CRYOEM 001 : INTRO TO CRYOEM

NCCAT Embedded Training — Master Class series

August 20, 2020

New York Structural Biology Center



SIMONS ELECTRON MICROSCOPY CENTER



NATIONAL CENTER FOR CRYOEM ACCESS & TRAINING

NCCAT CROSS-TRAINING



Training is teaching, or developing in oneself or others, any skills and knowledge that relate to specific useful competencies. Training has specific goals of improving one's capability, capacity, productivity and performance.

https://en.wikipedia.org/wiki/Training

NCCAT TRAINING DOCUMENTATION

https://nccat.nysbc.org/activities/nccat -cross-training/

https://nccat.nysbc.org/activities/nccat -remote-learning/

https://nccat.nysbc.org/activities/nccat -cross-training/remote-embeddedcross-training/



ACCESS

TRAINING NCCAT Workshops and Short courses > SPA Short course 2020 NCCAT Cross-training

HOME

ABOUT

NEW5

Programs NCCAT Remote Learning > Remote Office Hours > Online Classroom > Previous EM

Courses

Partners

> Curriculum

(C)



TRAINING

NCCAT provides access to state-of-the-art equipment, including high-end microscopes and direct detectors, as well as specimen preparation robots, screening microscopes and all the other ancillary equipment required to solve structures to the highest possible resolution using cryo electron microscopy (cryoEM) methods.

An already established cross-training program provides training across a wide variety of skill levels and career goals. Our workshops and forums provide opportunities to target specific areas of education and professional development.

Workshops & Courses

Embedded cross-training



JOBS

Learn with us.>

Join our community.>

ty.> Di

ACKNOWLEDGEMENTS





NCCAT CROSS-TRAINING RESOURCES



APPION WORKSHOPS — AN OVERVIEW



OO1 CRYOEM 101

\$4

CRYOEM 001 : SINGLE PARTICLE MASTERCLASS

Introduction to cryoEM: SPA

Building a cryoEM toolkit

EM compatible samples

EM support films and grids

Sample preparation

Tools of the trade: microscopes and detectors

Microscope operations Data collection strategies Data assessment & QC Data processing: cryoEM IT infrastructure On-the-fly feedback **3D** Reconstruction Visualization and validation

CRYOEM: TECHNOLOGY ON THE RISE

Single-particle cryo-electron microscopy (cryo-EM) is the Method of the Year 2015

nature methods

Review on CRISPR-Cas9 specificity Reconstruction of dense neural populations Photoswitchable probe for photoacoustic imaging A refined force field for DNA simulation: METHOD OF THE YEAR 2015

Chemistry Nobel prize 2017

The Royal Swedish Academy of Sciences has decided to award the **2017 NOBEL PRIZE IN CHEMISTRY**

Jacques Dubochet Joachim Frank Richard Henderson

"for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution"

microED Science breakthrough of the year runner-up 2018

Science





CRYOEM: TECHNOLOGY ON THE RISE

1986

2017

????





Frank (2017)



TBD (20??)

Resolution revolution

(~2012-2014)

Microscopes



Direct Detectors



Computers



14 independent structures





Leginon / SerialEM / EPU, ...

MotionCorr2, Unblur, ...

RELION, FREALIGN/cisTEM, cryoSPARC EMAN, Sparx, SPHIRE, XMIPP, ...

Software

WHAT IS POSSIBLE TODAY?

#2Å within a day

12 h

WHAT IS POSSIBLE TODAY?

#2Å within a day



IS THIS ROUTINELY DONE?

Aldolase	Glutamate dehydrogenase	Apoferritin	20S proteasome	60S/80S ribosome
D2	D3	0	D7	C1
~150kDa	334kDa	443kDa	750kDa	~2-4MDa
rabbit muscle	cow liver	horse spleen	Thermoplasma or Mycoplasma	human

HOW MANY IMAGES DO WE NEED?







WHERE ARE THE BOTTLENECKS?

technology aimed towards completely automating the processes involved in solving macromolecular structure using cryo-electron microscopy (cryoEM)







Sample preparation Data collection

(pre-)Processing











NCCAT CROSS-TRAINING FOCUS ON 4 AREAS



NCCAT CROSS-TRAINING FOCUS ON 4 AREAS



https://doi.org/10.1038/s41592-019-0395-x

THE FIELD IS CHANGING



https://doi.org/10.1002/pro.2989





~1.9Å



WHAT NEXT?

1 Barles at 3+ 23 () + 1

cryoEM 001 : Single Particle Masterclass

- 1. Building a cryoEM toolkit
- 2. EM compatible samples
- 3. EM support films and grids
- 4. Sample preparation
- 5. Tools of the trade: microscopes and detectors
- 6. Microscope operations
- 7. Data collection strategies
- 8. Data assessment & QC
- 9. Data processing:
 - cryoEM IT infrastructure
 - On-the-fly feedback
 - 3D Reconstruction
- 10. Visualization and validation