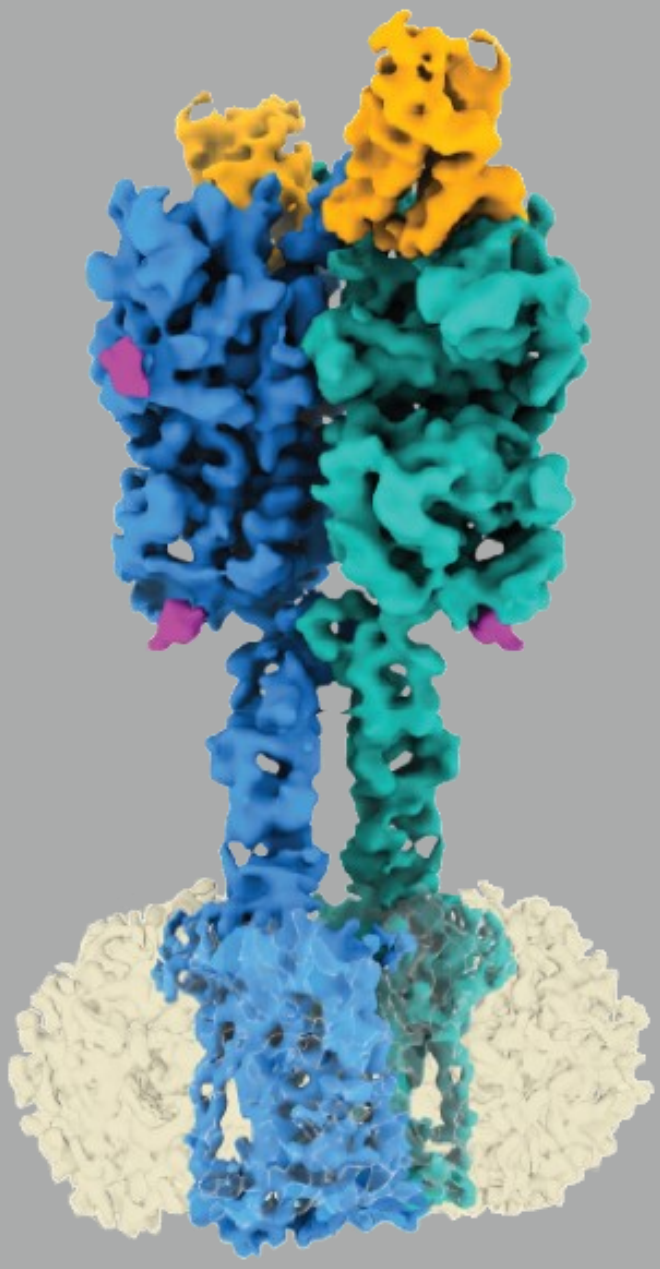
Stanford Bio-X CryoEM Workshop



September 24-25, 2019

James H. Clark Center Auditorium
Stanford University

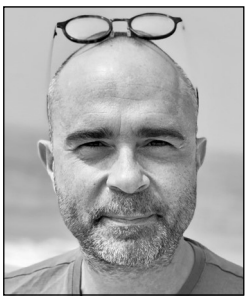
@STANFORDBIOX #BIOXCRYOEM

Tuesday, September 24th

8:00 AM

Registration

Clark Center courtyard



Georgios Skiniotis

8:30 AM

Introduction

Georgios Skiniotis

Professor of Molecular & Cellular Physiology, of Structural Biology, and of Photon Science, Stanford University

This workshop will provide a thorough introduction to cryoEM and related methodologies with an emphasis on single-particle analysis and reconstruction.

9:00 AM

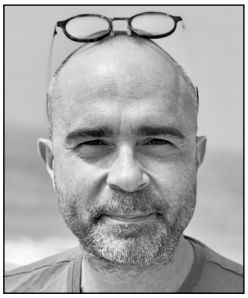
EM Image Formation and Data Collection

Yifan Cheng, Professor of Biochemistry & Biophysics, University of California, San Francisco

Yifan Cheng

Abstract:

The first part of my lecture concerns image formation in electron microscope. This includes a brief discussion of electron optic system of a standard electron microscope, image formation theory of weak-phase object, and contrast transfer function. In the second part, I will discuss low-dose imaging in single particle cryo-EM. Last, I will discuss approaches to correct beam-induced image motions.



Georgios Skiniotis

10:00 AM

Classification and 3D Reconstruction Workflow

Georgios Skiniotis, Professor of Molecular & Cellular Physiology, of Structural Biology and of Photon Science, Stanford University

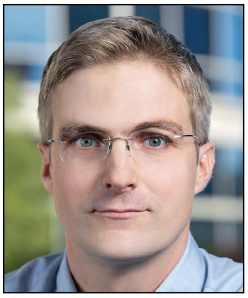
Abstract:

I will outline the principles and general workflow for image processing steps towards cryoEM 3D reconstructions with the main focus on single-particle analysis. Emphasis will be placed on image classification, i.e. the categorization of projections aiming to obtain subsets that can facilitate high resolution information but also different conformations adopted by a macromolecule.

11:00 AM

Image Processing with cisTEM

Alexis Rohou, Scientist, Department of Structural Biology, Genentech



Alexis Rohou

Abstract:

cisTEM is a free, open-source cryoEM image processing package which supports a complete workflow from raw movies to a refined three-dimensional map. It is notable for its ease of use and for its speed. It features a single-window graphical user interface, live graphical feedback during job execution, support for massively parallel job distribution across CPU clusters or workstations, and high-resolution refinement algorithms. I will give an overview of the package and introduce features to be released soon, as well as some of the fundamental algorithmic differences between cisTEM and other single-particle image processing packages.

12:00 PM

Lunch

Nexus Café Patio

Please wear your name tag to be checked in!

Daniel Southworth

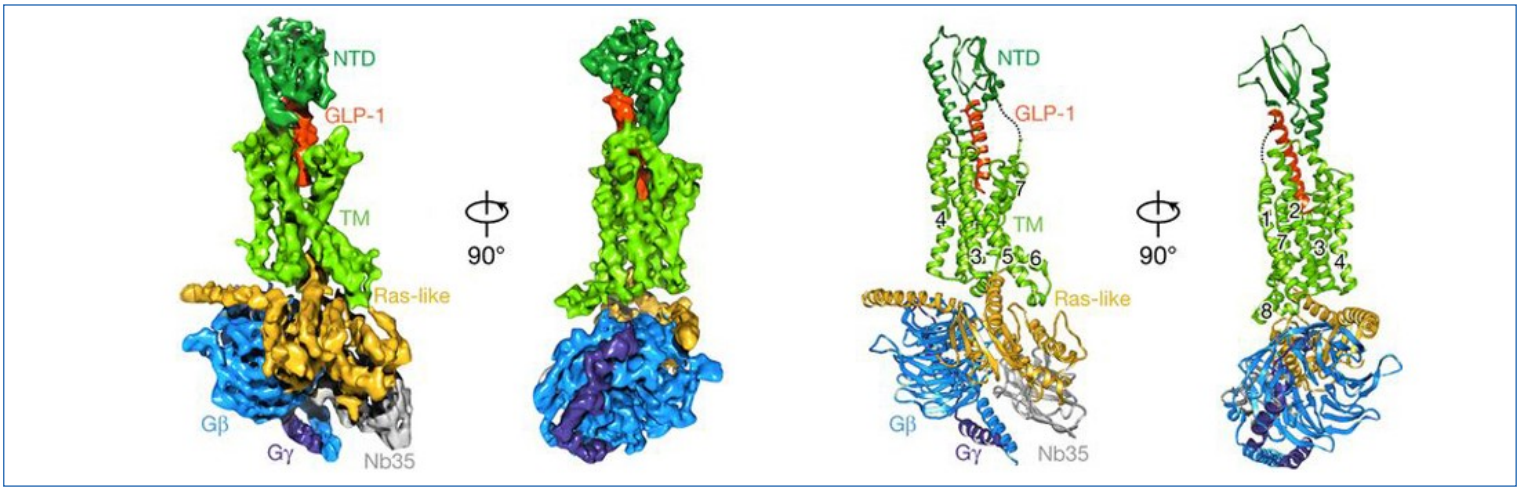
1:30 PM

Specimen Preparation for cryoEM

Daniel Southworth, Associate Professor of Biochemistry & Biophysics, University of California, San Francisco

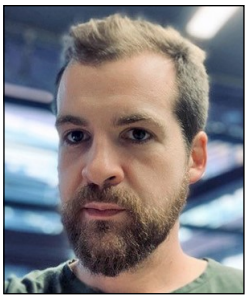
Abstract:

Advancements in cryo-EM continue to fuel an explosion of high-resolution macromolecular structures that have previously been unattainable. While these advancements have made atomic resolution feasible, and even routine for many cases, the challenges of preparing a biological sample in a way that is suitable for cryo-EM imaging remain significant. Major challenges include sample purity and instability, conformational and compositional heterogeneity, preferred orientation, and denaturation due to the vitrification process. The goal of this seminar is to broadly cover the major challenges in sample preparation and introduce both well-established and novel methods to improve samples for high resolution structure determination by cryo-EM.



2:30 PM

Image Processing with RELION



Cornelius Gati

Cornelius Gati, Panofsky Fellow at SLAC National Accelerator Laboratory and Stanford University

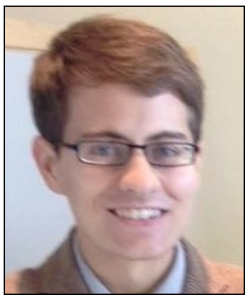
Abstract:

In recent years, the number of high resolution reconstructions from single particle cryoEM has sky-rocketed. Not only is it possible to obtain atomic resolution (!) reconstructions from isolated biomolecules — we also observe a trend towards ever smaller proteins that can be imaged, making the technique more amenable to a broad range of biomolecules. RELION is currently the most broadly used software package for high resolution reconstructions in single particle cryoEM. This workshop will cover the question 'I have my raw images, what now?', covering all basics of RELION, starting with raw images, motion correction, CTF estimation, classification to final reconstructions. It will also cover common problems and pitfalls during the process. After this course, participants should easily be able to go through a dataset by themselves and join the steadily growing community of electron microscopists.

3:30 PM

Break

Clark Center courtyard



Michael Robertson

3:45 PM

Modeling into cryoEM Maps

Michael Robertson, Postdoctoral Research Fellow, Molecular & Cellular Physiology, Stanford University

Abstract:

In recent years cryoEM has allowed for the determination of the structures of a wide variety of popular drug targets that have been impossible for other methods. However, modeling into cryoEM maps presents several unique challenges inherent to the method. We will discuss the current state of the art methods for model building and refinement into cryoEM maps. GemSpot, a pipeline for the robust identification of chemically reasonable poses of ligands in cryoEM maps, will also be presented. Finally, modeling and refinement techniques will be demonstrated with real world examples of several important drug targets.

4:45 PM

Reception and Poster Session
Nexus Café Patio

Posters Presented:

Engineering Strategies for Testing the Integration of Functional Systems in Living Cells
Anton Jackson-Smith[1], Will Roberts[1], Drew Endy[1]
Department of Bioengineering[1], Stanford University

Transcription Polymerase-Catalyzed Emergence of Novel RNA Replicons
Nimit Jain[1,2,3], Lucas R. Blauch[4], Michal R. Szymanski[5,6,7], Rhiju Das[8], Sindy K. Y. Tang[4], Y. Whitney Yin[5,6], Andrew Z. Fire[1,2]
Departments of Pathology[1], Genetics[2], Bioengineering[3], Mechanical Engineering[4], and Biochemistry[8], Stanford University; Department of Pharmacology & Toxicology[5] and Sealy Center for Structural Biology[6], University of Texas Medical Branch; Intercollegiate Faculty of Biotechnology of the University of Gdansk and Medical University of Gdansk[7]

Lipid-Dependent Gating of G-Protein Coupled Inwardly Rectifying Potassium (GIRK) Channels
Yamuna Kalyani Mathiharan[1,2], Ian Glaaser[3], Jacob Mahoney[2], Paul Slesinger[3], Georgios Skiniotis[1,2]
Departments of Molecular & Cellular Physiology[1] and Structural Biology[2], Stanford University; Department of Neuroscience[3], Icahn School of Medicine, Mount Sinai

Exploring Cryo-EM Latent Space with Variational Autoencoders
Nina Miolane[1], Frédéric Poitevin[2], Susan Holmes[1]
Departments of Statistics[1] and Structural Biology[2], Stanford University

Organisms and Substrates Enabling Community-Based Measurements of Wood-Degrading Fungi
Rolando Perez[1], Drew Endy[1]
Department of Bioengineering[1], Stanford University

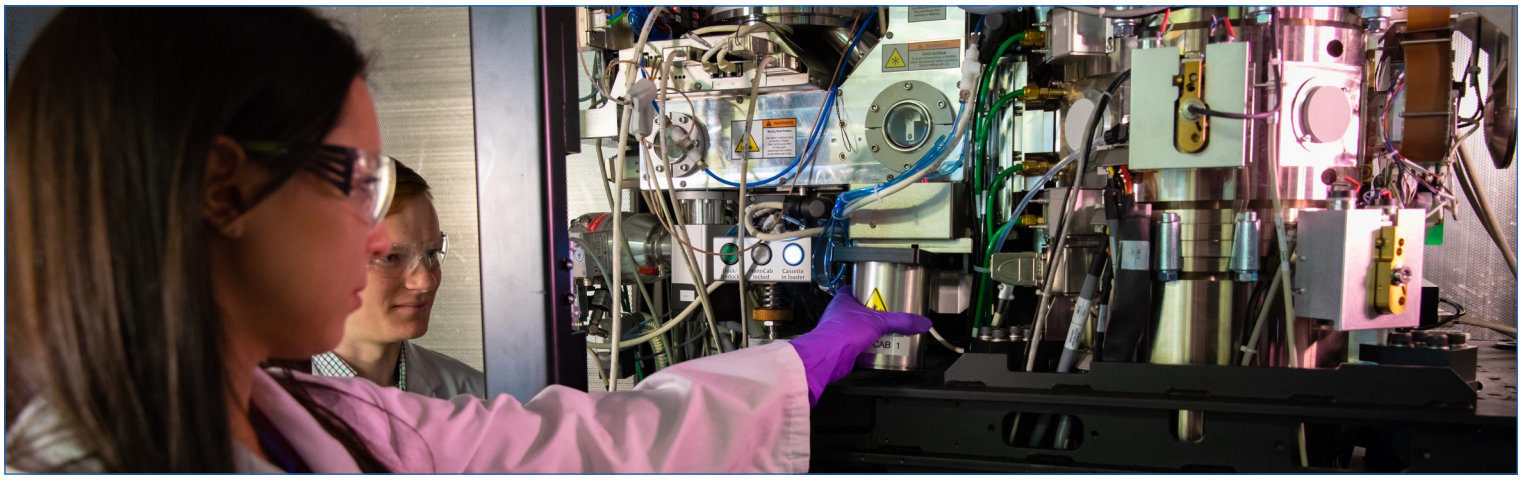
Convenience Tools to Explore Variability in CryoEM Data
Frédéric Poitevin[1], Yee-Ting Li[2], Nina Miolane[3], Cornelius Gati[4], Michael Levitt[1]
Departments of Structural Biology[1] and Statistics[3], Stanford University; Stanford-SLAC CryoEM Center[2] and SSRL-cryoEM[4], SLAC National Laboratory

Bridging the Resolution Gap from Atoms to Human Diseases: A Medical Imaging Perspective
Mirabela Rusu[1]
Department of Radiology[1], Stanford University

Elucidating the Structure and Function of *Toxoplasma gondii* Invasion Machinery
Li-av Segev-Zarko[1], Stella Y. Sun[1,2], Peter D. Dahlberg[3], Michael F. Schmid[1,2], Jesus Galaz-Montoya[1,2], W.E. Moerner[3], Wah Chiu[1,2], John C. Boothroyd[1]
Departments of Microbiology & Immunology[1], Bioengineering[2], and Chemistry[3], Stanford University

Unraveling Molecular Interactions of the Influenza A Virus Matrix Layer
Lisa Selzer[1], Zhaoming Su[2,3], Jasmine Moshiri[3], Wah Chiu[2,3], Karla Kirkegaard[1,3]
Departments of Genetics[1], Bioengineering[2], and Microbiology & Immunology[3], Stanford University

Widespread Differential Expression of Coding Region and 3'UTR Sequences-Functions in Embryonic Stem Cells
Ze Yang[1], Shaoyi Ji[1], Leonardi Gozali[1], Arif Kocabas[2], Mary Hynes[1]
Department of Biology[1], Stanford University; Rockefeller University[2]



Wednesday, September 25th

8:30 AM

Registration

Clark Center courtyard

9:00 AM

Wah Chiu

Overview of cryoEM Methodologies and Capabilities at SLAC

Wah Chiu, Professor of Photon Science, Bioengineering, and Microbiology & Immunology, Stanford University and SSRL, SLAC National Linear Accelerator Laboratory

Abstract:

We have established three NIH-supported programs for cryoEM research and user data collection facilities for the local, national and international scientific community. Using one 300 keV electron microscope, we are able to determine within a year over 20 near atomic resolution structures spanning from ion channels, chaperonins, toxins, viruses, RNA-protein complexes, RNAs and organic metallic compounds in different biochemical states. We also use our electron microscopes to study cells and small molecule crystals by electron cryo-tomography. Examples will be presented to illustrate these cryoEM applications.

Alpay Seven

10:00 AM

Computational Set-Up for cryoEM

Alpay Seven, Postdoctoral Research Fellow, Molecular & Cellular Physiology, Stanford University

Abstract:

Cryo electron microscopy is a powerful technique to study mechanism of macromolecular complexes. Recent detector, microscope and single particle processing software developments have made atomic resolution protein structure determination almost routine. Despite the popularity of this technique, the computational setup of the cryo-EM processing software on workstations and/or computer clusters can be challenging. In this workshop, I will demonstrate simple computational setups of some popular cryo-EM processing software. I will also introduce current solutions to tackle computational needs of fast evolving single-particle software platforms and how to efficiently run computationally expensive processing jobs.

10:45 AM

Break

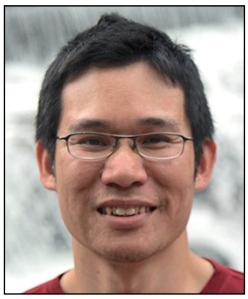
Clark Center Courtyard

11:00 AM

CRYOEM DATA MANAGEMENT

Yee-Ting Li, Science Solutions Architect, SLAC National Accelerator Laboratory

The advancement of new imaging methods and sensor technologies has lead to unprecedented increases in data rates and volumes. I shall present details of how the Stanford/SLAC CryoEM Centre organises and stores data to help create atomic resolution cryoEM maps.



YEE-TING LI

ELIZABETH MONTABANA

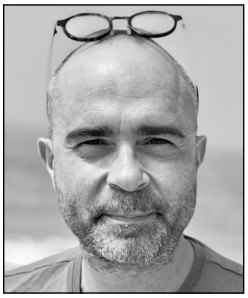
11:30 AM

ACCESSING STANFORD CRYOEM FACILITIES

Elizabeth Montabana, Cryo-Electron Microscopy Scientist, Molecular & Cellular Physiology, Stanford University

Abstract:

You are interested in pursuing a cryo-EM project at Stanford – what next? This session will cover the operations of Stanford's TEM screening facilities, plunge-freezing equipment, and microscope training. I will discuss scheduling, expectations, and timelines for a standard cryo-EM project. I will talk about applying for Krios time at Stanford-SLAC facility and how to connect with help before and after your data collection.



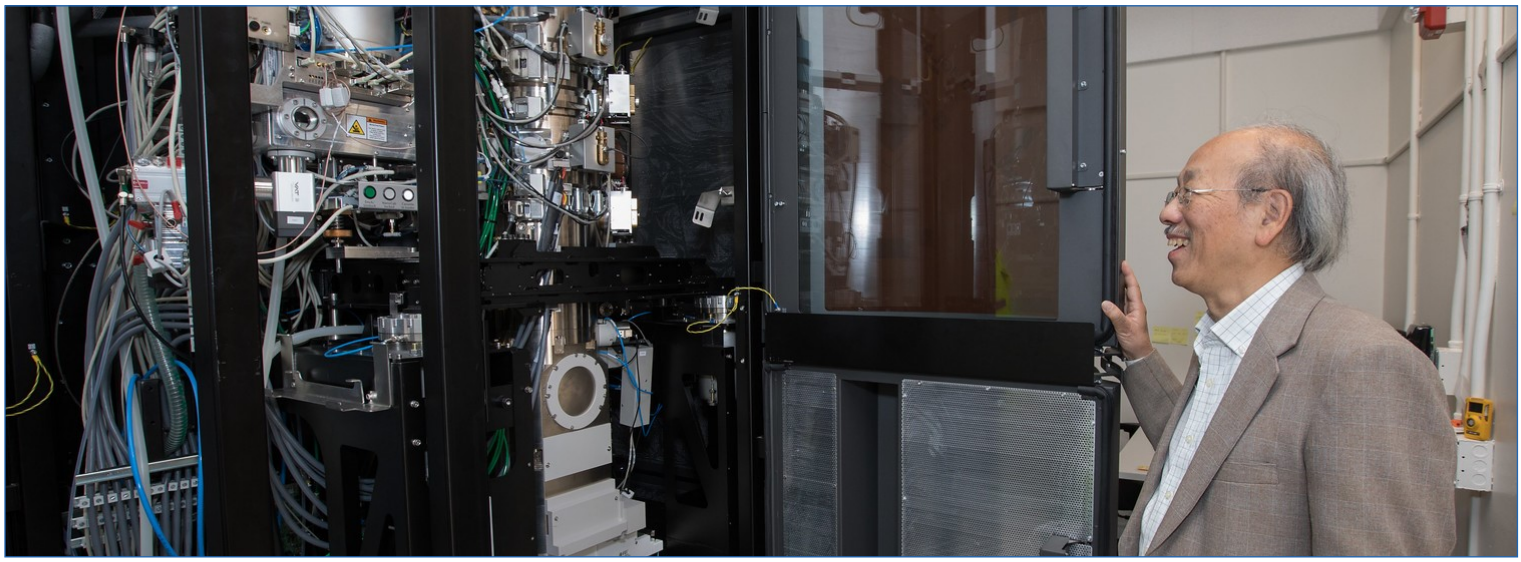
GEORGIOS SKINIOTIS

12:30 PM

CLOSING REMARKS

Georgios Skiniotis

Professor of Molecular and Cellular Physiology, of Structural Biology and of Photon Science, Stanford University



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A DIGITAL VERSION OF THIS BROCHURE

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