruction process is still complex, requiring many steps and elaborated optimization procedures. Therefore, the possibility to reach a wrong structure exists, justifying the need of robust statistical tests. Recently, some efforts to perform soft validation approaches have been made. In this talk we present recent methodologies to perform this task and some exemplifying cases in which these methods show their usefulness in providing an objective evaluation for maps that have recently been subject to a strong controversy in the field.

Validation of Helical Structures: Lies, Damned Lies and Resolution Statistics

Edward H. Egelman

Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, USA

The main metric that has been used in cryo-EM to assess the resolution of a reconstruction is the Fourier Shell Correlation (FSC). I will discuss why the FSC has never been, and never will be, a measure of resolution, but rather is a measure of self-consistency. As such, one may impose the wrong helical symmetry in generating a reconstruction, but as long as this is done consistently the “resolution” measured by the FSC is a complete artifact. Further, one can easily introduce errors in the reconstruction engine that improve the FSC, with the result that the more artifactual the reconstruction, the better the FSC. As single-particle and helical cryo-EM move to a nearly routine level of near-atomic resolution, a reality-based standard of resolution measured by comparison with a stereochemically constrained atomic model should emerge.

Optimizing Image Contrast for Cryo-EM

Nikolaus Grigorieff

Howard Hughes Medical Institute, Professor, Janelia Research Campus, Loudoun County, USA

The potential of the single-particle technique – imaging macromolecular machines of less than a hundred kD molecular mass at near-atomic resolution – is largely impeded by the loss of signal in the imaging process. Several factors contribute to this signal loss, including image blurring due to beam-induced sample motion, radiation damage, contrast transfer of the microscope and detector inefficiencies. In my talk, I will discuss how some of these problems can be addressed with current technology.

Validation of low-resolution EM models using statistics-based tools

Niels Volkmann

Professor of Bioinformatics and Systems Biology, Sanford Burnham Prebys, La Jolla, USA

Fitting of atomic-resolution structures into EM is routinely used to understand the structure and function of macromolecular machines. Despite the fact that a plethora of fitting methods has been developed over recent years, standard protocols for quality assessment and validation of these fits have not been established. In this talk I will describe how statistical tools are used to validate rigid-body fitting results and to test for the statistical significance of non-rigid conformational changes introduced by flexible fitting.

Poster Abstracts

Posters will be displayed throughout the conference at all times.

Poster Session I	Poster Session II
1. Aylett, Christopher	9. Grant, Timothy
2. Wan, William	10. Pintilie, Grigore
3. Castaño-Díez, Daniel	11. Greber, Basil
4. Veesler, David	12. Mills, Deryck
5. Fabre, Lucien	13. Johnson, Michael
6. Stagg, Scott	14. Louder, Robert
7. Fu, Tianmin	15. Jonic, Slavica
8. Rawson, Shaun	16. Kunz, Michael

Strategies to cope with variable global and local resolution and their application to the architecture of human mTORC1

Christopher H. S. Aylett, Daniel Boehringer, Marc Leibundgut, Nikolaus Schmitz & Nenad Ban

Postdoc, Biology, Institute of Molecular Biology and Biophysics, SWITZERLAND

Target of rapamycin (TOR), a conserved protein kinase and the central controller of cell growth, functions in two structurally distinct complexes, TORC1 and TORC2, which perform different, albeit related, functions within the signaling pathways of the eukaryotic cell. Dysregulation of mammalian TOR (mTOR) signaling is implicated in pathologies that include diabetes, cancer, and neurodegeneration, while the immunosuppressant macrolide rapamycin remains a front-line treatment of choice for tens of thousands of organ transplant and coronary stent recipients worldwide.

We resolved the architecture of human mTORC1 (mTOR with subunits Raptor and mLST8) bound to FK506 binding protein (FKBP)–rapamycin, by combining cryo-electron microscopy at 5.9 Å resolution with crystallographic studies of Chaetomium thermophilum Raptor at 4.3 Å resolution. Our structure explains how FKBP-rapamycin and architectural elements of mTORC1 limit access to the recessed active site. Consistent with its established role in substrate recognition and delivery, the conserved amino-terminal domain of Raptor was found to be juxtaposed to the kinase active site, and we propose a mechanism for its action in recruiting mTORC1 substrates.

In order to facilitate interpretation of electron cryo-microscopic data at the level of a molecular model using crystallographic software, we have developed software to perform figure of merit blurring over an MTZ (to prevent global over-fitting during atomic coordinate refinement) and real-space filtering to local resolution (to avoid local over-fitting during building and interpretation) of electron cryomicroscopy density maps. I will describe the application of these methods and demonstrate their validation using test datasets.

Developing subtomogram averaging methods for *in situ* structure determination of Marburg and Ebola viruses

William Wan[1], Mairi Clarke[1], Wim J. H. Hagen[1], Larissa Kolesnikova[2], Stephan Becker[2], John A. G. Briggs[1]

[1]Structural and Computational Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany; [2]Institut für Virologie, Philipps-Universität Marburg, Marburg, Germany

Filoviruses, including Marburg and Ebola viruses, are highly pathogenic, membrane-enveloped, filamentous viruses. Filovirus structure is organized in three layers: the inner nucleocapsid layer; the matrix layer under the membrane; and the glycoprotein layer above the membrane. Determining these structures within virions, i.e. in situ, will provide the most useful information, but is subject to a number of challenges. These include substantial amounts of overlapping densities, variable helical symmetries of the nucleocapsid, unknown organization of the matrix layer, and relatively small size of the glycoprotein. So far, these structures have not been amenable to high-resolution structure determination with standard cryo-EM methods such as helical reconstruction.

Through the development of cryo-electron tomography (cryo-ET) and subtomogram averaging, we have been able to determine the molecular structures of the filovirus proteins in virions and virus-like particles. These developments include adapting our subtomogram averaging workflow for direct detector data, using a new dual-walkup tilt-scheme for optimal tilt-series collection, improving the reconstructed tomograms using exposure filtering, and optimization of our subtomogram averaging scripts. These developments have allowed us to efficiently collect and process hundreds of high-quality tomograms. We are working towards higher resolution structures through improved processing methodologies and new technologies including phase-plates.

Geometrical tools and data management for particle picking in subtomogram averaging

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Dynamo Catalogue is a data management system for the organization of subtomogram averaging projects. This system manages tomographic data from multiple tomograms and allows visual feedback during all processing steps, including particle picking, extraction, alignment and classification. Command line commands, database queries and a set of GUIs give the user versatile control over the process. Here, we introduce a set of geometric tools for particle picking from simple (filaments, spheres, tubes, vesicles) and complex geometries (arbitrary 2D surfaces, rare instances on proteins with geometric restrictions, and 2D and 3D crystals). Dynamo Catalogue is part of the open source package Dynamo and includes tools to ensure format compatibility with the subtomogram averaging functionalities of other packages, such as Jsubtomo, PyTom, PEET, EMAN2, XMIPP and Relion.

Cryo-electron microscopy structure of a coronavirus spike glycoprotein trimer

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The tremendous pandemic potential of coronaviruses was demonstrated twice in the last decades by two global outbreaks of deadly pneumonia. Entry of coronaviruses into cells is mediated by the transmembrane spike glycoprotein S, which forms a trimer carrying receptor-binding and membrane fusion functions. S also contains the principal antigenic determinants and is the target of neutralizing antibodies. Here we present the structure of a murine coronavirus S trimer ectodomain determined at 4.0 Å resolution by single particle cryo-electron microscopy. It reveals the metastable pre-fusion architecture of S and highlights key interactions stabilizing it. The structure shares a common core with paramyxovirus F proteins, implicating mechanistic similarities and an evolutionary connection between these viral fusion proteins. The accessibility of the highly conserved fusion peptide at the periphery of the trimer indicates potential vaccinology strategies to elicit broadly neutralizing antibodies against coronaviruses. Finally, comparison with crystal structures of human coronavirus S domains allows rationalization of the molecular basis for species specificity based on the use of spatially contiguous but distinct domains.

Study of The Triclosan Efflux Transporter TriABC by Single Particle Electron Microscopy

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Antimicrobial resistance is one of the most serious public health threats to the treatment of infectious diseases worldwide. While antibiotics usually target ribosomes, other agents like SDS and Triclosan target membranes. To survive Gram-negative bacteria have developed multiple arrays of multidrug transporters to pump toxic compounds out of the cell. TriABC efflux pump is one of them and is capable to expel Triclosan. This protein complex belongs to the multidrug resistance or polyspecific transporters (MDRs) family and requires binding to an outer membrane factor (OMF) to function. TriC is the efflux pump, TolC/OpmH is the OMF. TriA and TriB are two periplasmic membrane fusion proteins (MFPs) that connect TriC to the OMF together. Based on the homologue AcrAB-TolC, transporters and MFPs exist in the inner membrane as dormant complexes. The activation of complexes is triggered by MFP binding to the outer membrane channel, which leads to a conformational change in the membrane proximal domain of MFP needed for stimulation of transporters. The activated MFP-transporter complex engages the outer membrane channel to expel substrates across the outer membrane. To date, no structure of the TriABC complex exists. To better understand the organization of the pump, we first used negative-stain electron microscopy. This technique allowed us to solve structures at low resolution and in combination with nanogold labeling, we were able to identify TriA, TriB and TriC. Now, we are using Cryo-EM to solve TriABC and TriC structures at high resolution. To date, our TriABC EM-map shows 8Å resolution. Comparison between the two EM-maps will provide critical information on the transporter activation and the efflux mechanism.

Tools for high-throughput high-resolution 3DEM

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In recent years cryo-EM has reached near-atomic resolution for many well-behaved samples. At the same time that structures are approaching atomic resolution using single particle reconstruction, tomographic reconstructions are also seeing large improvements in resolution with several structures reconstructed to better than 20Å resolution. Because both tomographic and single particle reconstruction are averaging techniques, they both benefit from high-throughput data collection. We have developed tools in the Leginon/Appion environment for facilitating throughput and for assessing data quality for both single particles and tomographic data. Here we will discuss the use of plots of resolution vs. number of particles for optimizing the quality of cryoEM data and 3D reconstructions for both tomographic and single particle data.

Three-dimensional snapshots of key intermediates in translation by time-resolved cryo-electron microscopy

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Motivation: Short-lived intermediates of a biological reaction are usually important for understanding mechanism of these reactions. Precise structural detail would benefit drug design and disease combat.

Problem statement: In the ribosome recycling process, dissociation of post-termination complex, promoted by ribosomal recycling factor (RRF) and elongation factor G (EF-G) is short lived (in the order of 60 ms) and hard to capture without nonhydrolyzble GTP analogs. A similar situation also exists in the initiation process. The association of 50S with 30S initiation complex is promoted by GTP hydrolysis on initiation factor 2 (IF2). After the two subunits have joined, IF2 will leave the 70S ribosome. What exactly happens between initial reactants and final products has remained mysterious.

Approach: To visualize key reaction intermediates and determine the order of events, we employed time-resolved cryo-electron microscopy using a mixing-spraying method, which allows us to investigate processes with reaction times in the tens to hundreds of milliseconds. Based on prior knowledge of the kinetics of the overall process, we carefully choose several time points to hunt for key intermediates.

Results: We captured a key intermediate PTC·EF-G·RRF in the ribosome recycling process before ribosome split. The splitting reaction follows quite well a calculation based on a parallel kinetics study. We also captured a key intermediate 70SIC·IF2 in the ribosome initiation process after 30SIC and 50S have associated but just before IF2 leaves 70S ribosome. These two intermediates virtually disappear within one second.

Conclusions: The structures of intermediate allow us to better understand the fundamental process of translation. The present results demonstrate the potential of time-resolved cryo-EM in studying short-lived intermediates of many other biological reactions.

Cryo-EM structures as a platform for structure based drug design

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Structure based drug design has traditionally been underpinned by X-ray crystallography and NMR. This poster discusses the power of single particle electron microscopy in informing drug design pipelines. From low (~25Å) to high (<4Å) resolution EM structures, it is possible to further our understanding of inhibitor binding. At a modest resolution Streptavidin tags we have used to label bound inhibitors permitting their localization within large protein complexes in a relatively rapid (weeks) timeframe using negative stain microscopy. Modest resolution EM structures ~<8Å are becoming more routine through advances in technology and data processing algorithms. This permits the identification of secondary structural elements and provides a constraint for producing structures based on known crystal structures or homology models. Moreover, with regards to large protein complexes, these models provide information on protein-protein interfaces, which can be targeted to modulate function. Finally, a number of <4Å EM structures have recently been published which permits not just the identification of secondary structural elements, but the position of side chains. Having solved a 3.25Å structure of an industrial herbicide target, we are using this to provide a foundation for in silico design of small molecule inhibitors.

Automatic estimation and correction of anisotropic magnification distortion in electron microscopes

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We demonstrate a significant anisotropic magnification distortion, found on an FEI Titan Krios microscope and affecting magnifications commonly used for data acquisition on a Gatan K2 Summit detector. We describe a program to automatically estimate anisotropic magnification distortion from a set of images of a standard gold shadowed diffraction grating. The distortion present on our Titan Krios microscope limits the resolution of a set of rotavirus VP6 images to ~7 Å, which increases to ~3 Å following estimation and correction of the distortion. We also use a 70S ribosome sample to demonstrate that in addition to affecting resolution, magnification distortion can also interfere with the classification of heterogeneous data.

Resolution and Probabilistic Structural Models of Subcomponents Derived from CryoEM Maps of Mature P22 Bacteriophage

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CryoEM continues to produce density maps of larger and more complex assemblies with multiple protein components of mixed symmetries. Resolution is not always uniform throughout a cryoEM map, and it can be useful to estimate resolution in specific molecular components of a large assembly. Here, we present procedures to 1) estimate resolution in subcomponents by gold-standard Fourier Shell Correlation (FSC), 2) validate modeling procedures, particularly at medium resolutions, which can include loop modeling and flexible fitting, and 3) build probabilistic models that combine high-accuracy priors (such as crystallographic structures) with medium-resolution cryoEM densities. As an example, we apply these methods to new cryoEM maps of the mature bacteriophage P22, reconstructed without imposing icosahedral symmetry. Resolution estimates based on gold-standard FSC show the highest resolution in the coat region (7.6Å), while other components are at slightly lower resolutions (portal: 9.2Å, hub: 8.5Å, tailspike: 10.9Å, and needle: 10.5Å). These differences are indicative of inherent structural heterogeneity and/or reconstruction accuracy in different subcomponents of the map. Probabilistic models for these subcomponents provide new insights and structural information while taking into account uncertainty given the limitations of the observed density.

Cryo-EM Analysis of the Conformational Flexibility of Transcription Initiation Complexes

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Transcription factor IIH (TFIIH) is a large transcription initiation factor complex composed of 10 subunits. TFIIH is responsible for melting of the promoter DNA prior to initiation of mRNA synthesis by RNA polymerase II (Pol II) and is additionally involved in DNA repair pathways. Human TFIIH has been visualized previously in the context of the Pol II pre-initiation complex (PIC), and its two ATPase subunits XPD and XPB could be localized in the density. However, the modest resolution and high flexibility of TFIIH precluded a more detailed molecular interpretation. This type of structural heterogeneity occurs in many molecular machines, which often need to access different conformational states to perform their function, and hampers the determination of structures at subnanometer or near-atomic resolution. Various sorting strategies implemented in different cryo-EM data processing software packages are available to address this issue. We aim to analyze the structural heterogeneity present in transcription PICs and TFIIH to determine the structure of TFIIH.

Structure of alcohol oxidase of *Pichia pastoris* by cryo-electron microscopy

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The first step in methanol metabolism in methylotrophic yeasts, the oxidation of methanol with O_2 to formaldehyde and hydrogen peroxide, is catalysed by alcohol oxidase (AOX), a 600-kDa homo-octamer containing eight FAD cofactors. We collected cryo-EM data on a JEOL 3200 FSC, using a Gatan K2 direct electron detector in a defocus range of 0.6-2.5 µm in counting mode with 30 0.2-s frames using an electron dose of 1.7 $e^-/Å^2$/frame. We calculated a 3.4 Å map using RELION from 49,000 particles, applying D4 symmetry. The structure was fitted in Coot and refined using Phenix. All residues of the 662-amino acid polypeptide as well as the FAD are well resolved. AOX shows structural homology to other members of the GMC family of oxidoreductases, which share a conserved FAD binding domain, but have different substrate specificities. AOX contains conserved residues with large aromatic side chains near the active site, explaining its specificity for small alcohols. Compared to the other GMC enzymes, AOX has a large number of amino acid inserts, the longest 75 residues. These inserts were found at the periphery of the monomer and make extensive intersubunit interactions that are responsible for the very stable octamer.

Focused Classification of a Late-Stage Eukaryotic Small Subunit Ribosome Assembly Intermediate

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Eukaryotic ribosomes are intricate arrangements of 78 proteins and 4 RNA molecules, and their assembly is mediated by over 200 assembly factors (AFs). Here we present cryo-EM data of a late-stage small subunit assembly intermediate. These data exhibited heterogeneity from multiple sources: occupational and conformational variations of the AFs, conformational variation in the RNA and protein core, and angular anisotropy due the particles assuming a preferred. The latter issue was resolved by collecting mages from grids tilted at a range of angles, and the former two issues through focused classification of seven different masked regions.

Structural characterization of the human transcription preinitiation complex by cryoEM

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In eukaryotes, the general transcription factor IID (TFIID) plays a central role in the initiation of RNA polymerase II (Pol II)-dependent transcription by nucleating pre-initiation complex (PIC) assembly at the core promoter. Despite its importance in coordinating transcription initiation, a detailed mechanistic understanding of TFIID's activities remains elusive. The high degree of conformational plasticity within the TFIID architecture has largely limited structural analysis to low-resolution electron microscopy (EM) studies. However, by improving the biochemical preparation of TFIID complexes and by exploiting the latest technological advances within the field of 3D cryo-EM, we have been able to visualize promoter binding by human TFIID in close-to-atomic-level detail. Our high-resolution structures have revealed which components of TFIID are involved in direct recognition of conserved core promoter sequences, and show how TFIID fulfills its primary function of properly positioning TBP on the core promoter, which ultimately determines the placement of Pol II relative to the transcription start site. Ultimately, our structures provide a framework for a molecular understanding of the complex mechanism underlying TFIID function, shedding new light into the overlapping roles of TFIID as both a coactivator and a general platform for PIC assembly in the coordination of transcription initiation.

StructMap: Elastic distance analysis of electron microscopy maps for studying conformational changes

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Single-particle electron microscopy (EM) has been shown to be very powerful for studying structures and associated conformational changes of macromolecular complexes. In the context of analyzing conformational changes of complexes, distinct EM density maps obtained by image analysis and three-dimensional (3D) reconstruction are usually analyzed in 3D for the interpretation of structural differences. However, graph visualization of these differences based on a quantitative analysis of elastic transformations (deformations) among density maps has not been done yet due to the lack of appropriate methods. Here, we present an approach that allows such visualization. This approach is based on statistical analysis of distances among elastically aligned pairs of EM maps (a map is deformed to fit the other map), and results in visualizing EM maps as points in a lower-dimensional distance space [1]. The distances among points in the new space can be analyzed in terms of clusters or trajectories of points related to potential conformational changes. The results of the method are shown with synthetic and experimental EM maps at different resolutions.

[1] Sorzano, C. O. S., A. L. Alvarez-Cabrera, M. Kazemi, J. M. Carazo, and S. Jonic (2016) StructMap: Elastic distance analysis of electron microscopy maps for studying conformational changes. *Biophysical Journal* (in print)

Contrast in cryo-electron tomography reconstruction methods

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Cryo-electron images usually suffer from low contrast due to the phase contrast recording modality. Two main reconstruction methods are well established in cryo-electron tomography for three dimensional reconstruction: Weighted back projection (WBP) and algebraic reconstruction techniques (ART). So far WBP has proven to be the method of choice when it comes to achieving high resolution in combination with sub-tomogram averaging. ART based methods on the other hand are used when improved contrast is necessary but have not been as successful in recovering high frequency information in the same amount as WBP. Interestingly, little is known about the reason for the contrast improvement and even more, what the contrast of an ideal reconstruction should look like. Here we will present a comparison of the contrast properties of WBP and ART based reconstruction methods by means of Dirac-impulse like images and their system response after reconstruction and compare re-projections of real data reconstructions with their original micrograph counterpart. Doing so, we will explain why ART based methods improve the visual contrast impression and further we will unify WBP and ART by deriving a weighting filter for WBP from ART reconstructions that has the same contrast enhancement properties. Finally we will show that a specific ART implementation, Simultaneous Algebraic Reconstruction Technique (SART), is capable of reconstructing an ideal contrast for sub-tomogram averaging and thus improve averaging quality and resolution.

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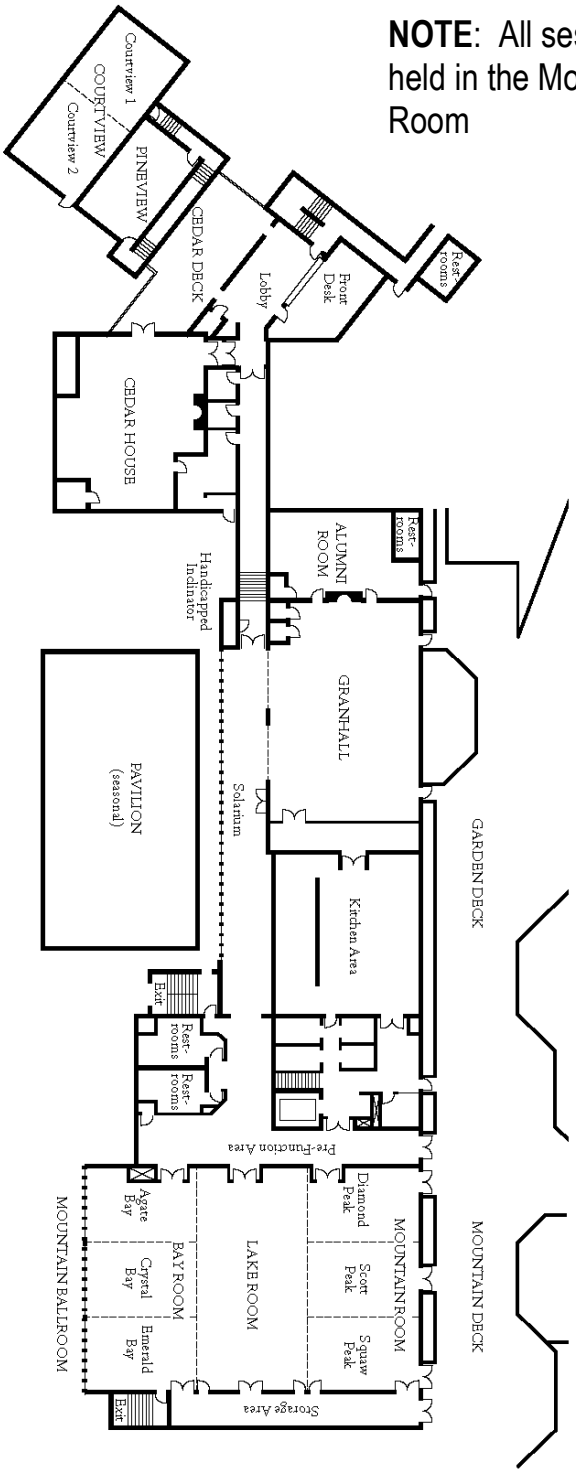
Meeting Room Diagram

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



MEETING ROOM DIAGRAM

Meeting Rooms	Size	Room Dimensions: Sq. Footage	Ceiling Height	Seating Capacity: Theatre	Classroom	Banquet	Reception
Granhall	57 x 40 ft.	2,982	9-14 ft.	200	150	250	250
Solarium	54 x 13 ft.	702	sloping	–	–	45	70
Alumni Room	20 x 30 ft.	600	9-14 ft.	50	32	40	–
Cedar House	45 x 25 ft.	1,125	A-frame	100	75	100	100
Courtview (opens)	48 x 22 ft.	1,056	10-17 ft.	80	60	–	–
Courtview 1	24 x 22 ft.	528	10-17 ft.	40	32	–	–
Courtview 2	24 x 22 ft.	528	10-17 ft.	40	32	–	–
Pineview	13 x 30 ft.	540	10-17 ft.	40	32	–	–
Decks:							
Garden-side Deck	freeform	7,000	–	–	–	–	700
Cedar Deck	30 x 60 ft.	1,800	–	–	–	100	120

Meeting Rooms	Size	Room Dimensions: Sq. Footage	Ceiling Height	Seating Capacity: Theatre	Classroom	Banquet	Reception
Mountain Ballroom (opens)	60 x 30 ft.	5,400	12-16 ft.	900	360	360	360
Mountain Room	60 x 30 ft.	1,800	12-16 ft.	180	120	120	180
Diamond Peak	20 x 30 ft.	600	14 ft.	50	32	32	50
Scott Peak	20 x 30 ft.	600	14 ft.	50	32	32	50
Squaw Peak	20 x 30 ft.	600	14 ft.	50	32	32	50
Lake Room	60 x 30 ft.	1,800	12-14 ft.	180	120	120	180
Bay Room	60 x 30 ft.	1,800	12-14 ft.	180	120	120	180
Agate Bay	20 x 30 ft.	600	14 ft.	50	32	32	50
Crystal Bay	20 x 30 ft.	600	14 ft.	50	32	32	50
Emerald Bay	20 x 30 ft.	600	14 ft.	50	32	32	50
Pavilion (seasonal)	40 x 80 ft.	3,200	16-20 ft.	180	120	120	180

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