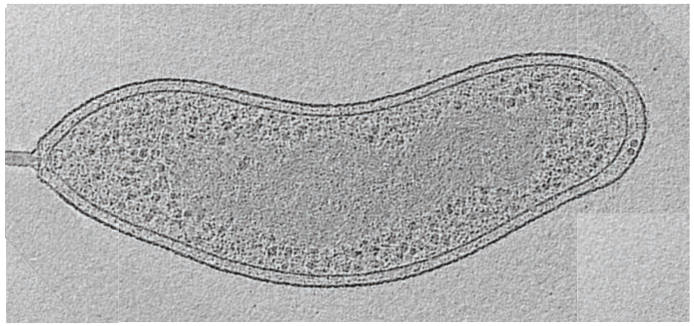
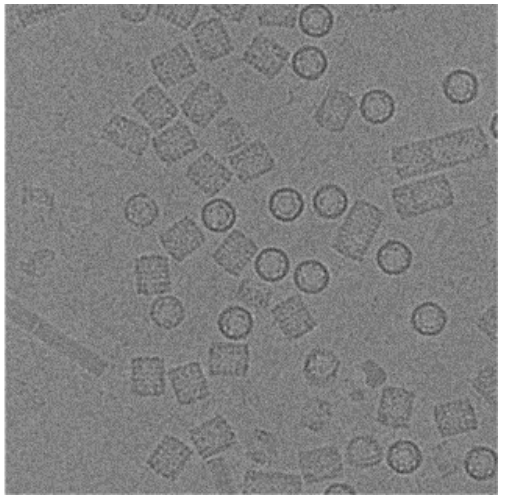
Part 6: Single particle analysis

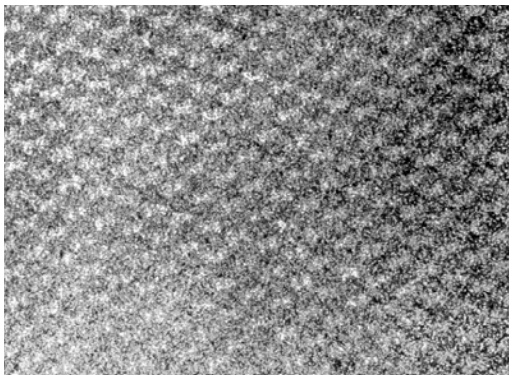
Basic approaches in 3-D EM



Tomography

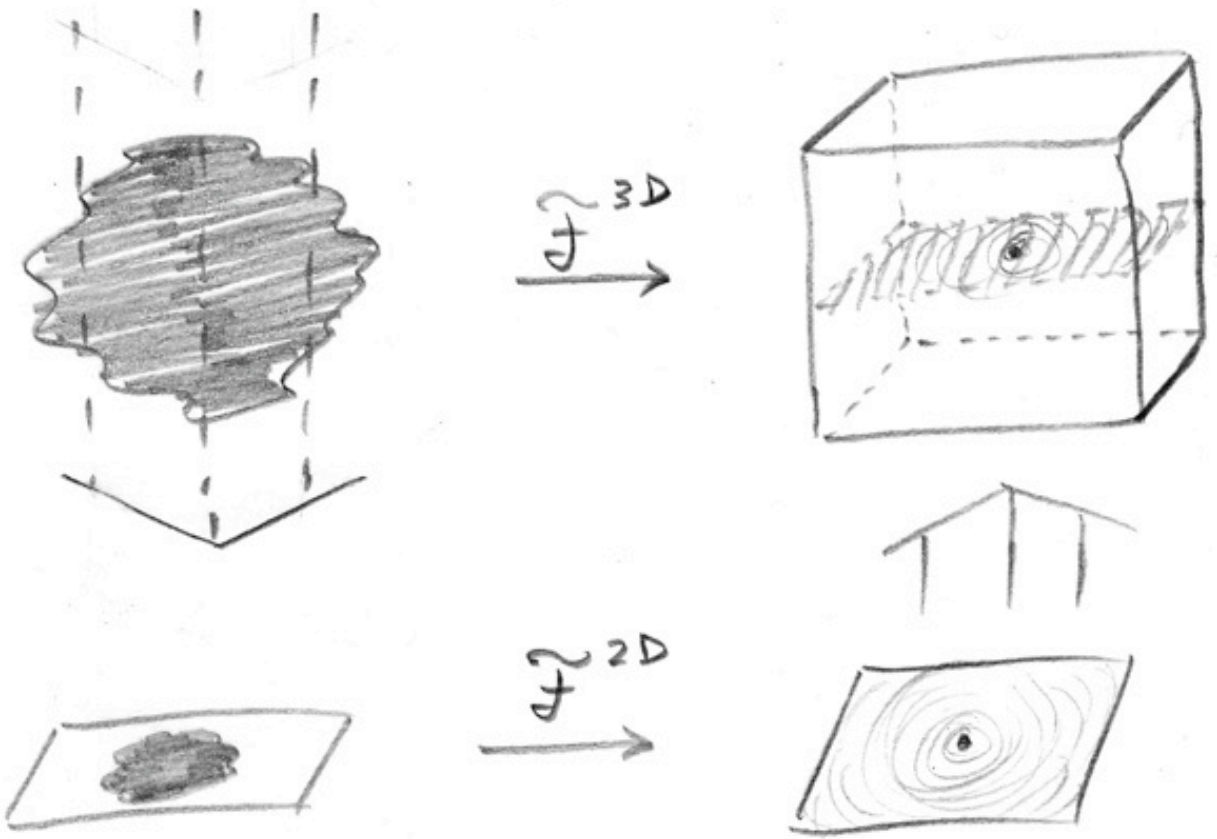


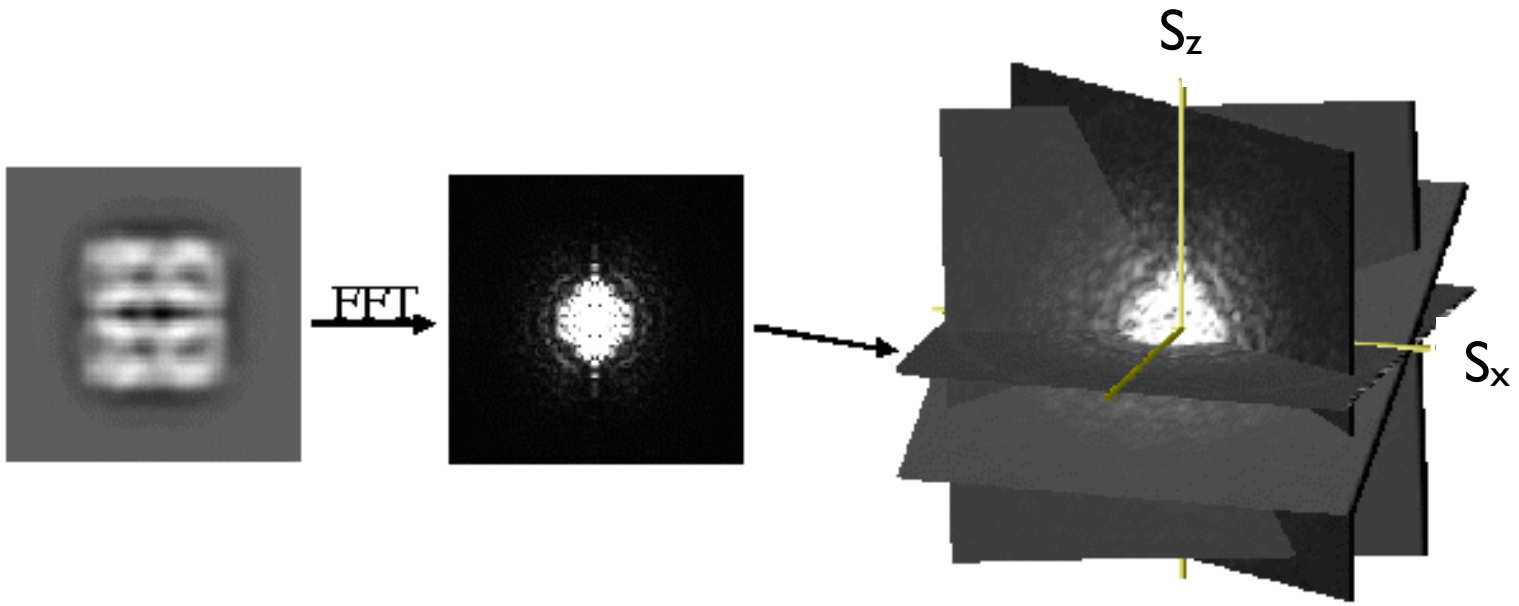
Single particle analysis



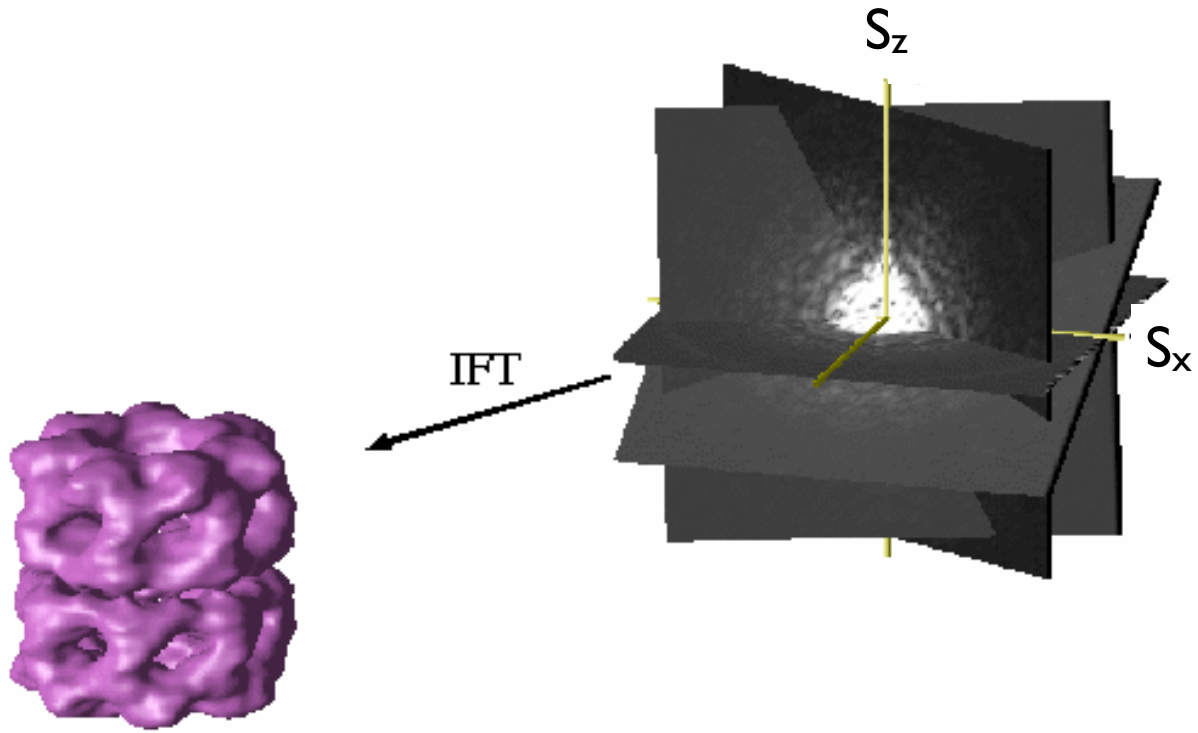
2D crystallography

The projection theorem



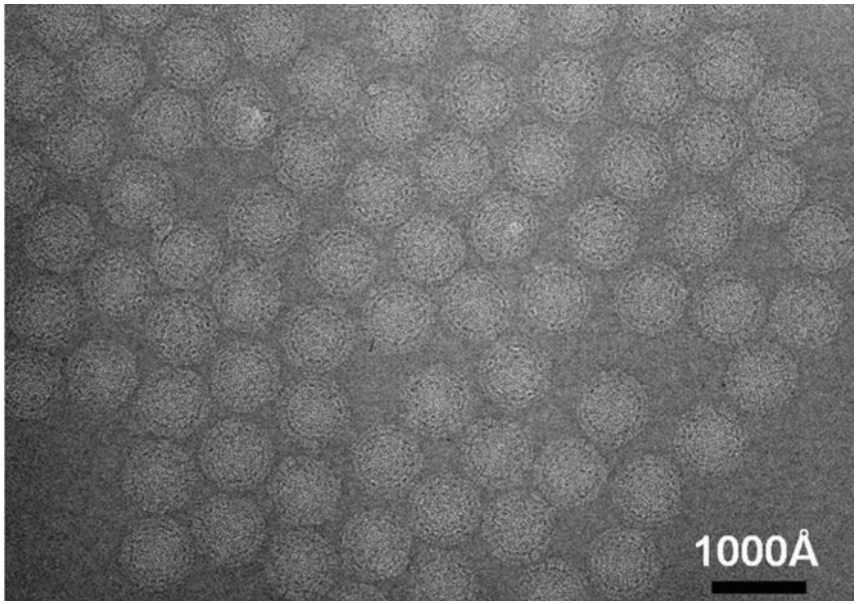


EMAN tutorial at http://ncmi.bcm.tmc.edu/~stevel/EMAN/doc/

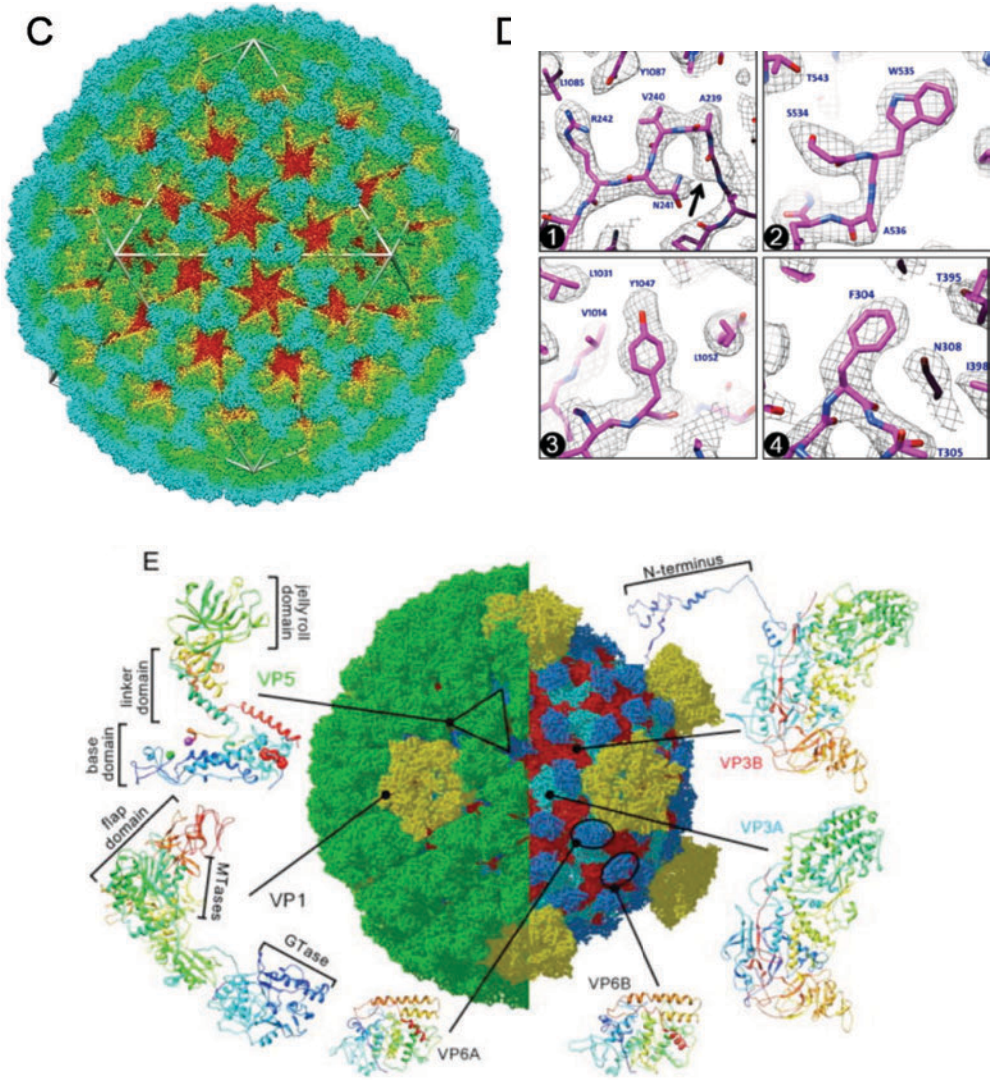


EMAN tutorial at http://ncmi.bcm.tmc.edu/~stevel/EMAN/doc/

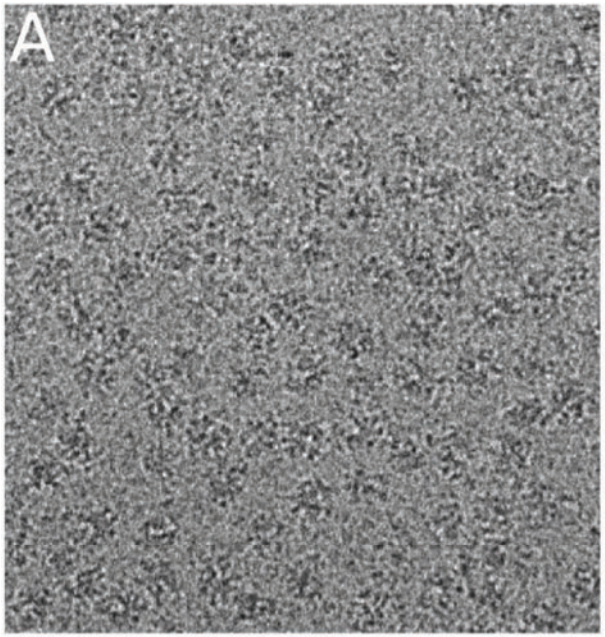
Example large object:
an icosahedral virus



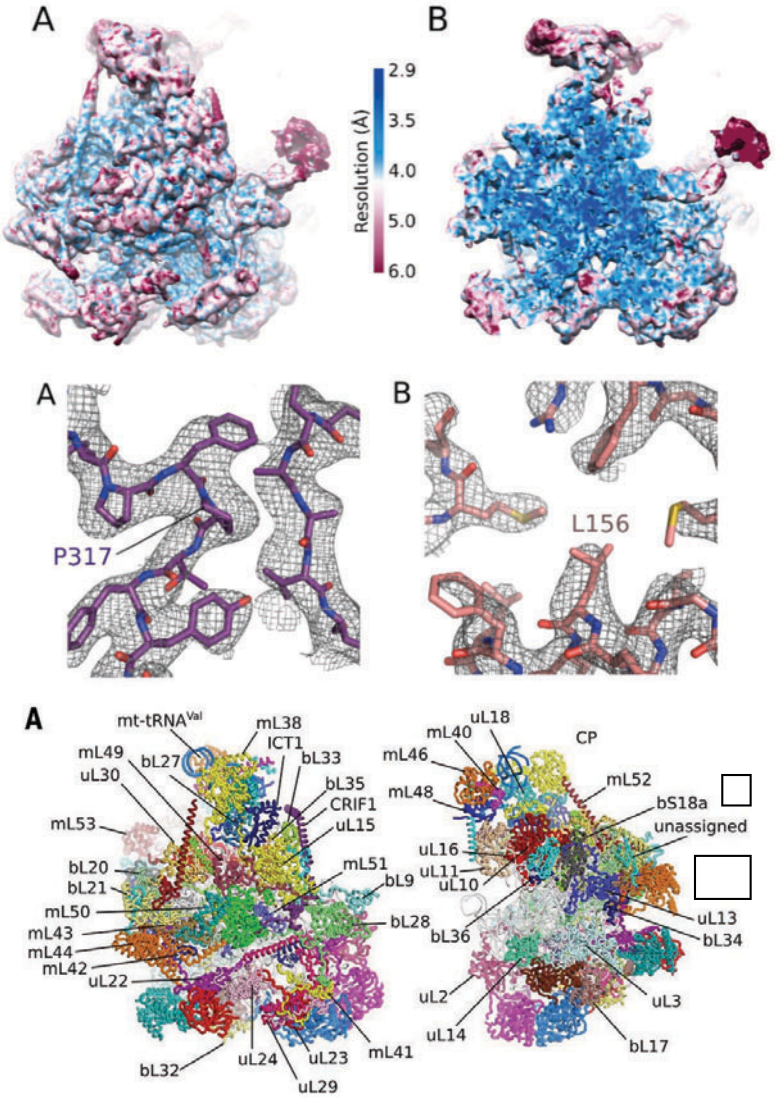
Zhang et al., Cell 2010



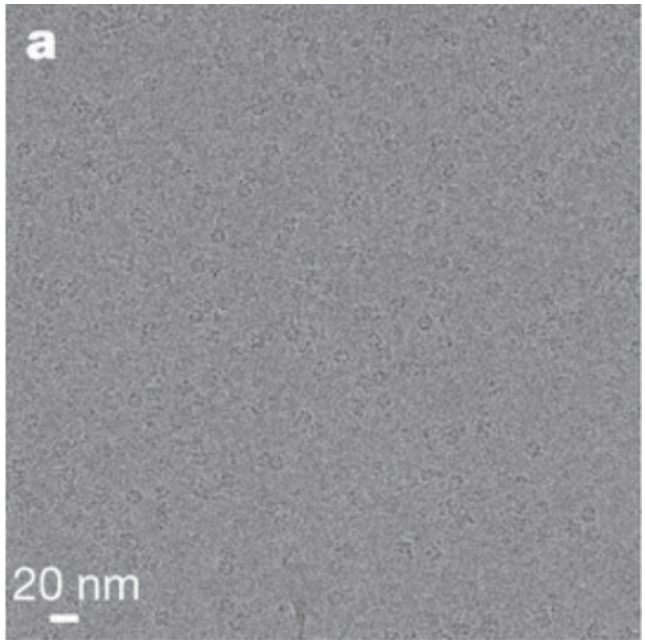
An example large macromolecular complex: the ribosome



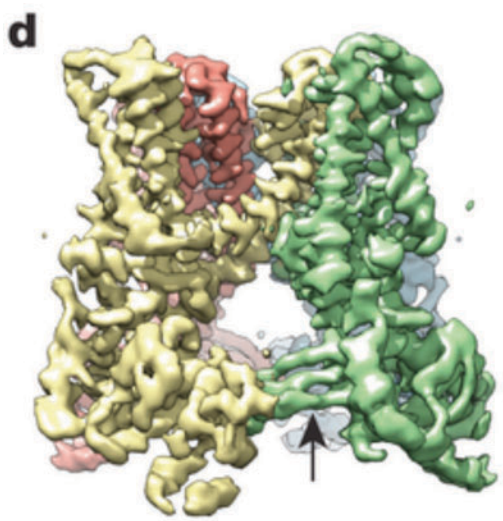
Brown et al., Science 2014

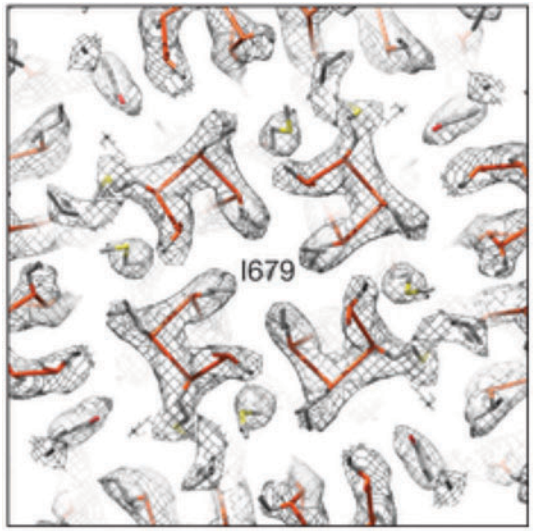


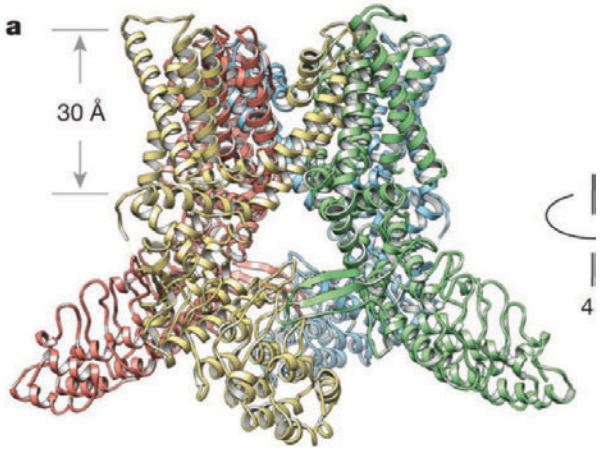
An example smaller complex:
a membrane (TRP) channel



Maofu et al., Nature 2013







Advantages of single particle analysis

- Does not require crystals
- Samples can be partially inhomogeneous
- Physiological conditions possible
- Requires small amount of sample
- Rapid - many steps automated
- May eventually even be possible in vivo

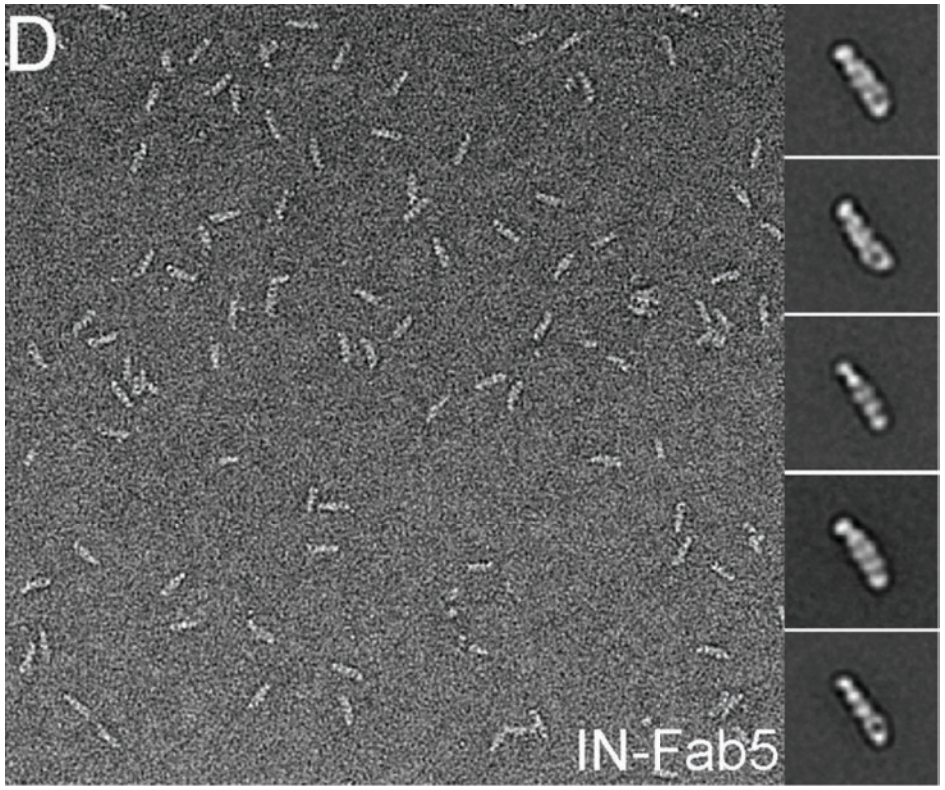
Limitations

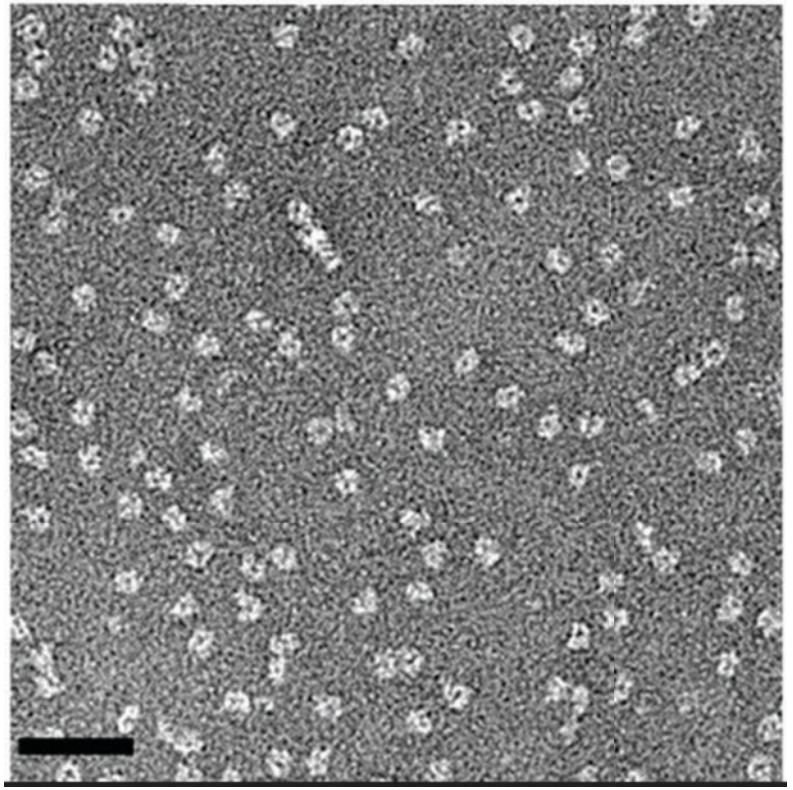
- Radiation damage
- Precision of image alignment
- Numbers of particles averaged
- Conformational heterogeneity
- Orientational preferences

Intro to single particle analysis
Concept check questions:

- Any two projection images of the same object share at least one feature - what is it?
- How can this fact be used to align particle images?
- What kinds of samples are amenable to single particle analysis, and what kinds of resolutions have been obtained in the best cases?
- Name 6 advantages of single particle analysis (as compared to other popular structural techniques like X-ray crystallography and NMR spectroscopy).
- Describe 5 limitations.

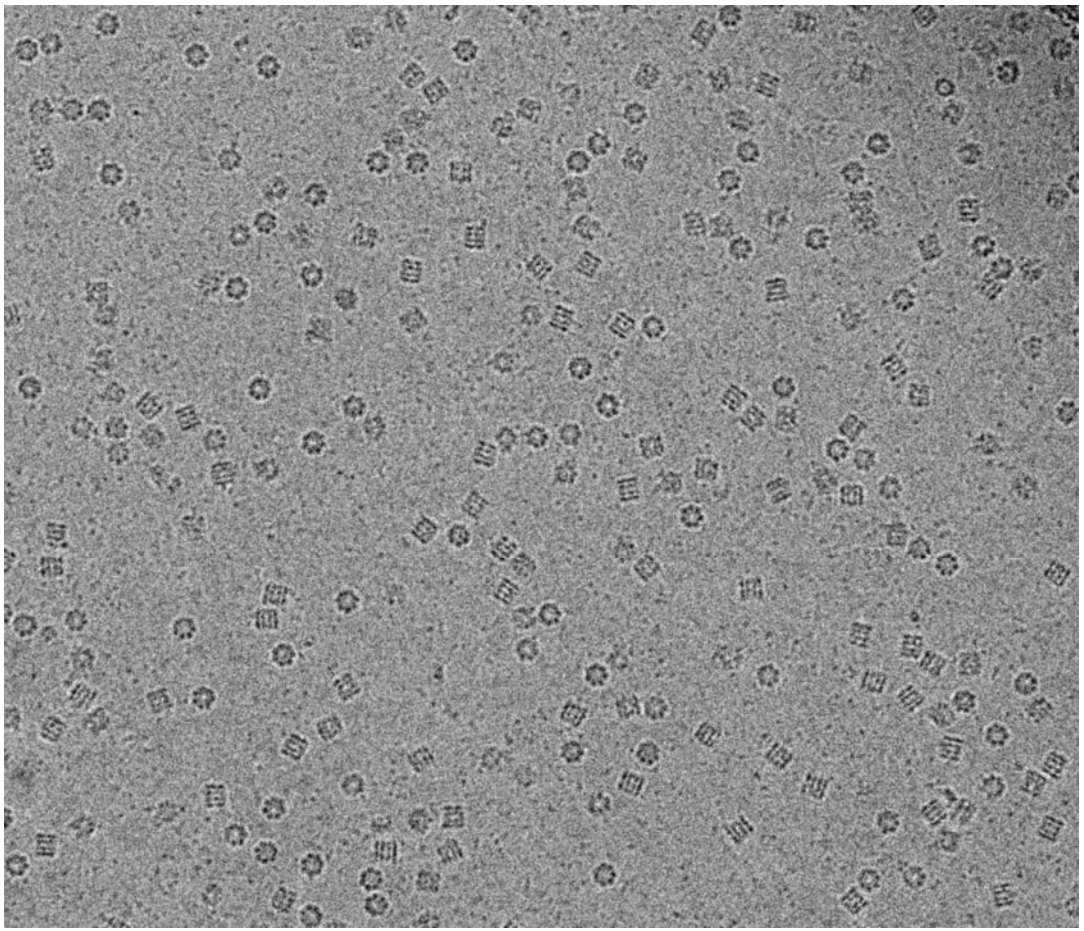
Most projects begin with negative staining - example images





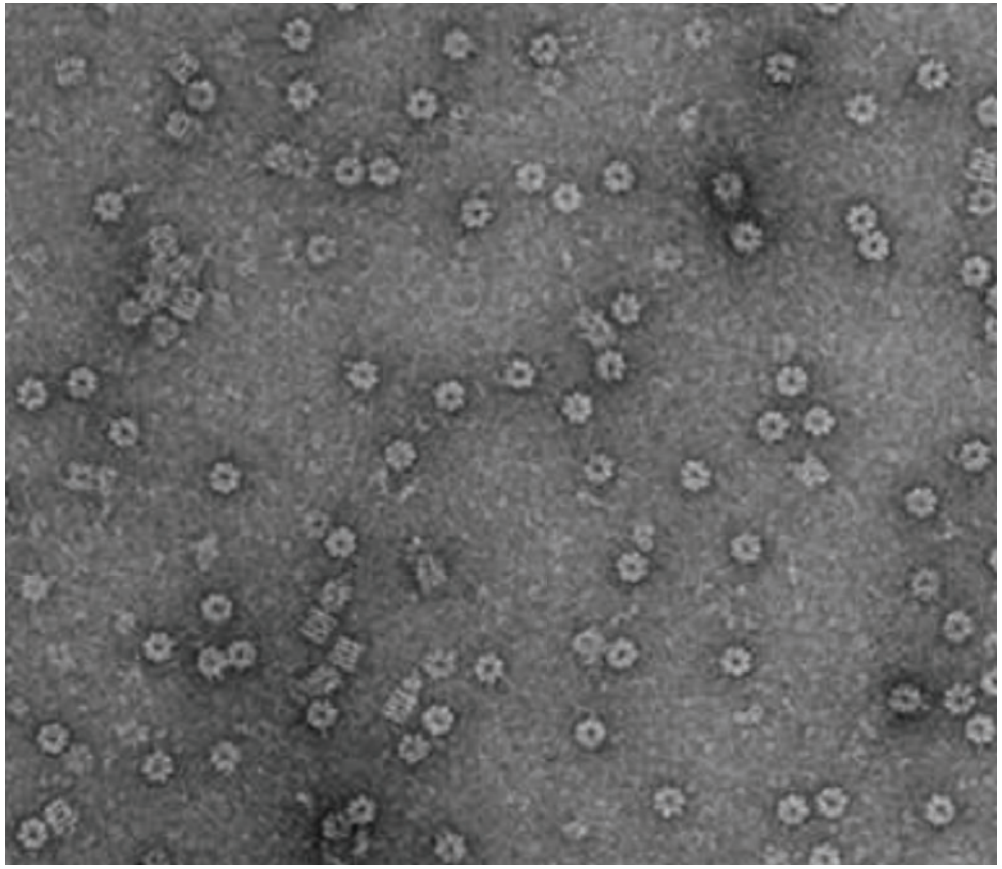
Then plunge-freezing

Example image of plunge-frozen GroEL

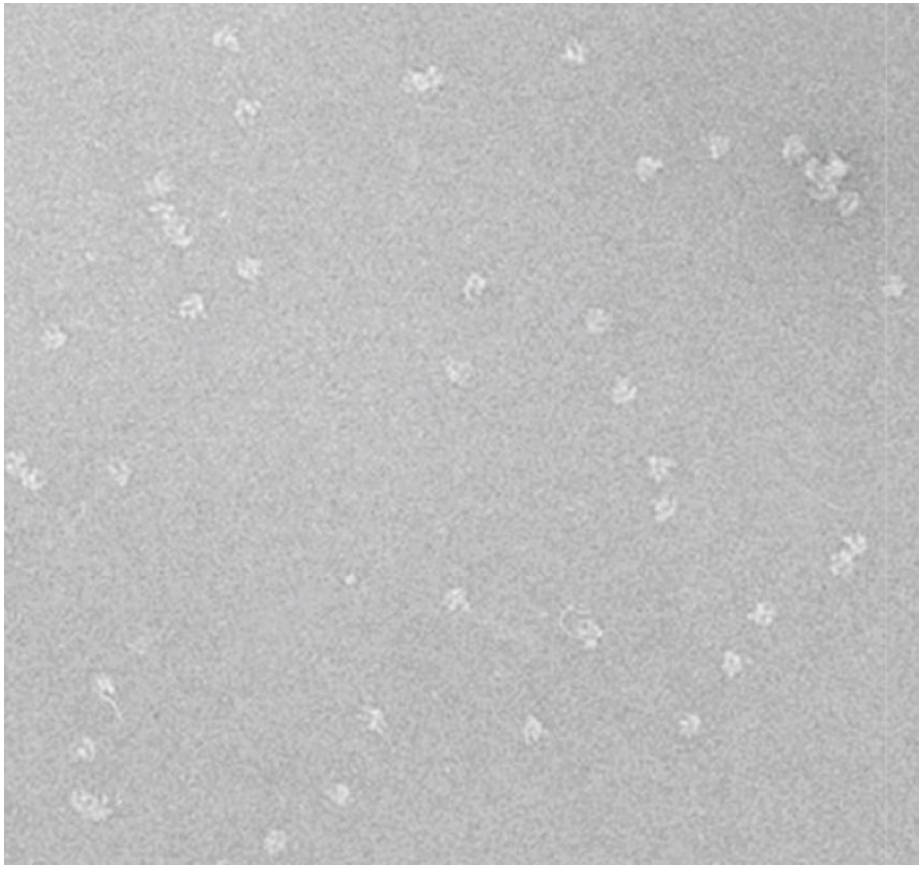


GroEL
http://en.wikipedia.org/wiki/Cryo-electron_microscopy

Cryo-negative staining

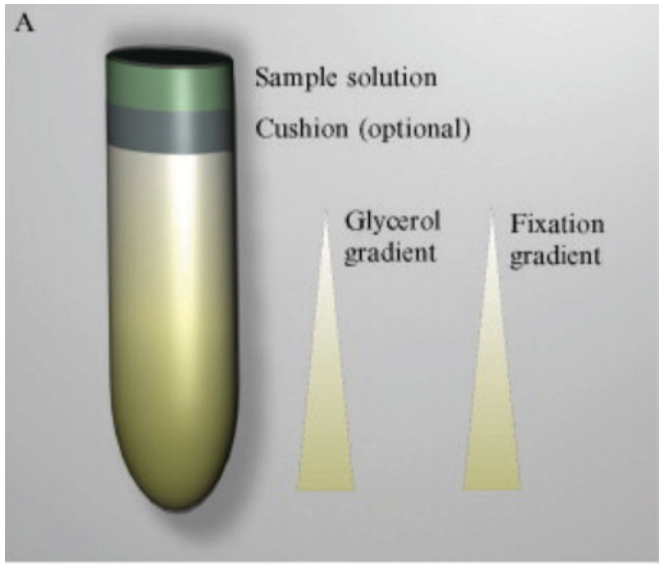


GroEL
described in De Carlo et al., Micron 2008

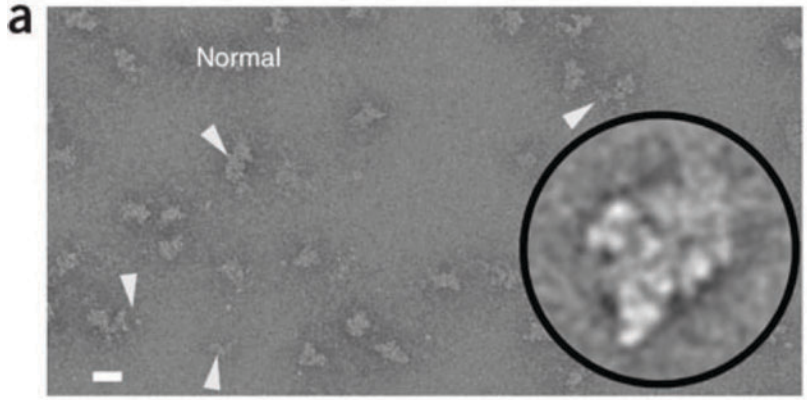


RNA polymerase II
described in Kostek et al., Structure 2006

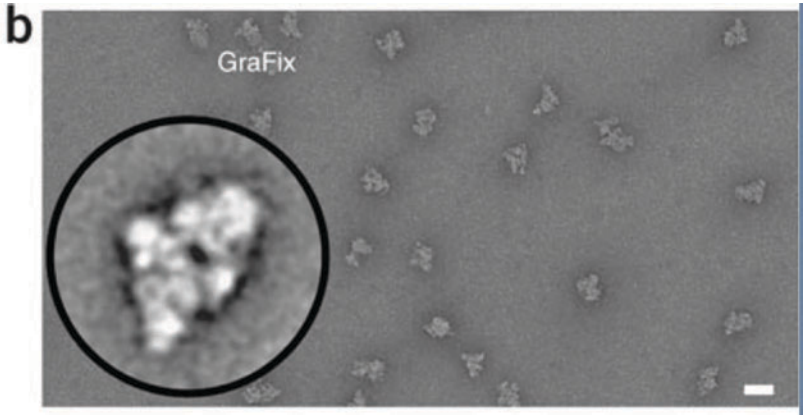
GraFix purification/stabilization



Kastner et al., Nature Methods 2008

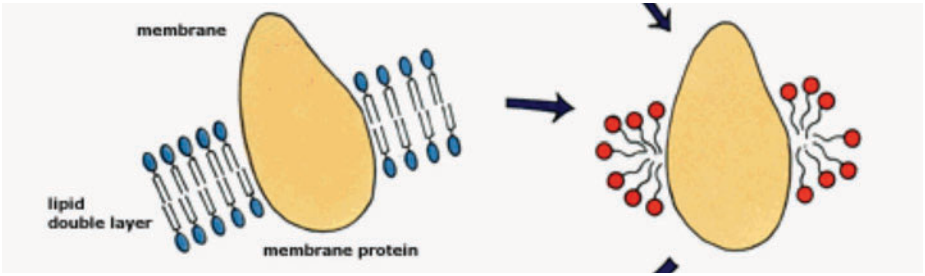


Splicesomes



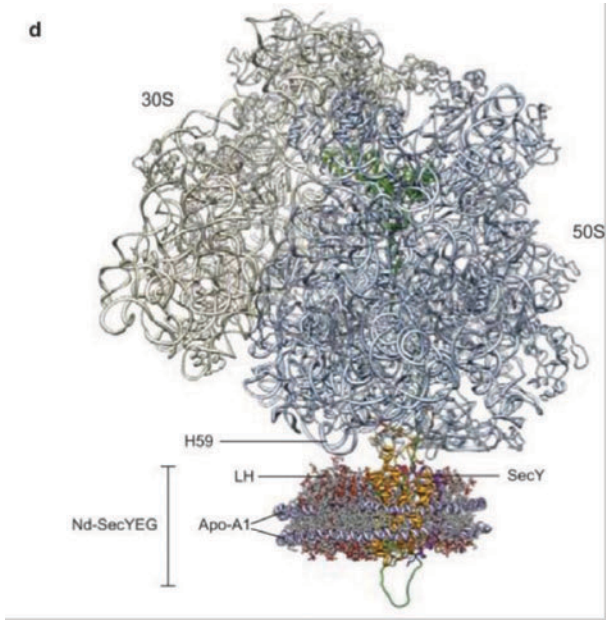
How to stabilize membrane proteins?

1. Detergent solubilization



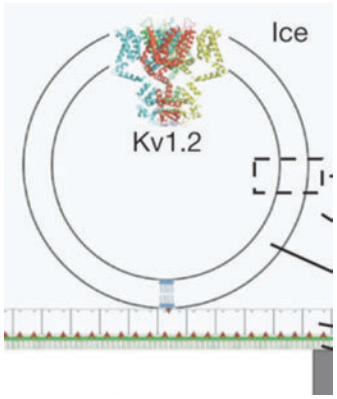
http://www.nobelprize.org/nobel_prizes/chemistry/laureates/1988/illpres/crystals.html

2. Nanodiscs

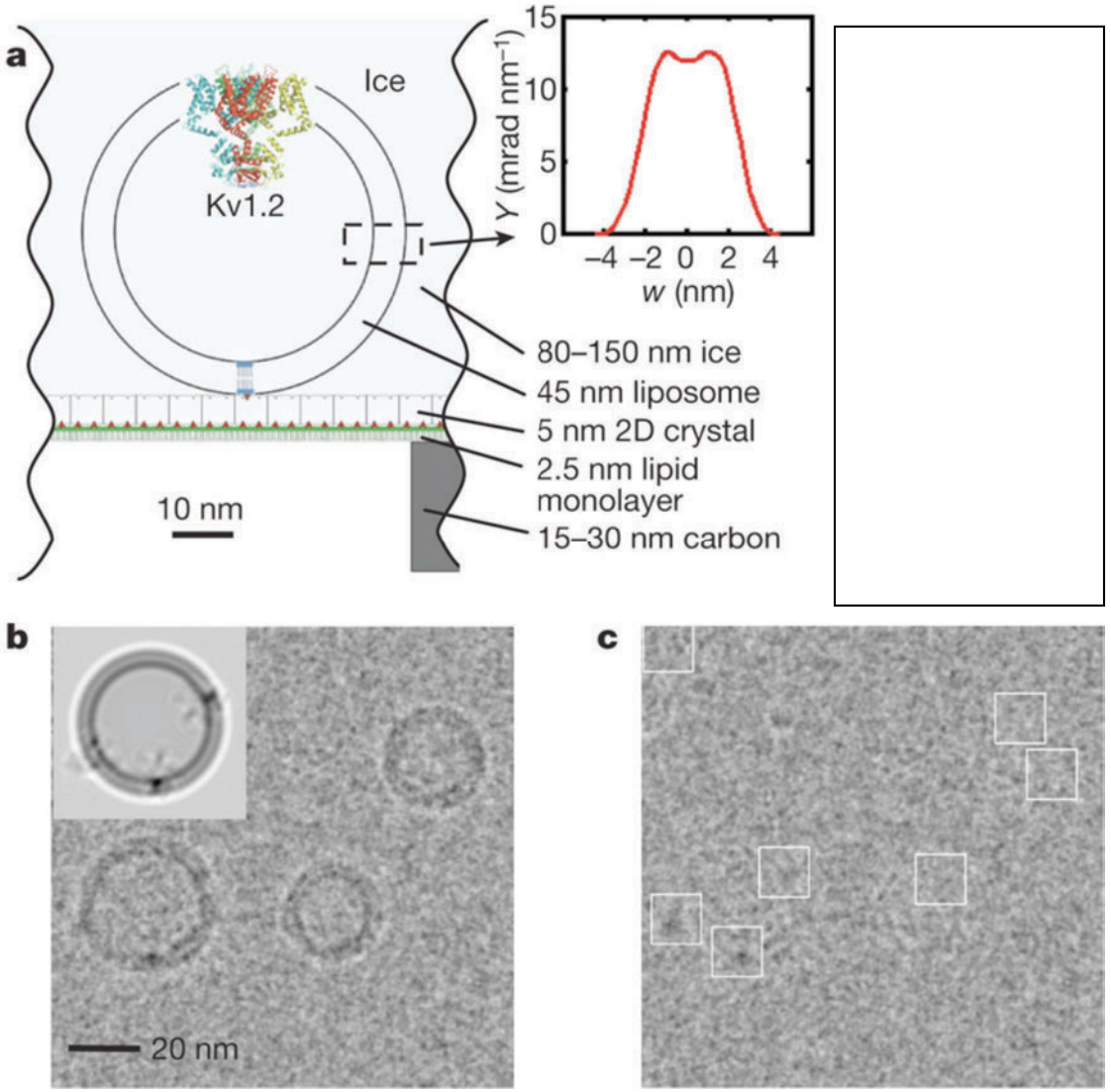


SecY/Sec61 complex in a Nanodisc
Frauenfeld et al., NSMB 2011

3. Embed in liposomes



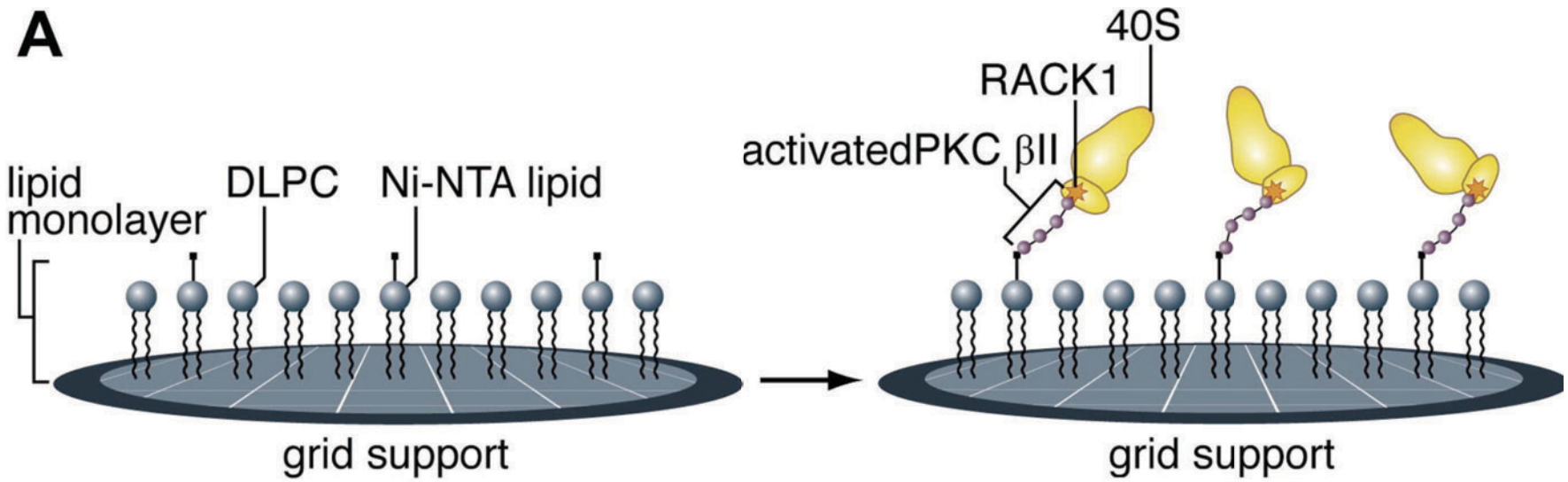
Wang and Sigworth,
Nature 2009



Spherically constrained single particle reconstruction

Wang and Sigworth,
Nature 2009

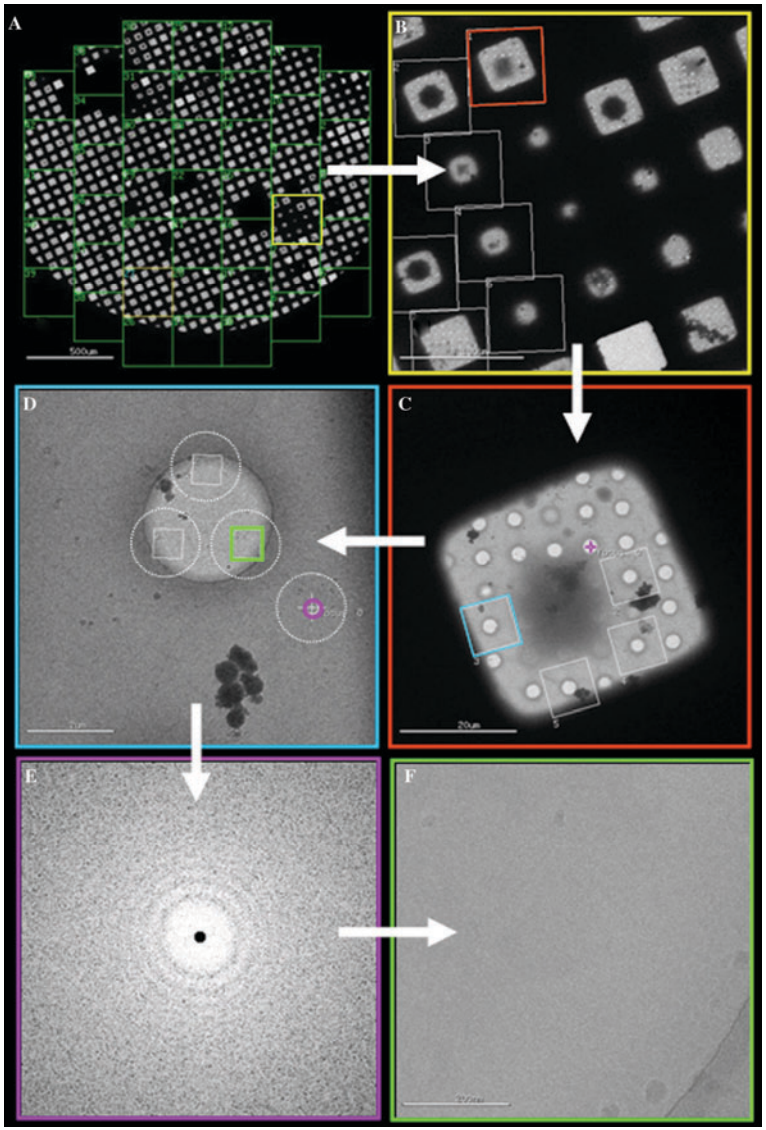
Affinity grid capture



Sharma et al., JSB 2013

Single particle analysis: Special sample prep issues
Concept check questions:

- Why do most projects begin with "negative staining"? What is "negative" about it?
- What is "cryo-negative staining," and why might one do it?
- Explain the "GraFix" method.
- Name three ways to stabilize membrane proteins for cryo-EM imaging.
- What is an "affinity grid"?



120x

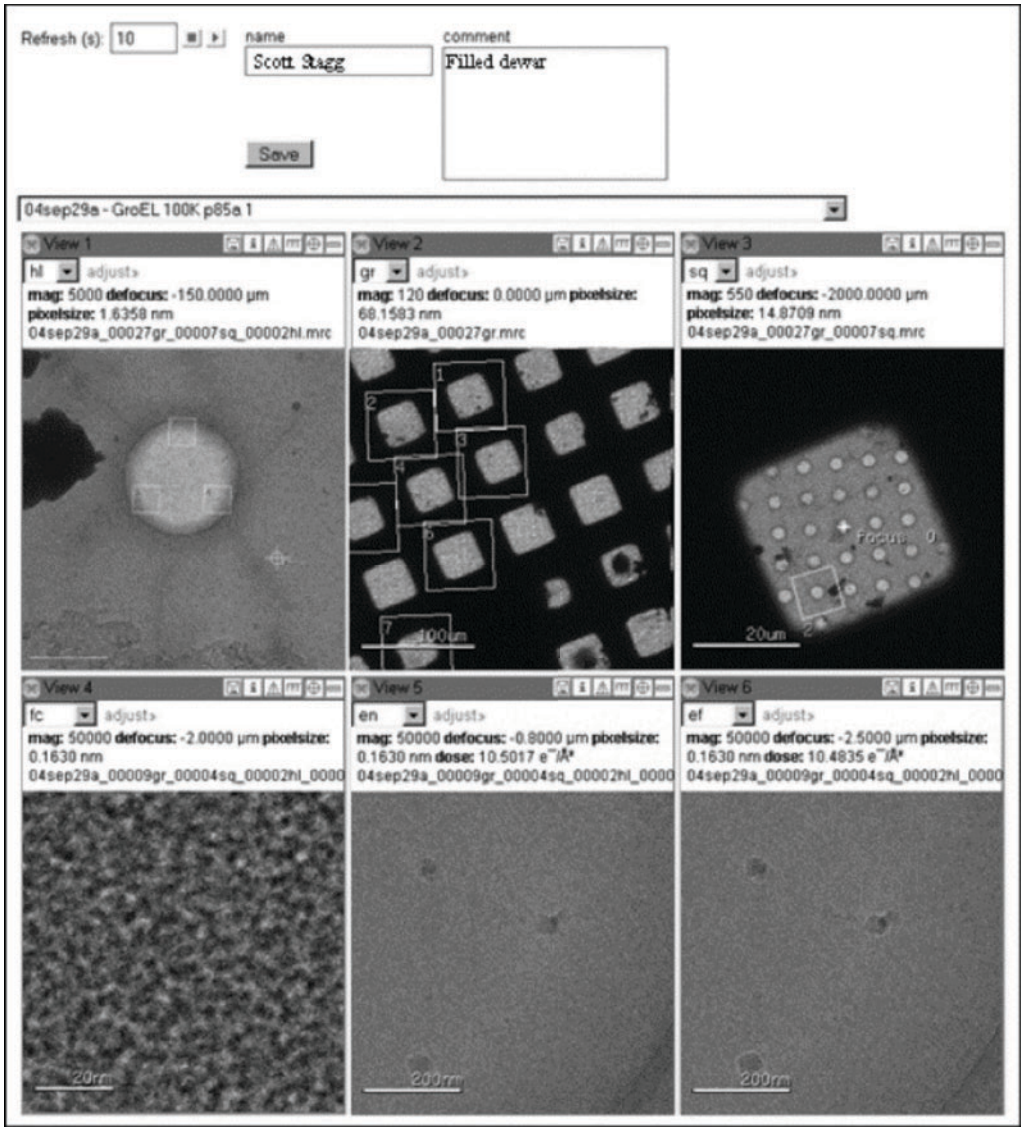
500x

5kx
1% dose

50kx

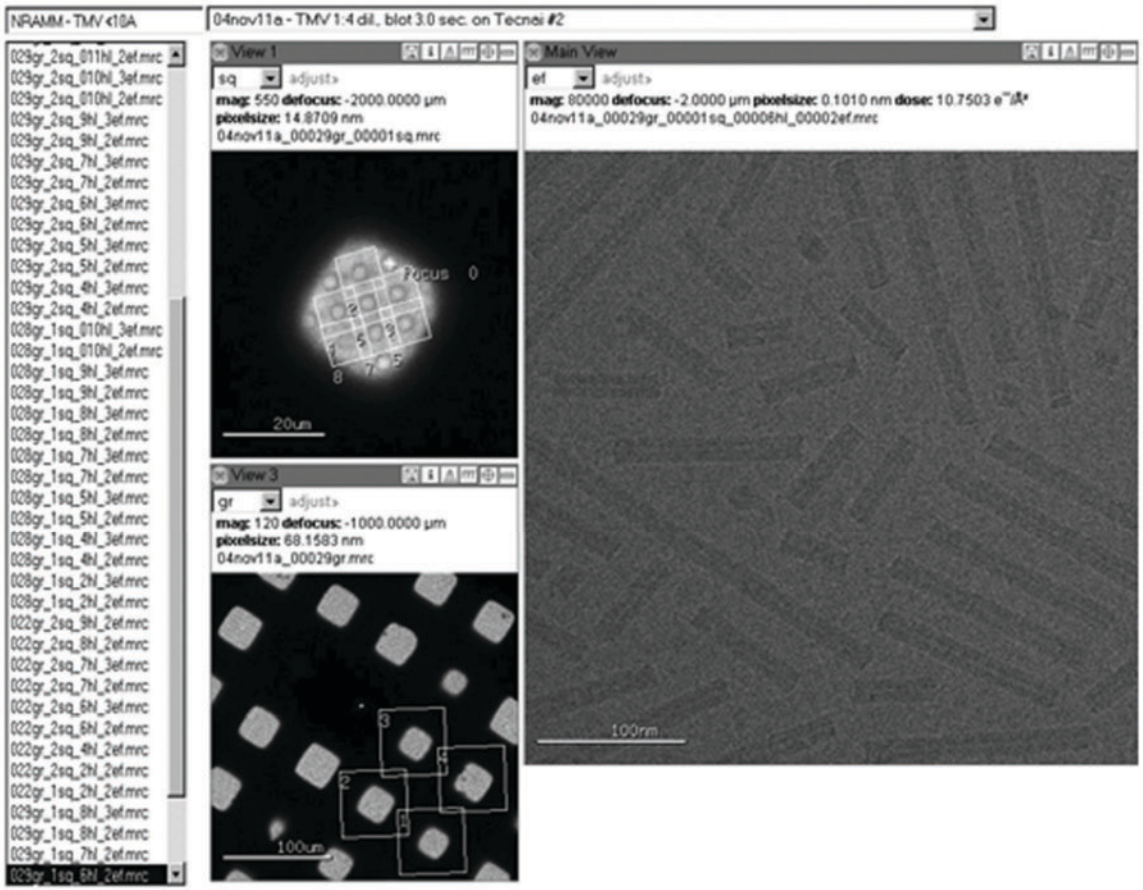
Leginon: a program for automatic data collection

Suloway et al., JSB 2005



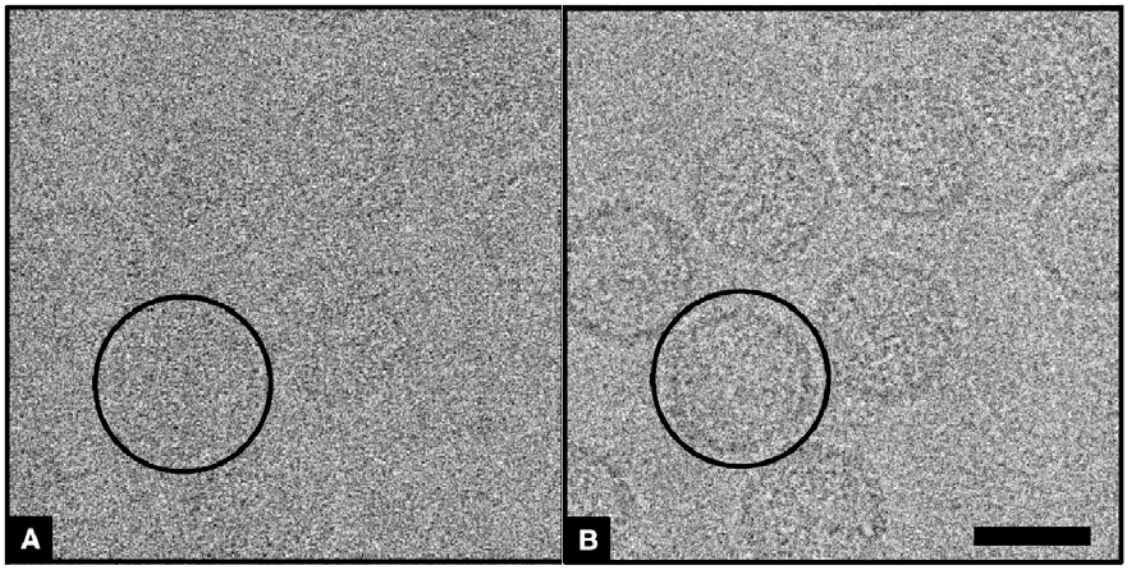
Leginon "Observer"

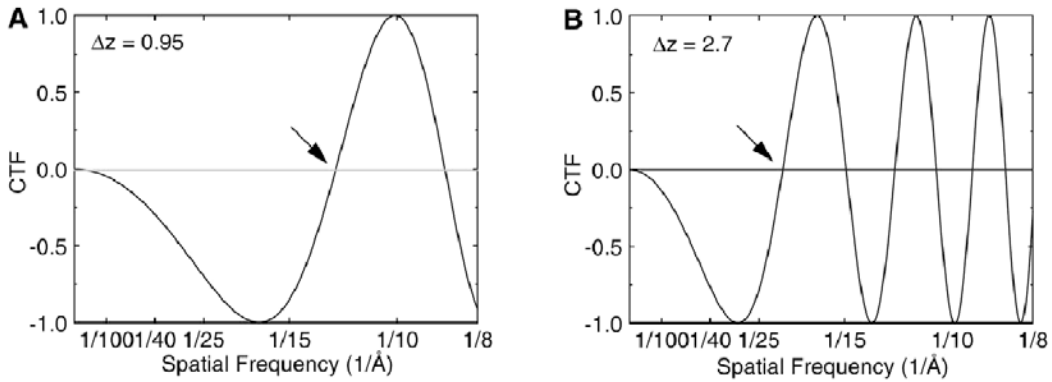
Suloway et al., JSB 2005



A Leginon web-based viewing tool

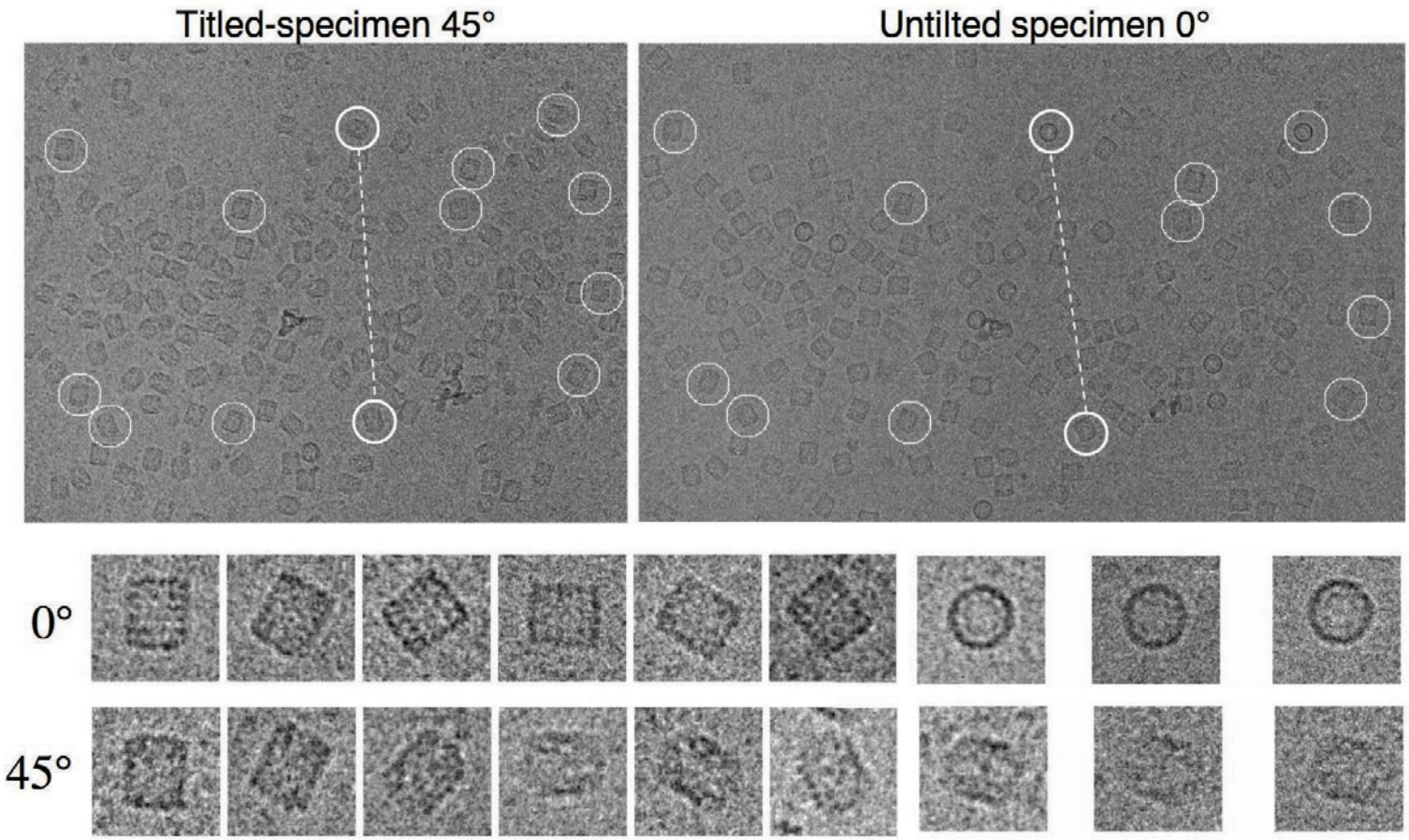
Suloway et al., JSB 2005





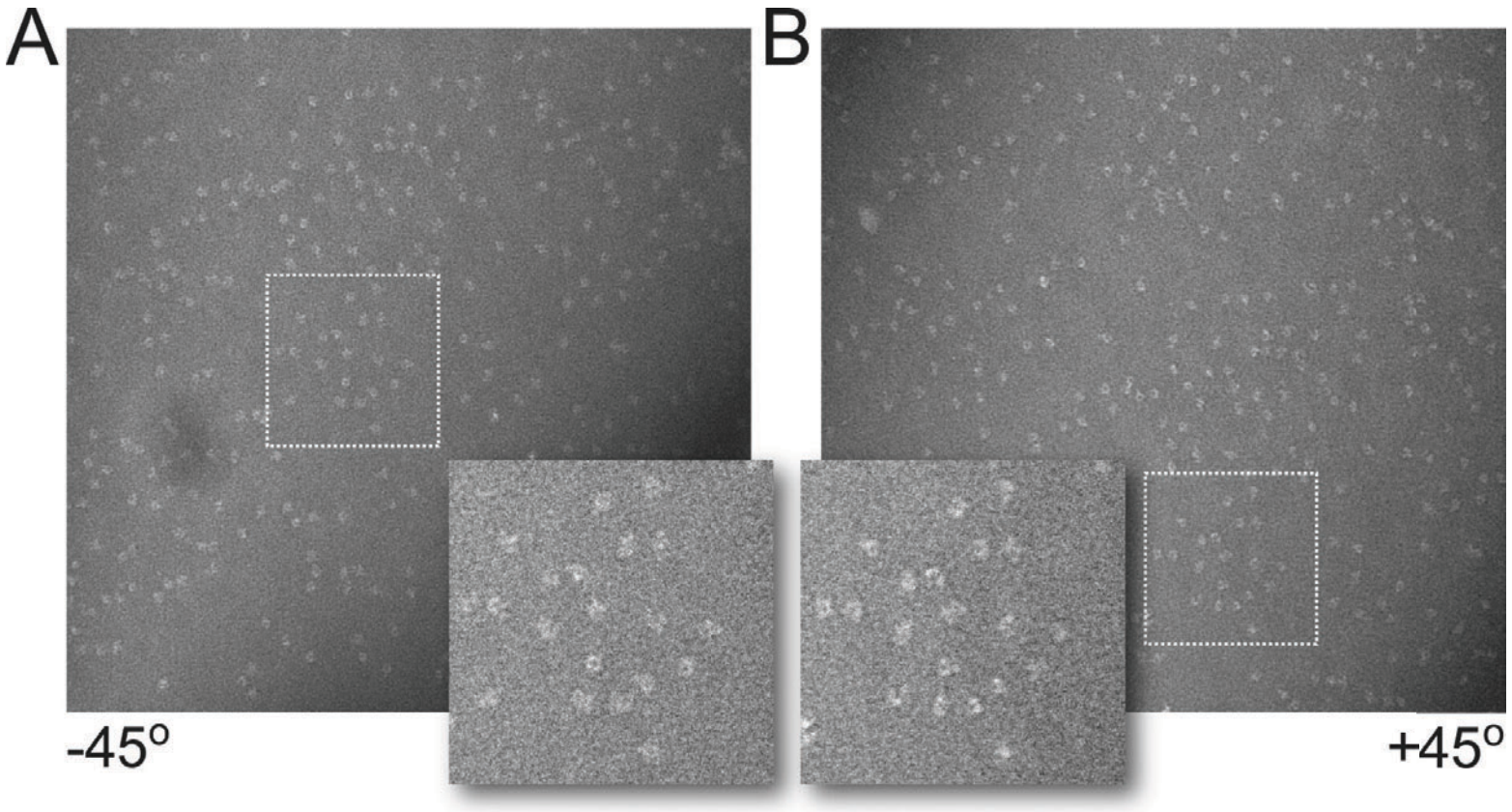
Thuman-Commike and Chiu, Micron 31:687

Sometimes focal pairs are recorded



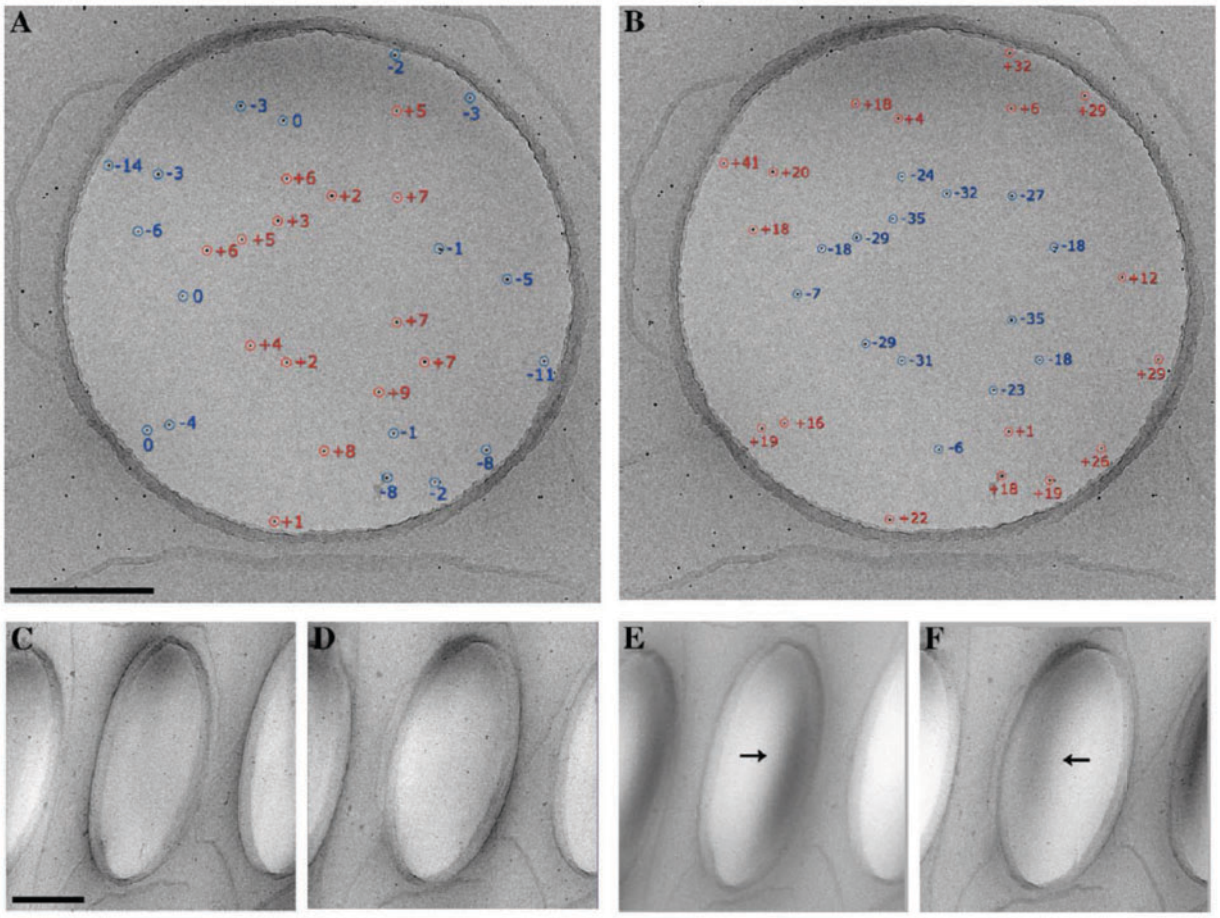
Sometimes tilt pairs are recorded

http://nramm.scripps.edu/wp-content/seminars/2005/cryoem/lectures/slides/Boisset-Random_Conical_Tilt-1up.pdf



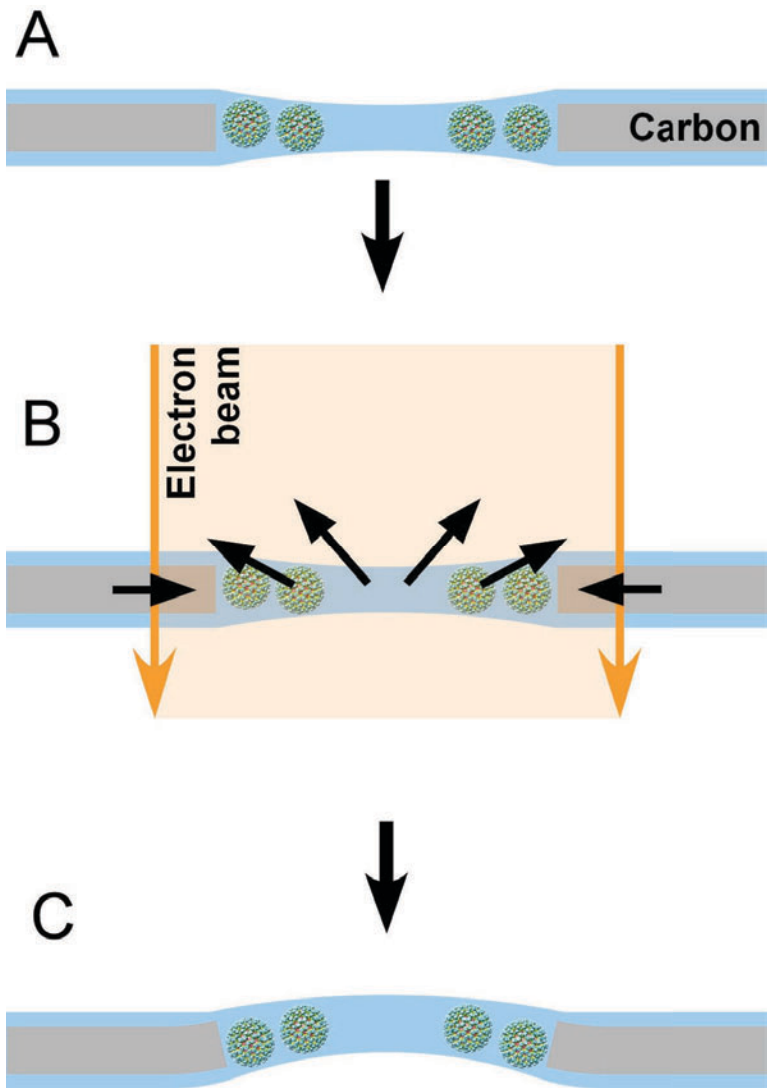
Sometimes tilt pairs are recorded

Chandramouli et al., JSB 2011



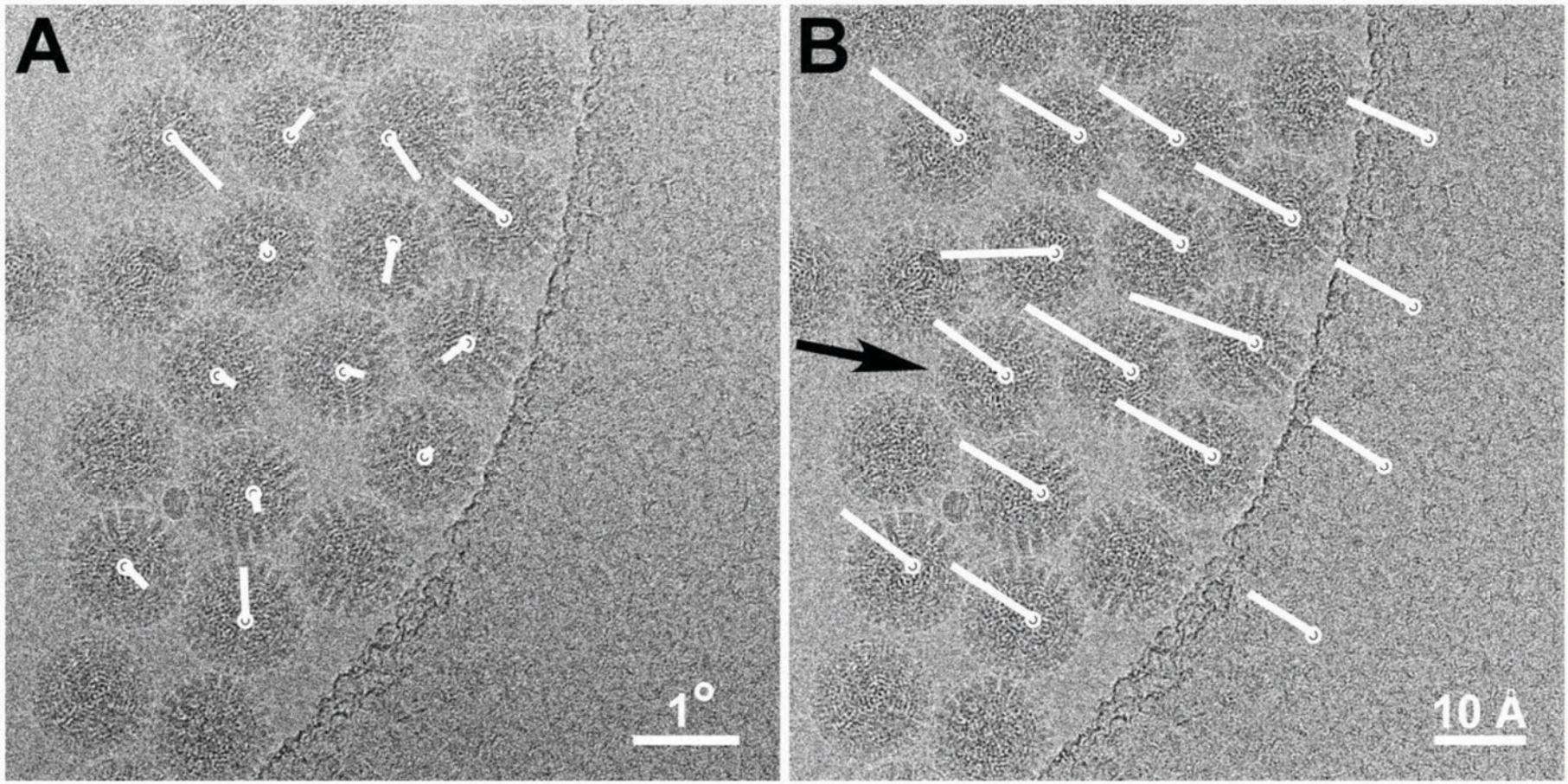
Wright et al., JSB 2006

Vitreous ice within holes moves and flows and bulges with exposure



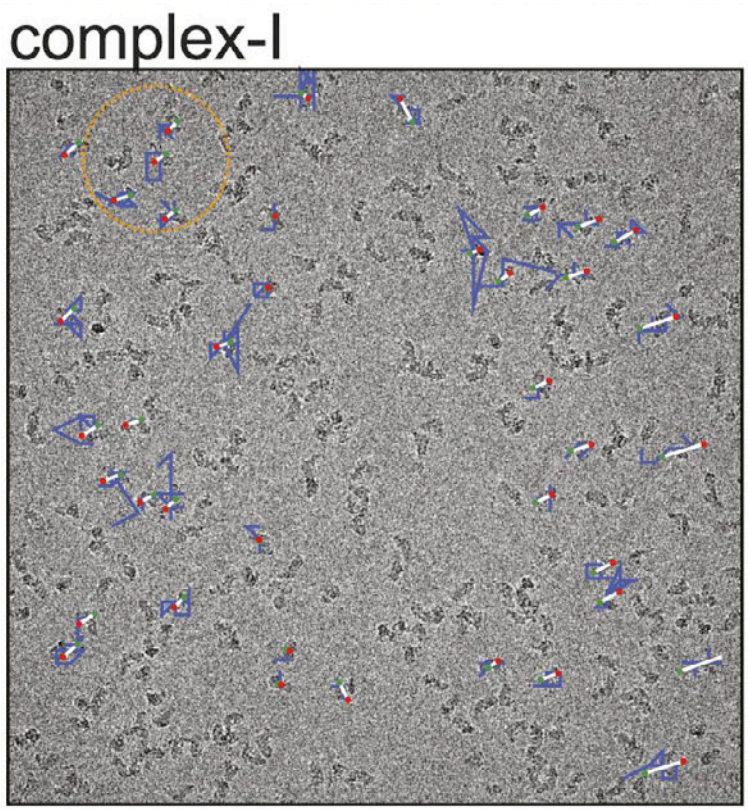
Brilot et al.,
JSB 2012

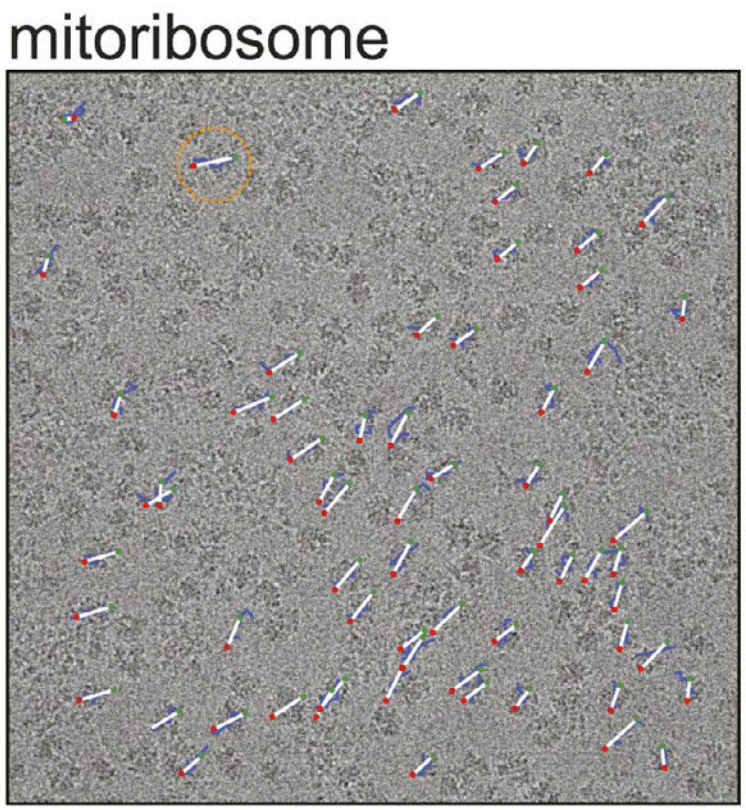
Beam-induced specimen movement



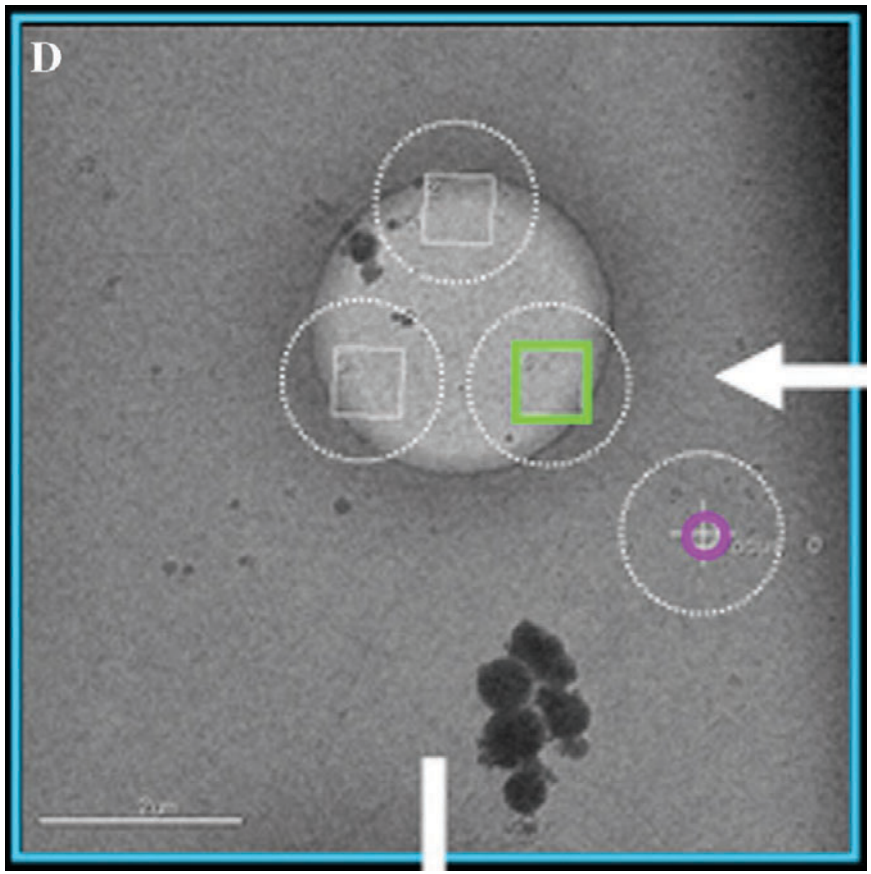
Campbell et al., Structure 2012

Beam-induced motion





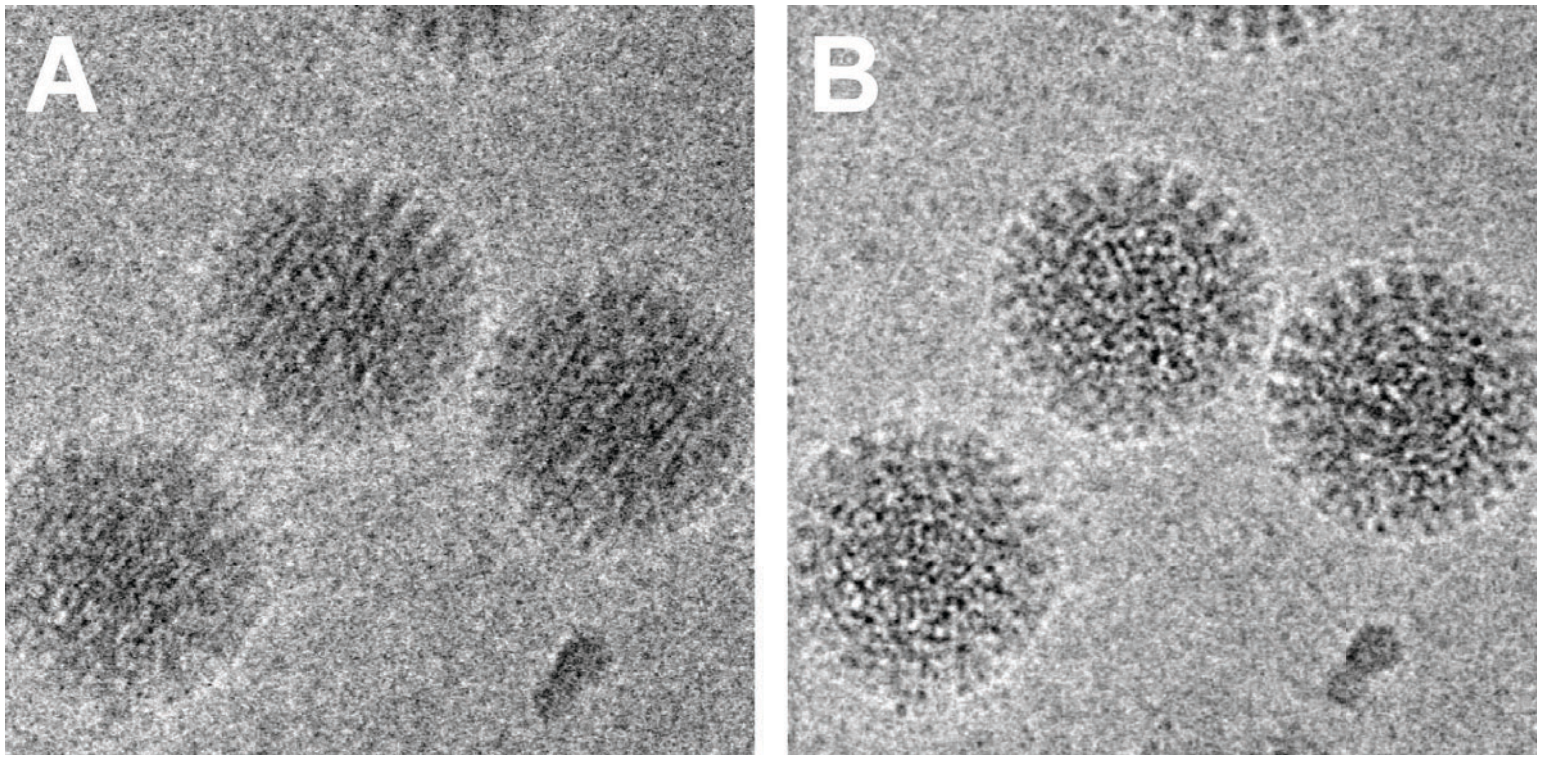
Scheres, eLife 2014



Ideas to reduce beam-induced specimen movement:

* include some carbon in each exposure
* use more rigid grid material

Images can also be motion-corrected



Brilot et al., JSB 2012

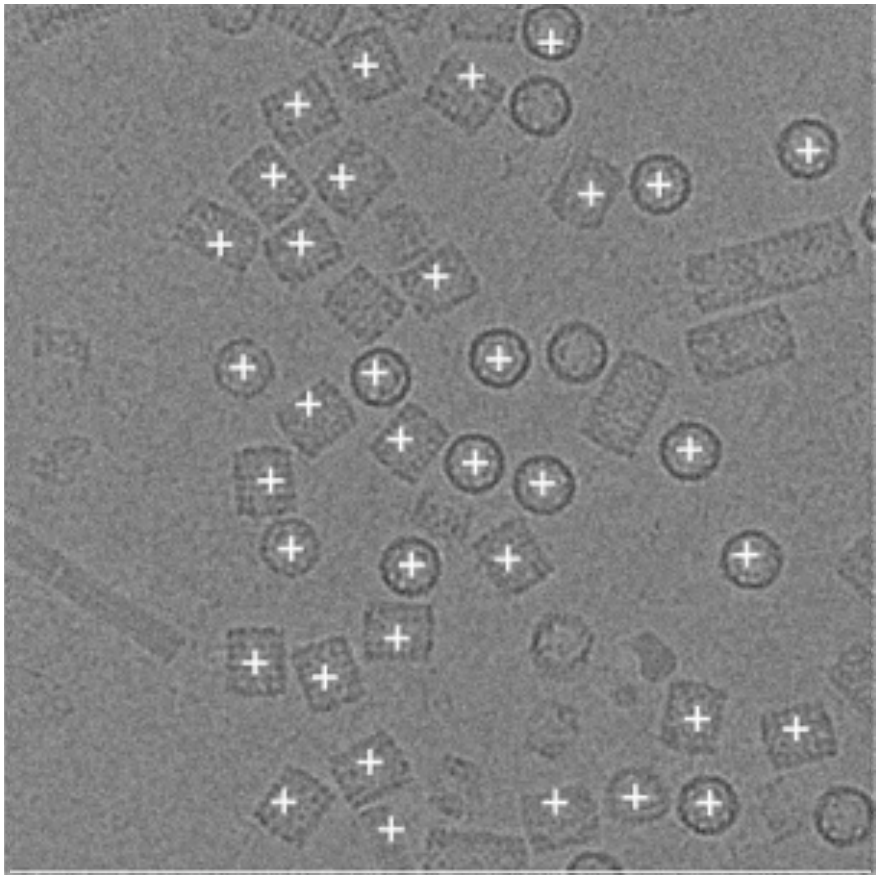
Single particle analysis - Data collection
Concept check questions:

- How do programs such as Leginon record good single particle images automatically (what is the sequence and logic of their operations)?

- In addition to single projection images, sometimes pairs of images are recorded. What kinds of pairs are recorded, and why?

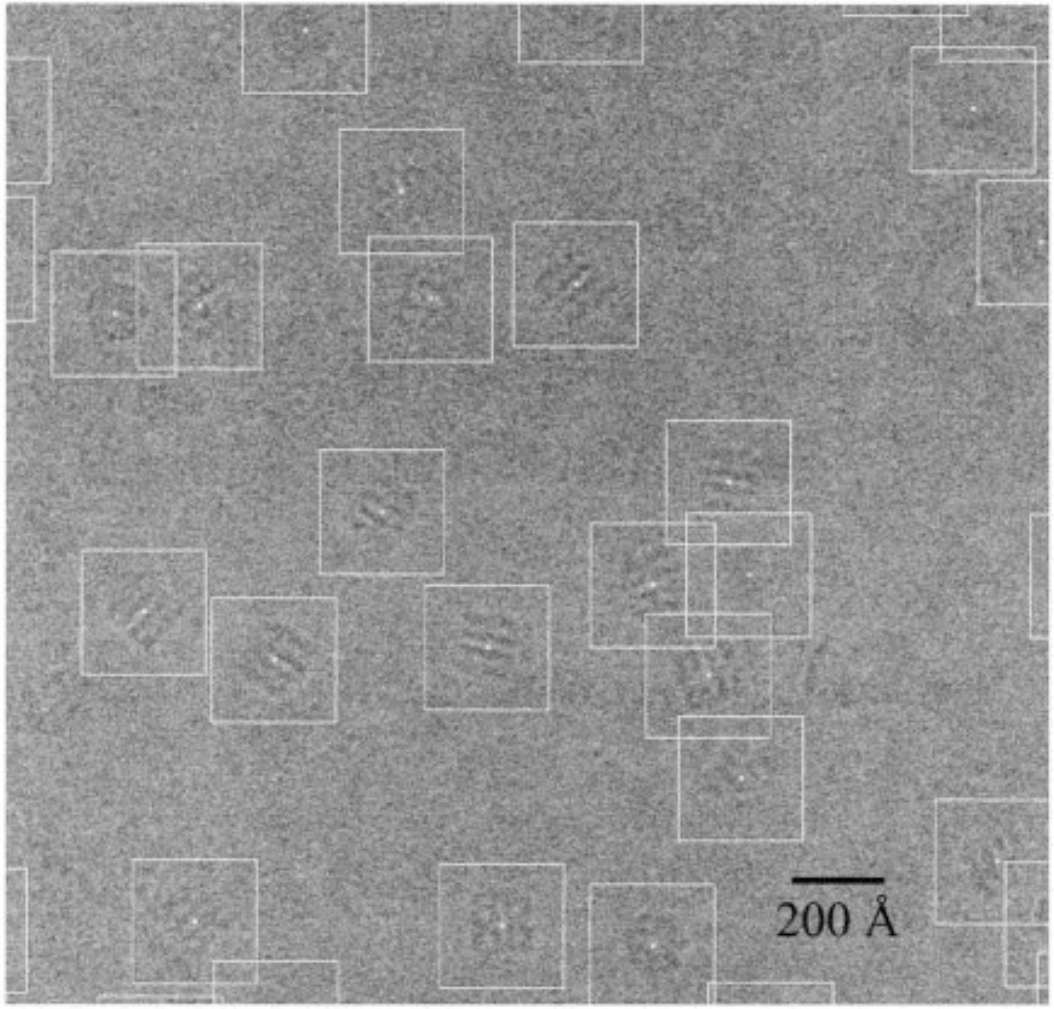
- What kinds of beam-induced specimen movement are common and what can be done to reduce or mitigate the problems this causes?

Step 1: Identify particles



Zhu et al., JSB 2004

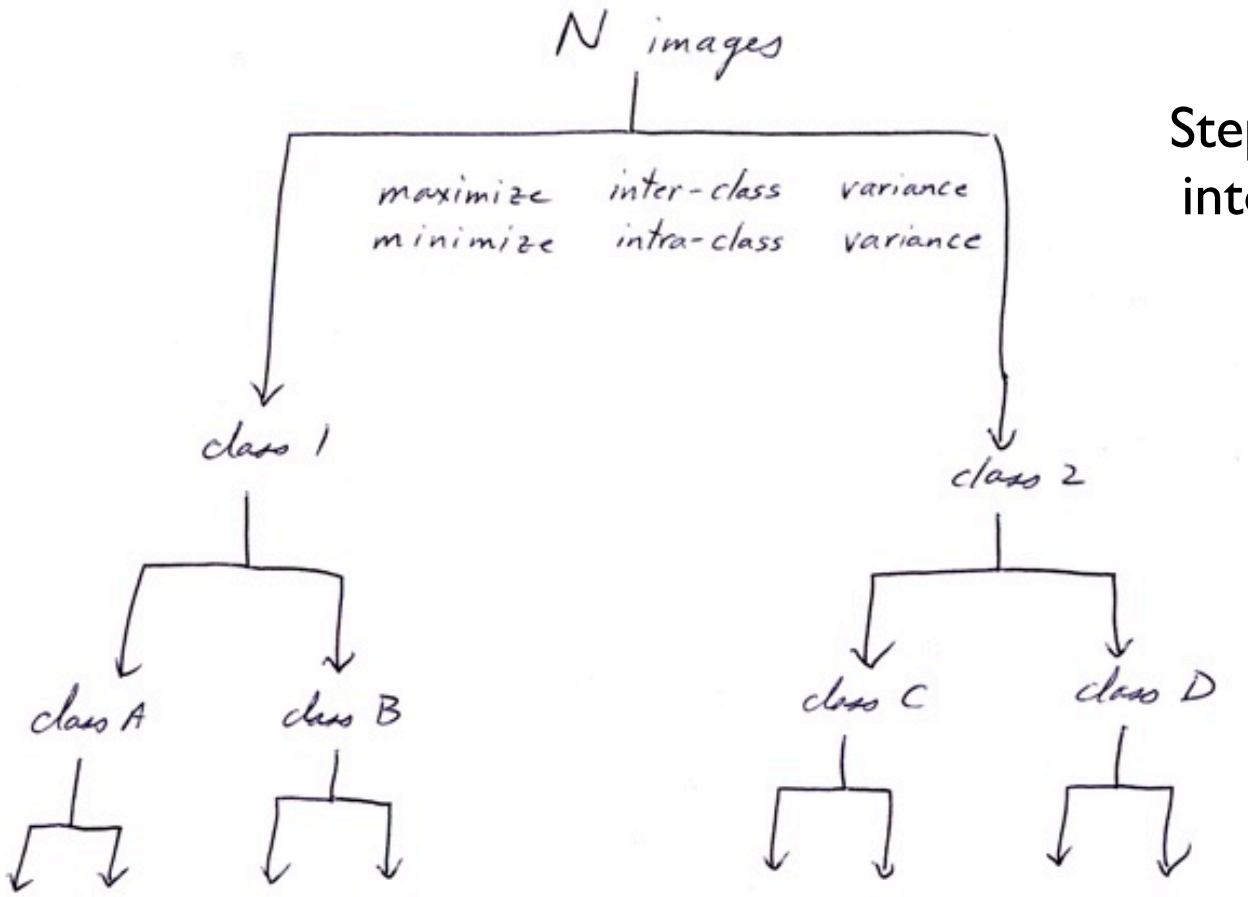
manual detection
cross-correlation
neural nets, edge-detection, ?



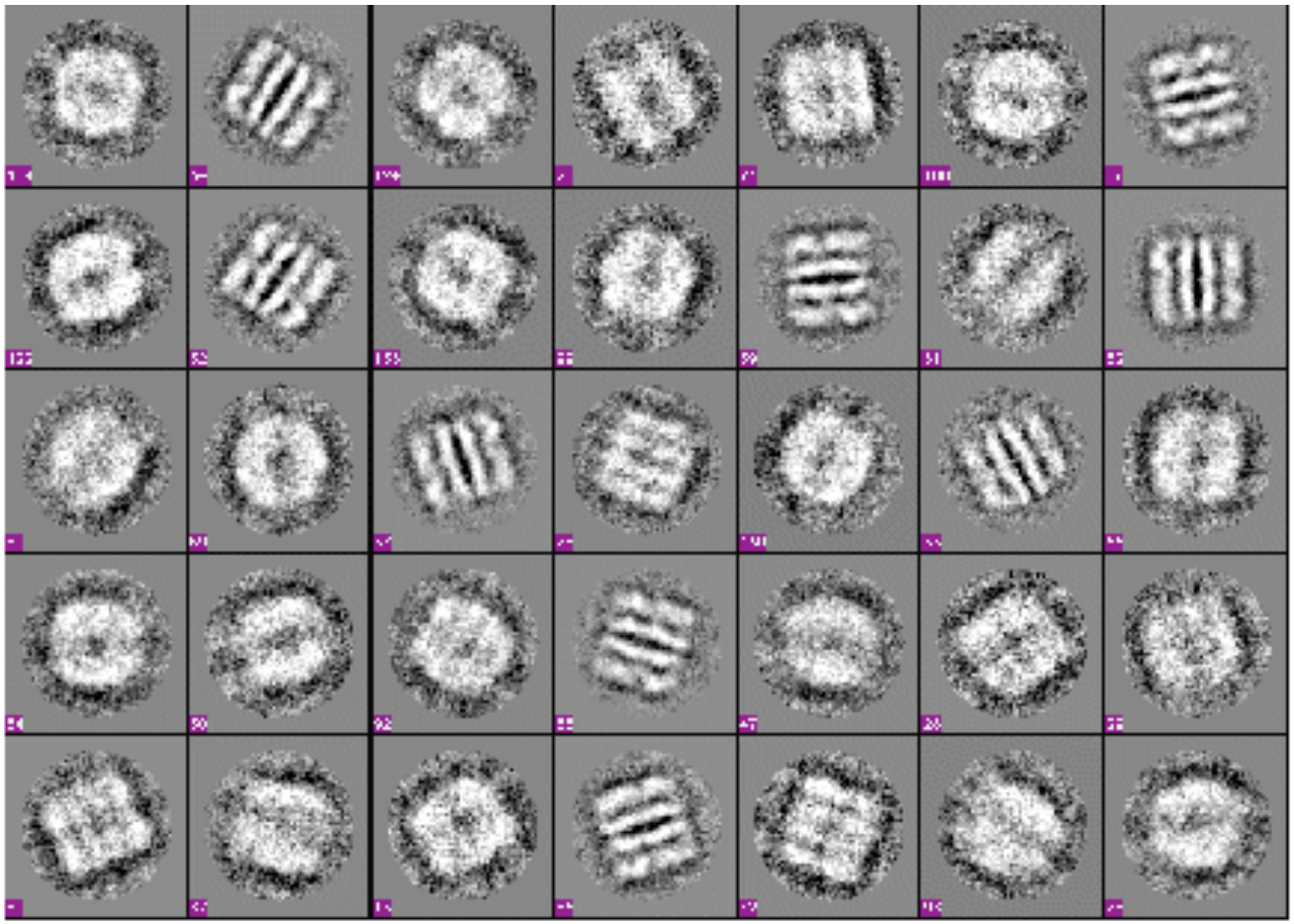
Ludtke et al., JMB 314:253 (2001)

Another example image where the particles are harder to see

Hierarchical Ascendant Classification



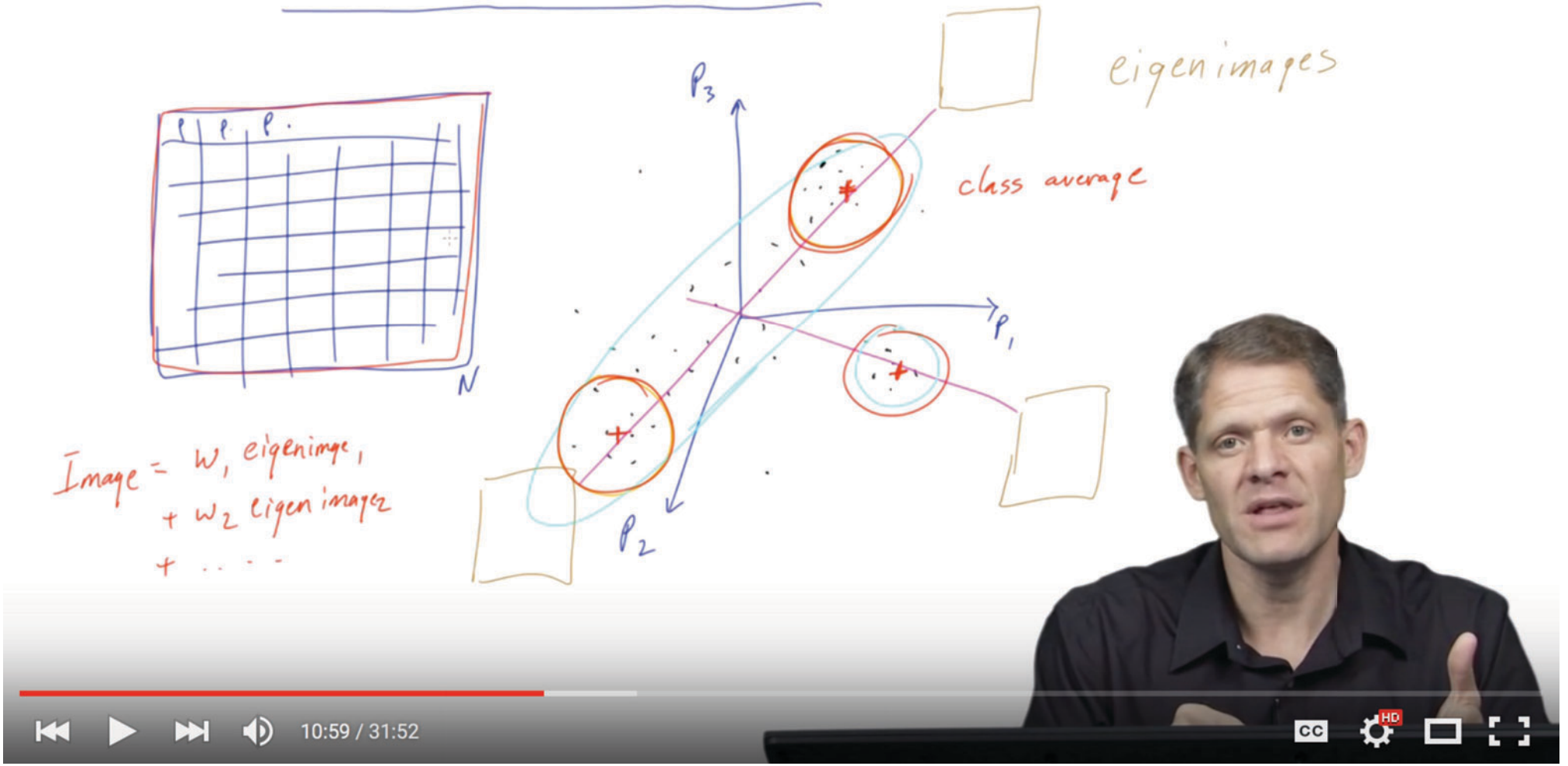
Step 2: classify the particles into homogeneous classes

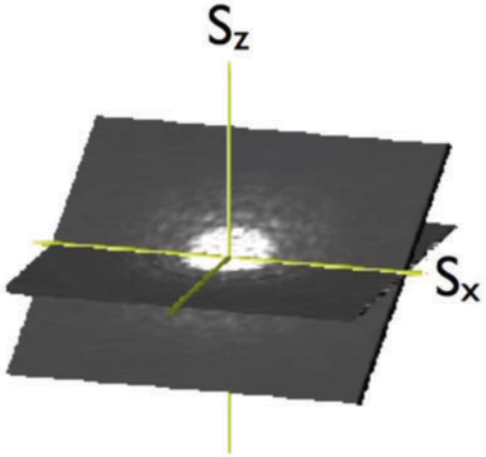


Example class averages

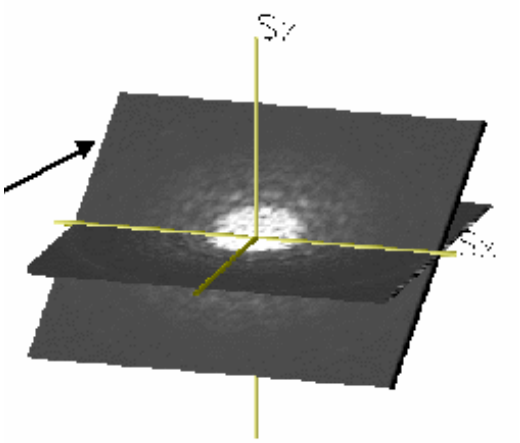
EMAN tutorial at http://ncmi.bcm.tmc.edu/~stevel/EMAN/doc/

Multivariate statistical analysis -another way to classify particles



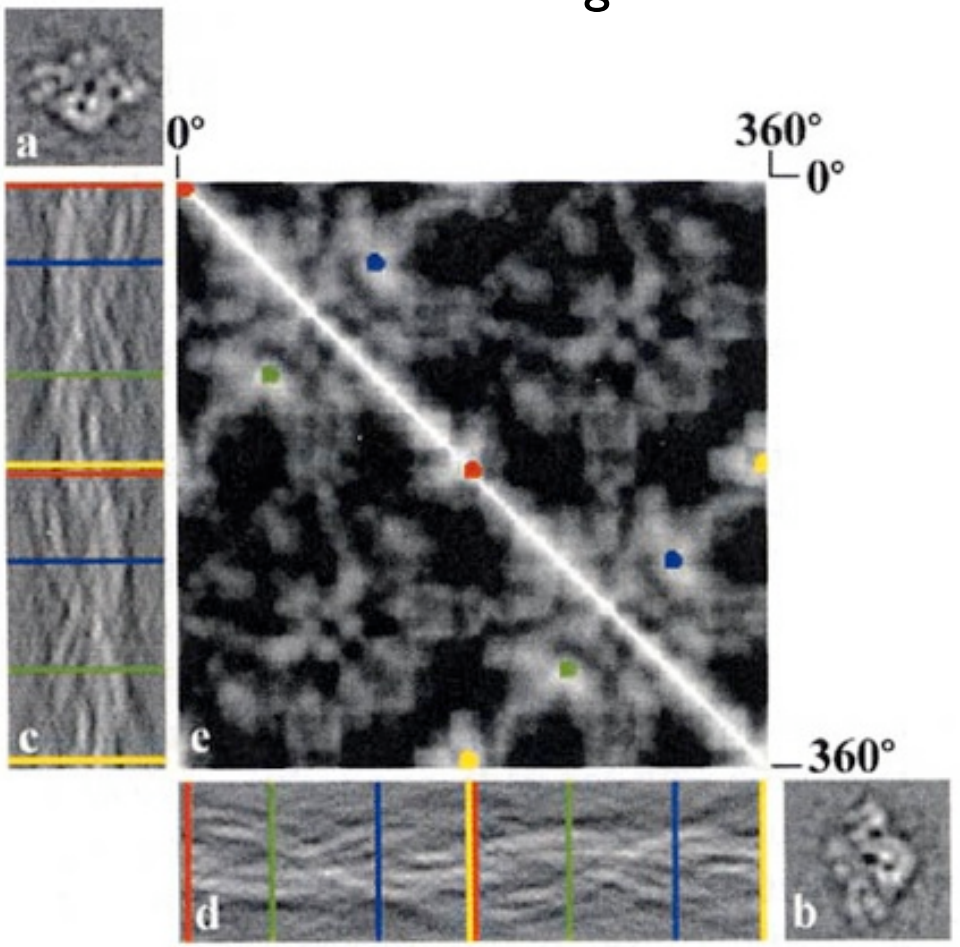


Sinograms can be used to find the relative orientations of class averages

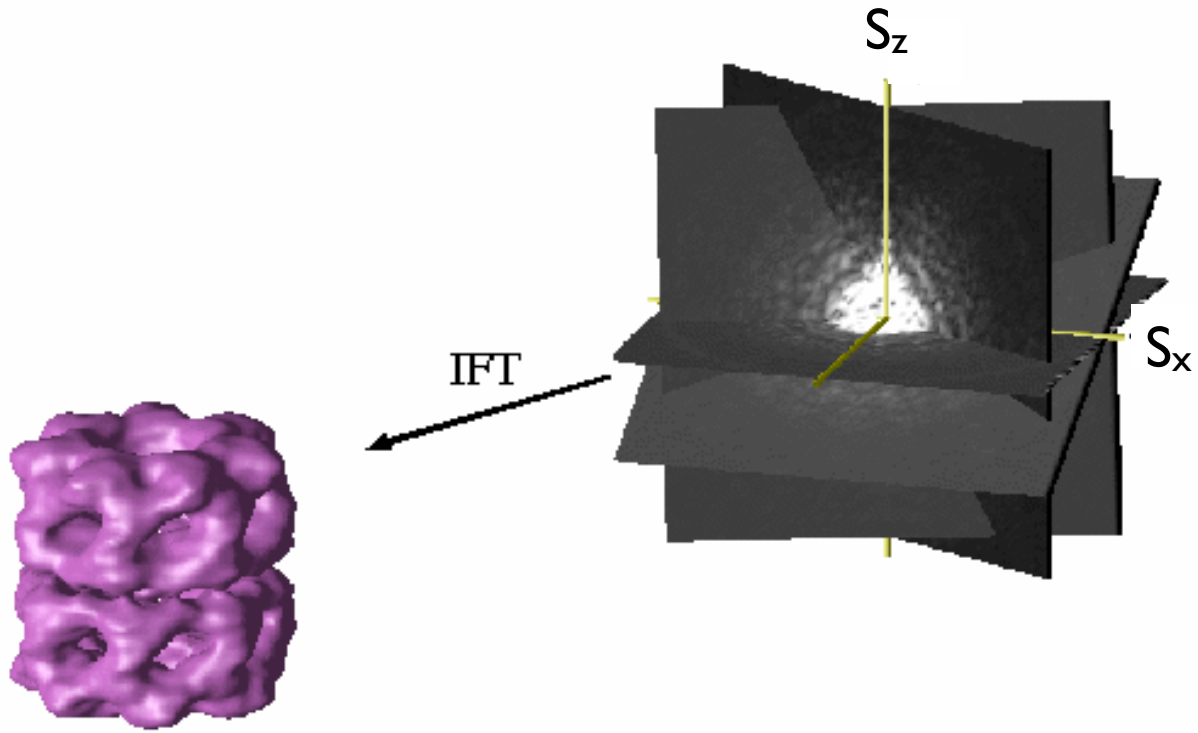


A real example sinogram

A "sinogram"

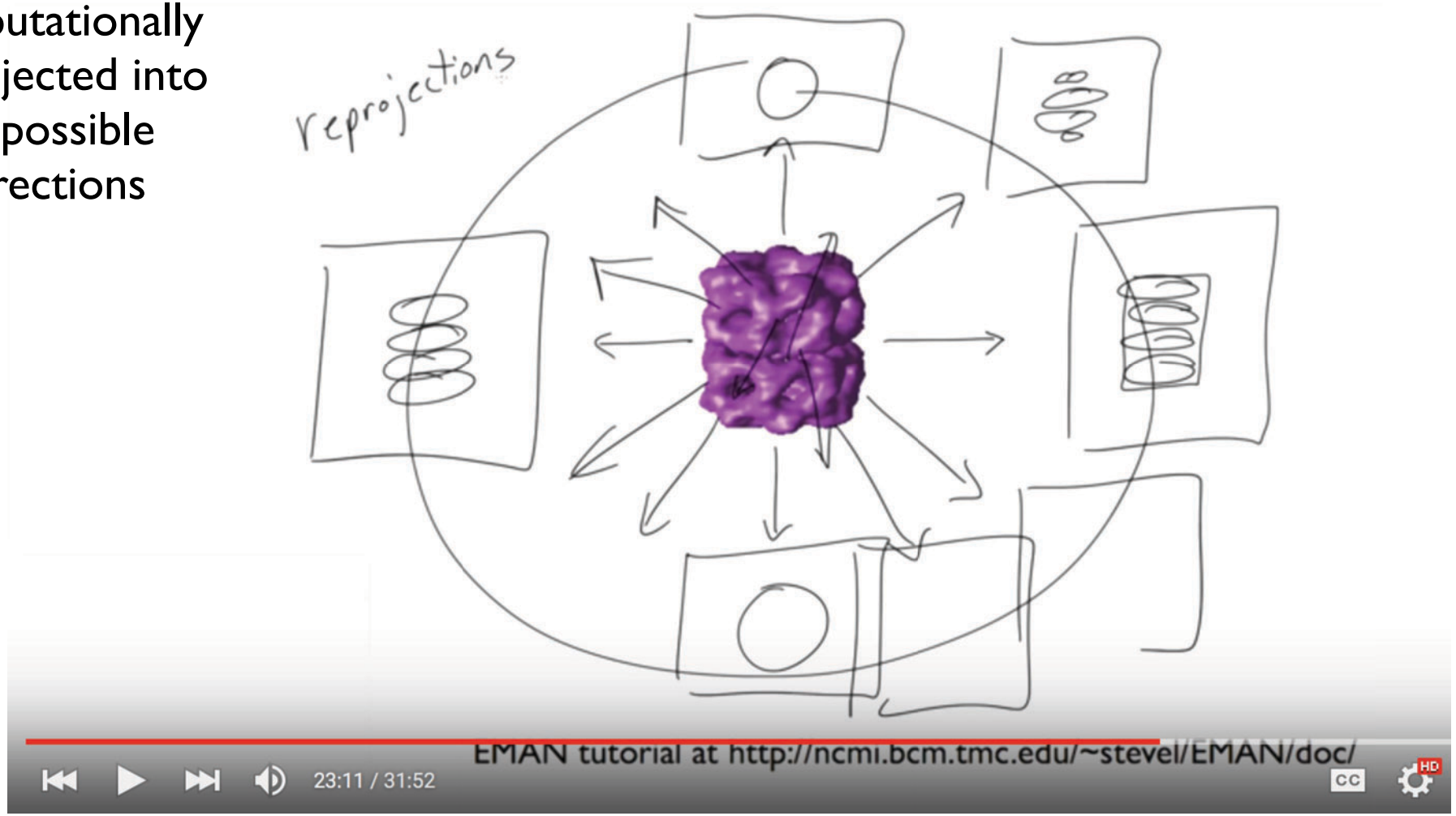


Ruprecht and Nield, Prog. Biophys. Mol. Biol. 75:121 (2001)

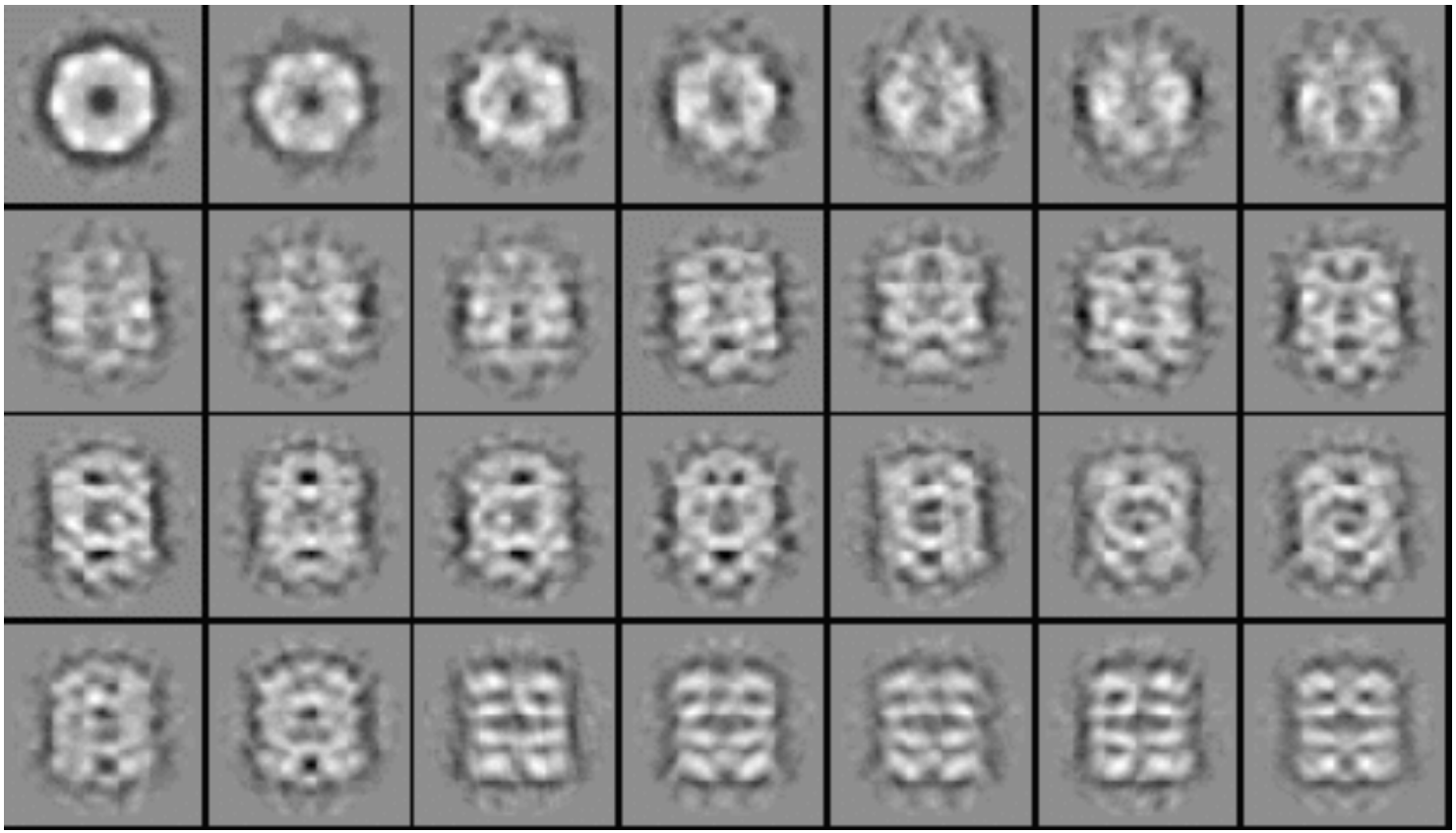


EMAN tutorial at http://ncmi.bcm.tmc.edu/~stevel/EMAN/doc/

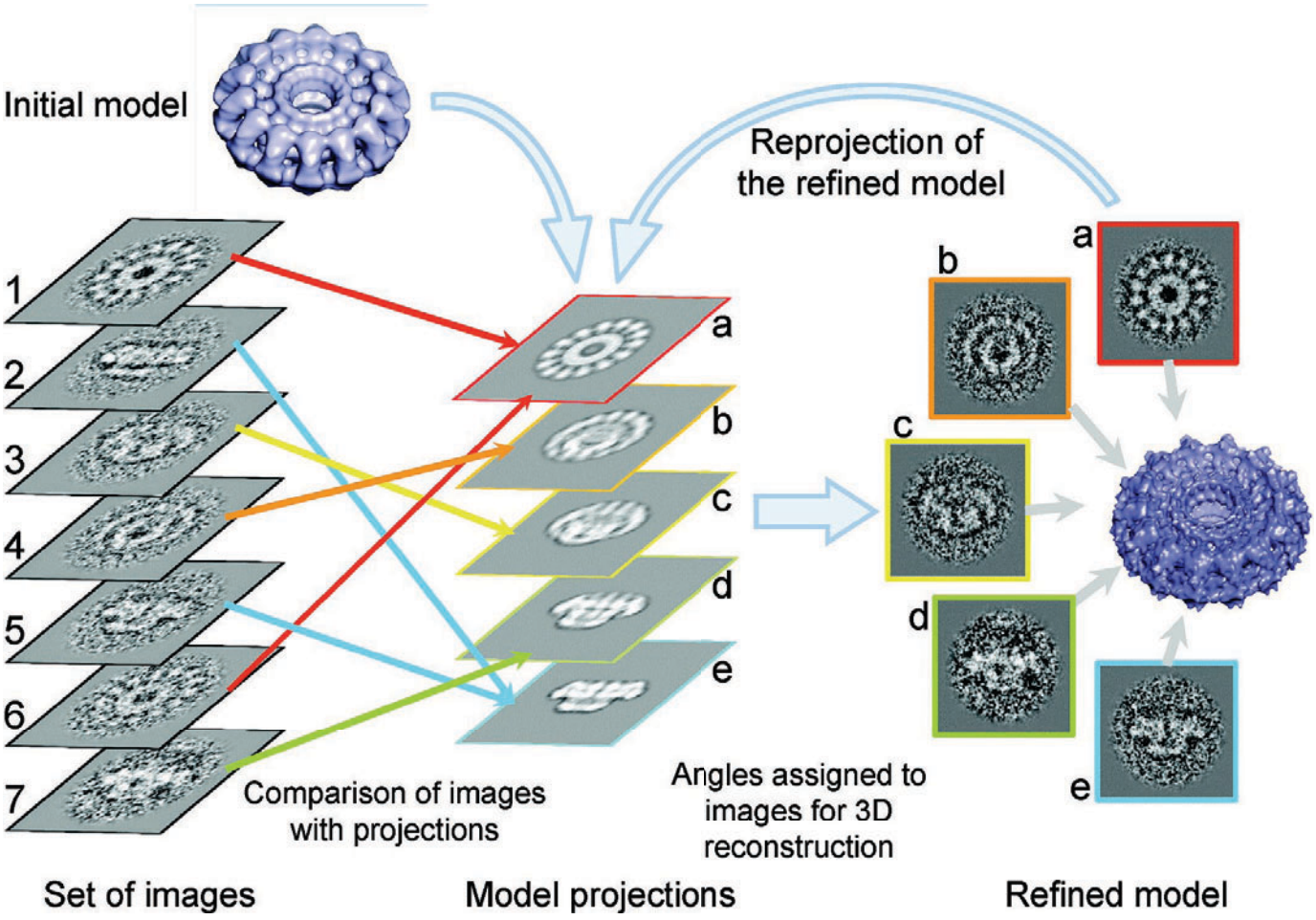
Next, the model is computationally reprojected into all possible directions



Reproject the model in all possible directions

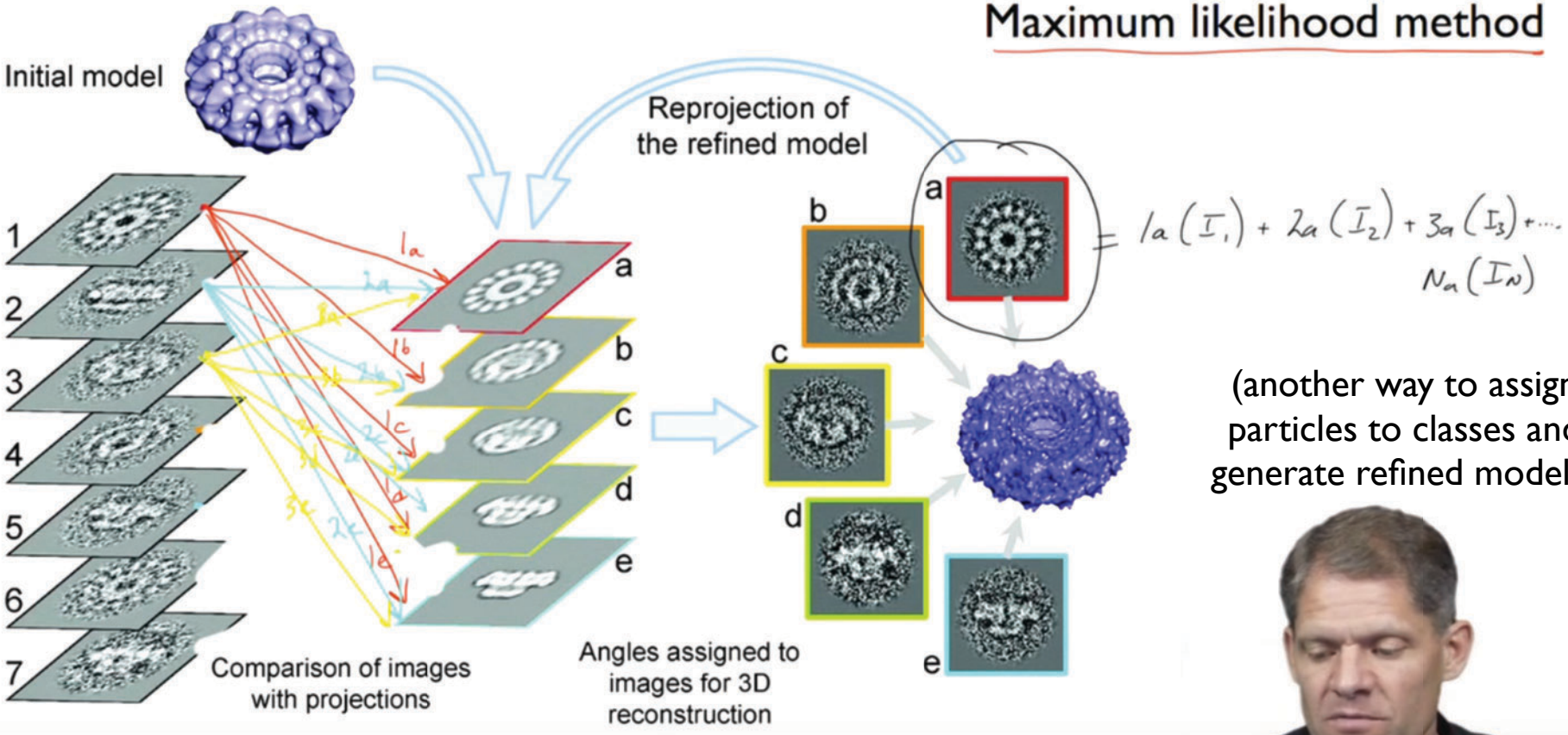


EMAN tutorial at http://ncmi.bcm.tmc.edu/~stevel/EMAN/doc/



Orlova and Saibil, Chemical Reviews 2011

Maximum likelihood method



(another way to assign particles to classes and generate refined models)

Orlova and Saibil, Chemical Reviews 2011

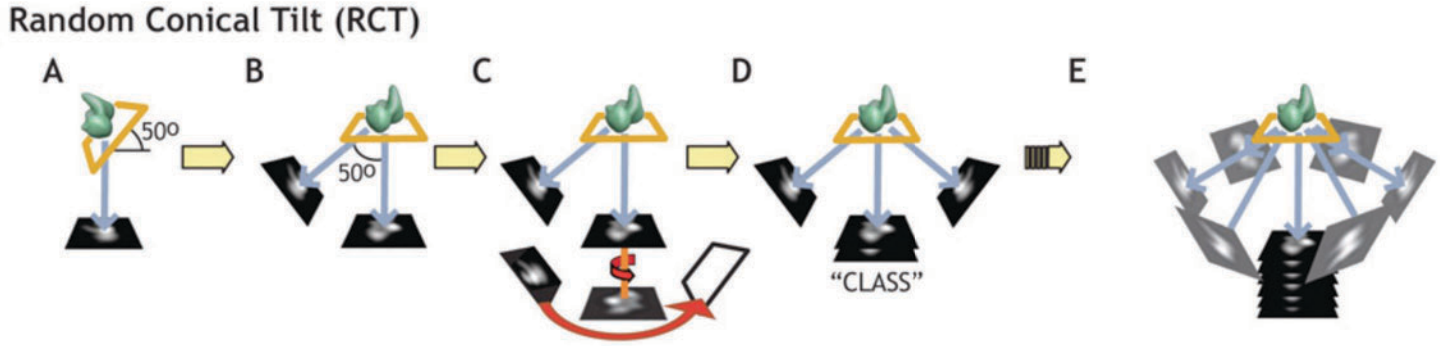
30:41 / 31:52

Single particle analysis - Reconstruction basic workflow Concept check questions:

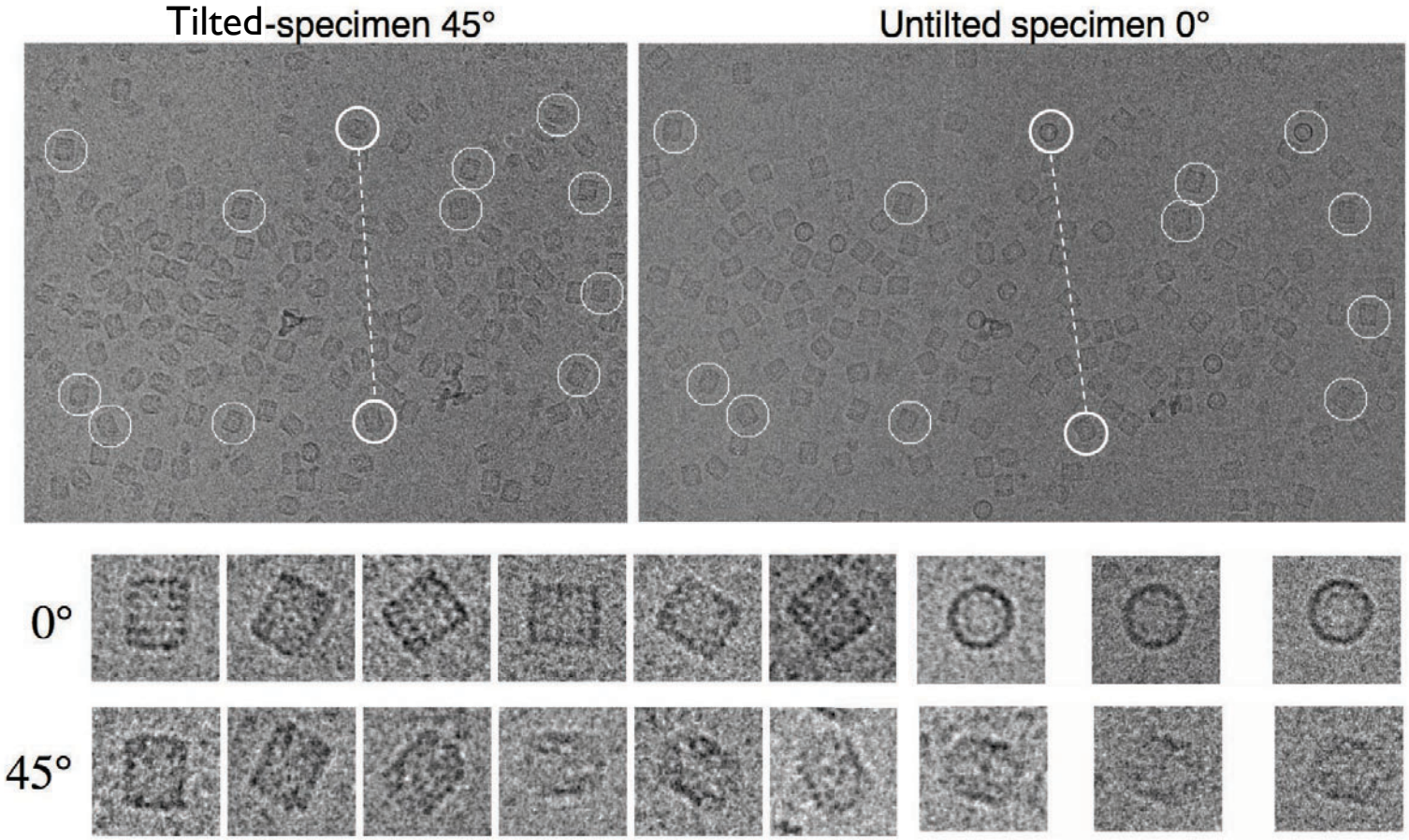
- What methods are used to identify individual particles in cryo-EM images?
- After particles are picked, the next step in single particle image processing is typically to classify the images. What factors make different particle images look different?
- What characteristics are the same and which are different about images in the same "class"?
- Describe two different methods to classify single particle images.
- What is an "eigenimage"?
- How can the relative orientations of different class averages be found?
- Single particle reconstruction is an iterative process. What are the basic steps being iterated? How does one know when to stop iterating?
- Describe one way "maximum likelihood" methods can be used in single particle reconstruction.

Methods to generate an initial model:

- de novo using common lines
- known partial structure
- random conical tilt/orthogonal tilt
- tomography/sub-tomogram average
- random balls and sticks



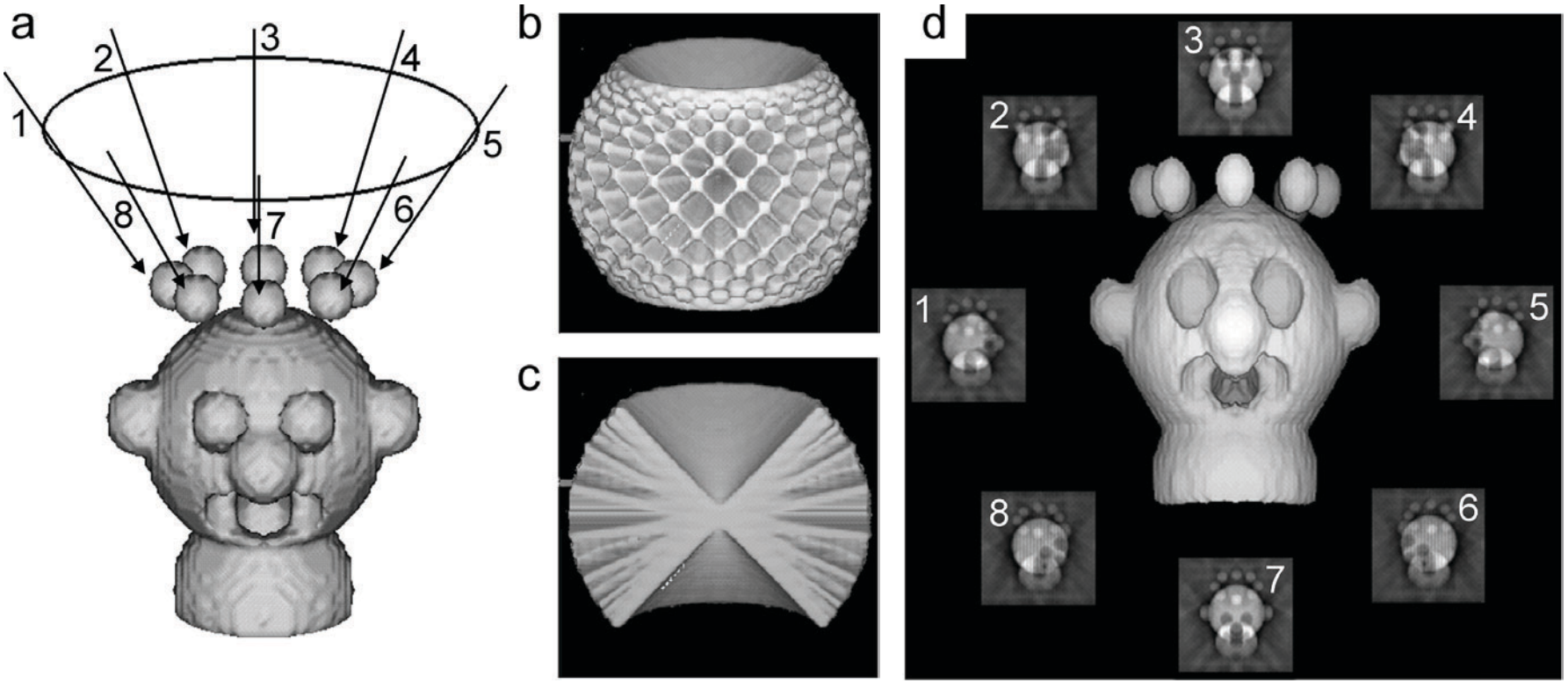
http://labs.mcb.harvard.edu/leschziner/research_methods.html



Example random conical tilt data

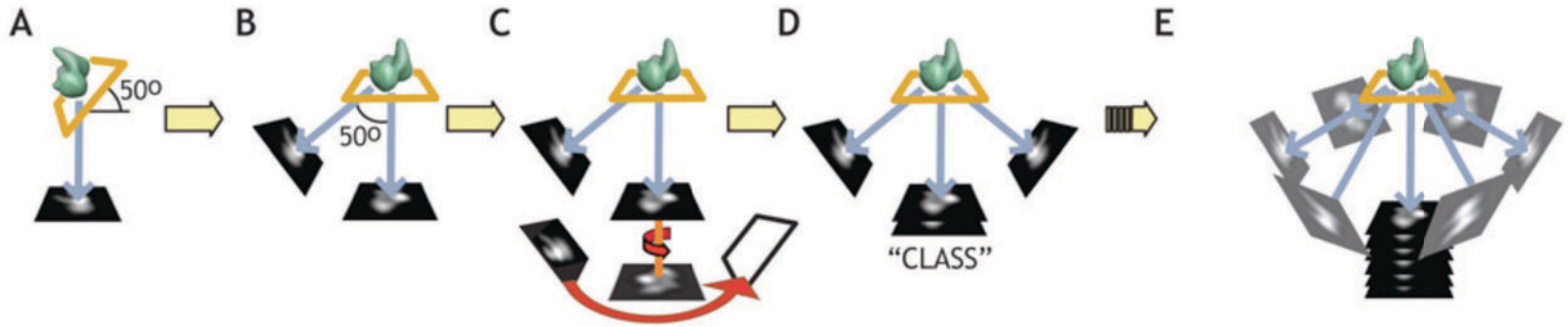
http://nramm.scripps.edu/wp-content/seminars/2005/cryoem/lectures/slides/Boisset-Random_Conical_Tilt-1up.pdf

The RCT method results in a missing wedge

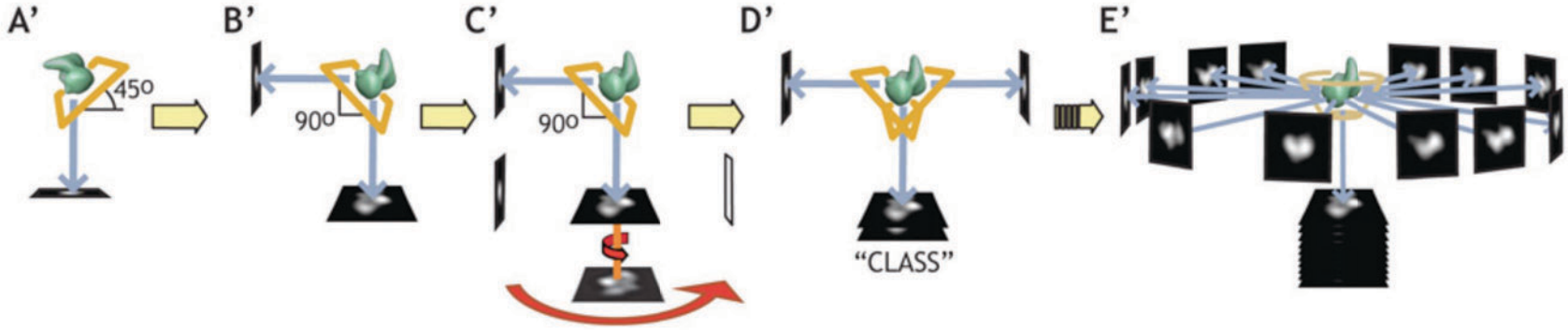


Boisset et al., Ultramicroscopy 1998

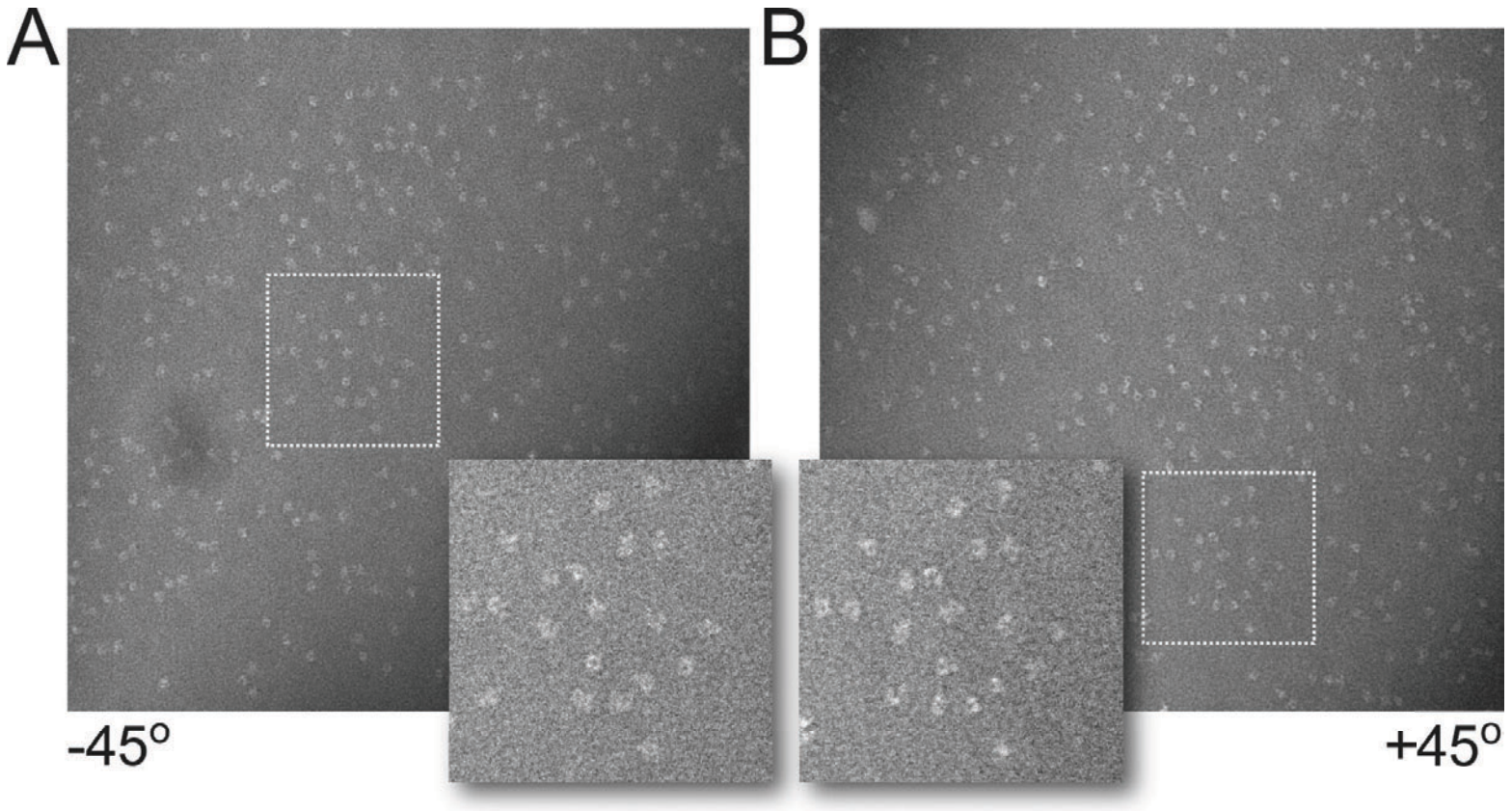
Random Conical Tilt (RCT)



Orthogonal Tilt Reconstruction (OTR)

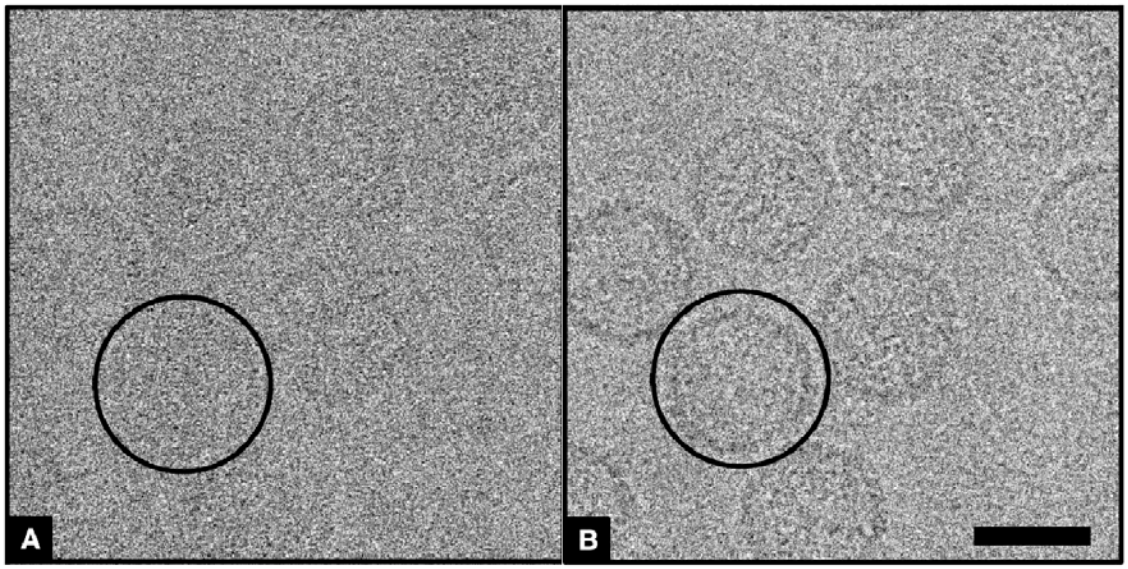


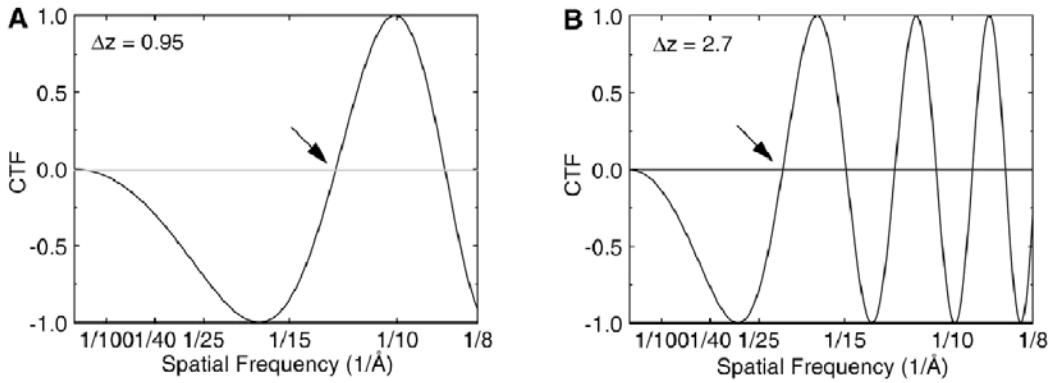
http://labs.mcb.harvard.edu/leschziner/research_methods.html



Example tilt-pairs for an orthogonal tilt reconstruction

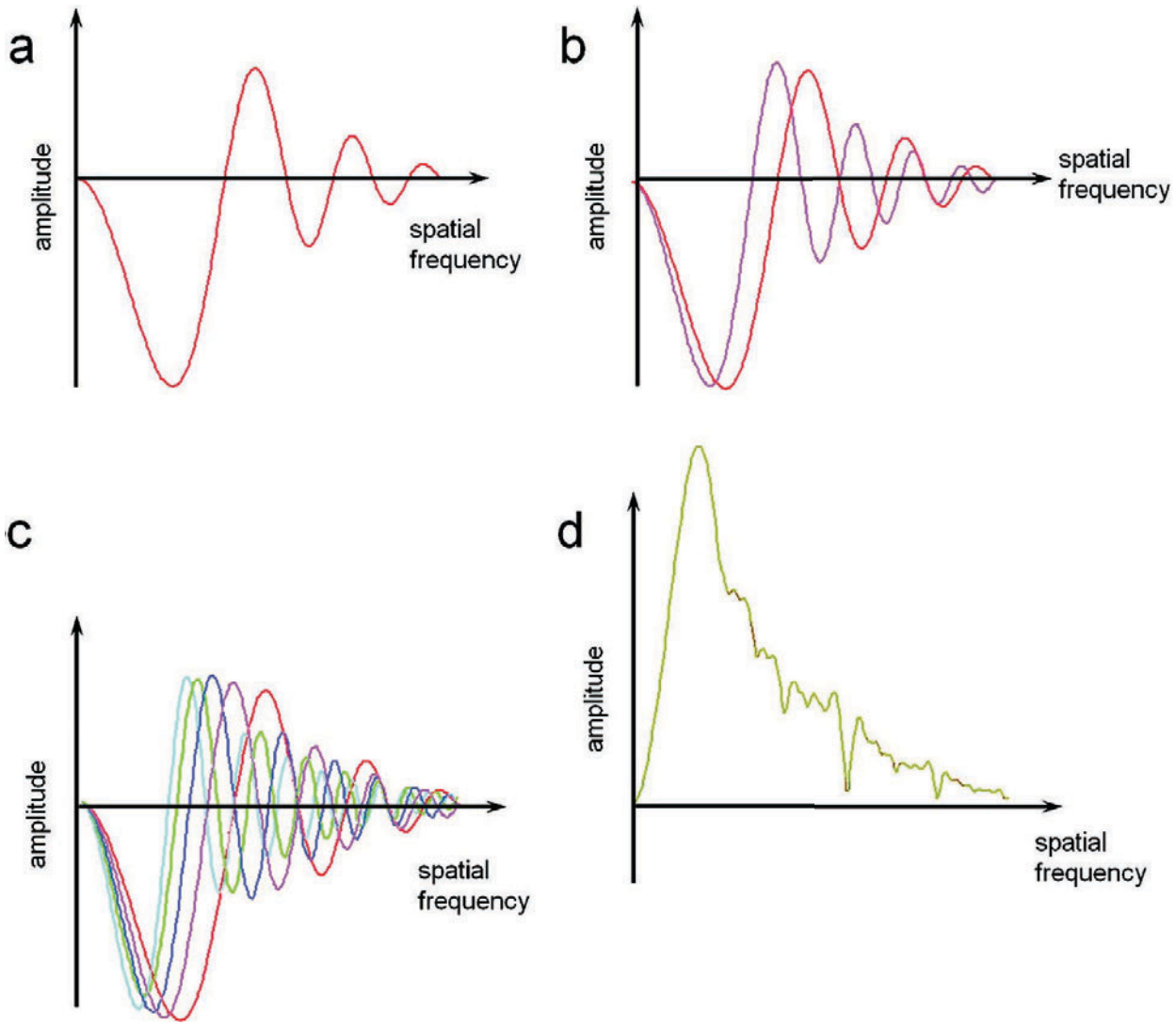
Chandramouli et al., JSB 2011





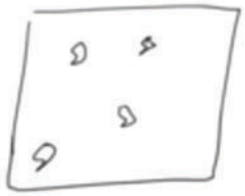
Thuman-Commike and Chiu, Micron 31:687

Including particles recorded with different defoci can fill in CTF gaps

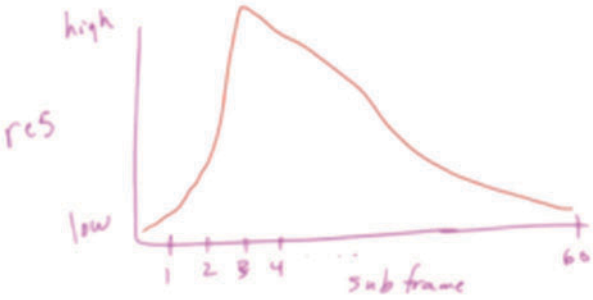


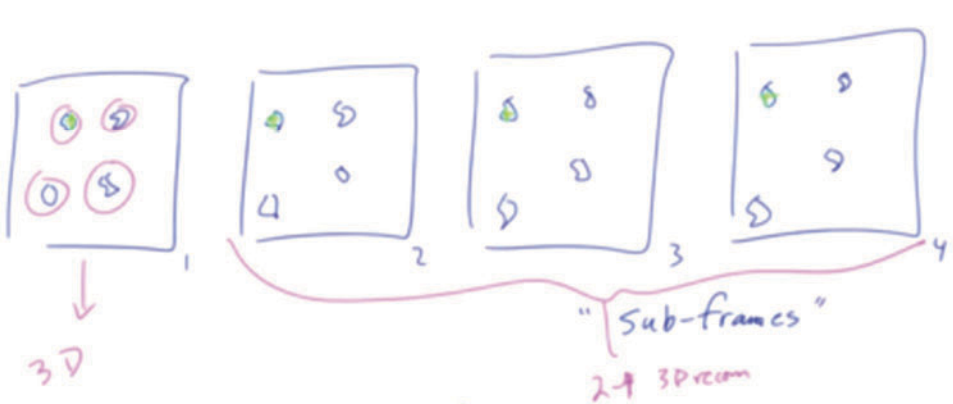
Orlova and Saibil,
Chemical Reviews 2011

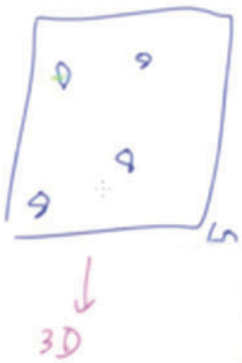
Taking advantage of sub-frames



20 e⁻/Å²
1 s
CCD

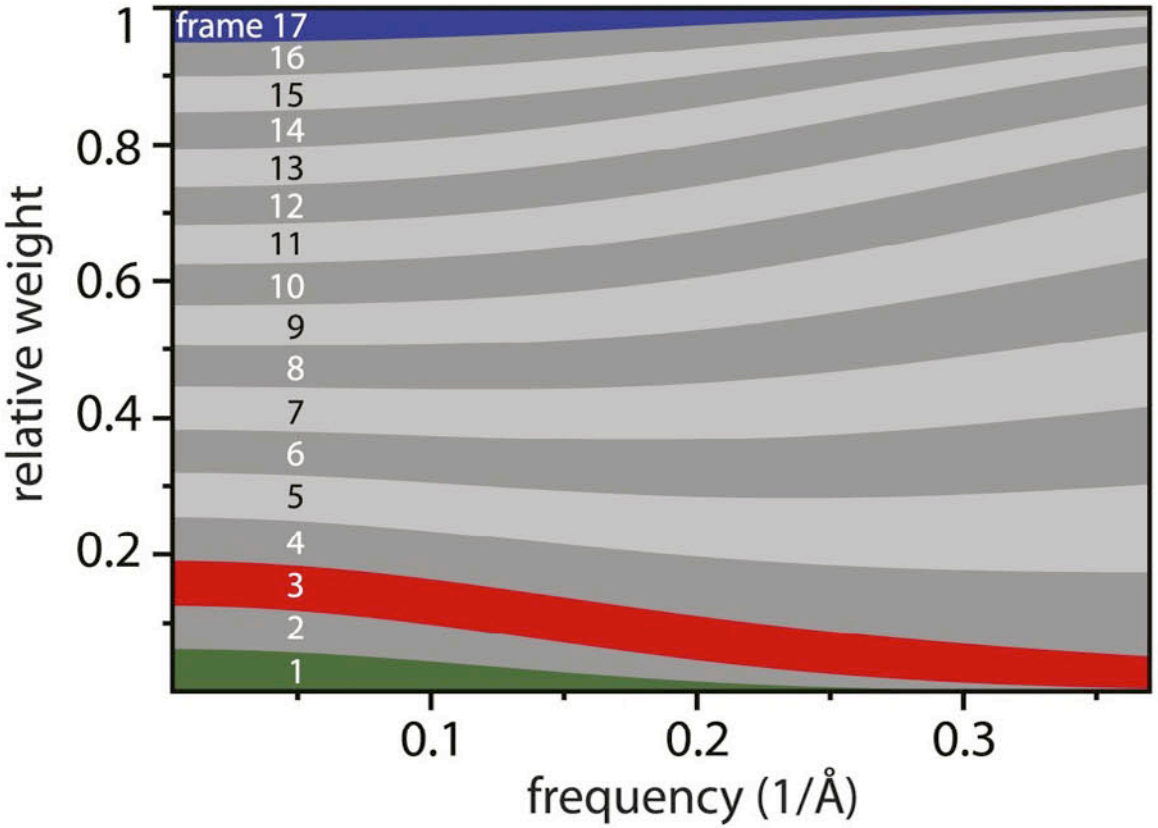






1/20 s
1 e⁻/Å²
direct detector
3 s → 60 e⁻/Å²

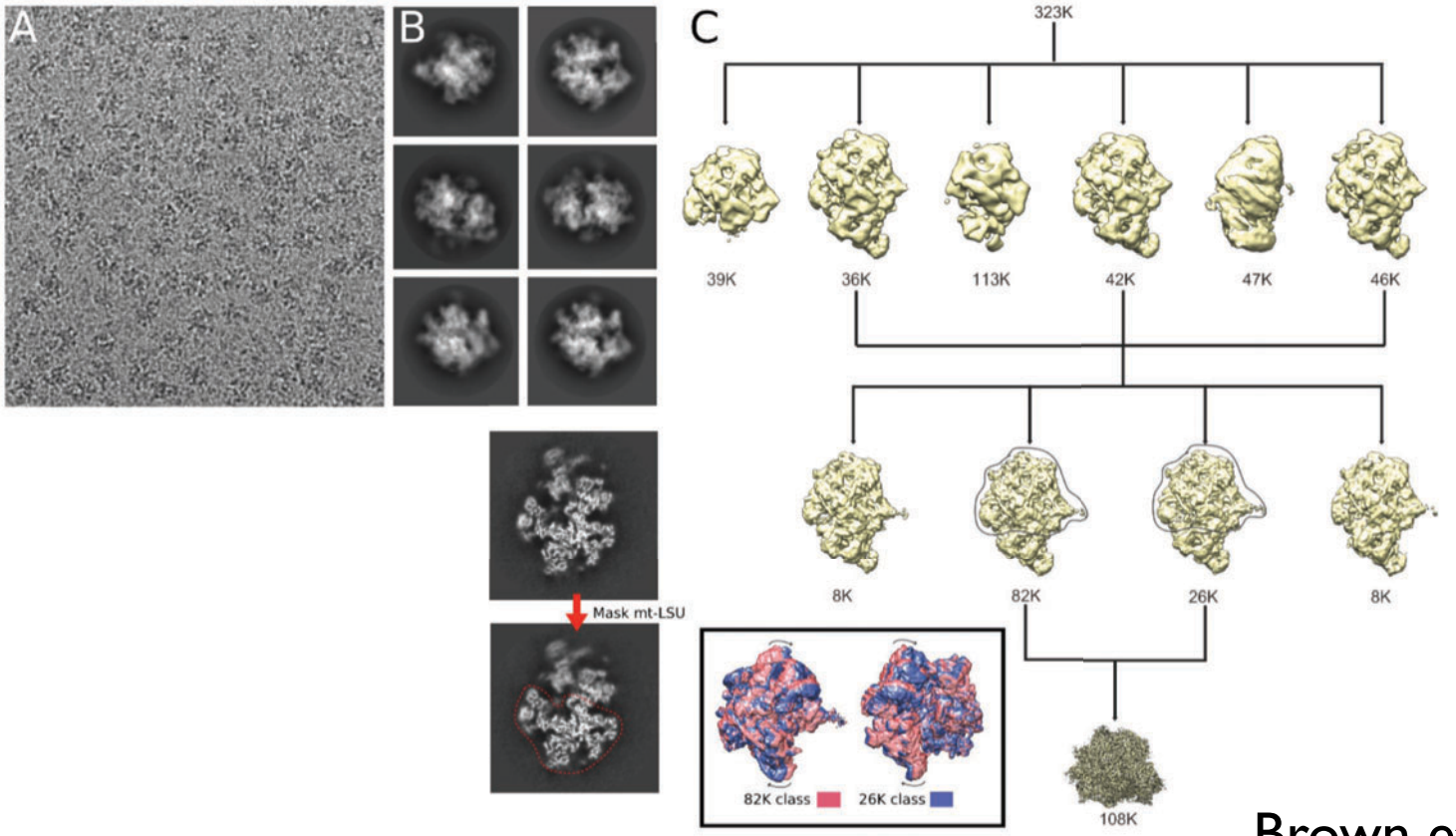
motion-correction
sub-frame-selected recons
band pass filters



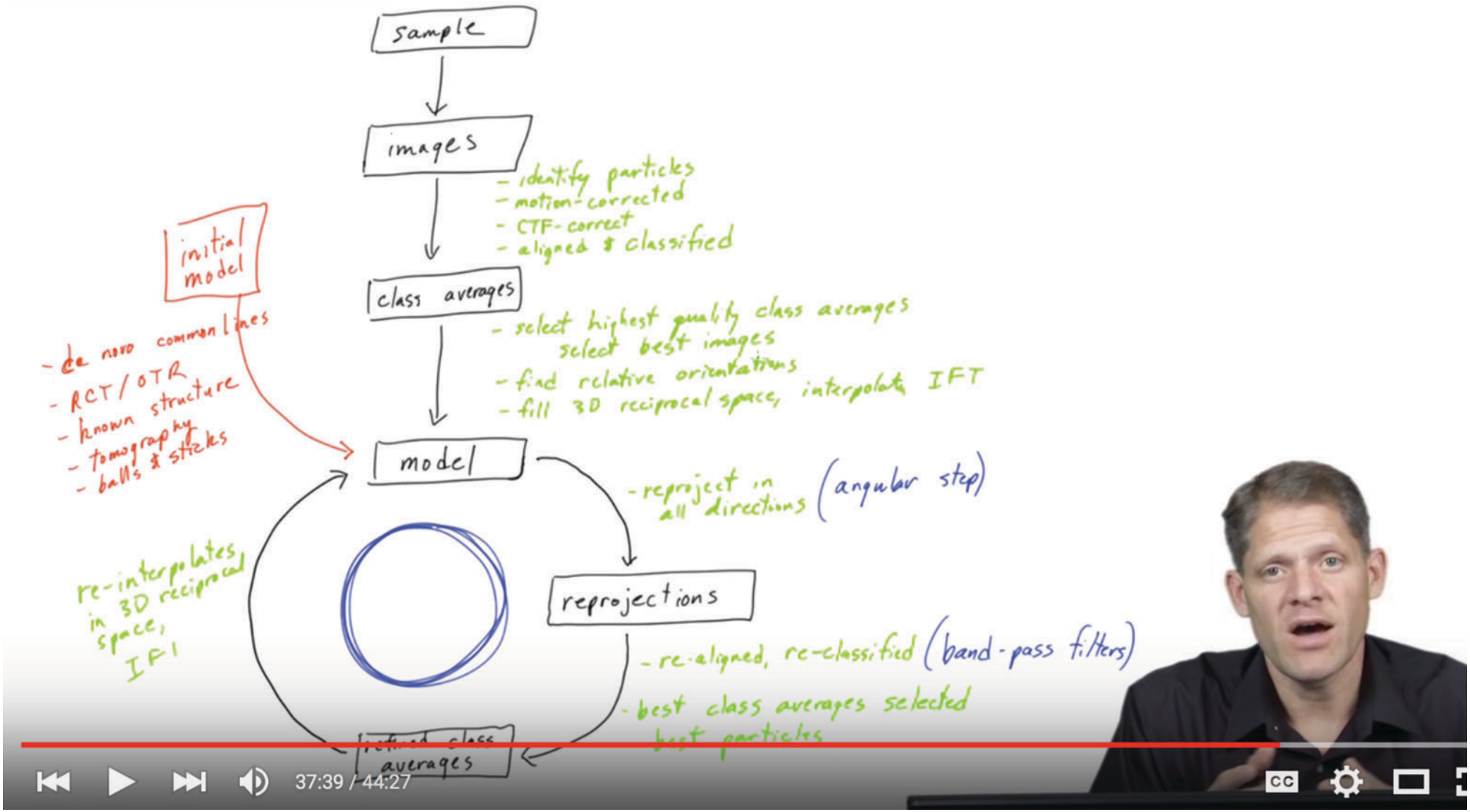
Scheres, eLife 2014

Each subframes can be band-pass-filtered to optimize its contribution to the final reconstruction

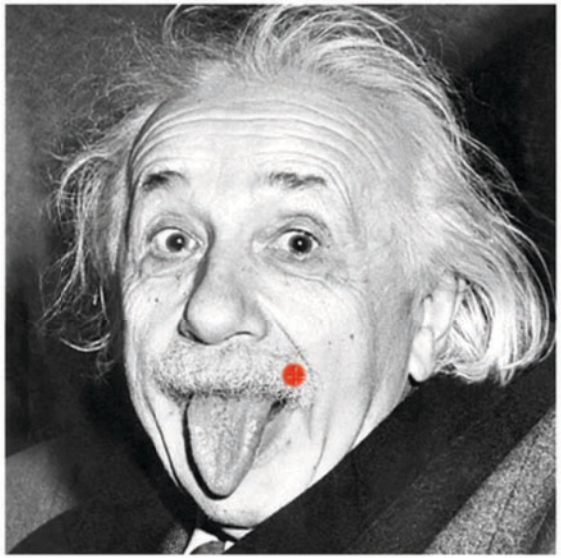
Example illustrating how single particle analysis can be used to sort out compositional and conformational heterogeneity

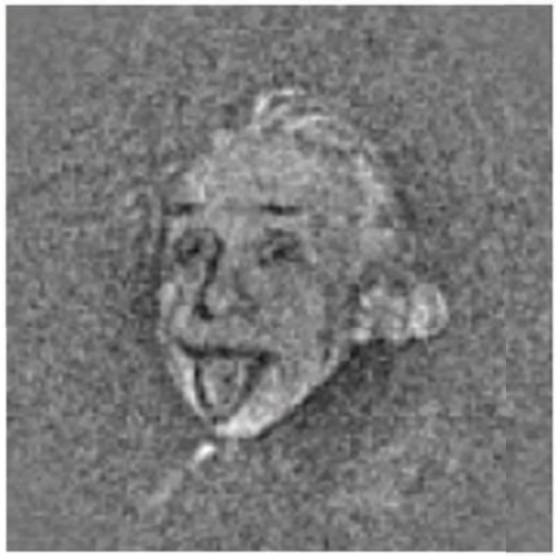


Brown et al., Science 2014

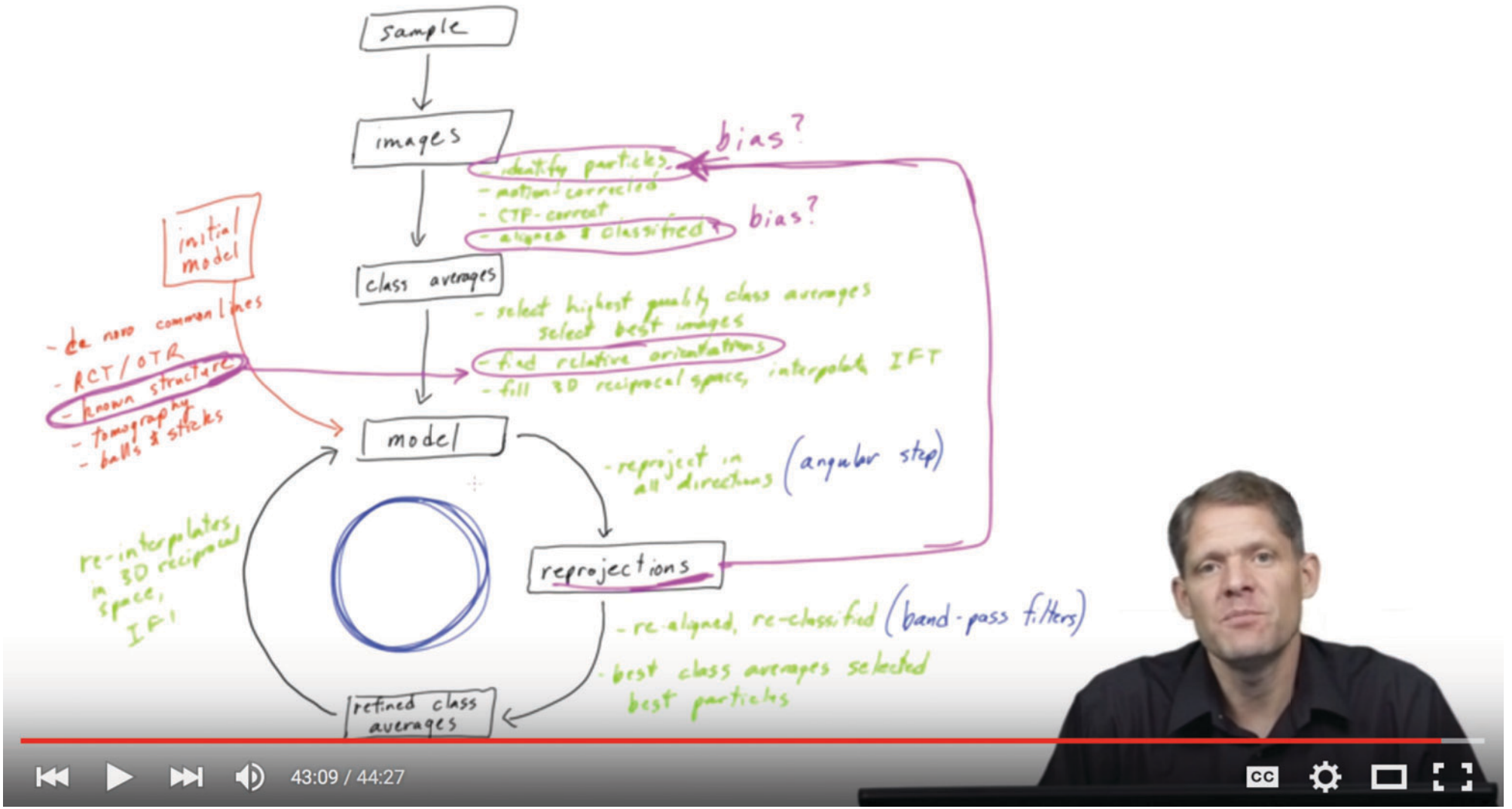

sample
images
- identify particles
- motion-corrected
- CTF-correct
- aligned & classified
initial model
- de novo common lines
- RCT / OTR
- known structure
- tomography
- balls & sticks
class averages
- select highest quality class averages
select best images
- find relative orientations
- fill 3D reciprocal space, interpolate IFT
model
- reproject in all directions (angular step)
re-interpolates in 3D reciprocal space, IFT
reprojections
- re-aligned, re-classified (band-pass filters)
- best class averages selected
best particles
refined class averages
37:39 / 44:27
CC

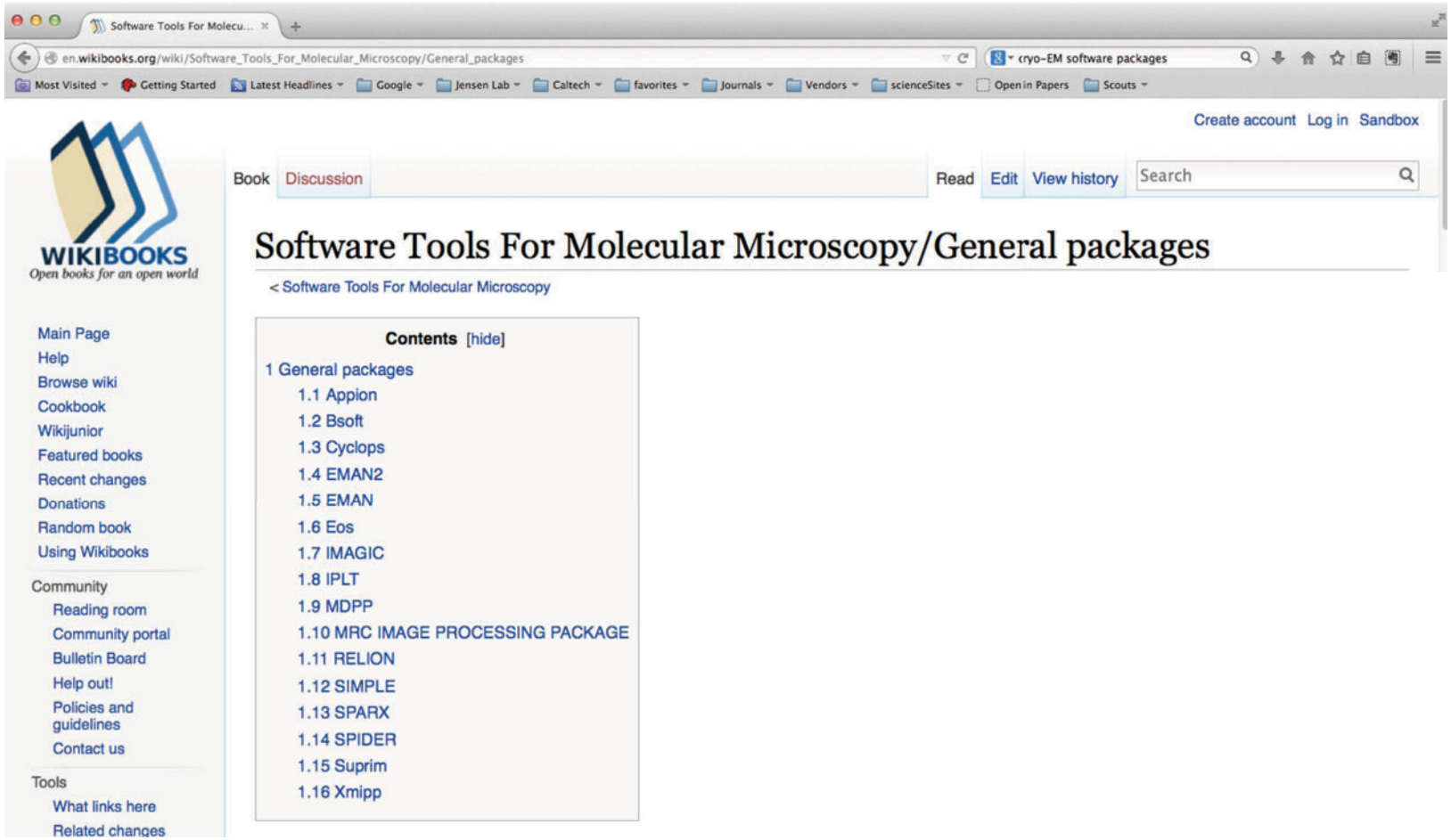
Reference bias





Shatsky et al., JSB 2009

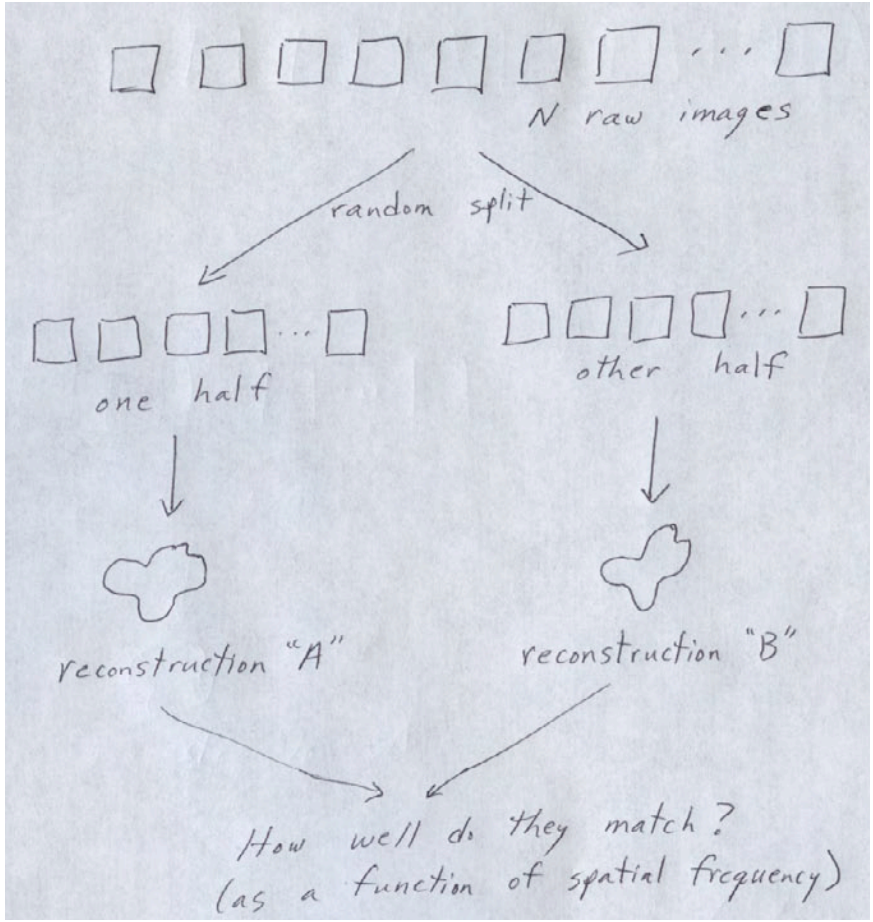

sample
images
- identify particles
- motion corrected
- CTF-corrected
- aligned & classified
bias?
bias?
class averages
- select highest quality class averages
select best images
- find relative orientations
- fill 3D reciprocal space, interpolate, IFT
initial model
- de novo common lines
- ACT / OTR
- known structure
- tomography
- balls & sticks
model
- reproject in all directions (angular step)
re-interpolates in 3D reciprocal space, IFT
reprojections
- re-aligned, re-classified (band-pass filters)
- best class averages selected
best particles
refined class averages
43:09 / 44:27
CC


Software Tools For Molecu...
en.wikibooks.org/wiki/Software_Tools_For_Molecular_Microscopy/General_packages
cryo-EM software packages
Most Visited
Getting Started
Latest Headlines
Google
Jensen Lab
Caltech
favorites
Journals
Vendors
sciencesites
Open in Papers
Scouts
Create account
Log in
Sandbox
WIKIBOOKS
Open books for an open world
Book
Discussion
Read
Edit
View history
Search
Software Tools For Molecular Microscopy/General packages
< Software Tools For Molecular Microscopy
Contents [hide]
1 General packages
1.1 Appion
1.2 Bsoft
1.3 Cyclops
1.4 EMAN2
1.5 EMAN
1.6 Eos
1.7 IMAGIC
1.8 IPLT
1.9 MDPP
1.10 MRC IMAGE PROCESSING PACKAGE
1.11 RELION
1.12 SIMPLE
1.13 SPARX
1.14 SPIDER
1.15 Suprim
1.16 Xmipp
Main Page
Help
Browse wiki
Cookbook
Wikijunior
Featured books
Recent changes
Donations
Random book
Using Wikibooks
Community
Reading room
Community portal
Bulletin Board
Help out!
Policies and guidelines
Contact us
Tools
What links here
Related changes

Single particle reconstruction - additional topics
Concept check questions:

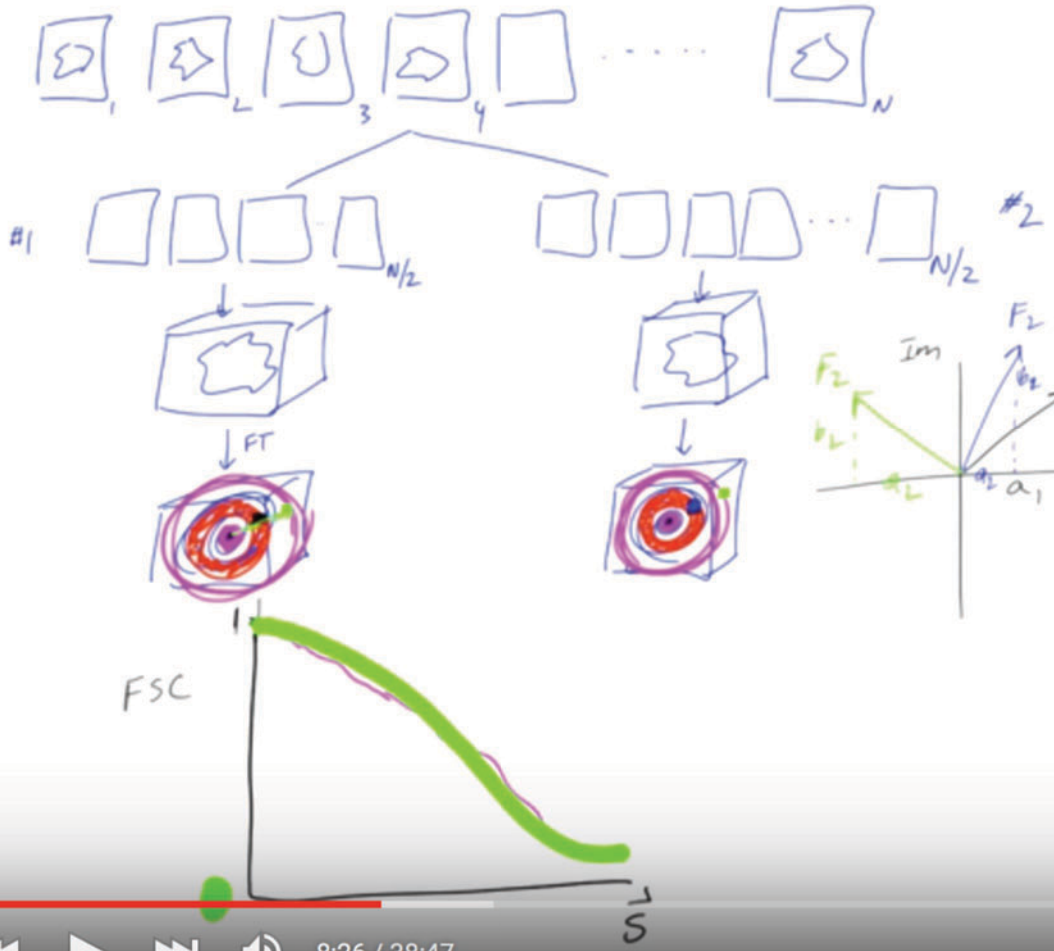
- Name 5 ways to generate an initial model.
- What are the differences between a "random conical tilt" and "orthogonal tilt" reconstruction, and why are these produced?
- Why are focal pairs of images sometimes recorded?
- Name three resolution-enhancing steps made possible by recording images on a direct detector in "movie-mode".
- Name two kinds of heterogeneity that can be detected and overcome with single particle methods.
- Given what you learned about how maximum likelihood methods could be used to produce a single particle reconstruction, how might they be used to sort out heterogeneity and produce multiple 3-D reconstructions from a single data set?
- What process parameters are typically changed from iteration to iteration in a single particle reconstruction?
- Where can a good list of single particle software packages be found?
- The "Einstein from noise" demonstration is famous in cryo-EM for illustrating what?
- Name three ways reference bias can be introduced into a single particle reconstruction, and how one can know if his/her structure is biased?

How to assess the resolution of a single particle reconstruction



$$\text{Fourier shell correlation: } \mathrm{FSC}(s, \Delta s) = \frac{\mathrm{Re}\left\{\sum_{[s,\Delta s]} \mathbf{F}_1(\mathbf{s})\mathbf{F}_2^*(\mathbf{s})\right\}}{\left\{\sum_{[s,\Delta s]} |\mathbf{F}_1(\mathbf{s})|^2 \sum_{[s,\Delta s]} \mathbf{F}_2(\mathbf{s})|^2\right\}^{1/2}}$$

How to assess the resolution of a single particle reconstruction



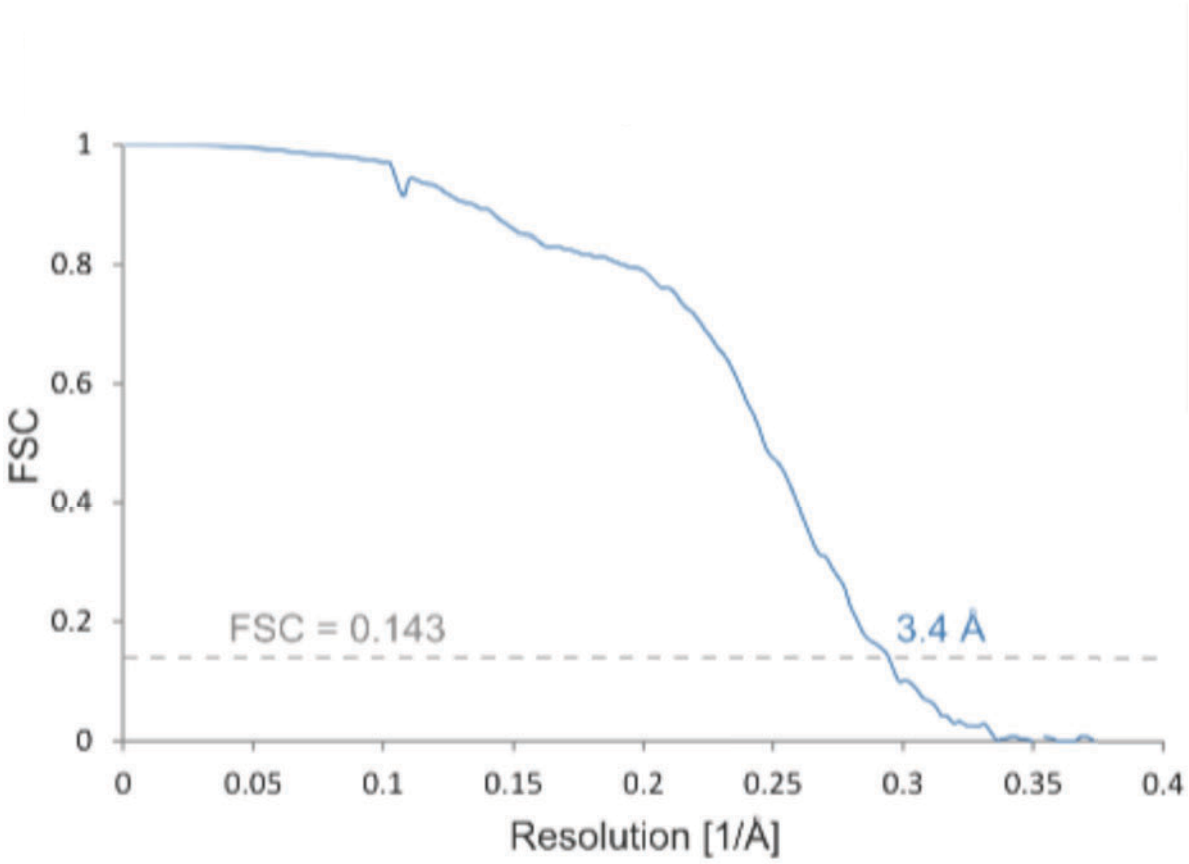
$$FSC = \frac{Re\left\{\sum_{shell} F_1(s)\cdot F_2^*(s)\right\}}{\left\{\sum_{shell}|F_1(s)|^2 \sum_{shell}|F_2(s)|^2\right\}^{1/2}}$$

$$\vec{F}_1 = a_1 + ib_1, \quad \vec{F}_2 = a_2 + ib_2$$

$$FSC = \frac{a_1a_2 + b_1b_2}{\sqrt{(a_1^2+b_1^2)(a_2^2+b_2^2)}}$$

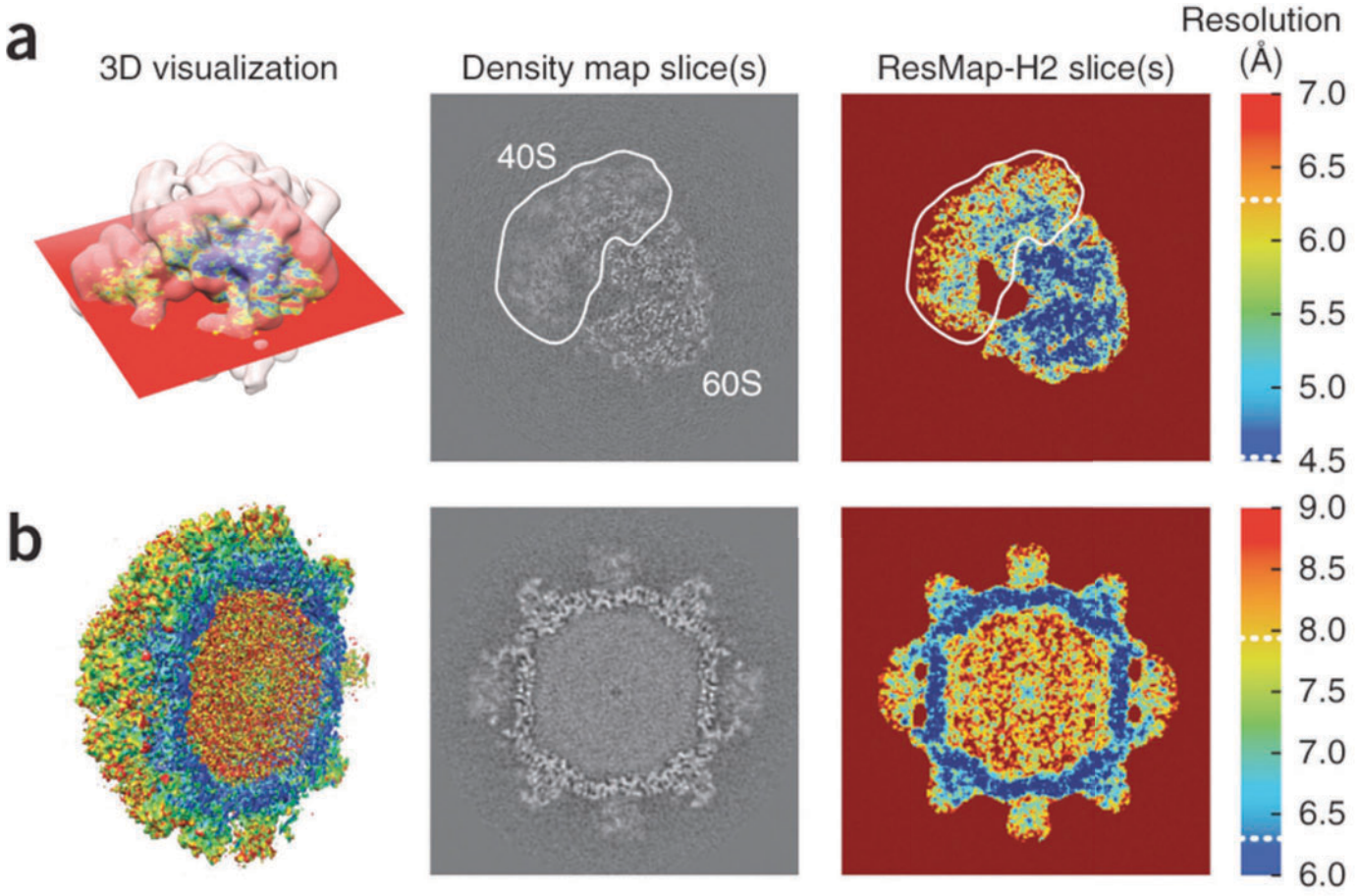
if $\vec{F}_1 = \vec{F}_2$, then $FSC = \frac{a^2+b^2}{a^2+b^2} = 1$

Example FSC curve

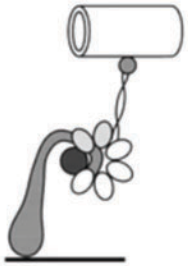


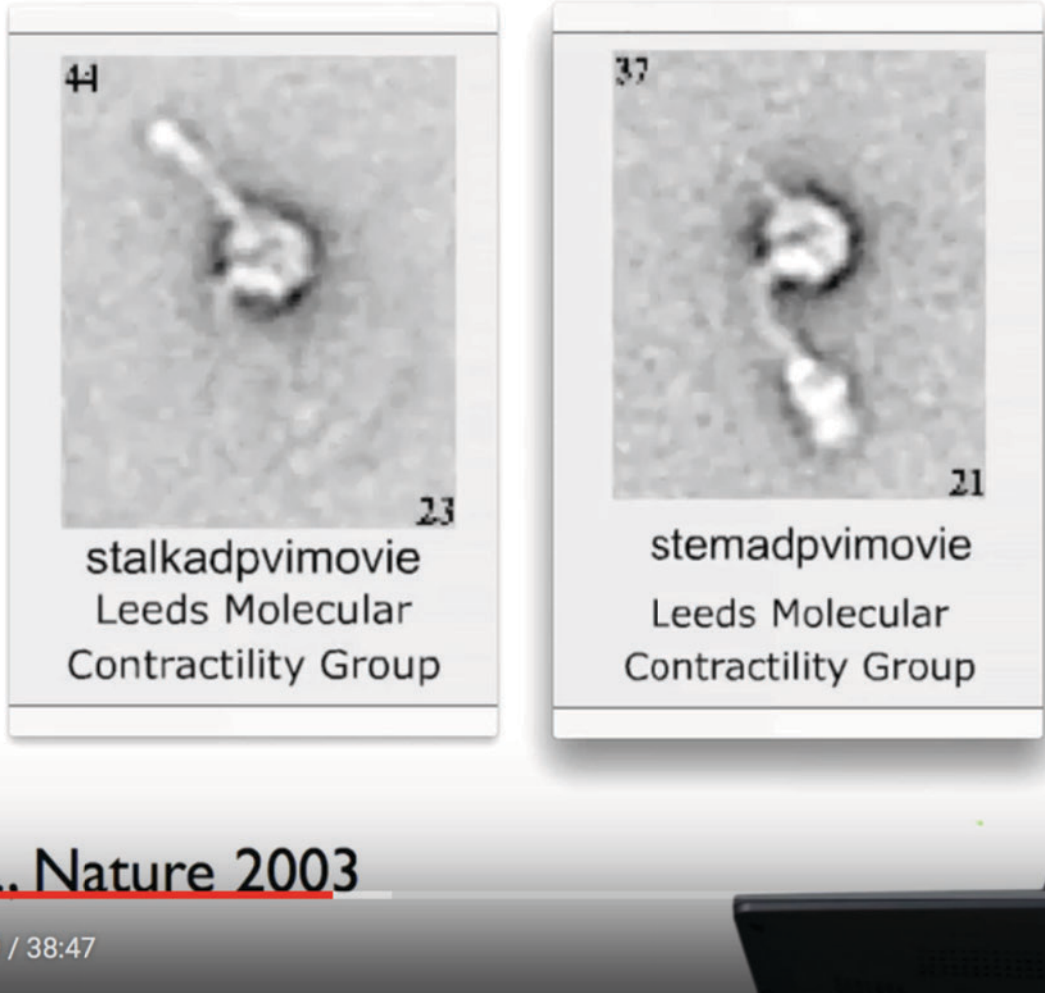
Brown et al., Science 2014

ResMap: a method to estimate local resolution

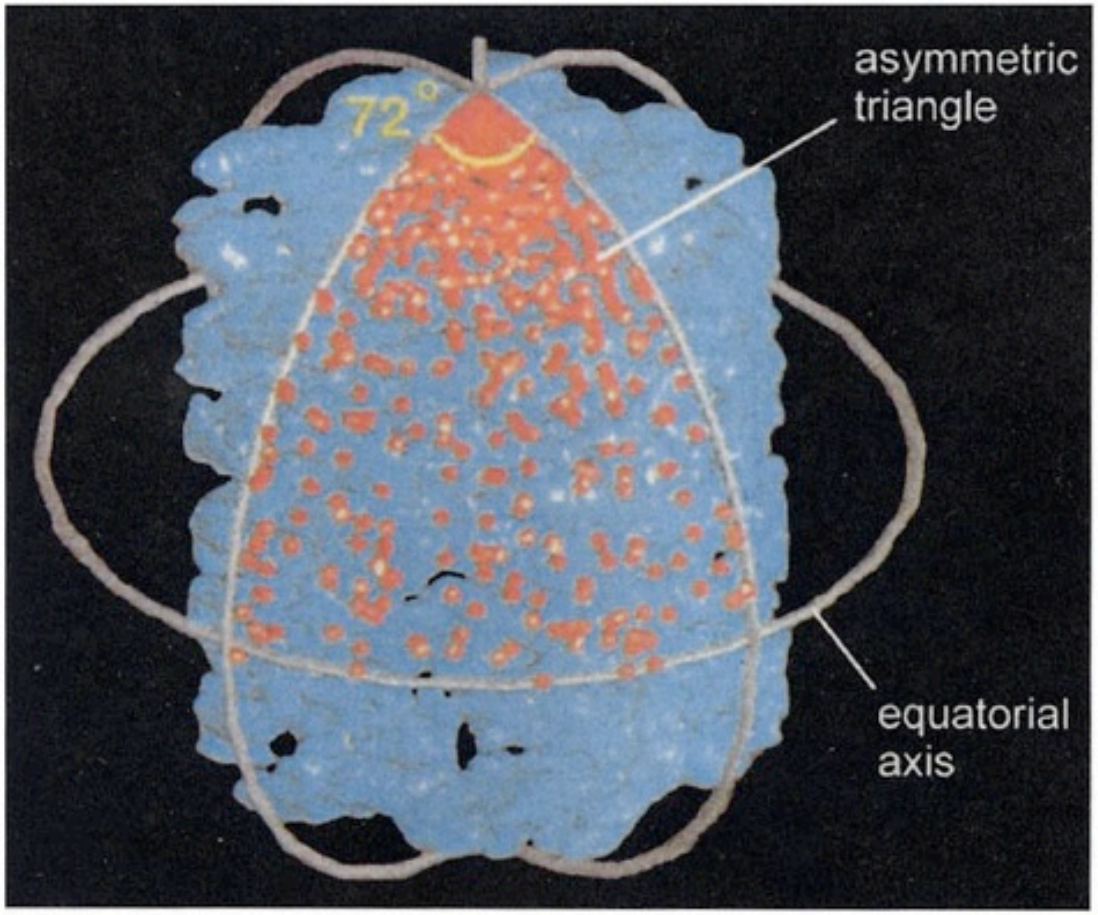


Kucukelbir et al., Nature Methods 2013





By aligning particles on one feature or another, conformational flexibility can be detected and characterized



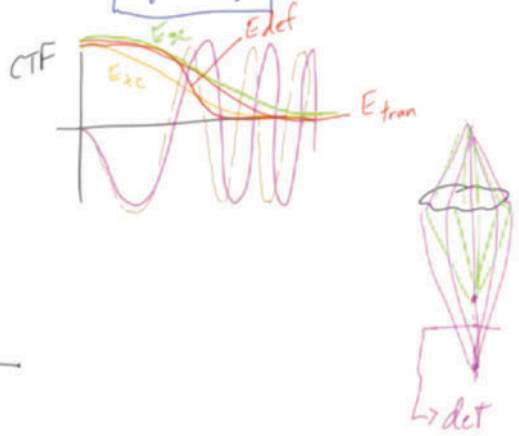
Preferred orientations lead to anisotropic resolution

Meissner et al. JMB 298:21 (2000)

Resolution limitations

particle homogeneity

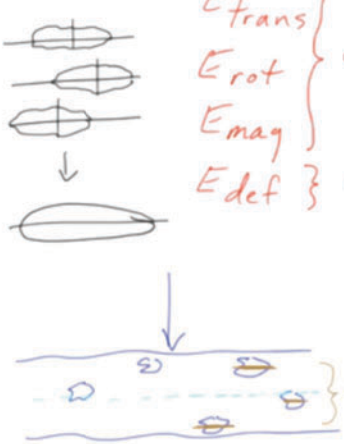
image quality



beam-induced specimen movement
modulation transfer function (MTF)
distortions, aberrations

$$E_{image\ quality} = E_{sc} E_{tc}$$

alignment precision



$$E_{trans} \} \propto e^{-ck^2}$$
$$E_{rot} \} \propto e^{-ck^2}$$
$$E_{mag} \} \propto e^{-ck^4}$$
$$E_{def} \} \propto e^{-ck^4}$$

$$E_{alignment\ precision} = E_{trans} E_{rot} E_{mag} E_{def}$$

$\sigma_{trans} < 1$ Å
$\sigma_{rot} < 1°$
$\sigma_{def} < 200$ Å

particles averaged

$$N_{views} = \pi D k$$

$$N_{images} = \pi D k \left(\frac{SNR_{desired}}{SNR_{present}} \right)^2$$

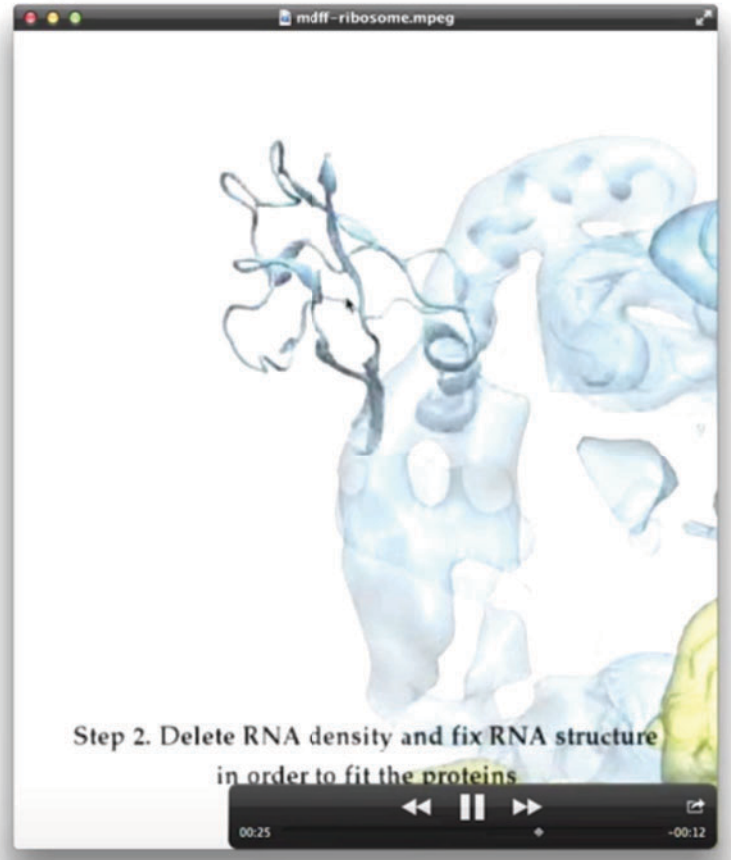
$$N_{images} = \pi D k \left(\frac{SNR_{desired}}{SNR_{ideal\ image} \cdot E_{image\ quality} \cdot E_{alignment\ precision} \cdot E_{particle\ heterogeneity}} \right)^2$$

N_{images}
10^3
10^6
10^7
10^4–10^6

Building a model:

- fit a known structure (Situs, Chimera, Modeller, Sculptor)
- molecular dynamics flexible fitting ("MDFF")
- build a new structure de novo
  - predict secondary structures in sequence
  - search for secondary structures in map
  - find path of backbone
  - fit side chains

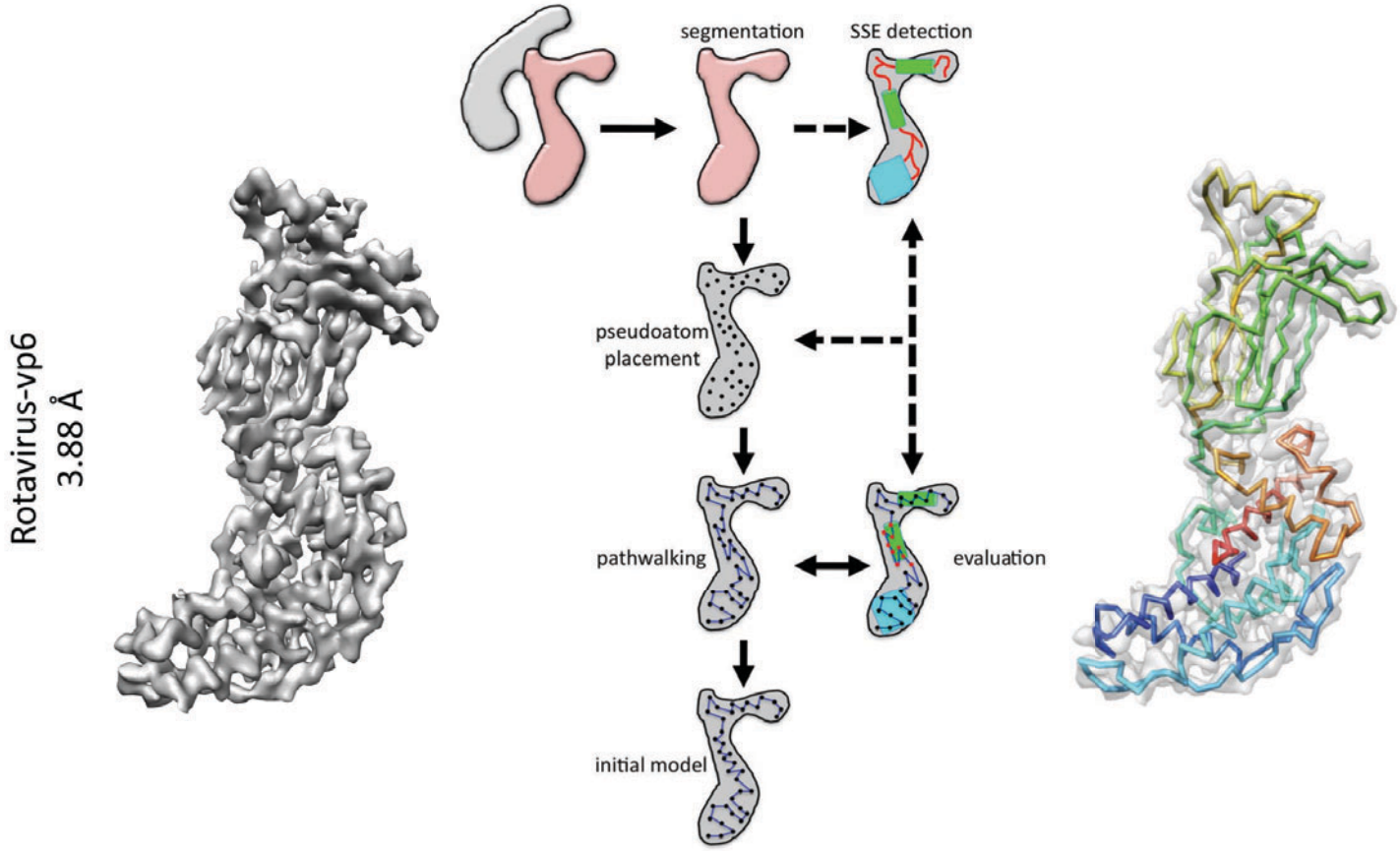
Molecular dynamics flexible fitting



http://www.ks.uiuc.edu/Research/mdff/method.html

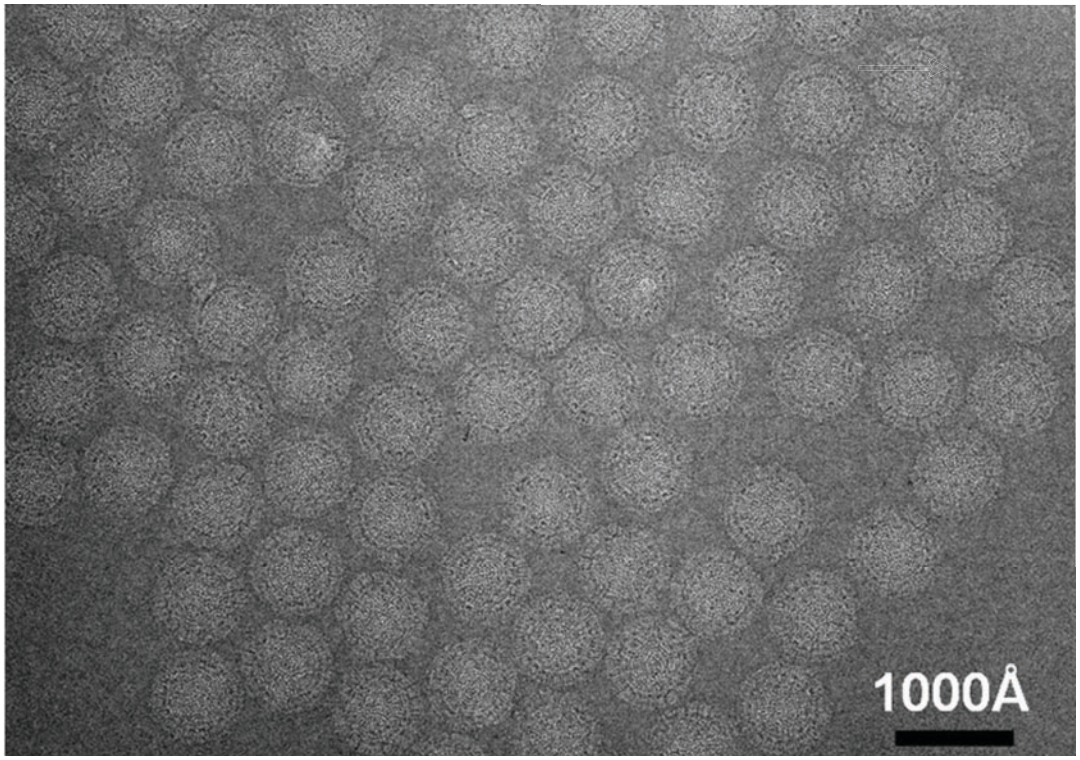
33:39 / 38:47

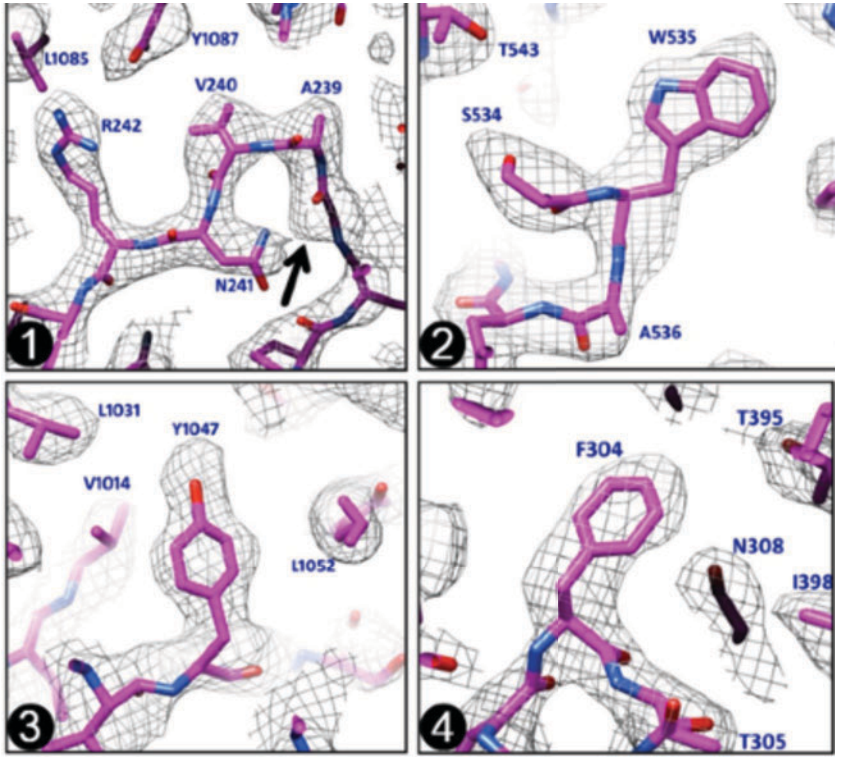
Finding secondary structures and building models



"Pathwalker" Baker et al., Structure 2012

Bulky side chains provide anchor points to align secondary structure elements to sequence





Zhang et al., Cell 2010

Single particle analysis - Interpretation and limitations
Concept check questions:

- How is the resolution of a single particle reconstruction measured?
- How can EM be used to characterize particle flexibility?
- When should one try to classify particles into distinct conformational states and solve structures of each one, and when should one conclude their particle is simply "flexible" (i.e. it exhibits a continuous range of motion)?
- Why do some particles exhibit preferred orientations? How can that problem be handled?
- Why can the effects of partial spatial coherence and translational alignment errors both be understood as envelopes?
- What is similar about partial temporal coherence and errors in defocus determination?
- How many images are typically needed for a near-atomic-resolution single particle reconstruction? What factors influence this number?
- What is "MDFF", and when is it used?