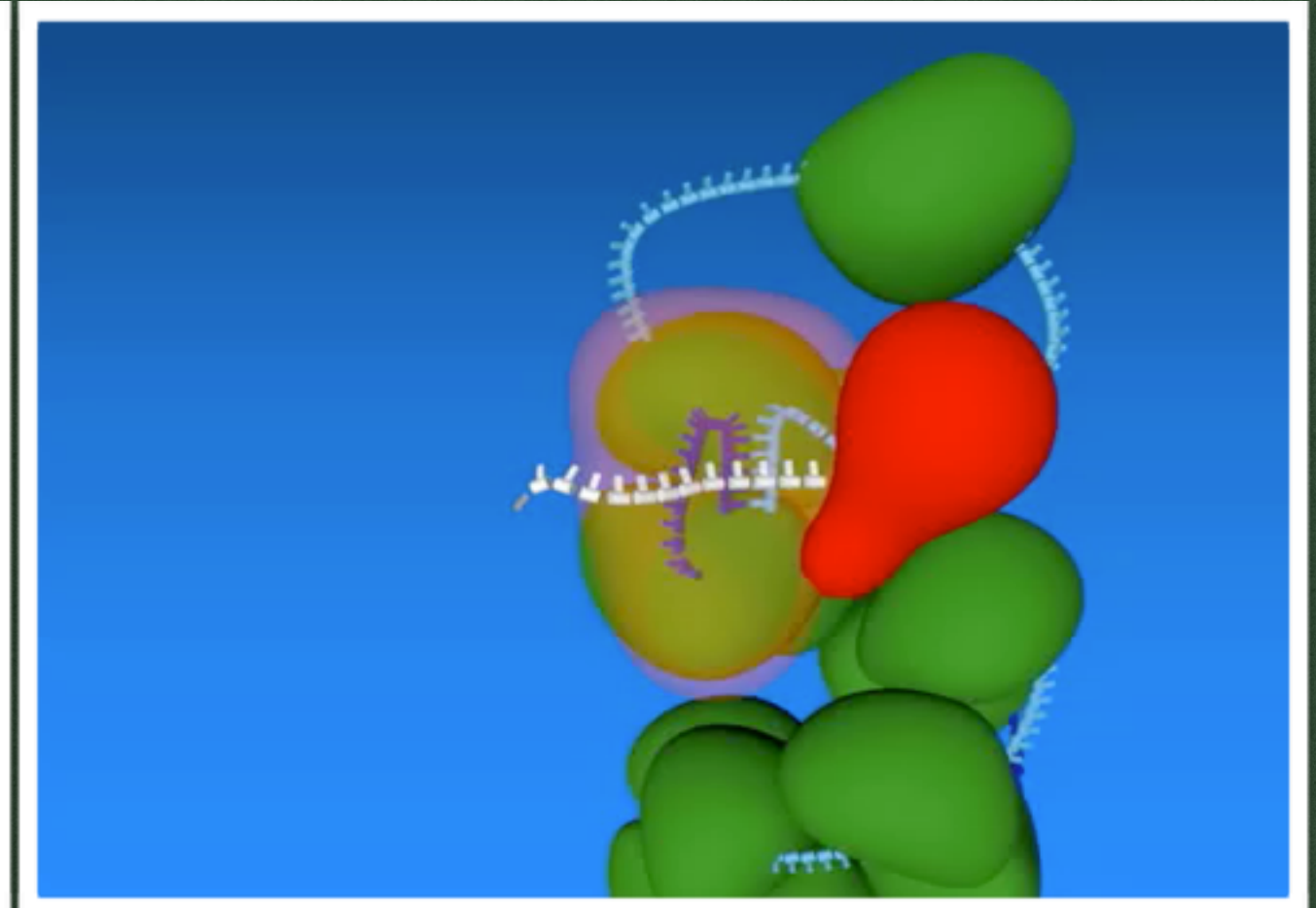
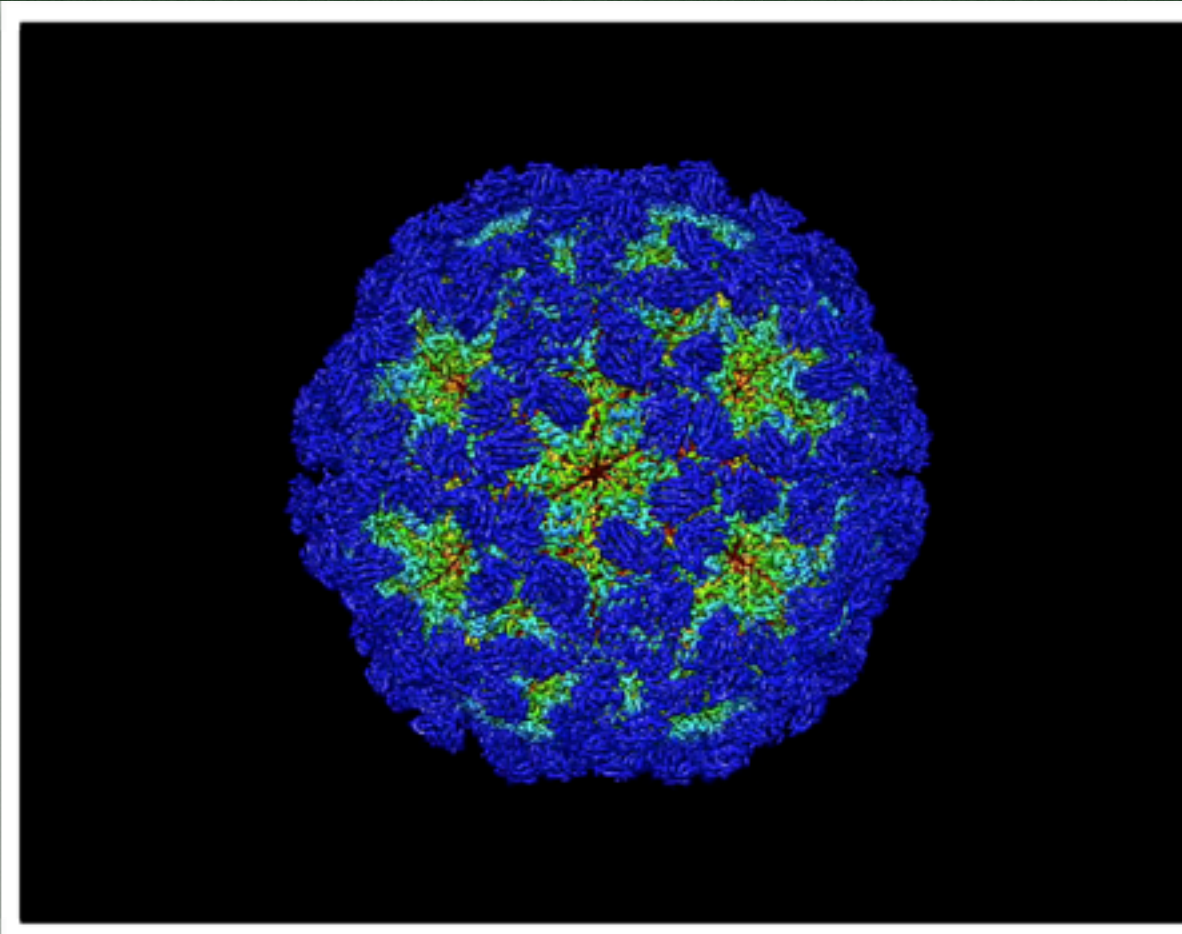
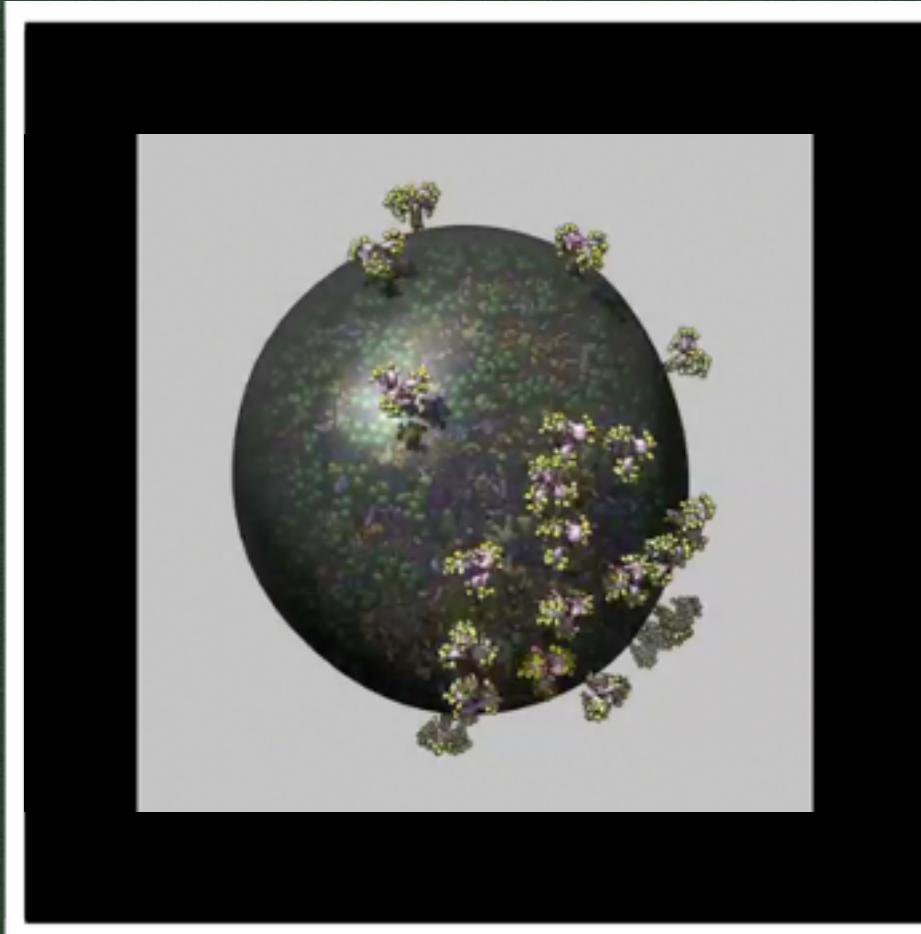
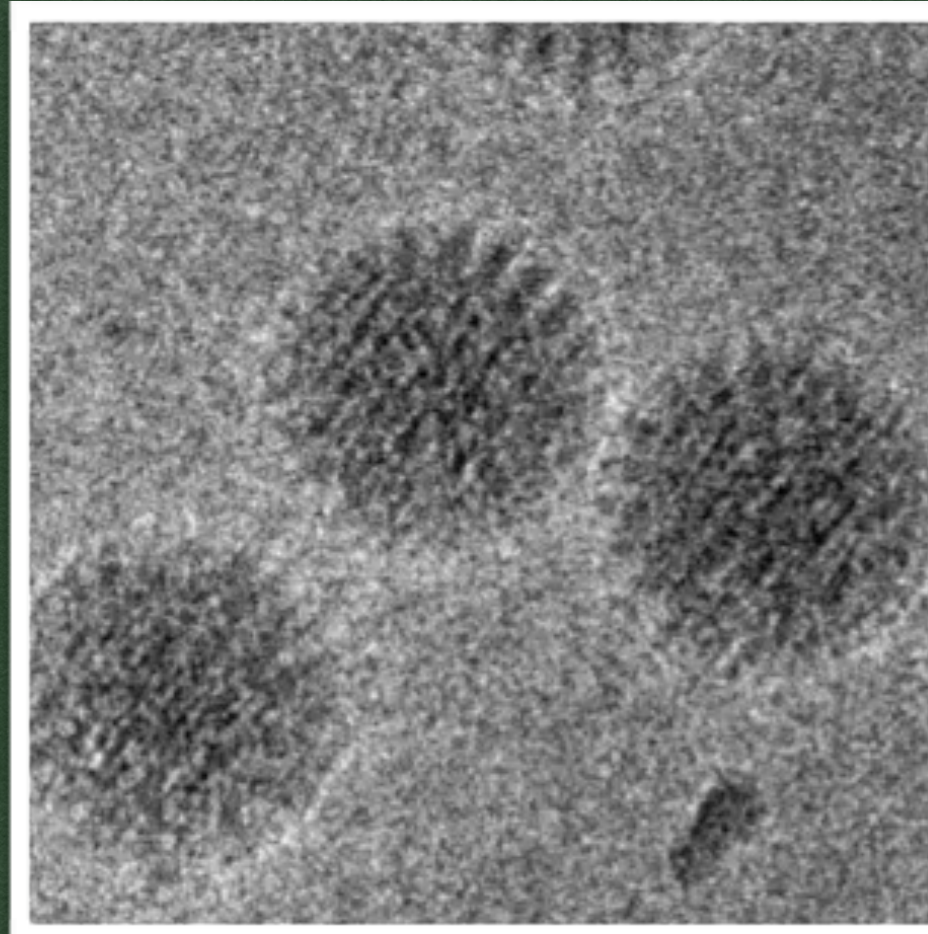




SIMONS ELECTRON  
MICROSCOPY CENTER

New York Structural  
Biology Center



animations courtesy of NRAMM



NATIONAL CENTER FOR  
CRYOEM ACCESS & TRAINING

Edward T Eng  
January 7, 2019

# 2019 WINTER EM COURSE

40 Min Intro + 20 min tour  
07 Jan 2019



# NEW YORK STRUCTURAL BIOLOGY CENTER

I 4th year of the course



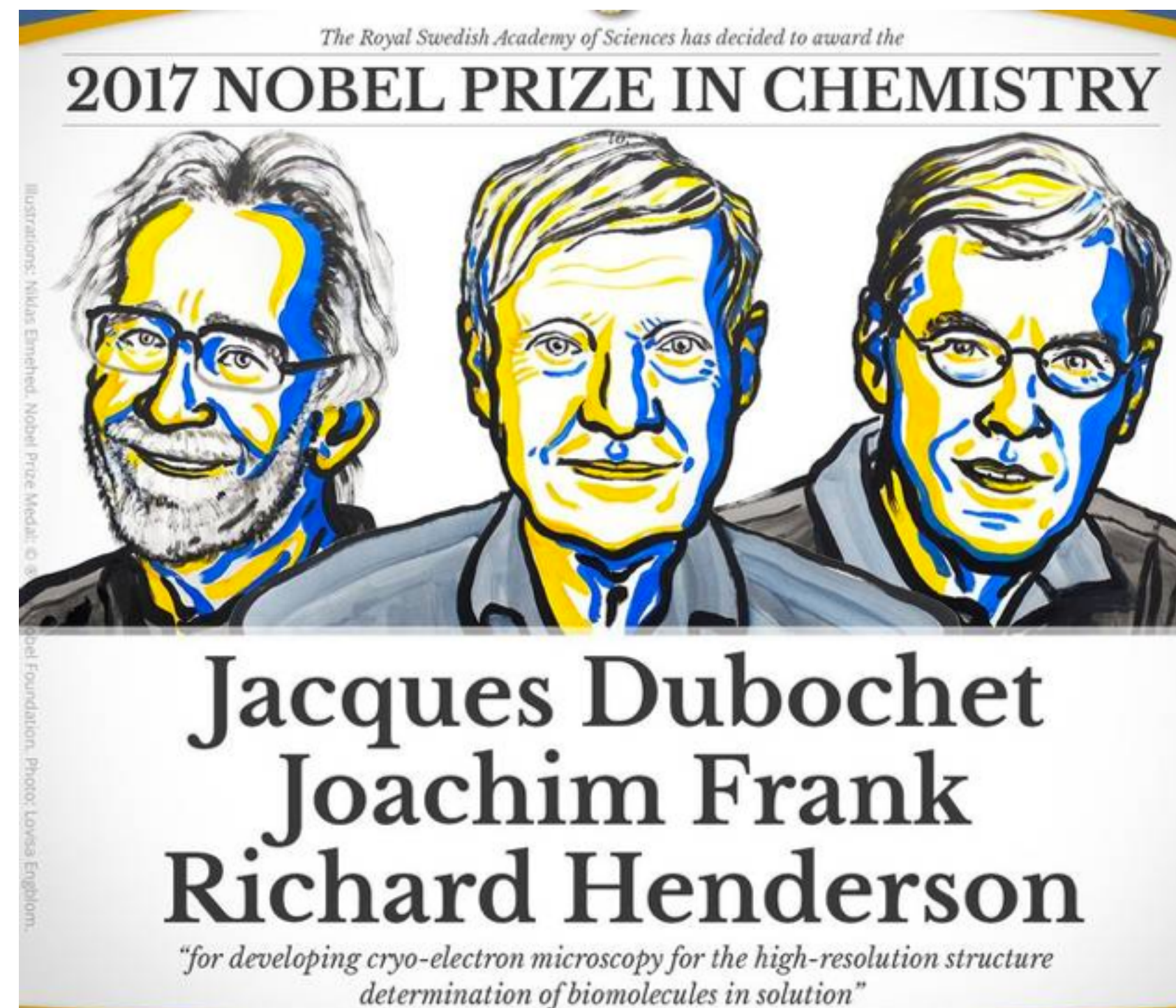


# TECHNOLOGY ON THE RISE

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



Chemistry Nobel prize 2017



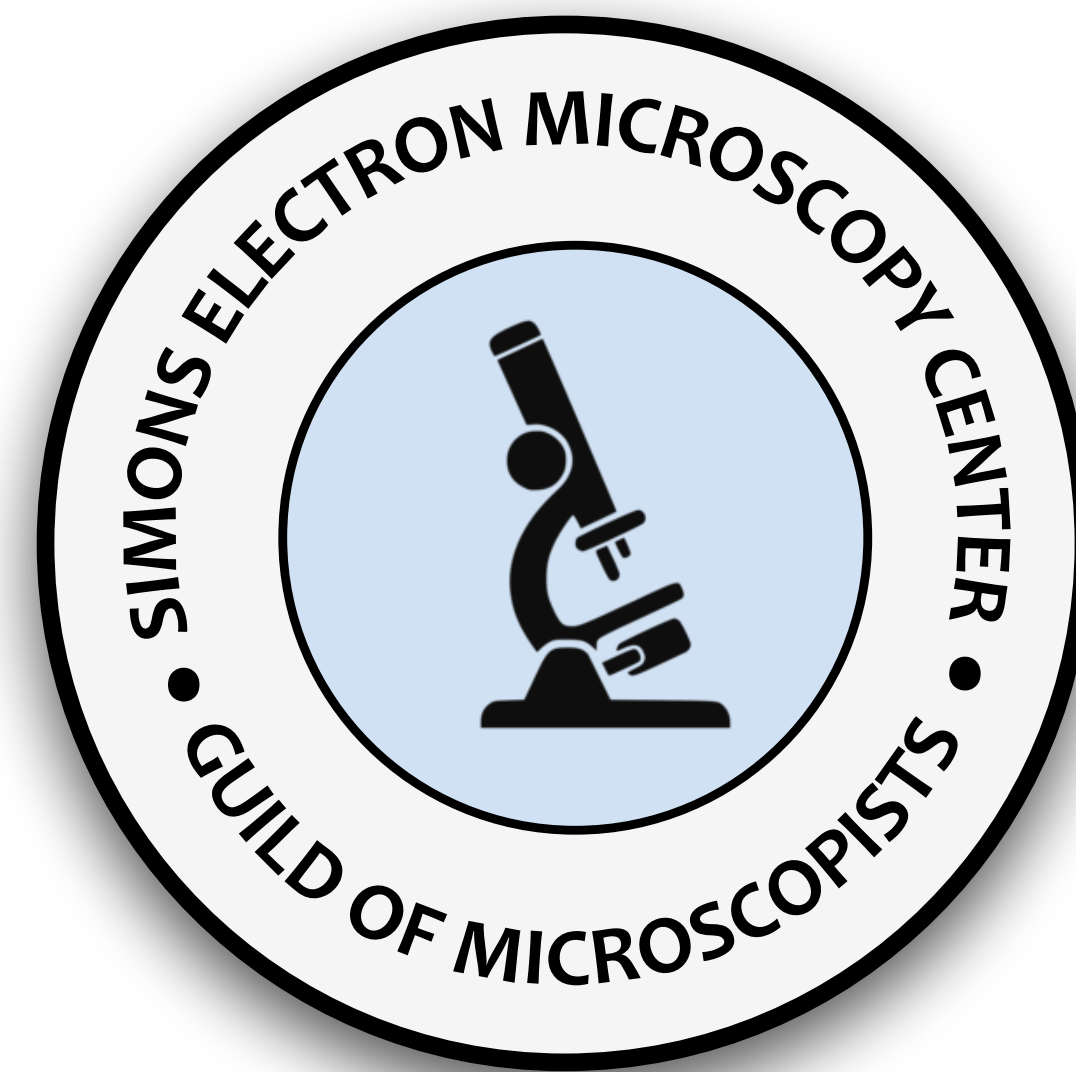
microED  
Science breakthrough of the year  
runner-up 2018





# COURSE INTRODUCTION

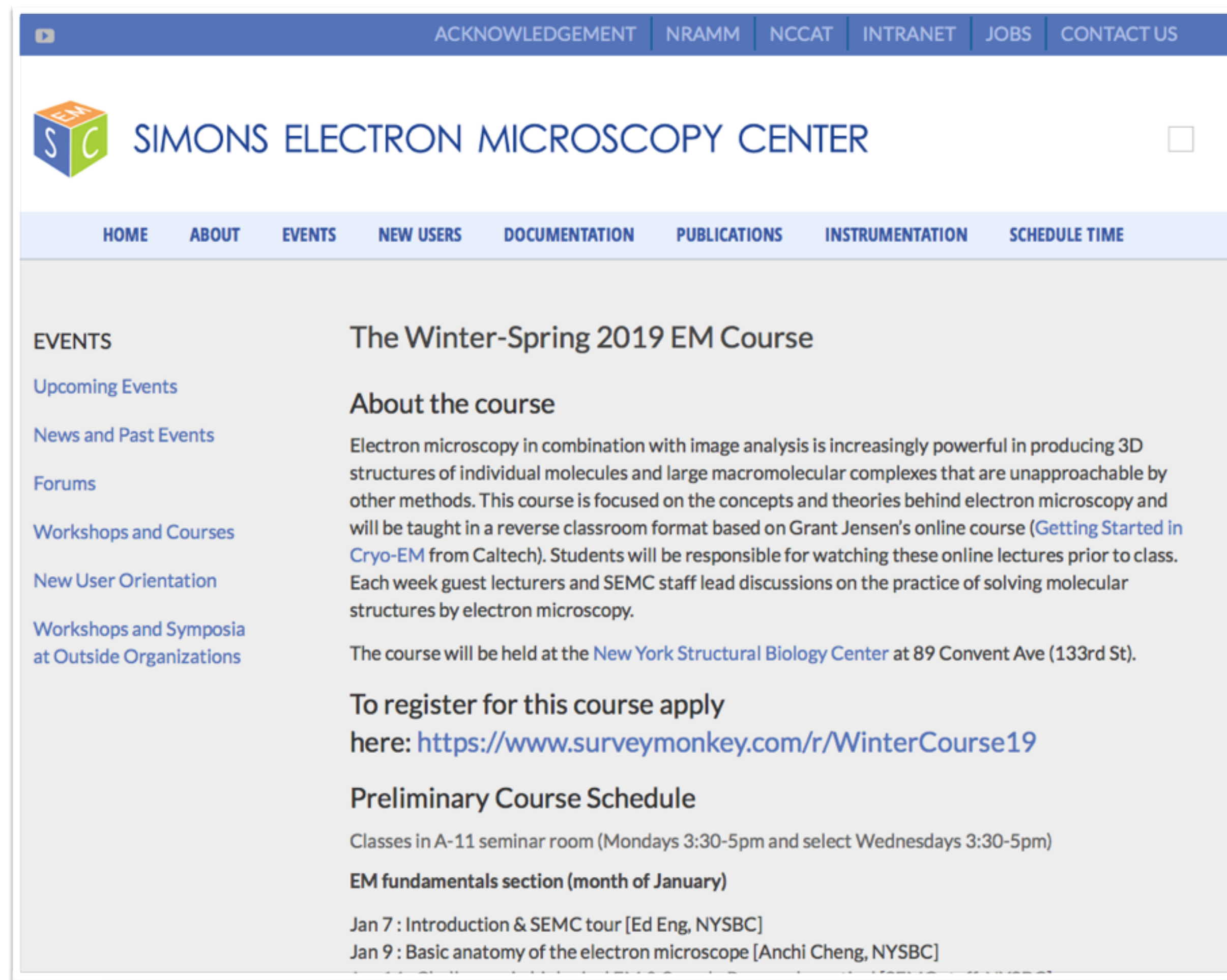
1. Welcome new students
2. Course logistics
3. Introduction to EM
4. Tour of the facility





# COURSE LOGISTICS

<http://semc.nysbc.org/the-winter-spring-2019-em-course/>



The screenshot shows the website of the SIMONS ELECTRON MICROSCOPY CENTER. The top navigation bar includes links for ACKNOWLEDGEMENT, NRAMM, NCCAT, INTRANET, JOBS, and CONTACT US. The main header features the center's logo and name. Below this is a secondary navigation bar with links for HOME, ABOUT, EVENTS, NEW USERS, DOCUMENTATION, PUBLICATIONS, INSTRUMENTATION, and SCHEDULE TIME. The main content area is titled "The Winter-Spring 2019 EM Course" and includes a sidebar with links to EVENTS, Upcoming Events, News and Past Events, Forums, Workshops and Courses, New User Orientation, and Workshops and Symposia at Outside Organizations. The main text describes the course as a reverse classroom format focusing on electron microscopy concepts and theories, based on Grant Jensen's online course "Getting Started in Cryo-EM from Caltech". It mentions that students will watch online lectures prior to class and that guest lecturers and SEMC staff will lead discussions on solving molecular structures. The course will be held at the New York Structural Biology Center at 89 Convent Ave (133rd St). Registration information is provided, including a link to a survey: <https://www.surveymonkey.com/r/WinterCourse19>. A preliminary course schedule is also listed, with classes in A-11 seminar room (Mondays 3:30-5pm and select Wednesdays 3:30-5pm). The EM fundamentals section (month of January) includes: Jan 7 : Introduction & SEMC tour [Ed Eng, NYSBC] and Jan 9 : Basic anatomy of the electron microscope [Anchi Cheng, NYSBC].

## Course Leader:

Ed Eng ([eeng@nysbc.org](mailto:eeng@nysbc.org))

## Classroom instructor:

Laura Yen ([lyen@nysbc.org](mailto:lyen@nysbc.org))

## Teaching Assistants:

Yong Zi Tan ([yztan@nysbc.org](mailto:yztan@nysbc.org))

Micah Rapp ([mar2294@columbia.edu](mailto:mar2294@columbia.edu))



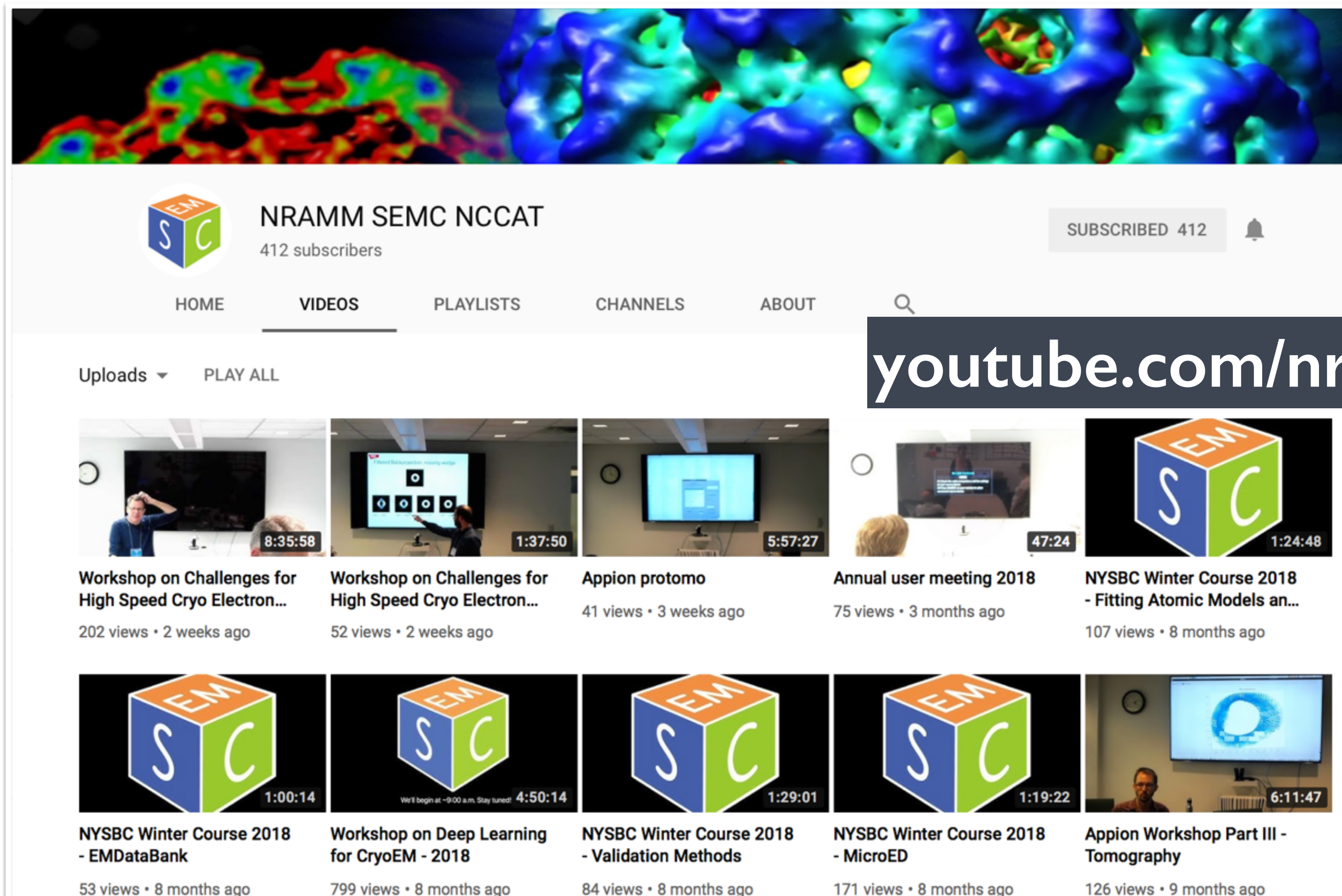
# COURSE LOGISTICS

- Questionnaire
  - [https://www.surveymonkey.com/r/SEMC\\_course\\_Registration](https://www.surveymonkey.com/r/SEMC_course_Registration)
  - email list
  - SEMC Winter EM Course 2019 Course Handbook
- Active research facility
  - public areas





# COURSE LOGISTICS



**NRAMM SEMC NCCAT**  
412 subscribers

SUBSCRIBED 412

HOME VIDEOS PLAYLISTS CHANNELS ABOUT

Uploads ▾ PLAY ALL

**Workshop on Challenges for High Speed Cryo Electron...**  
8:35:58  
202 views • 2 weeks ago

**Workshop on Challenges for High Speed Cryo Electron...**  
1:37:50  
52 views • 2 weeks ago

**Appion protomo**  
5:57:27  
41 views • 3 weeks ago

**Annual user meeting 2018**  
47:24  
75 views • 3 months ago

**NYSBC Winter Course 2018 - Fitting Atomic Models an...**  
1:24:48  
107 views • 8 months ago

**NYSBC Winter Course 2018 - EMDatabank**  
1:00:14  
53 views • 8 months ago

**Workshop on Deep Learning for CryoEM - 2018**  
4:50:14  
799 views • 8 months ago

**NYSBC Winter Course 2018 - Validation Methods**  
1:29:01  
84 views • 8 months ago

**NYSBC Winter Course 2018 - MicroED**  
1:19:22  
171 views • 8 months ago

**Appion Workshop Part III - Tomography**  
6:11:47  
126 views • 9 months ago

[youtube.com/nrammsemc](https://youtube.com/nrammsemc)



# COURSE TEXTBOOK

other courses & lectures ..

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

**Caltech** Getting Started in Cryo-EM

Welcome Course Overview Outline Lecture Videos Instructor Links

**WELCOME TO THE COURSE**

Before diving into the lecture videos, start by watching the [trailer](#) and reading the course [overview](#) and [outline](#).

We hope you enjoy learning about cryo-electron microscopy (cryo-EM)!

Getting Started in Cryo-EM with Professor Grant Jensen

CRYOELECTRON MICROSCOPY  
LABORATORY

Established with generous support from  
The Gordon and Betty Moore Foundation  
and  
The Agouron Institute

0:02 / 2:51

Caltech - Getting Started in Cryo-EM  
The Jensen Lab  
Email: [GettingStartedInCryoEM@gmail.com](mailto:GettingStartedInCryoEM@gmail.com)

LOG IN

LMB EM-course

**LMB cryo-EM course 2017**

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

**Mon June 19: Richard Henderson**  
Cryo-EM: past, present & future

**Tue June 20: Chris Russo**  
Microscopy physics and optics

**Wed June 21: Paula da Fonseca**  
Image formation, Fourier analysis, CTF

**Thu June 22: Christos Savva**  
Data acquisition

**Fri June 23: Lori Passmore**  
Sample preparation

**Mon June 26: Sjors Scheres**  
Image refinement in 2D and 3D

**Tue June 27: Rafael Fernandez-Leiro**  
Data processing strategy

**Wed June 28: Alan Brown**  
Atomic modeling & validation

**Thu June 29: John Briggs**  
Tomography

**Fri June 30: Shaoxia Chen & Giuseppe Cannone**  
Local setup and training

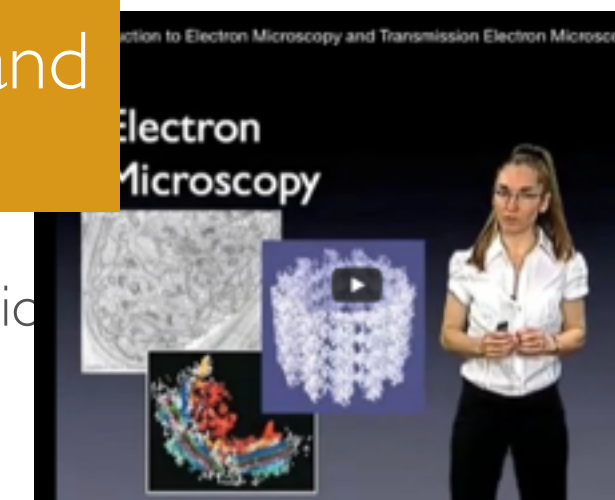
Enquiries: [scheres@mrc-lmb.cam.ac.uk](mailto:scheres@mrc-lmb.cam.ac.uk)

Lecture PDFs and professionally edited videos available on:

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

Introduction to Electron Microscopy and  
Transmission Electron Microscopy

<https://www.ibiology.org/techniques/transmission-electron-microscopy/>



CryoEM101

<https://cryoem101.org>

**CryoEM 101** HOME CHAPTERS ABOUT

**Cryo EM has emerged as a powerful tool for high-resolution structure determination.**

To aid the training efforts of newcomers to the field, we are creating a media-rich curriculum to augment your own hands-on training. The training material includes lecture videos, animations, and interactive simulations covering the major components of the cryo-EM workflow.

Chapter 1  
[Sample Purification](#)

Chapter 2  
Cryo-EM Grid Preparation

Chapter 3  
Grid Screening & Evaluation

Chapter 4  
Cryo-EM Data Collection





# COURSE STRUCTURE

Monday and select Wednesdays  
3:30-5pm - A-11 seminar room /  
SEMC conference room

1.5 hr class

Wednesdays  
Starts at 3:30 - SEMC conference room

Recitation section  
Journal club and practicals

## Recitation schedule

Jan 30: Microscope Practical  
Feb 6: Journal Club 1  
Feb 13: Tomography Practical  
Feb 20: Tomography Practical 2  
Feb 27: Journal club 2  
Mar 6: Single Particle Practical  
Mar 13: Journal club 3  
Mar 20: Journal club 4  
Mar 27: MicroEd Practical  
Apr 3: Journal club 5  
Apr 10: Journal club 6  
Apr 17: Journal club 7  
Apr 24: Journal club 8





# TAKING THE COURSE FOR CREDIT

Component	Percentage
Recitation	50%
Practical Worksheet	10% x 4
Attendance	10%

## Recitation schedule

- Jan 30: Microscope Practical**
- Feb 6: Journal Club 1
- Feb 13: Tomography Practical**
- Feb 20: Tomography Practical 2**
- Feb 27: Journal club 2
- Mar 6: Single Particle Practical**
- Mar 13: Journal club 3
- Mar 20: Journal club 4
- Mar 27: MicroEd Practical**
- Apr 3: Journal club 5
- Apr 10: Journal club 6
- Apr 17: Journal club 7
- Apr 24: Journal club 8



# CLASS ORGANIZATION

Section 1: EM fundamentals section

Section 2: Tomography section

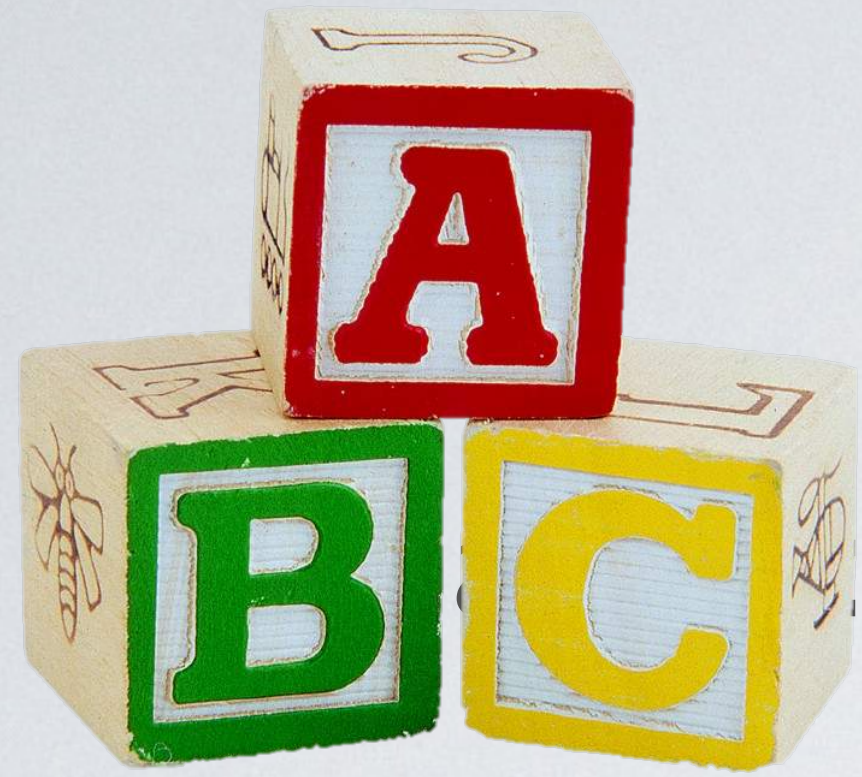
Section 3: Single particle section

Section 4: 2D crystallography section

Section 5: EM challenges and new frontiers







# EM FUNDAMENTALS

Introduction & SEMC tour

Jan 9 : Basic anatomy of the electron microscope

Jan 14 : Challenges in biological EM & Sample Prep

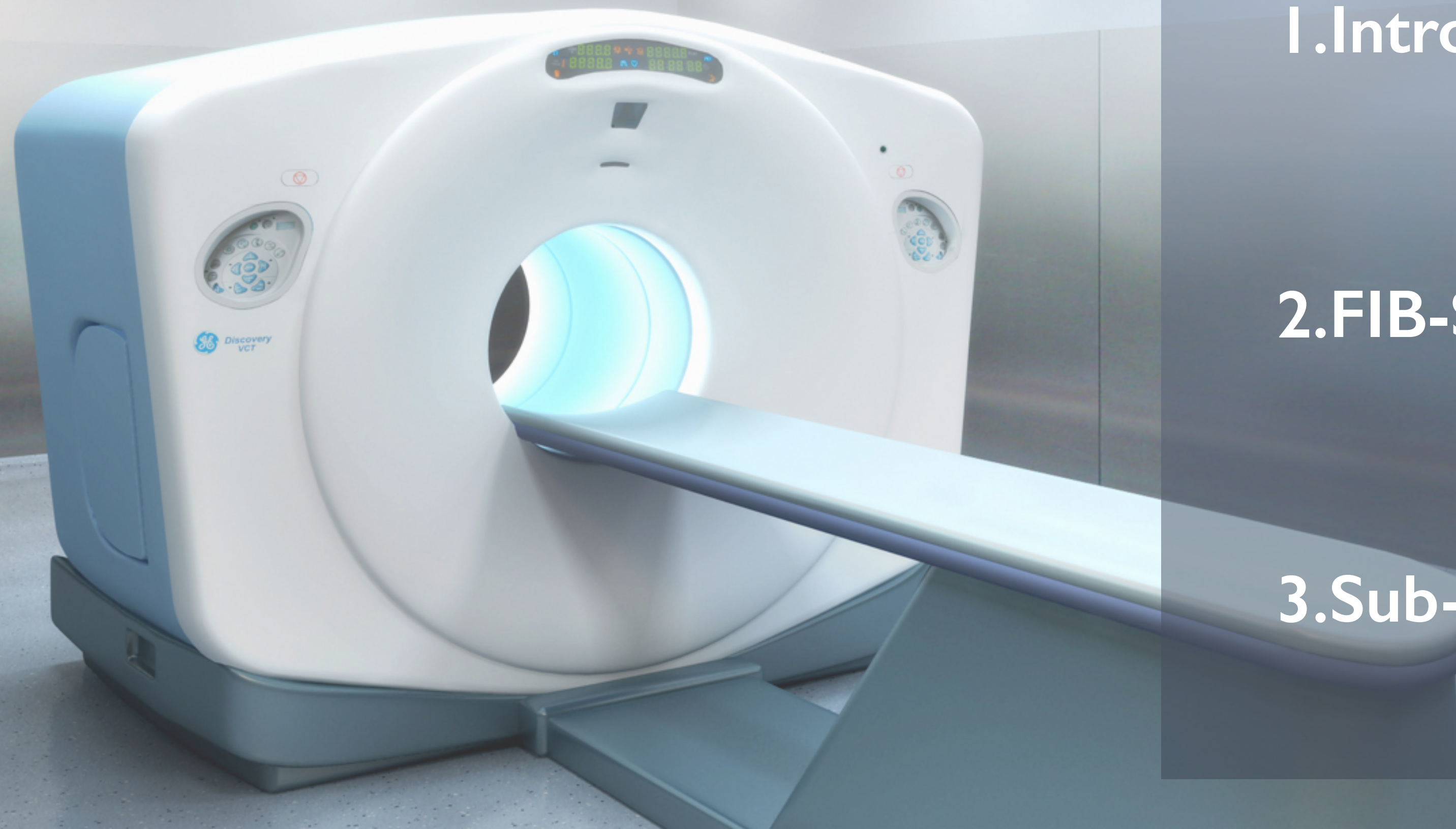
Jan 16 : Support films and practical

Jan 23 : Fourier transforms & Image Formation





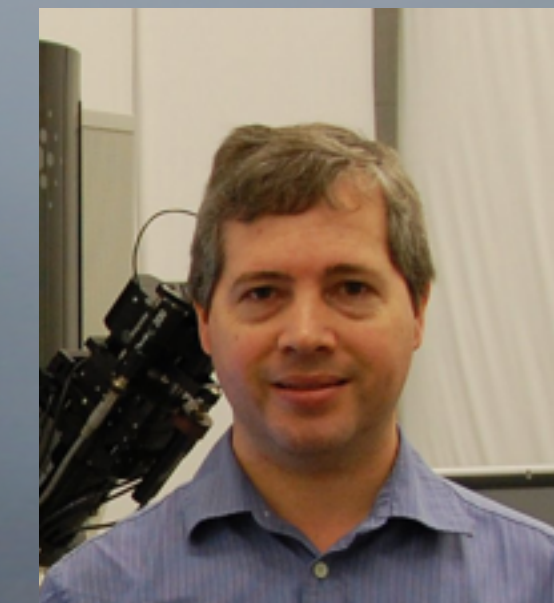
# TOMOGRAPHY SECTION



1. Intro and overview



2. FIB-SEM



3. Sub-tomogram averaging

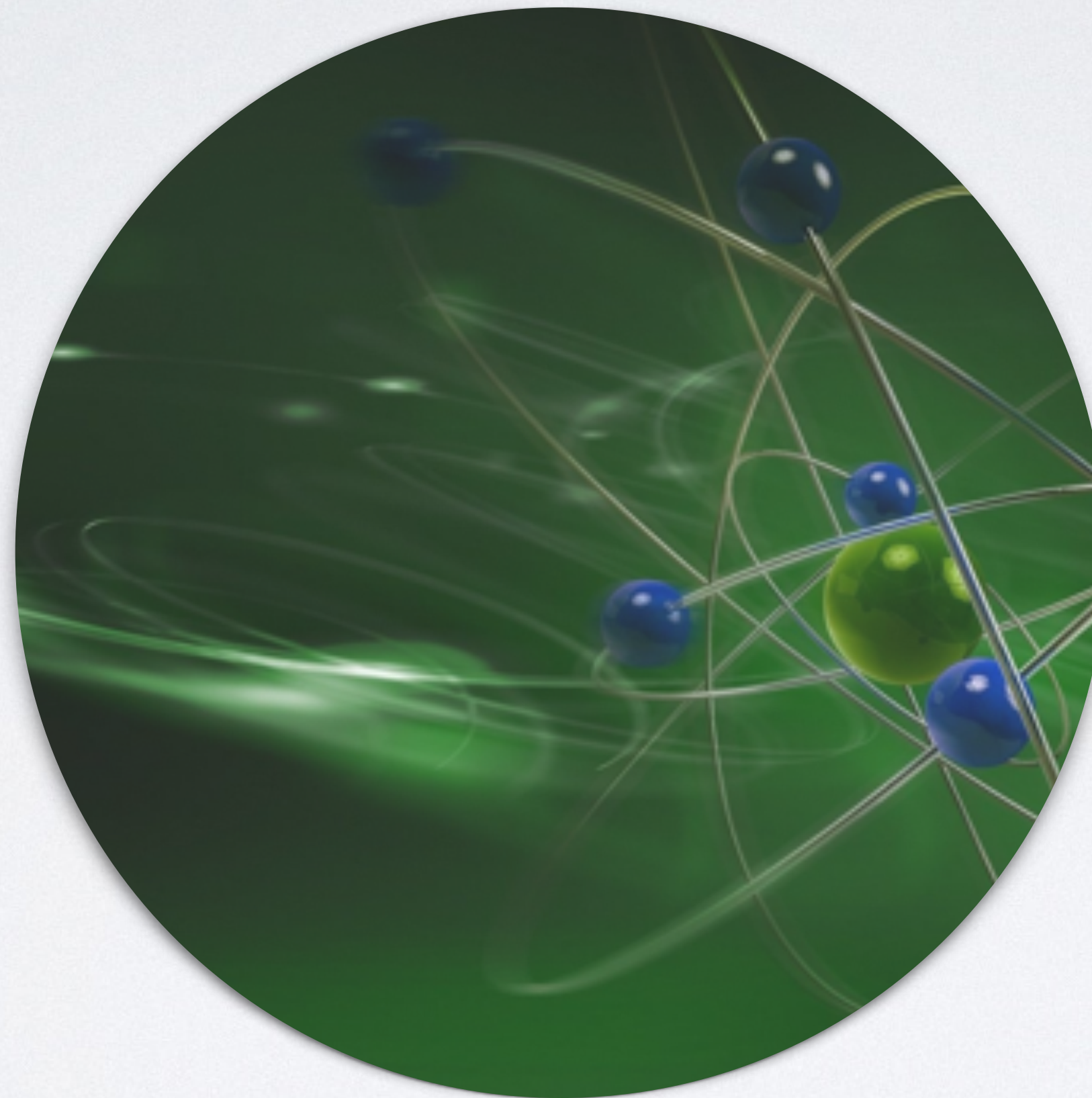




# SINGLE PARTICLE ANALYSIS



## 1. Intro to single particle



## 2. Data Analysis and reconstruction workflow



## 3. Interpretation and Limitations



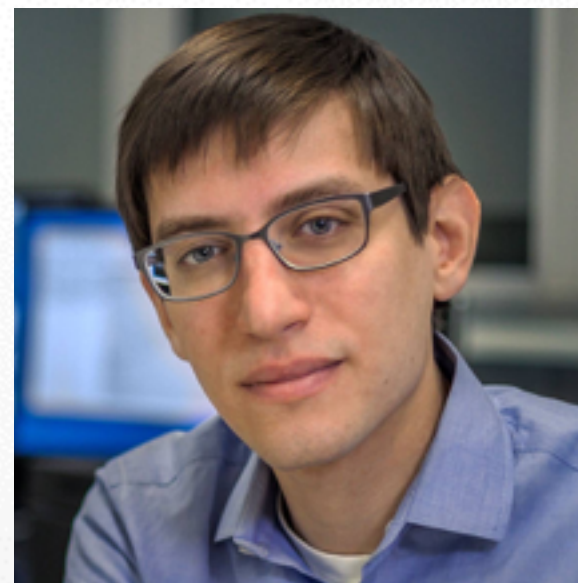


# 2D CRYSTALLOGRAPHY

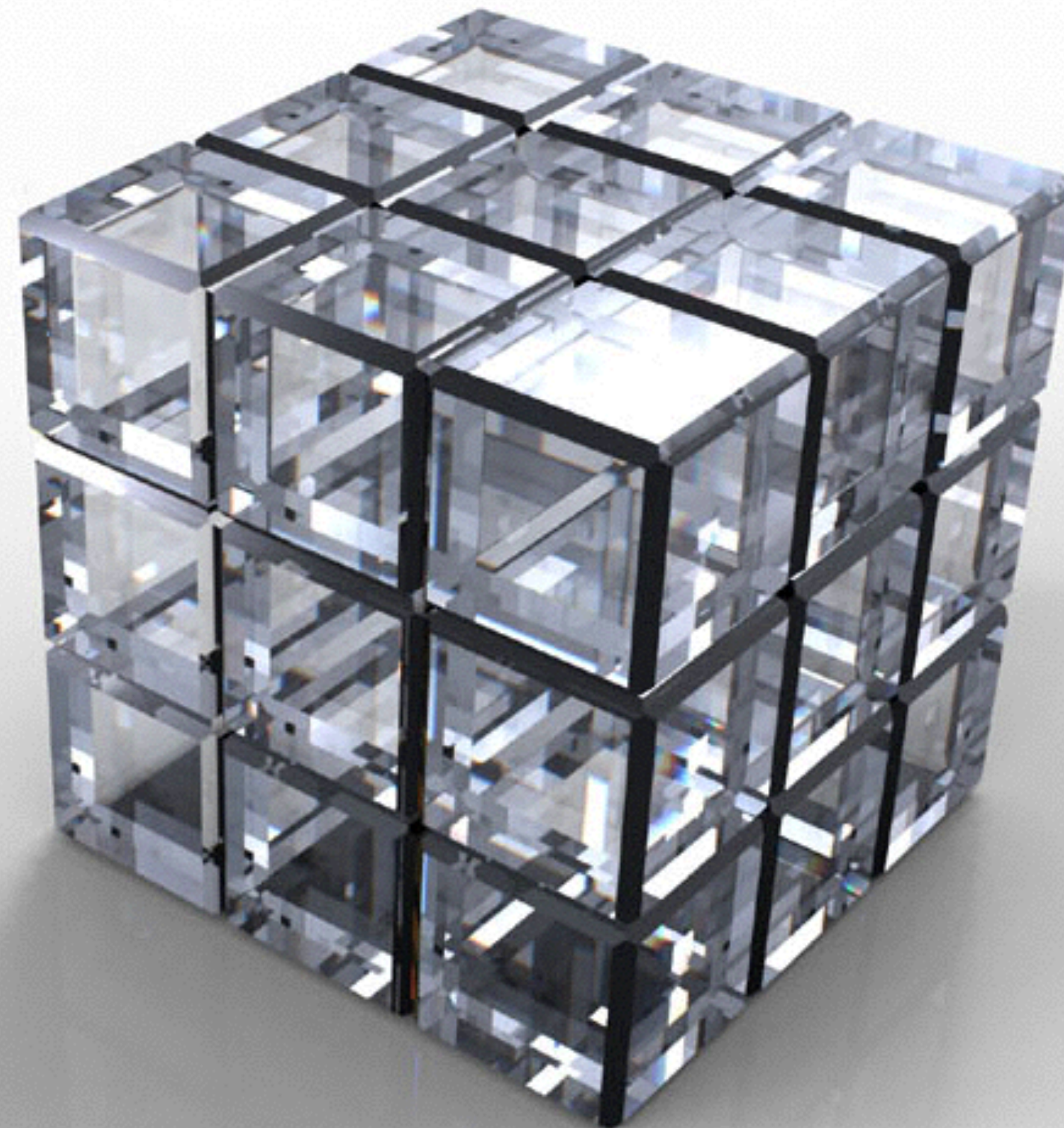
1. MicroED  
and 2D crystallography



2. Introduction to Helical



3. Helical (part II)





# EM CHALLENGES & NEW FRONTIERS



1. Validation  
methods

3. Moderate  
resolution  
interpretation &  
annotation



2. EMDataBank:  
Structure Data  
Archiving,  
Validation  
Challenges

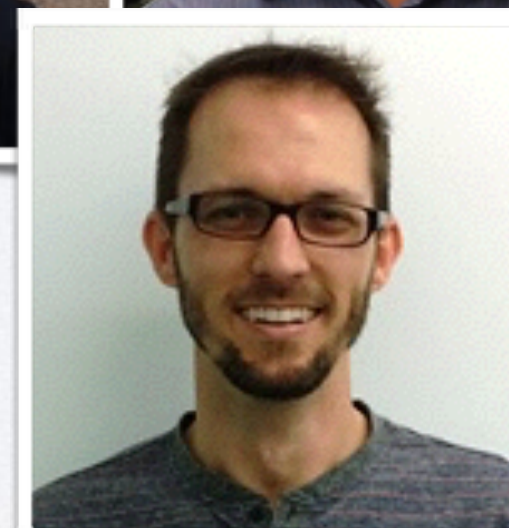
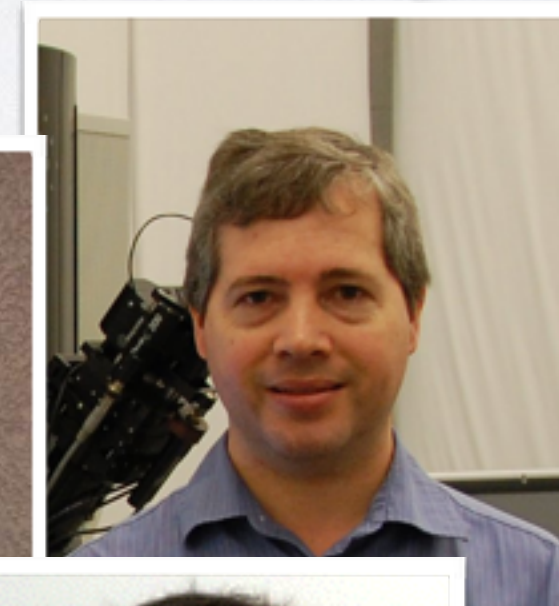
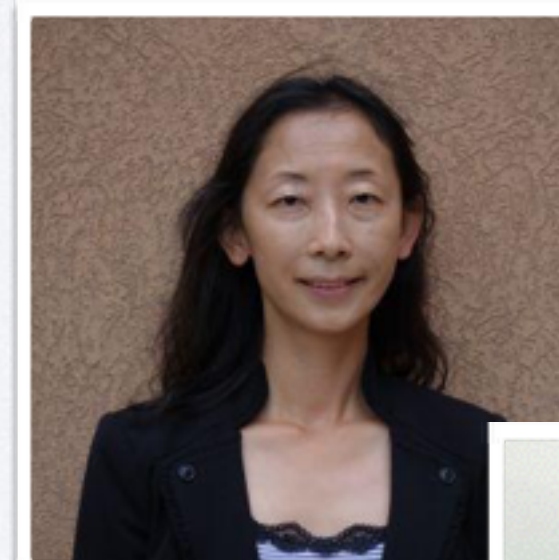
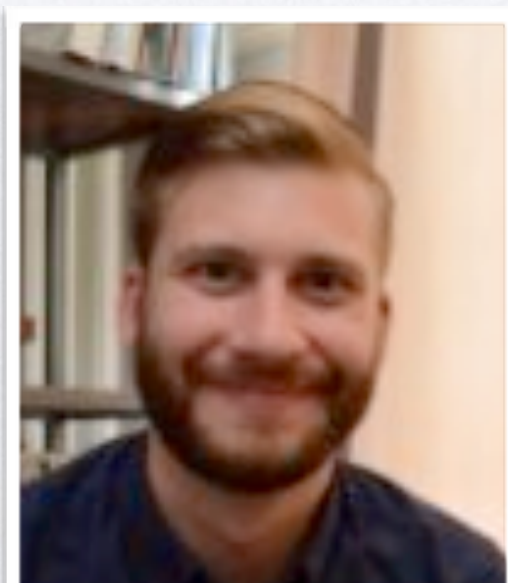
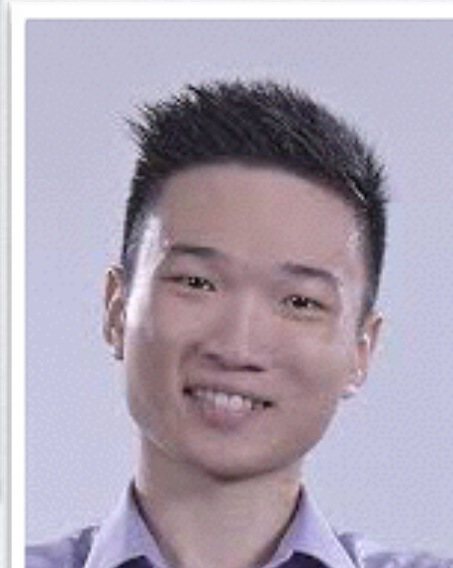
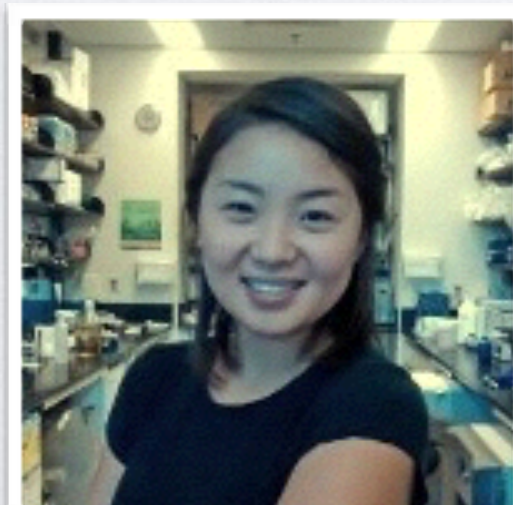
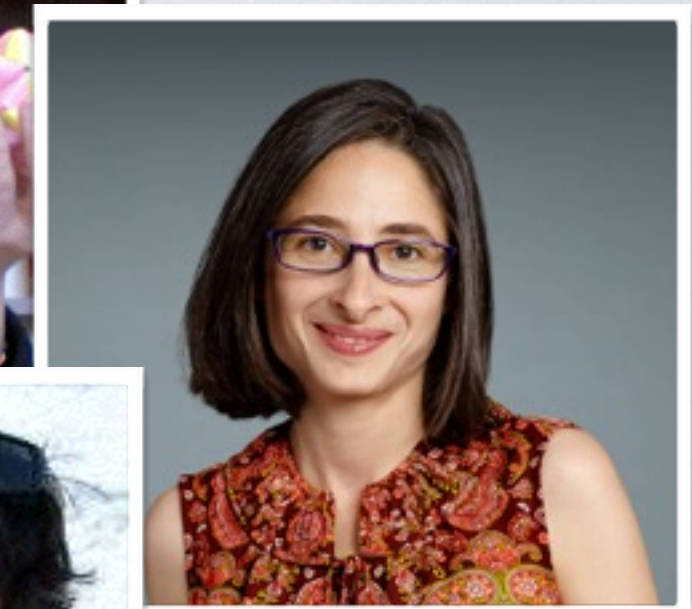
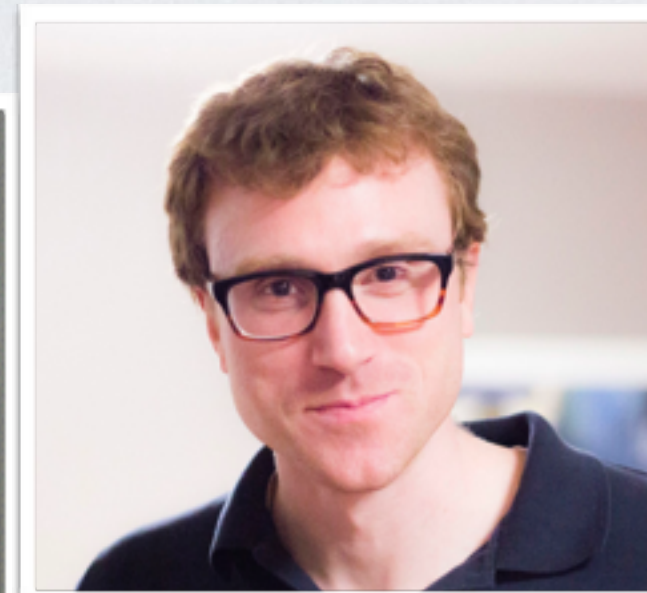


4. Fitting Atomic  
Models

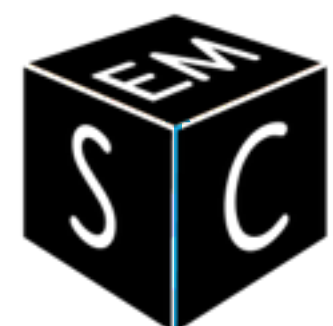




# TEAM OF INSTRUCTORS



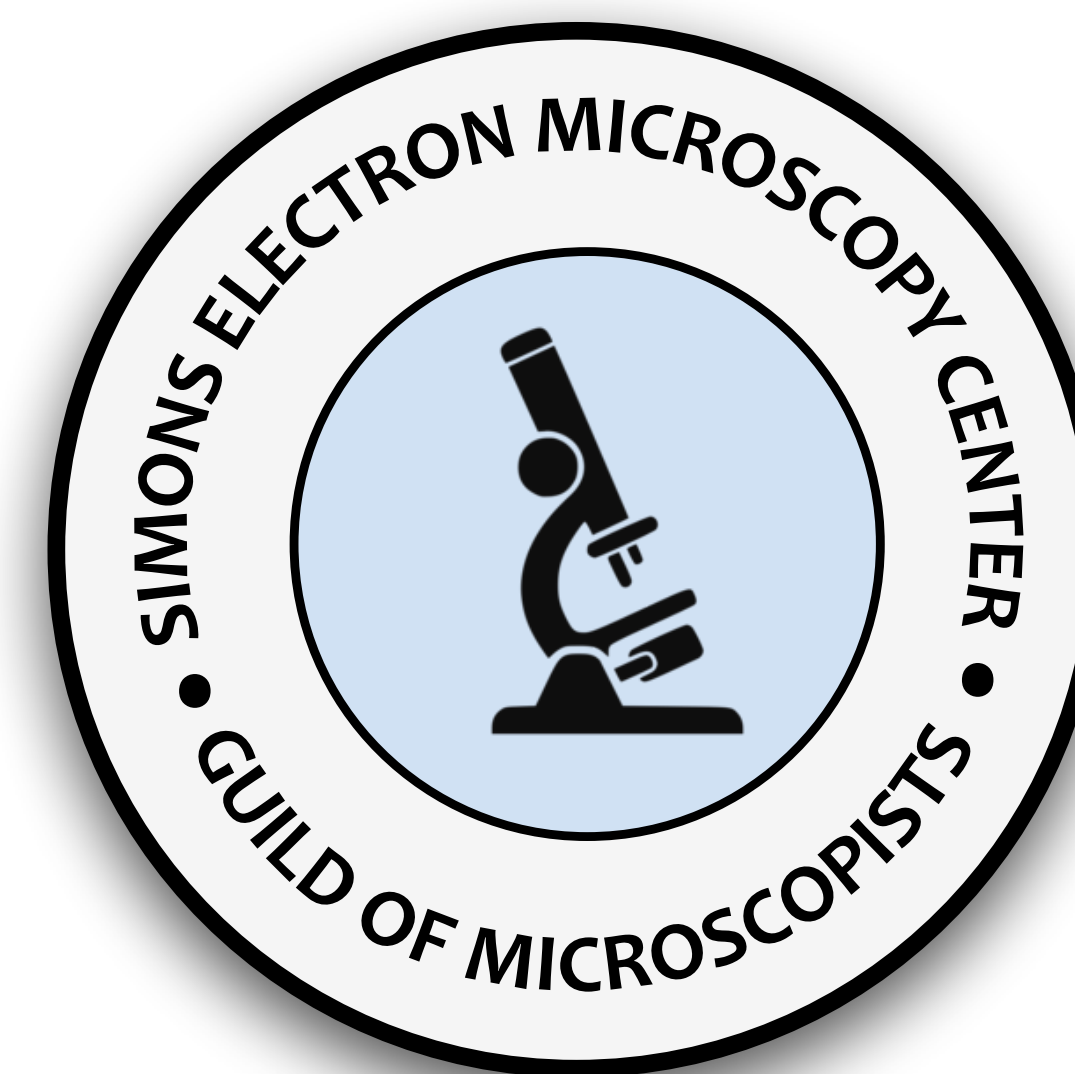




# SEMC WINTER EM COURSE

14th year of the course

1. Welcome new students
2. Course logistics
3. Introduction to EM
4. Tour of the facility



ACKNOWLEDGEMENT | NRAMM | NCCAT | INTRANET | JOBS | CONTACT US

 SIMONS ELECTRON MICROSCOPY CENTER

HOME | ABOUT | EVENTS | NEW USERS | DOCUMENTATION | PUBLICATIONS | INSTRUMENTATION | SCHEDULE TIME

## EVENTS

- Upcoming Events
- News and Past Events
- Forums
- Workshops and Courses
- New User Orientation
- Workshops and Symposia at Outside Organizations

## The Winter-Spring 2019 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](#) 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 this course apply here: <https://www.surveymonkey.com/r/WinterCourse19>

### Preliminary Course Schedule

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

#### EM fundamentals section (month of January)

Jan 7 : Introduction & SEMC tour [Ed Eng, NYSBC]  
Jan 9 : Basic anatomy of the electron microscope [Anchi Cheng, NYSBC]





SIMONS ELECTRON  
MICROSCOPY CENTER

New York Structural  
Biology Center



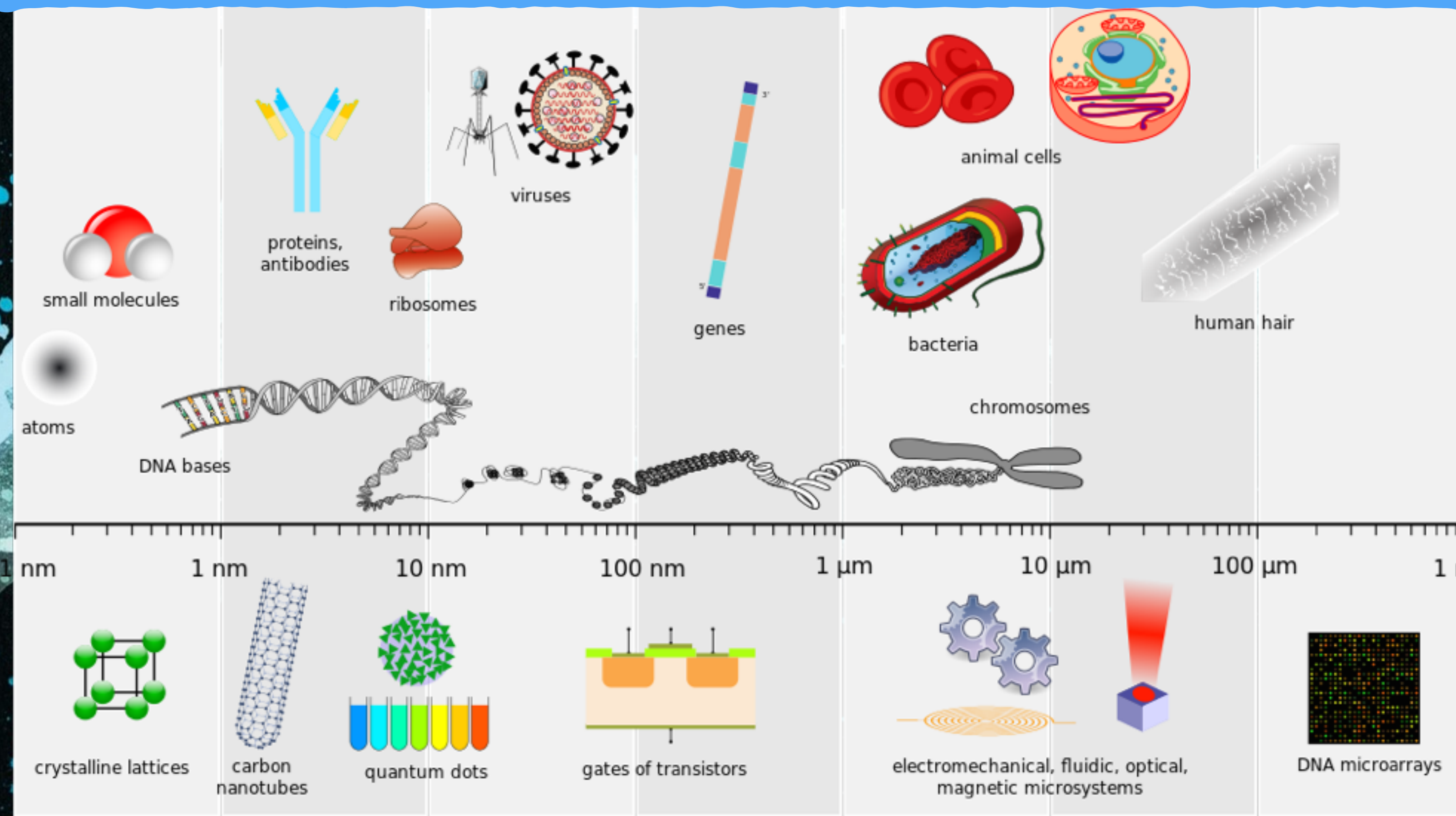
# INTRODUCTION TO (CRYO) EM



NATIONAL CENTER FOR  
CRYOEM ACCESS & TRAINING



# Electron Microscopy



X-ray

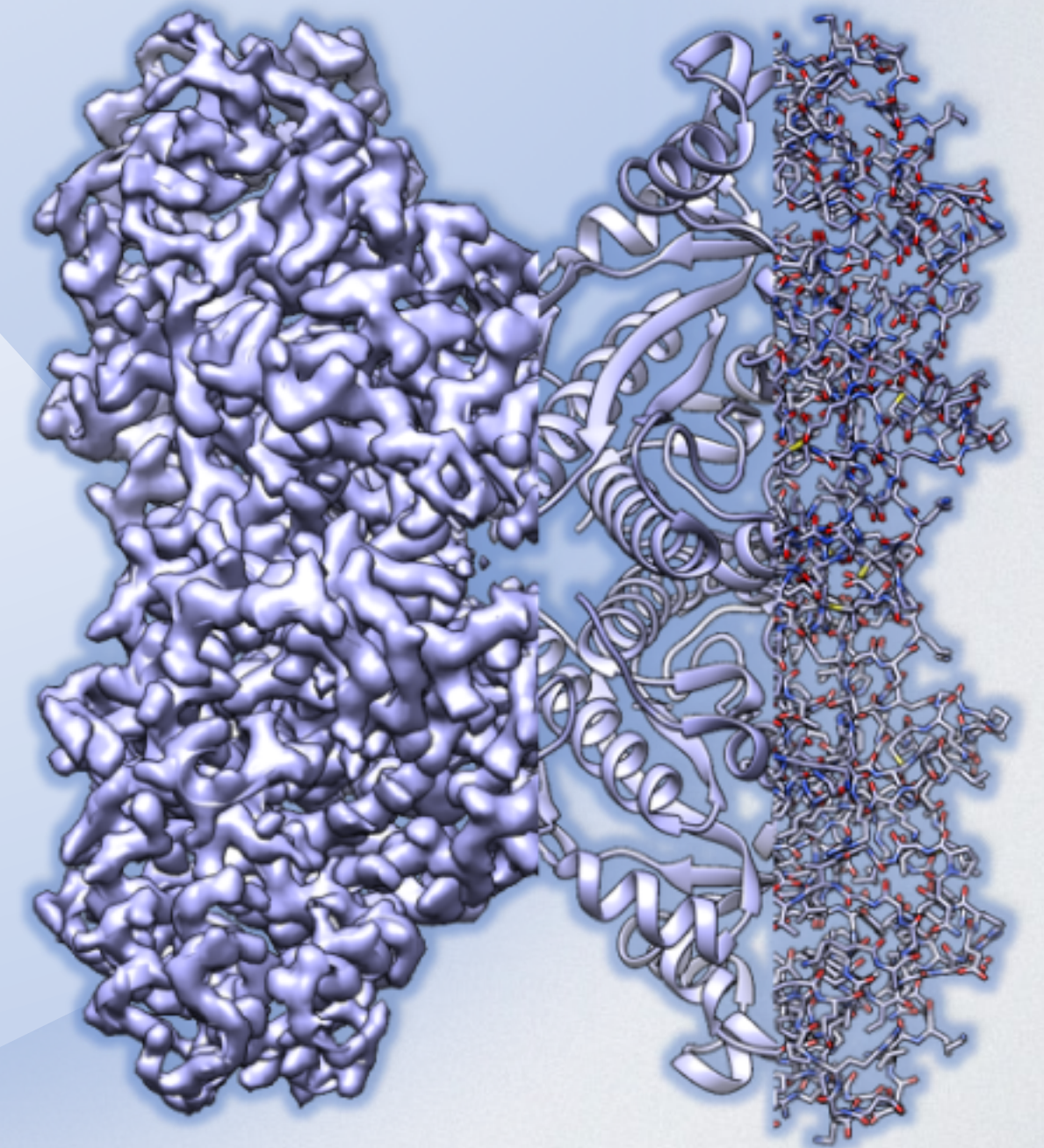
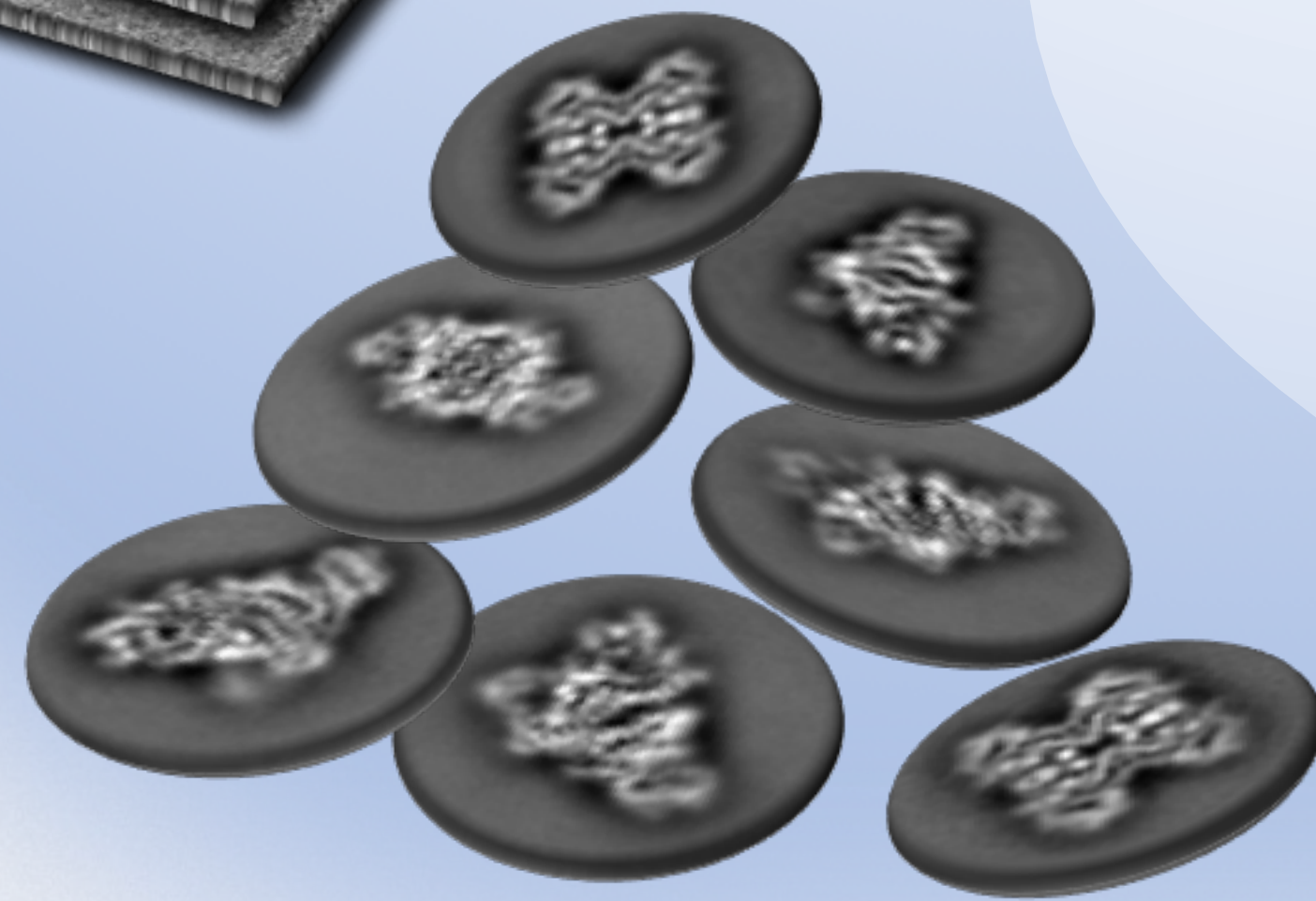
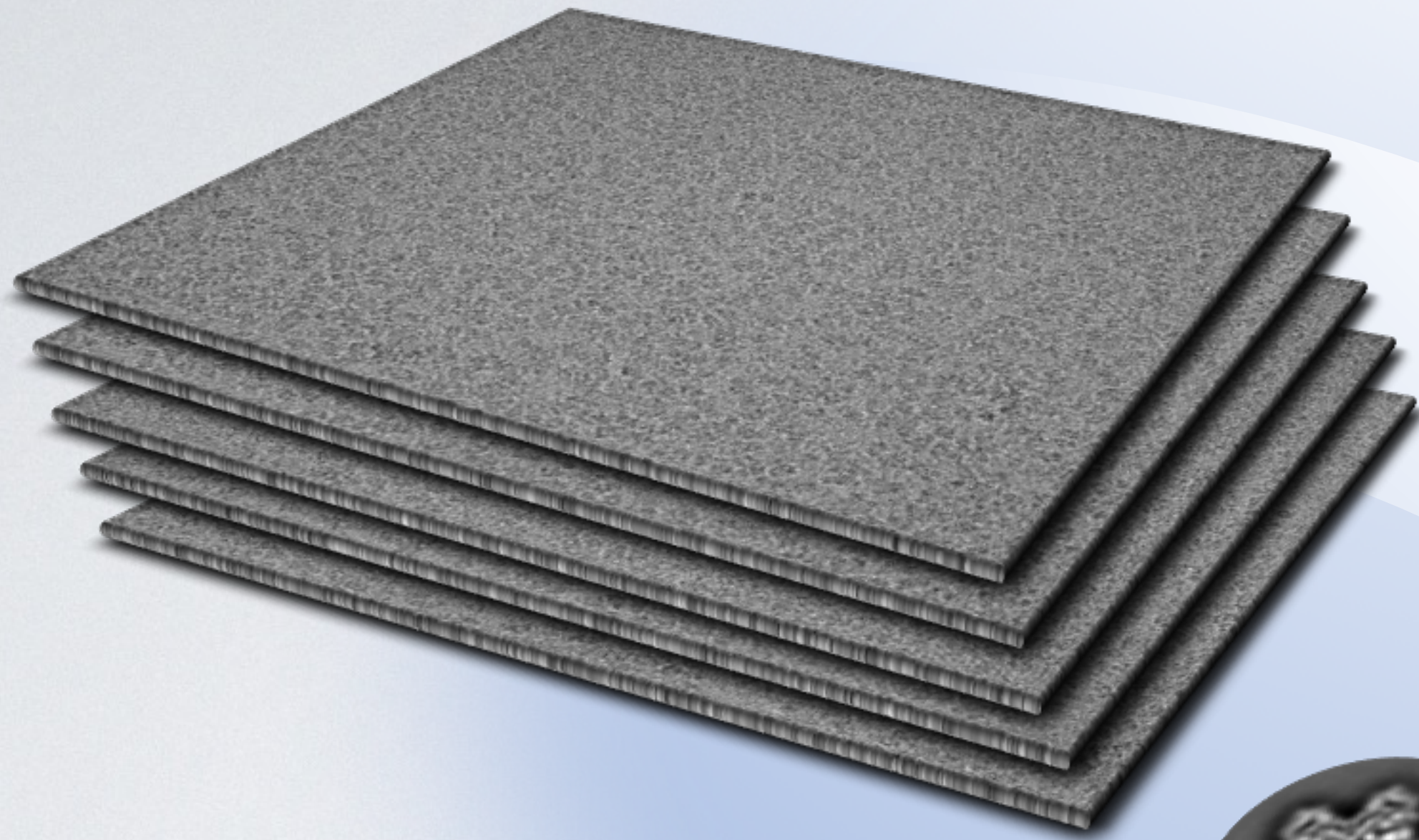
NMR

Light microscopy

Naked eye

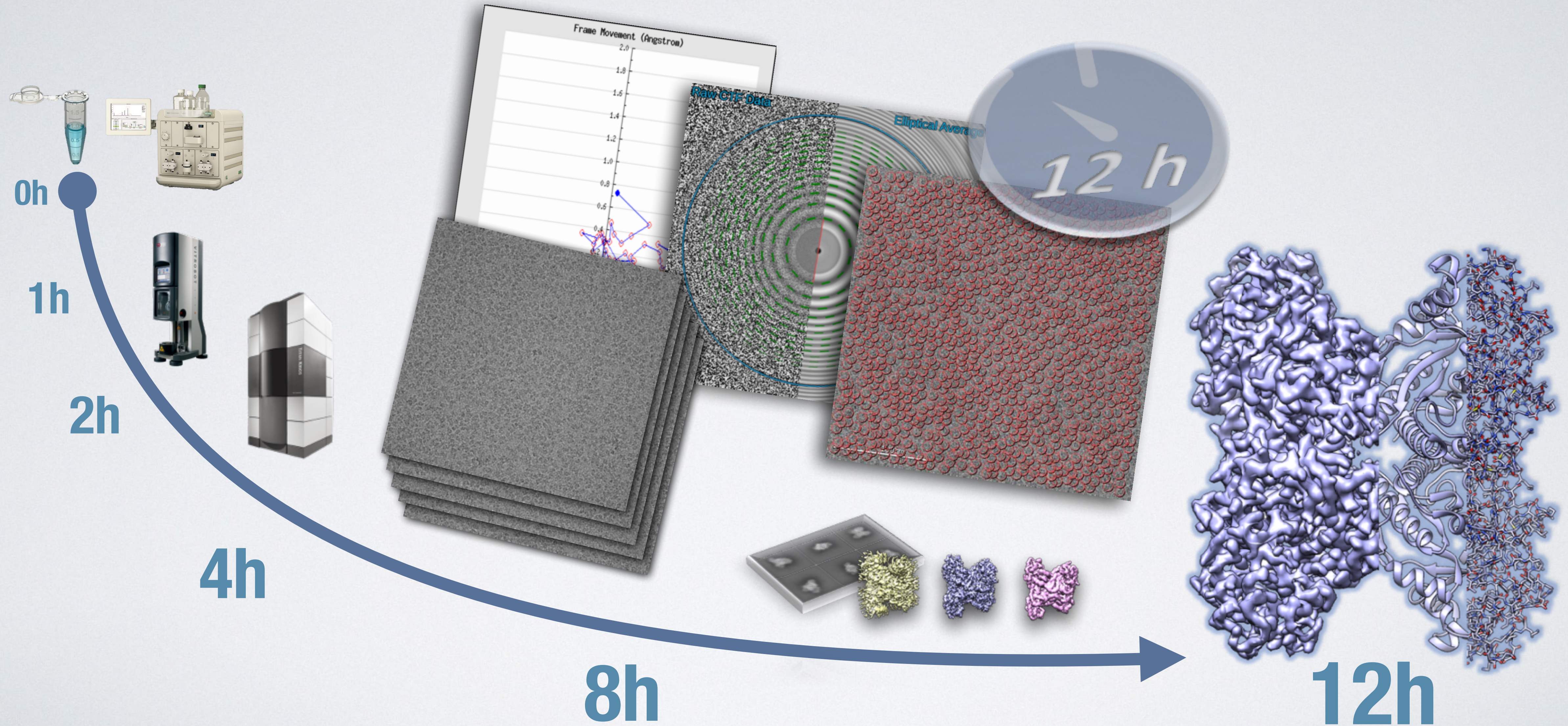


# WHAT IS POSSIBLE TODAY? 2Å within a day



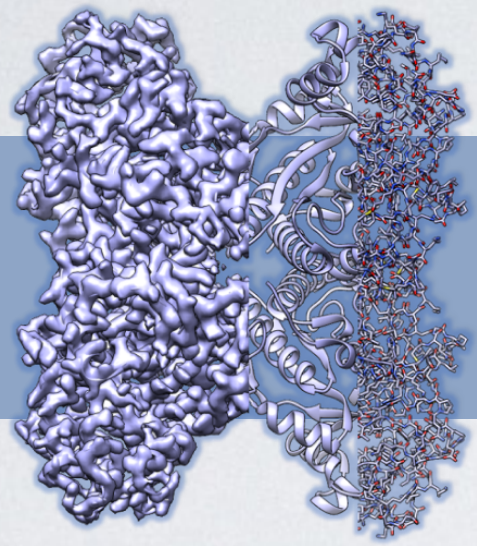


# WHAT IS POSSIBLE TODAY? 2Å within a day





# IS THIS ROUTINELY DONE?

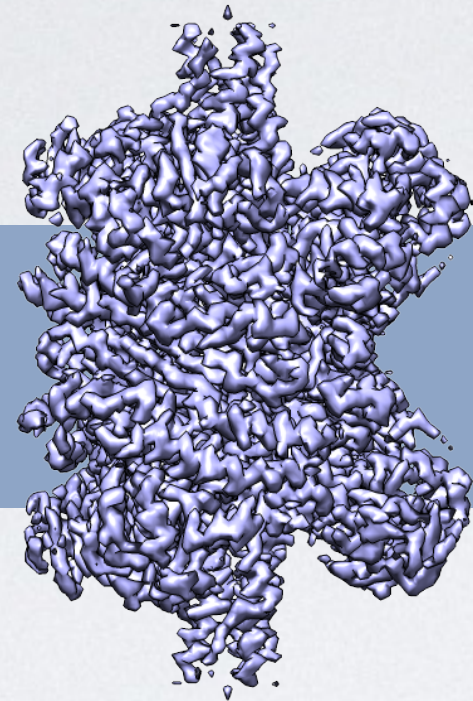


Aldolase

D2

~150kDa

rabbit muscle

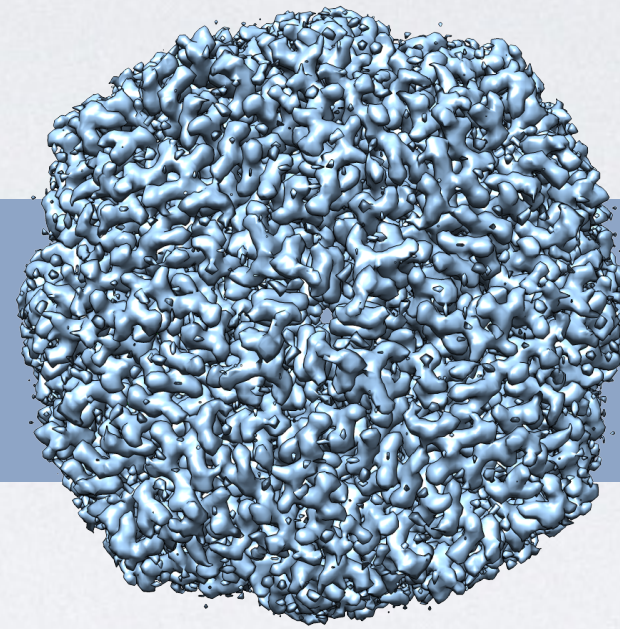


Glutamate  
dehydrogenase

D3

334kDa

cow liver

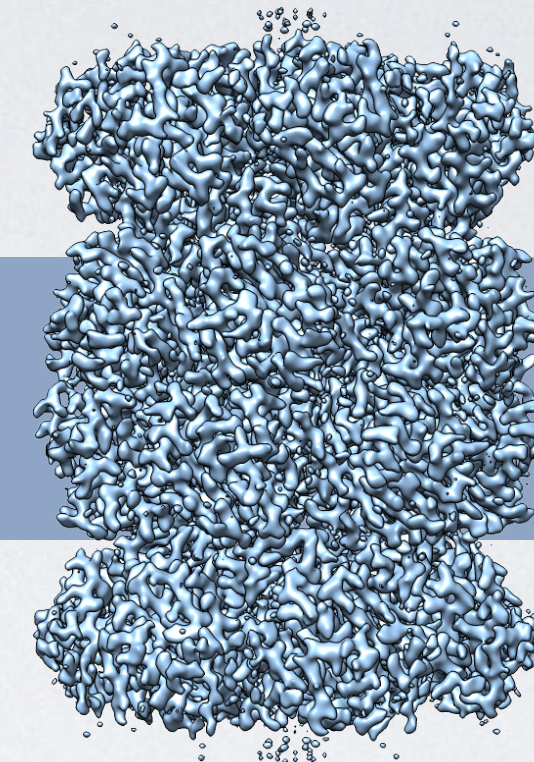


Apoferritin

O

443kDa

horse spleen

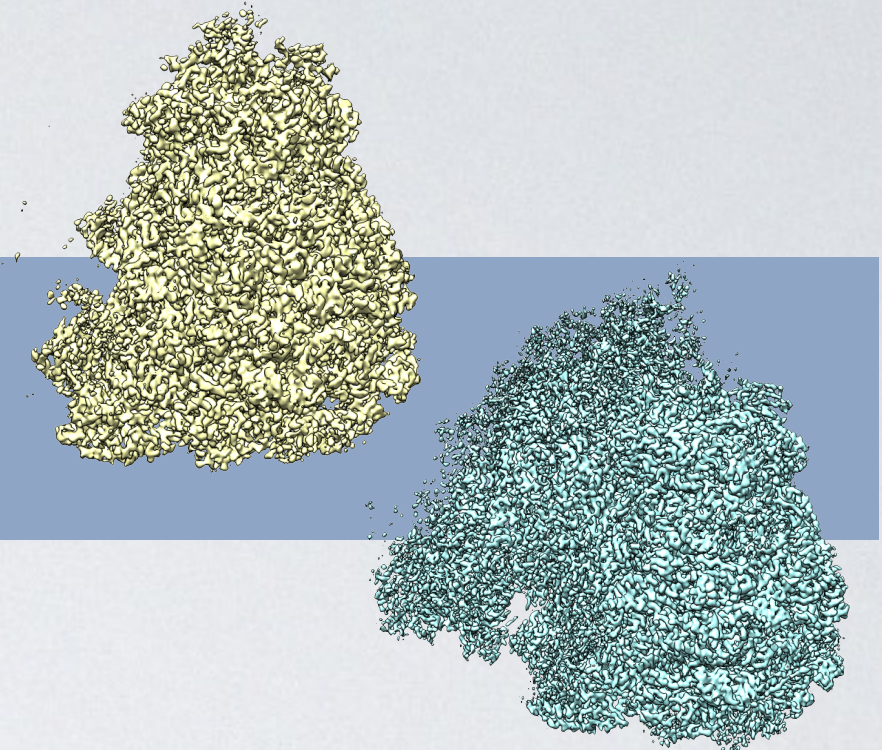


20S  
proteasome

D7

750kDa

Thermoplasma  
or Mycoplasma



60S/80S  
ribosome

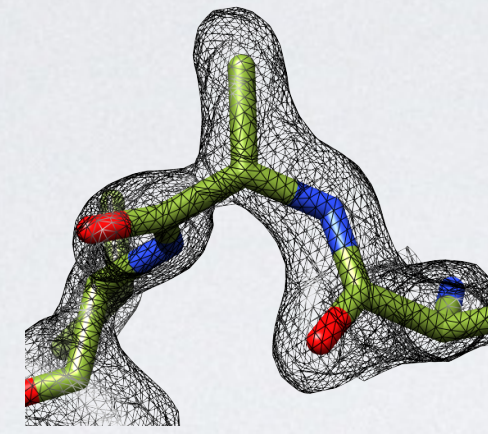
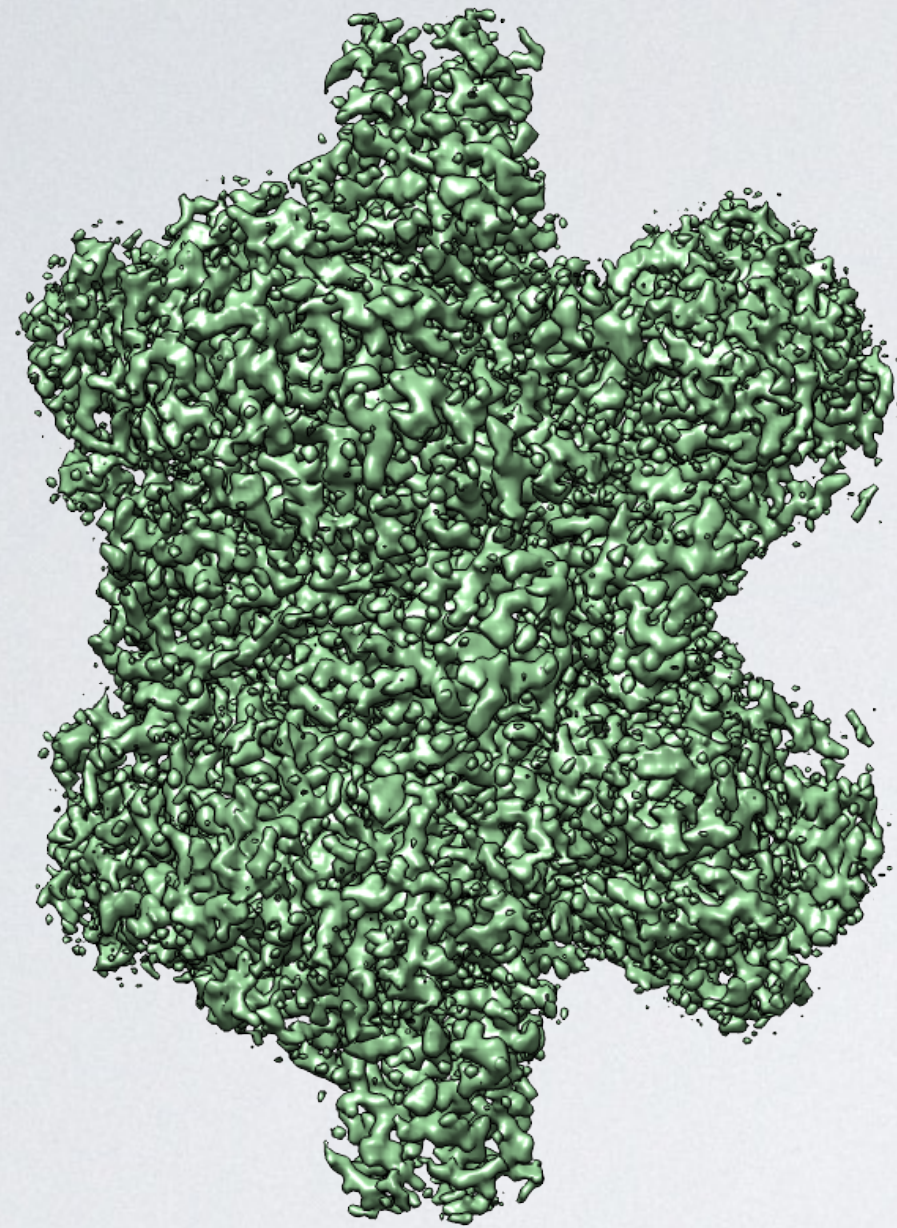
C1

~2-4MDa

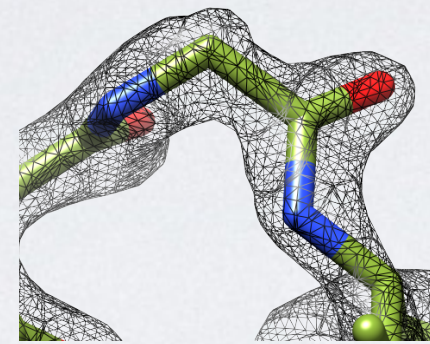
human



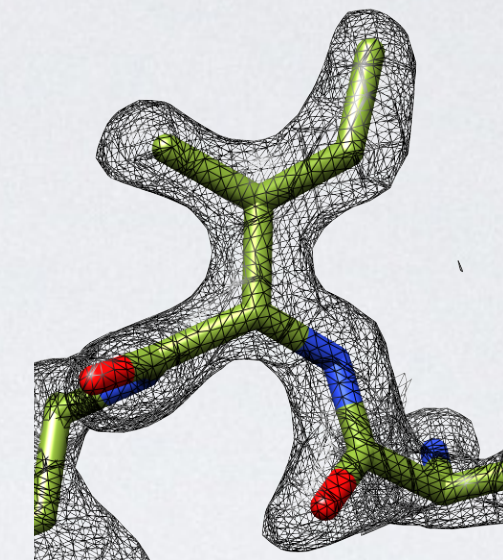
# IS THIS USEFUL?



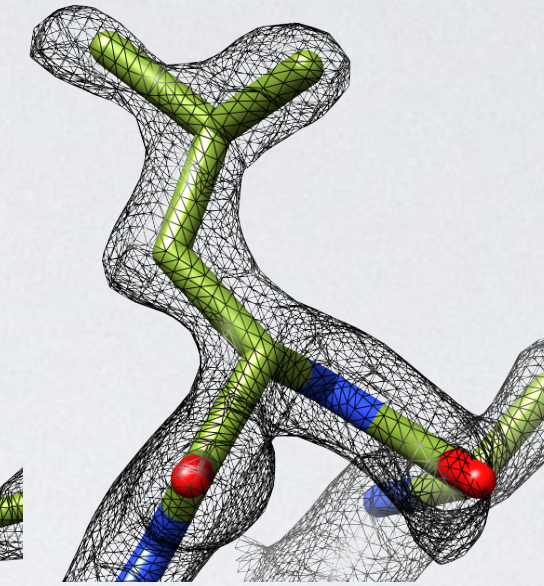
Alanine



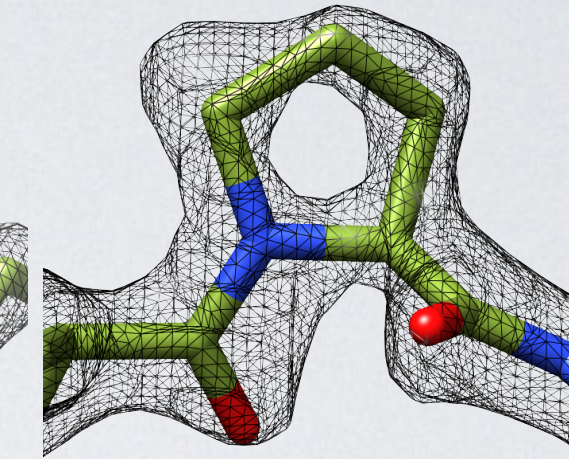
Glycine



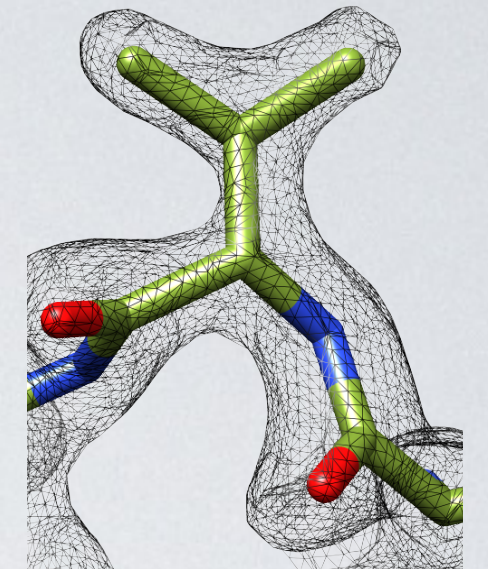
Isoleucine



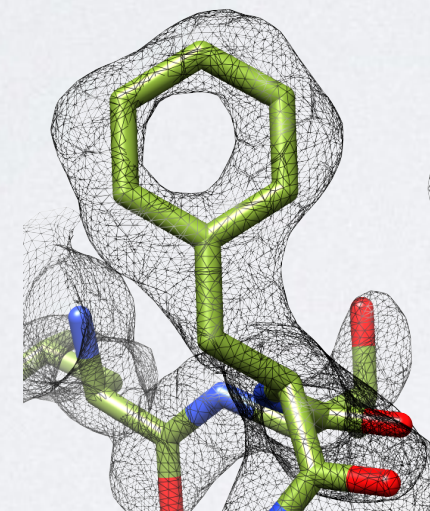
Leucine



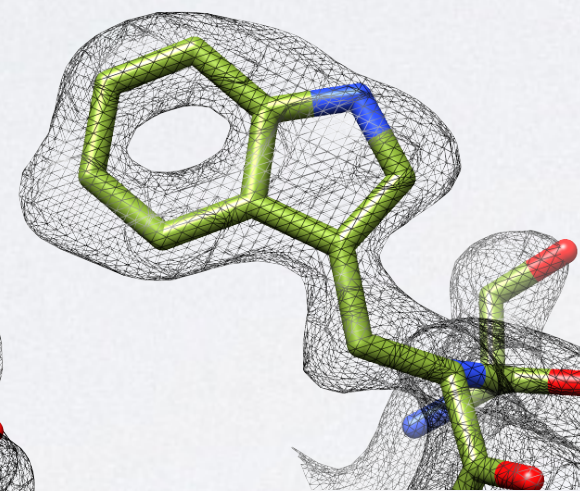
Proline



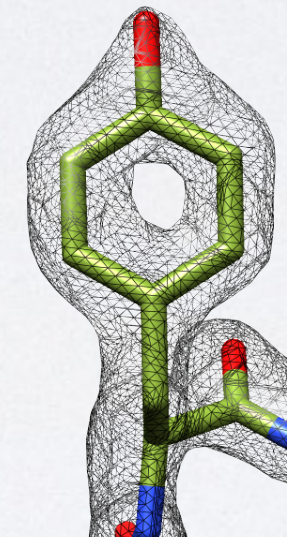
Valine



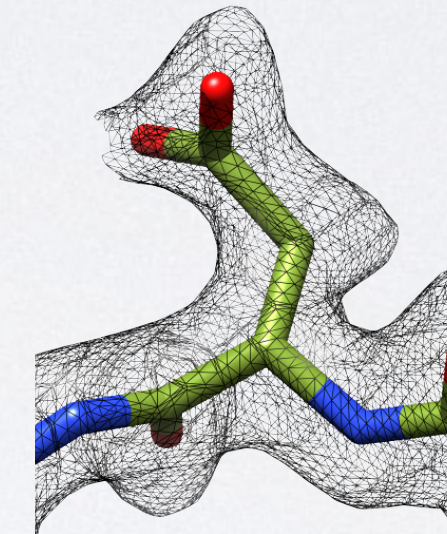
Phenylalanine



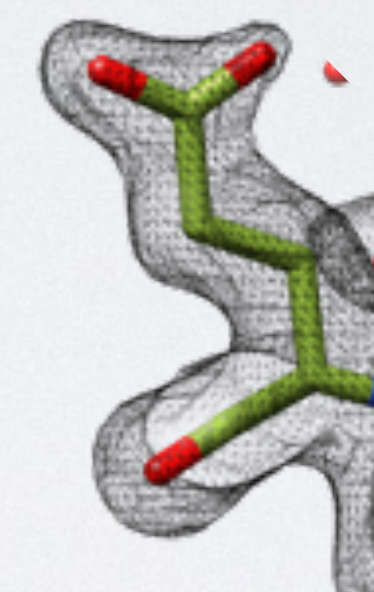
Tryptophan



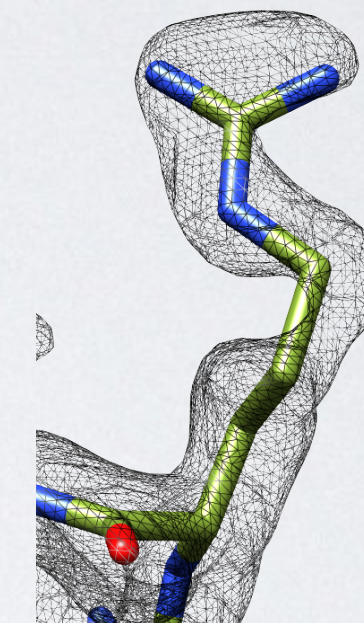
Tyrosine



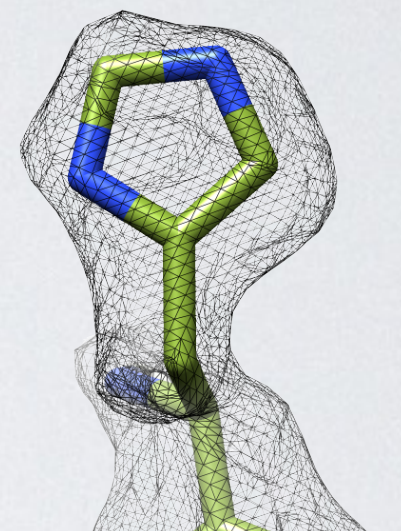
Aspartic acid



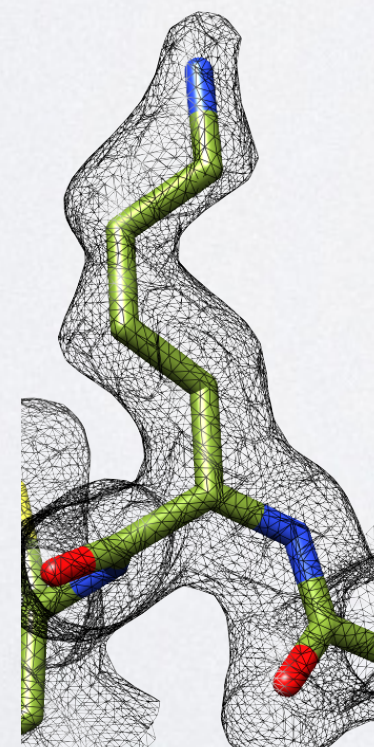
Glutamic Acid



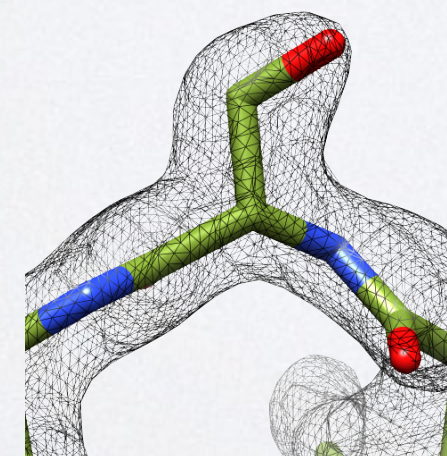
Arginine



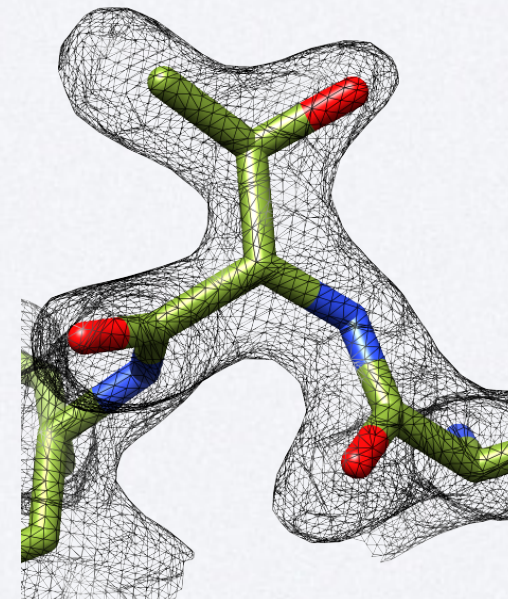
Histidine



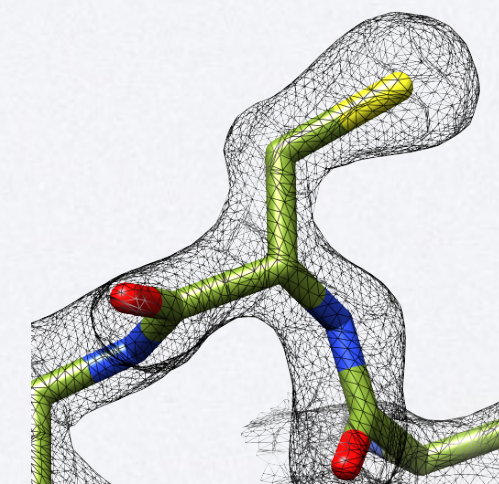
Lysine



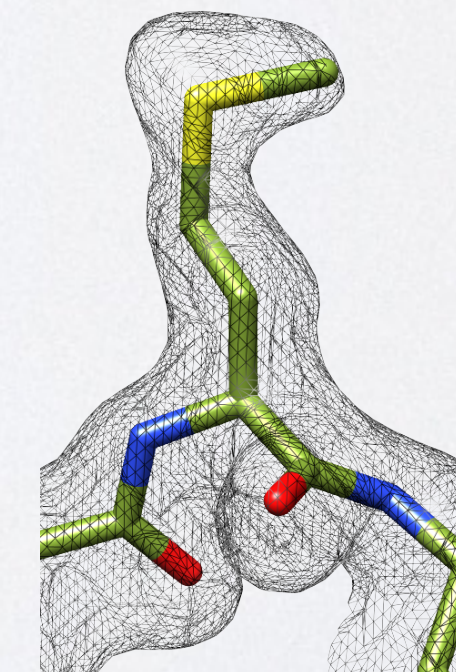
Serine



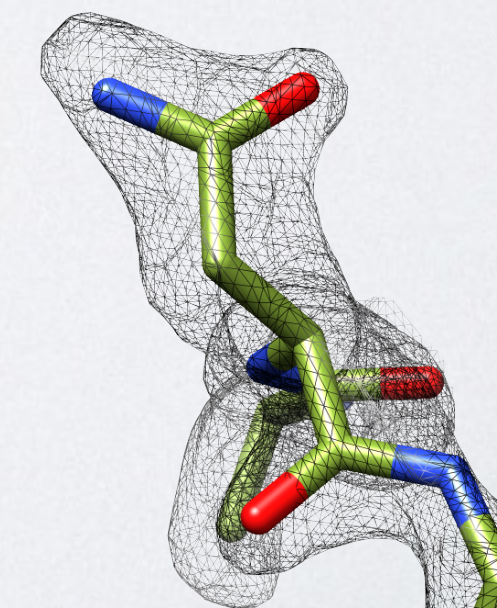
Threonine



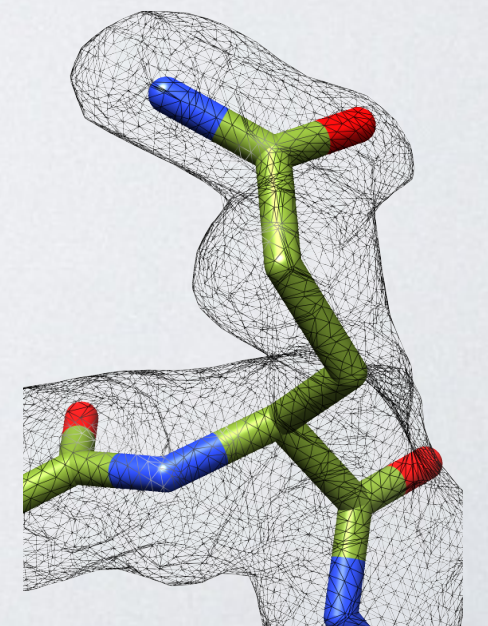
Cysteine



Methionine



Asparagine



Glutamine




# TECHNOLOGY TO ASSIST THE ADVANCEMENT OF BIOMEDICAL RESEARCH

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



Chemistry Nobel prize 2017



2017 NOBEL PRIZE IN CHEMISTRY  
"For the greatest benefit to mankind"  
Alfred Nobel

**Jacques Dubochet**  
**Joachim Frank**  
**Richard Henderson**

## The Nobel Prize in Chemistry 2017

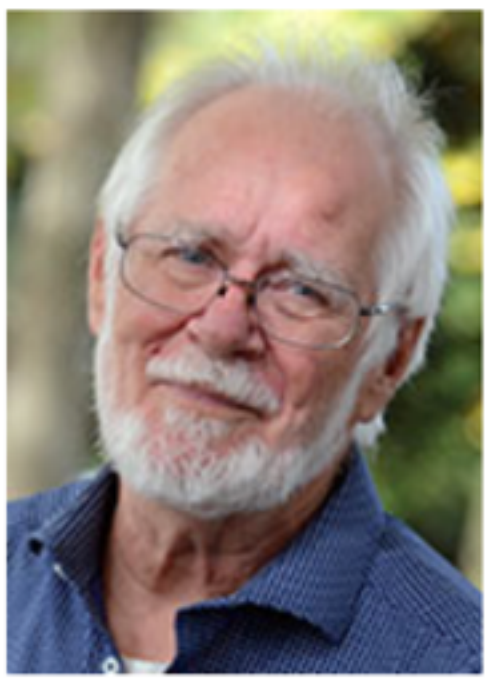


Photo: Félix Imhof © UNIL [CC BY-SA 4.0]  
**Jacques Dubochet**  
Prize share: 1/3




Photo: B. Winkowski © Columbia University Medical Center  
**Joachim Frank**  
Prize share: 1/3




Photo: MRC Laboratory of Molecular Biology  
**Richard Henderson**  
Prize share: 1/3

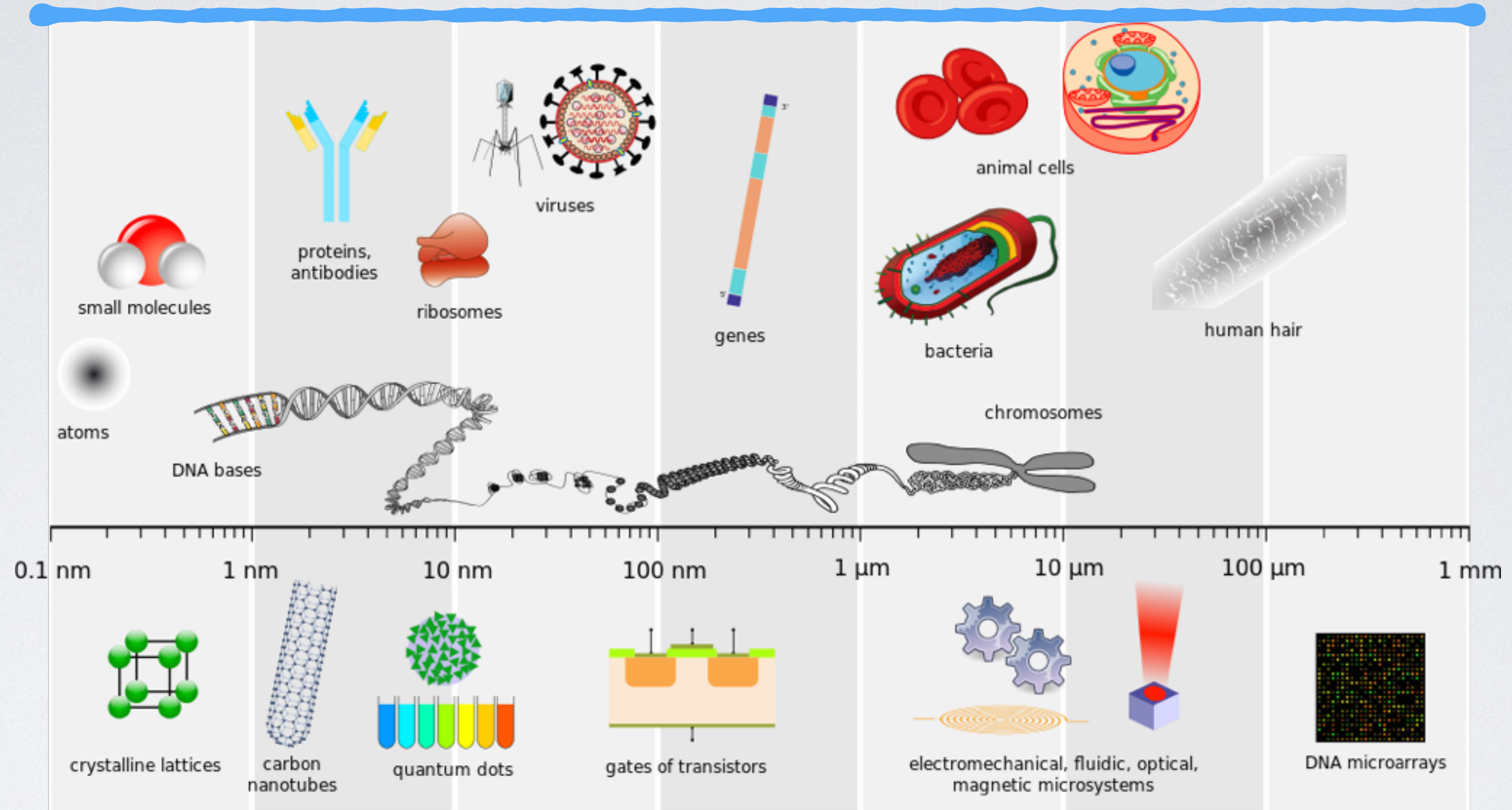
The Nobel Prize in Chemistry 2017 was awarded to Jacques Dubochet, Joachim Frank and Richard Henderson *"for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution"*.

[http://www.nobelprize.org/nobel\\_prizes/chemistry/laureates/2017/](http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2017/)



# SCALE OF EM

## Electron Microscopy



[https://en.wikipedia.org/wiki/Nanoscope\\_scale](https://en.wikipedia.org/wiki/Nanoscope_scale)

X-ray

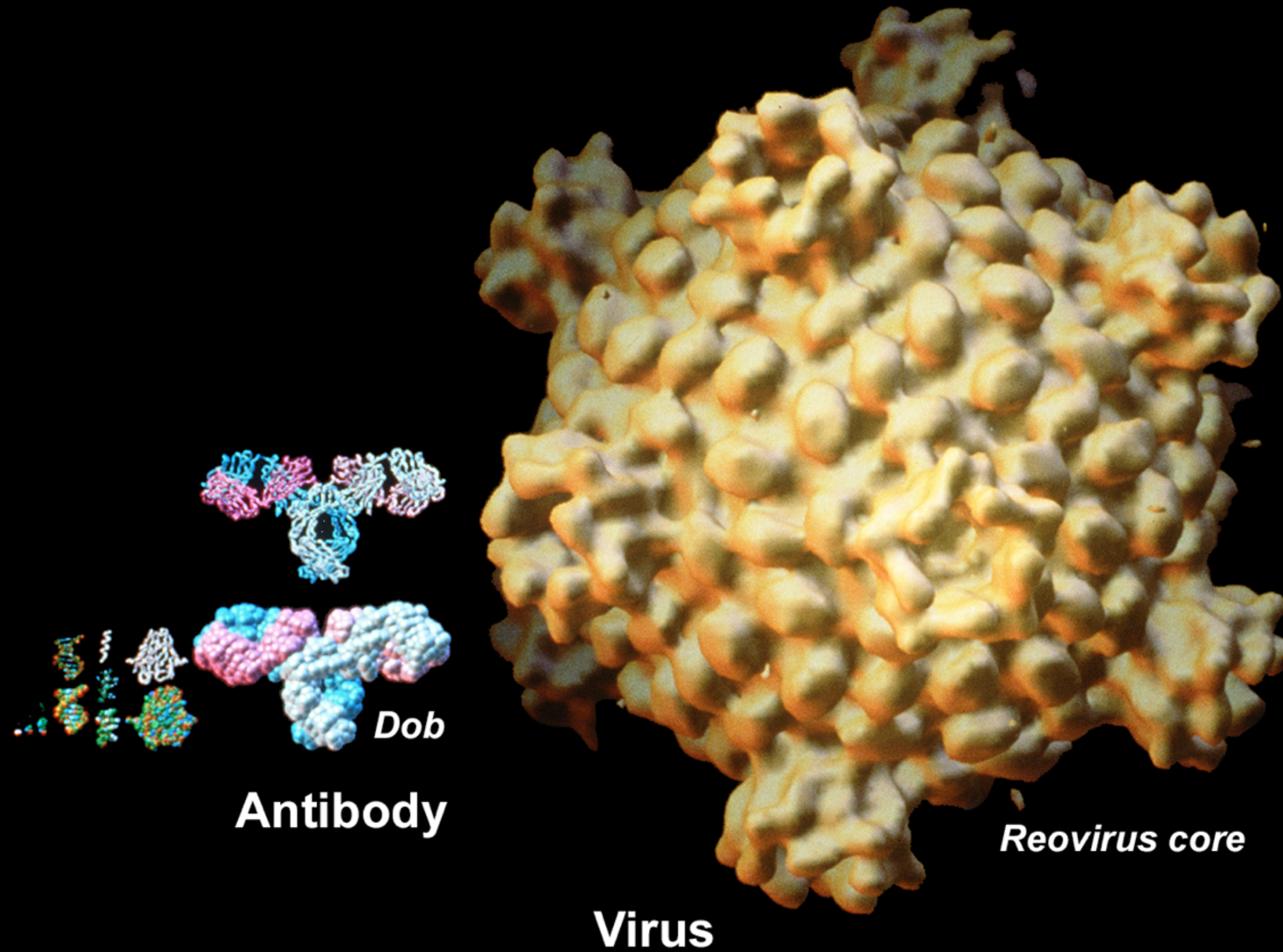
NMR

Light microscopy

Naked eye

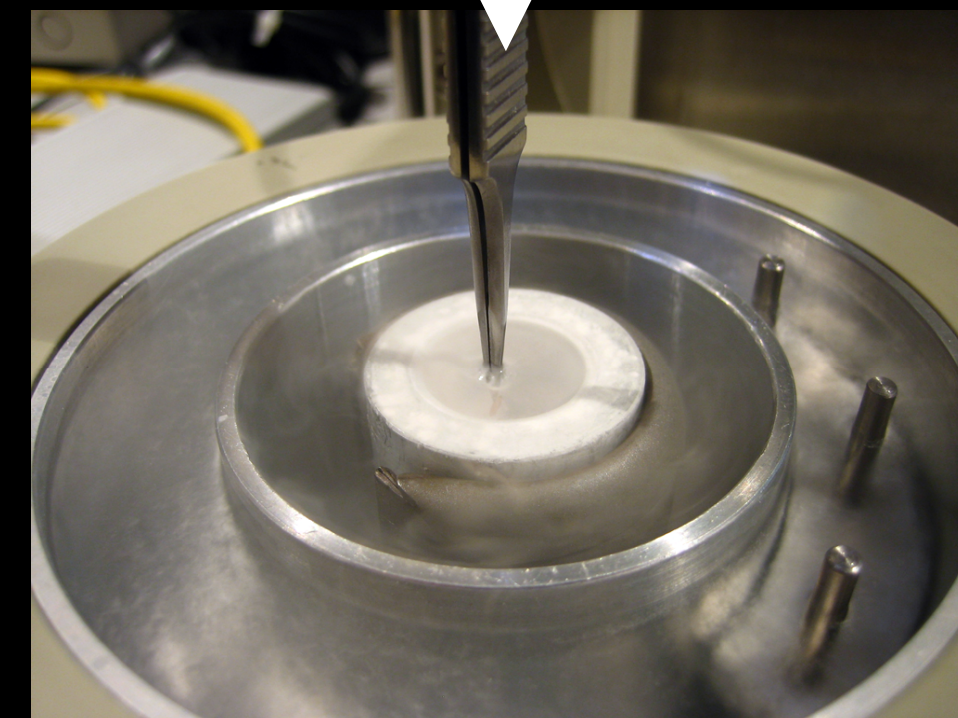
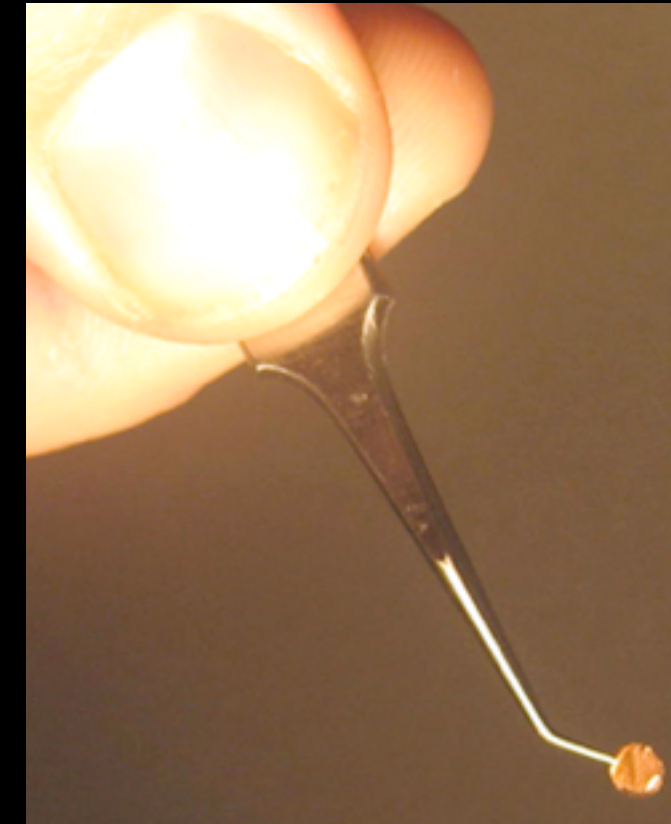
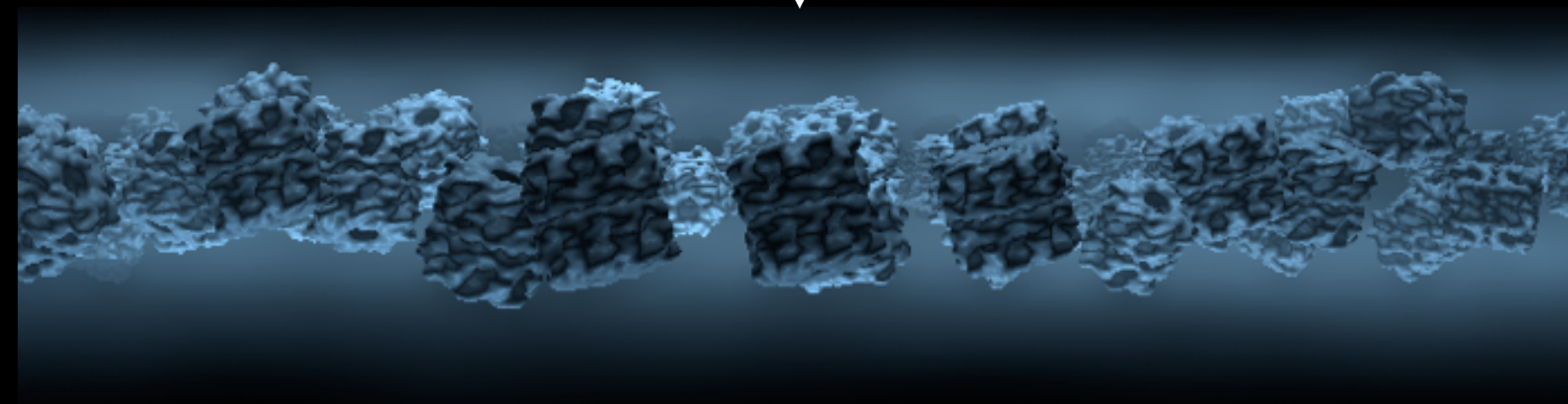
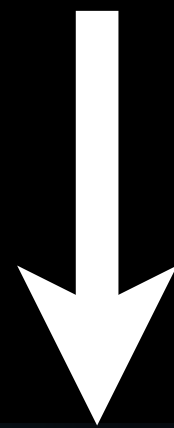
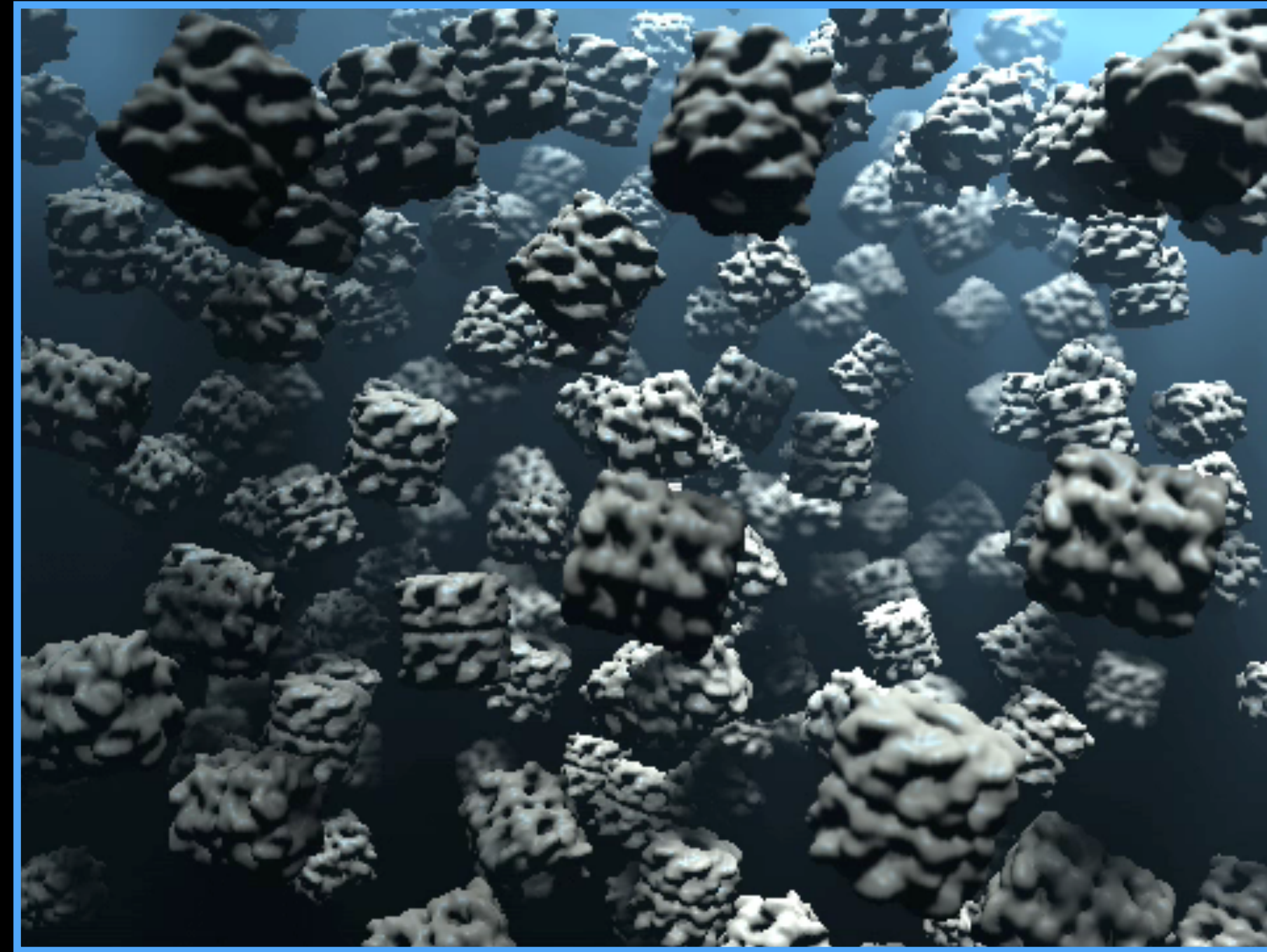


# BIOLOGICAL SAMPLES ARE AMENABLE TO EM

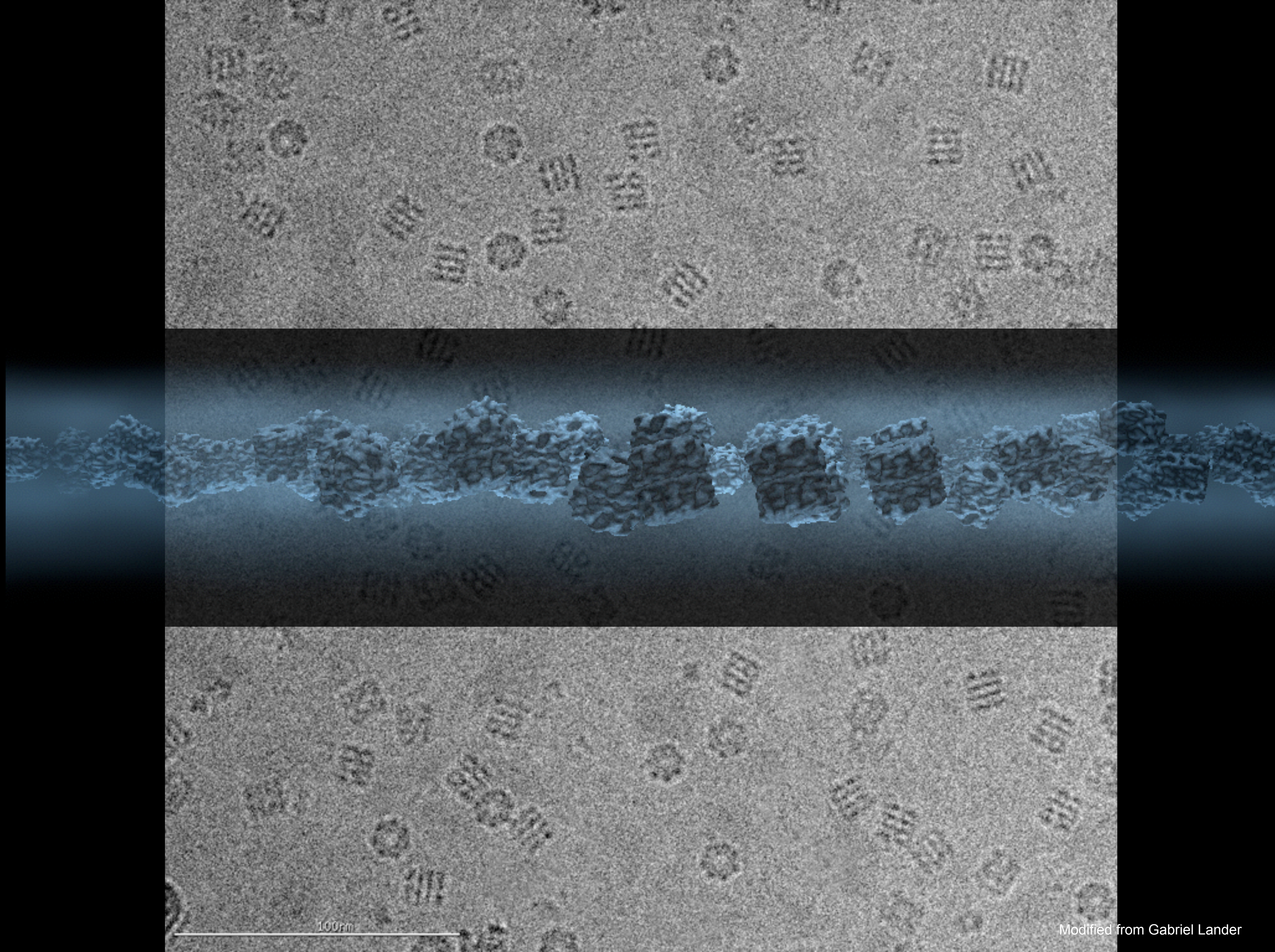




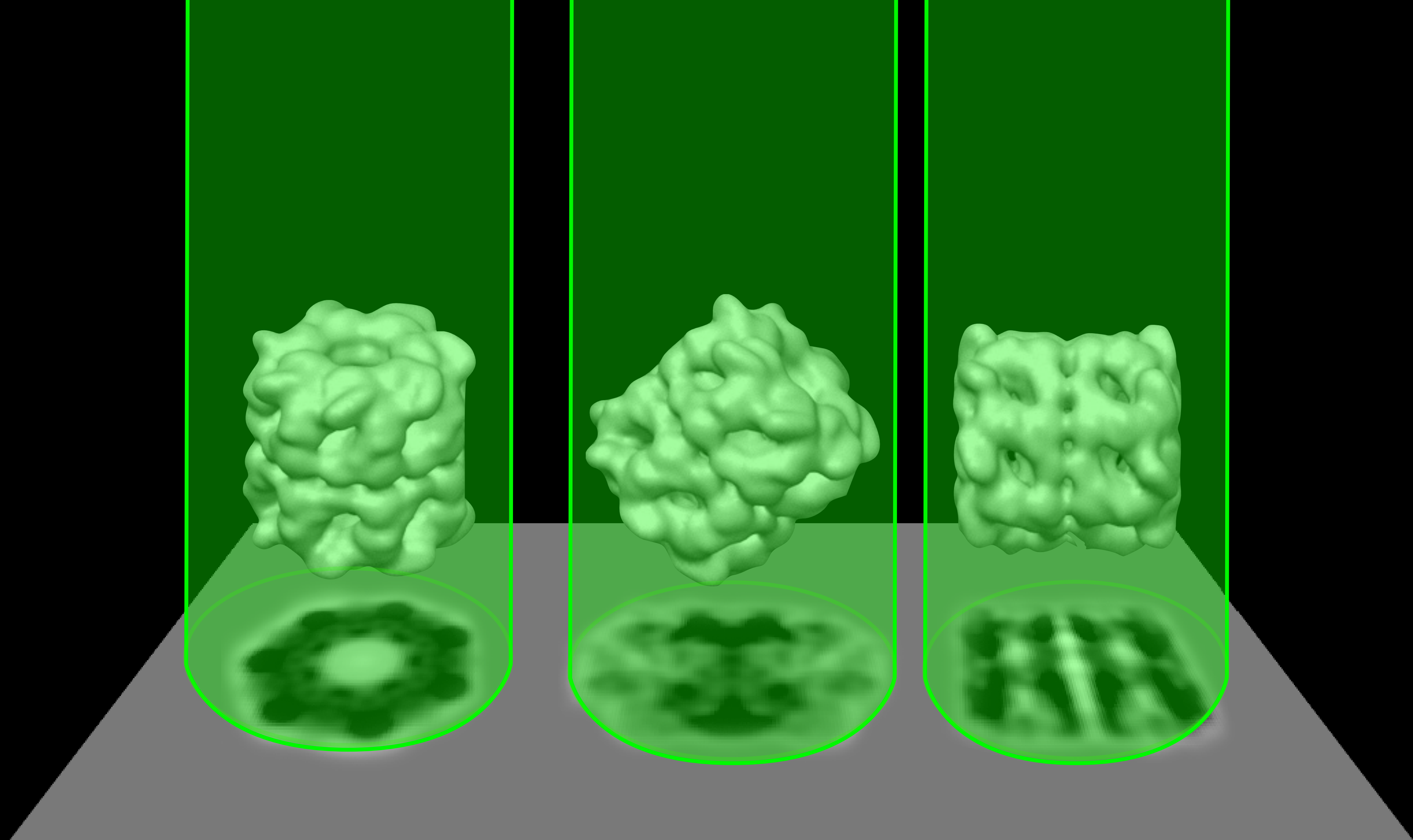
# PREPARING BIOLOGICAL SAMPLES FOR SPA





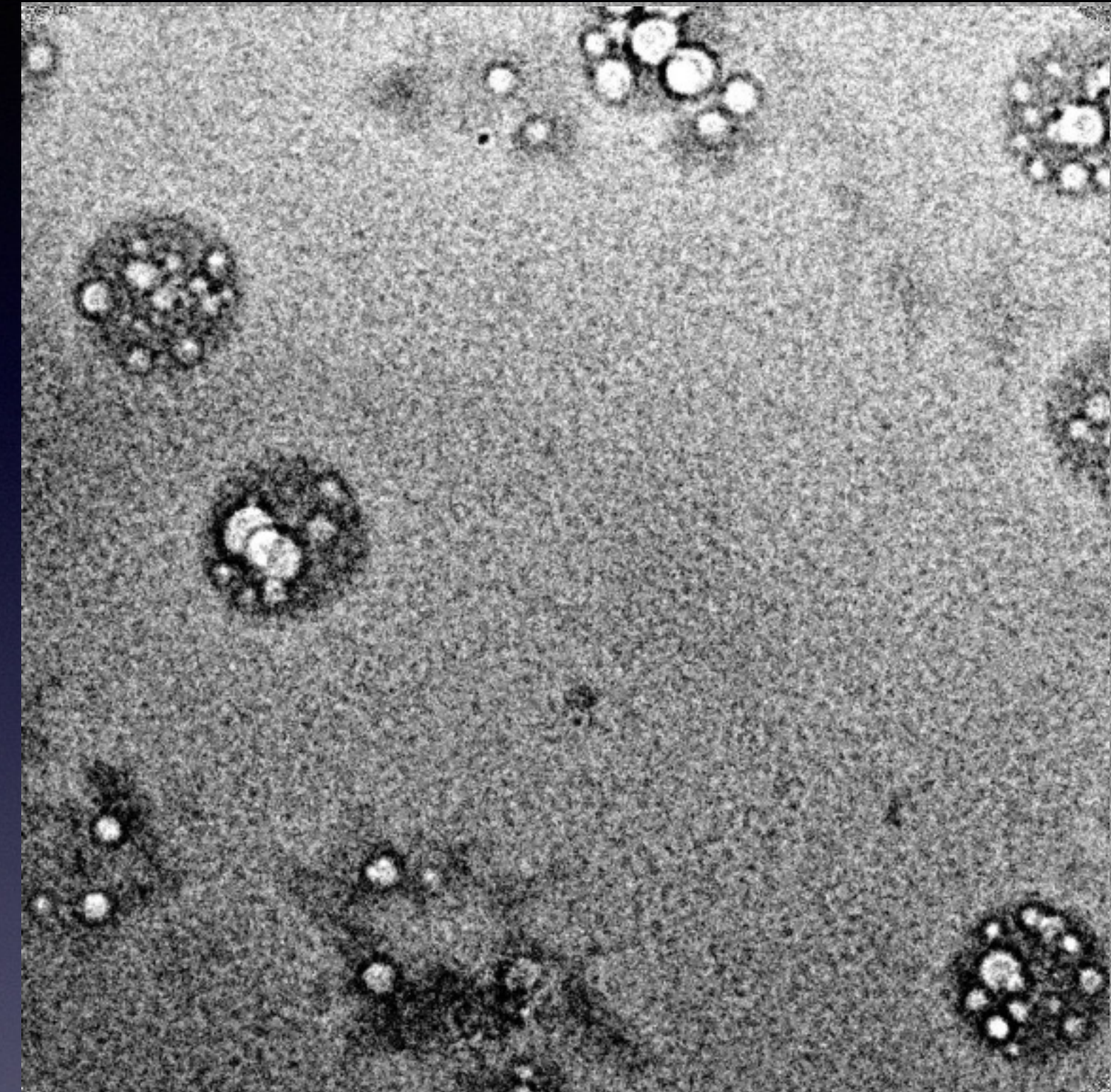
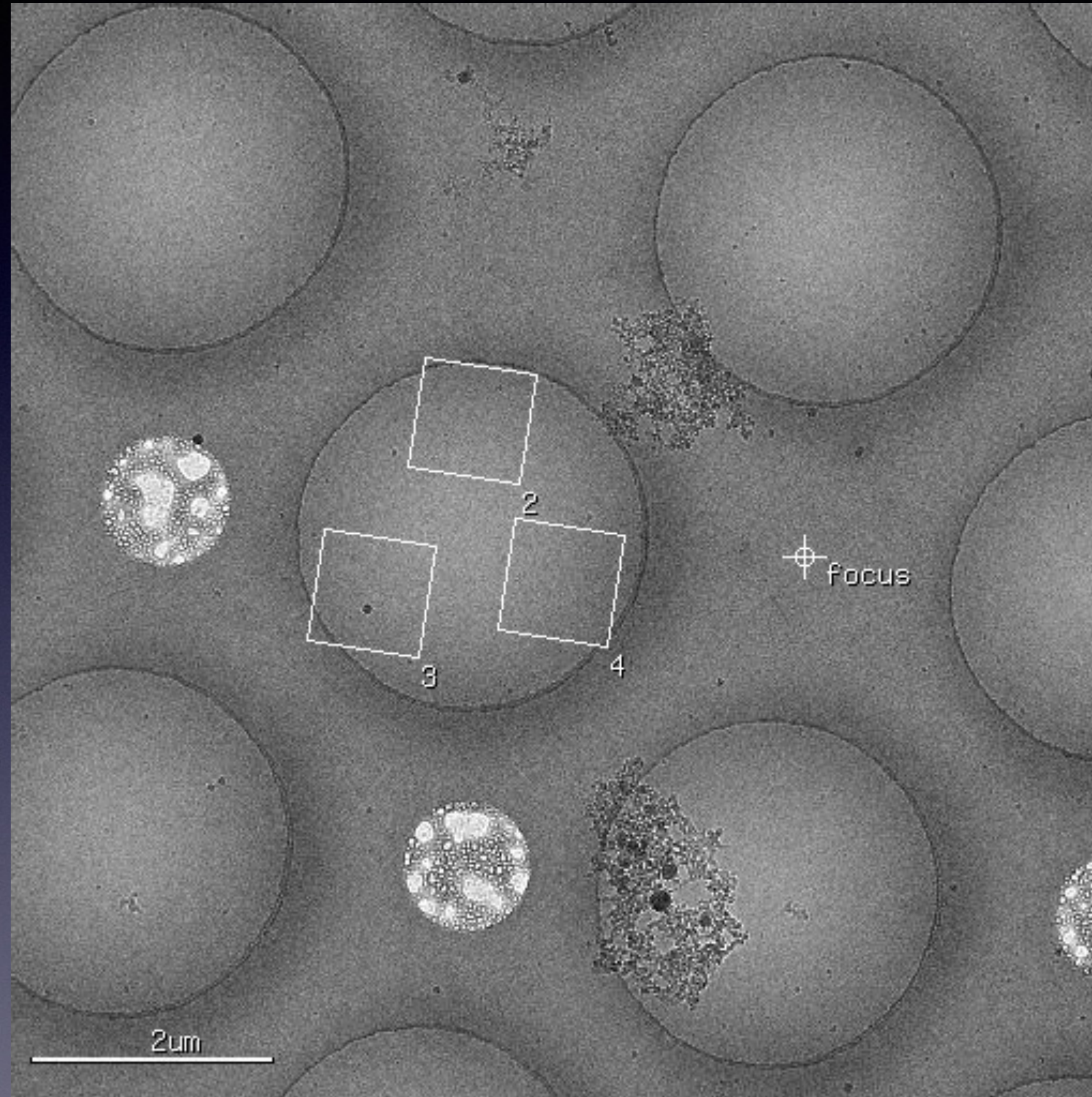






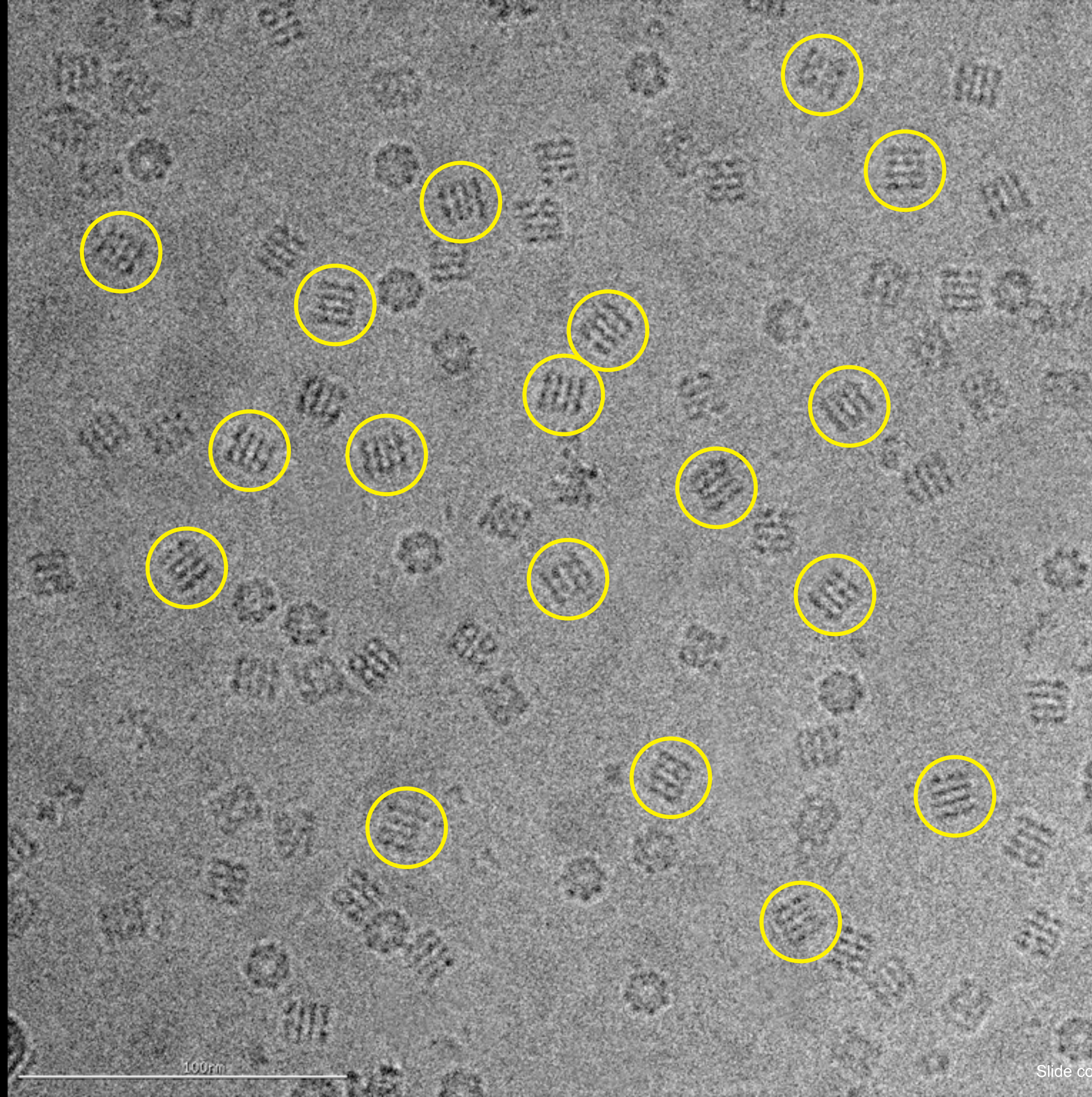


# CRYOEM CHALLENGE: RADIATION SENSITIVITY

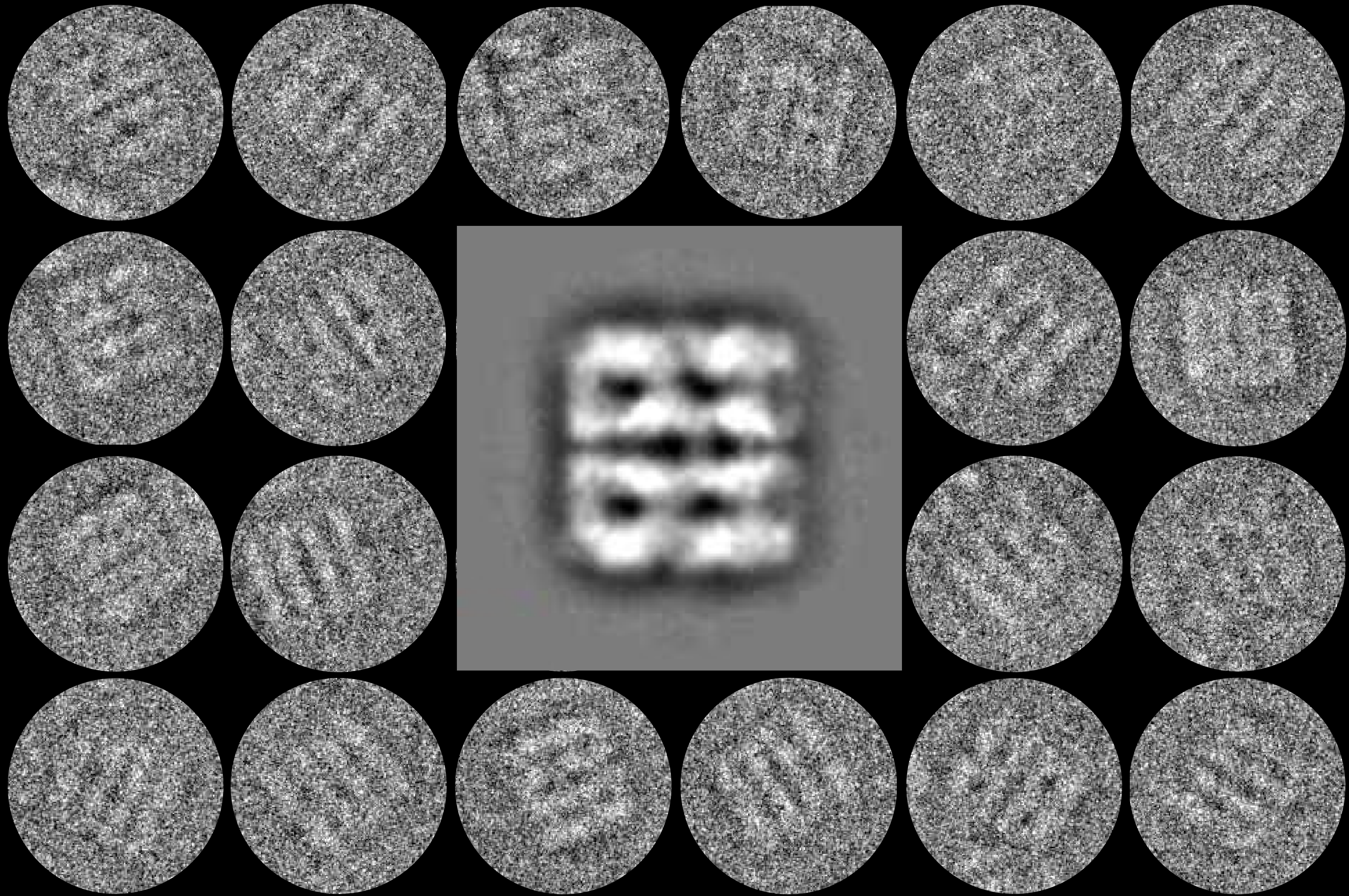


“Low-dose” imaging

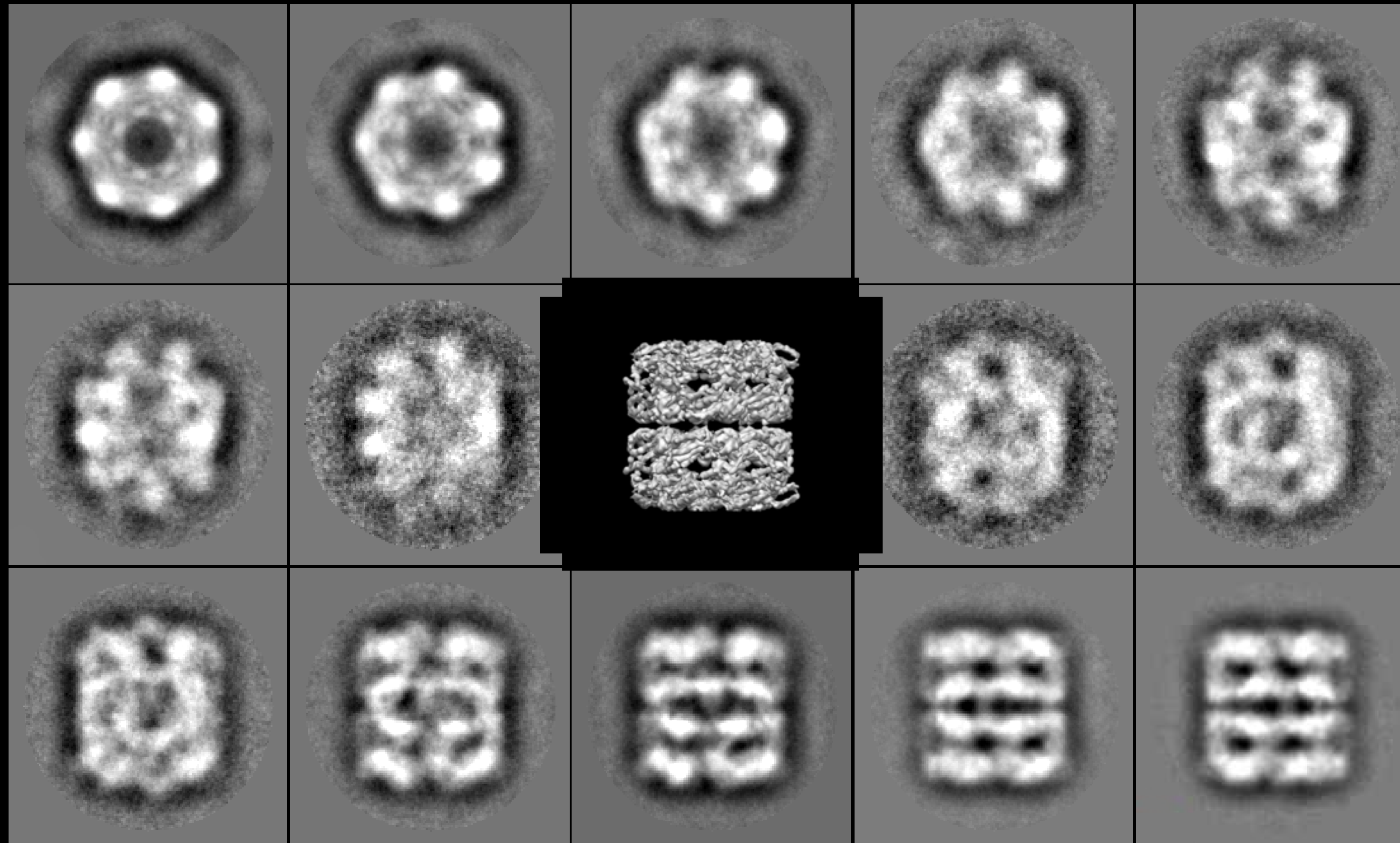




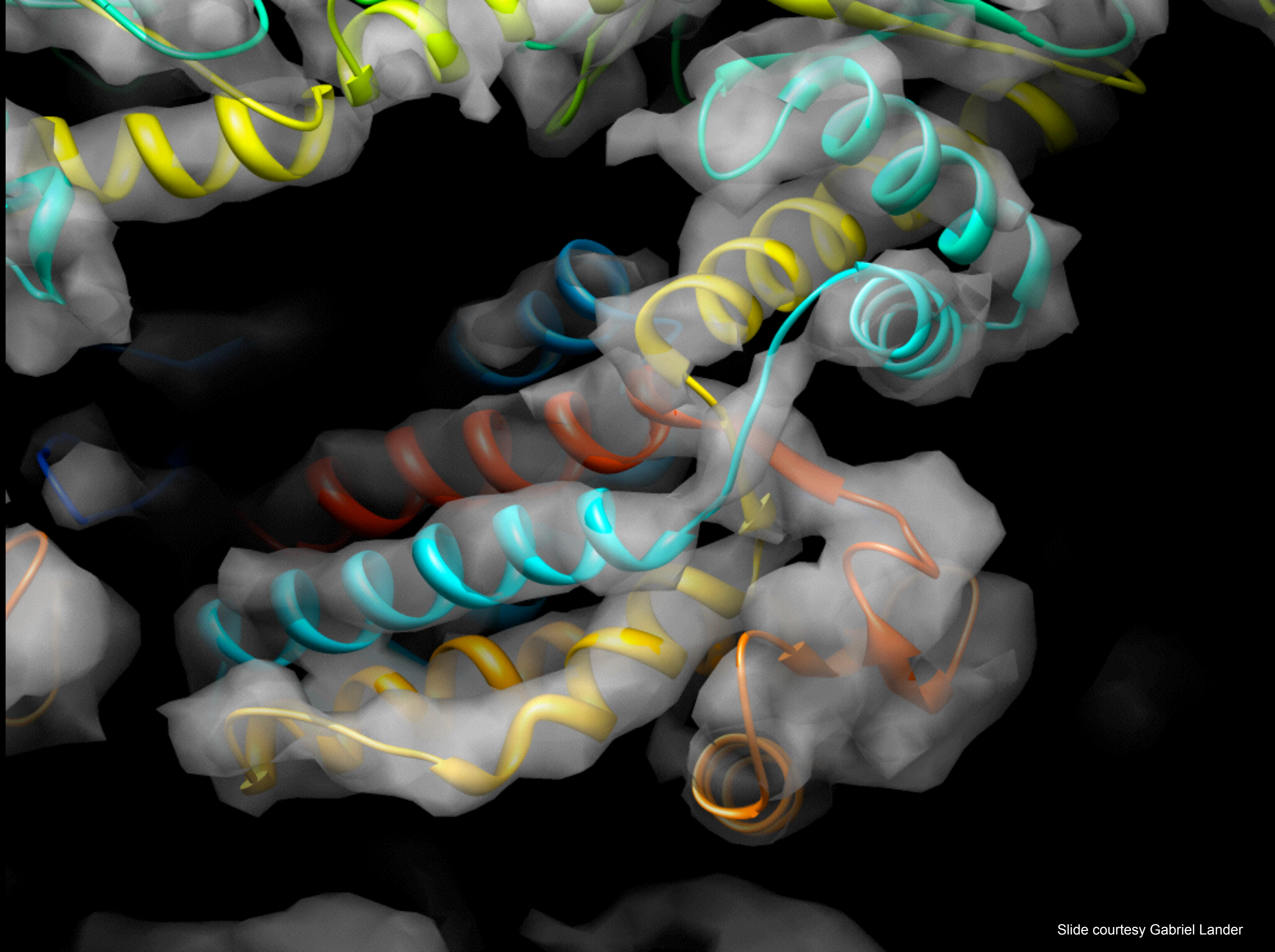






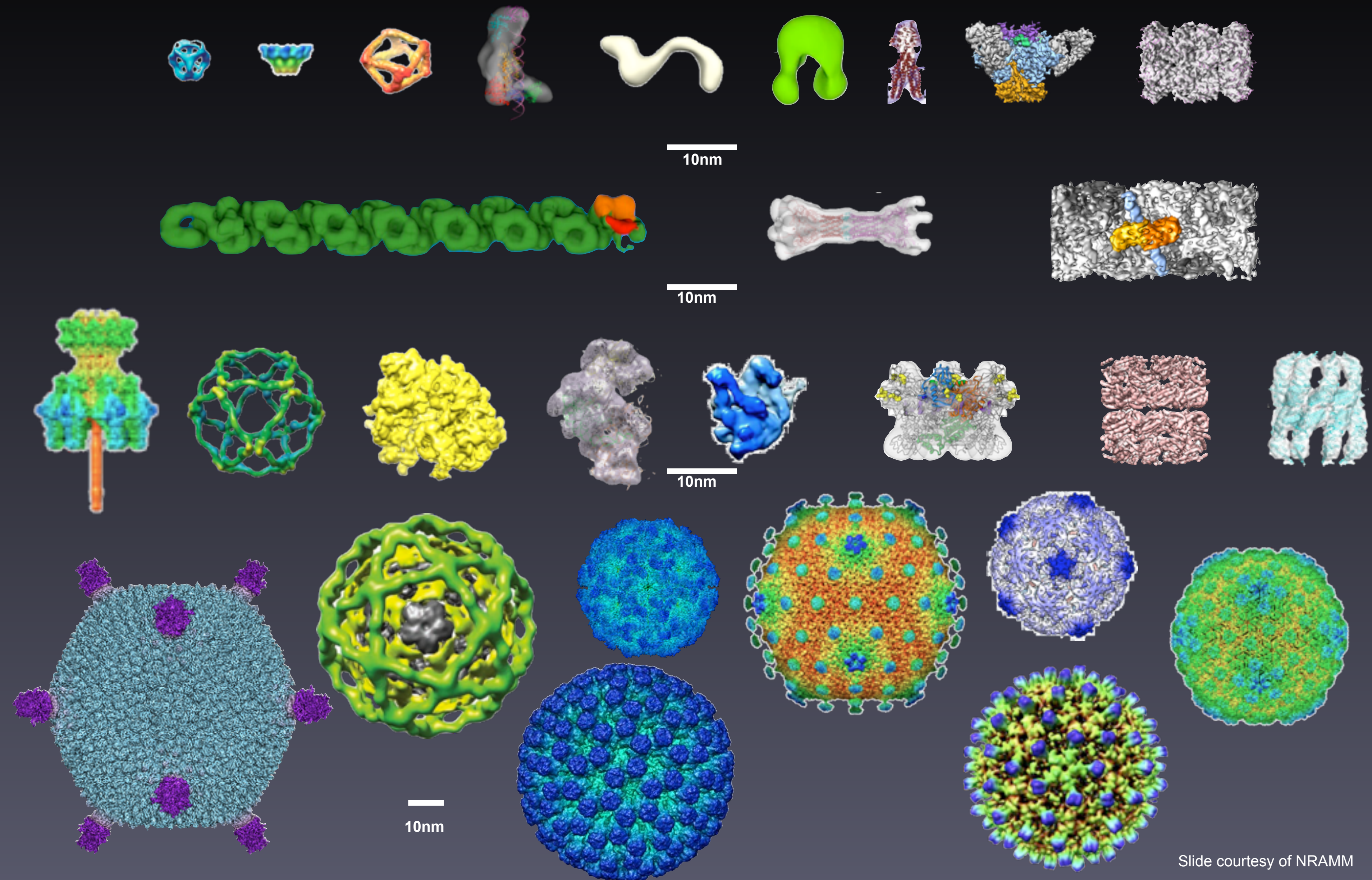






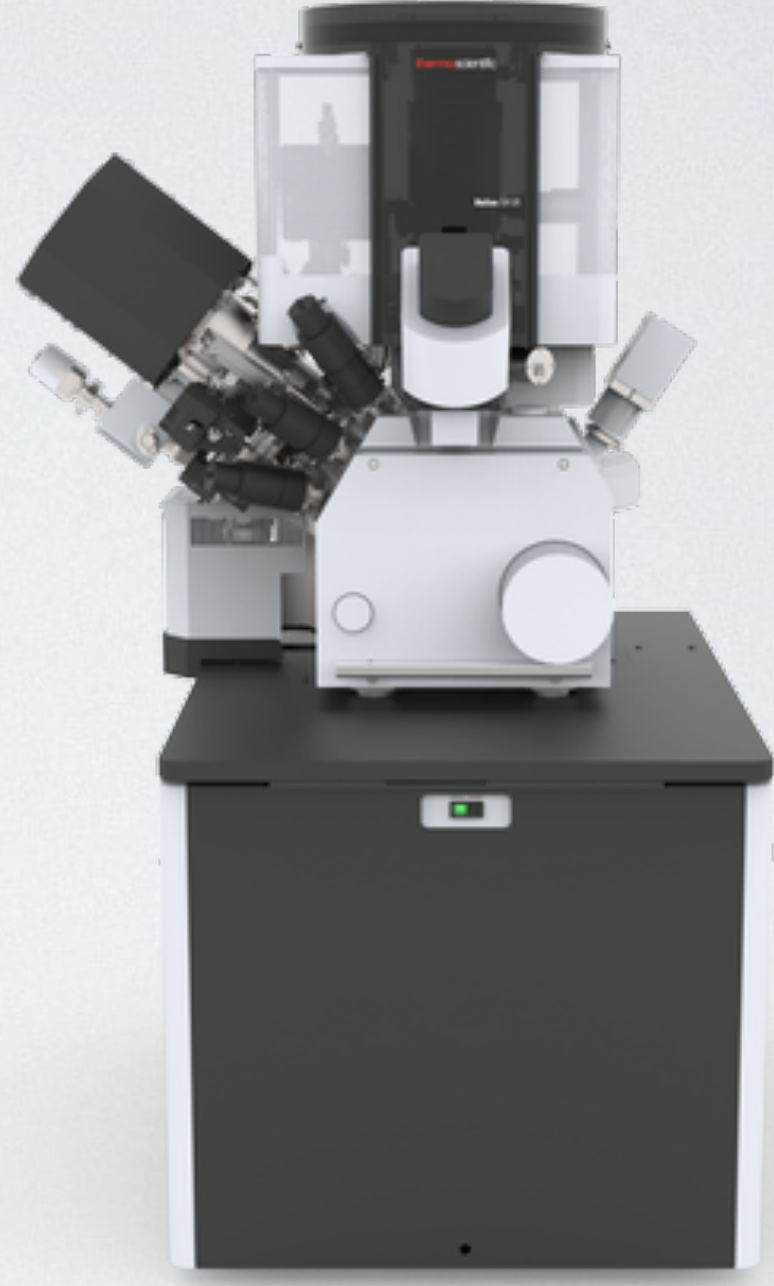


# BIOLOGICAL SAMPLES ARE AMENABLE TO EM





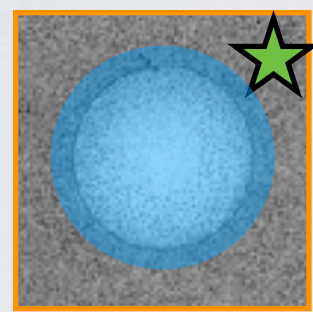
# TOOLS OF THE TRADE





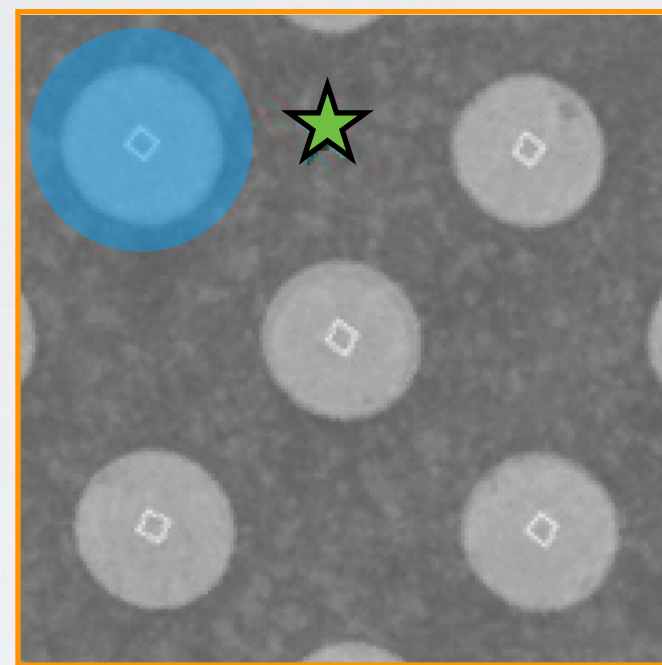
# INCREASING THROUGHPUT

1 target/setup  
80 s/image  
~1000 images/day



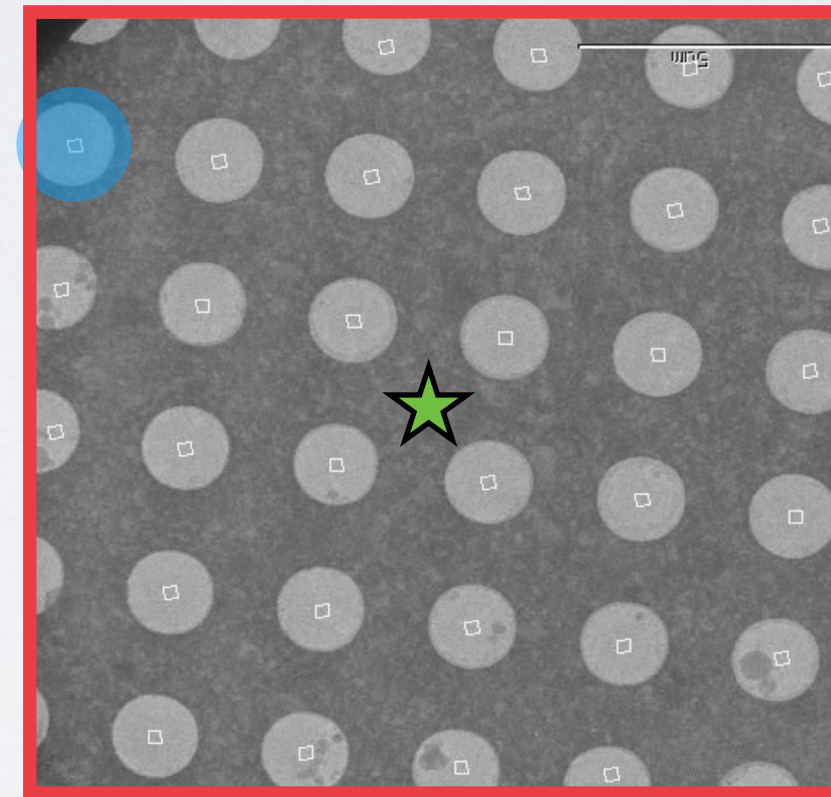
beam tilt  
0 mrad

5 targets/setup  
35 s/image  
~2500 images/day



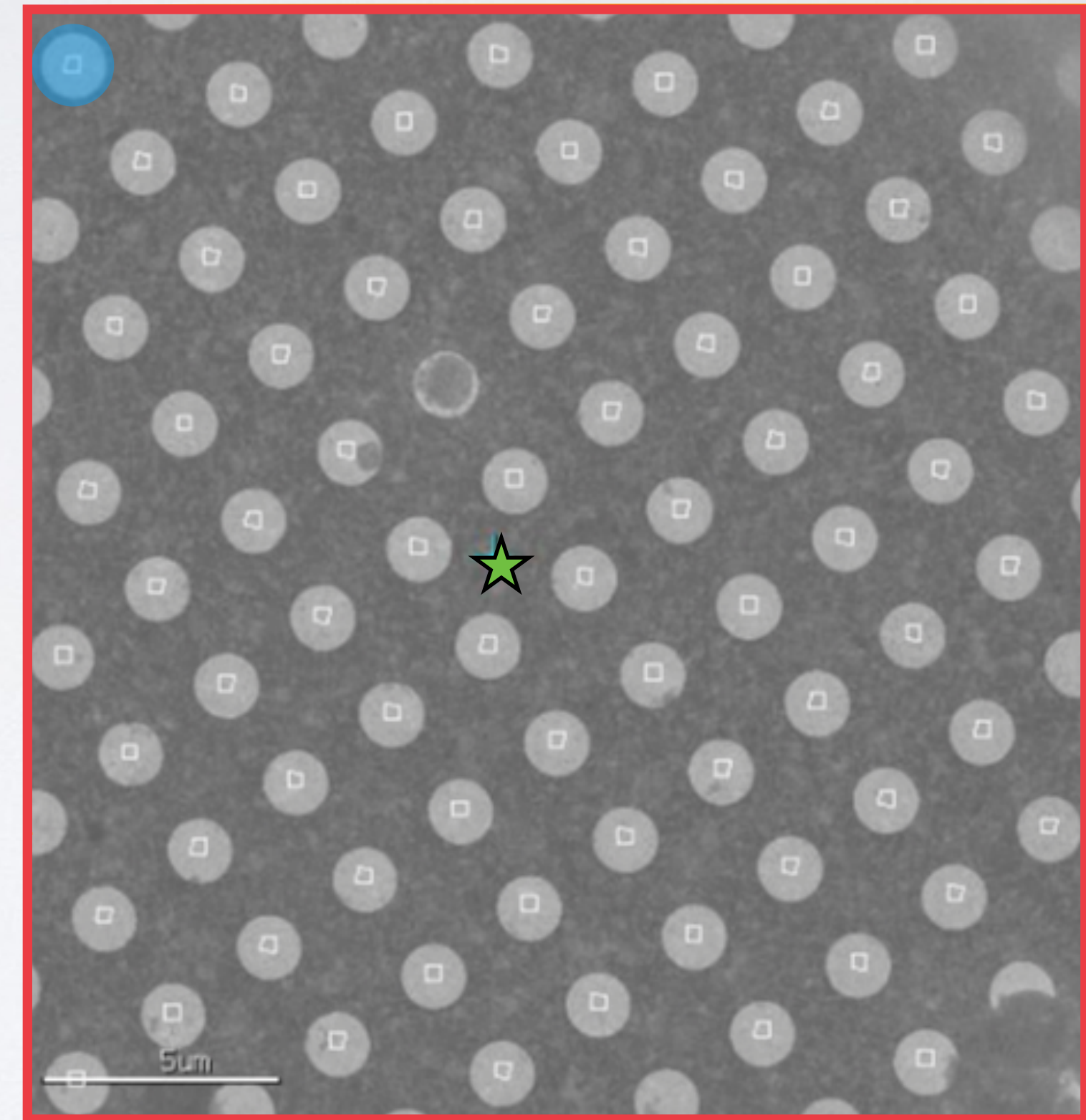
beam tilt  
0.5 mrad

30 targets/setup  
22 s/image  
~3800 images/day



beam tilt  
2 mrad

70 targets/setup  
21 s / image  
~ 4100 images/day



beam tilt  
~3 mrad

But... image shift induces beam tilt  
so... implement hardware coma correction

Overhead

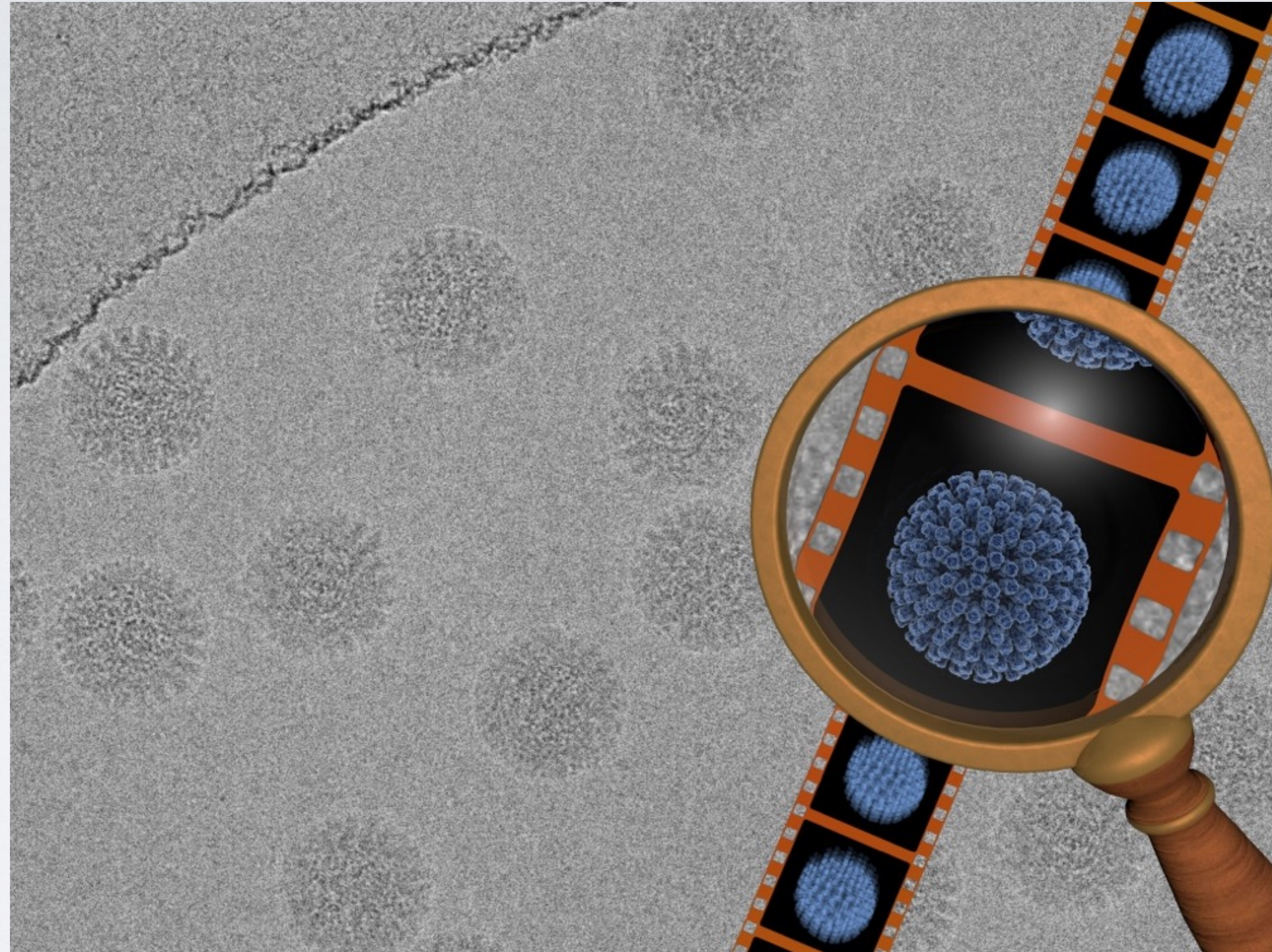
30 s stage move and settling

30 s focus and drift check

20s for K2 40 frame movie to save



# PUSHING RESOLUTION



Anchi Cheng



Melody  
Campbell

- Milazzo, A.C., Cheng, A., Moeller, A., Lyumkis, D., Jacovetty, E., Polukas, J., Ellisman, M.H., Xuong, N.H., Carragher, B., and Potter, C.S. (2011). Initial evaluation of a direct detection device detector for single particle cryo-electron microscopy. *J Struct Biol* 176, 404-408.
- Brilot, A.F., Chen, J.Z., Cheng, A., Pan, J., Harrison, S.C., Potter, C.S., Carragher, B., Henderson, R., and Grigorieff, N. (2012). Beam-induced motion of vitrified specimen on holey carbon film. *J Struct Biol* 177, 630-637.
- Campbell, M.G., Cheng, A., Brilot, A.F., Moeller, A., Lyumkis, D., Veessler, D., Pan, J., Harrison, S.C., Potter, C.S., Carragher, B., and Grigorieff, N. (2012). Movies of ice-embedded particles enhance resolution in electron cryo-microscopy. *Structure* 20, 1823-1828.

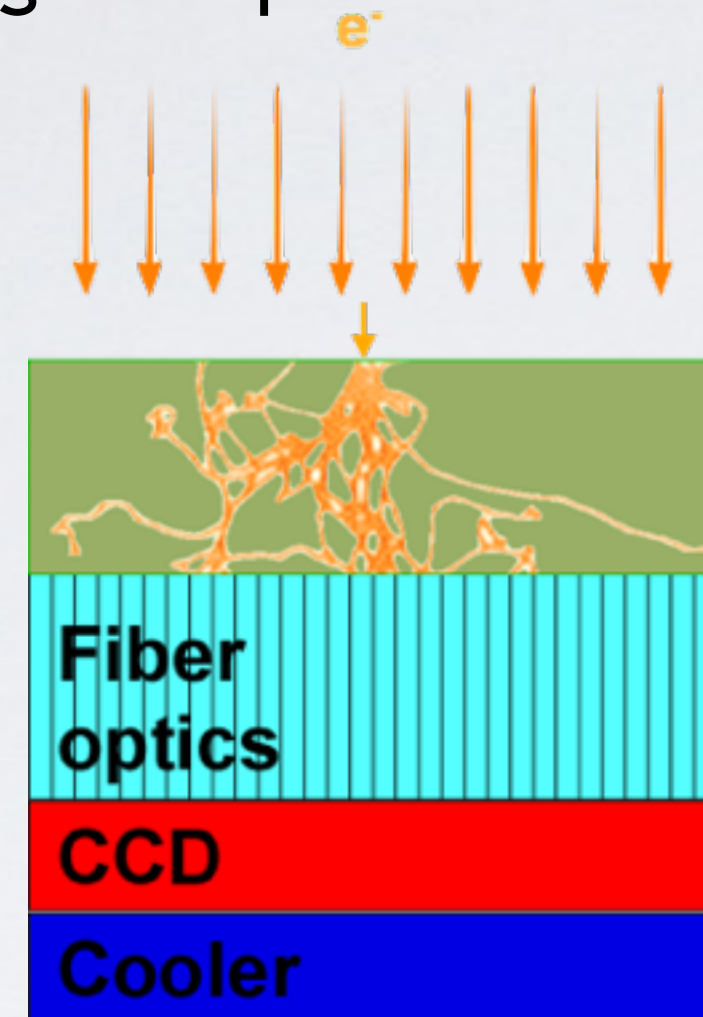


Niko Grigorieff

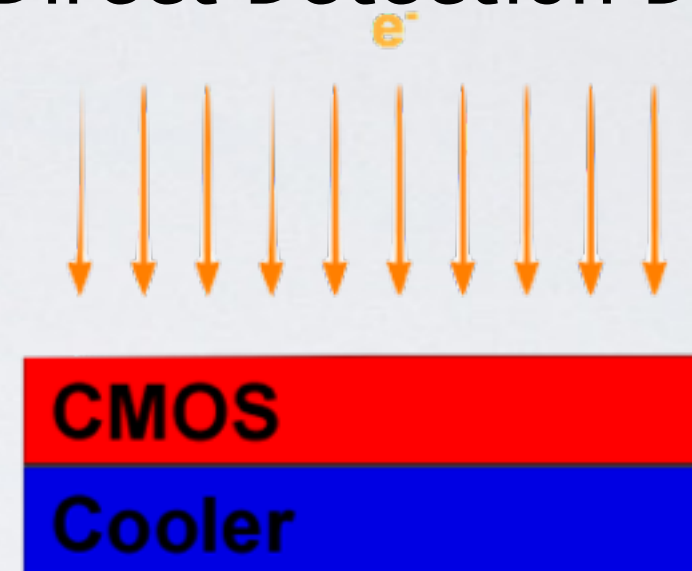
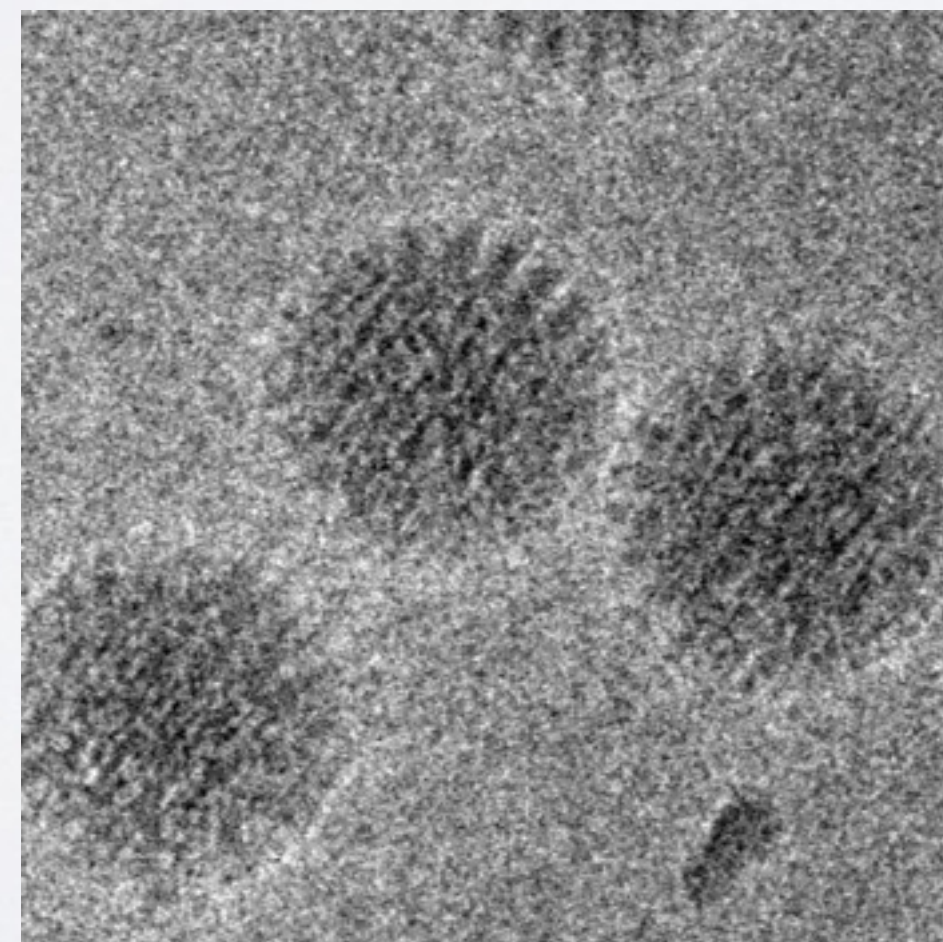


# PUSHING RESOLUTION

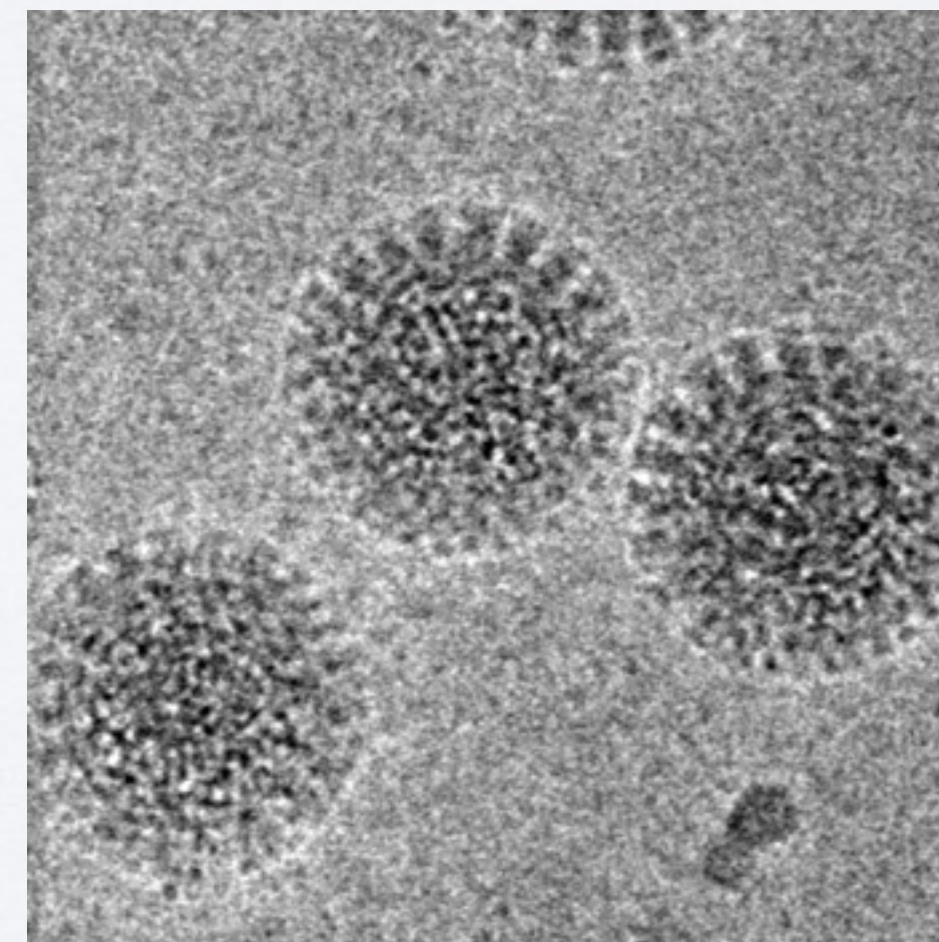
Charge Coupled Device (CCD)    Direct Detection Device (DDD)



60-frame average  
(translational alignment)

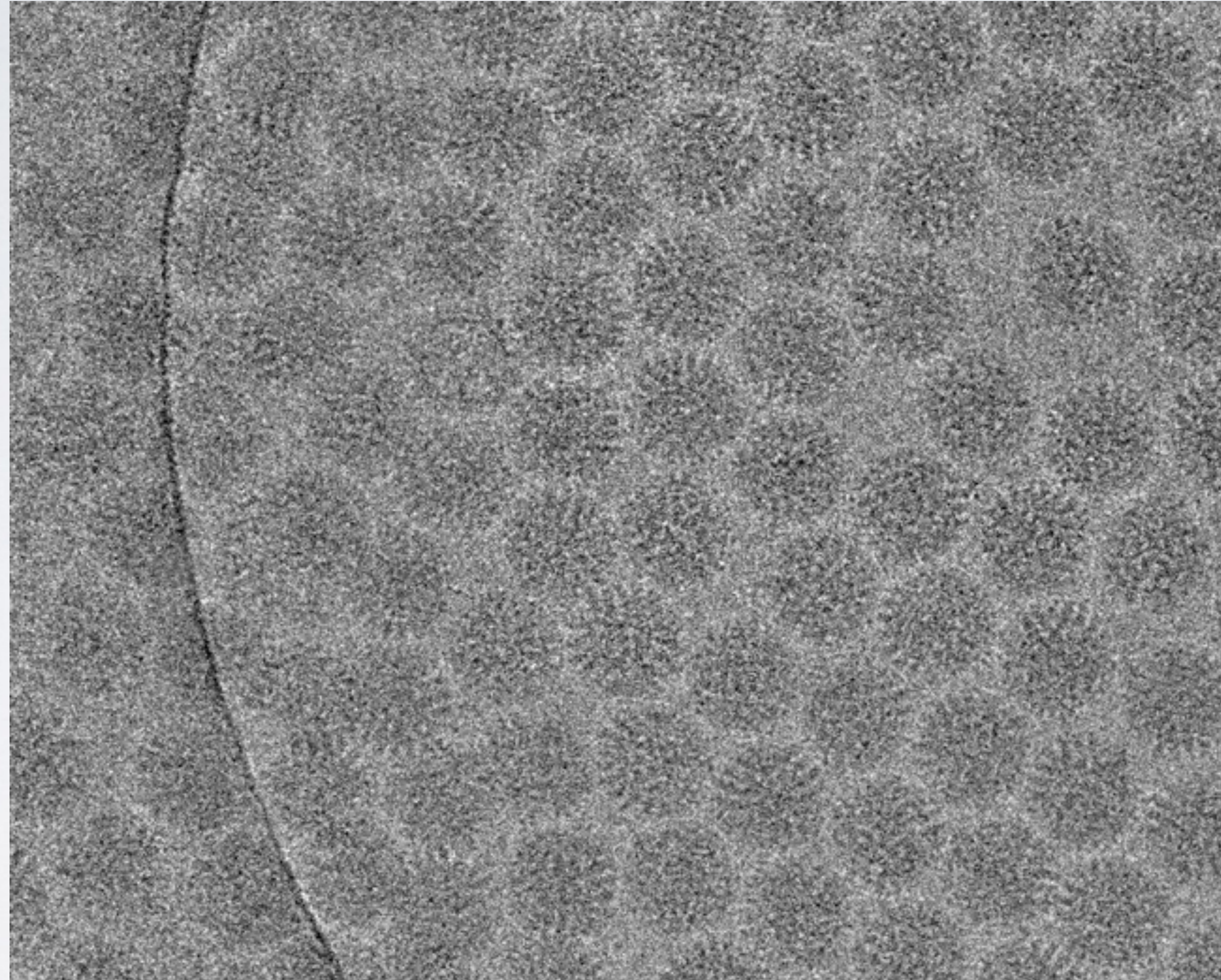


60-frame average  
(no alignment)





# PUSHING RESOLUTION



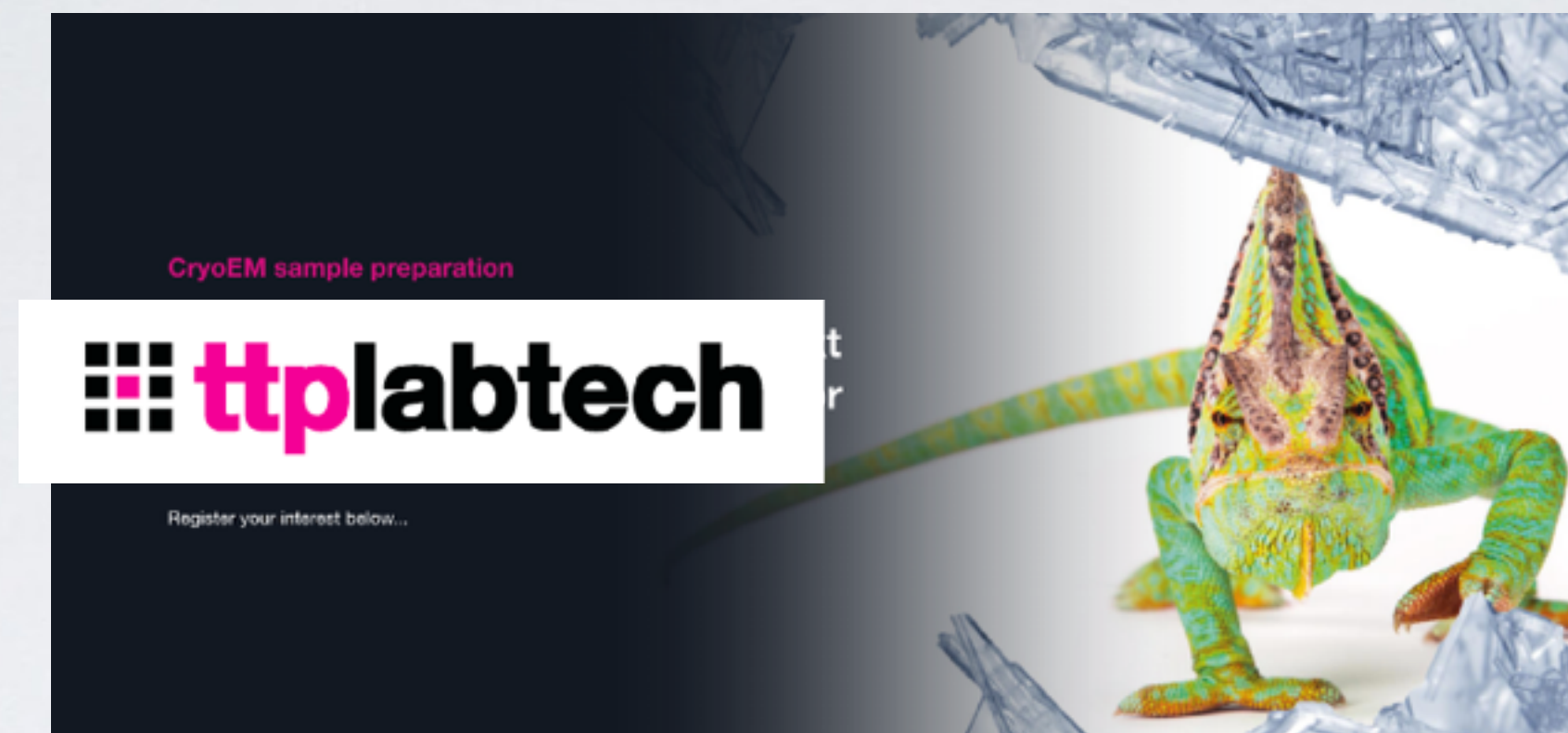
0.5 e<sup>-</sup>/Å<sup>2</sup>/frame

Image = Frame1 + Frame2 + Frame3 + Frame4 + Frame5

**We can use DDD movies to examine (and correct) “beam induced motion”**



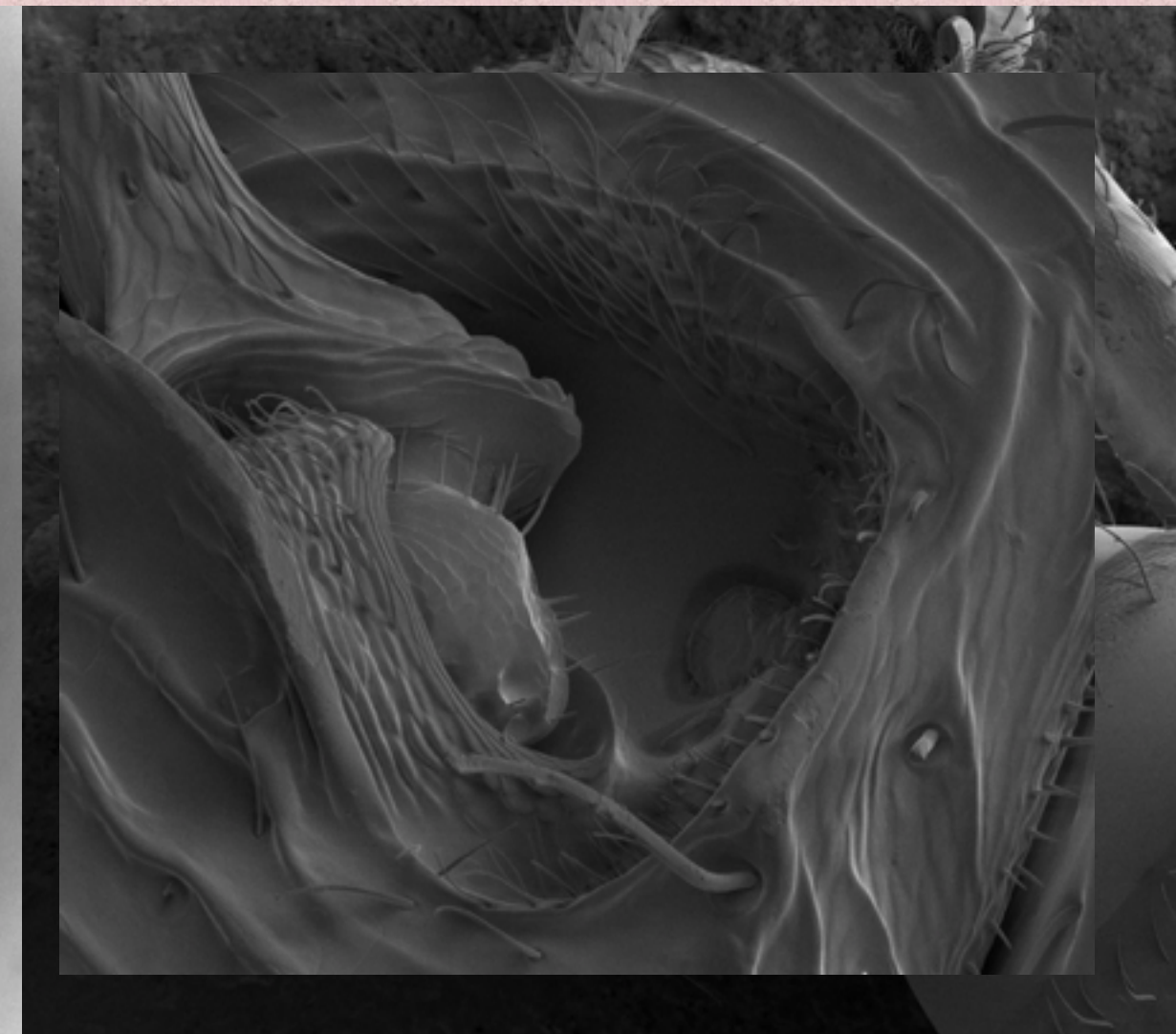
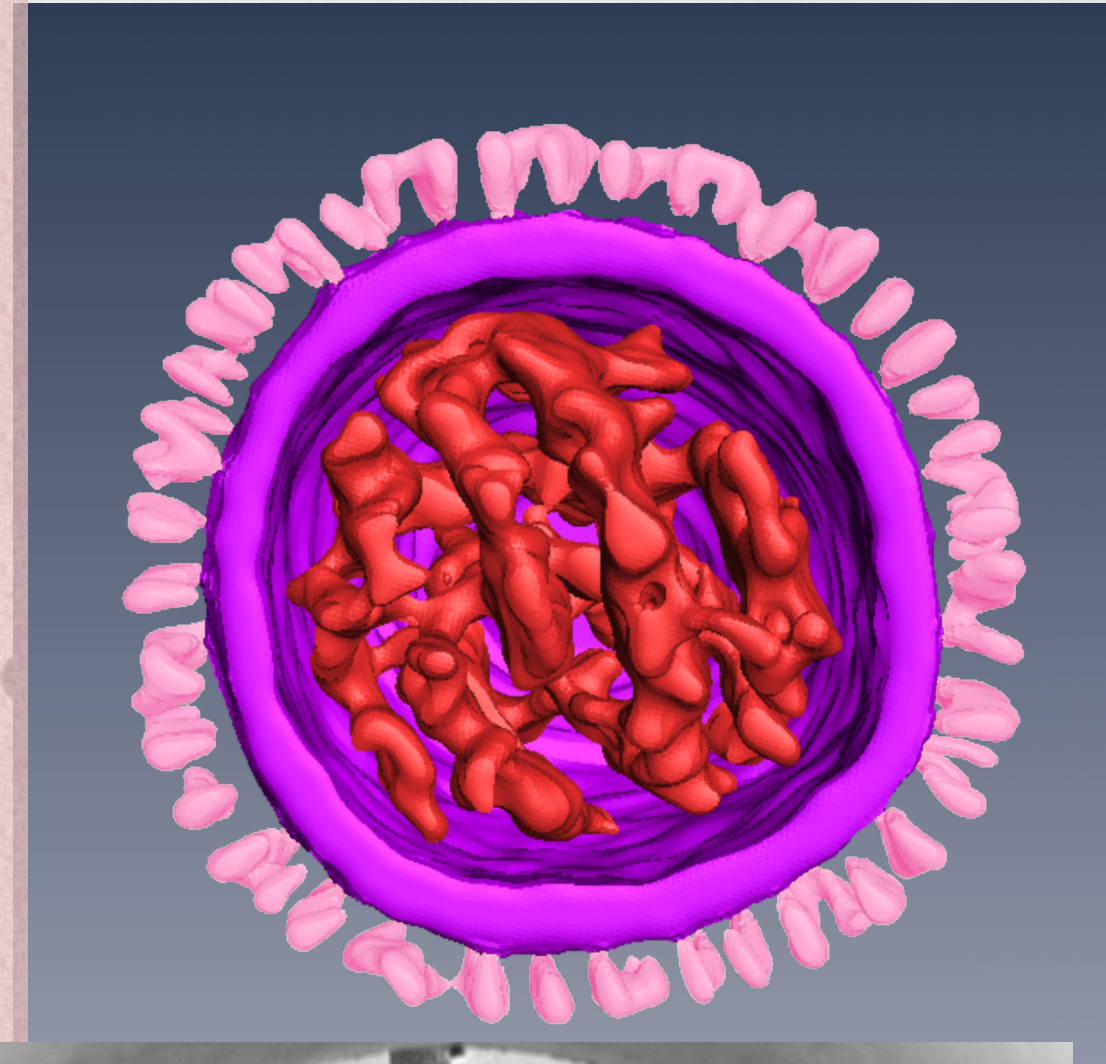
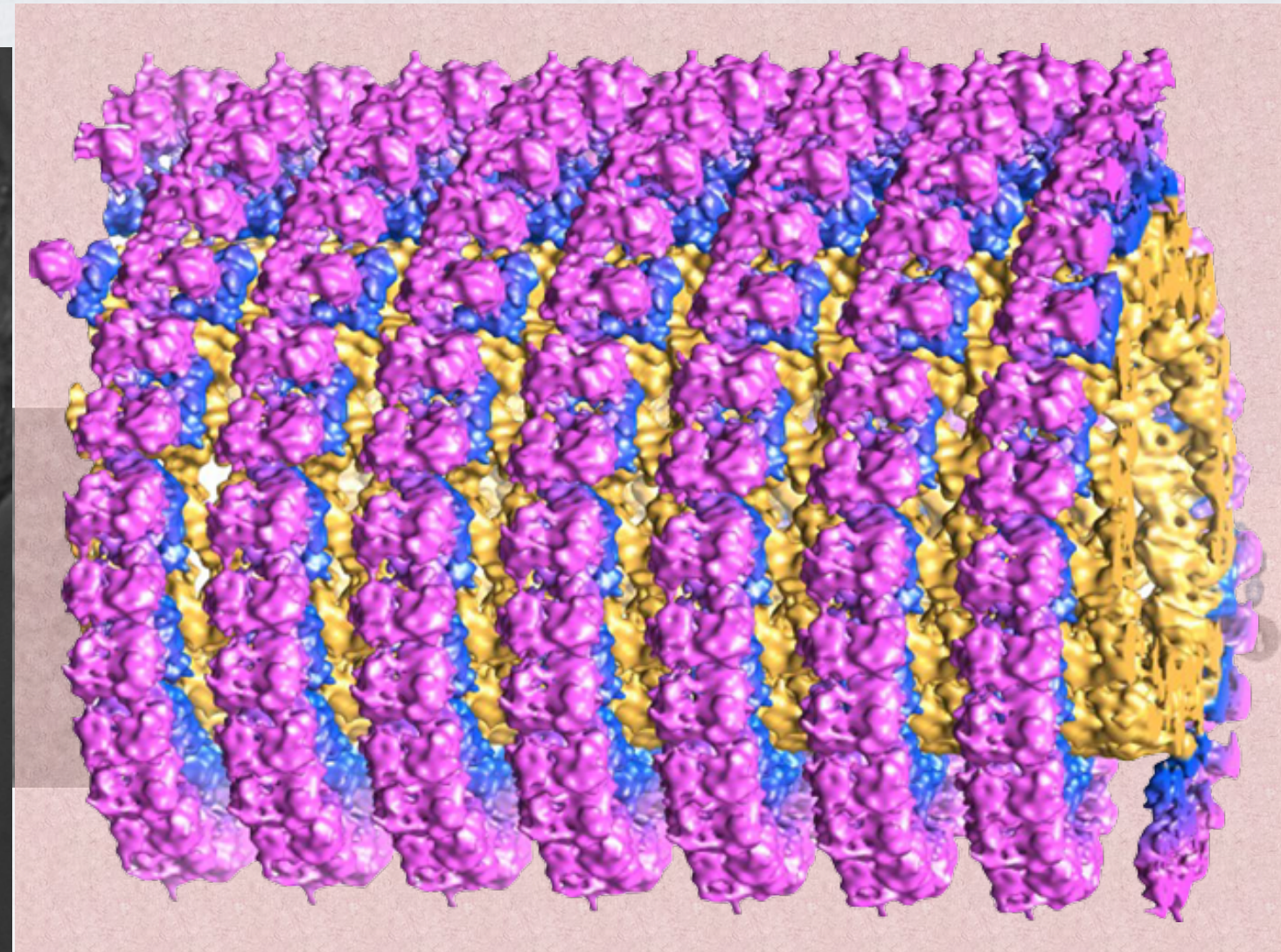
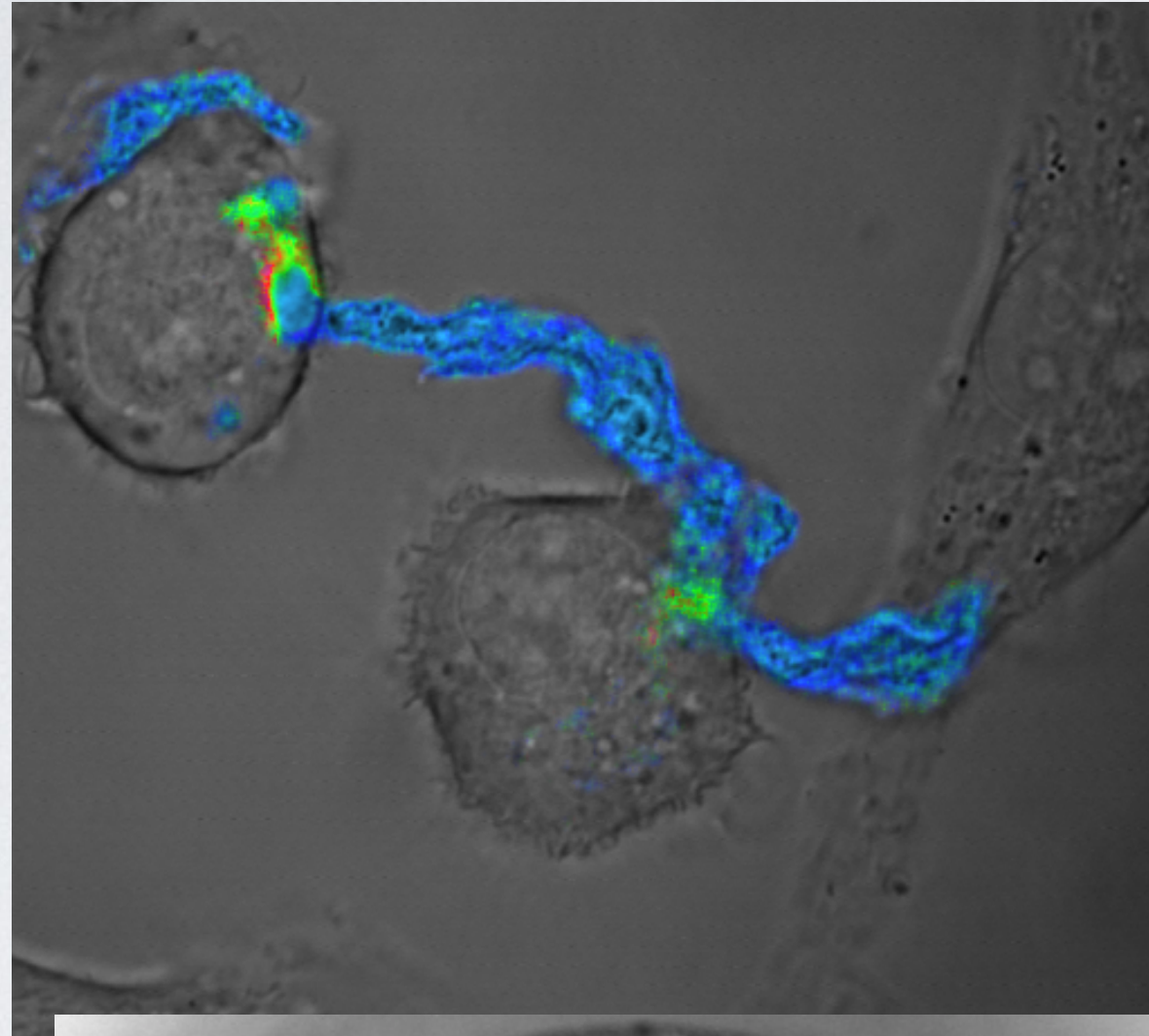
# CHAMELEON



SPOTITON EP2  
AUTOMATIC GRID PICKUP

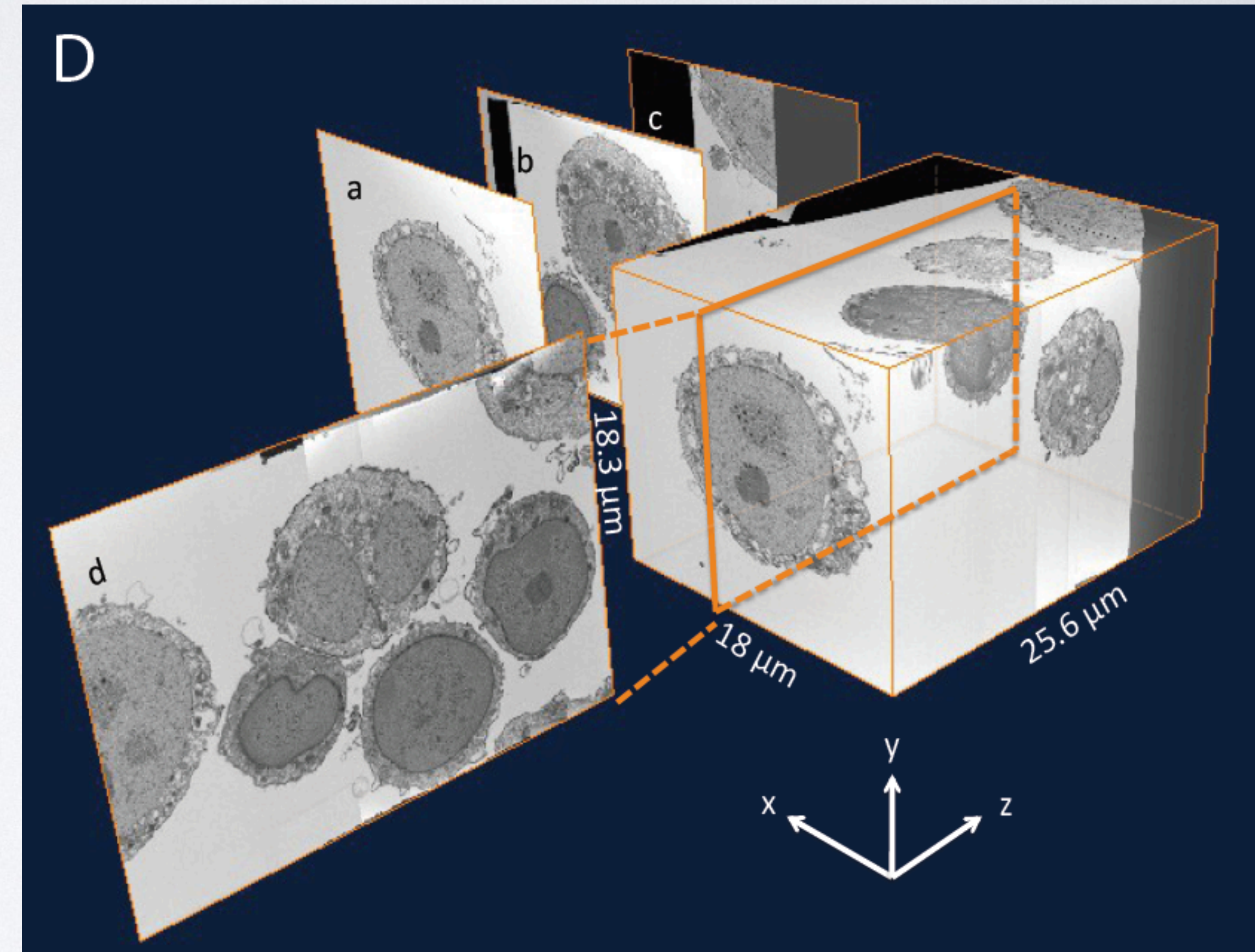
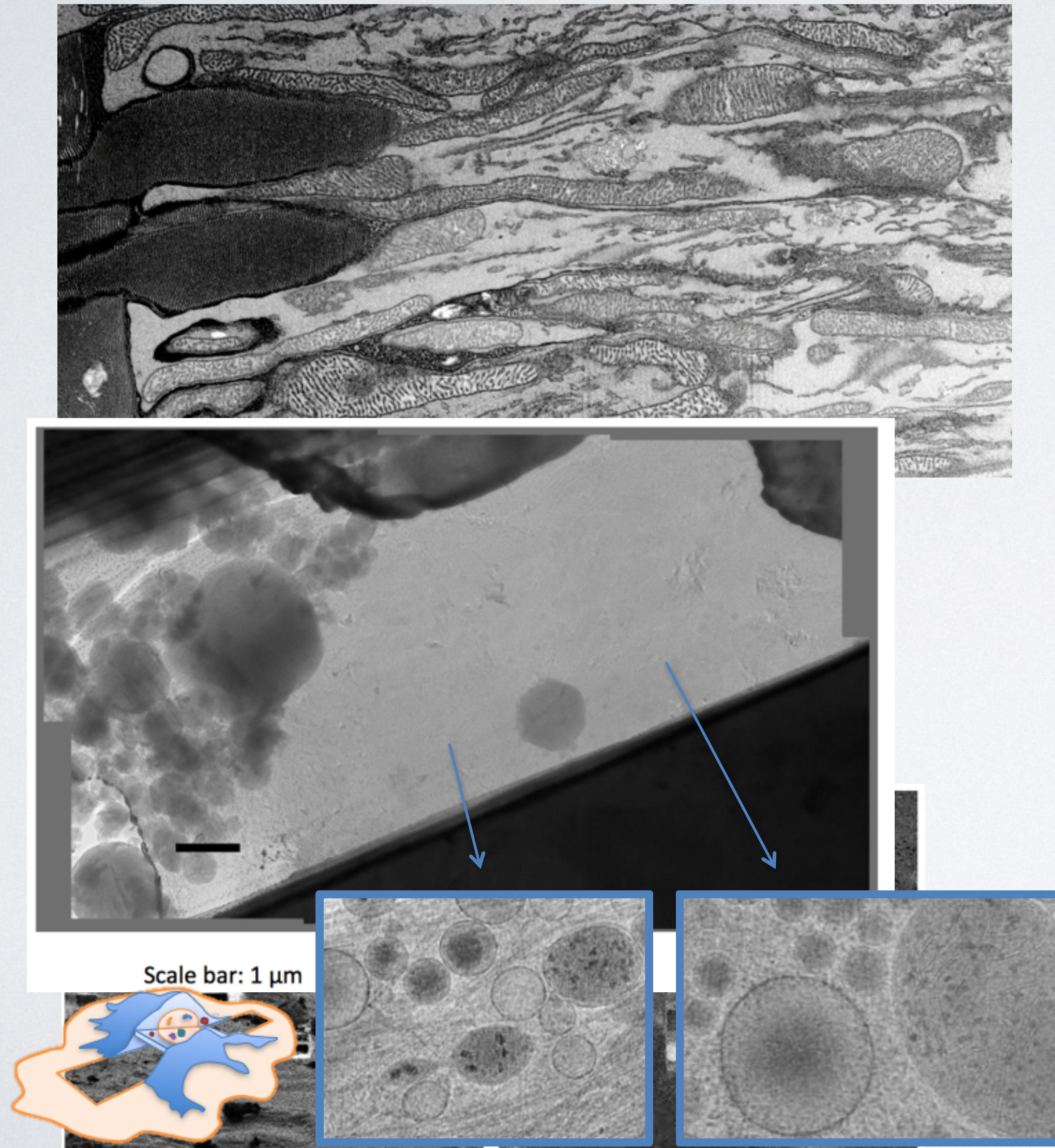


# THE NEXT CHAPTER



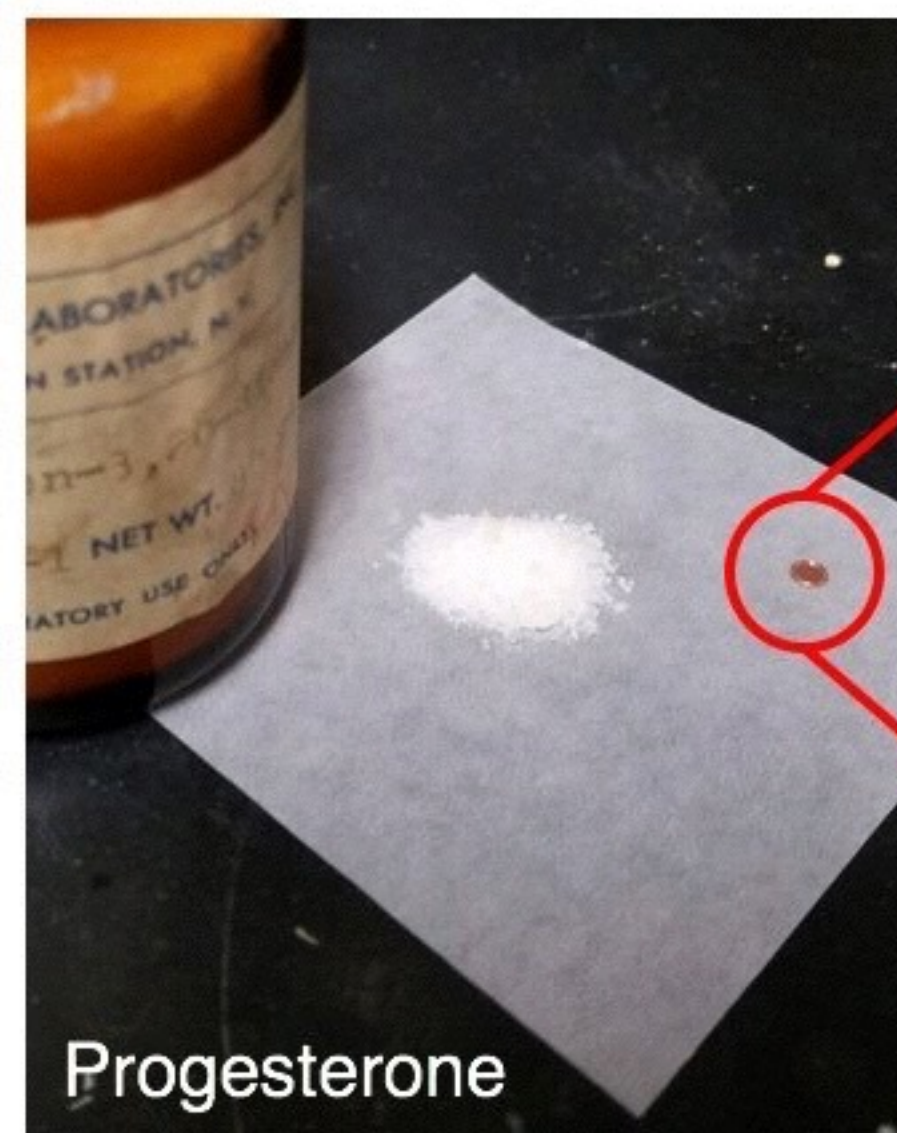


# THE NEXT CHAPTER





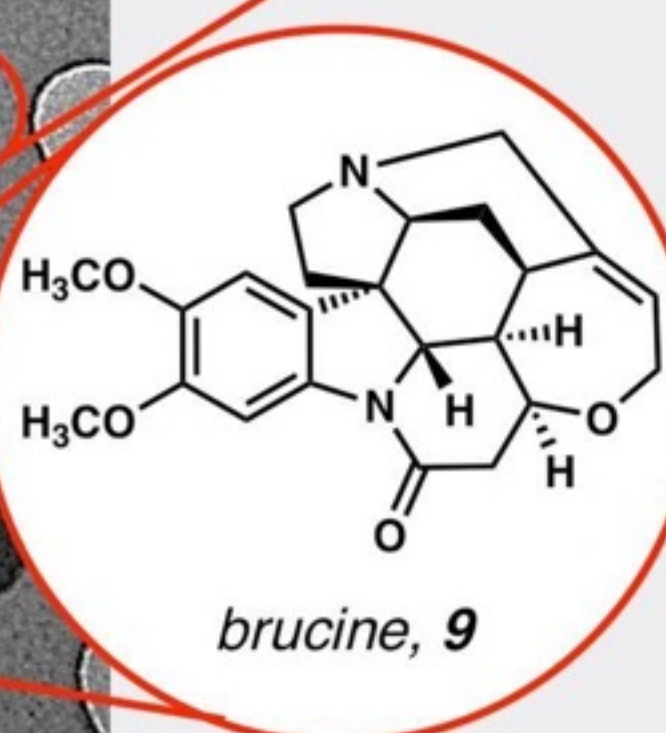
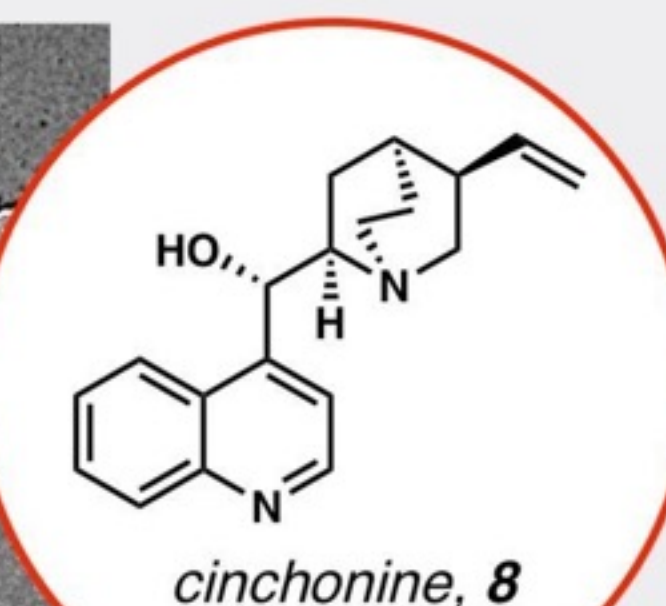
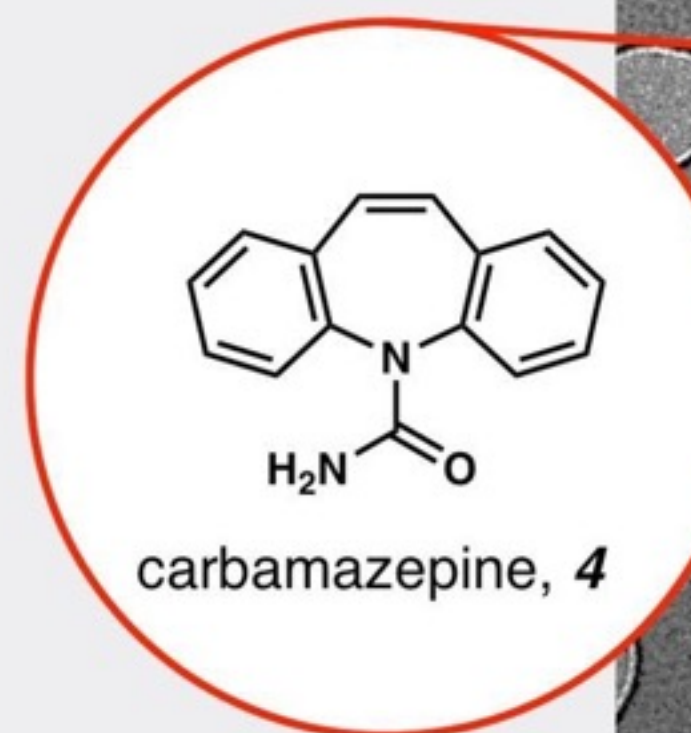
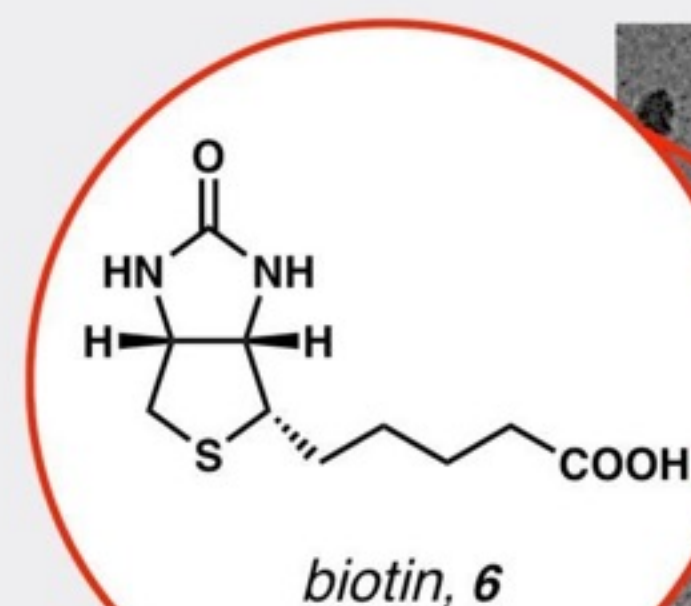
# ONE INSTRUMENT HAS SEVERAL APPLICATIONS



Powder  
 $\sim 10^{-1}$  g

Grid – nanocry  
 $\sim 10^{-15}$  g

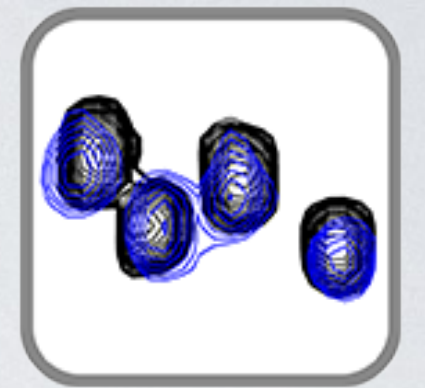
G. Jones et al./ACS Central Science 2018



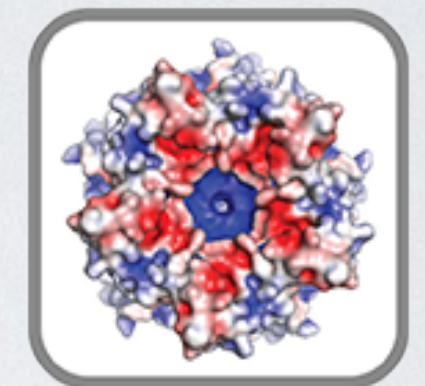
C G Jones et al DOI: 10.26434/chemrxiv.7215332.v1



# THE NEW YORK STRUCTURAL BIOLOGY CENTER



NMR  
Spectroscopy



X-Ray  
Crystallography


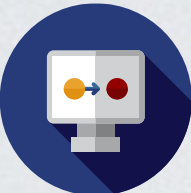
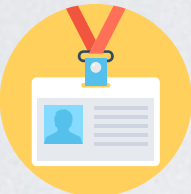




Electron  
Microscopy





# TRAINING SCHEDULING

Type	Frequency	Title	Description
 knowledge	yearly	SEMC EM Course	Theory behind EM <i>Graduate level course</i>
 computation	quarterly	SEMC Appion workshop	Data processing workshops
 access & certification	monthly	SEMC New User Orientation	Facility use and Safety Training <i>Leginon intro/use of screening microscopes</i>
 troubleshooting	weekly	User Project Discussion Meetings	<i>Appointments: Tue @ 3pm, Thurs @3pm &amp; @ 3:30pm</i>
 instrumentation	daily	Advanced Leginon use / 1-1 training appointments	Training for independent use of the microscopes and other SEMC resources



# TRAINING RESOURCES



**Bill Rice**  
Senior Scientist,  
EMG Manager



**Anchi Cheng**  
Res. Staff Scientist



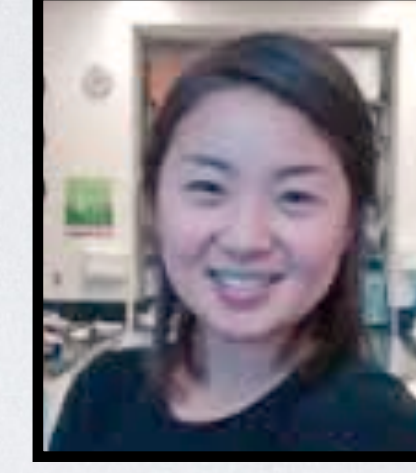
**Lorenzo Finci**  
Staff Scientist



**Alex Noble**  
Post Doc.



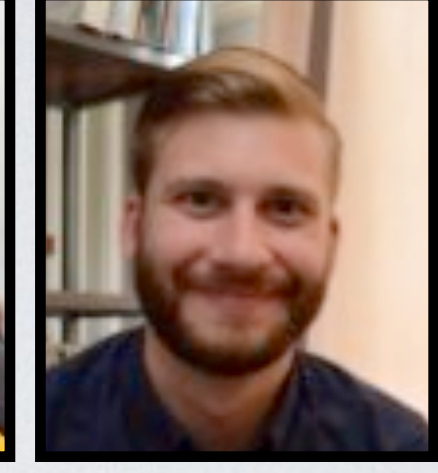
**Ed Eng**  
Scientist,  
NCCAT Manager



**Laura Kim**  
Scientist



**Yong Zi Tan**  
Grad. Student



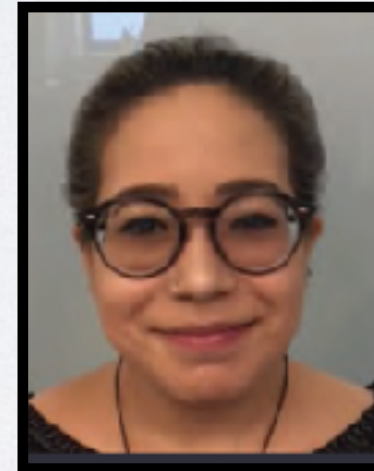
**Micah Rapp**  
Grad Student



**Misha Kopylov**  
Research Associate



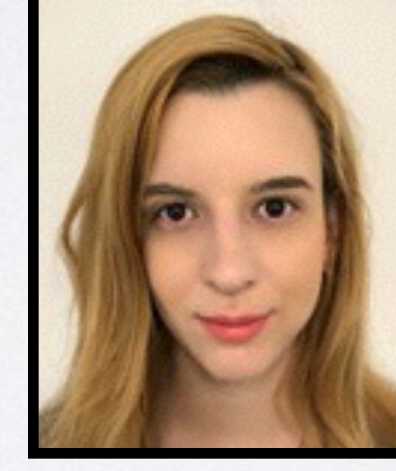
**Ashleigh Raczkowski**  
Senior Technician



**Daija Bobe**  
Technician



**Carolina Hernandez**  
Technician



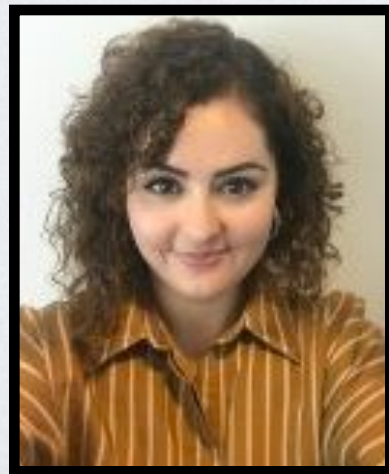
**Bruna Fitipalti**  
Intern



**Swapnil Bhatkar**  
Sys. Admin



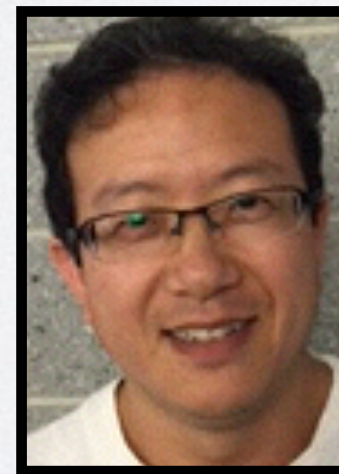
**Shakar Kris**  
Res. Programmer



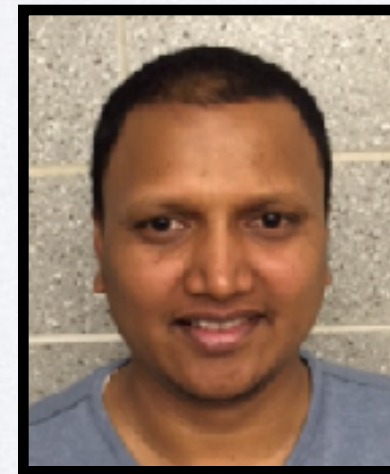
**Elina Kopylov**  
Traffic Controller



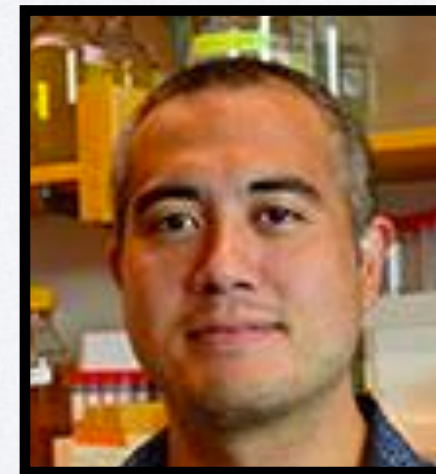
**Sargis Dallakyan**  
Res. Programmer



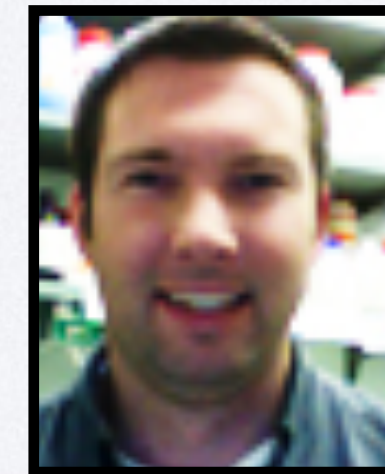
**Alex Wei**  
Technician



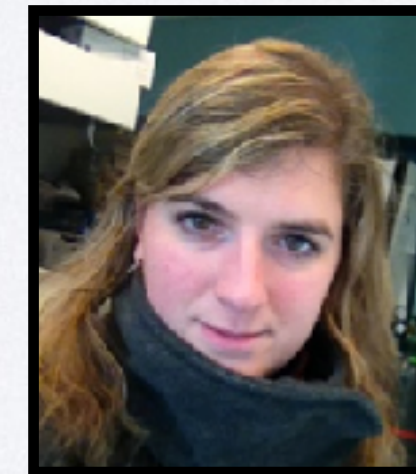
**Venkat Dandey**  
Post Doc.



**Kotaro Kelley**  
Post Doc.



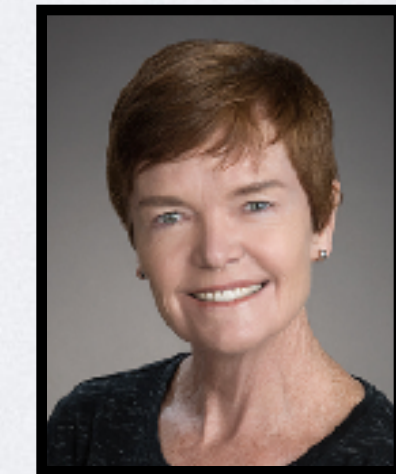
**Jason Gorman**  
Embedded Post Doc.



**Julia Brasch**  
Embedded Post Doc.



**Clint Potter**  
Director



**Bridget Carragher**  
Director



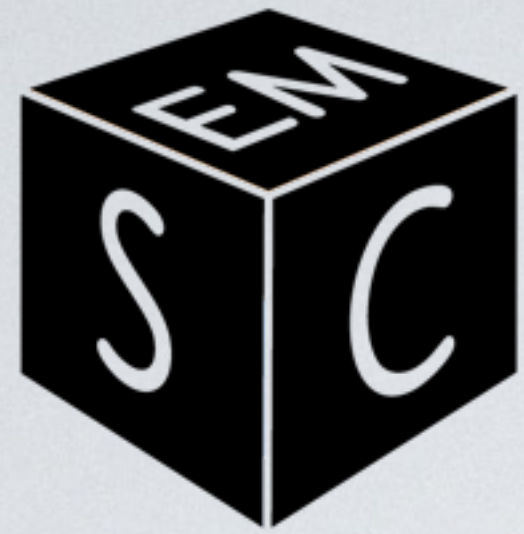
# NATIONAL CENTER *FOR* CRYOEM ACCESS *AND* TRAINING

NCCAT



New York  
Structural  
Biology  
Center



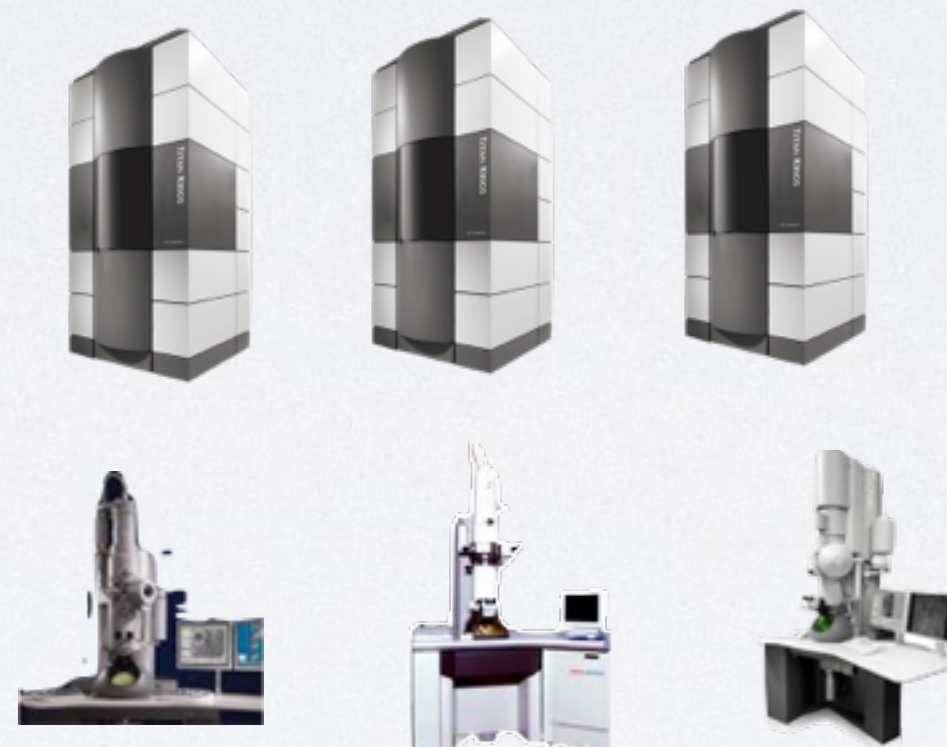


# Simons Electron Microscopy Center

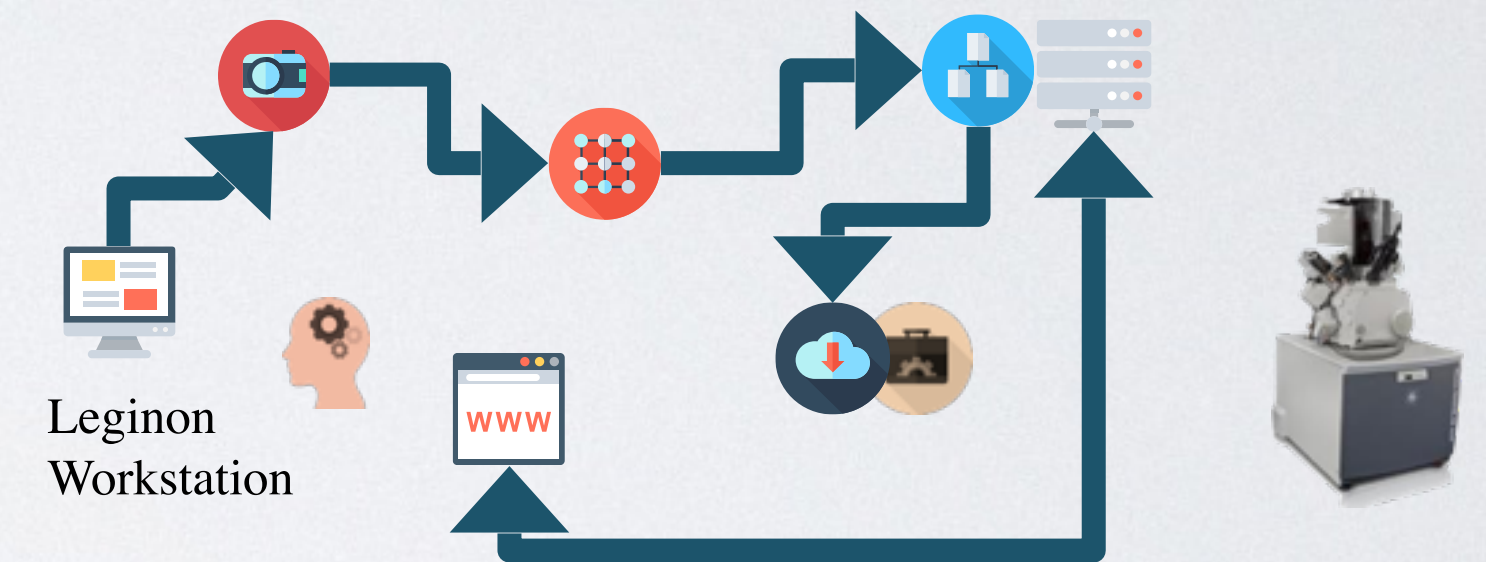
## Tour



Automated sample prep



Automated image acquisition



Automated image processing

