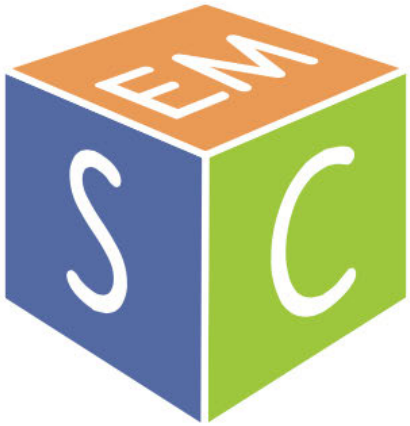



SIMONS ELECTRON MICROSCOPY CENTER

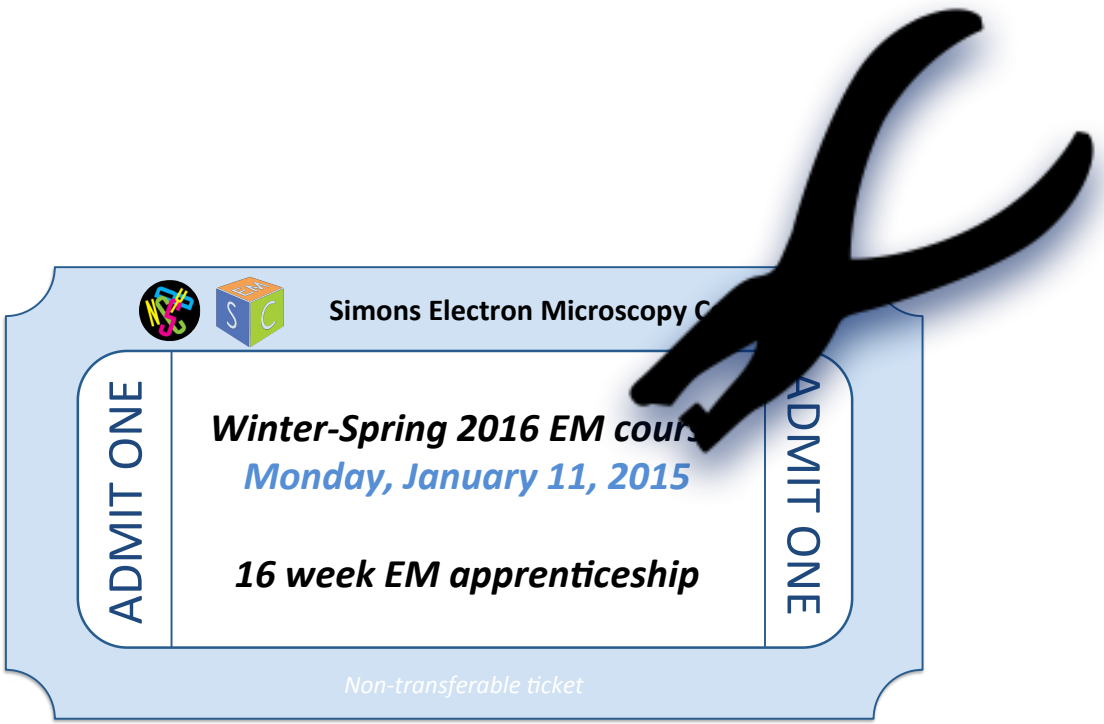
NEW YORK STRUCTURAL BIOLOGY CENTER

# Winter-Spring 2016 EM Course

40 Min Intro + 10 min tour
11 Jan 2016

# Art is science made clear.

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



# Art is science made clear.

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

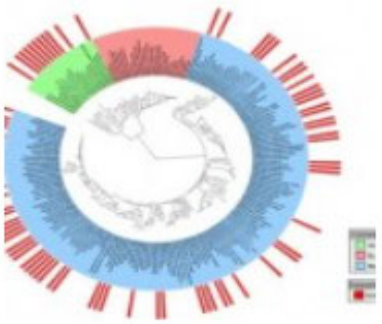
Painter's Studio
by Jan van der Straet (Stradanus) (Dutch, 1523-1604)

# NEW YORK STRUCTURAL BIOLOGY CENTER


NMR

X-ray

EM



Protein Production

# Driven by….

Albert Einstein College of Medicine of Yeshiva University

Columbia University in the City of New York

CUNY The City University of New York

Memorial Sloan-Kettering Cancer Center

Mount Sinai

NYU

The Rockefeller University

New York State Wadsworth Center, New York State Department of Health

Weill Cornell Medical College, Cornell University

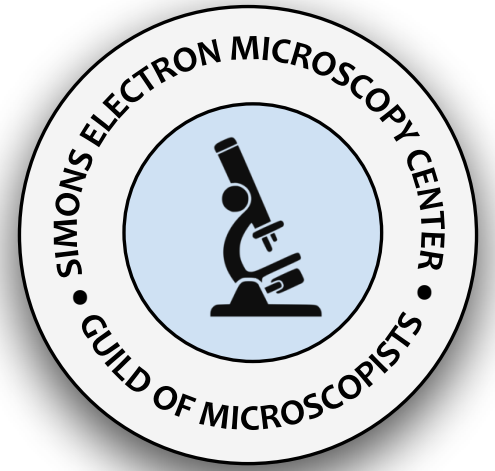
NYSBC non-member users

NRAMM

**National Resource for Automated Molecular Microscopy**

Simons Foundation

# Schedule




- *Welcome new students*

- Course logistics
  - Questionnaire

- Introduction to EM and the course

- Simons Electron Microscopy Center
  - SEMC training programs
  - NYSBC tour

# Handouts

Logistics

Questionnaire

Syllabus

# 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
- Starting
- Out
- Training
- Best Usage

Documentation
Publications
Instrumentation

## Winter-Spring 2016 EM Course

### Simons Electron Microscopy Center EM 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).

Classes will be Mondays from 3:30-5:00 pm. On Wednesday at 3:30 pm we will screen the Jensen lectures that will be covered the following week.

To register for the course please fill out the following application form.

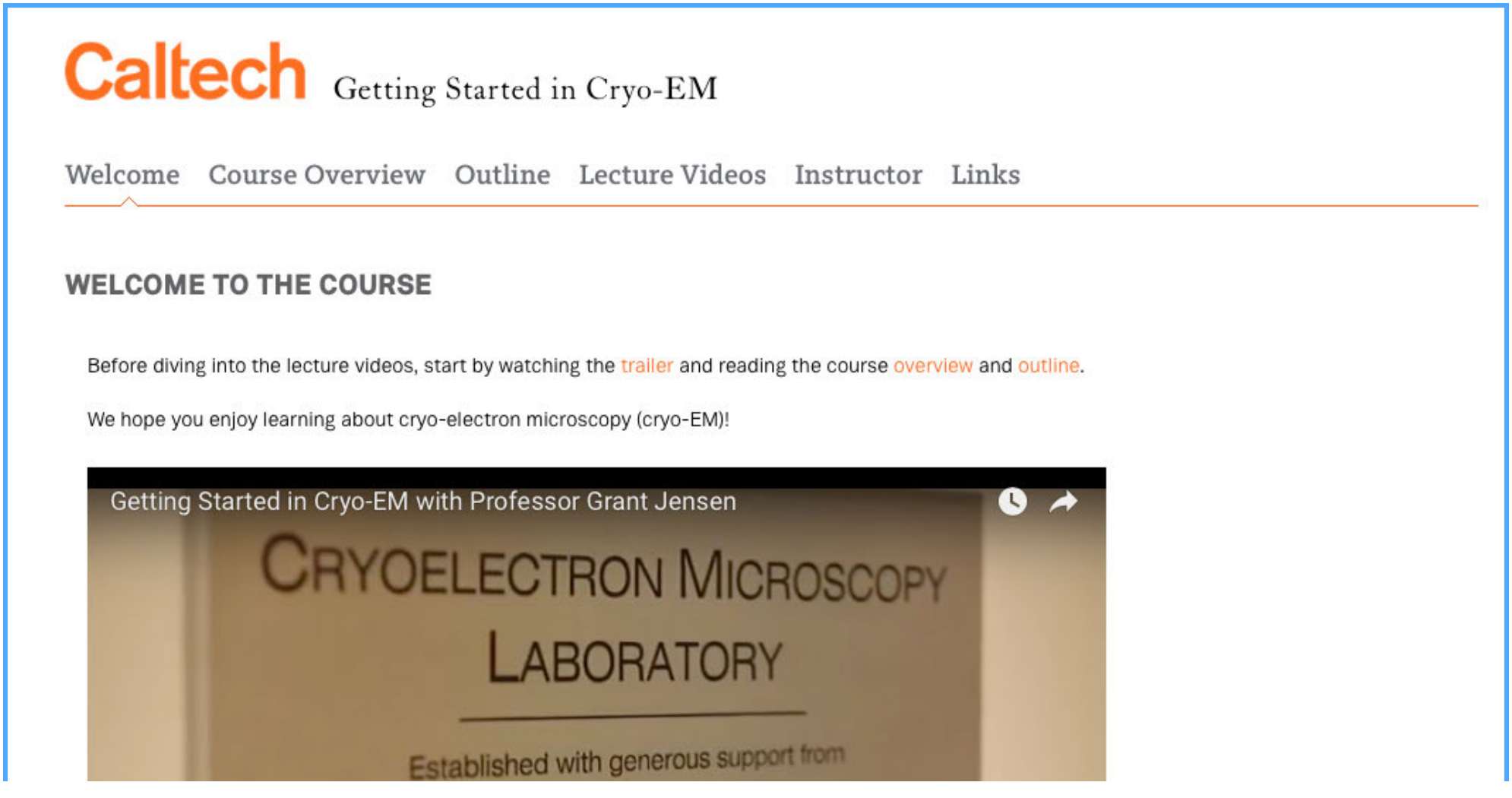
### Course Syllabus:

#### Classes in A-11 seminar room (Mon 3:30-5pm)

- **Jan 11** : Introduction & SEMC tour (short class) [Ed Eng & SEMC staff, NYSBC]
- **Jan 18** : No class - Martin Luther King
- **Jan 25** : Basic anatomy of the electron microscope [Christoph Wigge & Anchi Cheng, NYSBC]
- **Feb 1** : Fourier transforms and Image Formation [Bill Rice, NYSBC]

# Video lectures

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



# Class organization

## Monday

3:30-5pm - A11 seminar room

### 1.5 hr class

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

## Wednesday *(optional)*

Starts at 3:30 *(or earlier if possible)* - SEMC conference room

### Video screening/Recitation section

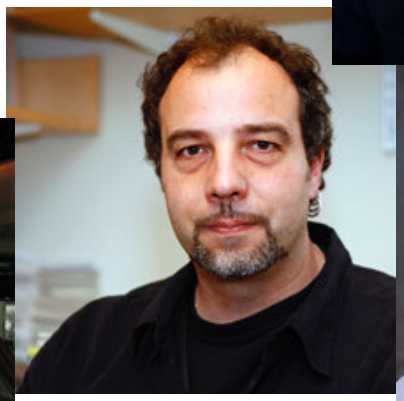
Jensen lectures that will be covered the next week will be played
SEMC lecturers will be available to assist with lecture topics

# Team of Instructors

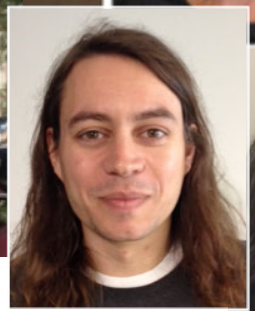
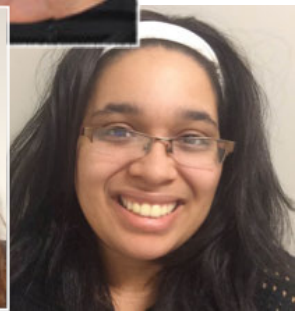


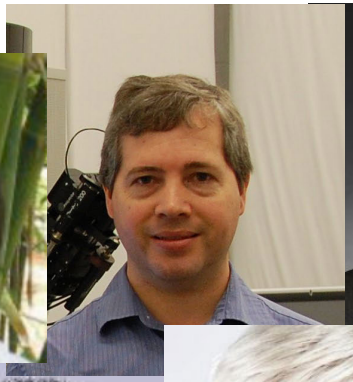
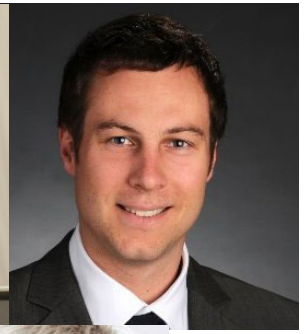


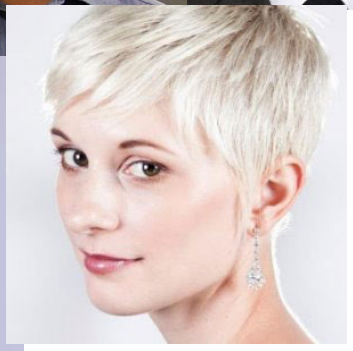






# Class structure

Section 0: Course overview / Intro to EM

Section 1: Anatomy of an EM

Section 2: Fourier transforms and Image Formation

Section 3: Challenges in EM & Sample prep

Section 4: Tomography
*Part I: ET*
*Part II: FIB-SEM*

Section 5: Single Particle
*Part I: SP Analysis & Sample prep*
*Part II: Data collection & Reconstruction*
*Part III: Additional topics & limitations*

Section 6: 2D crystallography
*Part I: 2dx*
*Part II: Helical*

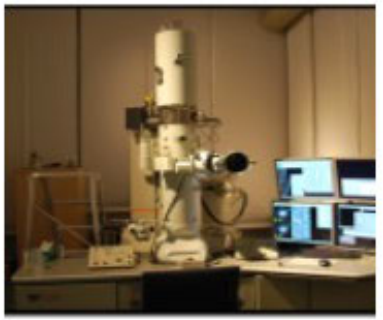
Section 7: EMDB, Validation methods & Fitting Atomic models

# Question:

What biological systems are you interested in?

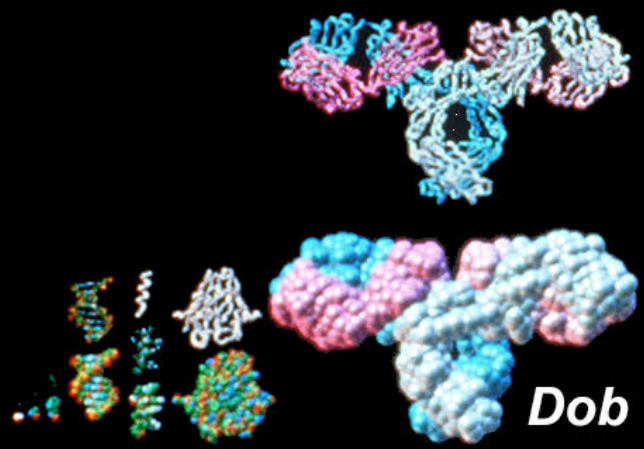
NMR

X-ray

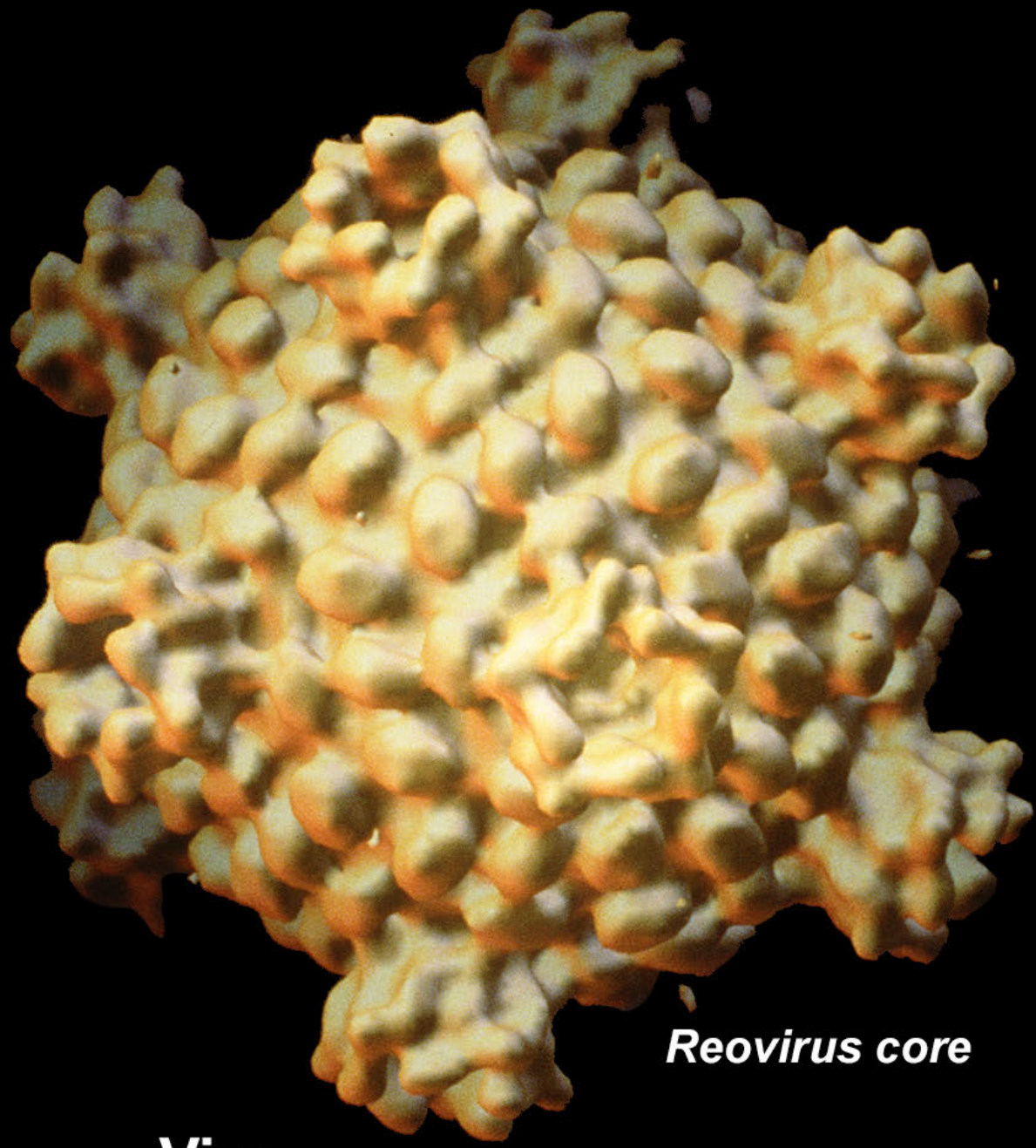


EM

# The Scale of Biological Structures

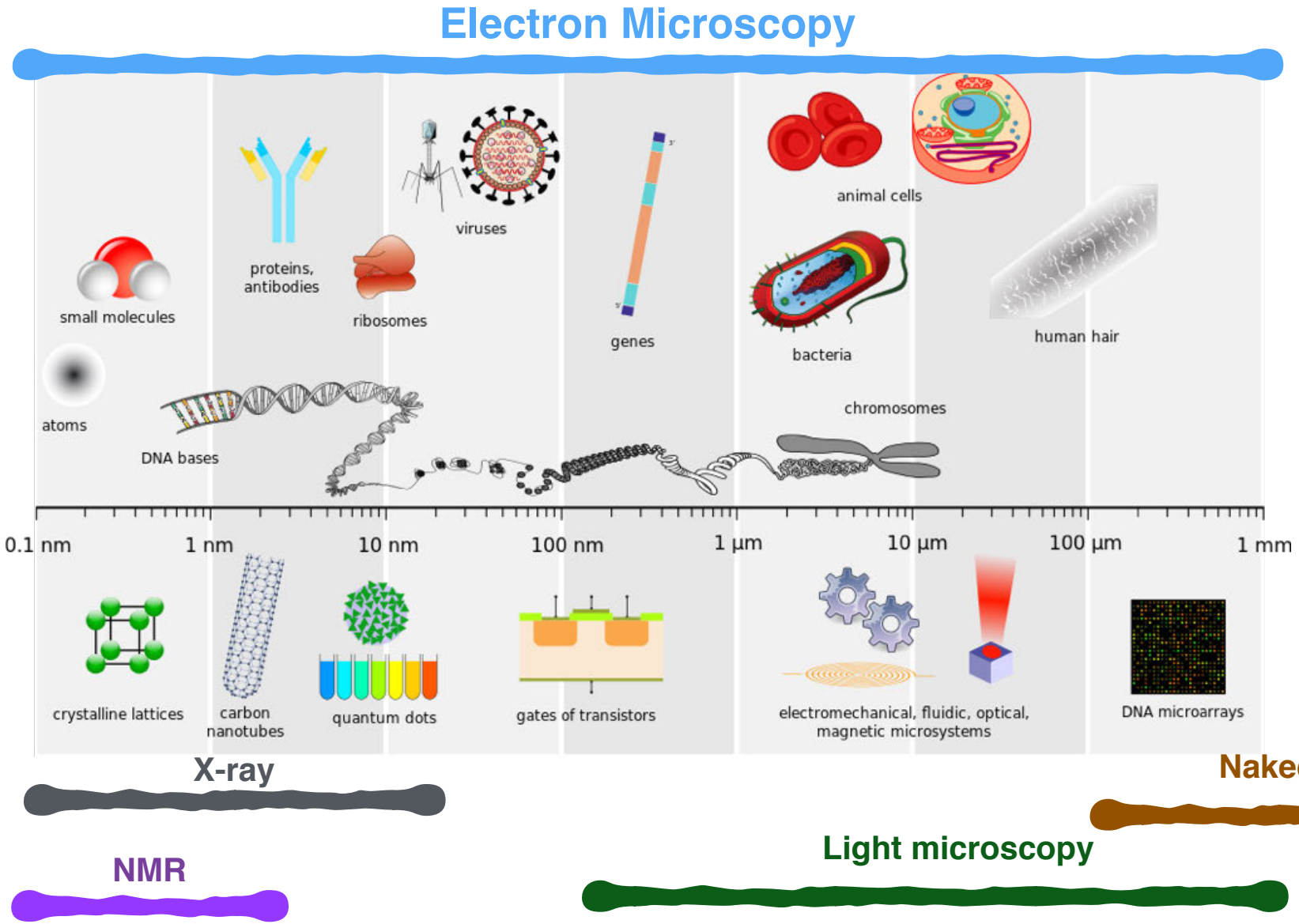



Antibody




Virus

# Nanoscale: The scale of biological structures




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

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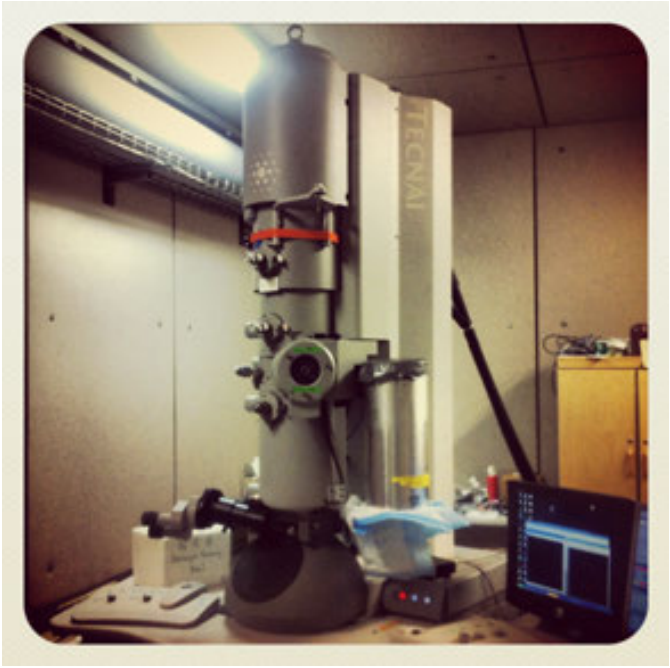
# Comparison of a light microscope, TEM & SEM

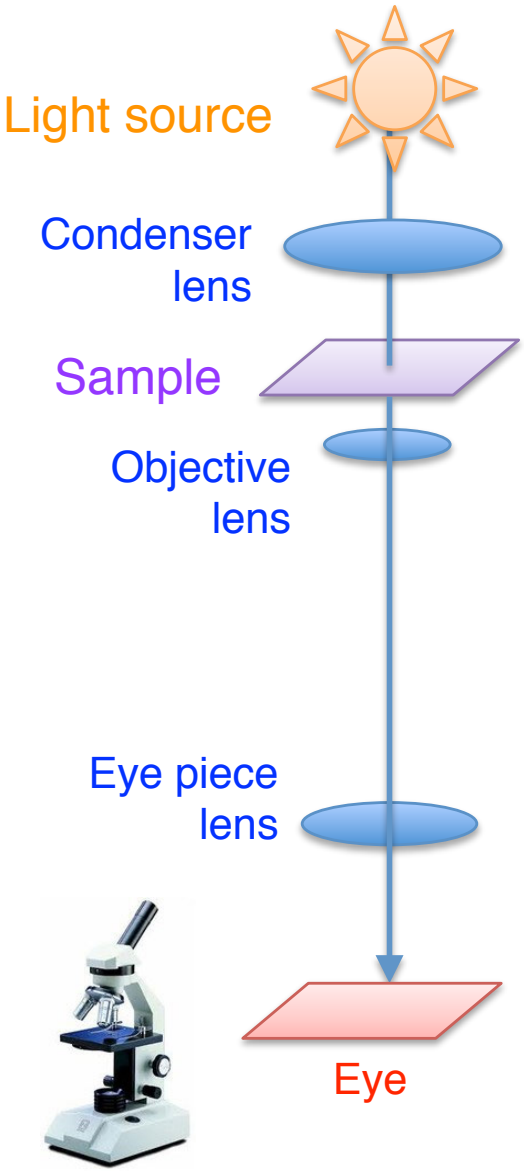
## Section 1: Anatomy of an EM

*Christoph Wigge & Anchi Cheng [NYSBC]*

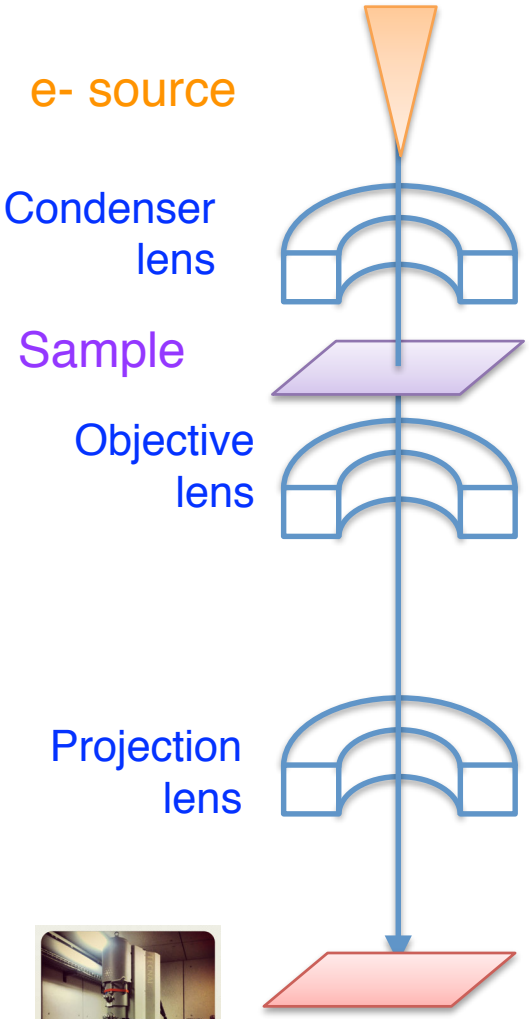
# Comparison of a light microscope, TEM & SEM

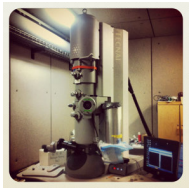


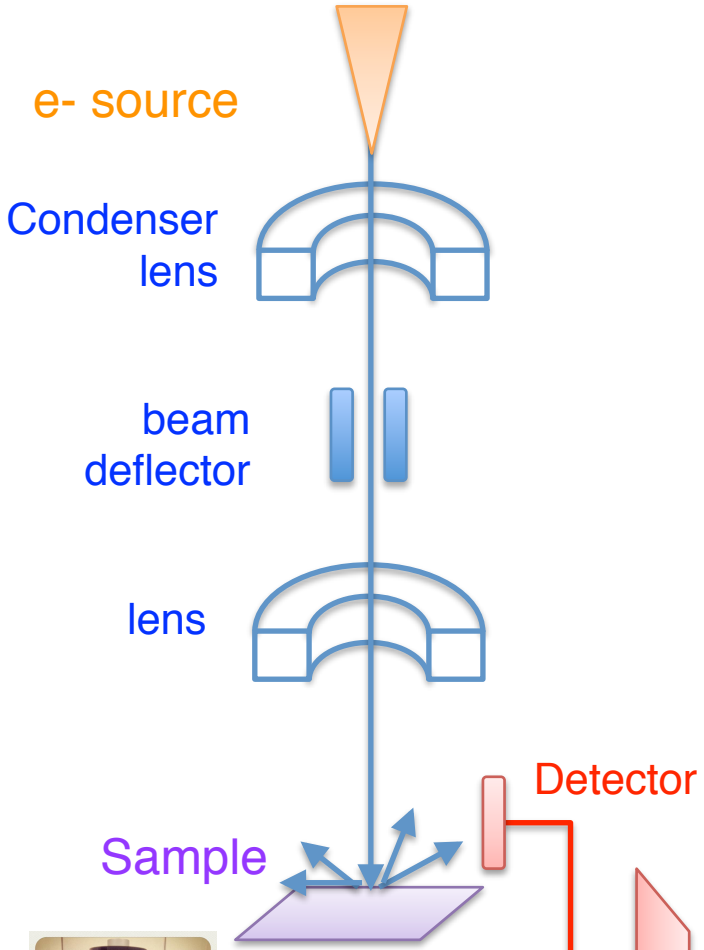

# Comparison of a light microscope, TEM & SEM

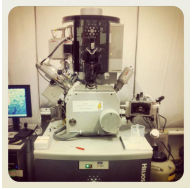






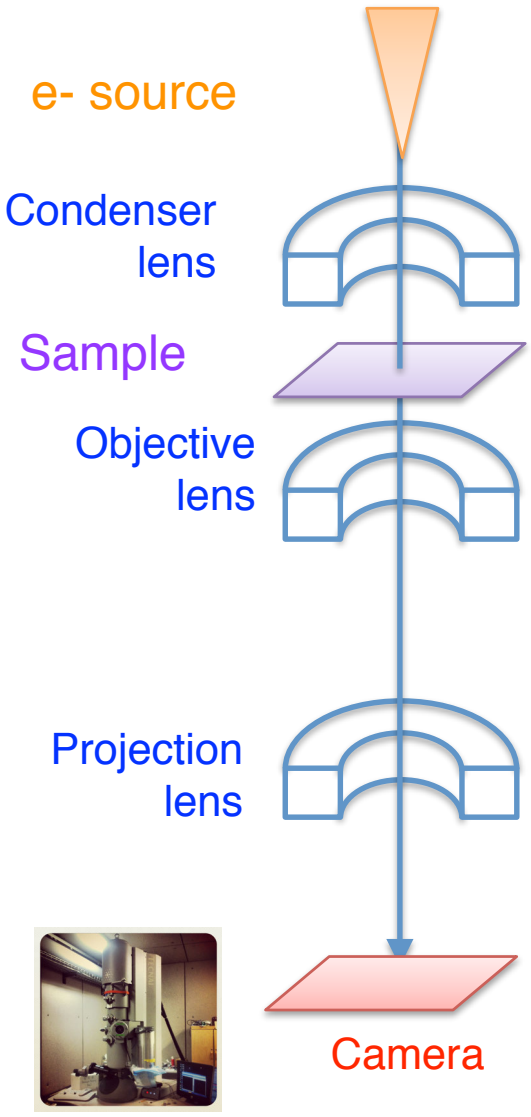



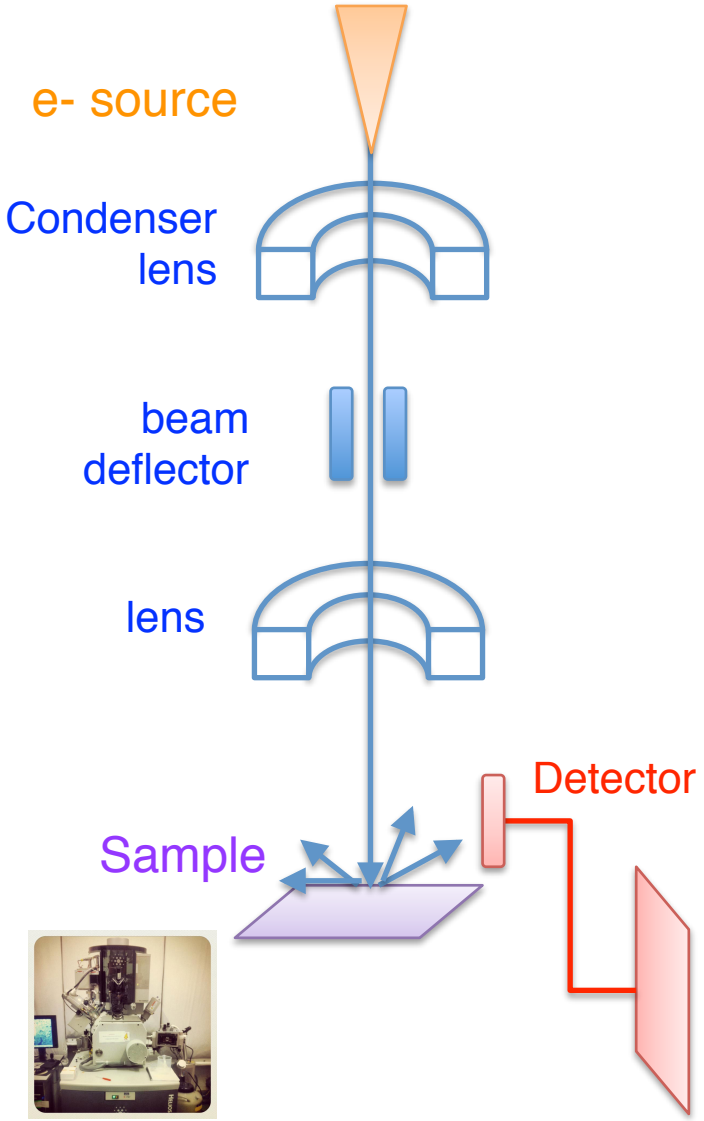
# Obtaining a 3D structure from a 2D image

## Section 2: Fourier transforms and Image Formation

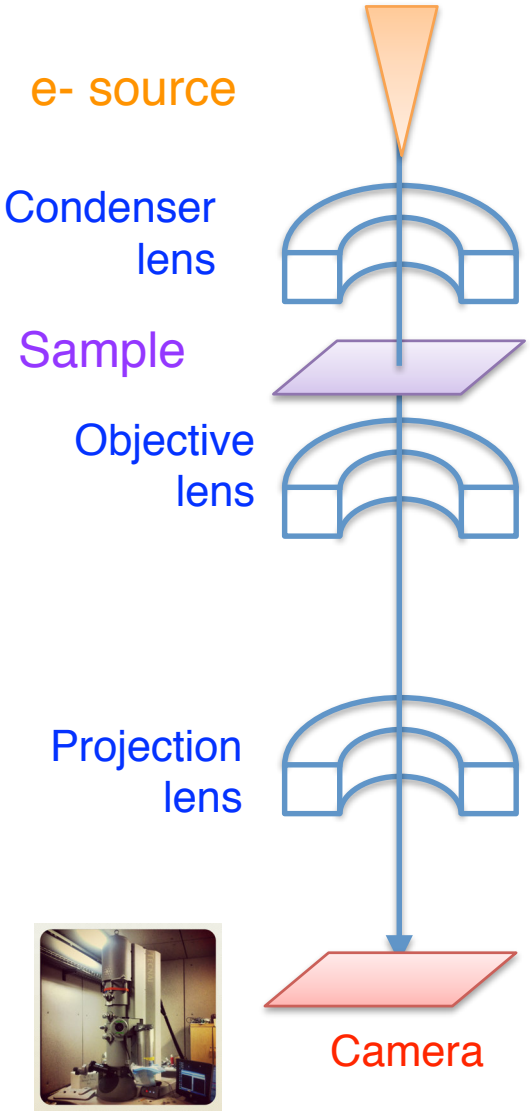
***Bill Rice [NYSBC]***

# Obtaining a 3D structure from a 2D image

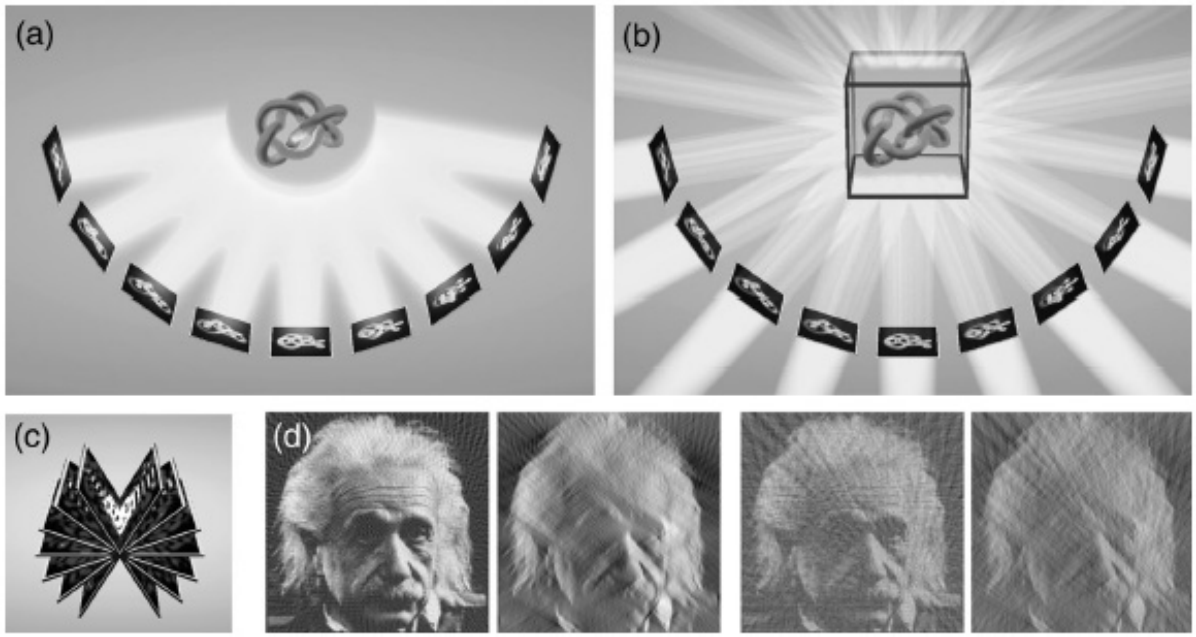


# Obtaining a 3D structure from a 2D image

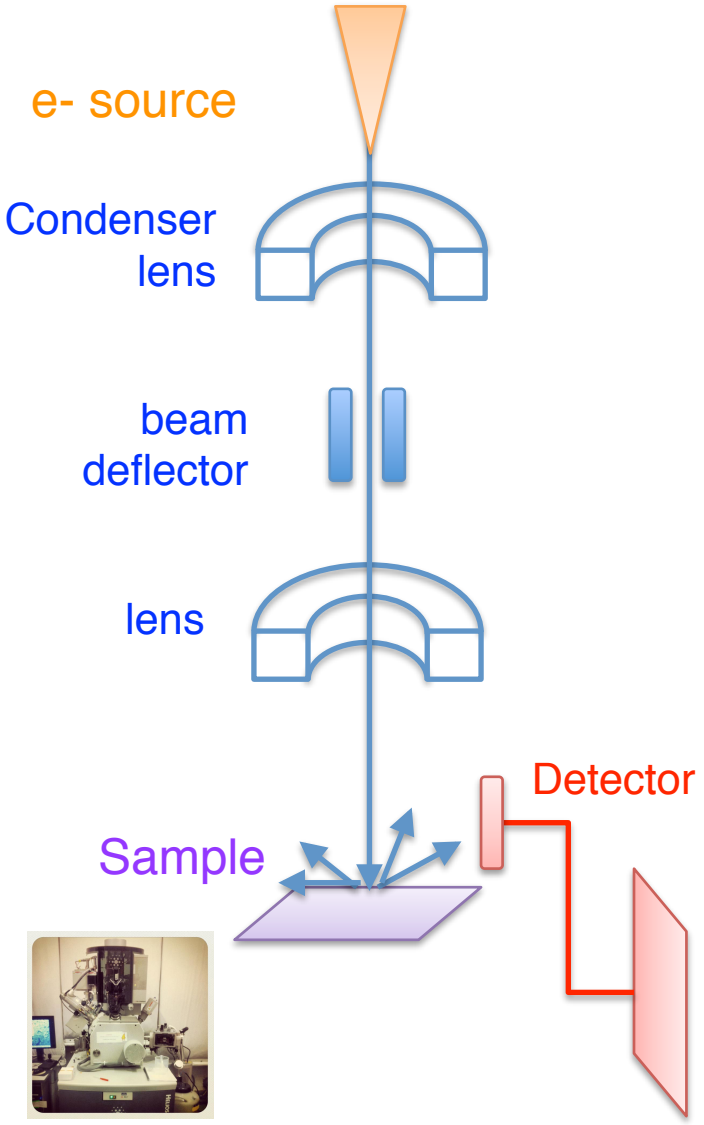


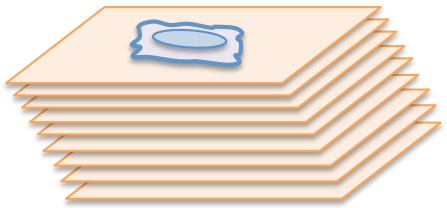



From W. Baumeister et al. Trend in Cell Biology 9(1999)81

# Obtaining a 3D structure from a 2D image



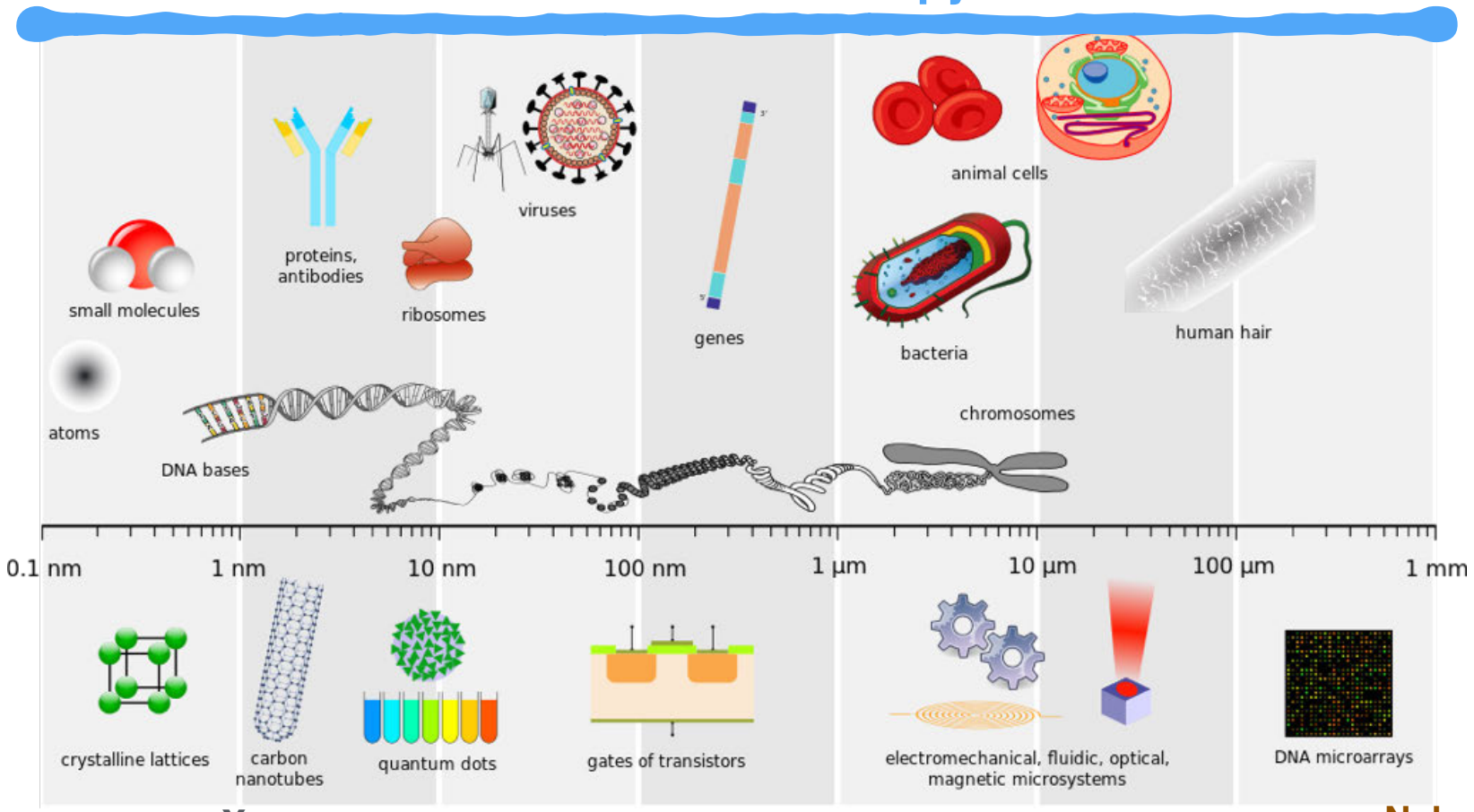
# How to make an EM ready sample

## Section 3: Challenges in EM & Sample prep

***Ed Eng & Ashleigh Raczkowski [NYSBC]***

# How to make an EM ready sample

**Electron Microscopy**




**X-ray**

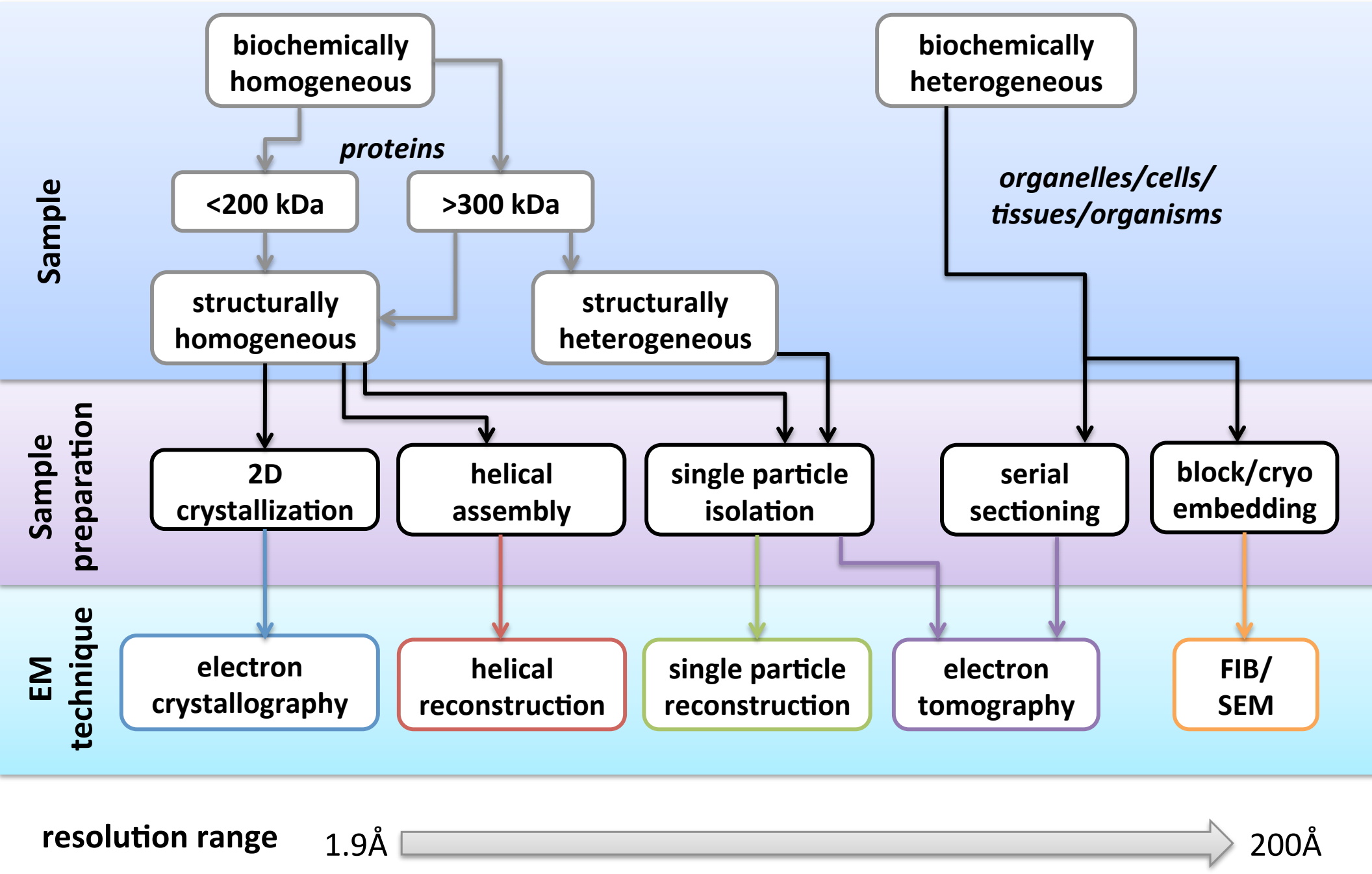
**Naked eye**

**Light microscopy**

**NMR**

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

# EM modalities



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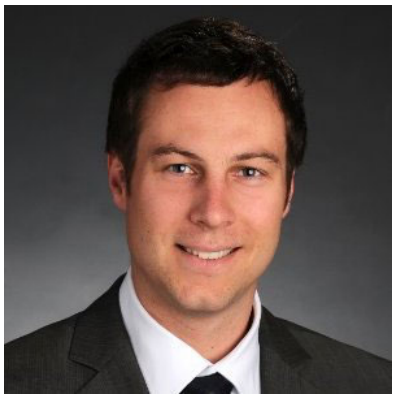
# Looking at biochemically unique/heterogeneous (pleiomorphic) samples

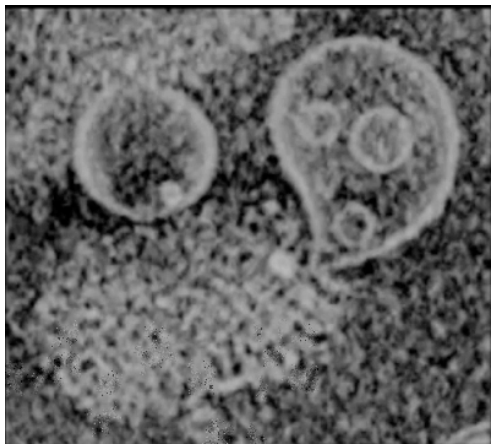
## Section 4: Tomography

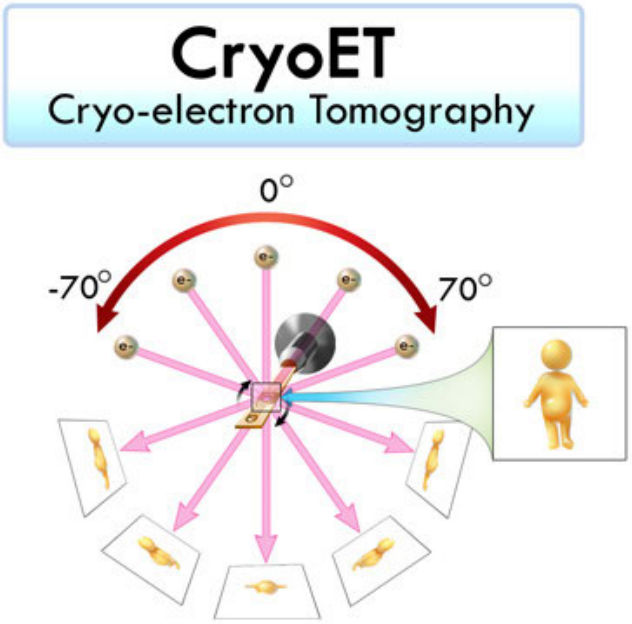
### *Part I: David Stokes [NYU]*

### *Part II: Bill & Christoph [NYSBC]*

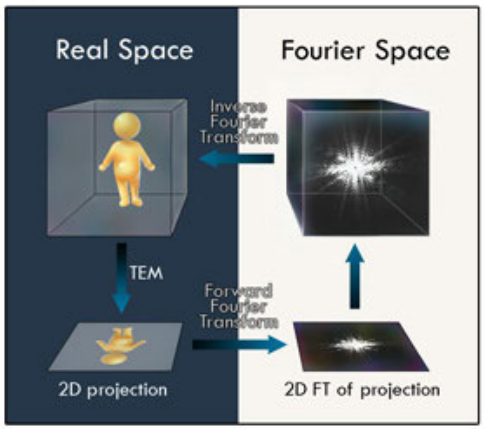


# Looking at biochemically unique/heterogeneous (pleiomorphic) samples






eicn
Electron Imaging Center for Nanomachines



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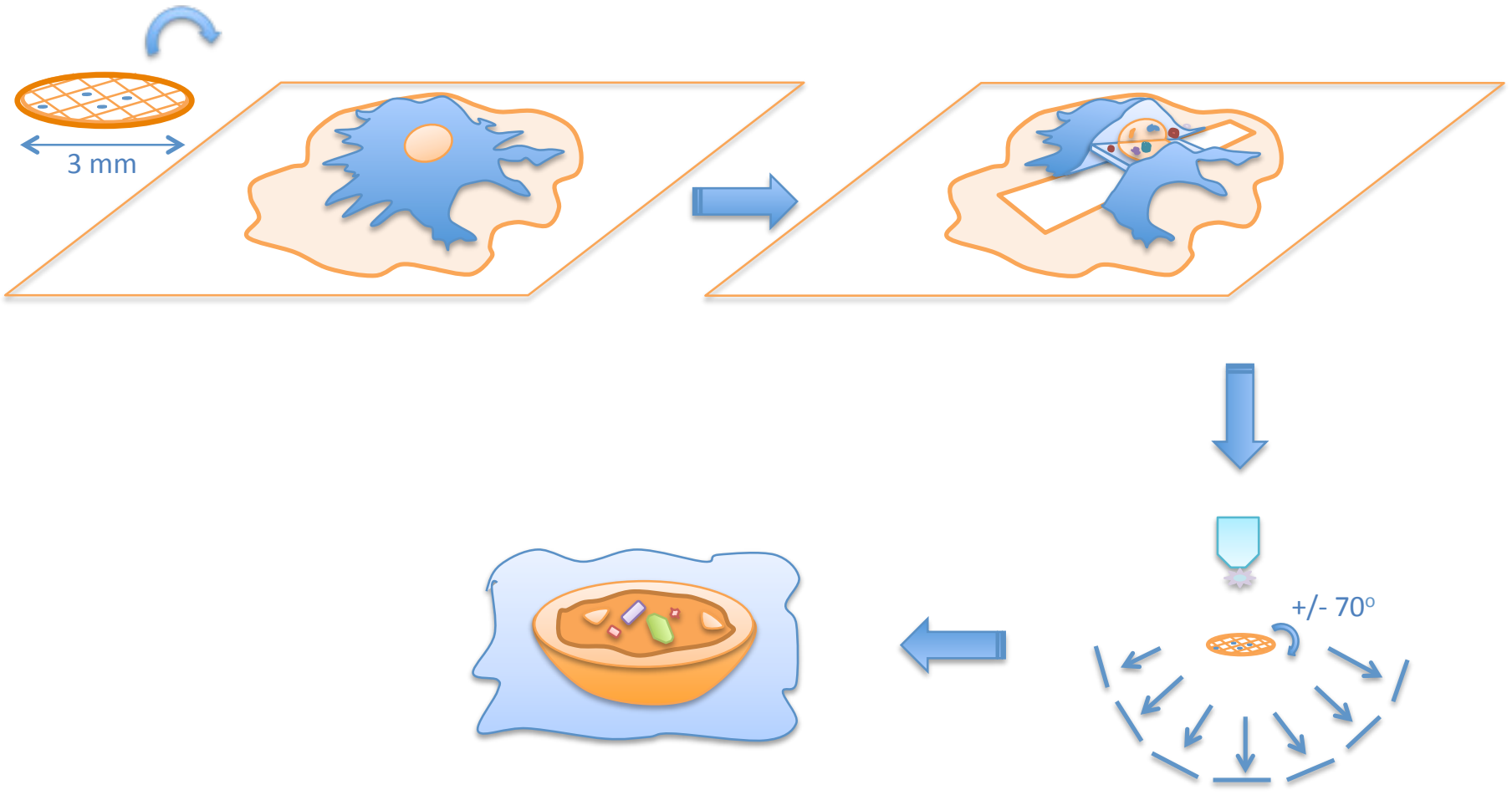

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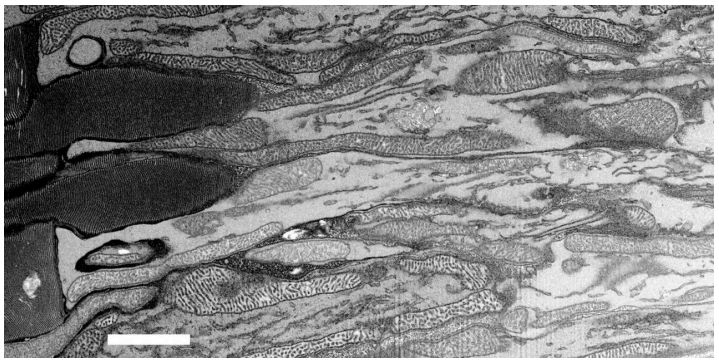
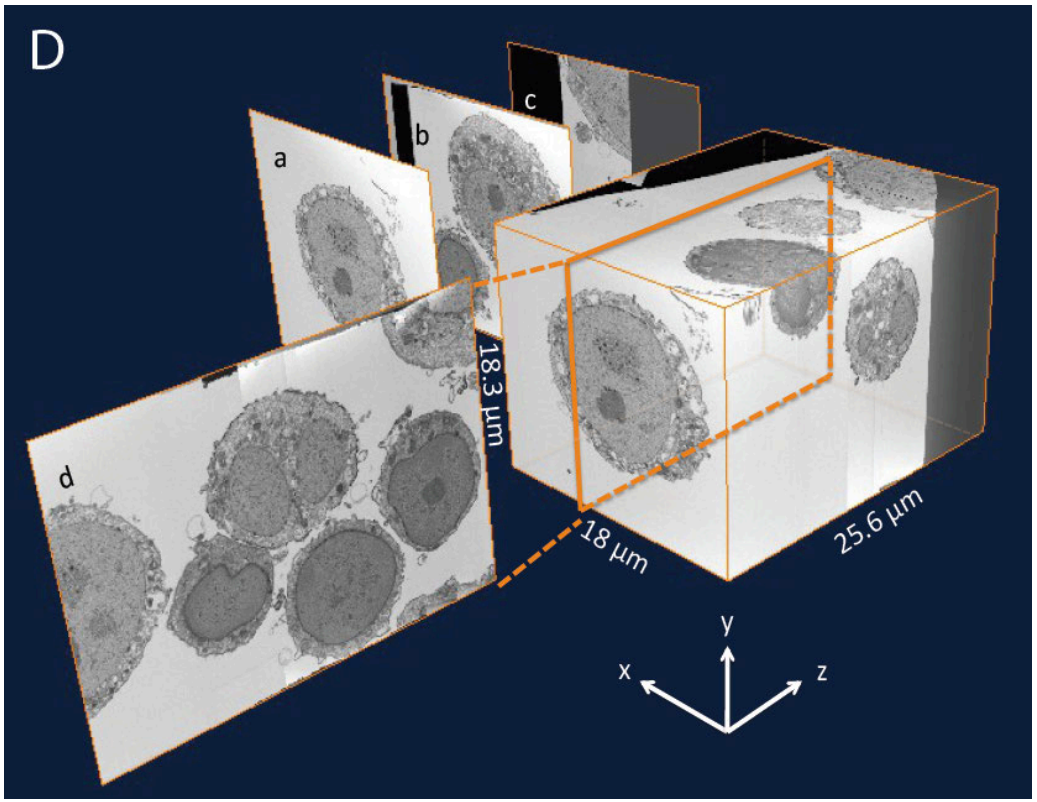




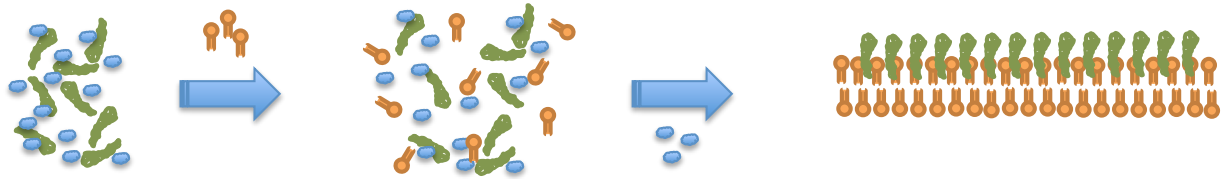
Looking at ordered arrays and small macromolecules

## Section 6: 2D crystallography

### *Part I: Iban Ubarretxena [MSSM]*

### *Part II: Hernando Sosa [AECOM]*

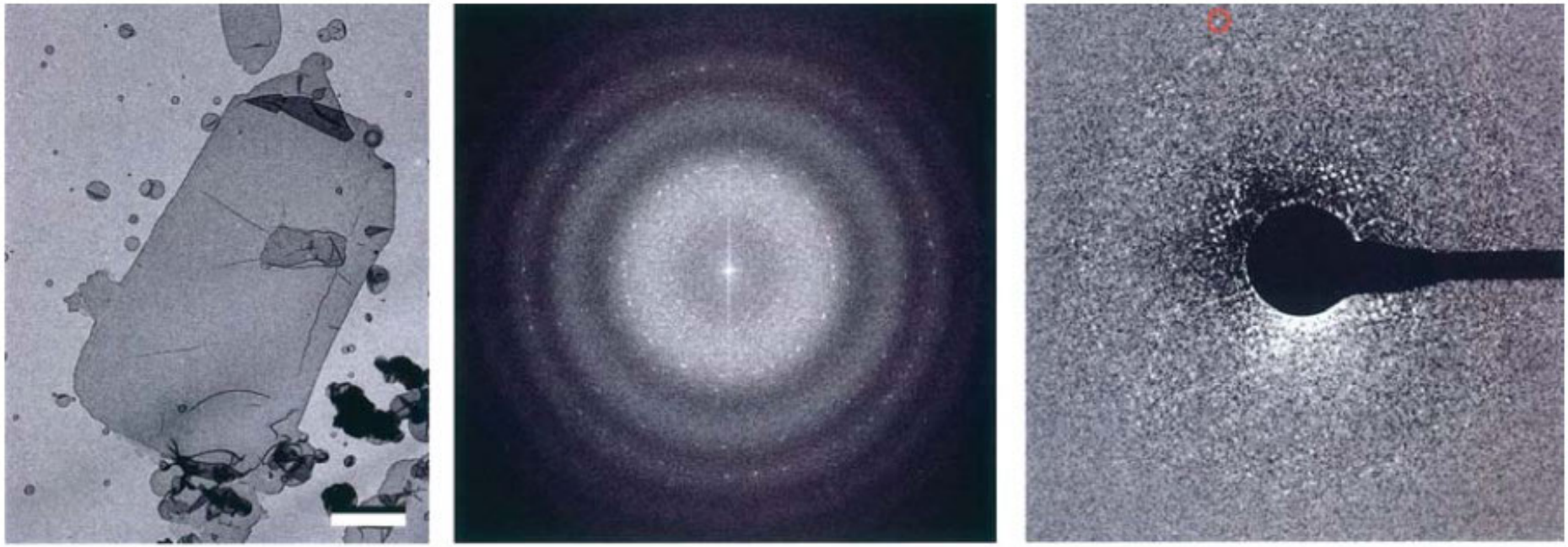
# Looking at ordered arrays and small macromolecules



Membrane protein purified in detergent

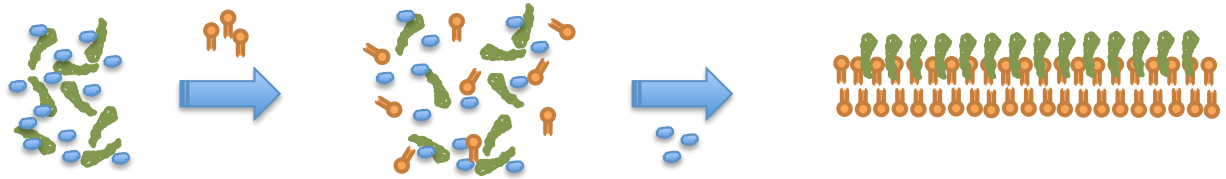
Detergent solubilized lipids added

Different buffer components screened and detergent removed



doi: 10.1038/npg.els.0003044

# Looking at ordered arrays and small macromolecules



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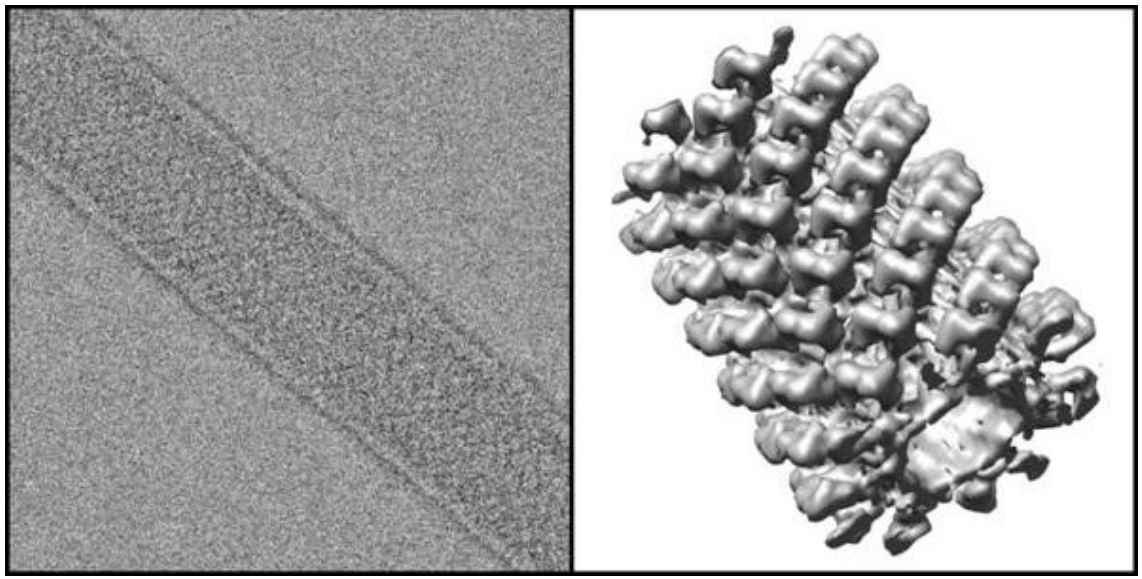
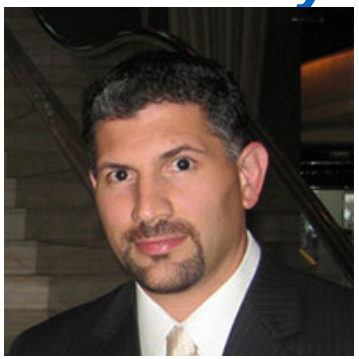


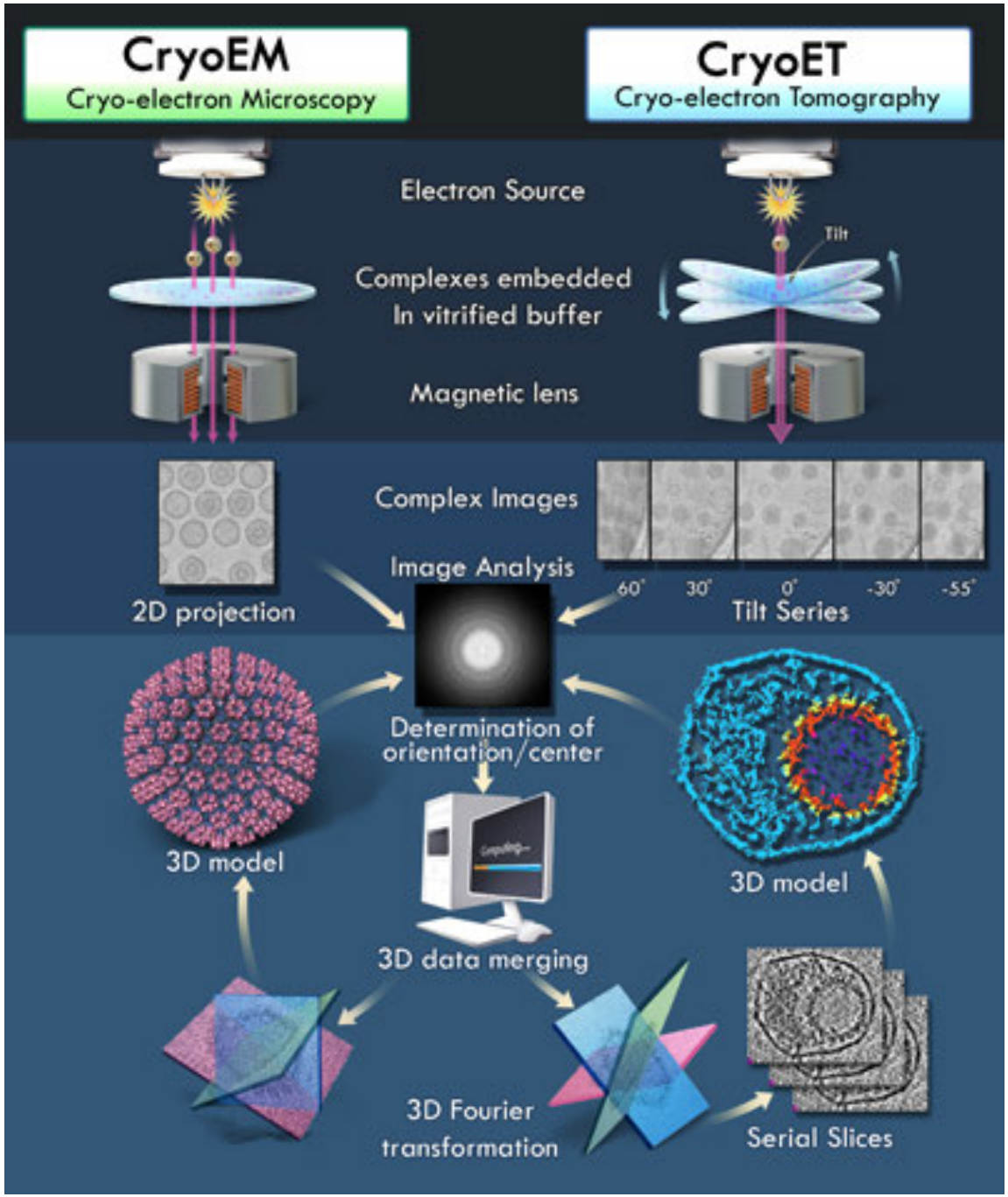
# Section 5: Single Particle

## *Part I: Joachim Frank [CU]*

## *Part II: Amedee Des Georges & Reza Khayat [CUNY]*

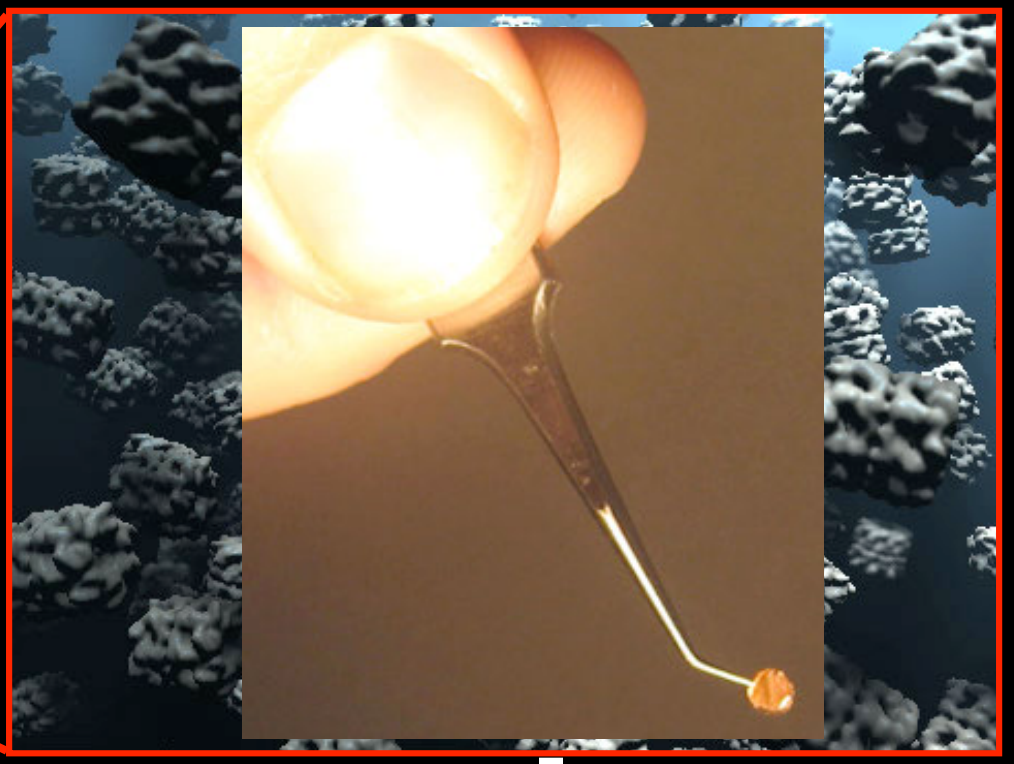


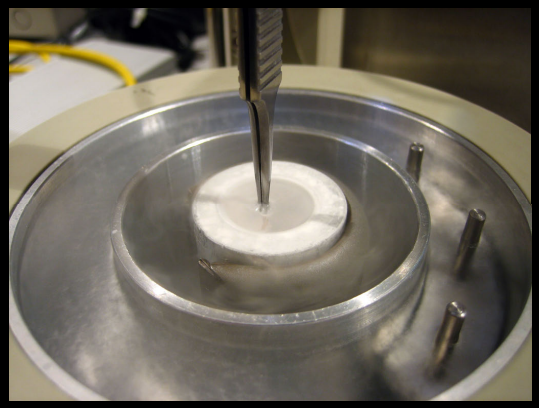
## *Part III: Yong Zi Tan, [CU/NYSBC]*

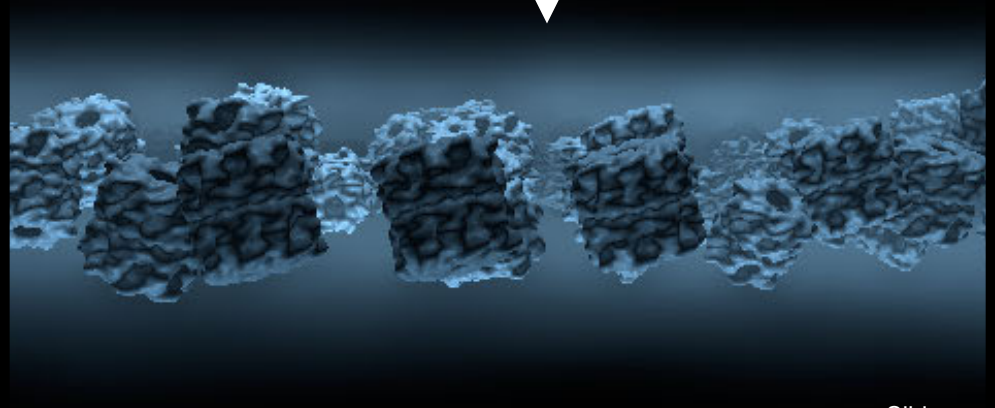



eicn
Electron Imaging Center for Nanomachines

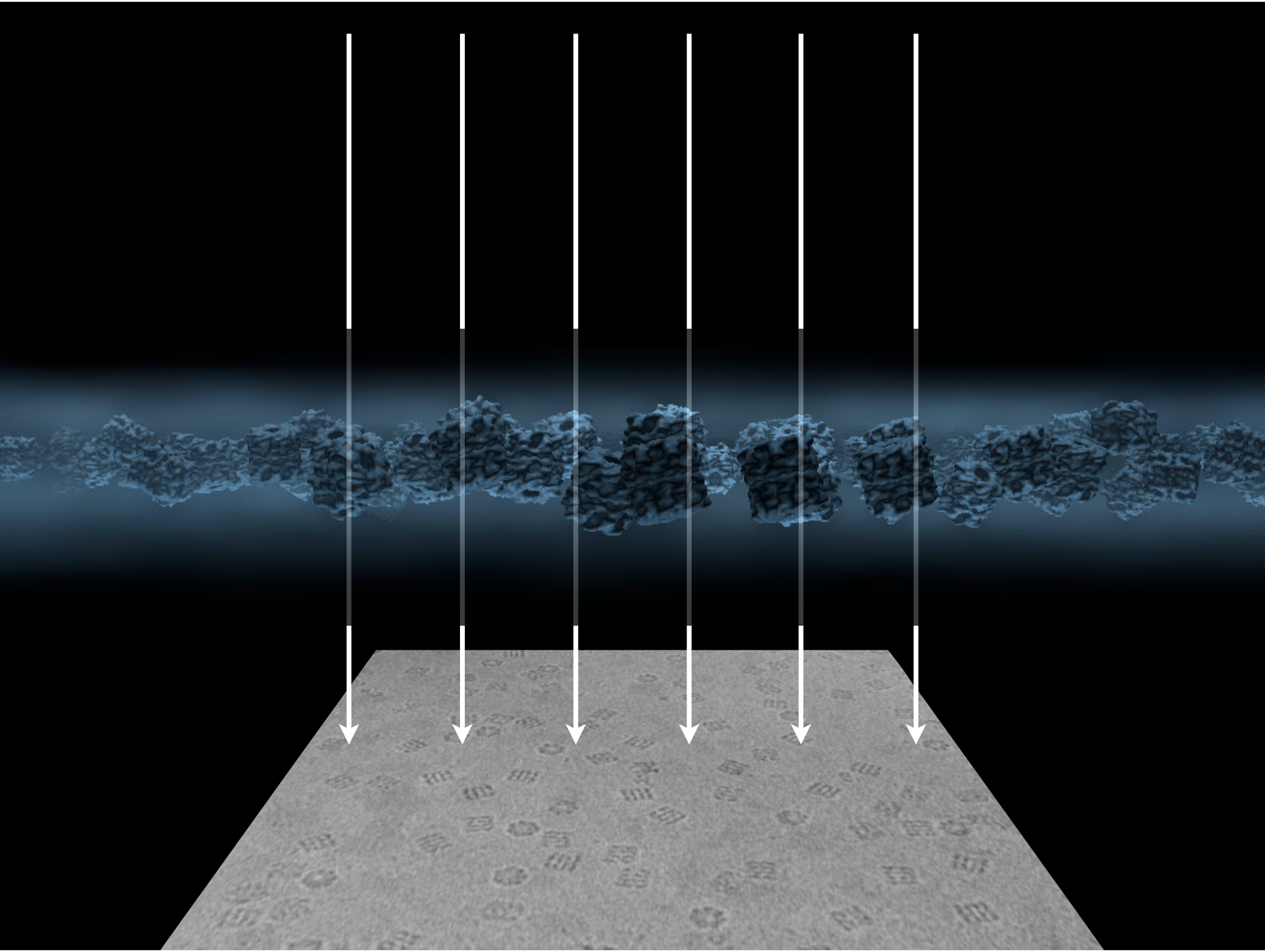
# Vitrification process for CryoTEM

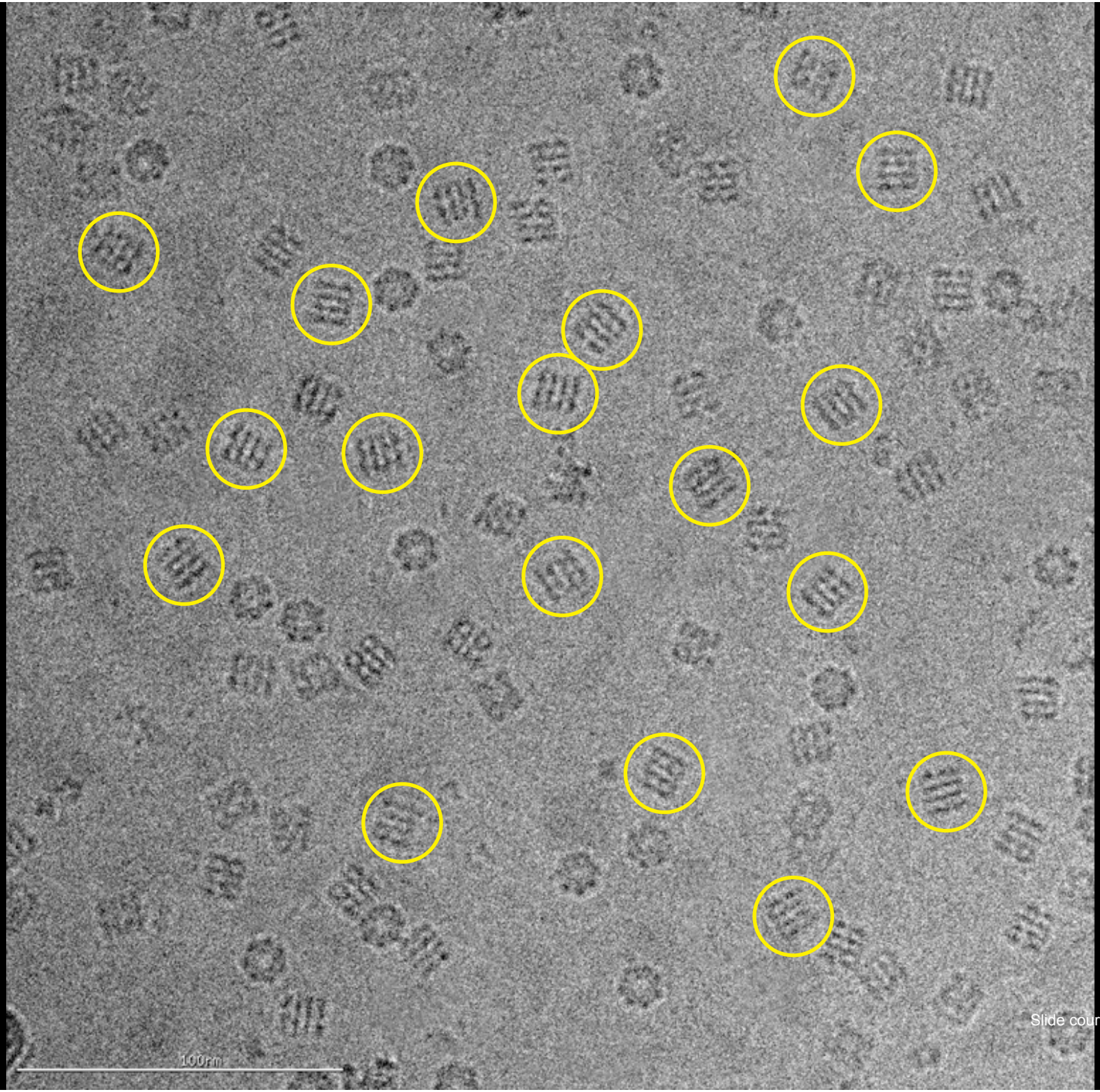




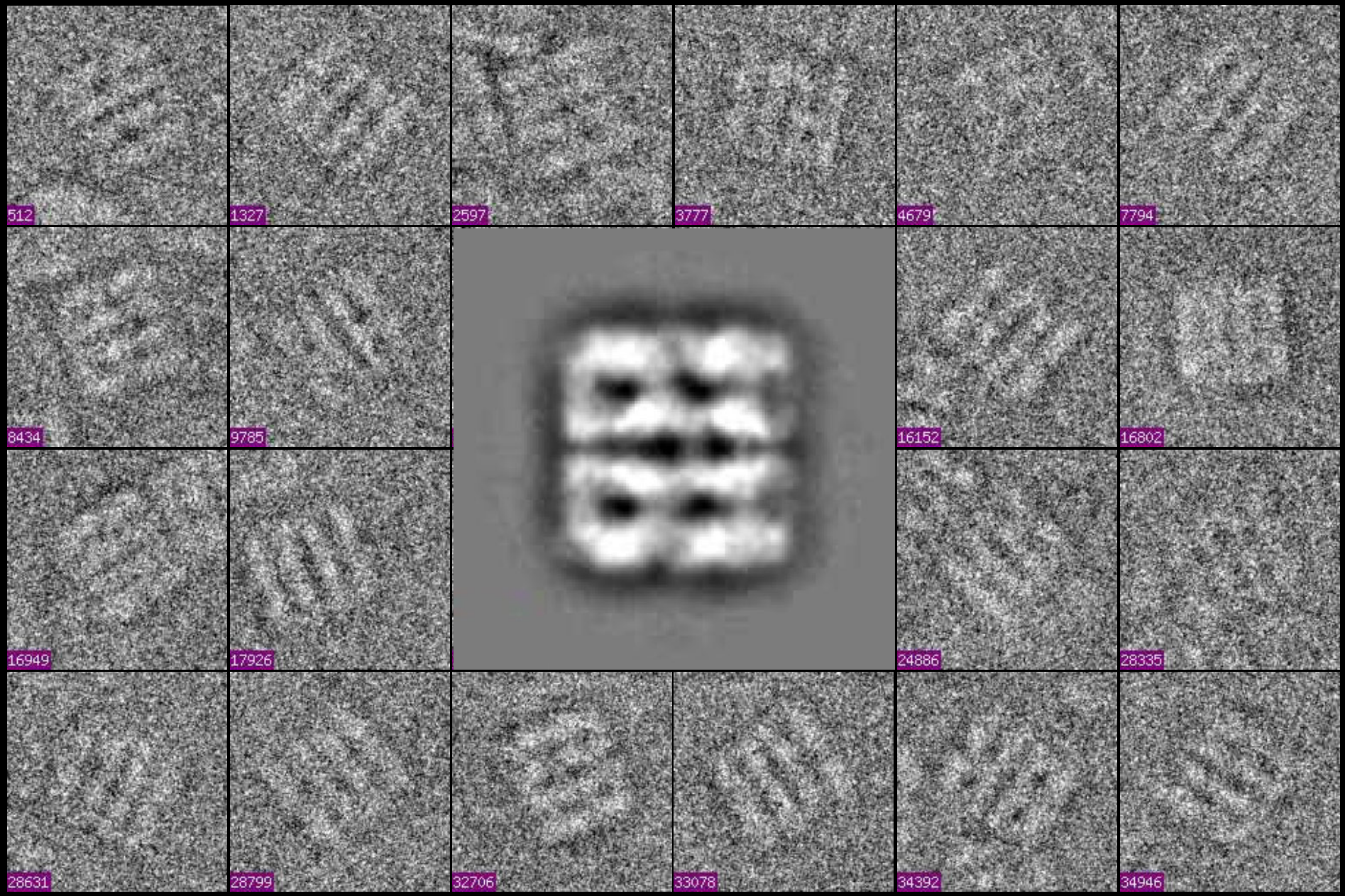


Slide courtesy Gabriel Lander

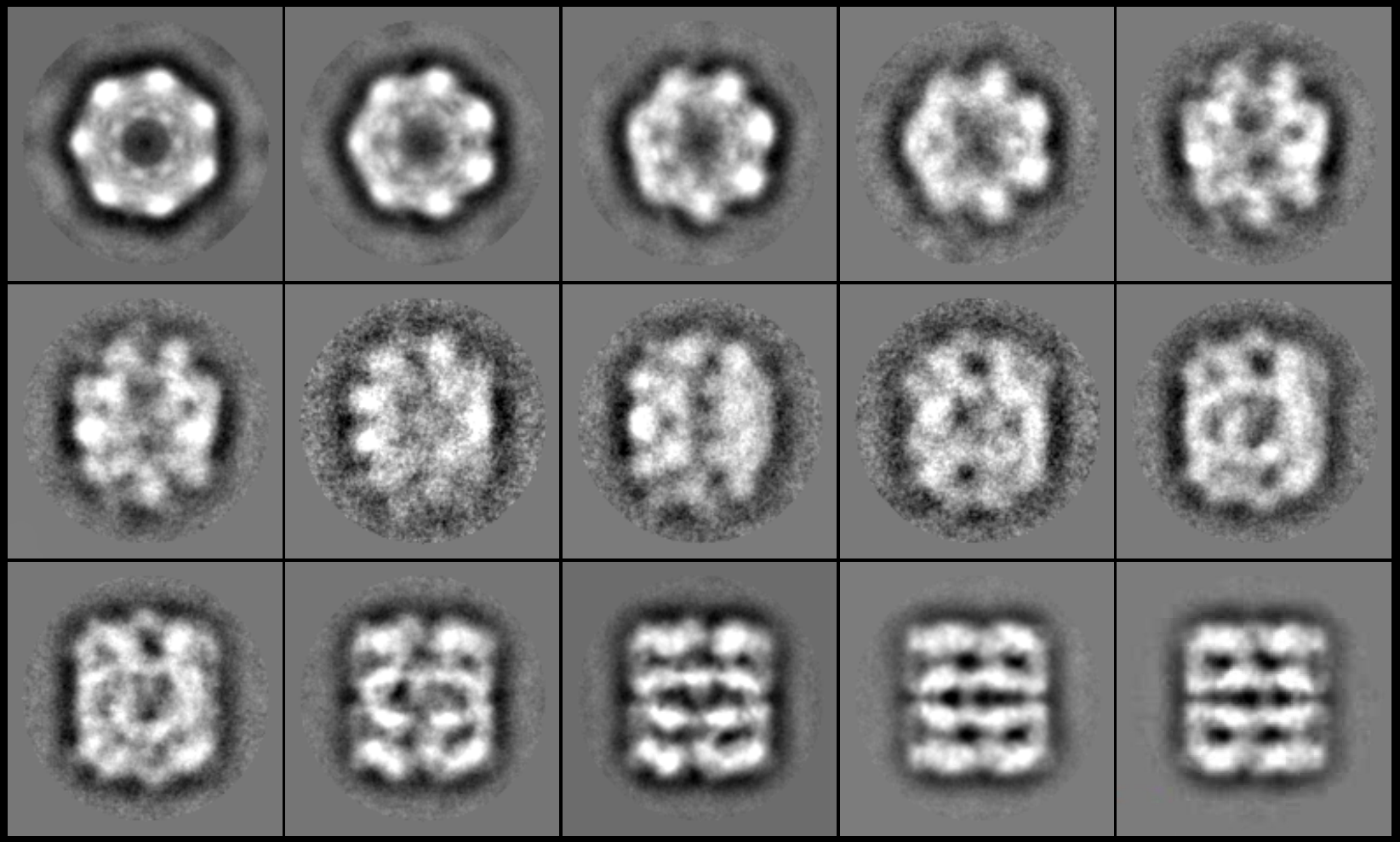




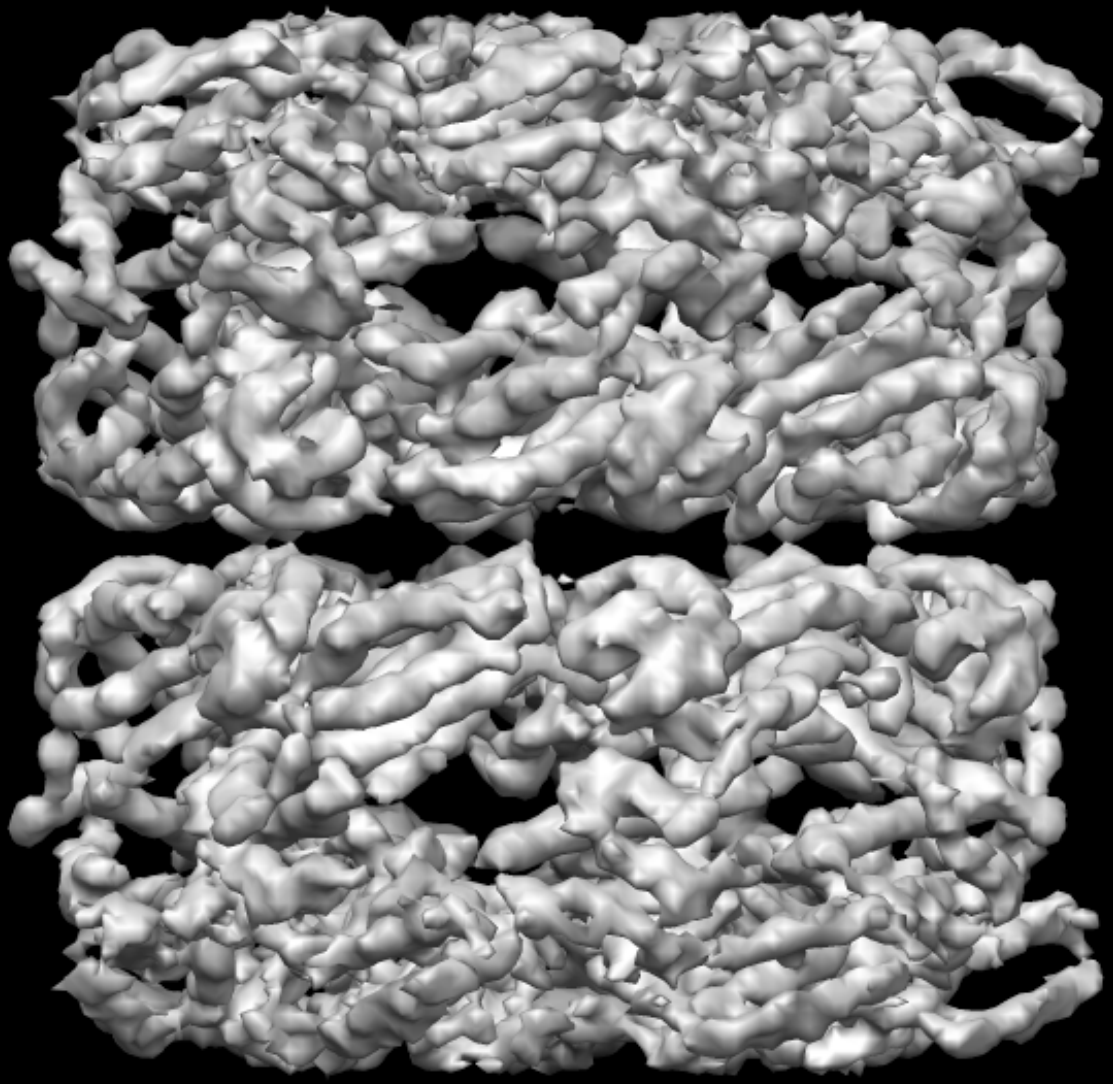

Slide courtesy Gabriel Lander



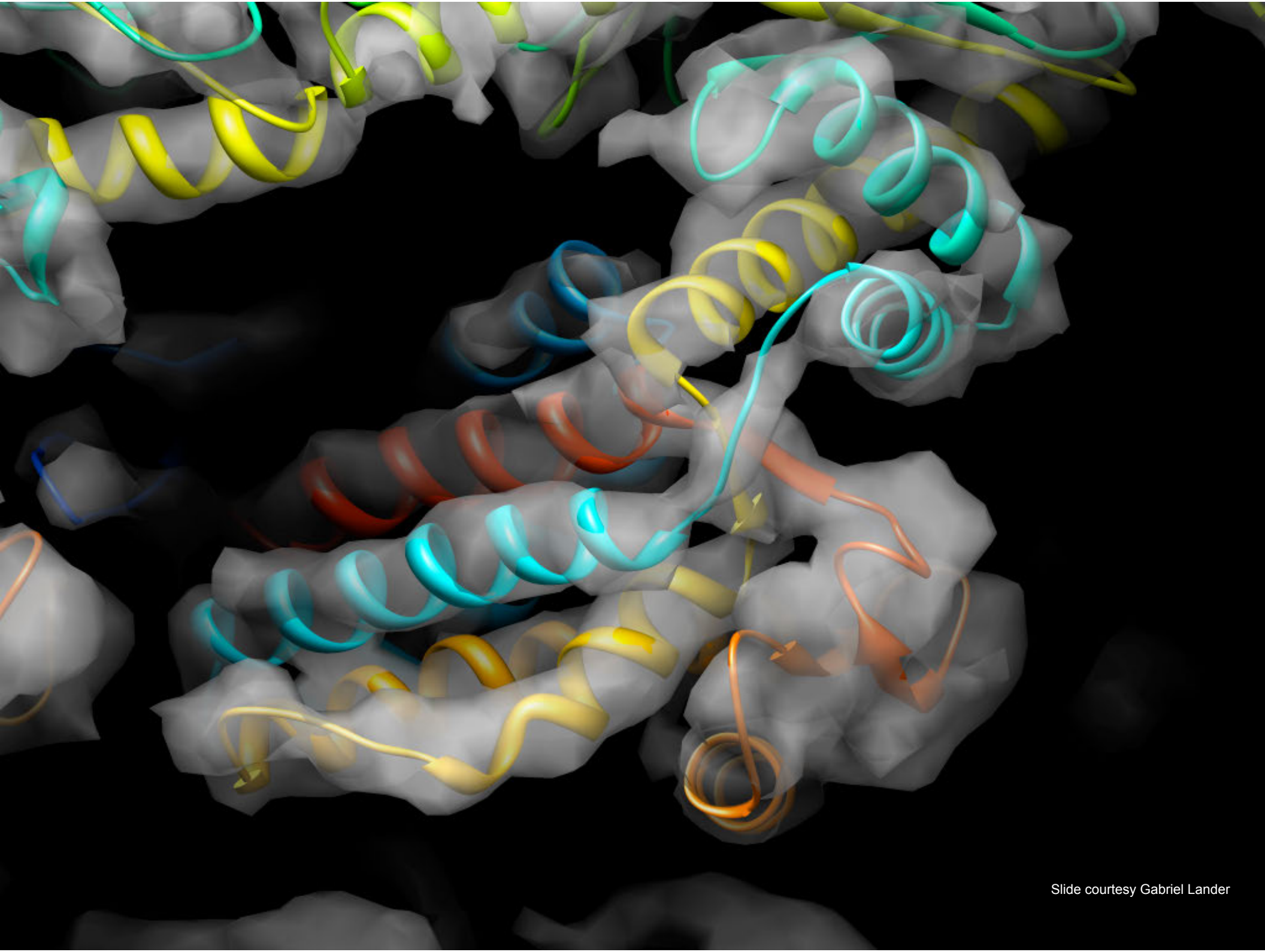

Slide courtesy Gabriel Lander



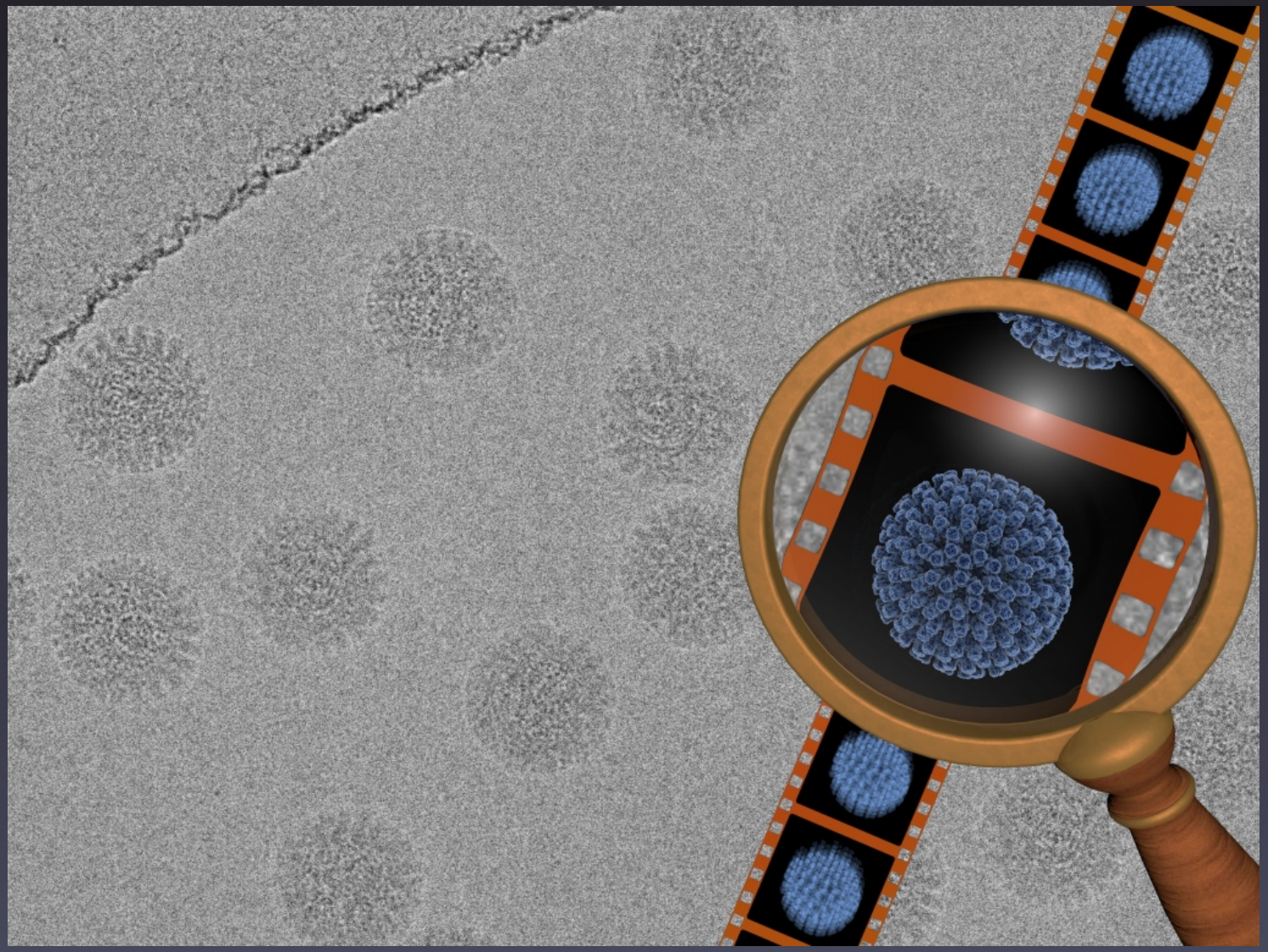
Slide courtesy Gabriel Lander



Slide courtesy Gabriel Lander


Slide courtesy Gabriel Lander

# The Resolution Revolution!



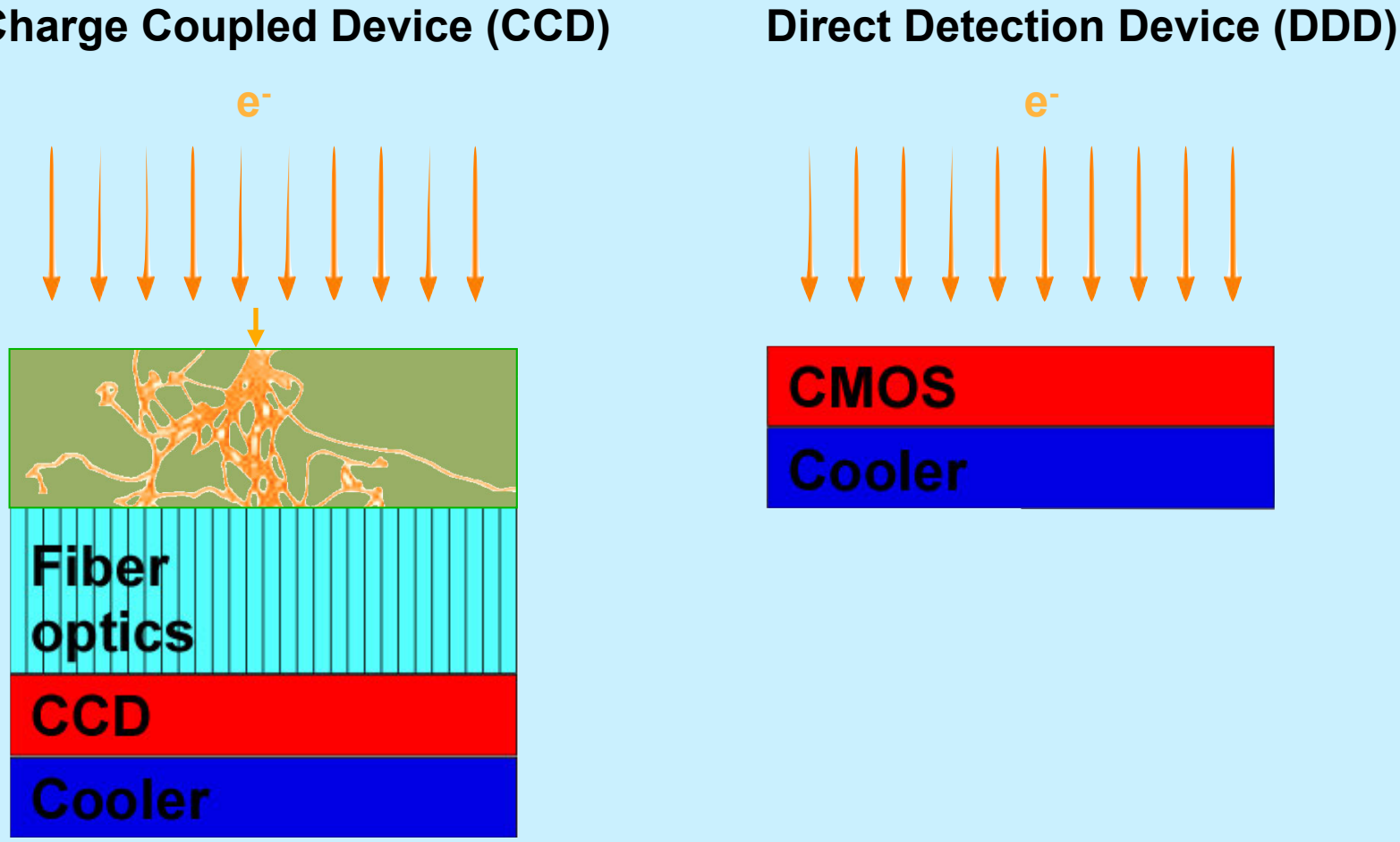
Anchi Cheng

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., Veesler, 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.

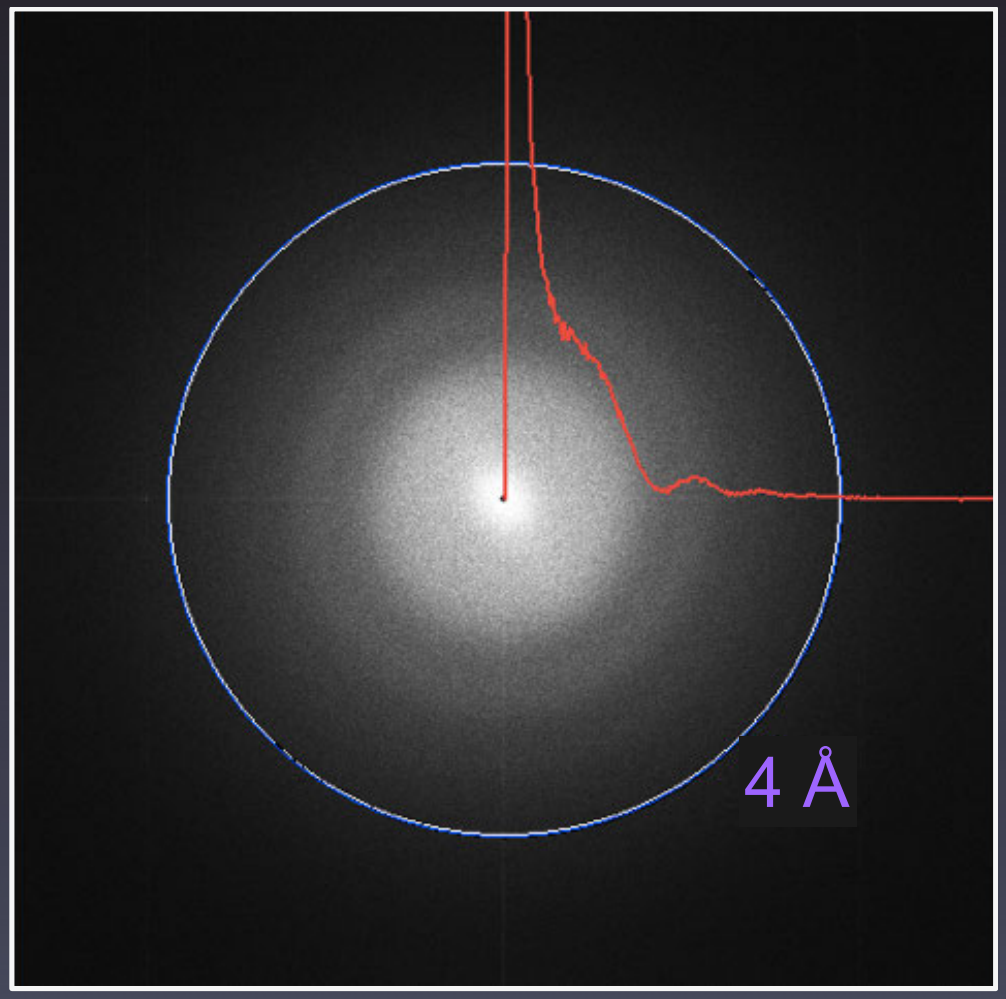

Niko Grigorieff

# A new generation of cameras has improved the resolution and the efficiency of data collection



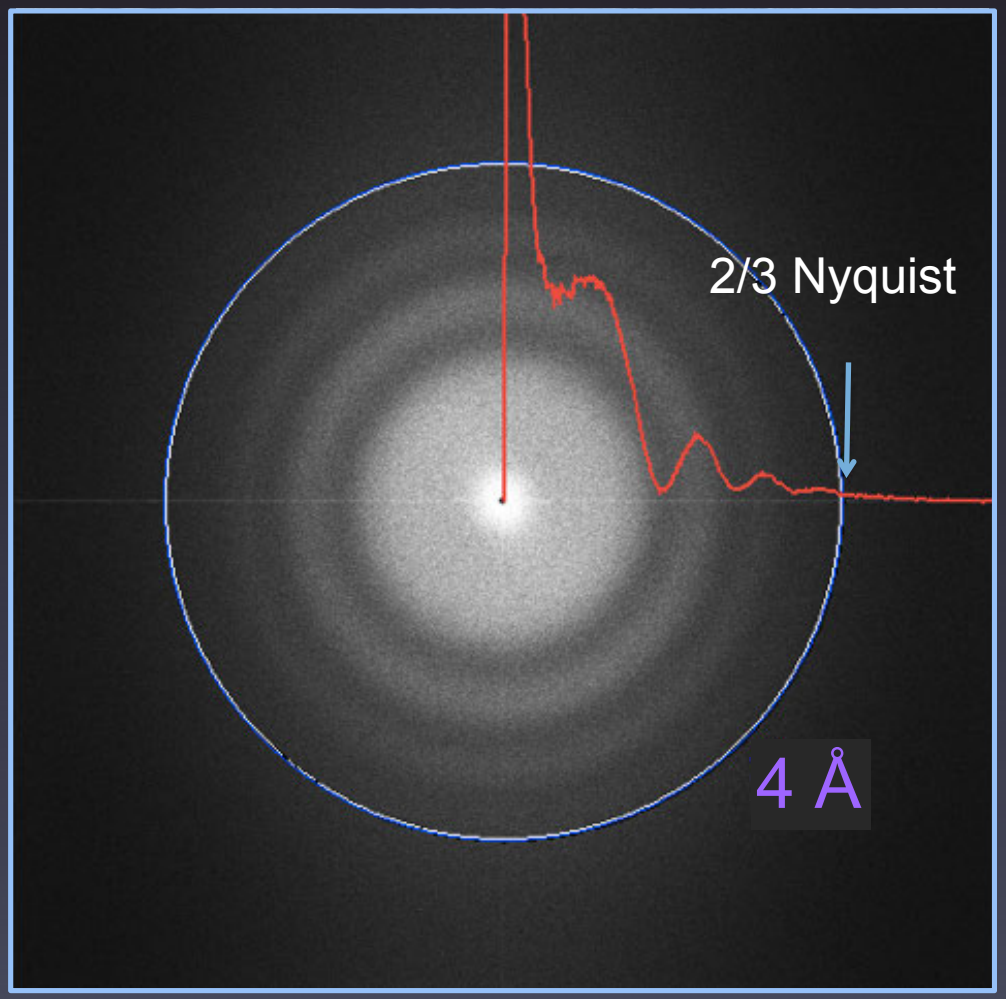
# Improving the resolution: Detecting electrons instead of photons

CCD




1.37 Å/pixel

DDD

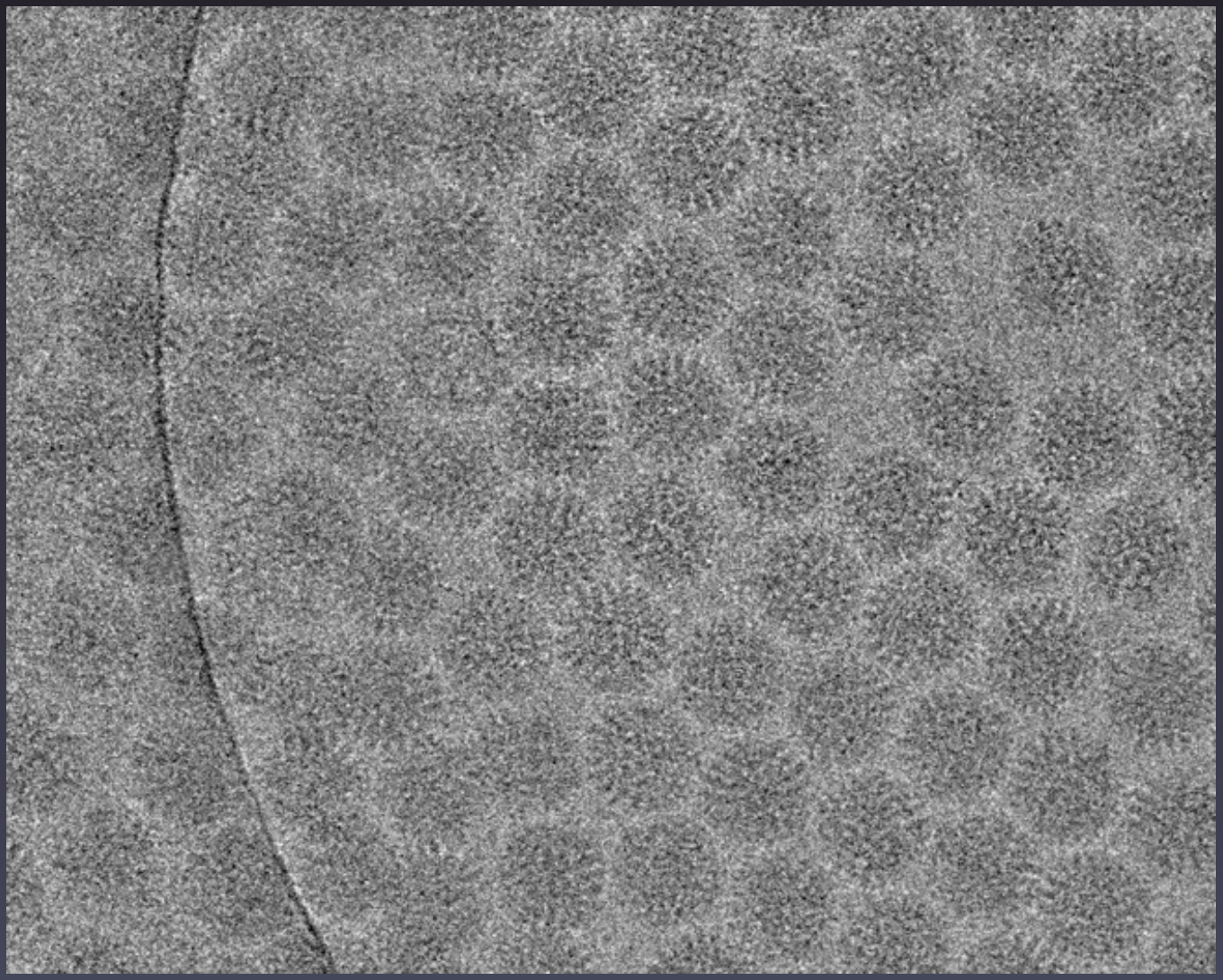



1.38 Å/pixel

200KeV ; 20 $e^-/Å^2$ ; carbon film ; 3k x 3k image

# Improving the resolution:
# Correcting for drift and beam induced motion

# Improving the resolution:
# DDD records a set of frames ("movie")



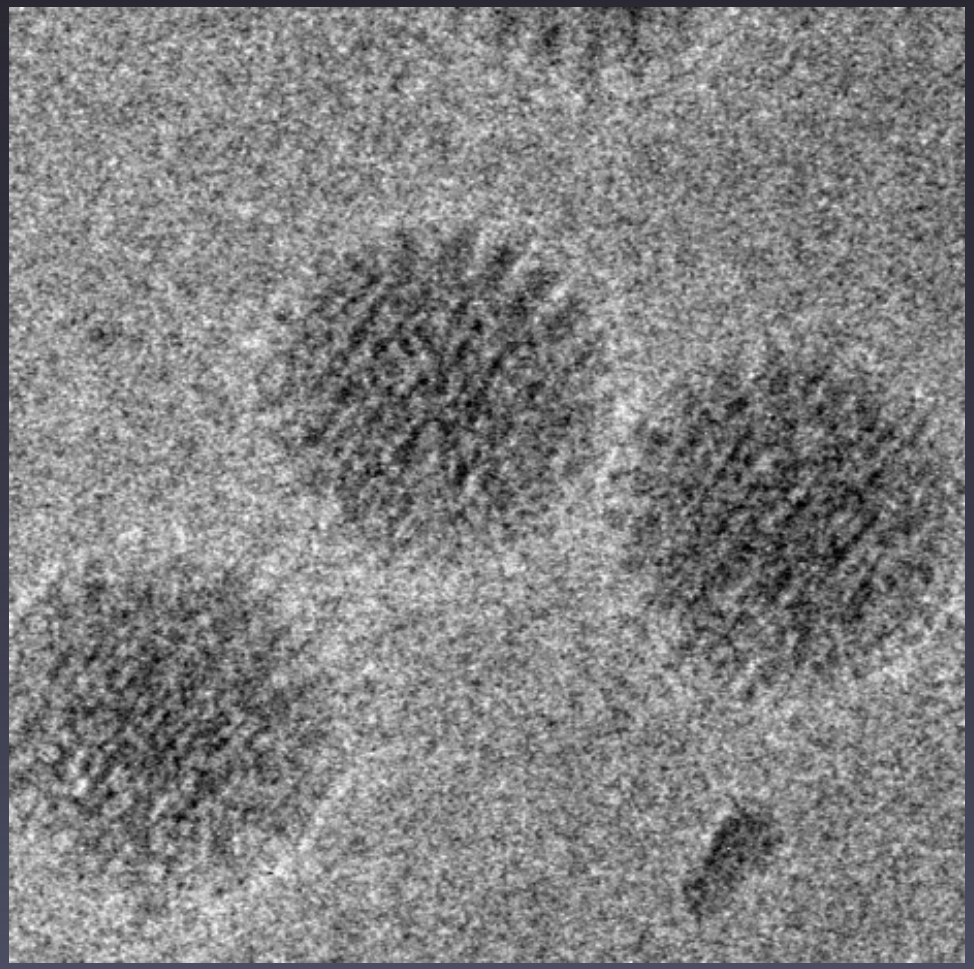
0.5 $e^-/Å^2$/frame

Image = Frame1 + Frame2 + Frame3 + Frame4 + Frame5

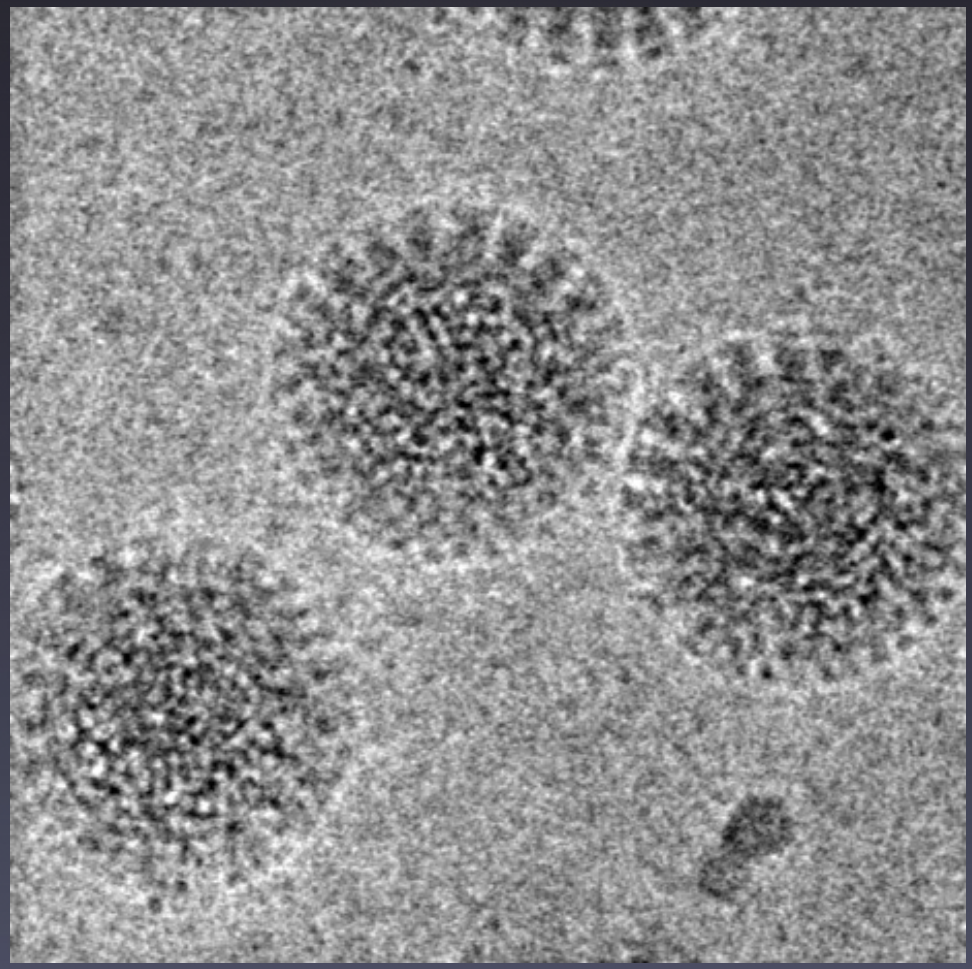
We can use DDD movies to examine (and correct) "beam induced motion"

# Correcting for beam induced movement

60-frame average
(no alignment)

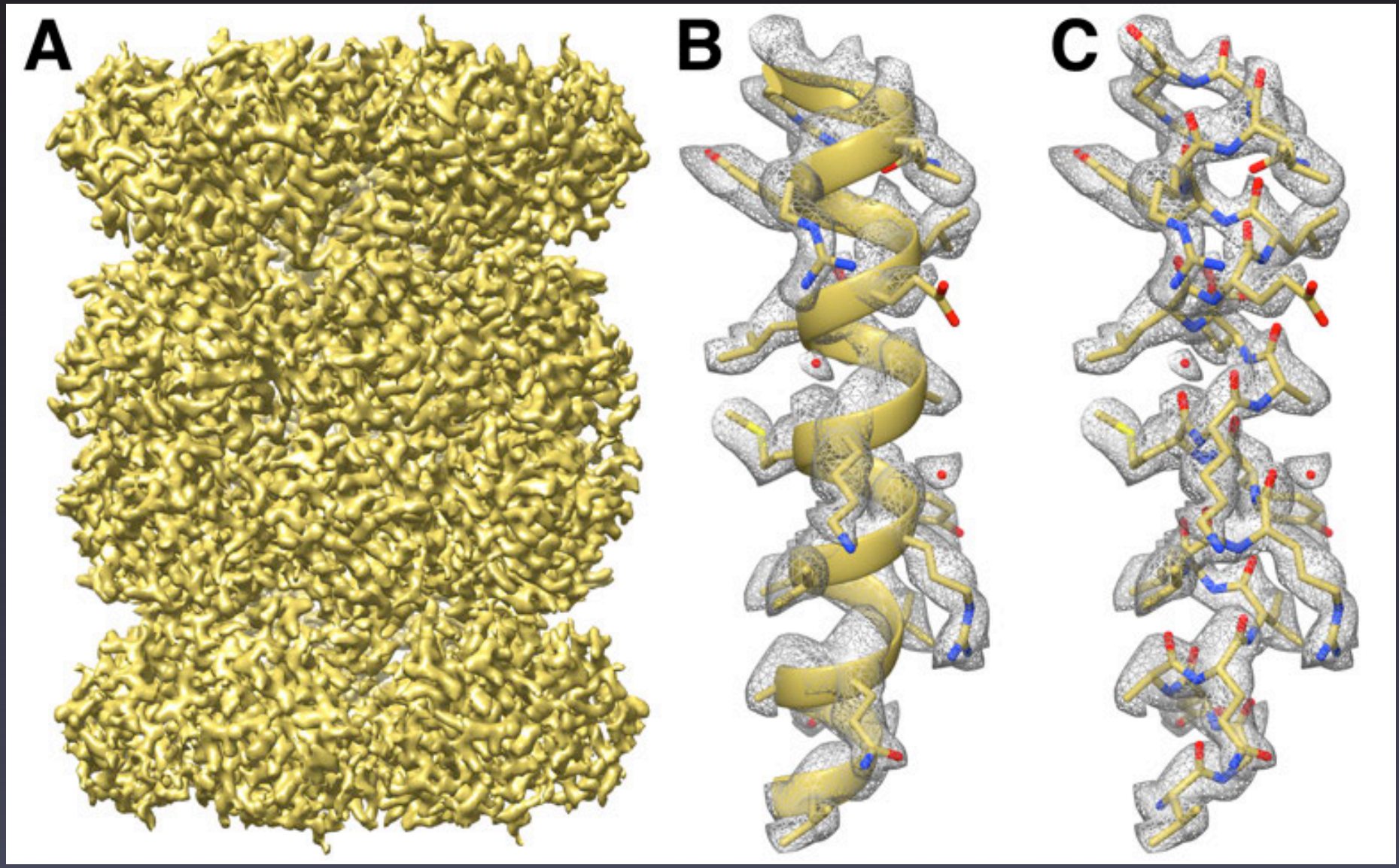


60-frame average
(translational alignment)



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

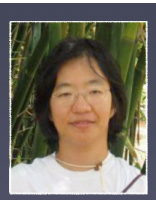
# 2.8 Å resolution reconstruction of the 20 S proteasome





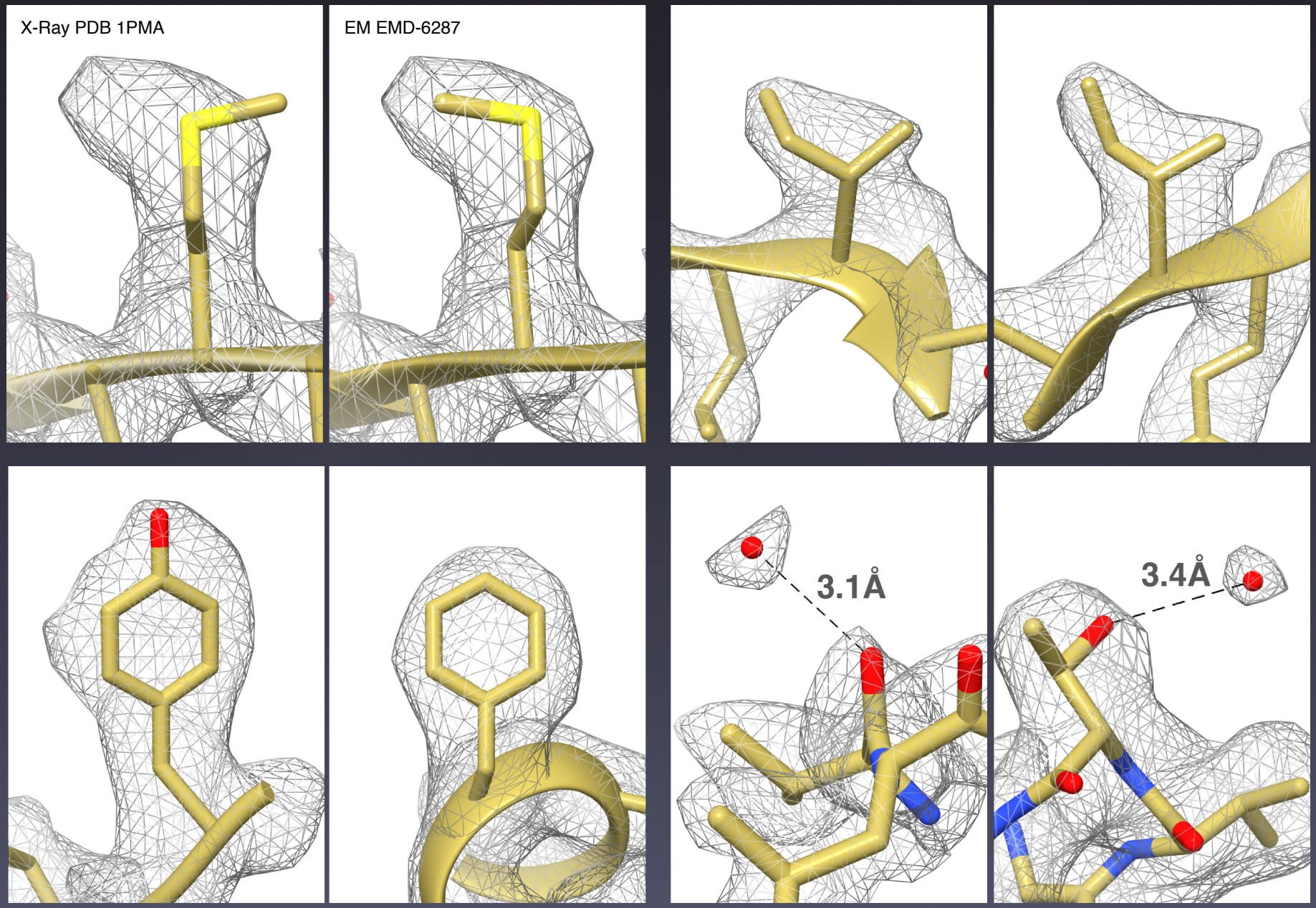
Melody Campbell


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 20 S proteasome



# Class structure

Section 1: Anatomy of an EM
*Christoph Wigge & Anchi Cheng [N*

Section 2: Fourier transforms and Image Form
*Bill Rice [NYSBC]*

Section 3: Challenges in EM & Sample prep
*Ed Eng & Ashleigh Raczkowski [NYSBC]*

Section 4: Tomography
*Part I: David Stokes [NYU]*
*Part II: Bill & Christoph [NYSBC]*

Section 5: Single Particle
*Part I: Joachim Frank [CU]*
*Part II: Amedee Des Georges &*
*Reza Khayat [CUNY]*
*Part III: Yong Zi Tan, [COLU/NYSBC]*

Section 6: 2D crystallography
*Part I: Iban Ubarretxena [MSSM]*
*Part II: Hernando Sosa [AECOM]*

Section 7:

EMDataBank
Unified Data Resource for 3DEM

EMDB,
Cathy Lawson [Rutgers]

Validation methods
*Tom Walz [RU]*

situs.biomachina.org

Fitting Atomic models
*Willy Wriggers*
*[Old Dominion University]*

***SEMC Forum***
***April 26 - noon***

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

NEW YORK STRUCTURAL BIOLOGY CENTER

## 9 member institutions

Albert Einstein College of Medicine of Yeshiva University

Columbia University in the City of New York

The City University of New York

Memorial Sloan-Kettering Cancer Center

Mount Sinai

NYU

The Rockefeller University — Science for the Benefit of Humanity

Weill Cornell Medical College, Cornell University

New York State Wadsworth Center — New York State Department of Health

### Member Institution Services

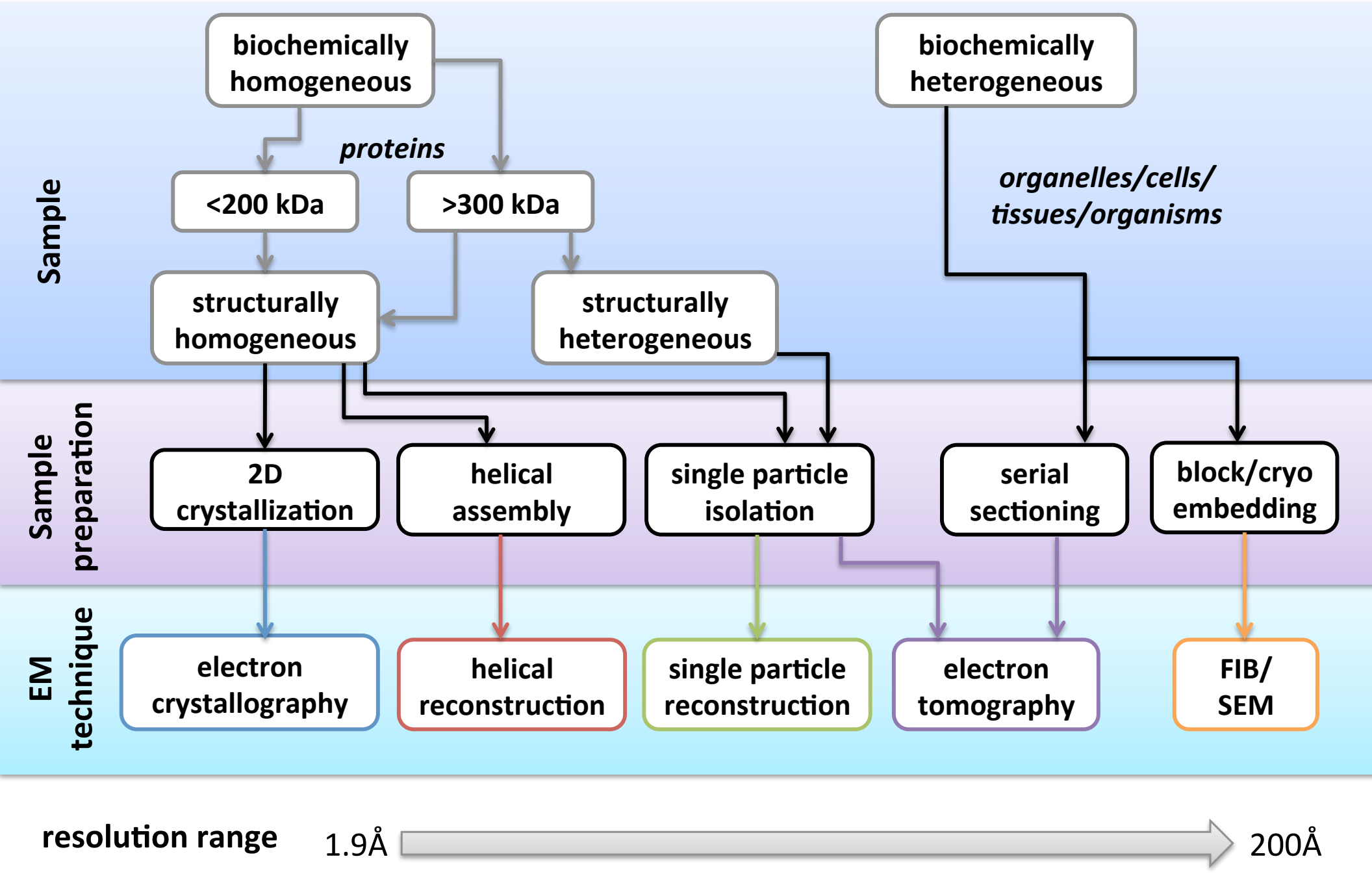
- Training in instrumentation and techniques, and individual instruction
- Assigned, dedicated instrument time

### Additional services

*(offered on a fee-for-service basis)*

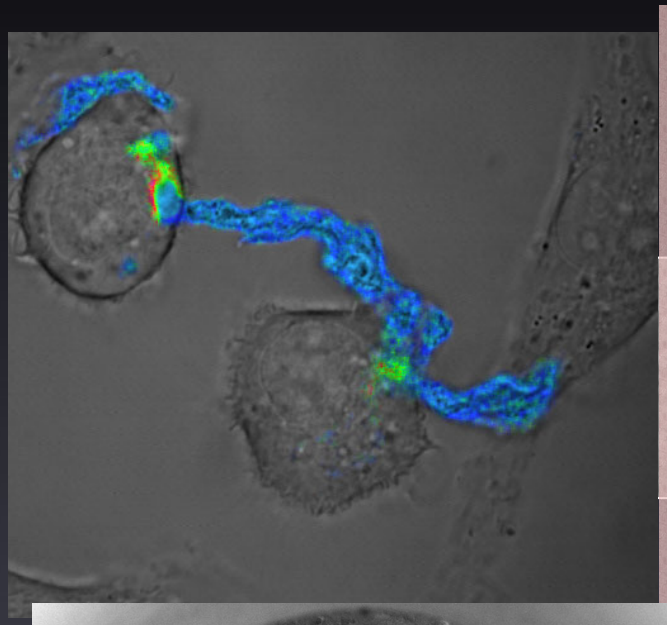
- Additional EM time (members) or access to EMs with staff assistance (non-members).
- EM starter kits and consumables

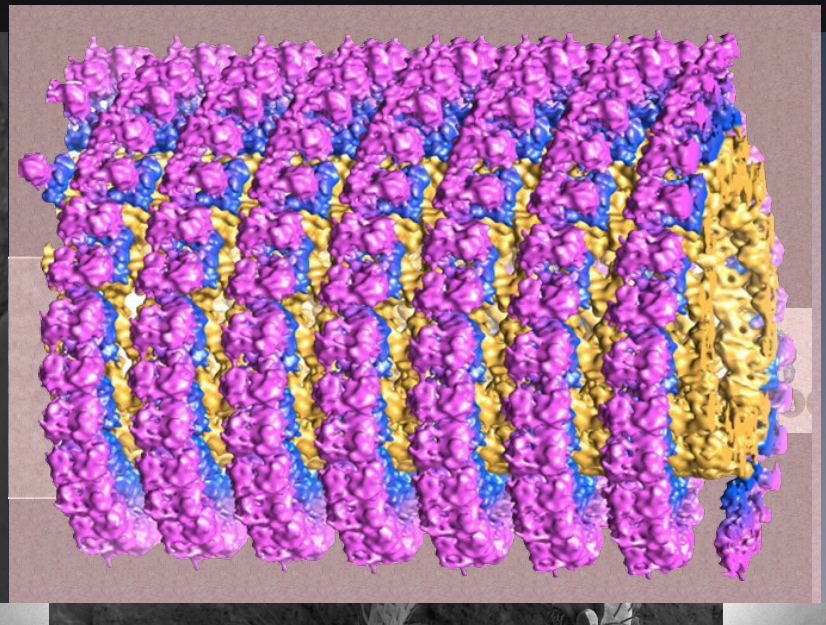
# SEMC mission

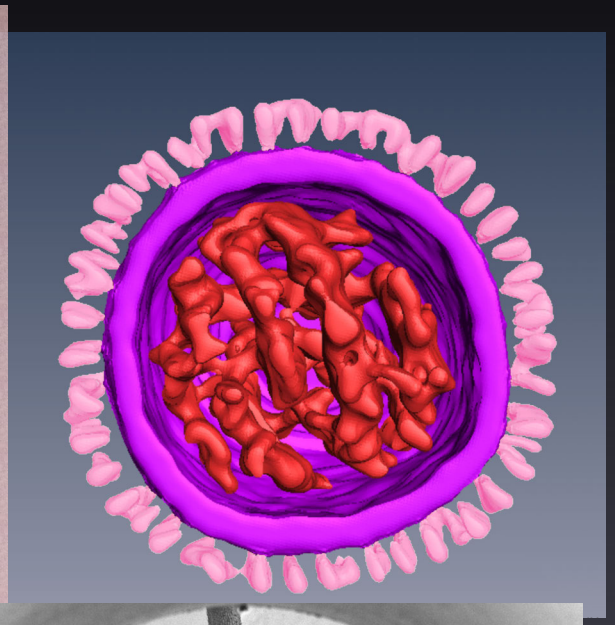


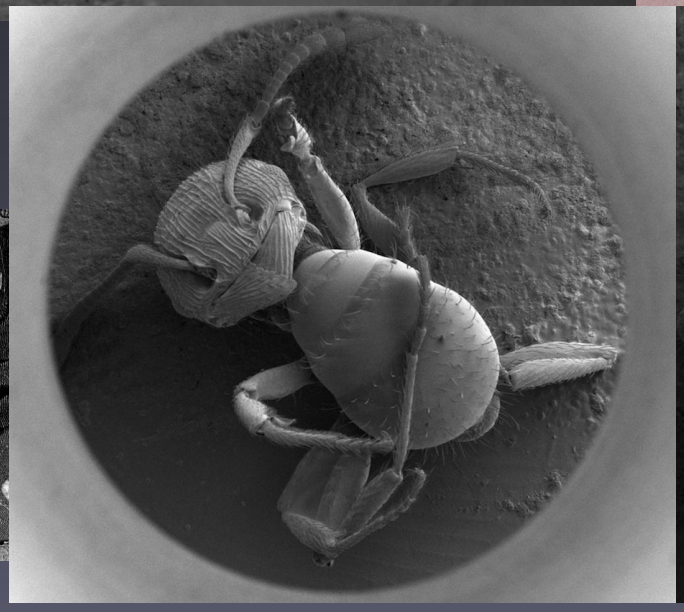
# The Simons Electron Microscopy Center

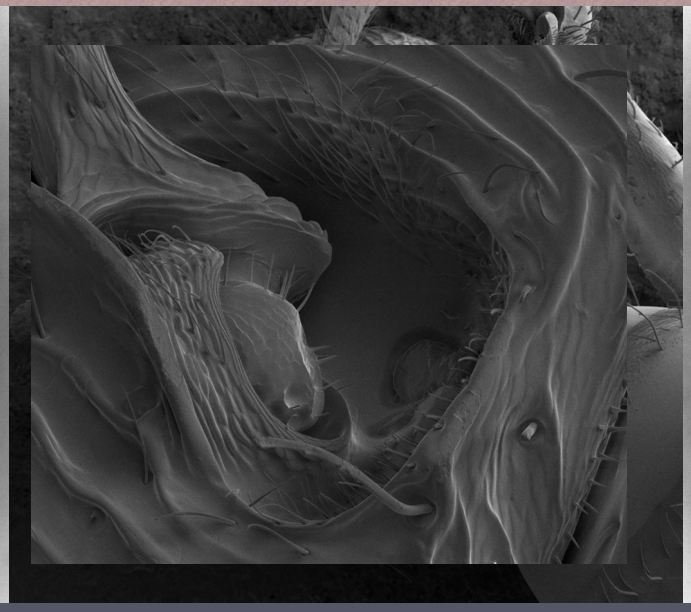
## Focus on:







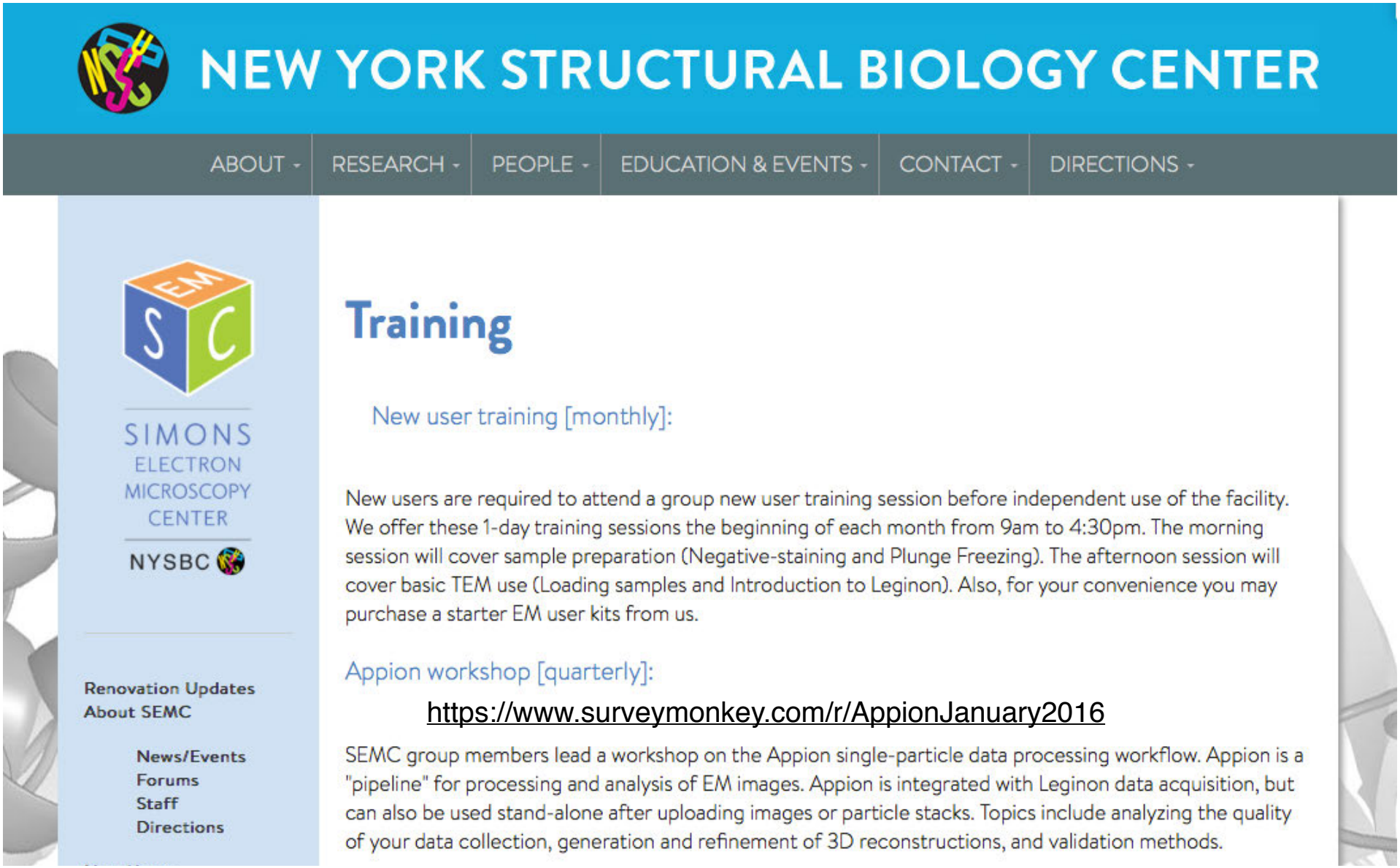




# SEMC Training Programs

| | | |
|---|---|---|
| yearly | SEMC EM Course | Theory behind EM<br>*starts Jan 11* |
| quarterly | SEMC Appion workshop | Appion data processing practical<br>*Jan 28* |
| monthly | SEMC New User Training | Sample preparation [neg-stain & cryo]<br>*Leginon intro/use of screening microscopes* |
| | 24-hr access test | All hours access test<br>*Safety training* |
| weekly | User Project Discussion Meetings | Mon @ 11am<br>*Thurs @3pm & @4pm* |
| daily | Advanced Leginon use | Training for independent use of the microscopes |

# Online registration



# Online materials

*http://semc.nysbc.org/start.html*

SIMONS ELECTRON MICROSCOPY CENTER

NEW YORK STRUCTURAL BIOLOGY CENTER

## EM Directors

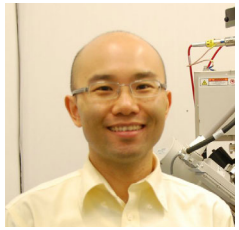
Bridget Carragher, Ph.D.

Clint Potter, Ph.D.

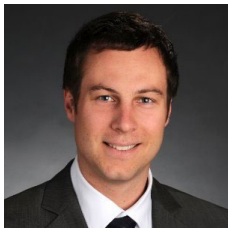
EMG@nysbc.org

## EM Staff

Bill Rice, Ph.D.
EM Manager



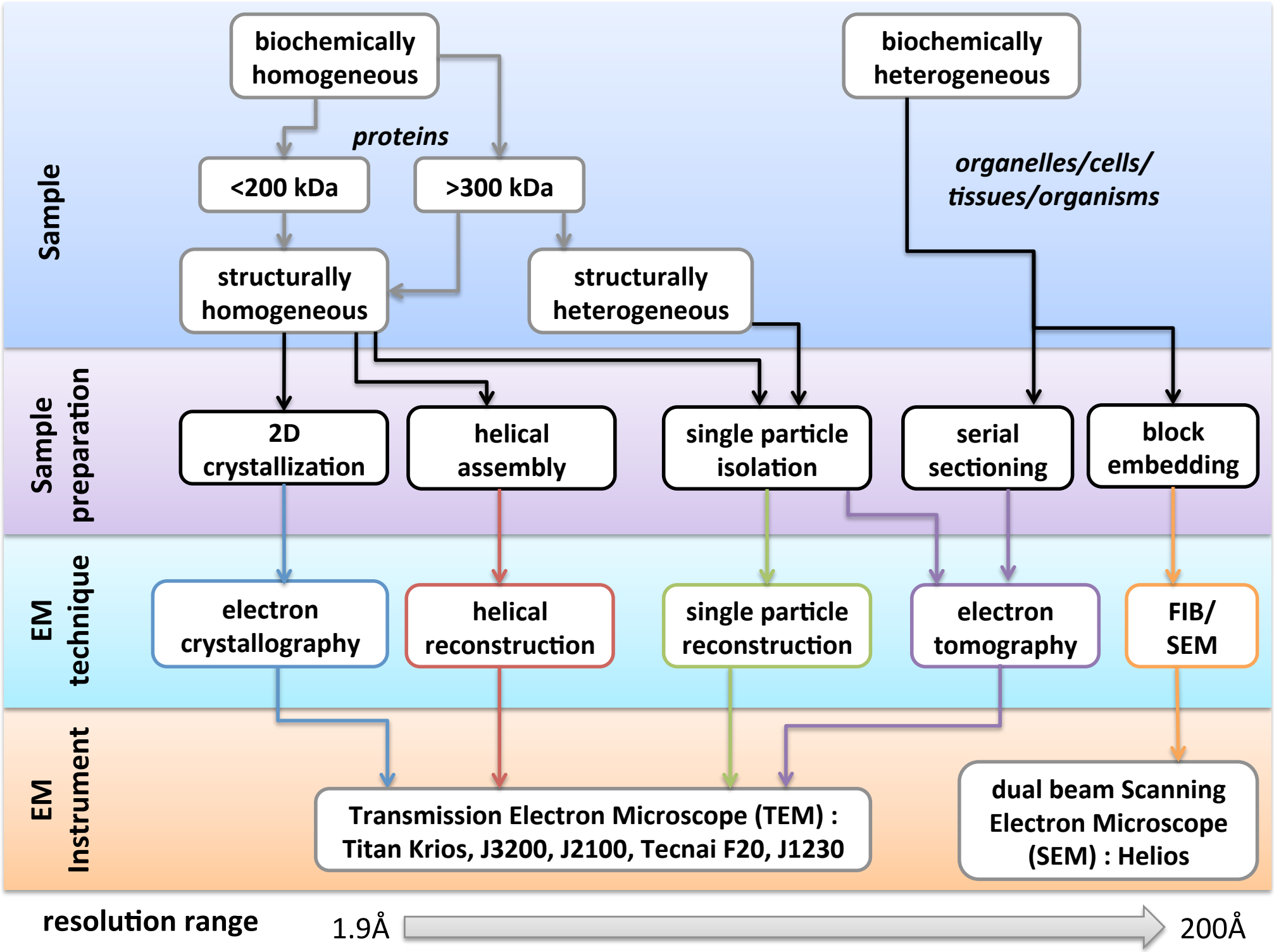
Ed Eng, Ph.D.
Scientist



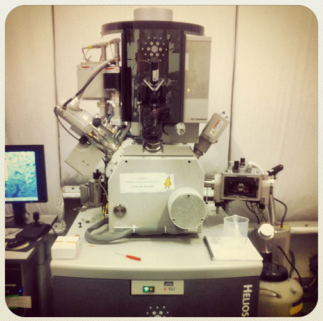
Christoph Wigge, Ph.D.
Scientist

Ashleigh Raczkowski, B.S.
Technician

Kelsey Jordan, B.S.
Technician

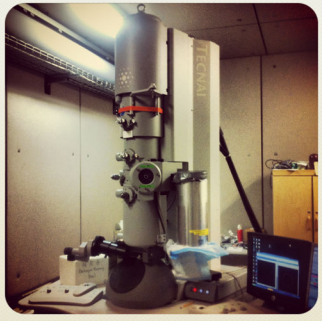

Sample
biochemically homogeneous
biochemically heterogeneous
proteins
<200 kDa
>300 kDa
organelles/cells/ tissues/organisms
structurally homogeneous
structurally heterogeneous
Sample preparation
2D crystallization
helical assembly
single particle isolation
serial sectioning
block embedding
EM technique
electron crystallography
helical reconstruction
single particle reconstruction
electron tomography
FIB/ SEM
EM Instrument
Transmission Electron Microscope (TEM) : Titan Krios, J3200, J2100, Tecnai F20, J1230
dual beam Scanning Electron Microscope (SEM) : Helios
resolution range
1.9Å
200Å

# Instrumentation

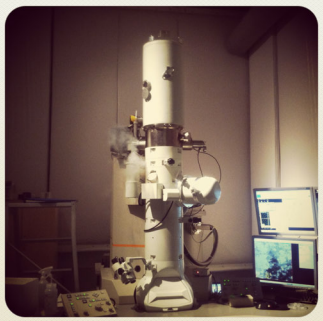


FEI Helios 650
+
4K ETD, TLD, ICE

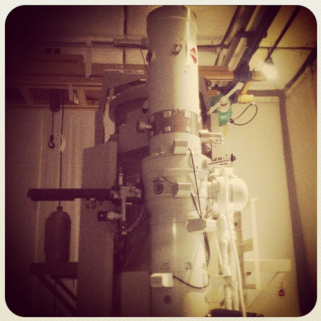
JEOL 1230
+
Gatan US4000
4K CCD



FEI Tecnai F20
+
TVIPS F416
4K CMOS



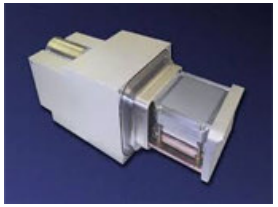
JEOL 2100F
+
Gatan
K2 Summit
*Direct Detector*



JEOL 3200FSC
+
Direct Electron
DE-20
*Direct Detector*

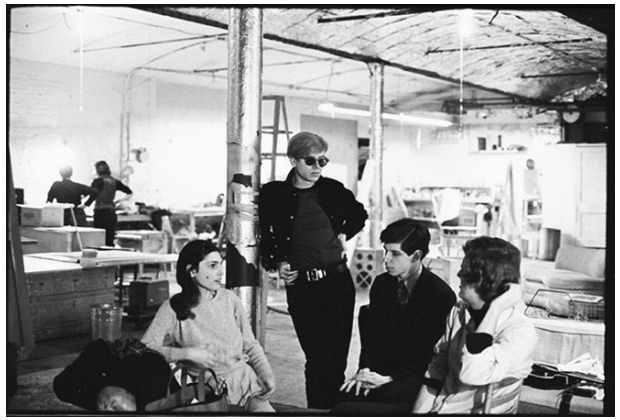
FEI Titan Krios
+
FEI
Falcon 2->3
*Direct Detector*

*Coming soon*



# Schedule

- *Welcome new students*
- *Course logistics*
  - *Questionnaire*
  - *Other SEMC training programs*
- *Introduction to EM*
- **NYSBC tour**



Andy Warhol's New York studio circa 1960s

Jeff Koons's New York studio