SIMONS ELECTRON MICROSCOPY CENTER

NEW YORK STRUCTURAL BIOLOGY CENTER

Winter-Spring 2017 EM Course

35 Min Intro + 20 min tour
09 Jan 2017

Schedule

Welcome

Logistics

"CryoEM"

Introduction to the course

1. Welcome new students
2. Course logistics
   – Questionnaire
3. Introduction to EM &
   the course schedule

Simons Electron Microscopy Center

1. SEMC training programs
2. Tour of the facility

New York Structural Biology Center

Welcome

Logistics

"CryoEM"

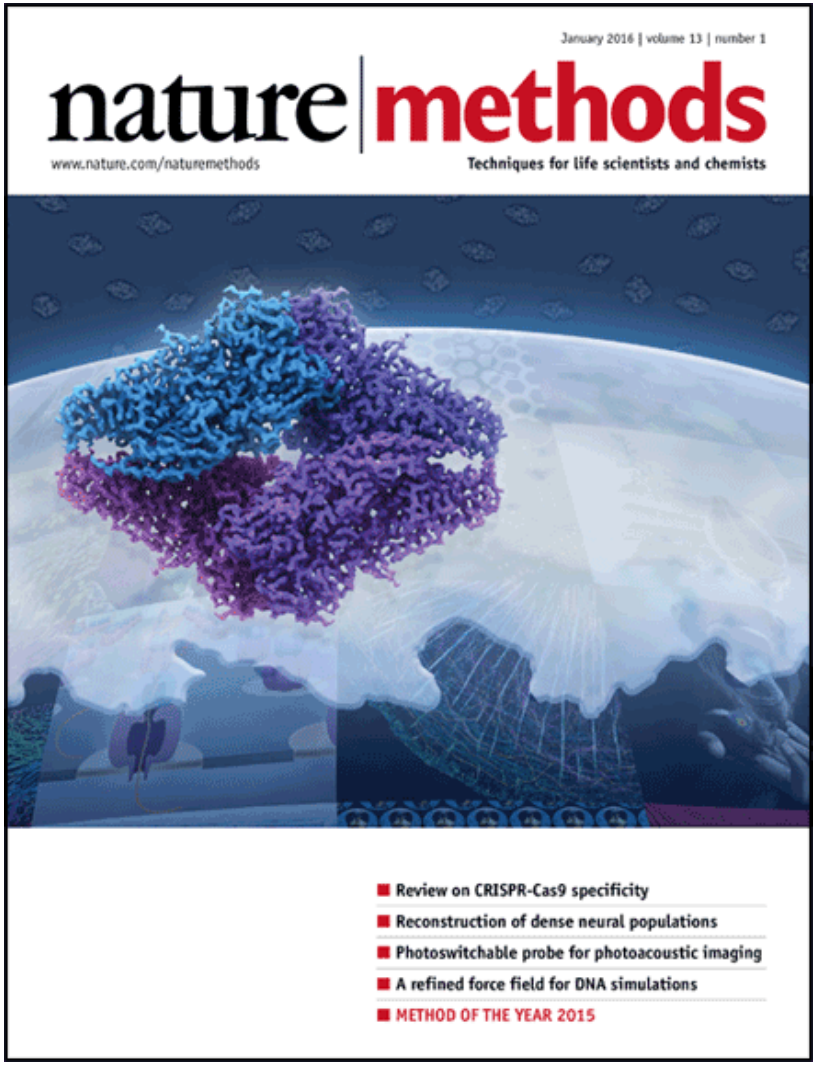
Method of the year

Welcome

Logistics

"CryoEM"

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



From a niche method to a usable workflow

Welcome

Logistics

"CryoEM"

Art is science made clear.

from *Le coq et L'arlequin* by Jean Cocteau (1889 - 1963)

Painter's Studio
by Jan van der Straet (Stradanus) (Dutch, 1523-1604)

Course logistics

Welcome

Logistics

"CryoEM"

Questionnaire
Syllabus

Course logistics

Welcome

Logistics

"CryoEM"

http://semc.nysbc.org/course.html

SIMONS ELECTRON MICROSCOPY CENTER

NYSBC

Renovation Updates
About SEMC

- **News/Events**
- **Forums**
- **Staff**
- **Directions**

New Users

- **Overview**
- **Prior to Start**
- **TO START**
- **Training**
- **Best Usage**

Documentation
Publications
Instrumentation
Schedule Time
NRAMM
Intranet
Additional Links

The Winter-Spring 2017 EM Course.

About the course:

Electron microscopy in combination with image analysis is increasingly powerful in producing 3D structures of individual molecules and large macromolecular complexes that are unapproachable by other methods. This course is focused on the concepts and theories behind electron microscopy and will be taught in a reverse classroom format based on Grant Jensen's online course (Getting Started in Cryo-EM [https://cryo-em-course.caltech.edu/] from Caltech). Students will be responsible for watching these online lectures prior to class. Each week guest lecturers and SEMC staff lead discussions on the practice of solving molecular structures by electron microscopy.

The course will be held at the New York Structural Biology Center at 89 Convent Ave (133rd St).

To register for the course go to: https://www.surveymonkey.com/r/WinterCourse17 .

Course Syllabus:

Classes in A-11 seminar room (Mondays 3:30-5pm and select Wednesdays 3:30-5pm)

EM fundamentals section (month of January)

- **Jan 9** : Introduction & SEMC tour [SEMC staff, NYSBC]
- **Jan 11** : (Video Screenings for introductory lectures)
- **Jan 16** : No class - Martin Luther King
- **Jan 18** : Challenges in biological EM & Sample Prep [Ed Eng & Ashleigh Raczkowski/Kelsey Jordan, NYSBC]
- **Jan 23** : Basic anatomy of the electron microscope [Laura Kim & Ed Eng, NYSBC]

Live stream

Welcome

Logistics

"CryoEM"

youtube.com/nrammsemc

Remote enrollment

youtube.com/nrammsemc

Welcome

Logistics

"CryoEM"

Reverse classroom

Welcome

Logistics

"CryoEM"

http://cryo-em-course.caltech.edu/videos

Other courses

Welcome

Logistics

"CryoEM"

LMB EM-course 2014

Daily in the MPLT from 9:30-10:30am

Mon May 12: Tony Crowther
Course introduction with a historical perspective

Tue May 13: Sjors Scheres
Image formation, Fourier analysis, CTF theory

Wed May 14: Chris Russo
Microscopy physics and optics

Thu May 15: Lori Passmore
sample preparation

Fri May 16: Paula da Fonseca
Initial data analysis

Mon May 19: Sjors Scheres
Image refinement in 2D and 3D

Tue May 20: Tanmay Bharat
Tomography and sub-tomogram averaging

Wed May 21: Richard Henderson
Map validation

Thu May 22: David Barford & Alan Brown
Low- and high-resolution modeling

Thu May 22: Shaoxia Chen, Christos Savva & others
(11am-12pm) Local setup and training & 2 example applications

Enquiries: scheres@mrc-lmb.cam.ac.uk

Lecture PDFs and professionally edited videos available on:

ftp://ftp.mrc-lmb.cam.ac.uk/pub/scheres/EM-course

Class structure

Welcome

Logistics

"CryoEM"

Monday and select Wednesdays

3:30-5pm - SEMC conference room

1.5 hr class

30 min - Introduction by guest lecturer
15 min - Coffee break/informal conversation
45 min - Open ended discussion

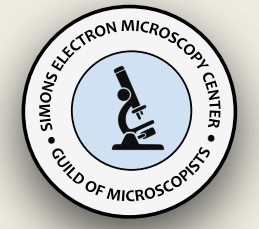
Wednesdays

Starts at 3:30 - SEMC conference room

Video screening/Recitation section

Jensen lectures that will be covered the next week will be played
SEMC lecturers will be available to assist with lecture topics

Class organization



Welcome

Logistics

"CryoEM"

Section 1: EM fundamentals section

1. Introduction & SEMC tour
2. Challenges in biological EM & Sample Prep
3. Basic anatomy of the electron microscope
4. Fourier transforms
5. Image Formation

Section 2: Tomography section

1. Intro and overview
2. FIB-SEM
3. Sub-tomogram averaging

Section 3: Single particle section

1. Intro and types of samples
2. Data Analysis and reconstruction workflow
3. Interpretation and Limitations

Section 4: 2D crystallography section

1. Intro and overview
2. Helical

Section 5: EM challenges and new frontiers

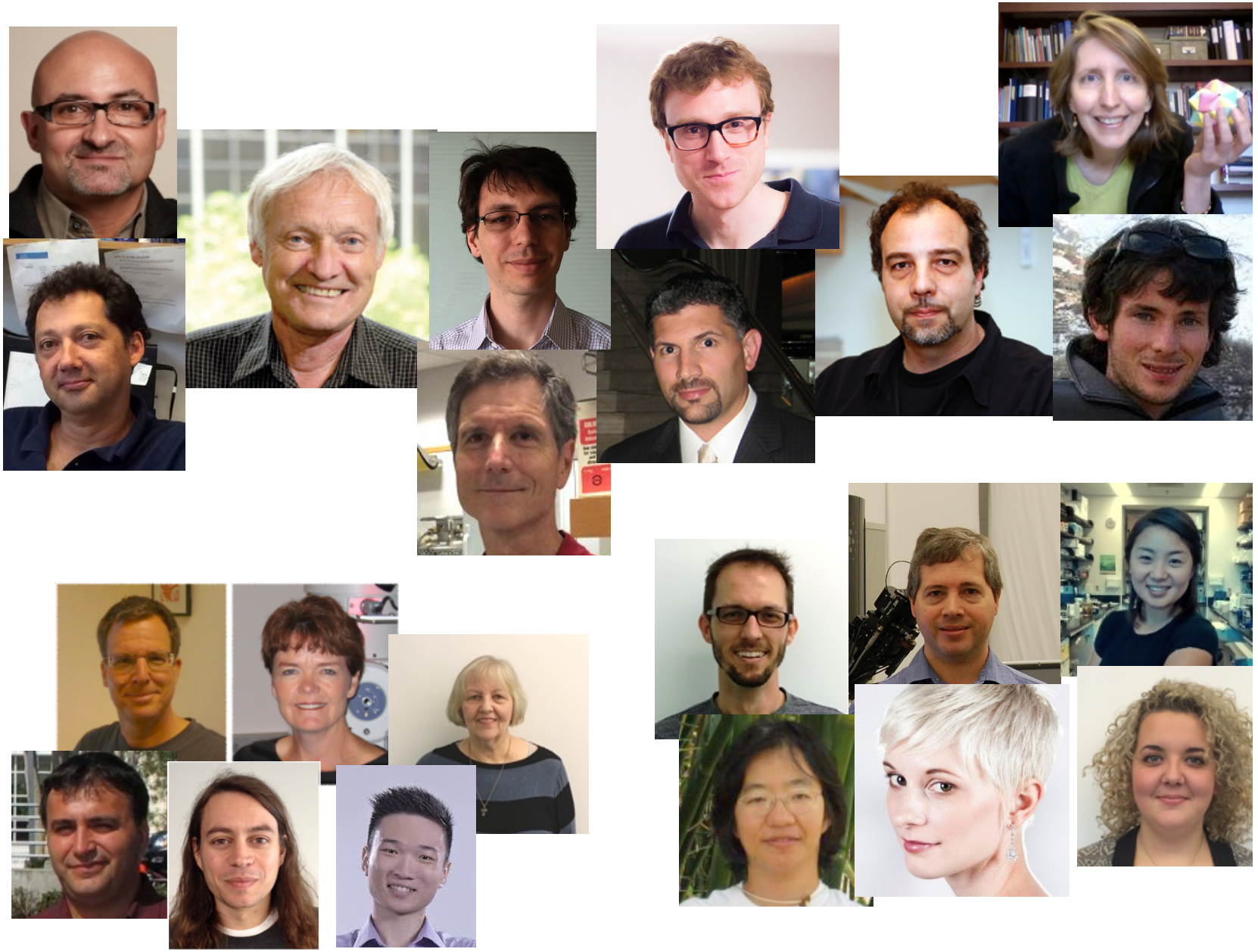
1. EMDataBank: Structure Data Archiving, Validation Challenges
2. Validation methods
3. Fitting Atomic Models
4. Conclusion & open discussion

Team of instructors

Welcome

Logistics

"CryoEM"



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What biological systems are you interested in?

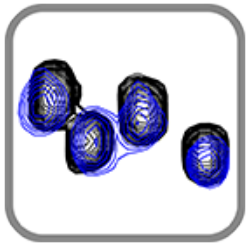
"Cryo-EM"

Scope of EM

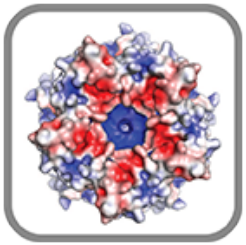
Foundational lectures

EM modalities

Challenges and frontiers

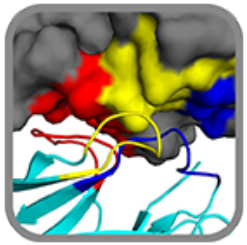


NMR Spectroscopy



X-Ray Crystallography

Electron Microscopy



Special Projects Group

Membrane Proteins

The scale of biological structures

"Cryo-EM"

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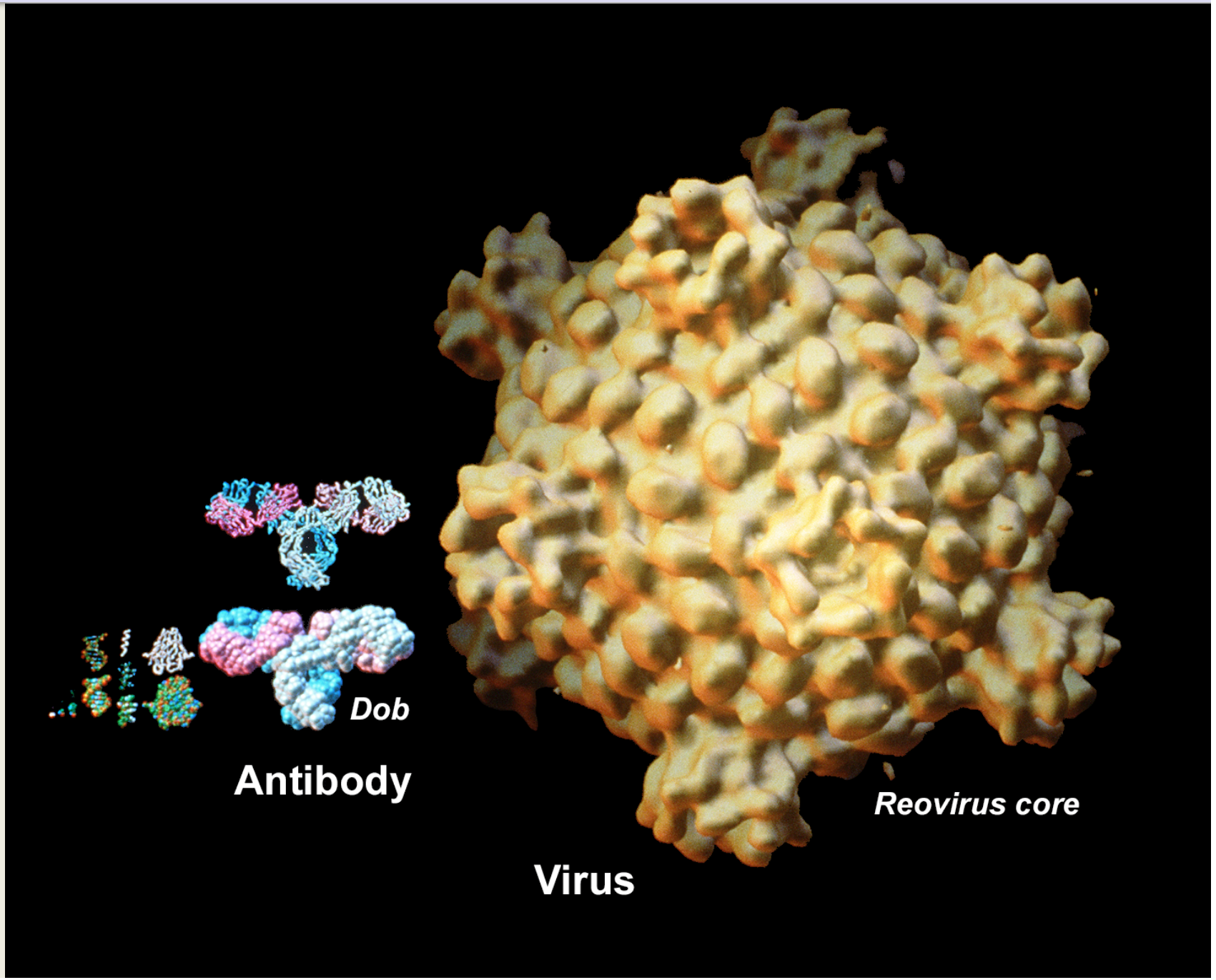

Nanoscale: the scale of biological structures

"Cryo-EM"

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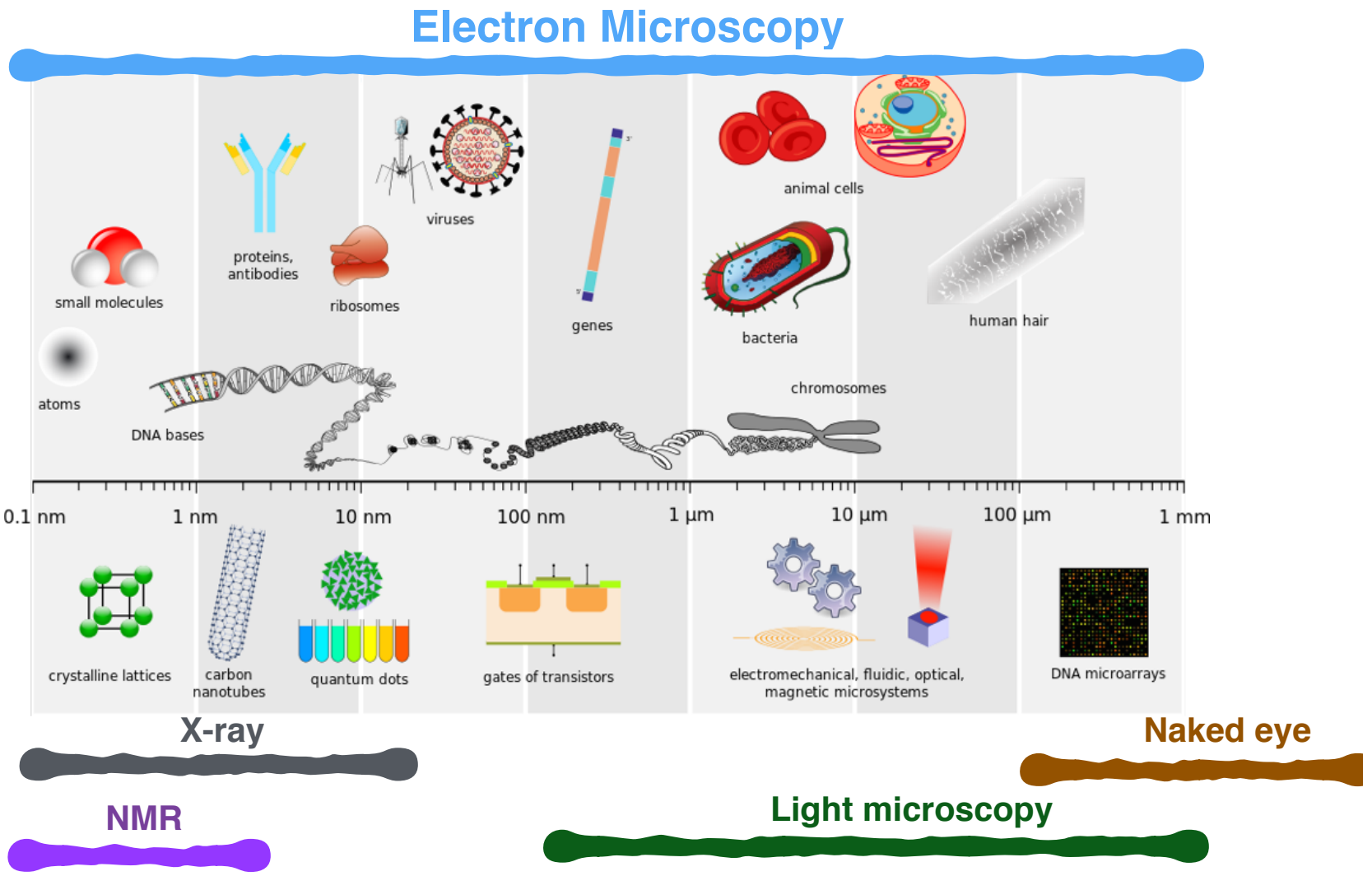

https://en.wikipedia.org/wiki/Nanoscopic_scale

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"Cryo-EM"

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How to make an EM ready sample

"Cryo-EM"

Scope of EM

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Challenges and frontiers

Section 1-2:
Challenges in biological EM & Sample Prep

Ashleigh Raczkowski/ Kelsey Jordan [NYSBC]

How to make an EM ready sample

"Cryo-EM"

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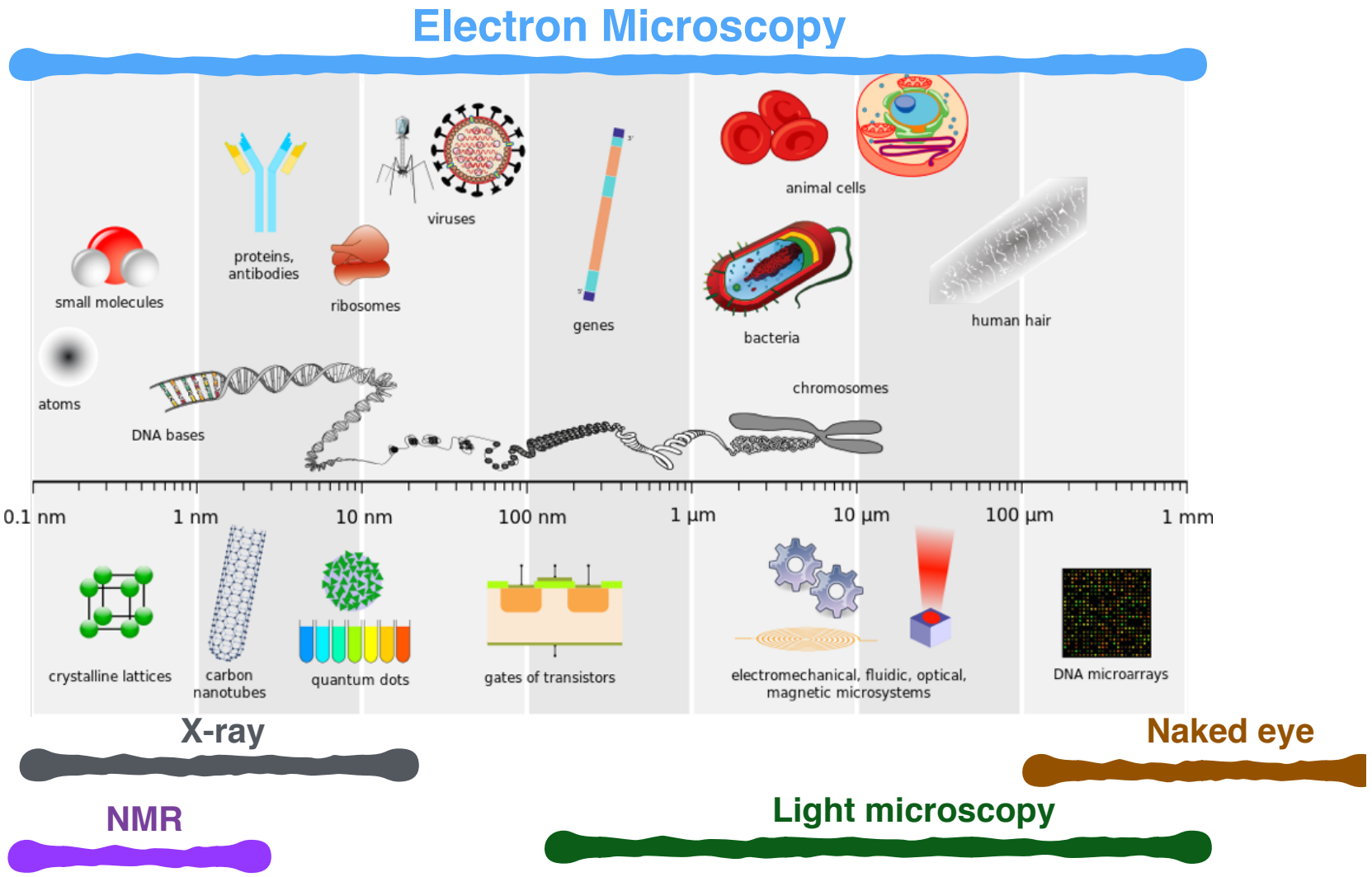

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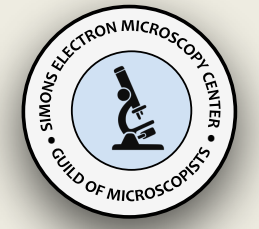
EM modalities



"Cryo-EM"

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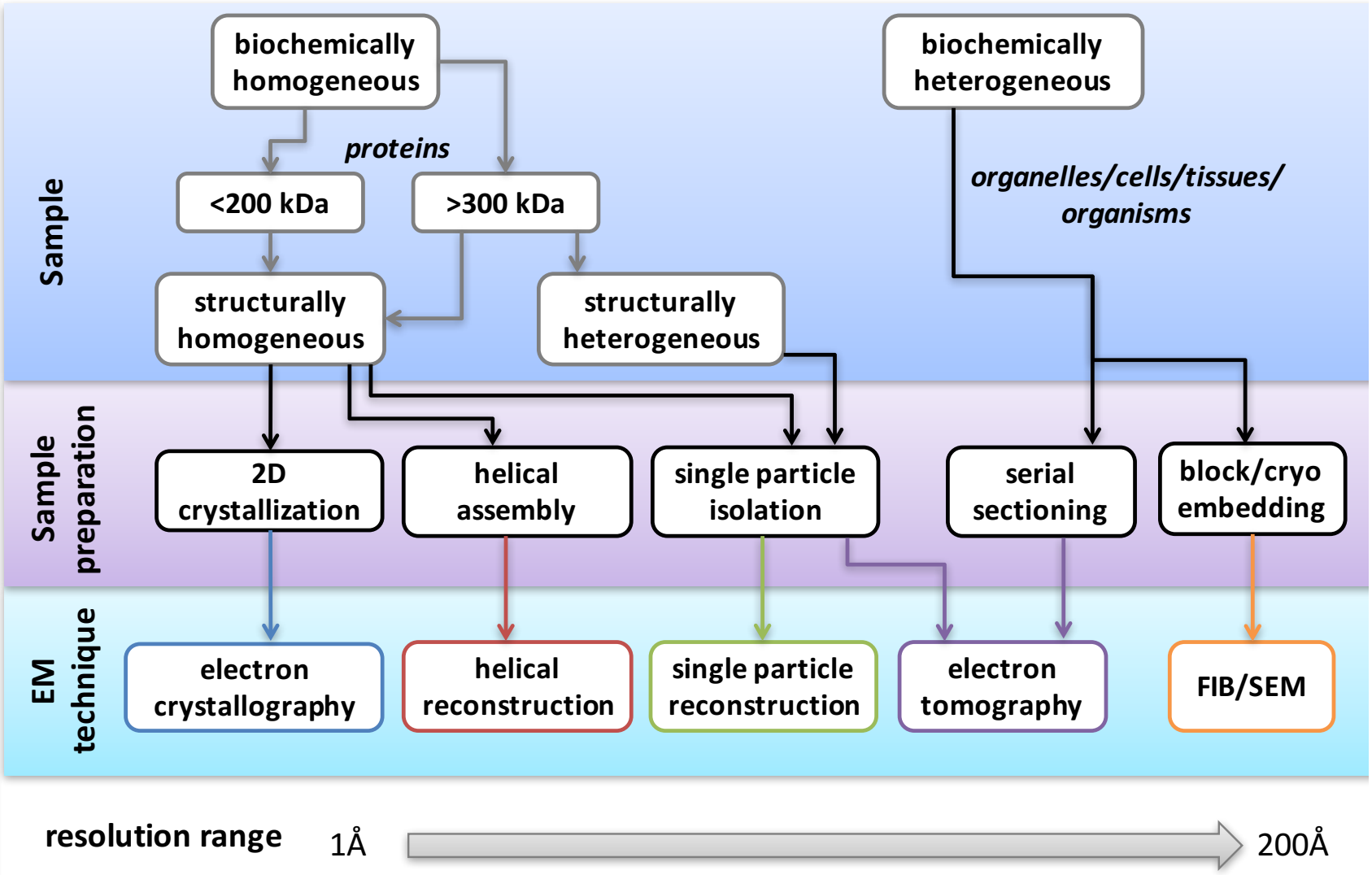

Comparison of a light microscope, TEM & SEM

"Cryo-EM"

Scope of EM

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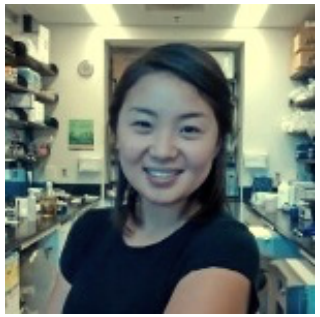
Section 1-3:
Basic anatomy of the electron microscope

Laura Kim [NYSBC]



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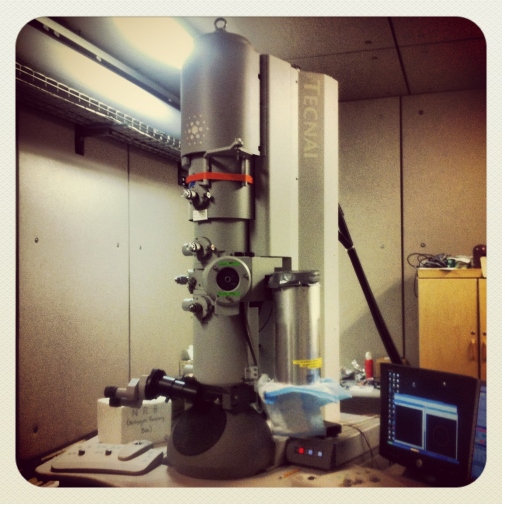

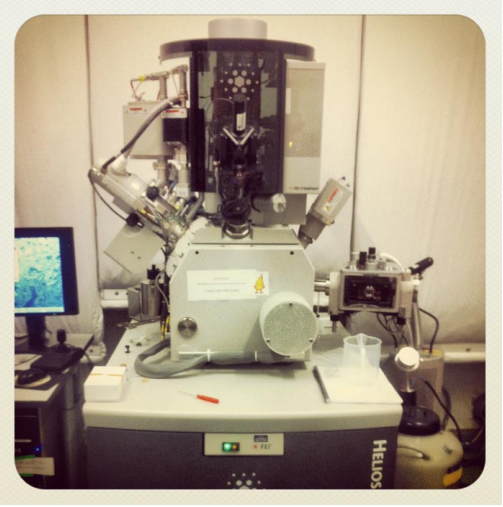

Comparison of a light microscope, TEM & SEM

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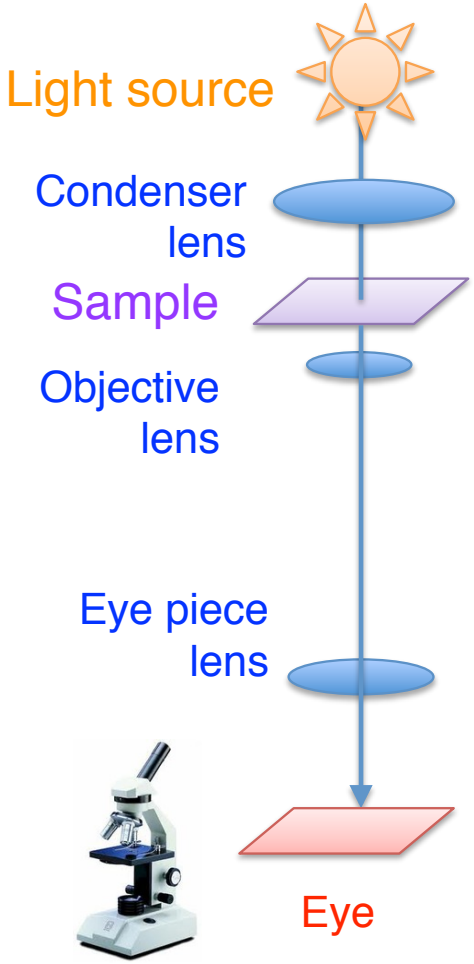
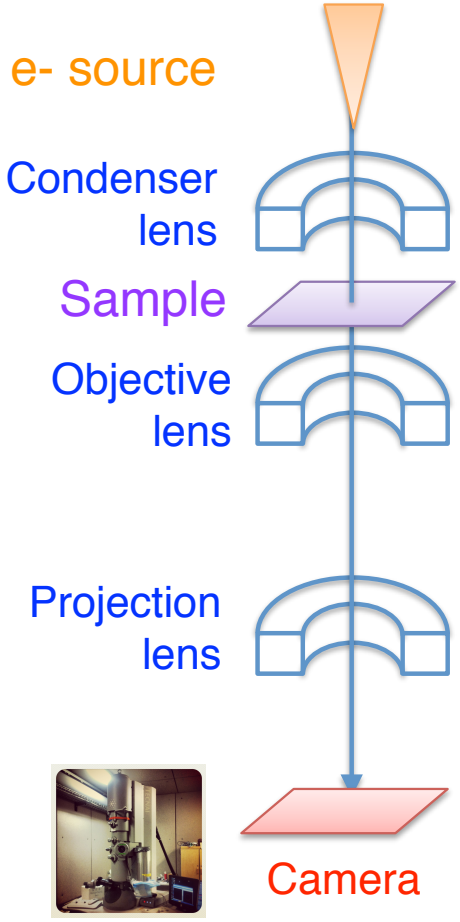
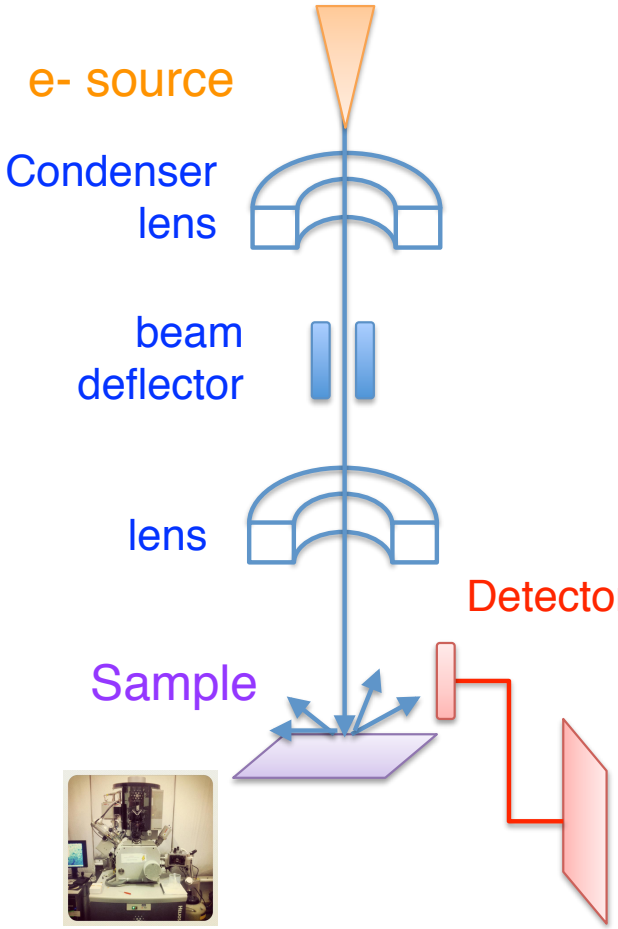
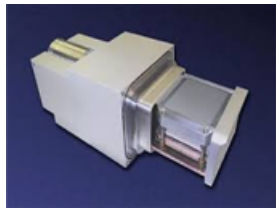
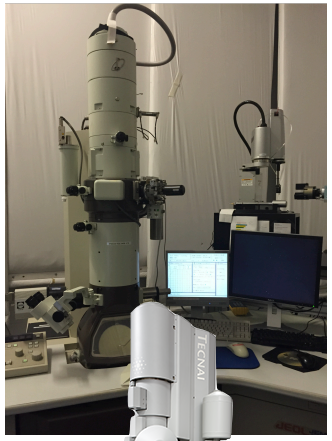


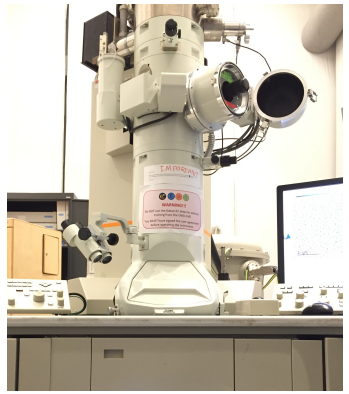


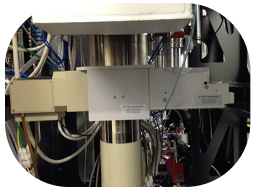


What equipment is needed for EM









"Cryo-EM"

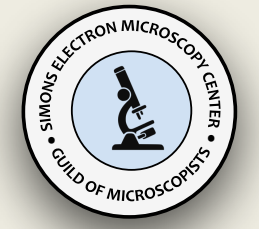
Scope of EM

Foundational lectures

EM modalities

Challenges and frontiers

What equipment is needed for EM



"Cryo-EM"

Scope of EM

Foundational lectures

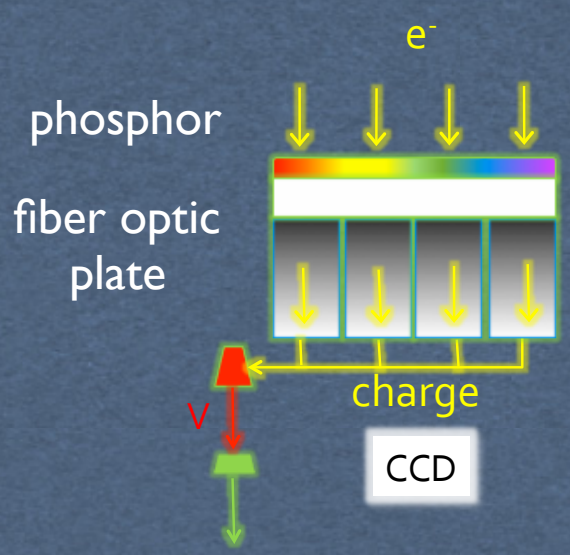
EM modalities

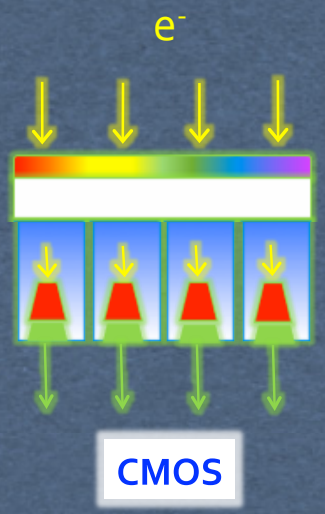
Challenges and frontiers

- Photon converted
- Direct sensing

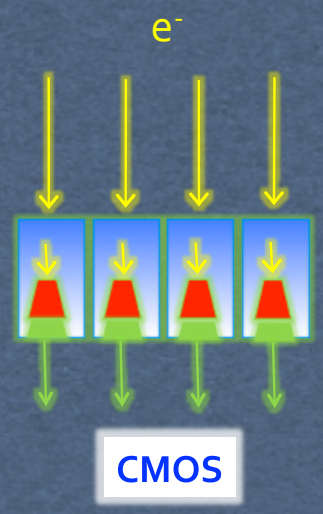
?

- CCD
  Charge Coupled Device
- CMOS
  Complementary Metal Oxide Semiconductor





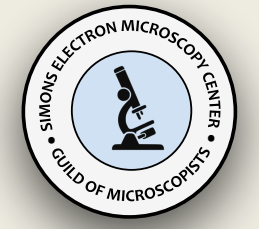
high dose rate



Direct Detectors

Slide courtesy Anchi Cheng

Obtaining a 3D structure from a 2D image



"Cryo-EM"

Scope of EM

Foundational lectures

EM modalities

Challenges and frontiers

Section 1-4: Fourier transforms

Section 1-5: Image formation

Bill Rice & Anchi Cheng [NYSBC]

Obtaining a 3D structure from a 2D image

"Cryo-EM"

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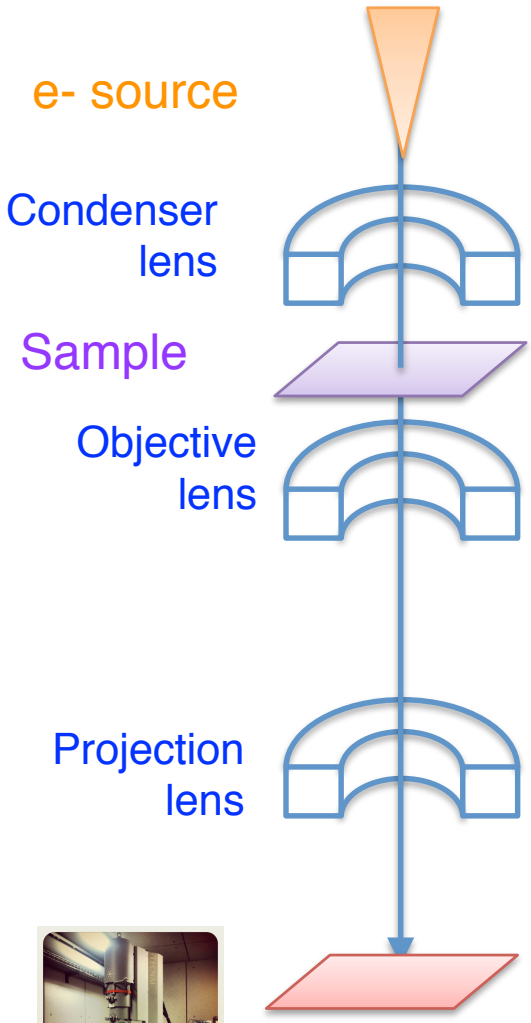

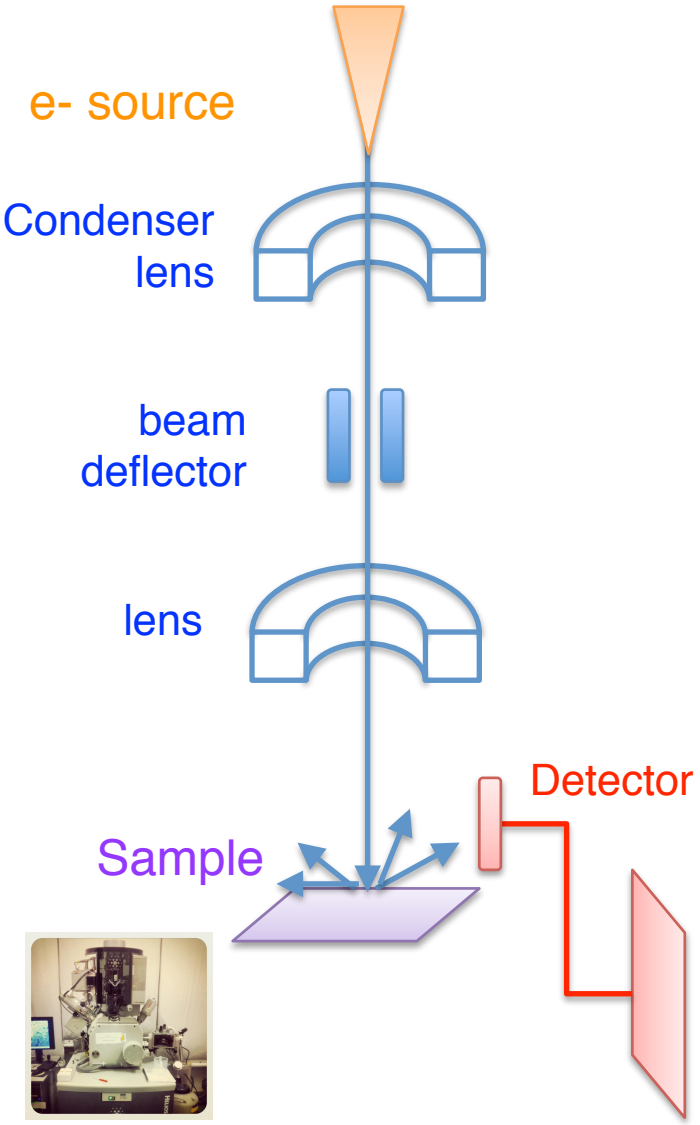

Obtaining a 3D structure from a 2D image

"Cryo-EM"

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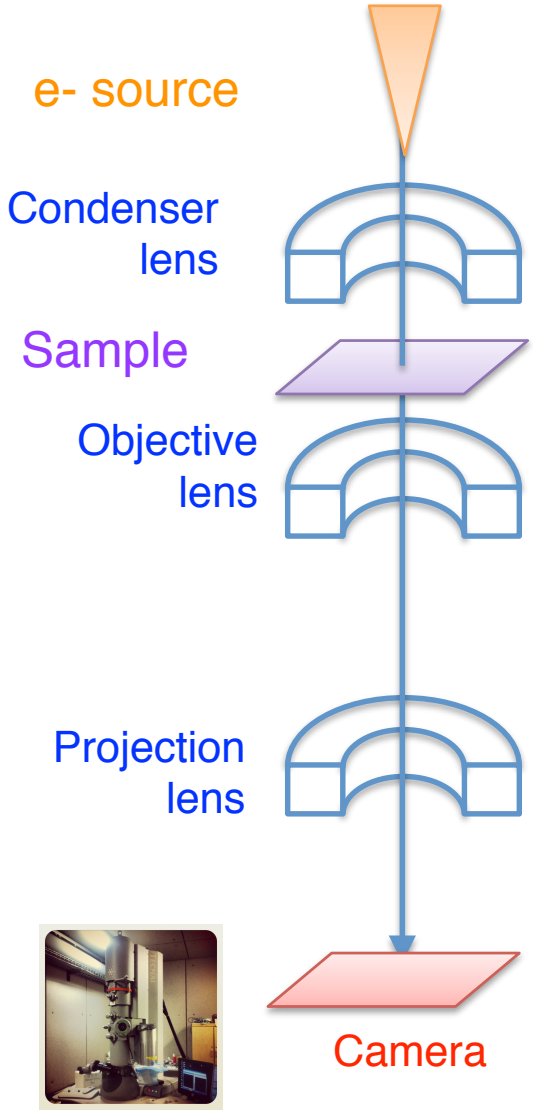

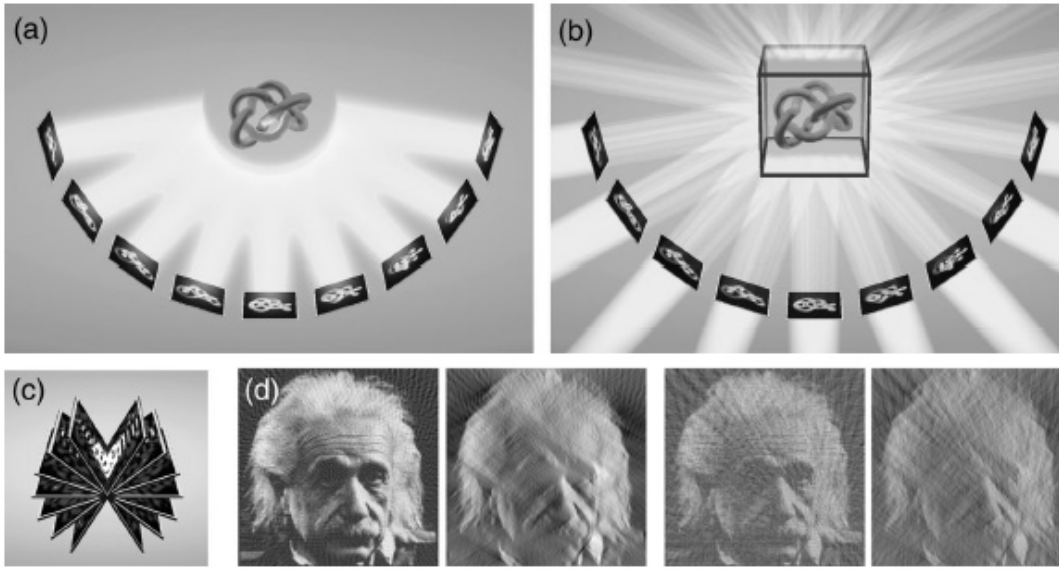

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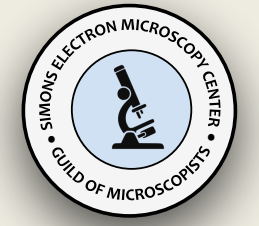

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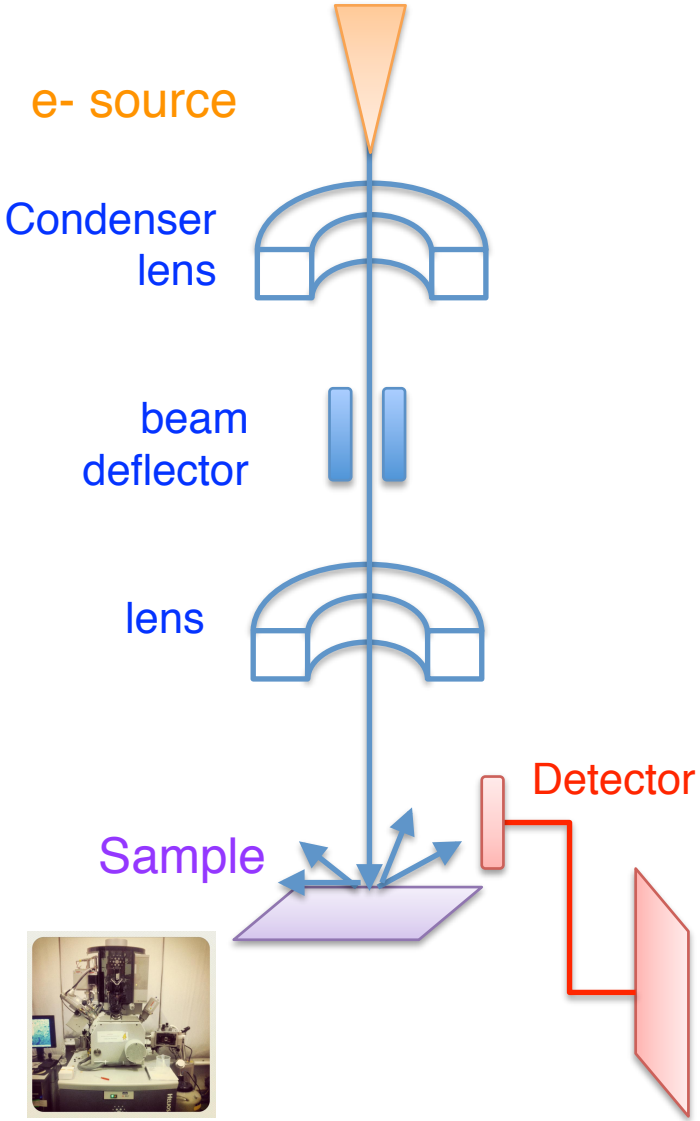
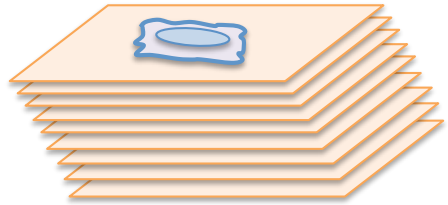
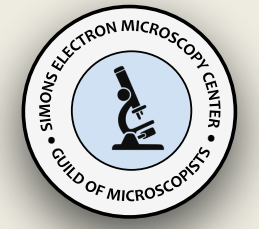




Class schedule



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Looking at biochemically unique/heterogeneous (pleomorphic) samples

"Cryo-EM"

Scope of EM

Foundational lectures

EM modalities

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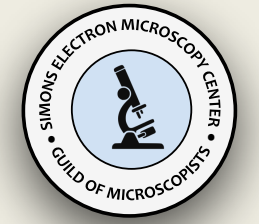
Section 2: Tomography

2-1: David Stokes [NYU]

2-2: Bill Rice & 2-3: Alex Noble [NYSBC]

Looking at biochemically unique/heterogeneous (pleomorphic) samples



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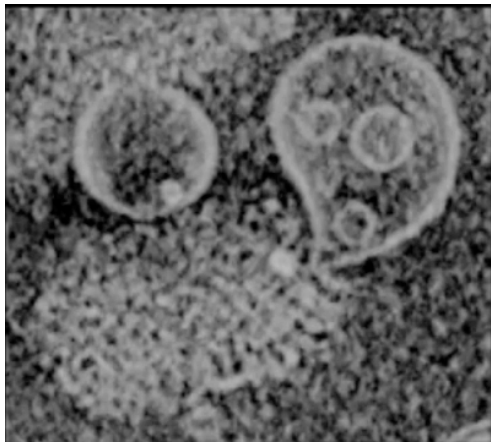
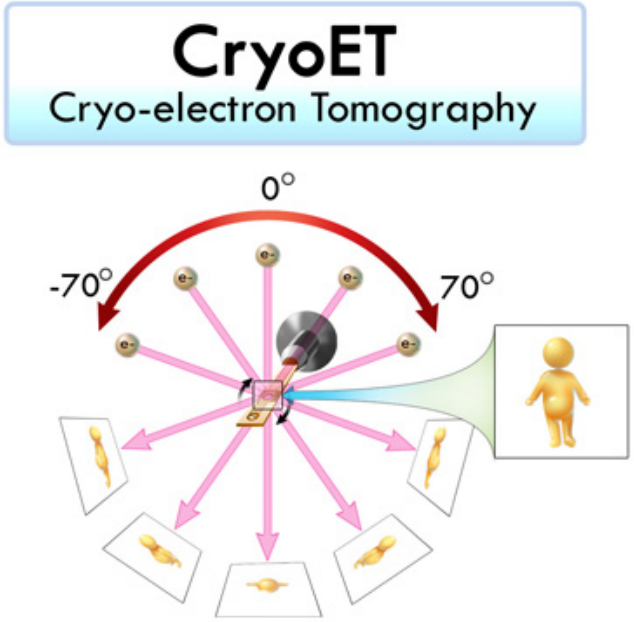
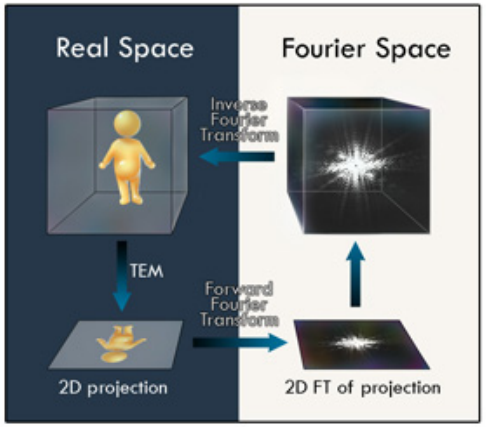
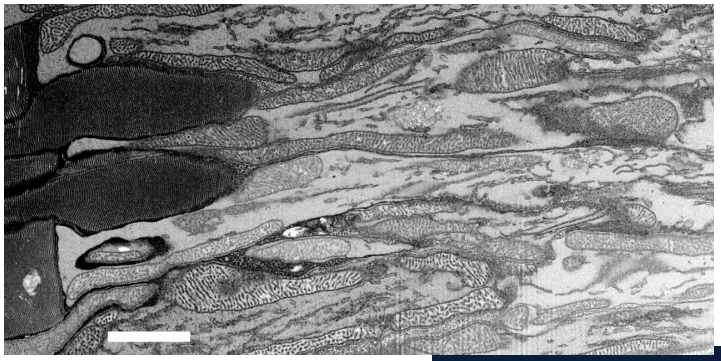


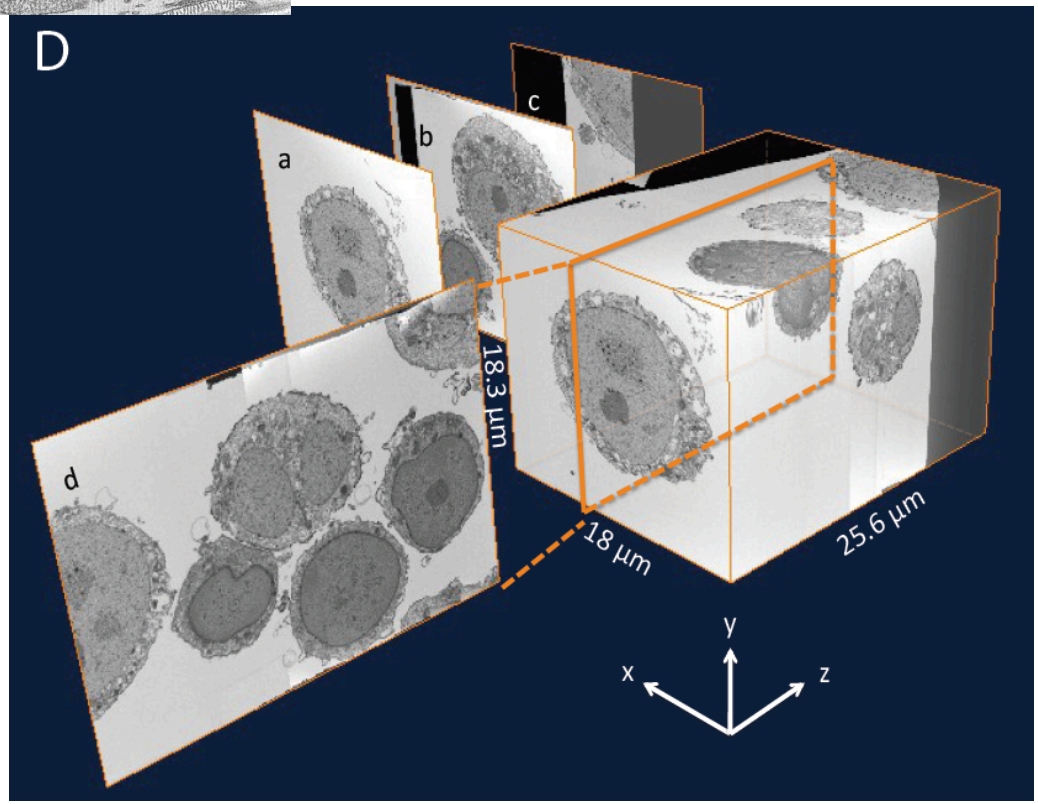


eicn
Electron Imaging Center for Nanomachines



Looking at biochemically unique/heterogeneous (pleomorphic) samples





"Cryo-EM"

Scope of EM

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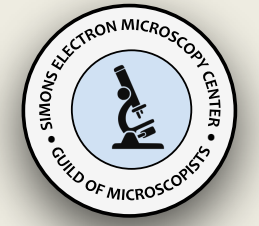
EM modalities

Challenges and frontiers

Looking at biochemically unique/heterogeneous (pleomorphic) samples



"Cryo-EM"

Scope of EM

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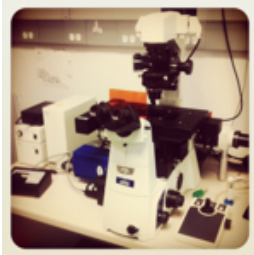
EM modalities

Challenges and frontiers

Cryo-Focused Ion Beam and Electron Tomography (Cryo-FIB-ET)

Nikon Ti-U

Light microscope

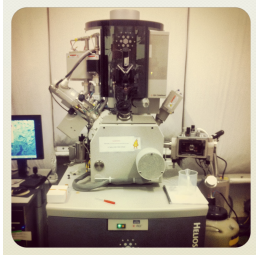


Epi-Fluorescence Module

CCD

FEI Helios

Ga ion/30kV FIB-SEM



FEG

ETD, TLD, ICE detectors

Krios

300kV, TEM

FEG

K2 or Falcon3

Looking at biochemically unique/heterogeneous (pleomorphic) samples

"Cryo-EM"

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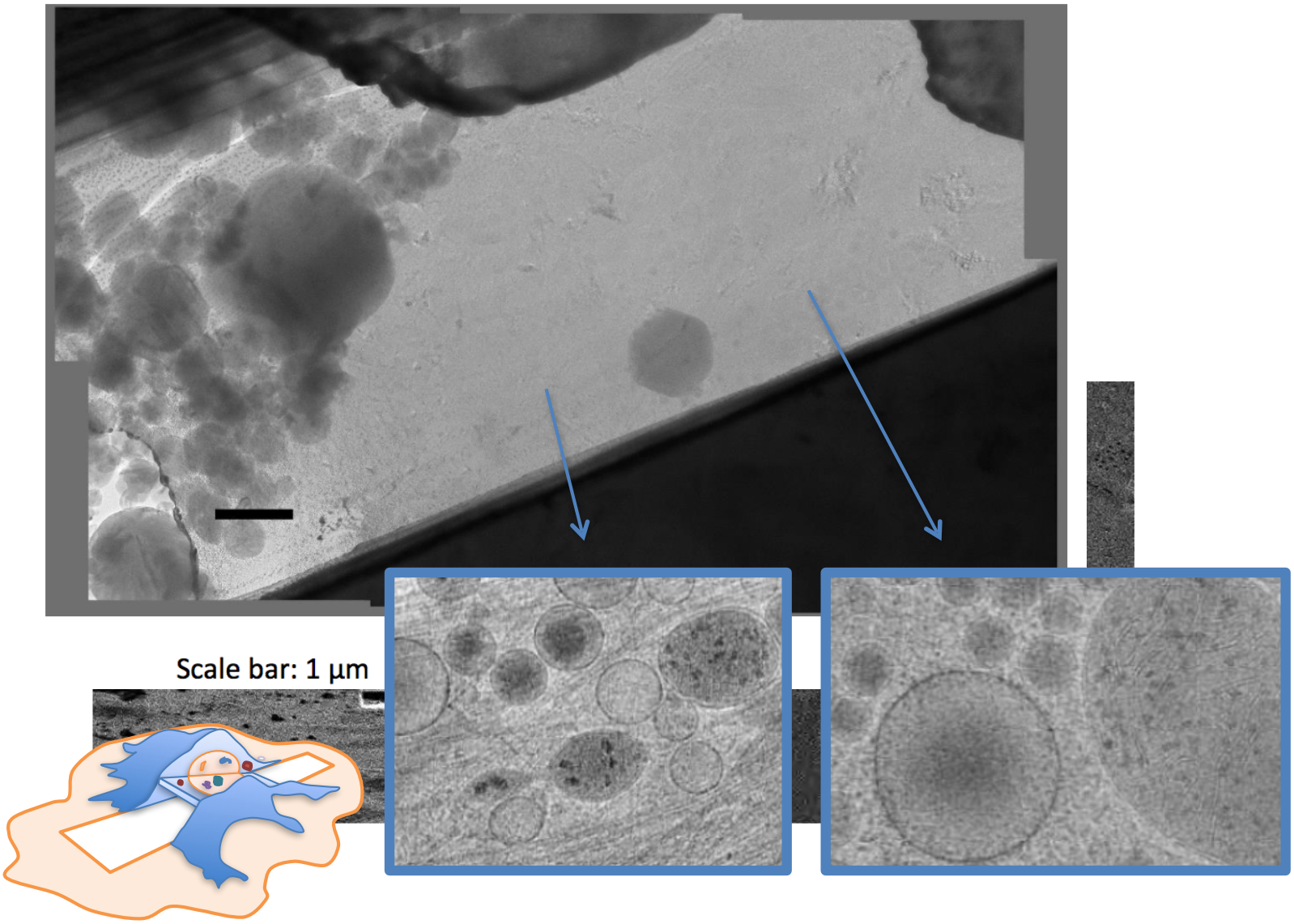
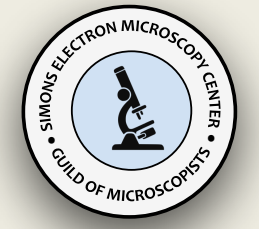


Class schedule



"Cryo-EM"

Scope of EM

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Section 1: EM fundamentals section

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Section 5: EM challenges and new frontiers

1. EMDataBank: Structure Data Archiving, Validation Challenges
2. Validation methods
3. Fitting Atomic Models
4. Conclusion & open discussion

Looking at biochemically homogeneous samples

"Cryo-EM"

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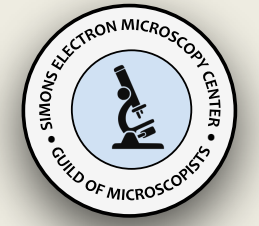
Section 3: Single Particle

3-1: Joachim Frank [CU]

3-2: Amedee Des Georges & Reza Khayat [CUNY]

3-3: Rich Hite [MSKCC]

Looking at biochemically homogeneous samples



"Cryo-EM"

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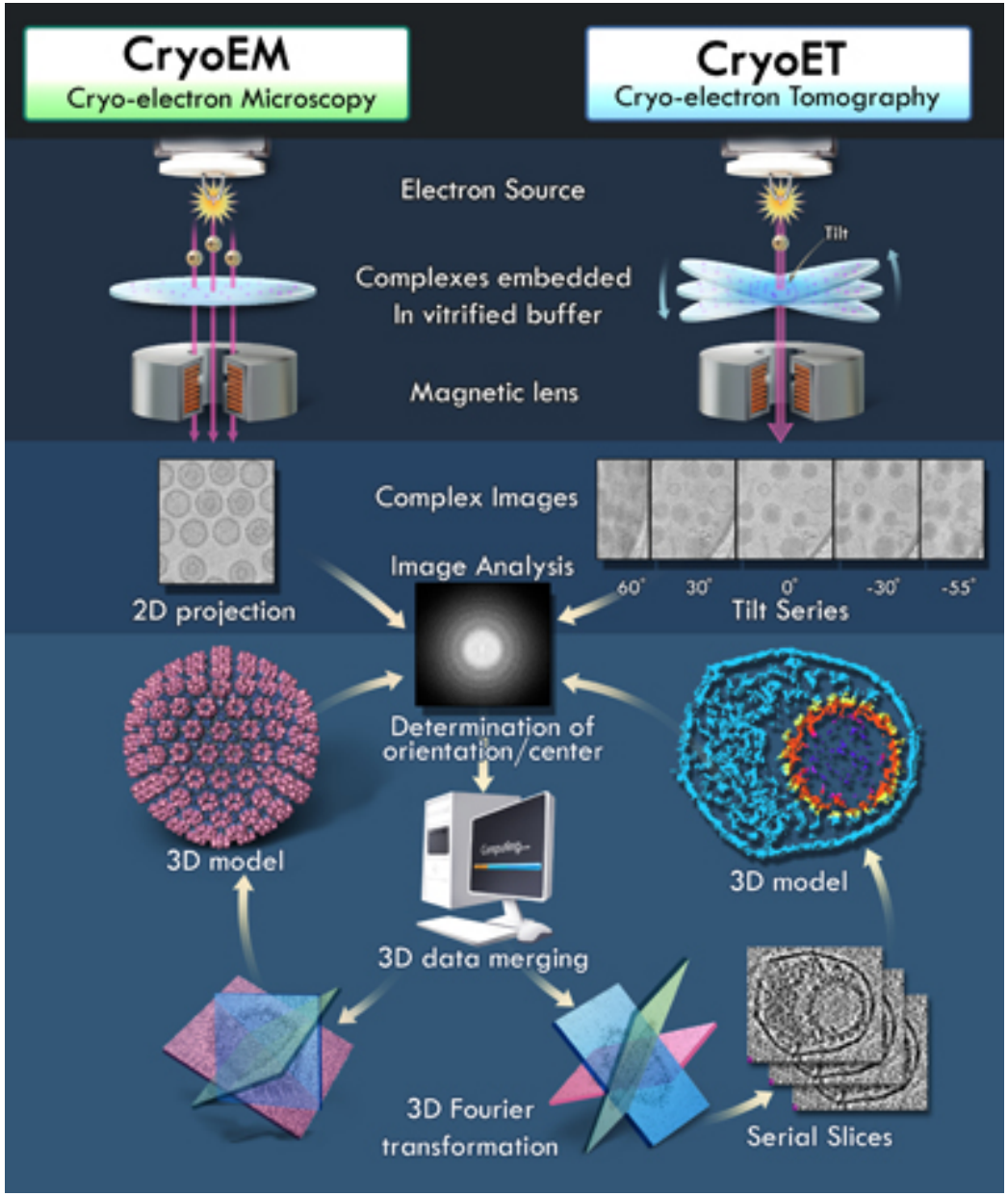
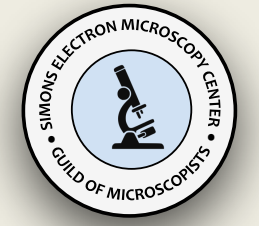


eicn
Electron Imaging Center for Nanomachine

Looking at biochemically homogeneous samples



"Cryo-EM"

Scope of EM

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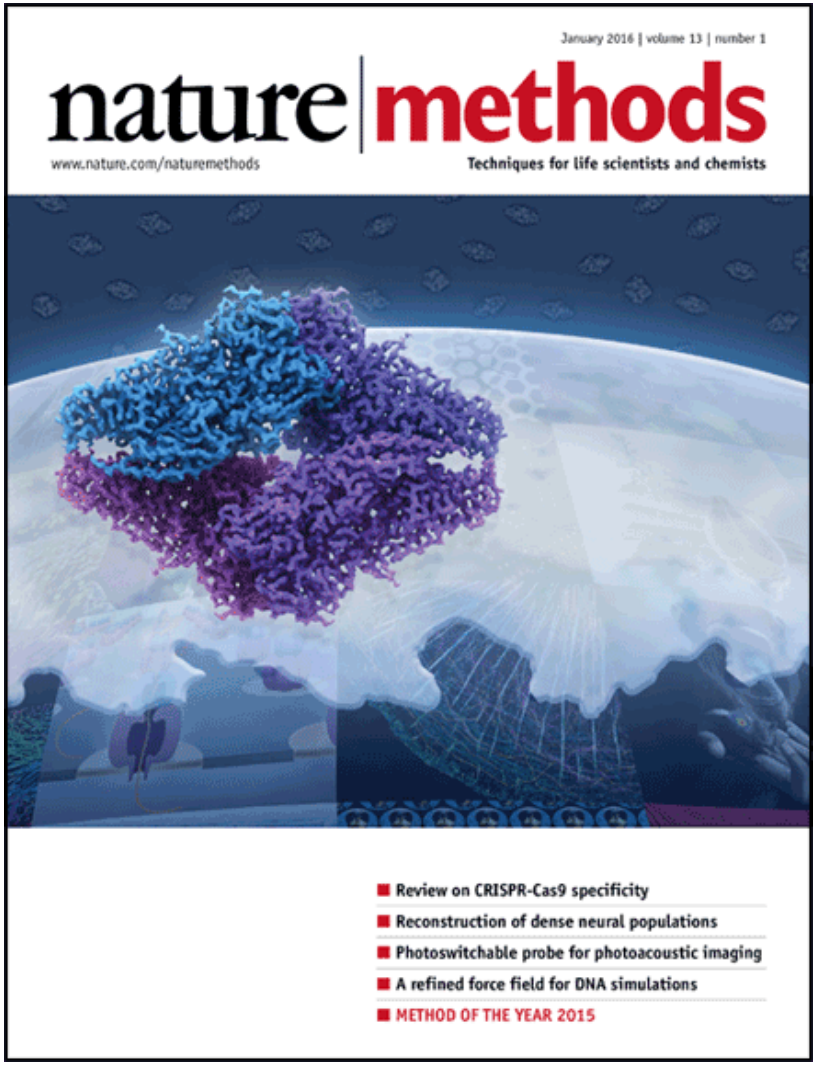
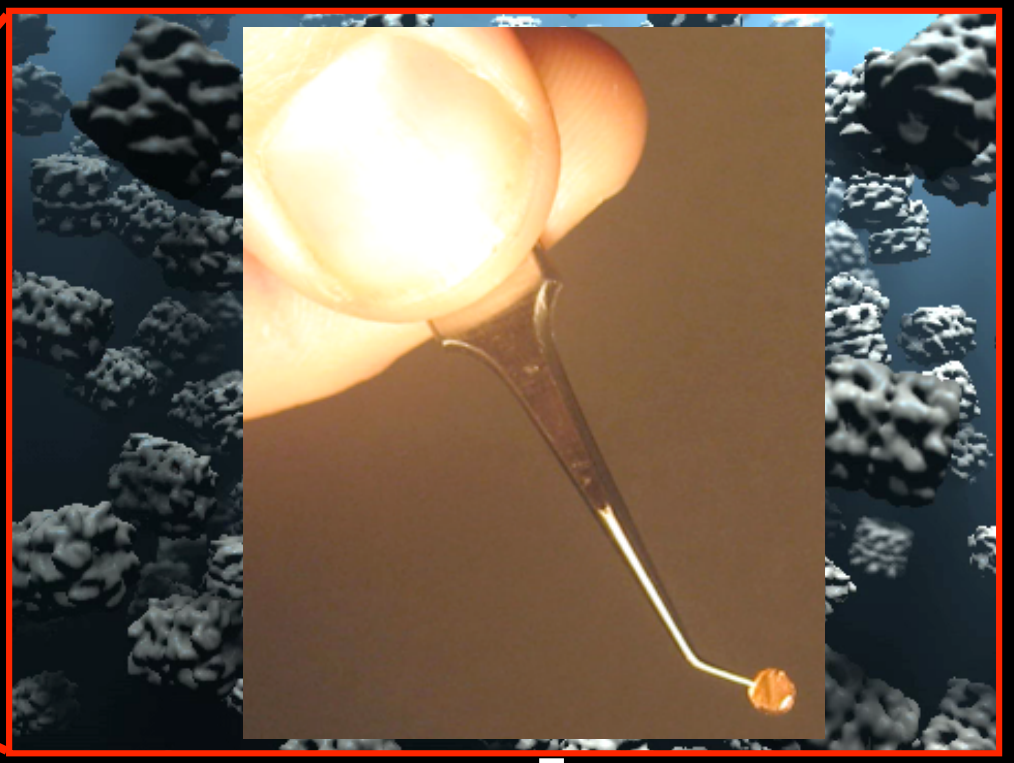
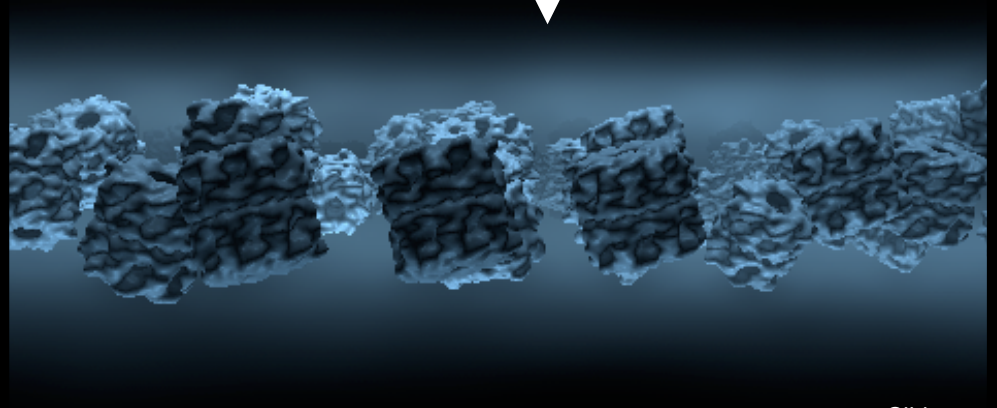
Single-particle cryo-electron microscopy (cryo-EM) is the Method of the Year 2015

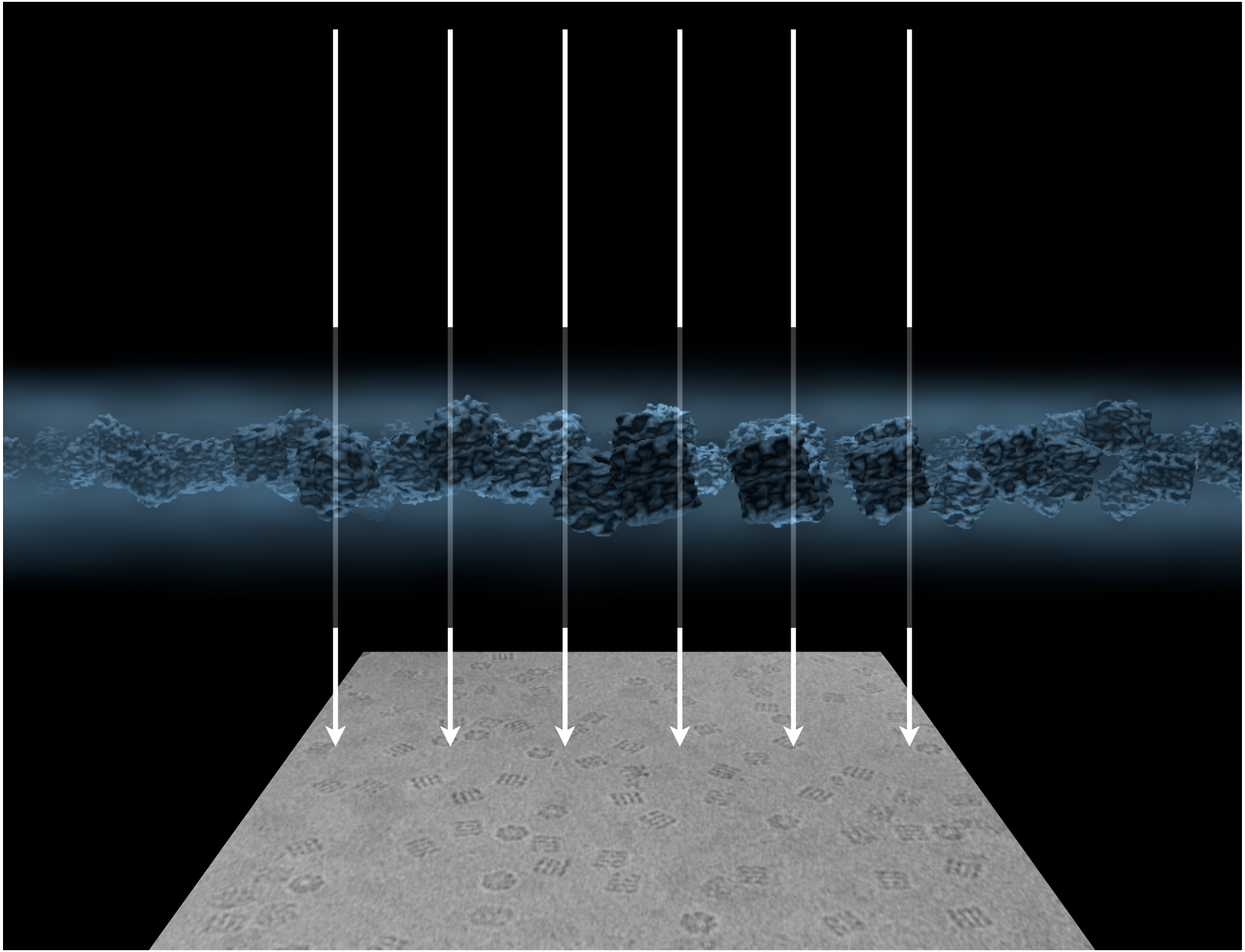


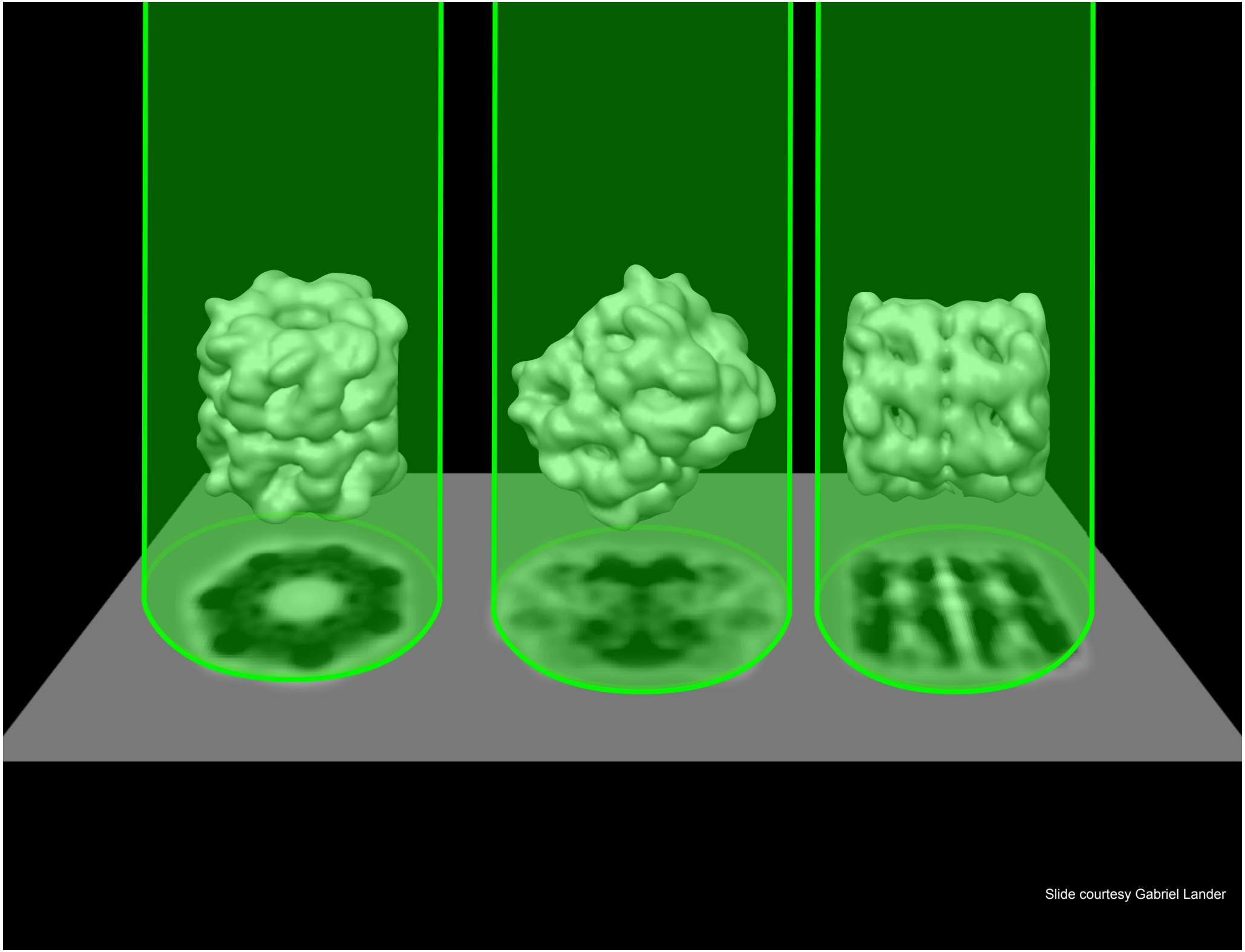
Vitrification process for CryoTEM

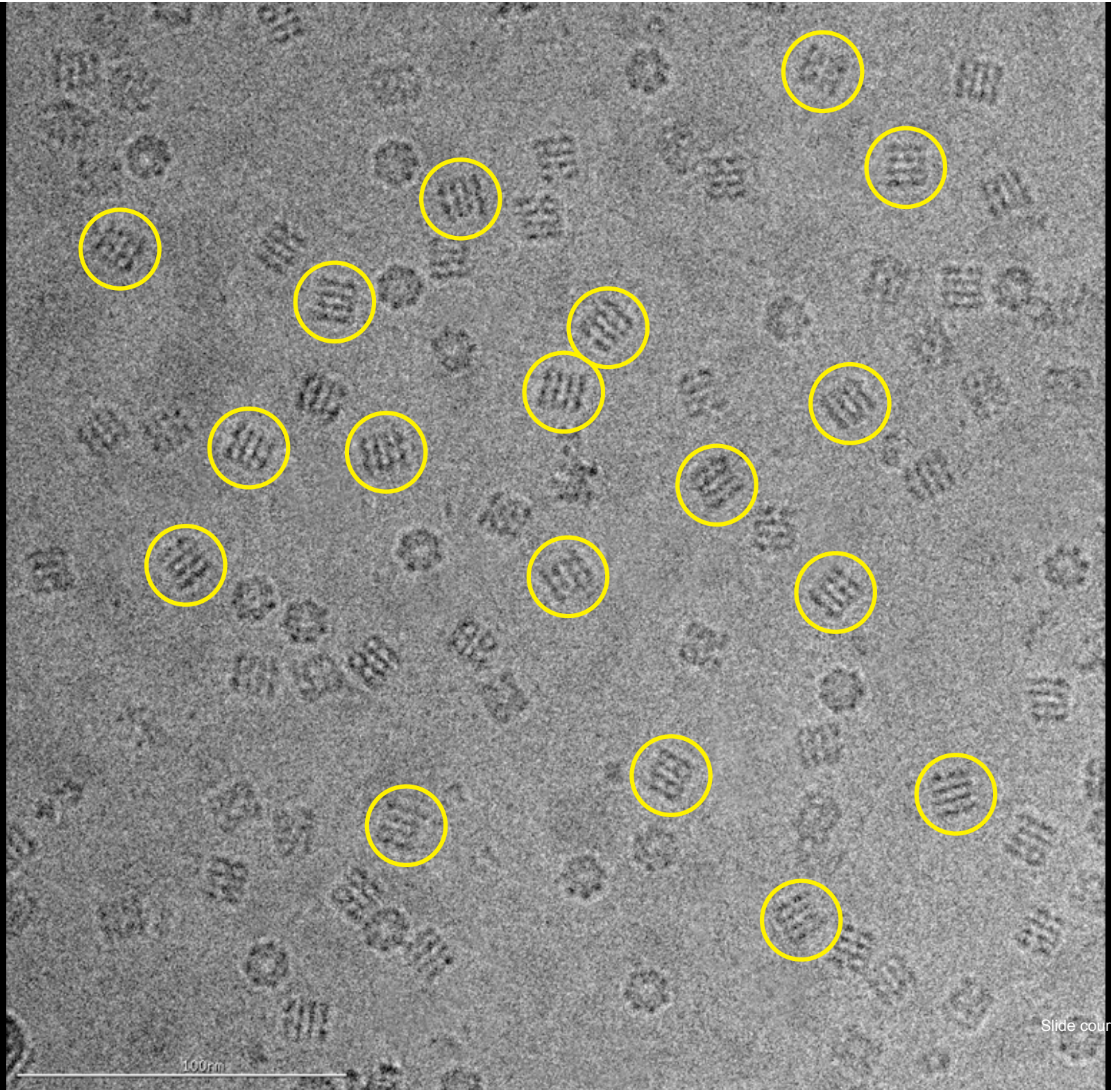


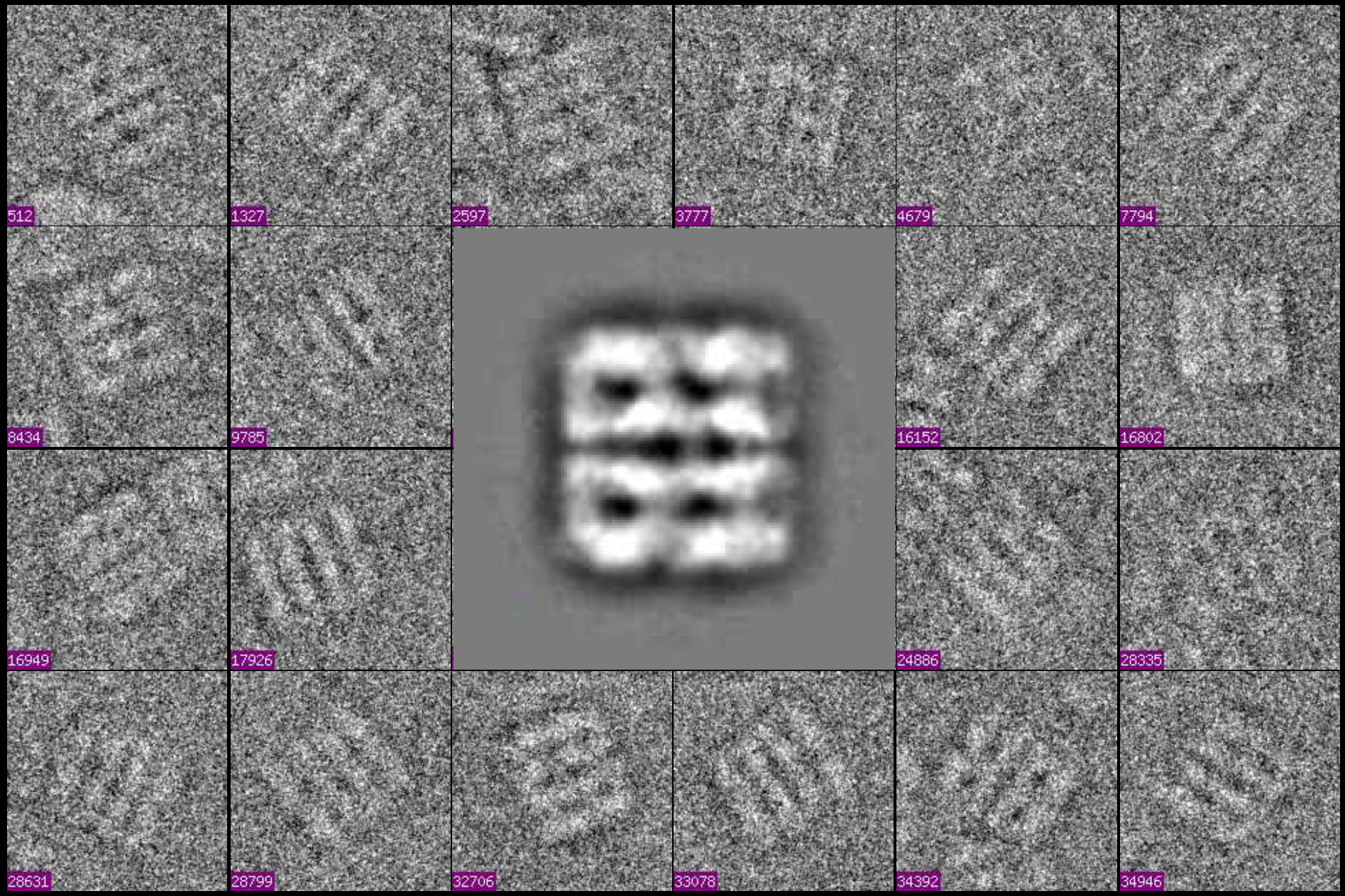


Slide courtesy Gabriel Lander

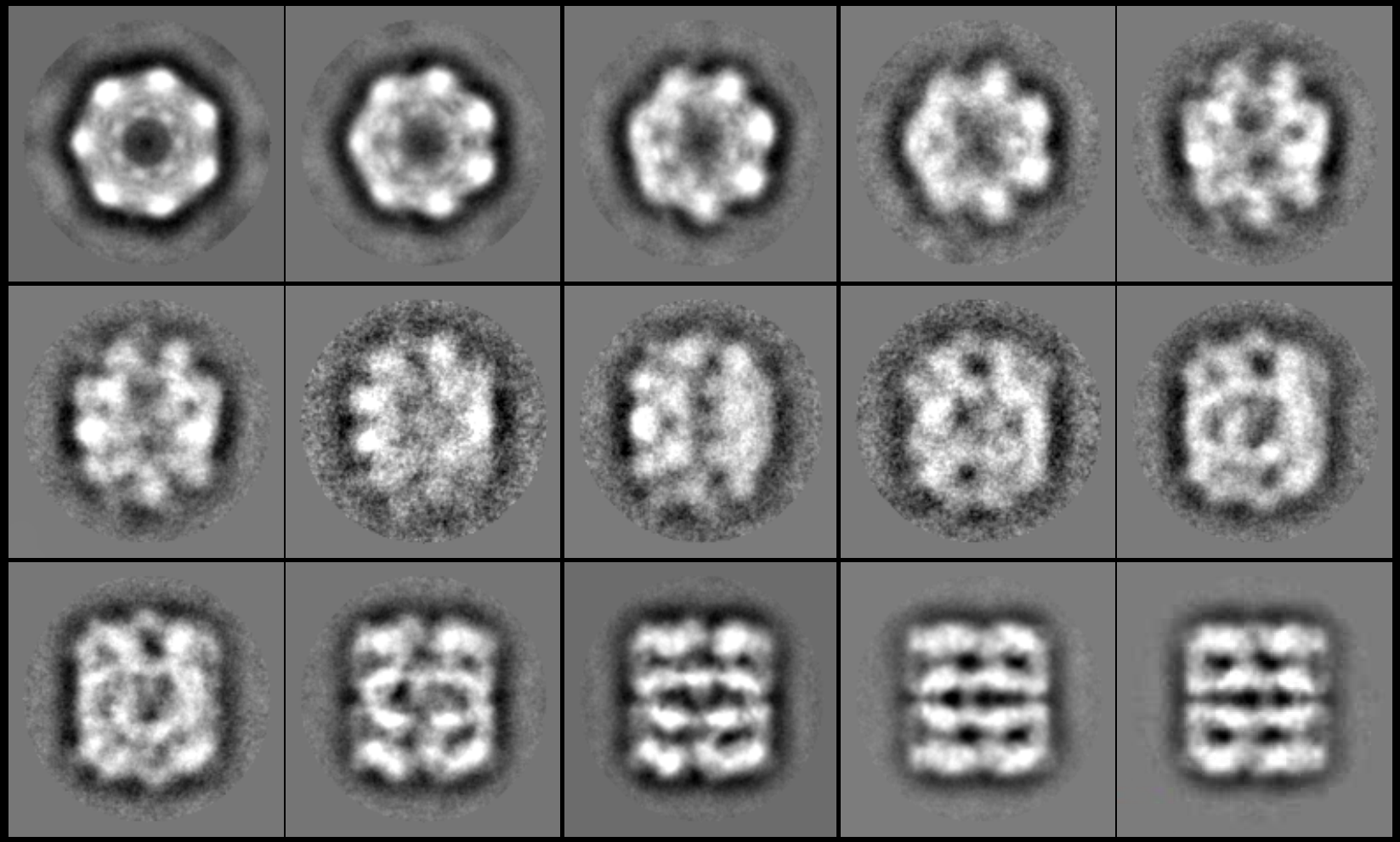



Slide courtesy Gabriel Lander

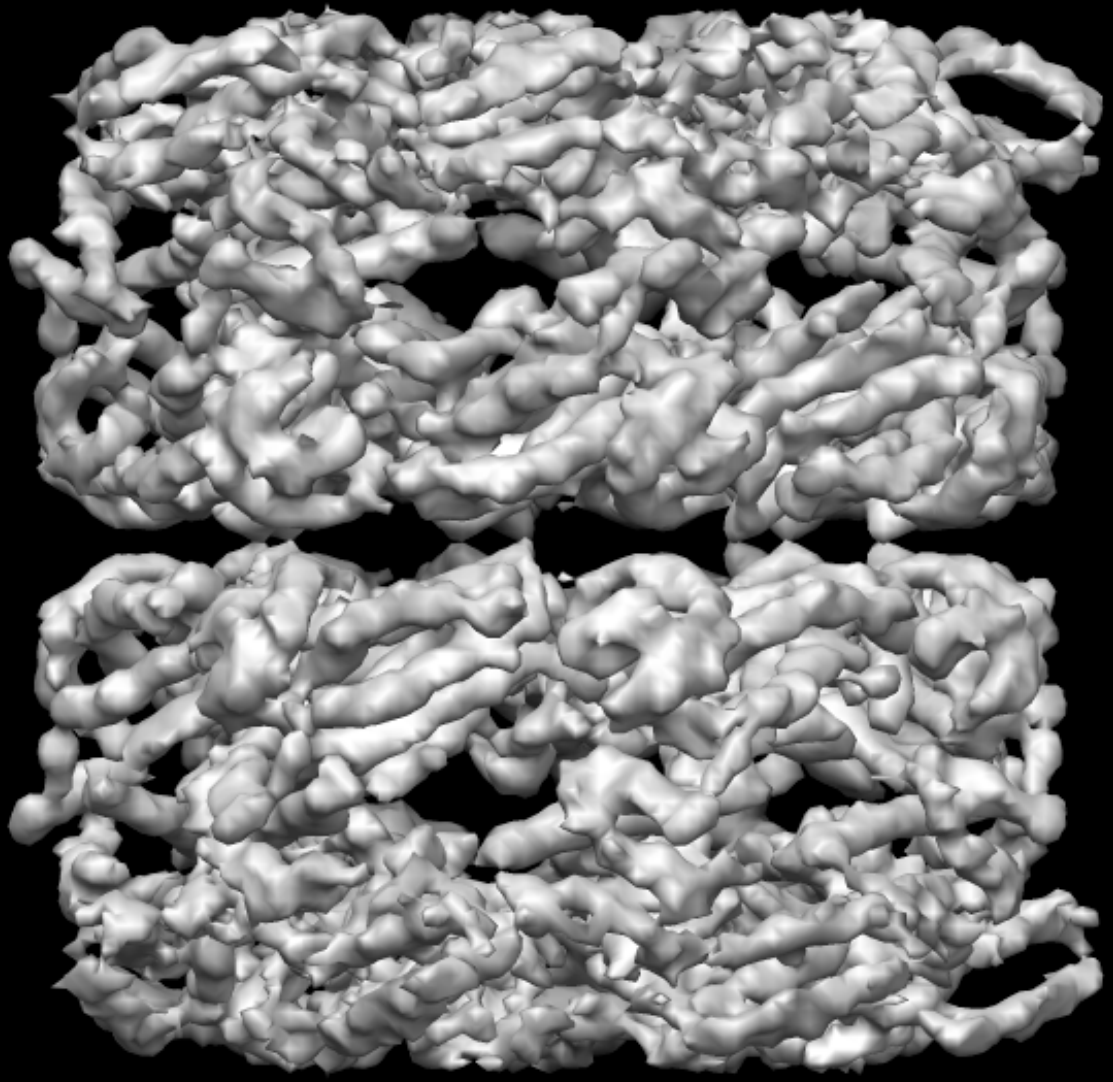

100nm
Slide courtesy Gabriel Lander



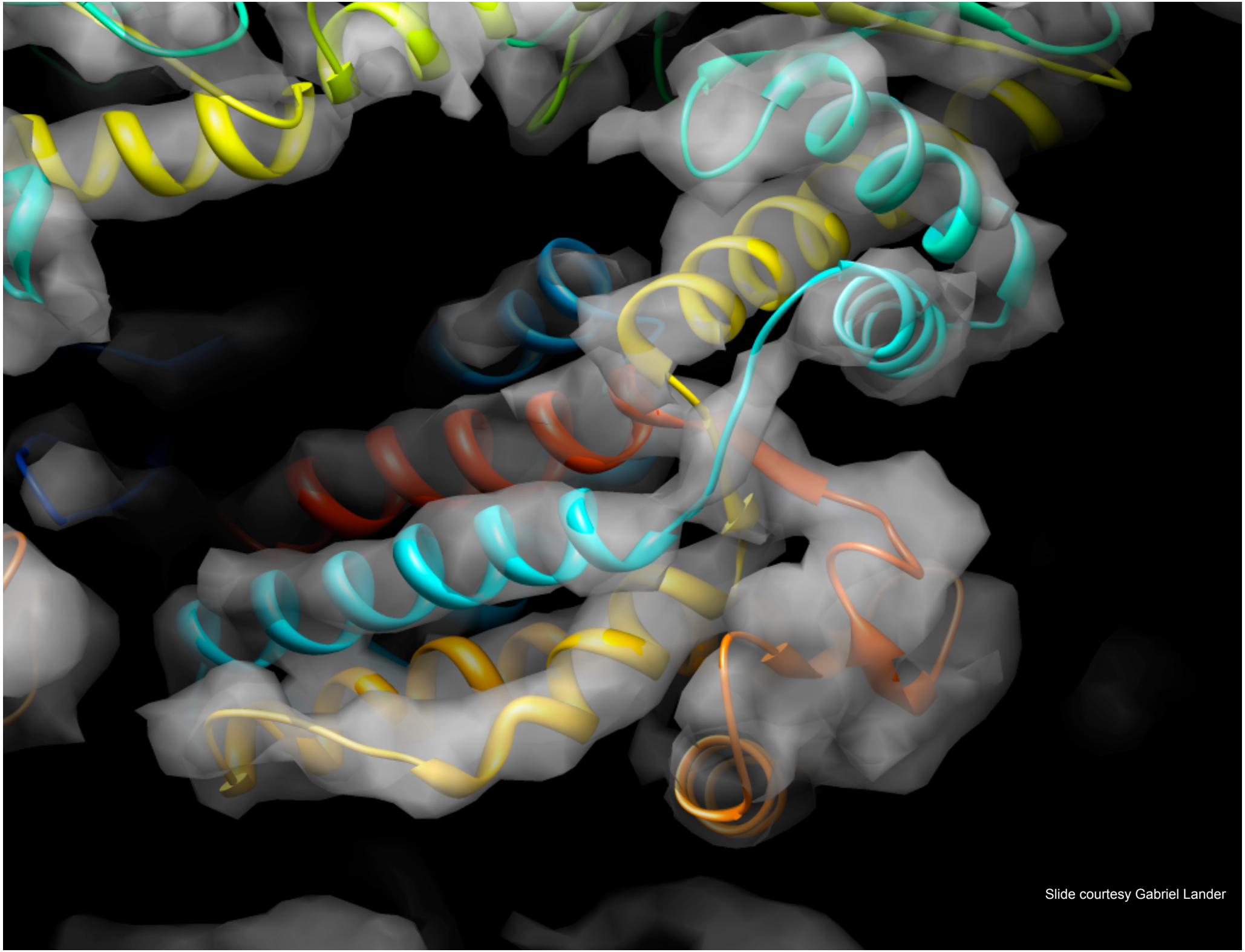
Slide courtesy Gabriel Lander



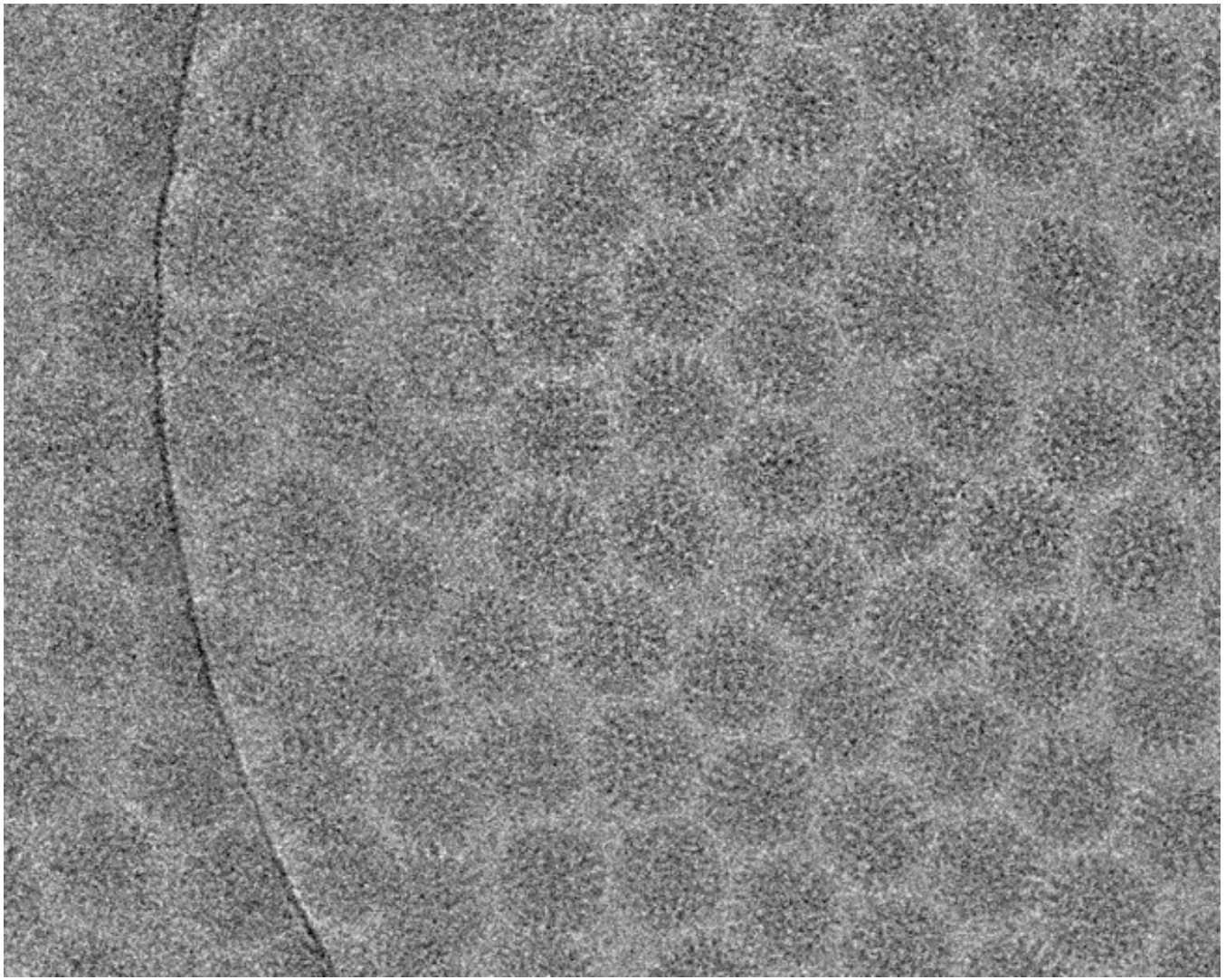
Slide courtesy Gabriel Lander



Slide courtesy Gabriel Lander


Slide courtesy Gabriel Lander

How does a direct detector improve EM performance?



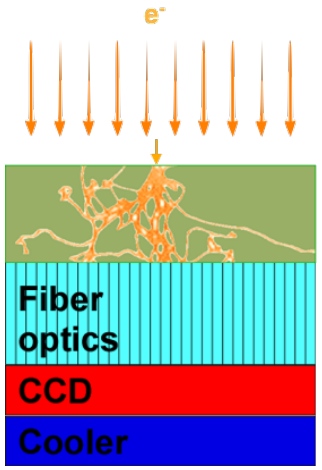
0.5 $e^-/Å^2$/frame

Image = Frame1 + Frame2 + Frame3 + Frame4 + Frame5

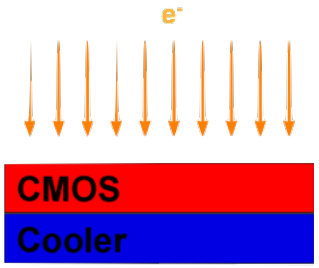
We can use DDD movies to examine (and correct) "beam induced motion"

How does a direct detector improve EM performance?

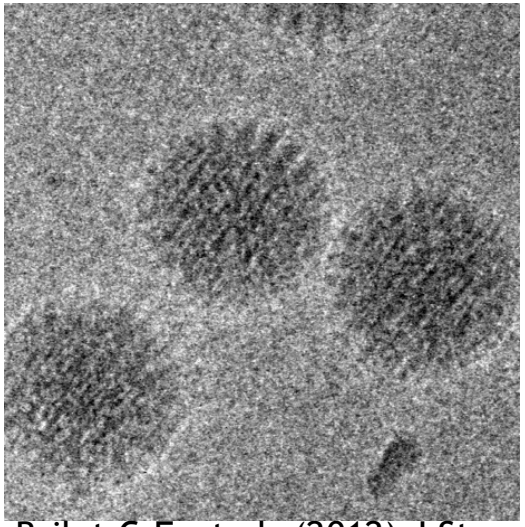
Charge Coupled Device (CCD)



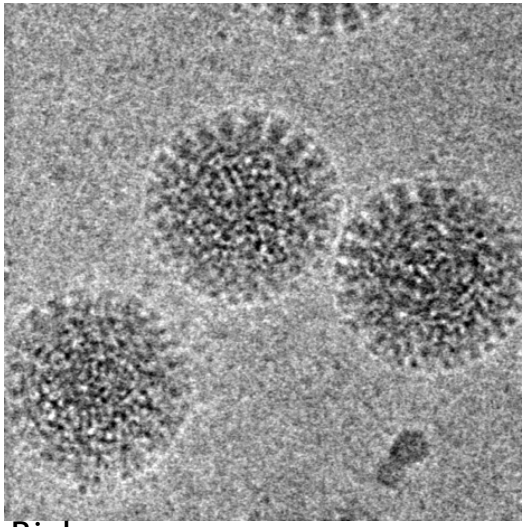
Direct Detection Device (DDD)



60-frame average (translational alignment)



60-frame average (no alignment)



Brilot C.F. et al. (2012) J Struct Biol.

2.8 Å resolution reconstruction of the 20 S proteasome

"Cryo-EM"

Scope of EM

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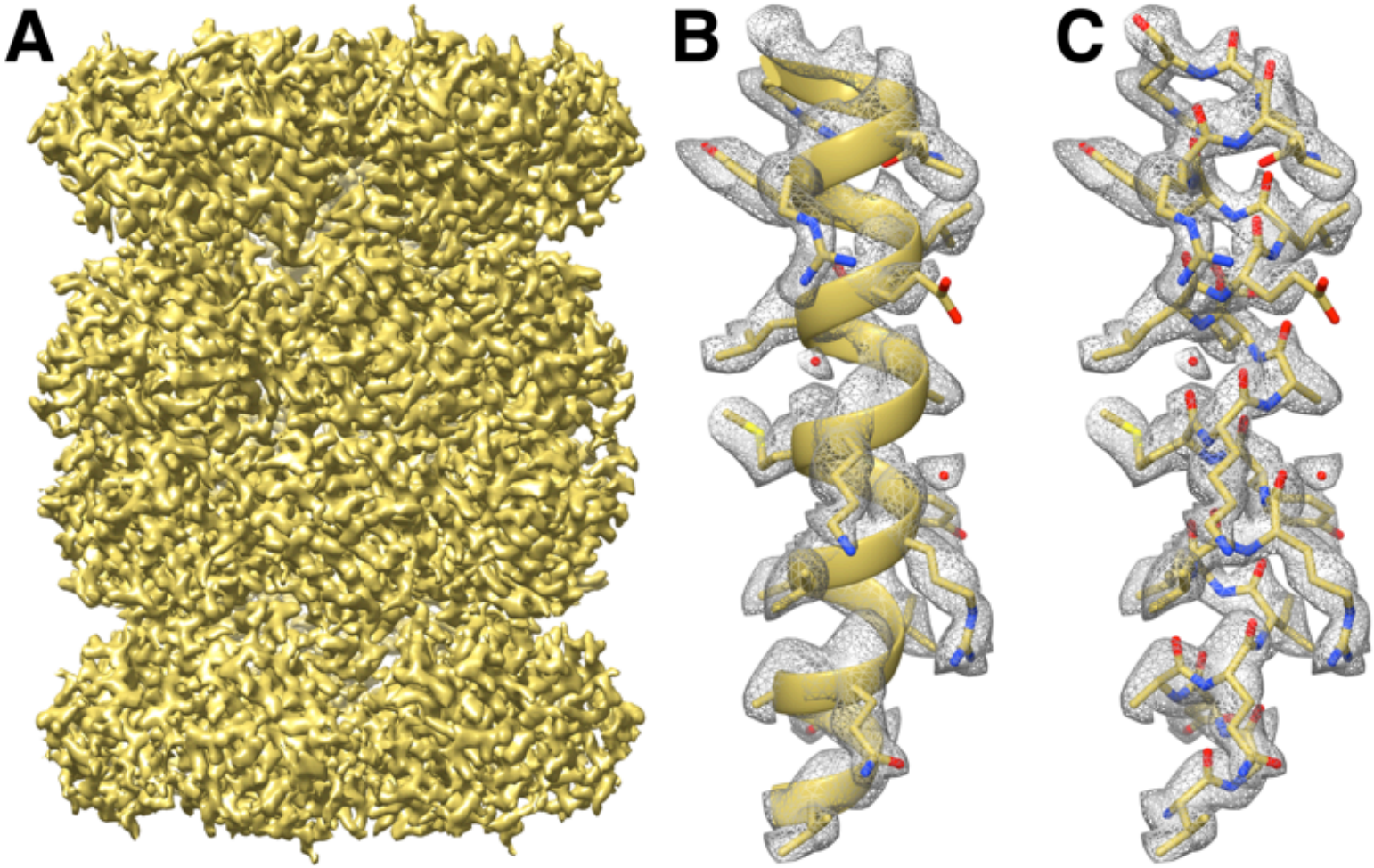
Challenges and frontiers



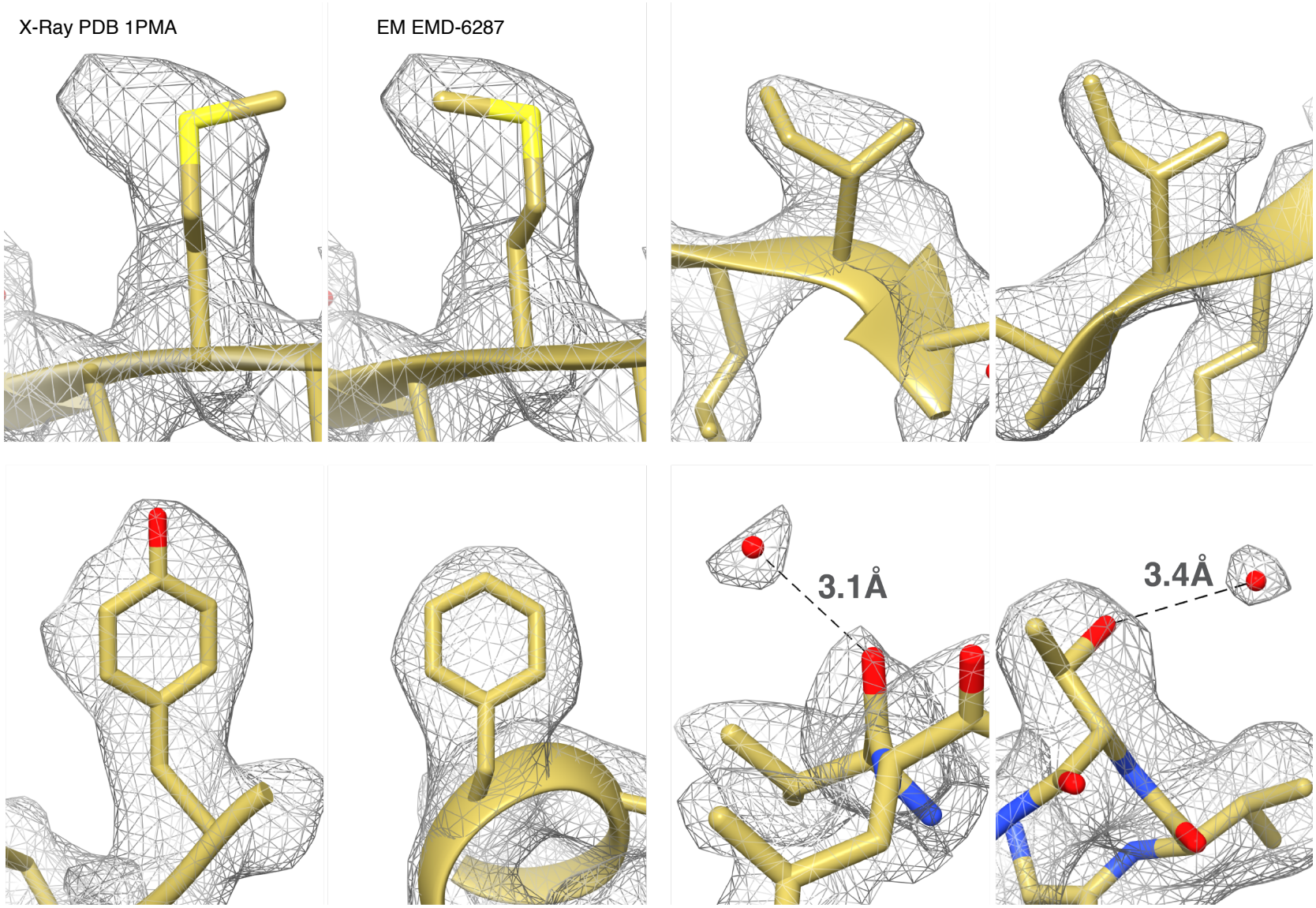

Melody Campbell


David Veesler

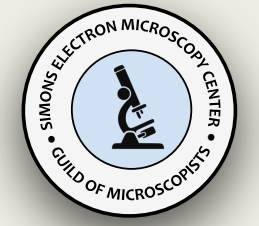

Anchi Cheng

• Melody Campbell, David Veesler, Anchi Cheng, Bridget Carragher, and Clinton S. Potter (2015). 2.8 Å resolution reconstruction of the Thermoplasma acidophilum 20 S proteasome using cryo-electron microscopy. eLife.

2.8 Å resolution reconstruction of the 20 S proteasome



Looking at ordered arrays and small macromolecules



"Cryo-EM"

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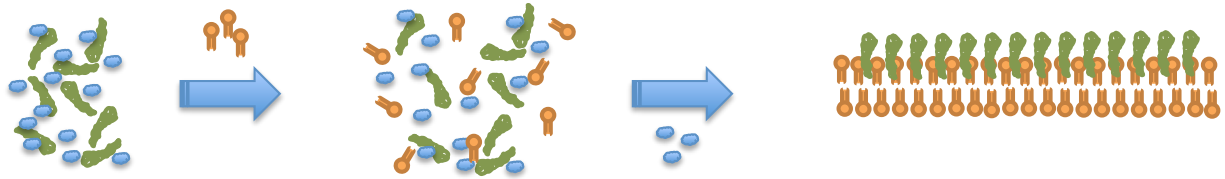
Section 4: 2D crystallography

4-1: Iban Ubarretxena [MSSM]

4-2: Hernando Sosa [AECOM]

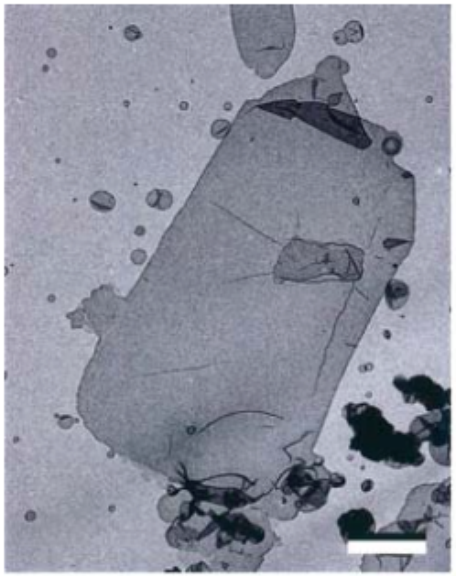
Looking at ordered arrays and small macromolecules

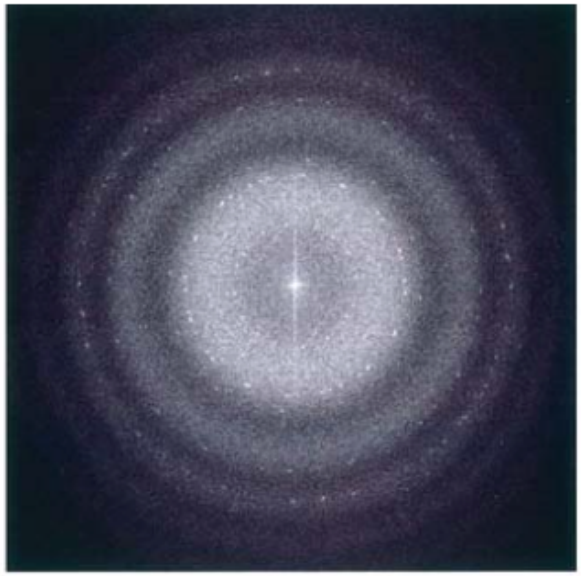


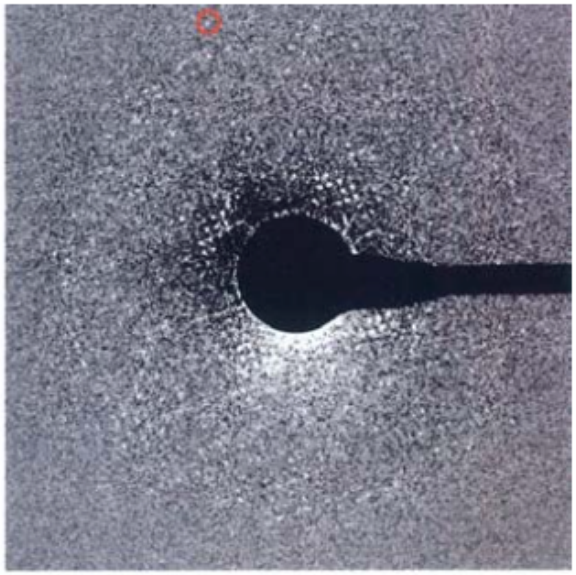
Membrane protein purified in detergent

Detergent solubilized lipids added

Different buffer components screened and detergent removed

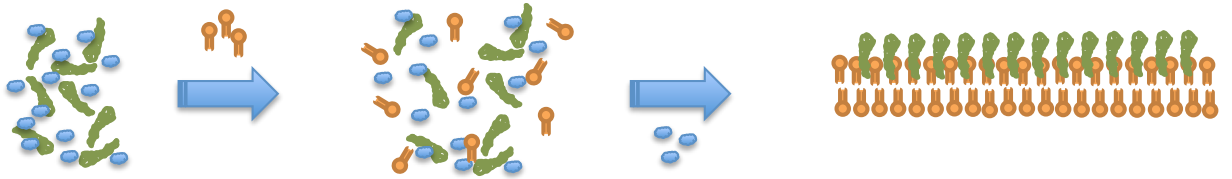






doi: 10.1038/npg.els.0003044

Looking at ordered arrays and small macromolecules



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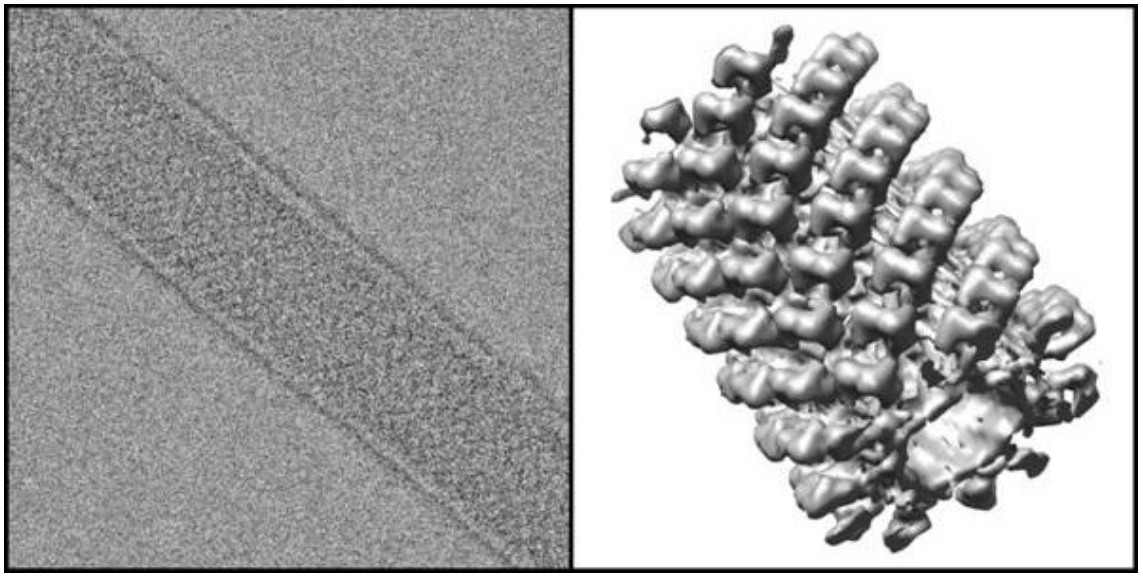
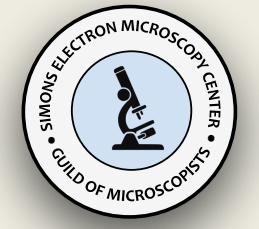


Class schedule



"Cryo-EM"

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EM modalities

Challenges and frontiers

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Section 5: EM challenges and new frontiers

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2. **Validation methods**
3. **Fitting Atomic Models**
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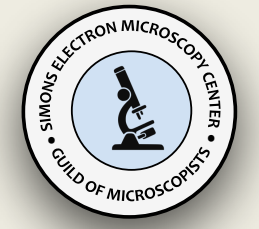
The next steps



"Cryo-EM"

Scope of EM

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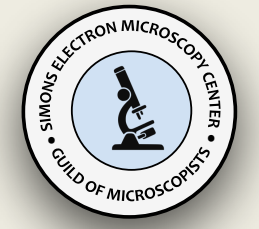
Section 5: EM challenges and new frontiers

5-1: EMDB,
Cathy Lawson [Rutgers]

5-2: Validation methods
Tom Walz [Rockefeller University]

5-3: Fitting Atomic models
Oli Clarke [Columbia University]

Class organization



Welcome

Logistics

"CryoEM"

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Schedule

Welcome

Logistics

"CryoEM"

Introduction to the course

1. Welcome new students
2. Course logistics
   – Questionnaire
3. Introduction to EM &
   the course schedule

Simons Electron Microscopy Center

1. SEMC training programs
2. Tour of the facility