Cryo-EM Course at the Laboratory for BioMolecular Structure (LBMS)

Cryo-Electron Tomography: Day 1 - Tuesday 14 June 2022 Speaker Liguo Wang (BNL) Introduction to LBMS David DeRosier Uthe evolution, deficiencies, and promise of cryo-electron (Brandeis)rtment of Microscopy Chen Xu (Umess) Introduction to electron microscopes and cameras MICrobial Sciences Institute Tamir Gonen (UCLA) Alcroeb? theory application and available software Liguo Wang (BNL) Introduction to negative staining and cryo-electron micro Coffee break @junliulab



uchin Lu (DNU)





Occupient (BNL) Coffee break Guobin Hu (BNL) EPU single particle data collection tutorial and demonstration

Day 3 - Thursday 16 June 2022

Time (EDT) 10:00-11:00 11:00-12:00

Speaker Jun Liu (Yale) Digvijay Singh (UCSD)

12:00-13:00

13:00-14:50

14:50-15:00

15:00-17:00

Jianfeng Lin (Yale)

Jun Liu (Yale)

Topic

Lunch break

Coffee break

demonstration

Day 4 - Friday 17 June 2022				
Time (EDT)	Speaker	Торіс		
10:00-11:00	Raphael Park (Yale)	Tomograp		
11:00-13:00	Muyan Chen (BCM)	Subtomog		
<i>©junliulab</i>	Qun Liu (BNL)	Discussion		
	Yong Xiong (Yale)			

- Introduction to Cryo-electron tomography
- cryo-FIB to prepare cryo-FIB samples
- Cryo-ET sample preparation tutorial and demonstration
- Cryo-ET data collection and reconstruction tutorial and

hic data segmentation tutorial and demonstration. raphy averaging tutorial and demonstration in EMAN2





Do you have any questions about cryo-ET? What's cryo-electron tomography (cryo-ET)? What's the difference between cryo-ET and single

- particle cryo-EM?
- How should I prepare samples for cryo-ET?
- How should I collect cryo-ET data?
- How should I analyze cryo-ET data?
- How could I use cryo-ET effectively?



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- What's electron tomography
- The power of cryo-ET
- Key steps in cryo-ET:

 - Data collection (Jun Liu)
 - Image analysis (Raphael Park & Muyuan Chen)
- Some classic cryo-ET applications



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Outline



Sample preparation (Digvijay Singh & Jianfeng Lin)



What's electron tomography?



Thermo Fisher



Principle of Electron Tomography



2D projection images from different views



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Back-projection to generate 3D reconstruction

Koster et al., JSB 1997



Key parameters to consider



-90 - 90 deg -80 - 80 deg -70 - 70 deg -60 - 60 deg -50 - 50 deg









original image



5 deg increment

2 deg increment

Koster et al. 1997



Key parameters to consider

4. Tilt range



original image





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- 1. Sample thickness (< 300nm) 2. Total doses (< 100e/Å2)
- 3. Tilt increment









2 deg increment

Koster et al. 1997



The power of cryo-ET

 Providing 3D snapshots of unique environments.

microscopy and near-atomic resolution techniques (such as cryo-EM or X-ray crystallography).



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biomedical complexes in their functional

Bridging the information gap between light





Ruska and his first electron microscope

Ernst Ruska & Max Knoll 1931





The Nobel Prize in 1986 awarded to Ruska for designing electron microscope

1u

Helmut Ruska 1938, 1940







Electron microscopy in Cell Biology





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Keith Porter et al. 1944



Electron microscopy in Cell Biology





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Albert Claude



Christian de Duve

George E. Palade

won the nobel prize in 1974 for their discoveries concerning the structural and functional organization of the cell



A single 2D image is not sufficient







John O'brien 1991



3D reconstruction from 2D images









David DeRosier Brandeis University

lug Prize in Chemistry 1982 ded to Aaron Klug "for his ent of crystallographic icroscopy ... "





ET provides unique 3D information





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CryoET — bridging the information gap

The Nobel Prize in Chemistry 2017



© Nobel Media. III. N. Elmehed Jacques Dubochet Prize share: 1/3



© Nobel Media. III. N. Elmehed Joachim Frank Prize share: 1/3



© Nobel Media. Ill. N. Elmehed **Richard Henderson** Prize share: 1/3

The Nobel Prize in Chemistry 2017 was awarded to Jacques Dubochet, Joachim Frank and Richard Henderson "for developing cryo-electron" *microscopy for the high-resolution structure determination of* biomolecules in solution".





The Nobel Prize in Chemistry 2014



© Nobel Media AB. Photo: A. Mahmoud Eric Betzig Prize share: 1/3



© Nobel Media AB. Photo: A. Mahmoud Stefan W. Hell Prize share: 1/3



Mahmoud Prize share: 1/3

The Nobel Prize in Chemistry 2014 was awarded jointly to Eric Betzig, Stefan W. Hell and William E. Moerner "for the development of super-resolved fluorescence microscopy."



CryoET — bridging the information gap

Cryo-EM NMR Crystallography

Alphafold2





Kellogg et al., Science 2018

Cryo-ET

(Cryo-FIB, Cryo-CLEM, Subtomogram averaging)

Super-resolution microscopy

Huang et al., Science 2008









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Some classic cryo-ET applications





Outline

Sample preparation (Digvijay Singh & Jianfeng Lin)



Sample preparation is the key for cryo-ET

Thin specimen (less than 0.3µm) is required for high-resolution cryo-ET imaging.

> Protein, virus, thin cell (< 10 μ m) Fast freezing to achieve a frozen hydrated state

> > Large cell, tissue (> 10 μ m) High-pressure freezing

Cryo-section & Focused ion beam milling









Frozen hydrated specimen preparation



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Large protein complexes

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Cheng et al., JMB 2007

Herpes Simplex Virus

Bacteria

Liu et al., JB 2009

Thin periphery of large cells

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Most cells are too large for cryo-ET imaging

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Schaffer et al., 2016

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Cryo-ET data collection

Cryo-ET data collection is tedious

Frank: Electron Tomography

Automation is the key

Frank: Electron Tomography

Many cryo-ET data acquisition packages

UCSF Tomo Leginon FEI tomography EM-Manu

SerialEM

Automated electron microscope tomography using robust prediction of specimen movements

David N. Mastronarde*

Boulder Laboratory for Three-Dimensional Electron Microscopy of Cells, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309, USA

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Seria EM

SerialEM provides a flexible interface. The script capability provides a relatively easy way to add commands requested by users.

2005

SerialEM (David Mastronarde)

File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help

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✓ Low Dose Mode View 2300x Sp6 026147% ✓ Continuous update of mag 3 beam Perfore position of area ● None ← Focus ← Trial Pactor on tilt evis ↓ 000 µm Measure of the second		- D Low Dose Control 21	
Blanks BEAM when screen down Blanks dublank Offsets for View Defocus: 60 + Shift Set Zerol Mormalize beam through View V Keep Focus and Trial identical Copy current area mag & beam to V F T R Center Unshifted Balance Shifts Rototate intercarea.covis O deg Isourge Construction Auto Survey Center Unshifted Balance Shifts Rototate intercarea.covis O deg Isourge Construction Color Blue P Draw Rotate when load For anchor state #1 Note: Sec 0-montage01.st Acquire (A) Tilt series New file et item New file et argent Set Demontage01.st Acquire (A) Tilt series Note: Sec 0-montage01.st Registration 1 + Oraw all reg. Add Stage Pos Registration 1 + Oraw all reg. Add Points Collapse groups Show Acquire area Add Points Collapse groups Show Acquire area <td< td=""><td></td><td> ✓ Low Dose Mode ✓ Low Dose Mode ✓ View: 2300x Sp 6 C2 61.41% ✓ Continuous update of mag & beam Define position of area ○ None ○ Focus ○ Trial Position on tilt axis: ○ 0.00 um Maximum area separation: -0.71 um Additional beam shift ○ Set Reset ○ 0.00, 0.00 Area to show when screen down ○ Vie. ○ Foc. ○ Tri. ○ Rec. ○ Sea. </td><td>Intensity K2 Direct Detection K2 Direct Detection Mode: Counted HW Processing HW Processing Background Subtraction Gain Correction Update HW Dark Reference Health Status</td></td<>		 ✓ Low Dose Mode ✓ Low Dose Mode ✓ View: 2300x Sp 6 C2 61.41% ✓ Continuous update of mag & beam Define position of area ○ None ○ Focus ○ Trial Position on tilt axis: ○ 0.00 um Maximum area separation: -0.71 um Additional beam shift ○ Set Reset ○ 0.00, 0.00 Area to show when screen down ○ Vie. ○ Foc. ○ Tri. ○ Rec. ○ Sea. 	Intensity K2 Direct Detection K2 Direct Detection Mode: Counted HW Processing HW Processing Background Subtraction Gain Correction Update HW Dark Reference Health Status
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		Move Item	

Selecting targets (SerialEM)

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+

Collecting tilt series (SerialEM)

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FastTomo: A SerialEM Script for Collecting Electron Tomography Data

Albert Xu, Chen Xu[†] Department of Biochemistry and Molecular Pharmacology & Cryo-EM Core Facility University of Massachusetts Medical School Email: albert.t.xu@gmail.com, [†]Chen.Xu@umassmed.edu

"It achieves a speedup over conventional tracking methods by minimizing the usage of off-target tracking shots, and instead applies proportional control to the specimen images."

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Image analysis — Alignment & Reconstruction

MotionCorr2

IMOD

. . .

EMAN2 Appion-Protomo AreTomo

Contents lists available at **ScienceDirect**

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Automated tilt series alignment and tomographic reconstruction in IMOD

David N. Mastronarde *, Susannah R. Held

Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309, United States

Alignment and reconstruction (IMOD)

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IMOD EMAN2

Amira

Segmentation (IMOD)

EMAN2 - Raphael & Muyuan

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Chen et al., Nat Methods 2017, 2019, 2021

Image analysis — Sub-tomogram averaging

EMAN2 13 Peet Dynamo Relion EmClarity

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Picking 3-D sub-tomograms

Liu et al. Nature 2006

Sub-tomogram averaging

20nm

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Liu et al. Nature 2006

Salmonella Secretion Machine

Hu et al. Cell 2017

HIV Capsid at 3.9Å Resolution

(D25A)

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~300,000 copies were used to determine the 3.9Å resolution structure. Schur et al., Science 2016 tructural features in the immature CA-S

Routine single particle CryoEM sample and grid characterization by tomography

Alex J Noble¹, Venkata P Dandey^{1†}, Hui Wei^{1†}, Julia Brasch^{1,2}, Jillian Chase^{3,4}, Priyamvada Acharya^{1,5}, Yong Zi Tan^{1,2}, Zhening Zhang¹, Laura Y Kim¹, Giovanna Scapin^{1,6}, Micah Rapp^{1,2}, Edward T Eng¹, William J Rice¹, Anchi Cheng¹, Carl J Negro¹, Lawrence Shapiro², Peter D Kwong⁵, David Jeruzalmi^{3,4,7,8}, Amedee des Georges^{3,4,8,9}, Clinton S Potter^{1,2}, Bridget Carragher^{1,2}*

-36:21:3°, Gold Quantifoil, Krios, K2

NPC

"Science presents three papers that piece together this giant jig-saw puzzle, revealing a near-atomic picture of the massive human NPC. These studies build on decades of painstaking work of biochemical reconstitution, x-ray crystallography, mass spectroscopy, mutagenesis, and cell biology; use substantially improved cryo-electron tomography reconstructions of the entire human NPC; and leverage artificial intelligence to accurately model components."

The future of cryo-ET is bright!

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