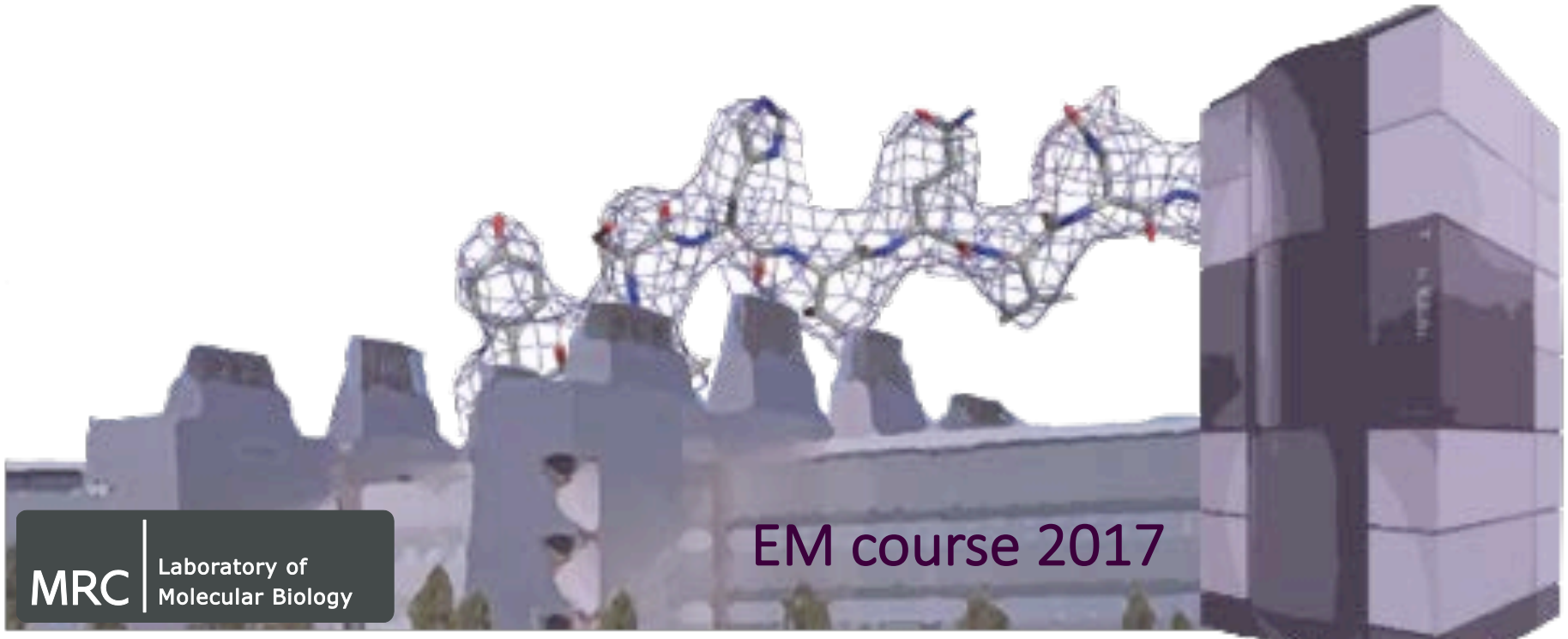
Tomography and Subtomogram Averaging

John Briggs



What do we need to get a 3D structure?

Sample preparation methods

A transmission electron microscope

Different views of our object of interest

Computational approaches for producing a 3D reconstruction from 2D projections

Methods for validation and interpretation of the 3D structure

What do we need to get a 3D structure?

Sample preparation methods

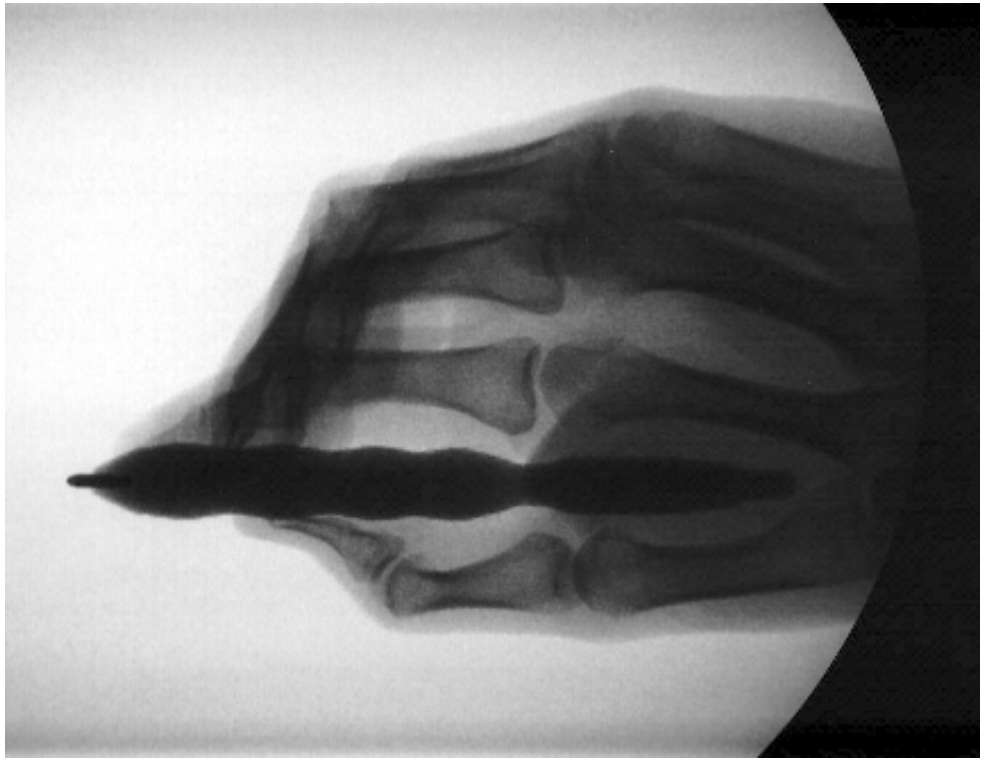
A transmission electron microscope

Different views of our object of interest

Computational approaches for producing a 3D reconstruction from 2D projections

Methods for validation and interpretation of the 3D structure

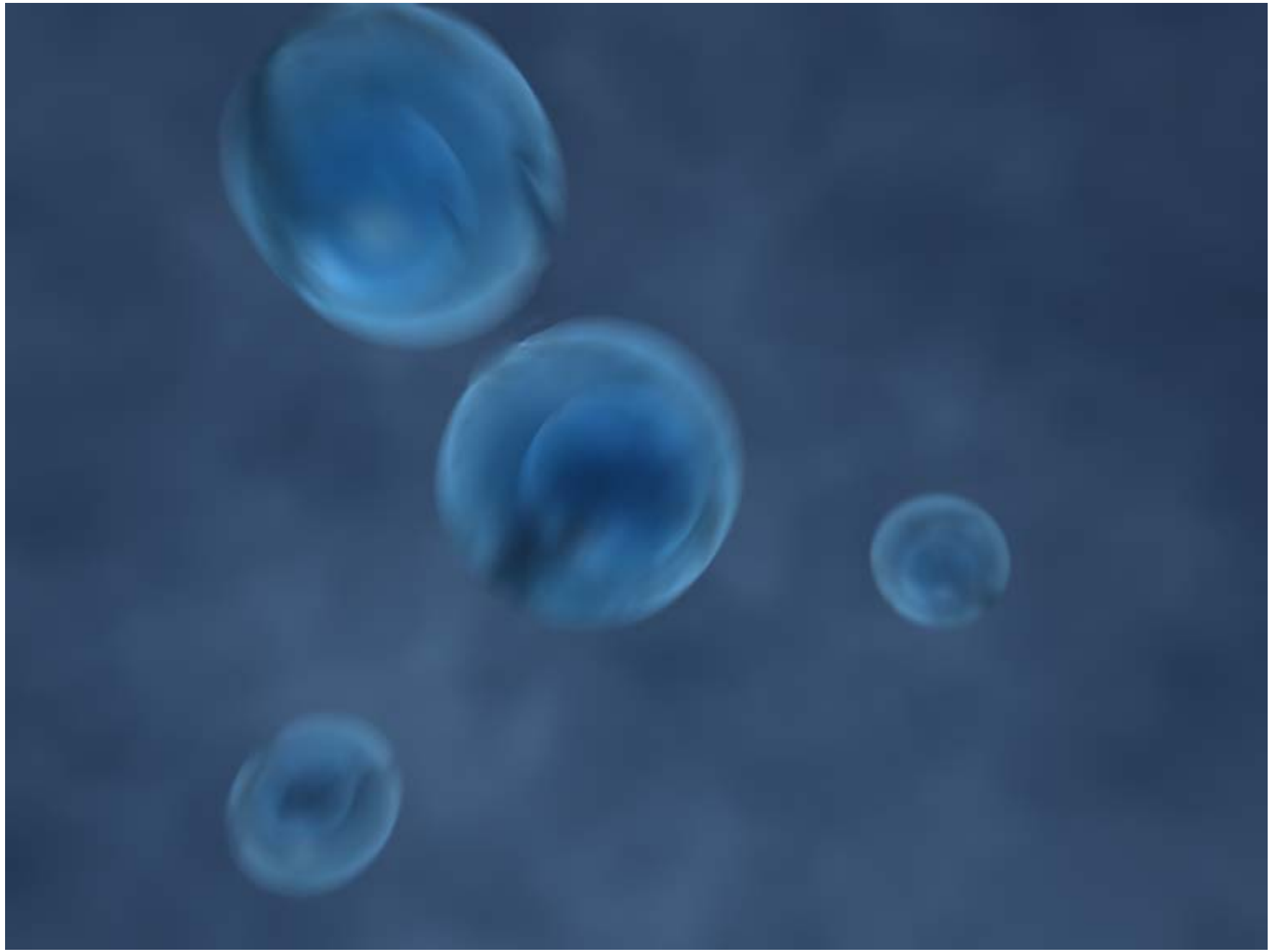
We need different views of our object



Tomography?

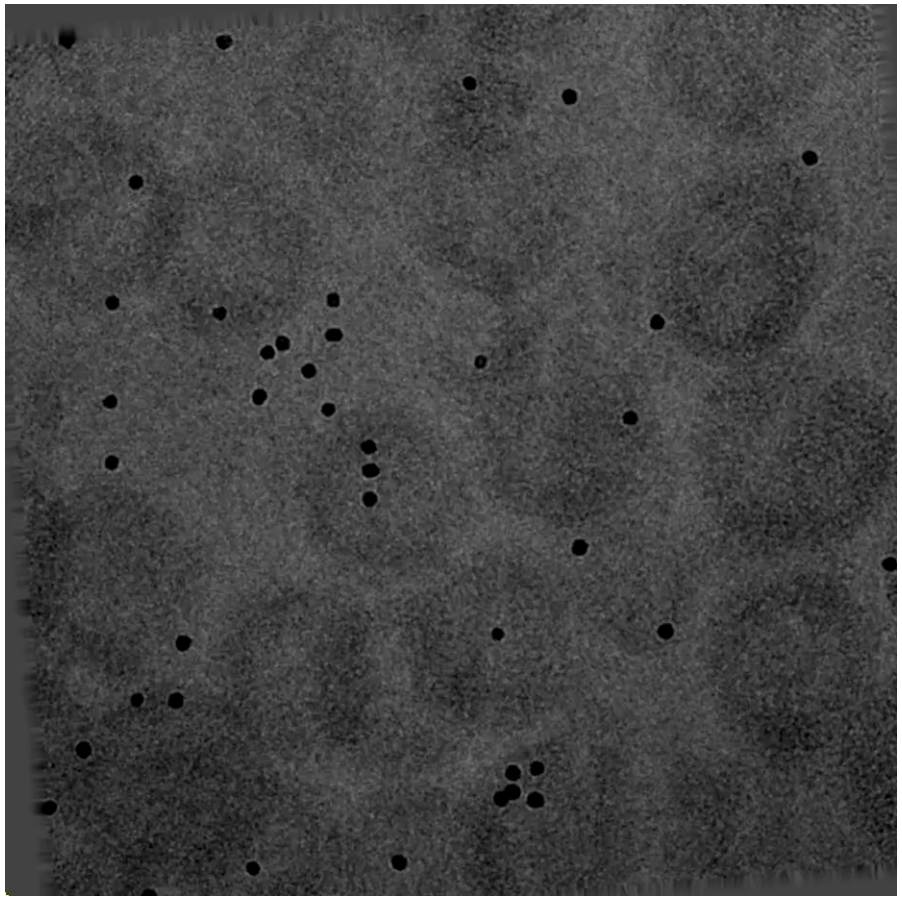
In single-particle methods we obtain different views of our object of interest by imaging many different copies that are oriented differently relative to the electron beam.

In tomography we obtain different views by physically rotating the sample in the microscope

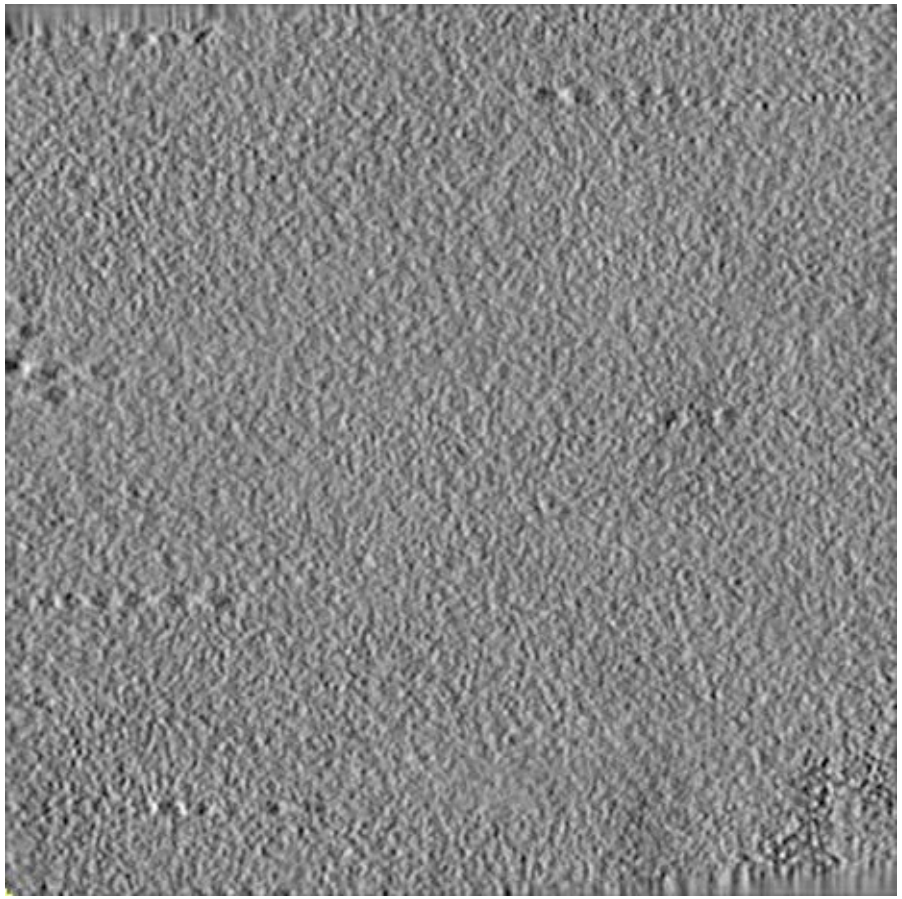


Movie from the Baumeister lab, Max-Planck Institute for Biochemistry

Tilt series and tomogram



Tilt-series



Tomogram

Why do tomography?

Because our sample is a unique structure (bits of cells, tissues, viruses etc.)

Because our sample is within a complex environment

Topics to be covered

Sample preparation methods

Data collection and microscope requirements

Alignment and reconstruction

Subtomogram averaging

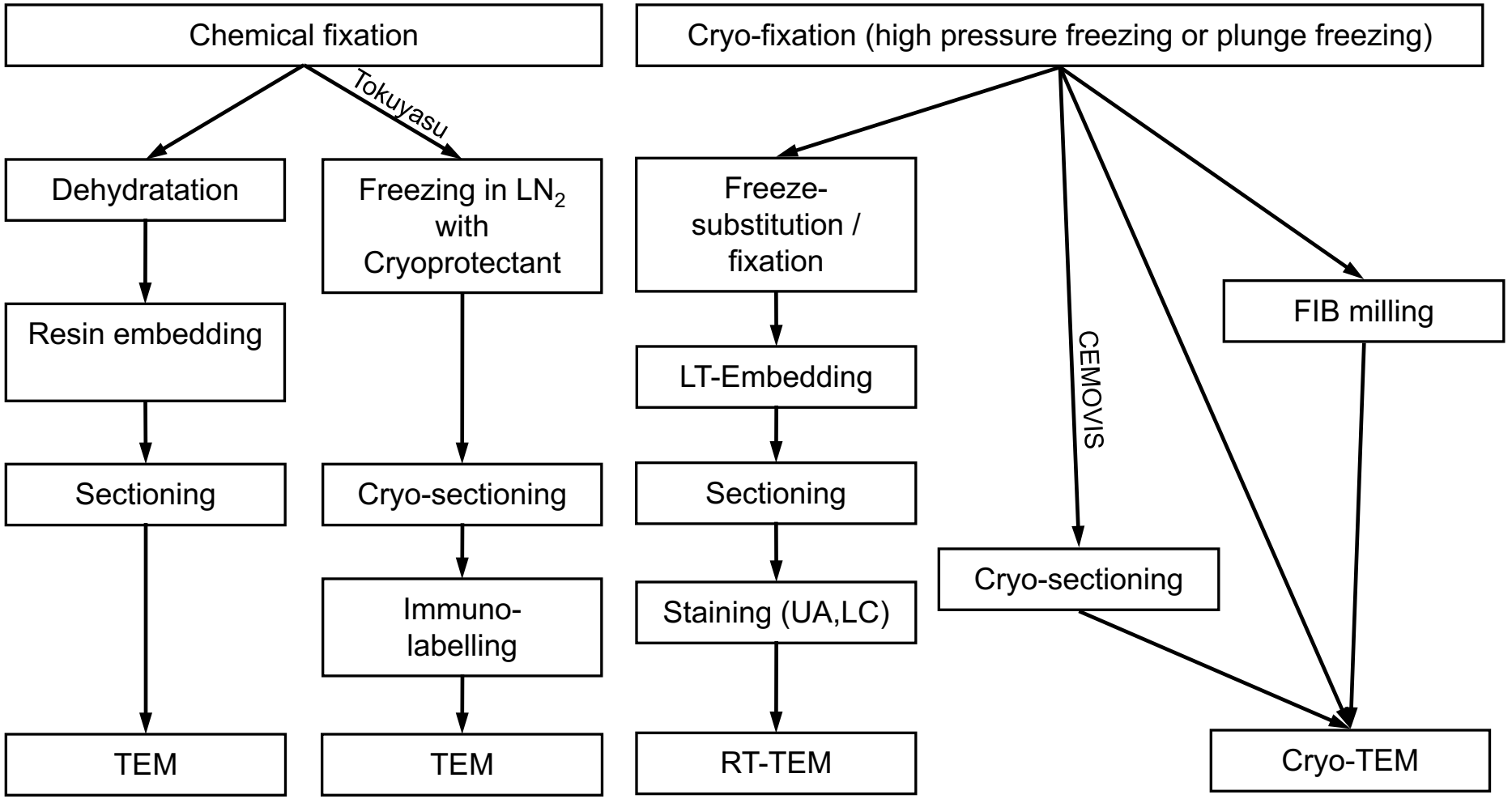
Sample preparation

Sample must have structure preserved

Sample must be thin

Sample must be stable in vacuum

Cellular sample preparation methods

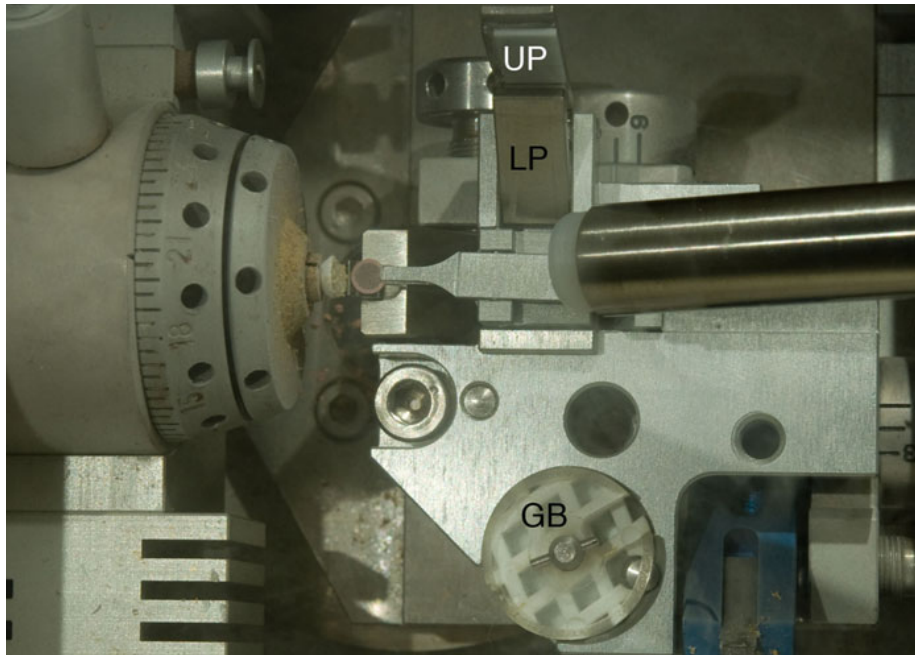


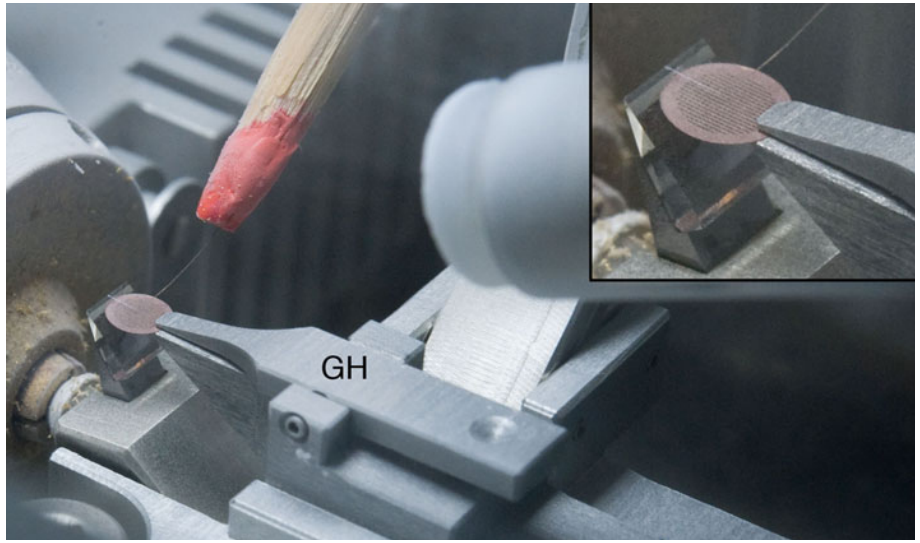
Cryo-sectioning (CEMOVIS)

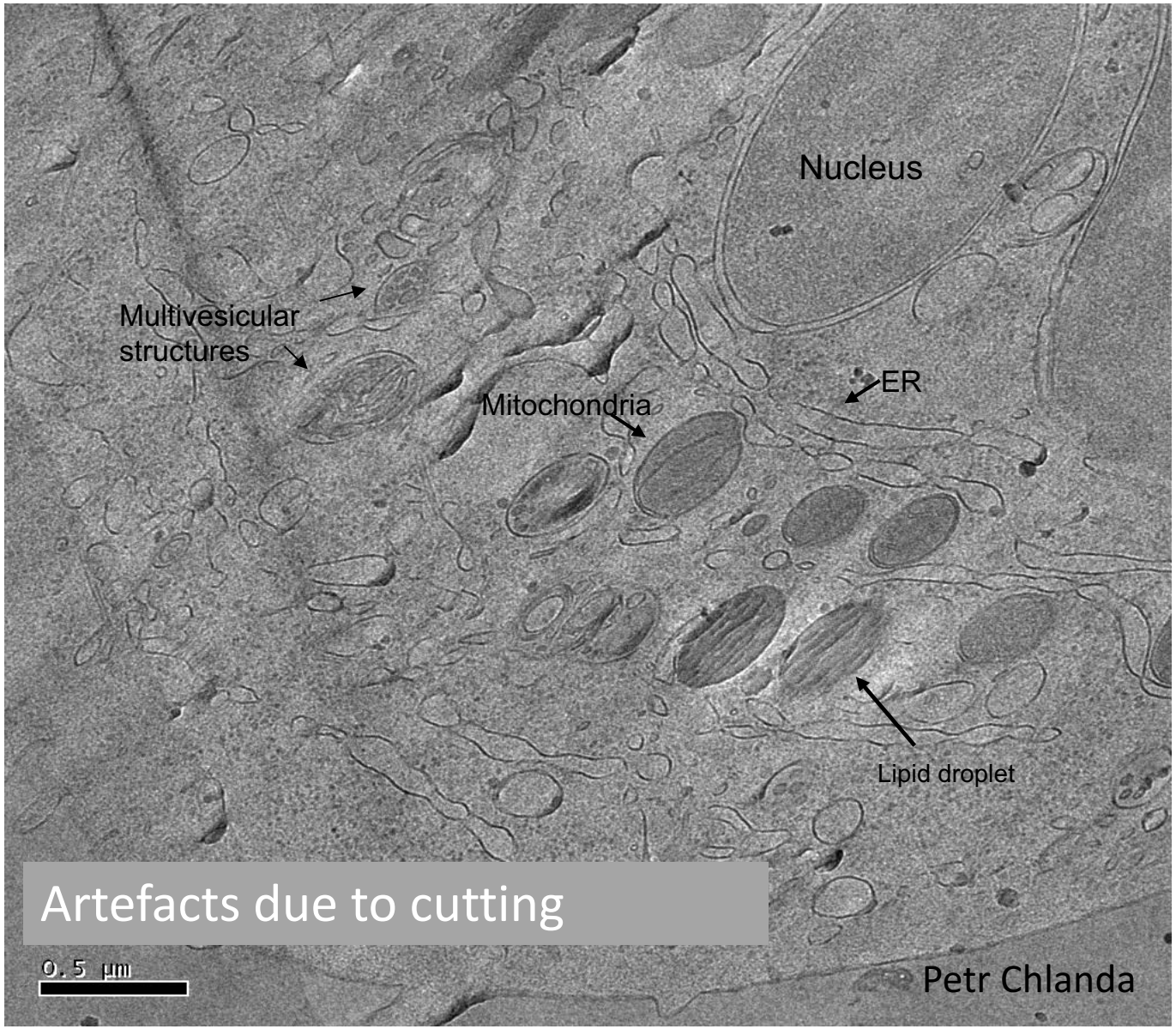
High-pressure freezer

Cryo-microtome

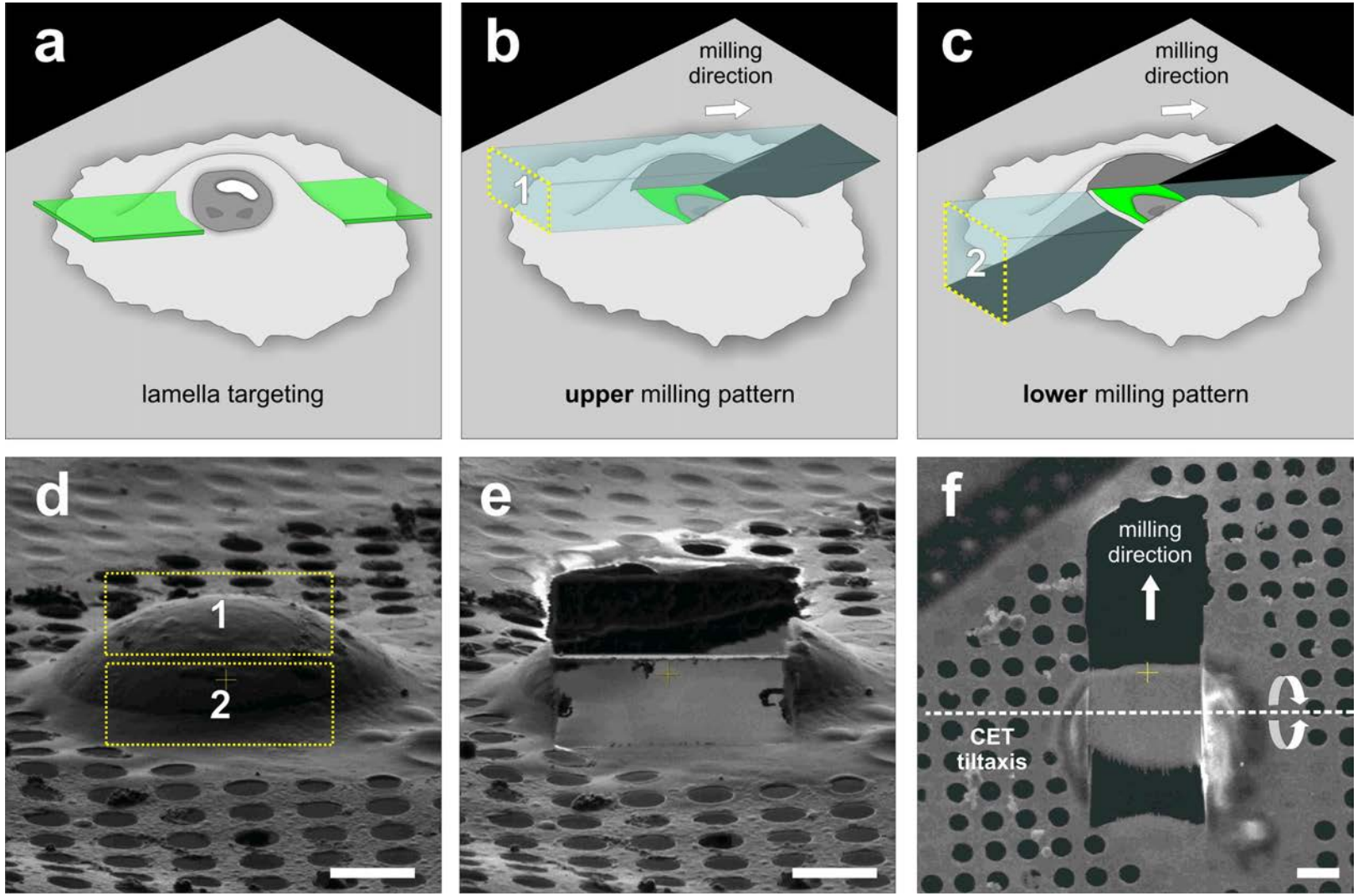
Cryo-sectioning (CEMOVIS)





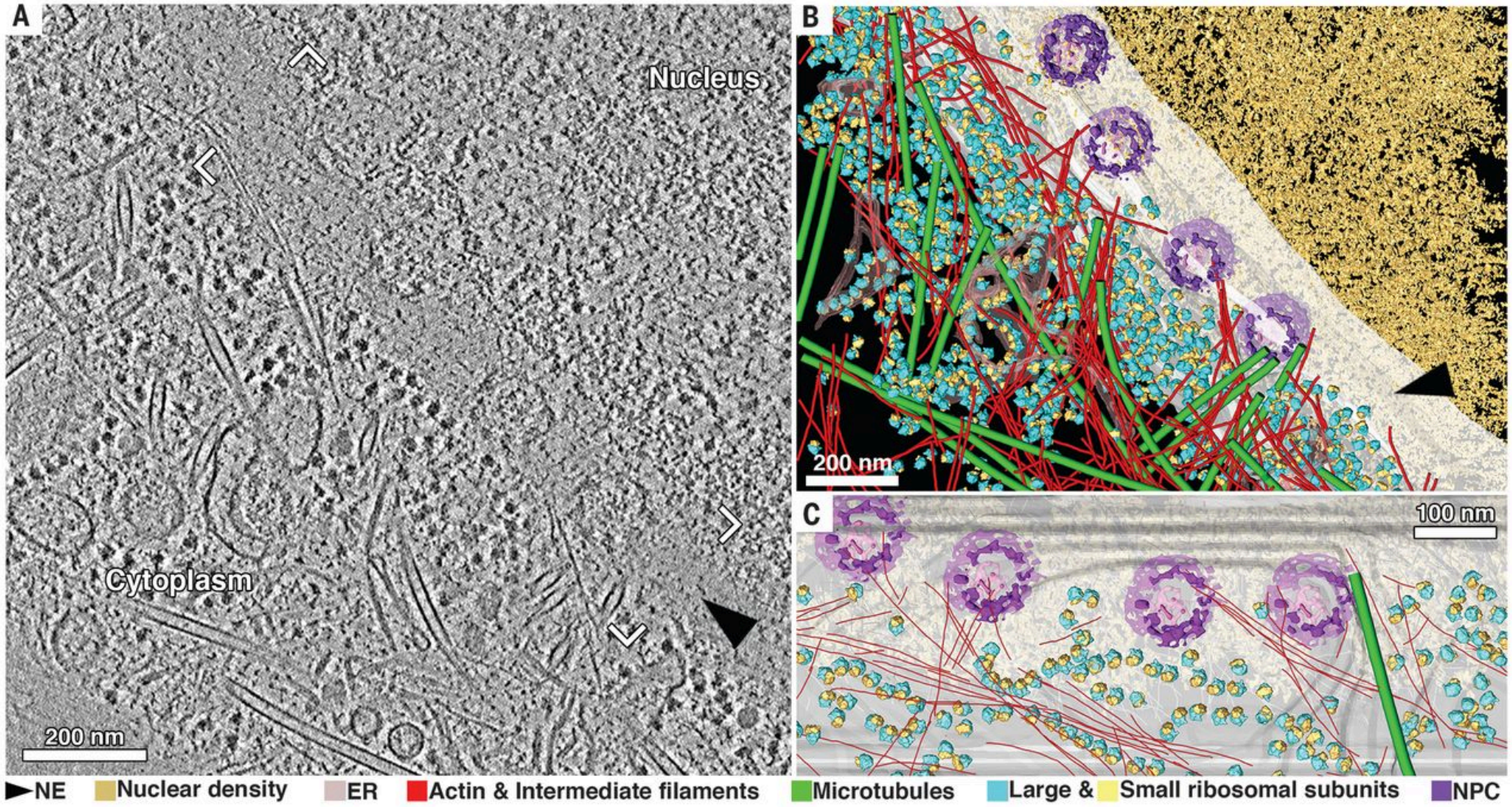


Sample preparation - FIBSEM



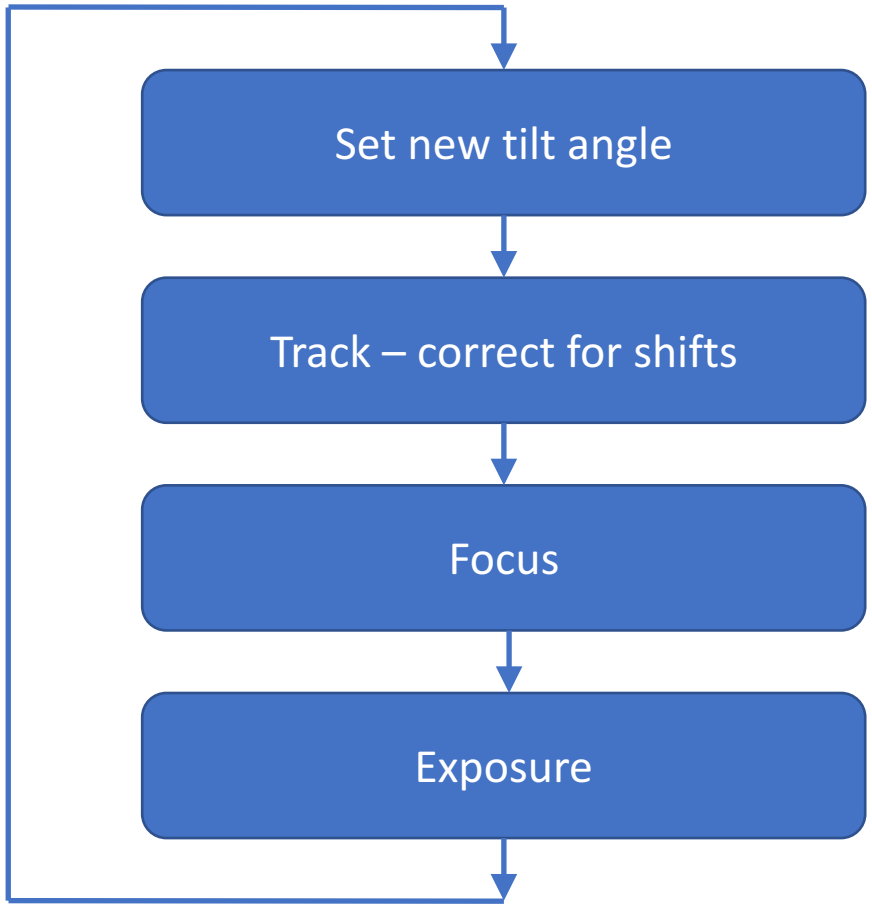
Rigort and Plitzko 2015

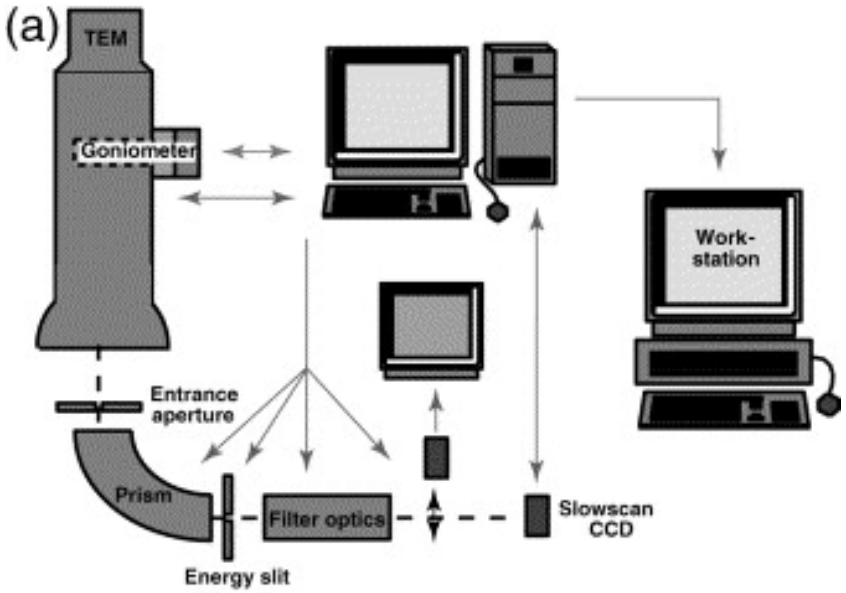
Sample preparation - FIBSEM

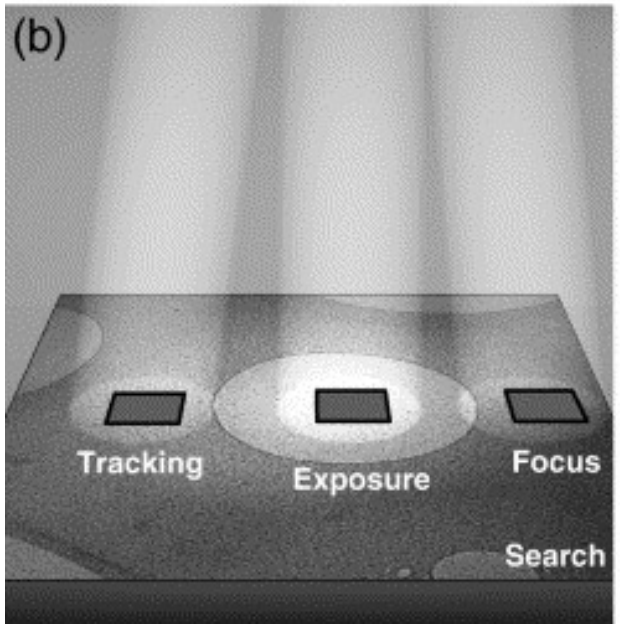


Mahamid et al, Science 2015

Data collection







Baumeister et al. Trends in Cell Biology 1999

Microscope requirements

(for cryo-electron tomography of thicker samples)

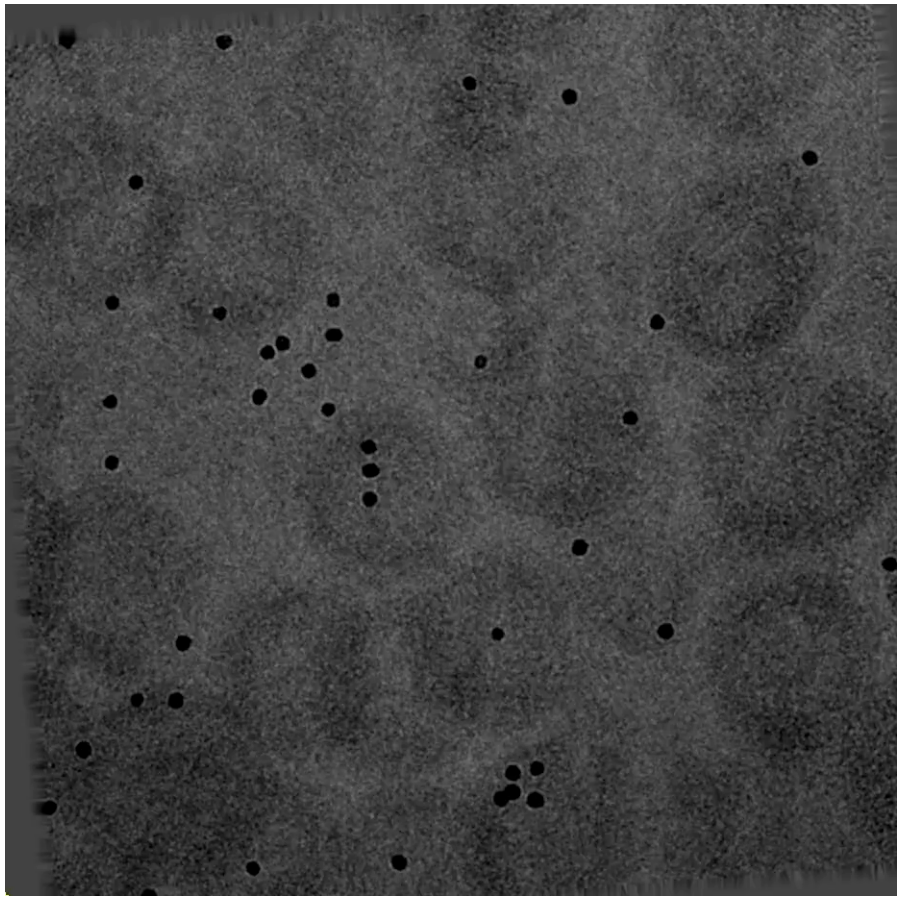
High-voltage (ideally 300kV)

Energy filter

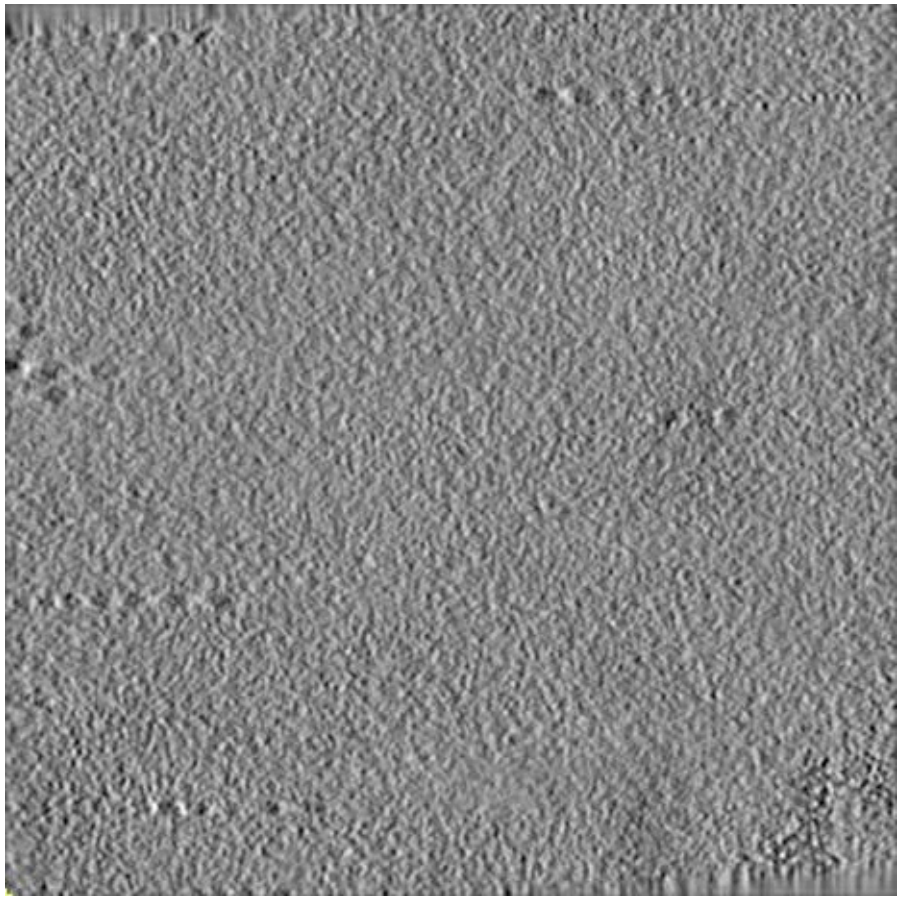
Very stable and well-calibrated microscope stage

Appropriate software

Tilt series and tomogram



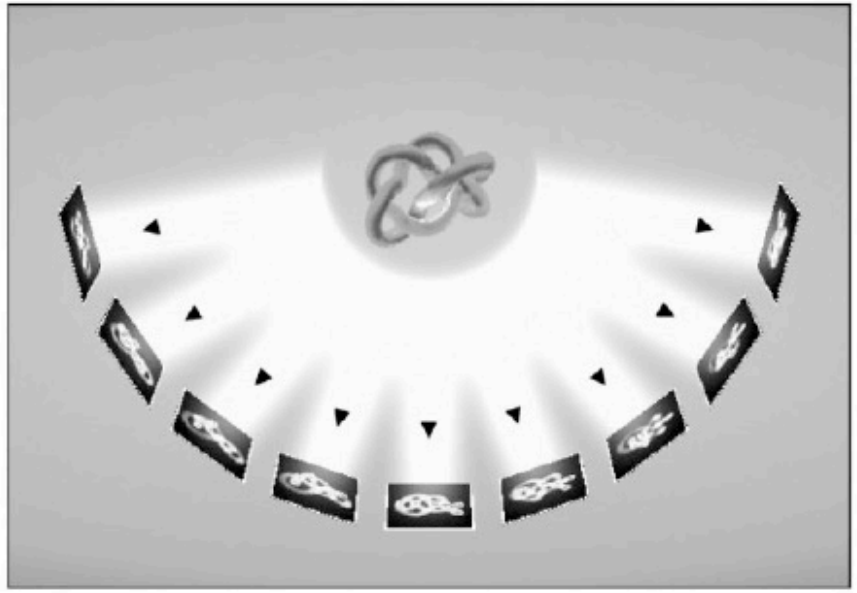
Tilt-series



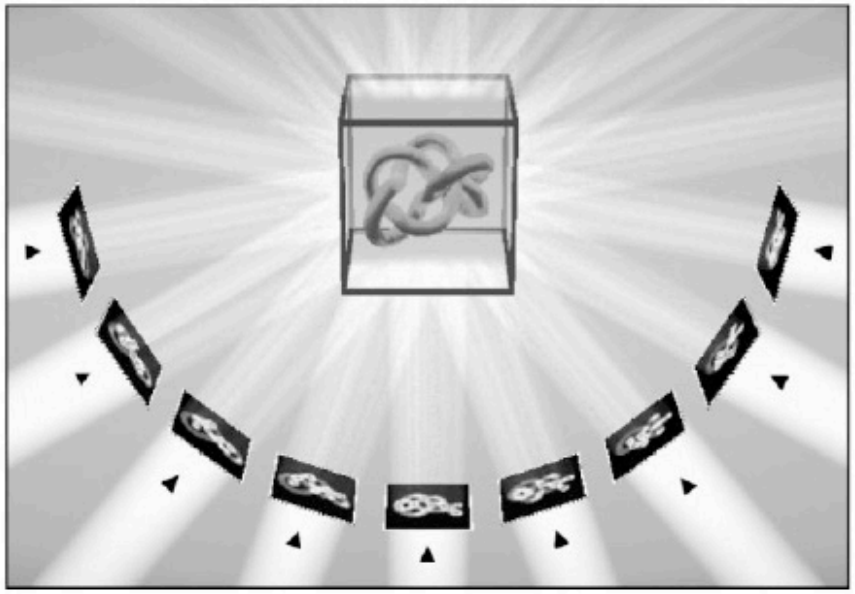
Tomogram

Forward and back projection

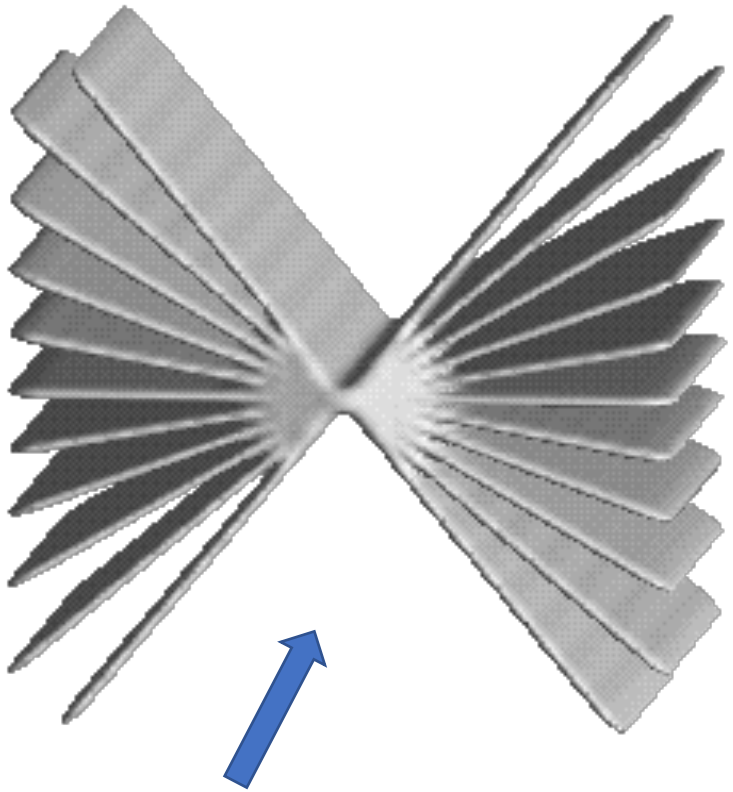
3D object -> 2D projections



2D projections -> 3D reconstruction

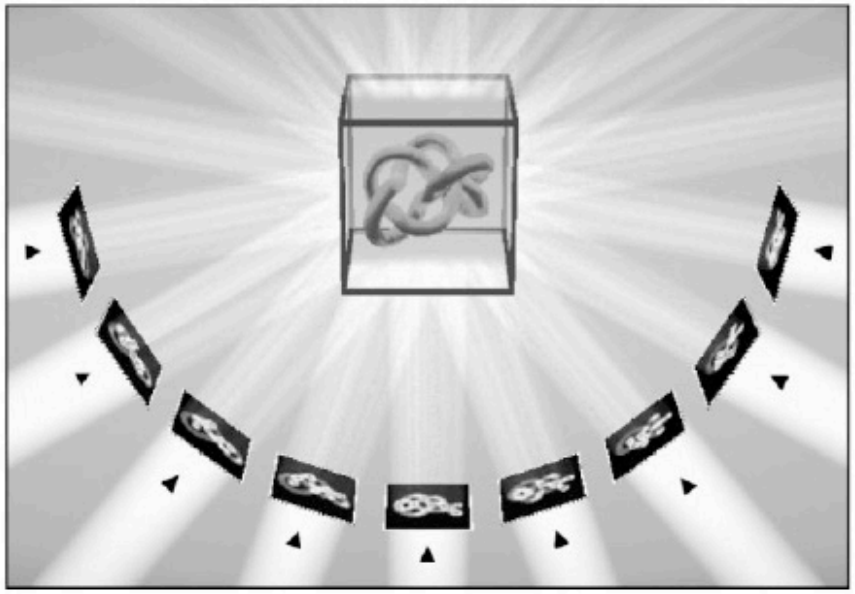


The data is incomplete: the missing wedge

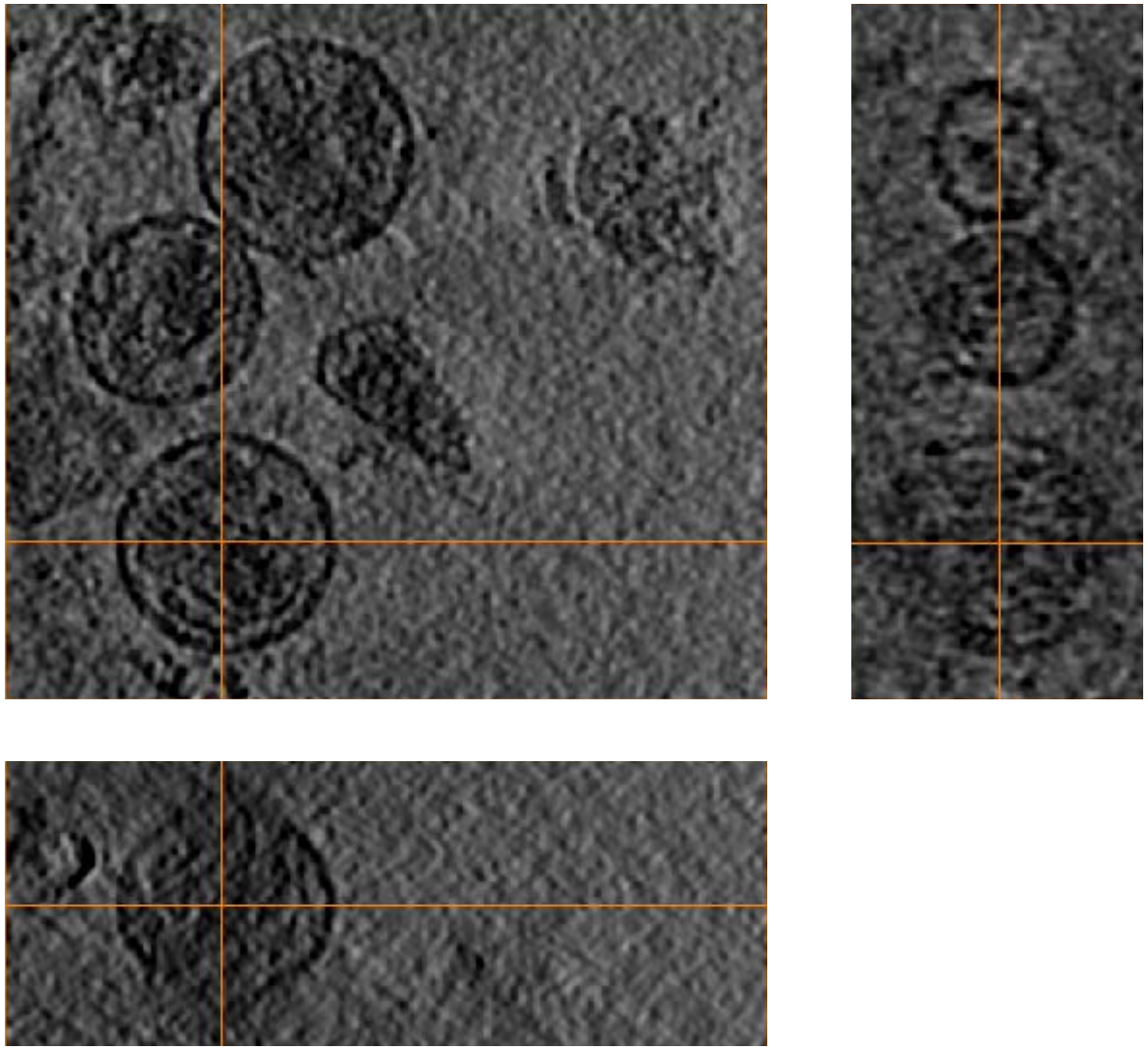


Missing information

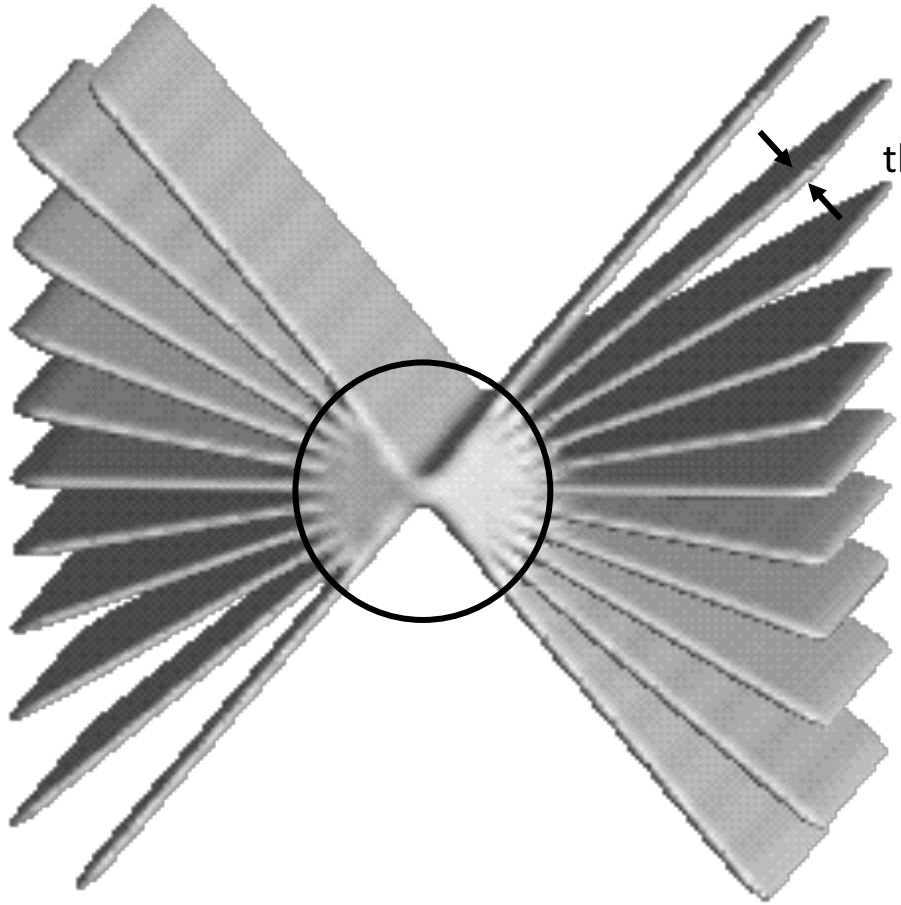
2D projections -> 3D reconstruction



The missing wedge leads to smearing in z



Missing information due to discrete sampling

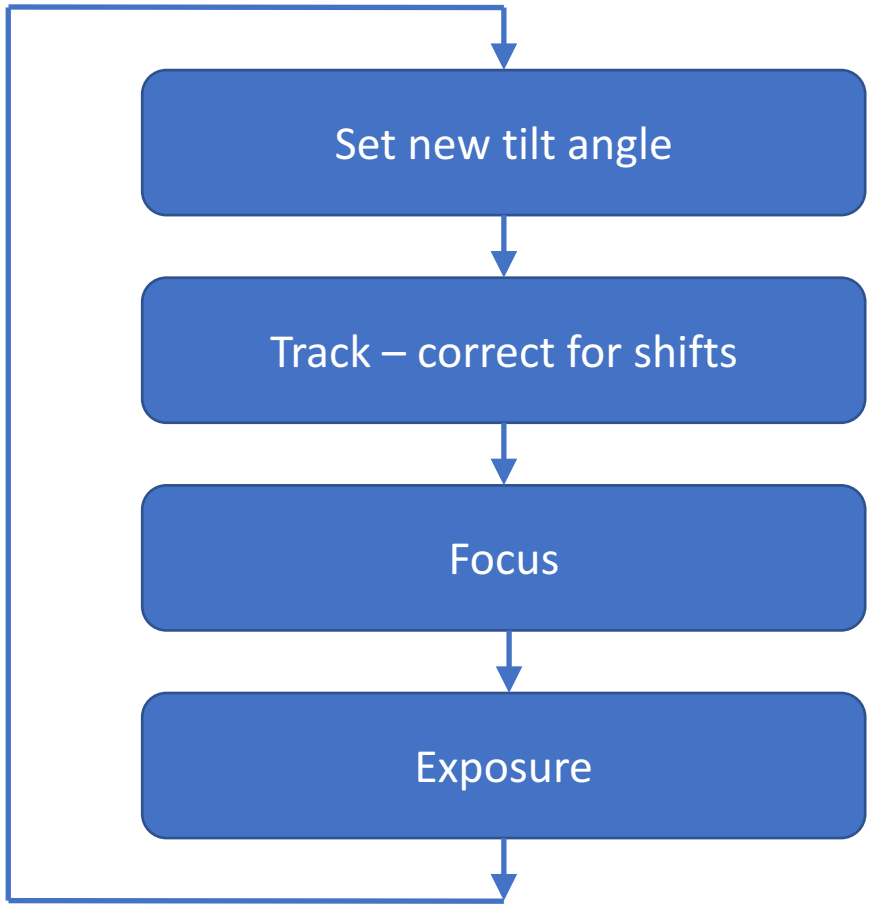


Crowther Criterion

$$r = \pi D/N$$

r: Resolution limit
N: Number of projections
D: Object thickness

Data collection



What questions should we ask before data collection?

Data collection

What total dose? Resolution vs signal-to-noise

What tilt range? Completeness of information vs dose and speed

What angular increment? Resolution vs dose and speed

What order to collect the images? Speed, reliability, optimal dose, sample distortion…

What magnification? Resolution and DQE vs field of view

What defocus? High-frequency information vs low frequency information

Alignment and Reconstruction

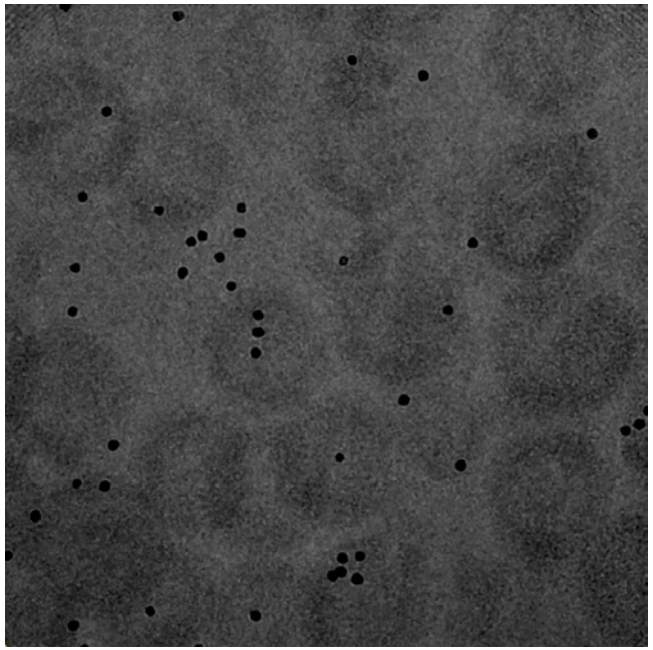
Once we have collected the data how do we reconstruct a tomogram?

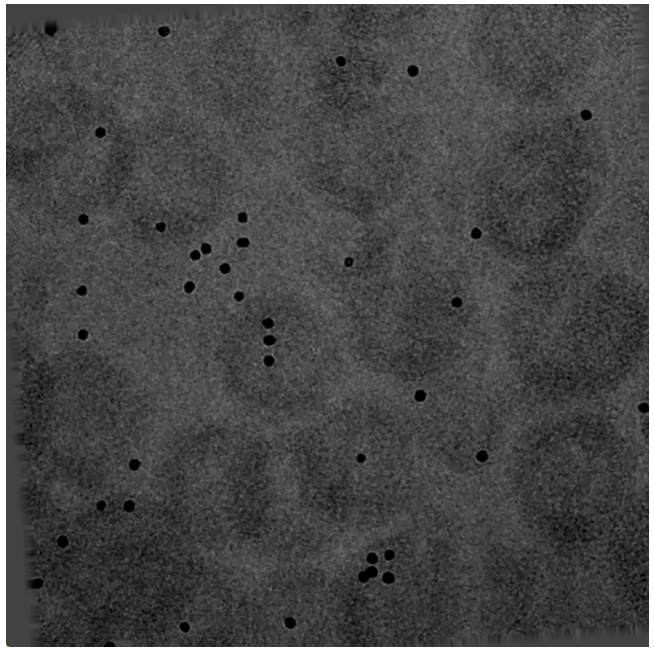
Alignment and Reconstruction

We need to know how the projections relate to each other: the angles and shifts between the projections.

We have defined the angles in the microscope by tilting around a defined axis by defined increments.

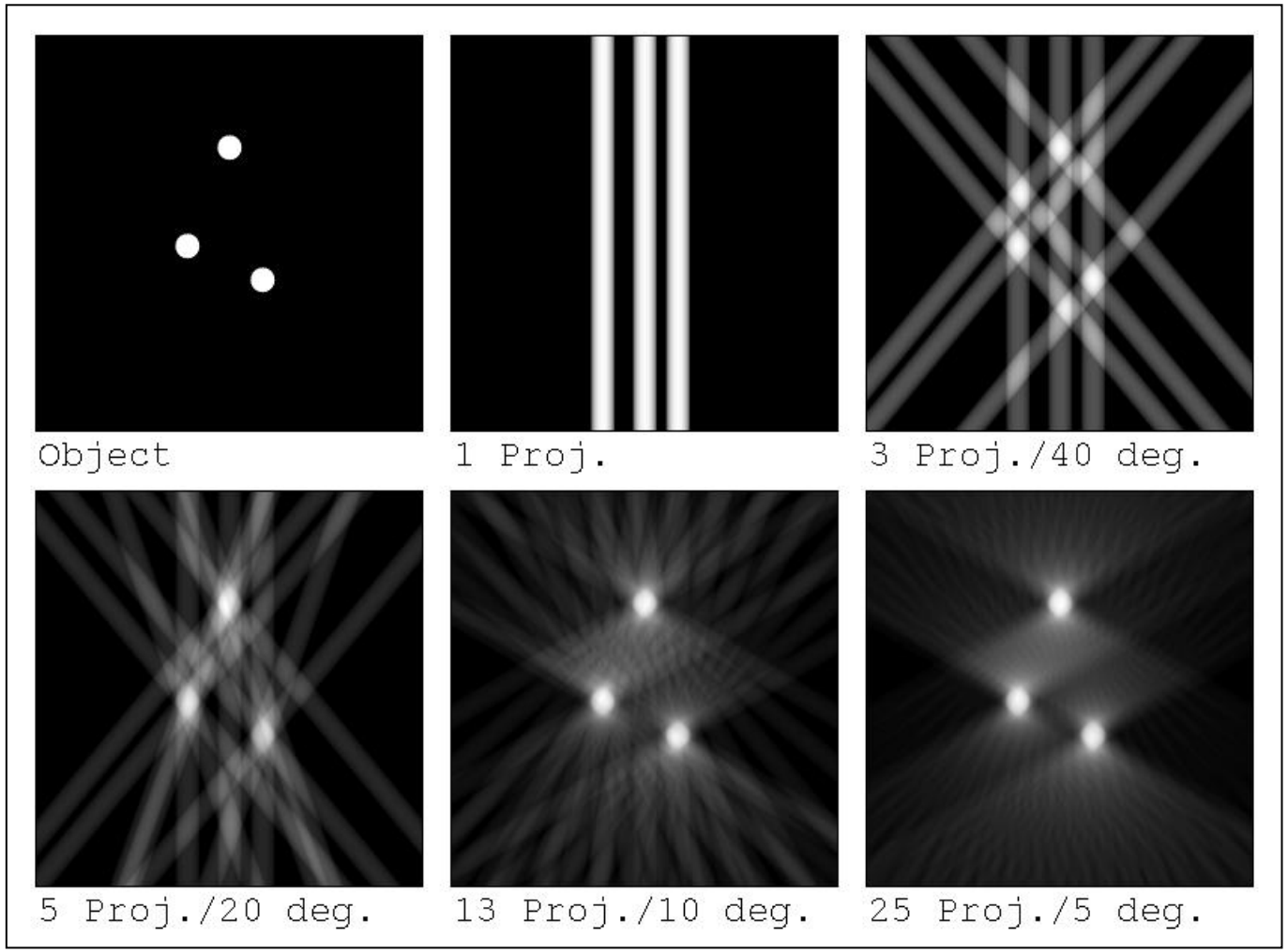
Alignment is necessary to deal with the shifts in the image.
(at larger fields of view, other distortions may become important)



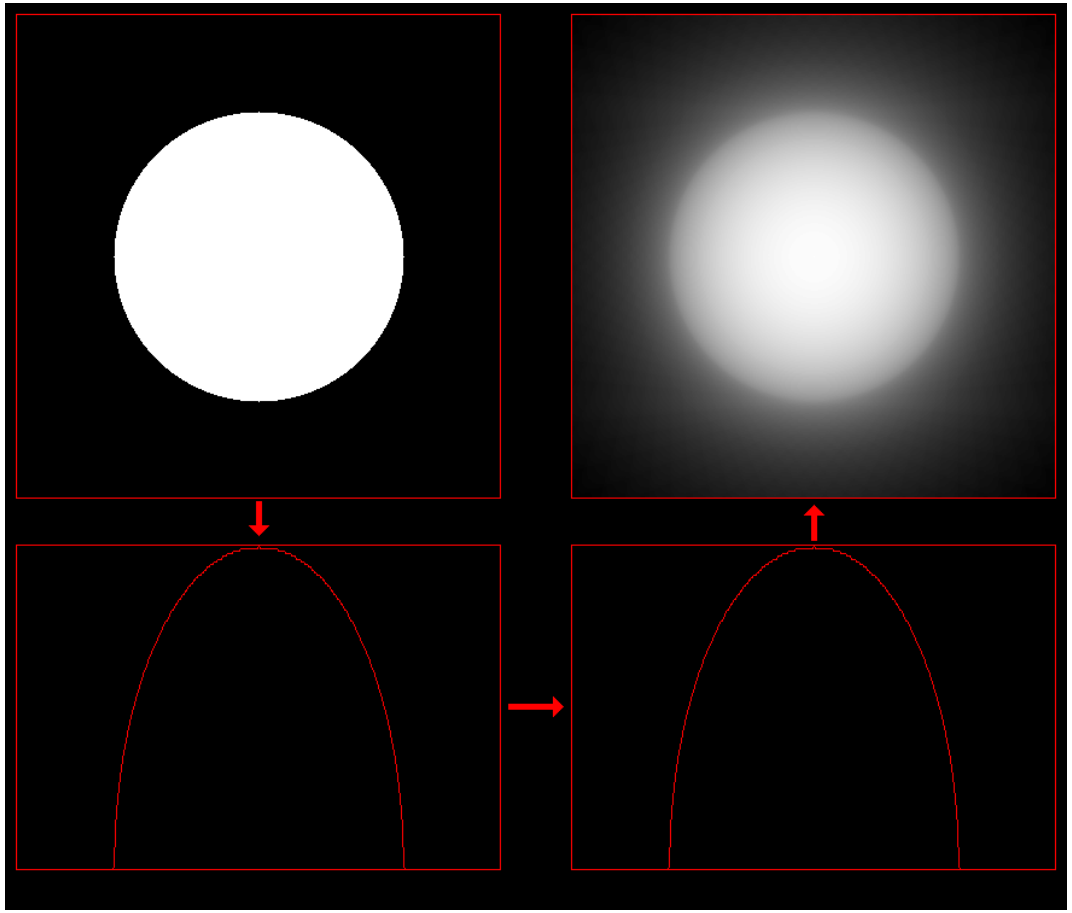


Alignment and Reconstruction

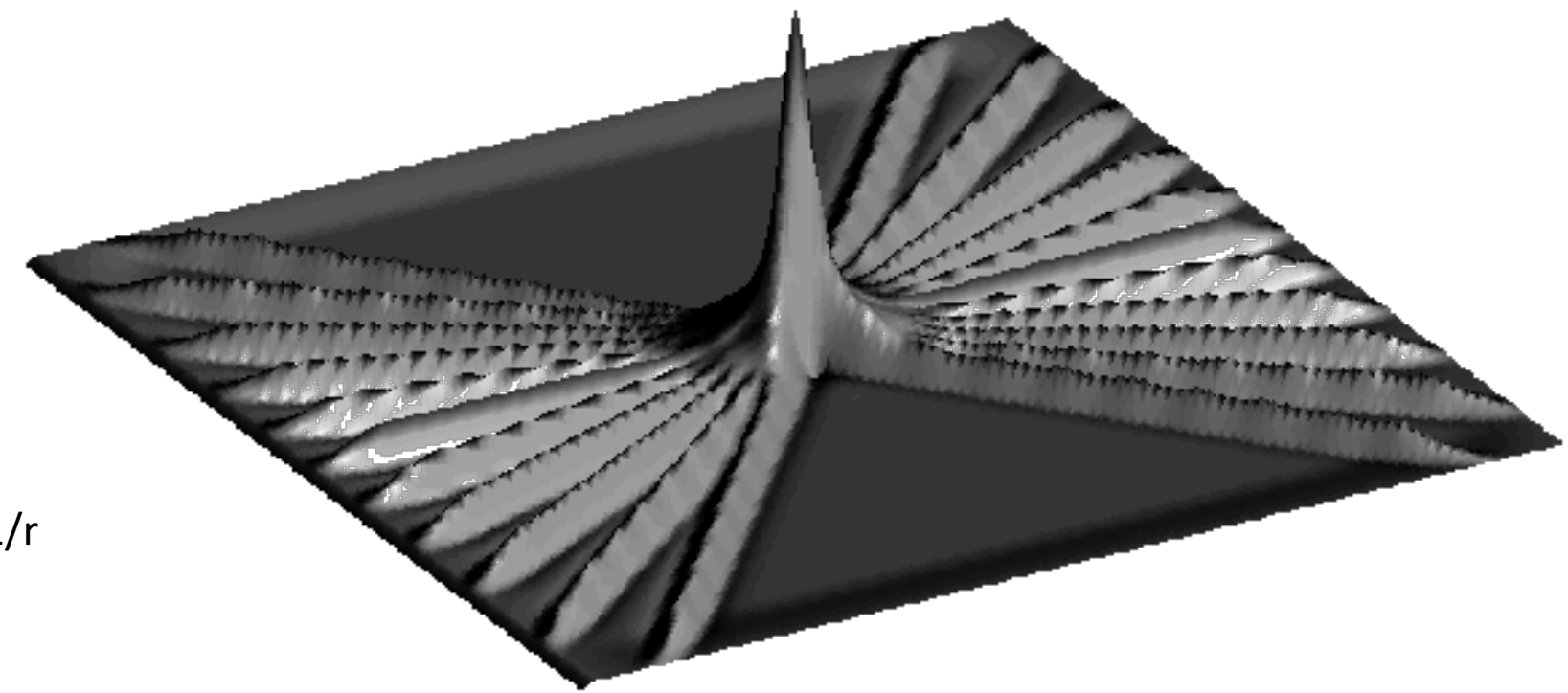
- Reconstruction by weighted back projection



Back-projection

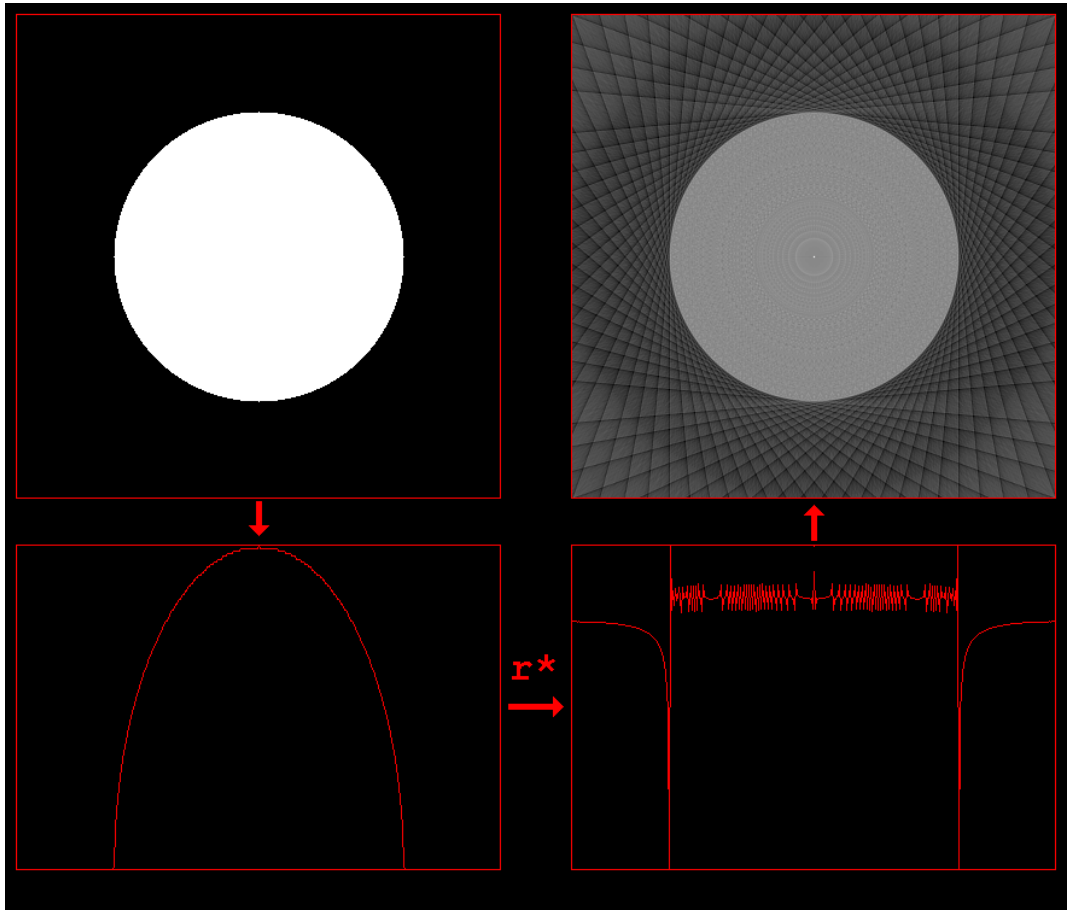


Back-projection



1/r

Back-projection



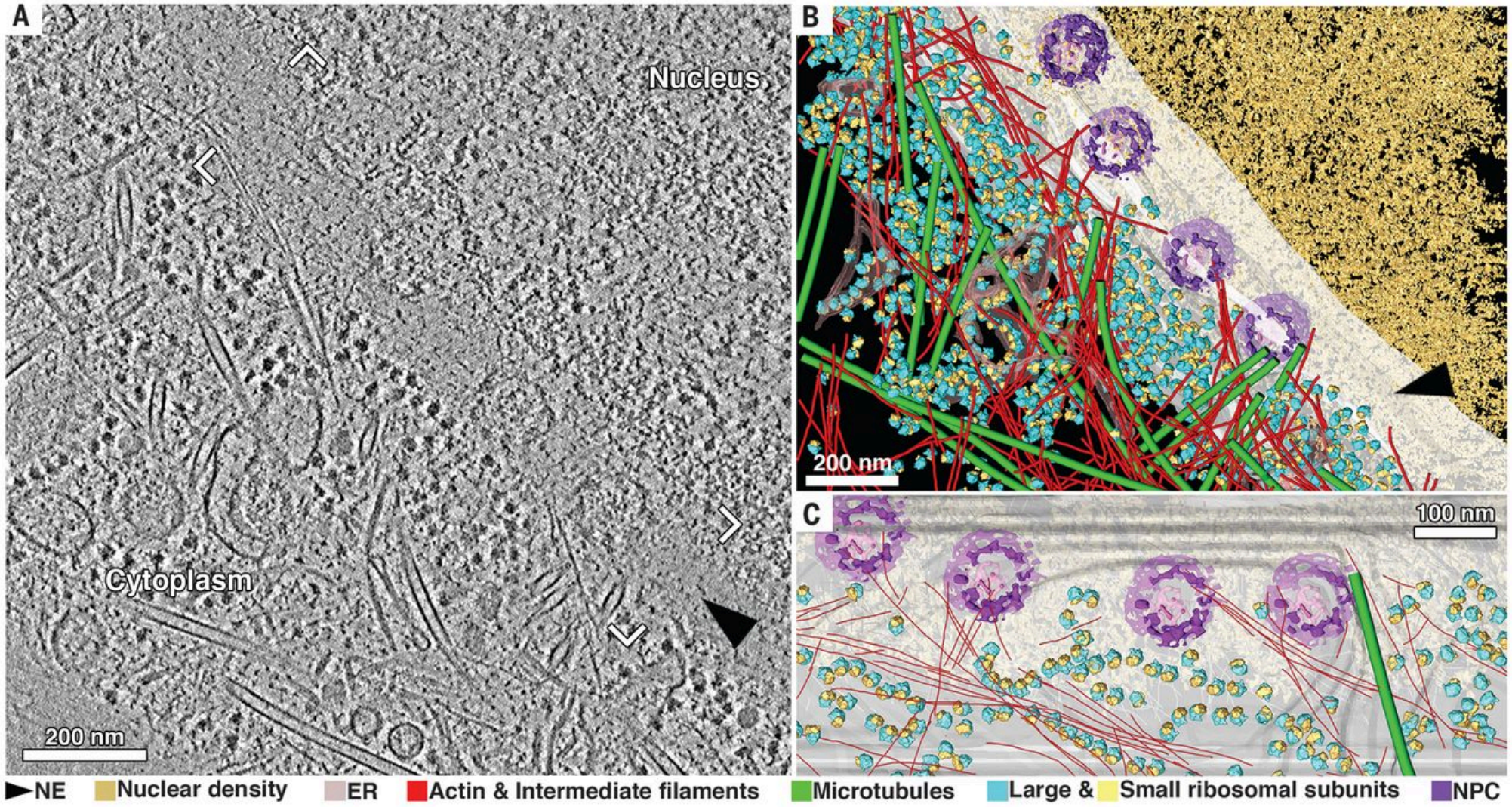
Software

Data Collection: **SerialEM**, FEI Tomo, Leginon…

Tomogram reconstruction: **IMOD**, TOM, protomo, PyTOM…

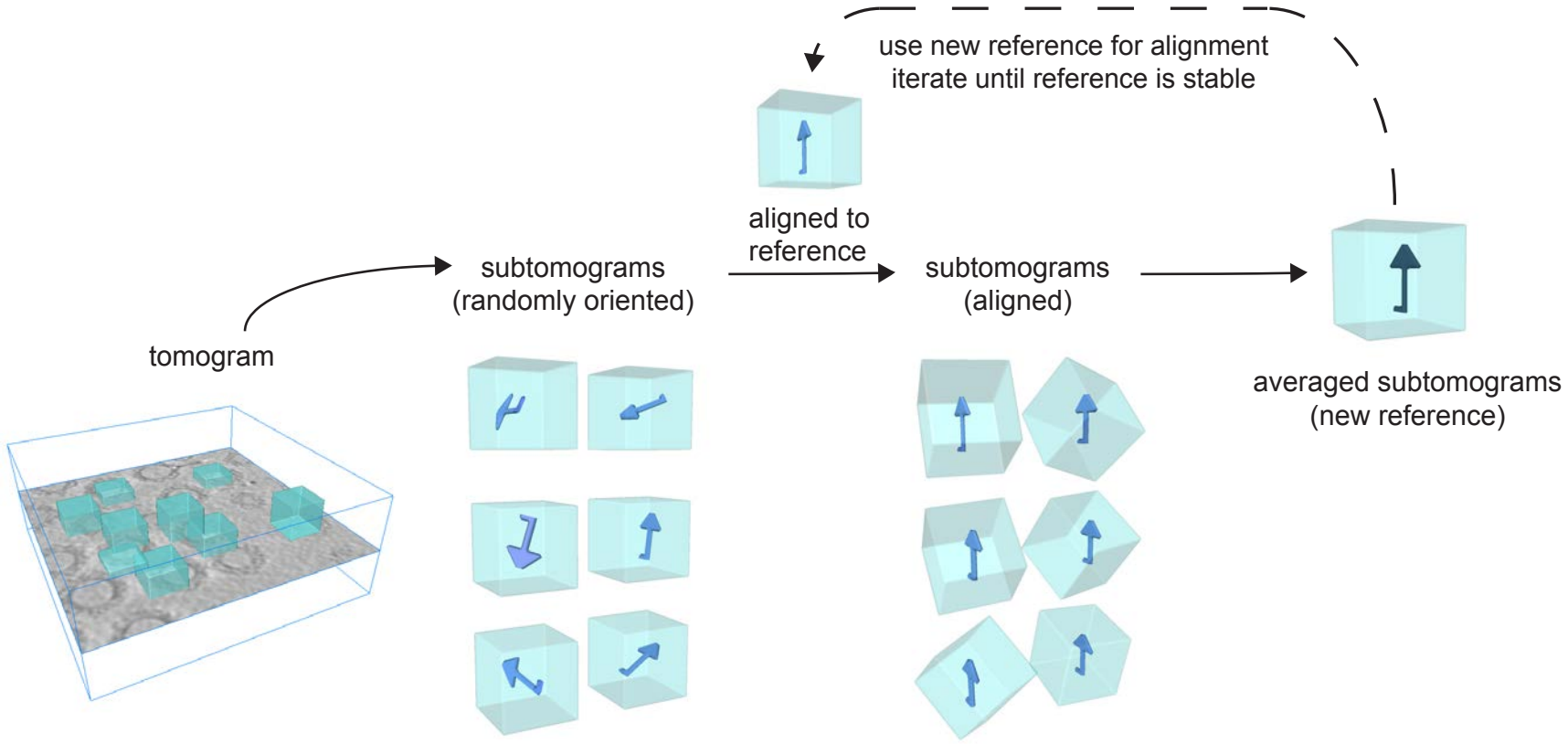
Subtomogram averaging

Subtomogram averaging

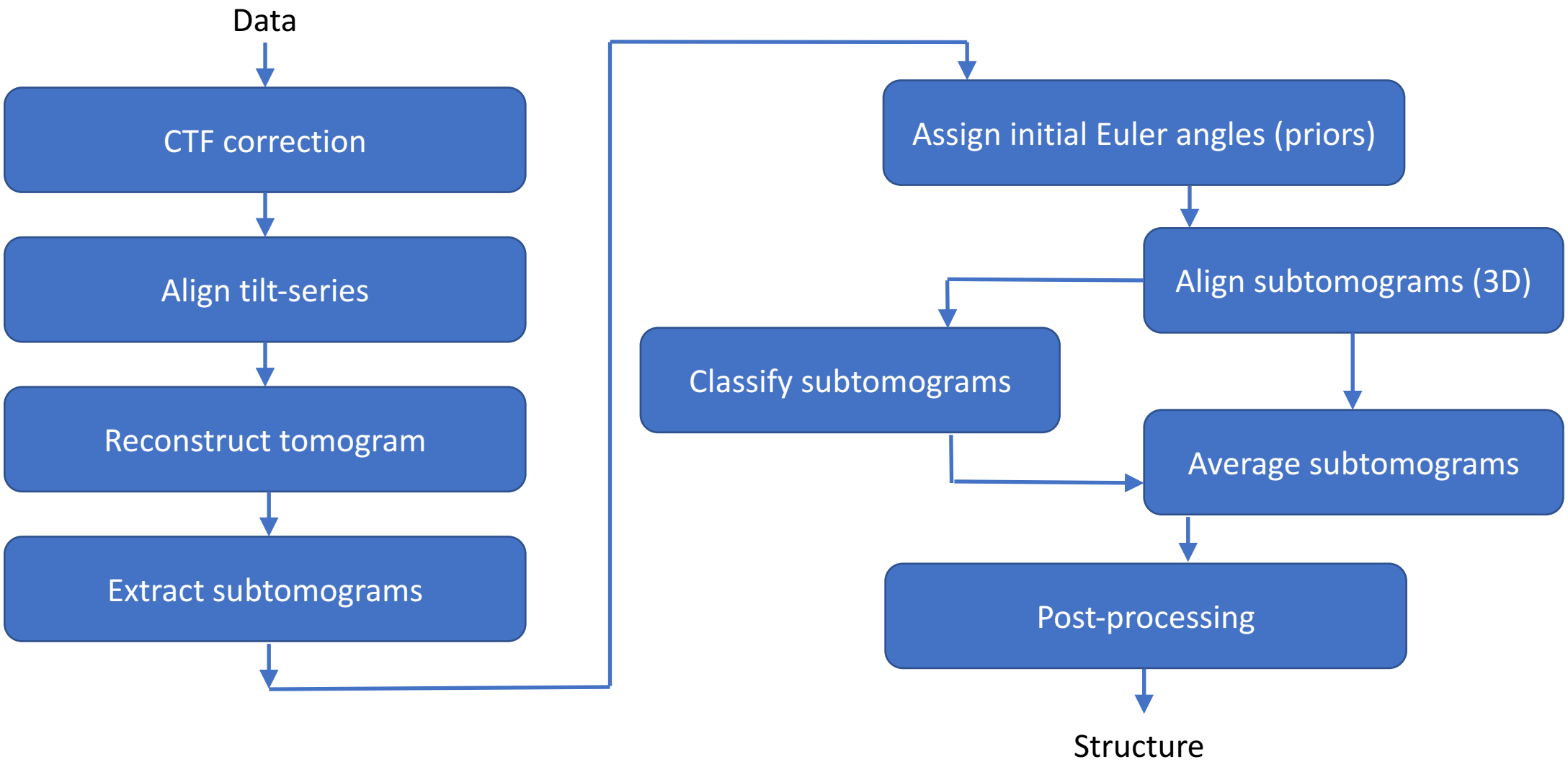


Mahamid et al, Science 2015

Subtomogram averaging



Subtomogram averaging process



Software for subtomogram averaging

Dynamo (Castano-Diez, Basel)

PEET (Heumann and Mastronarde, Boulder)

PyTOM (Foerster, Utrecht)

RELION (Bharat and Scheres, LMB)

Maximum cross-correlation or Maximum likelihood

Subtomogram averaging

Why not just do single particle reconstruction? (if you can, then do it!)

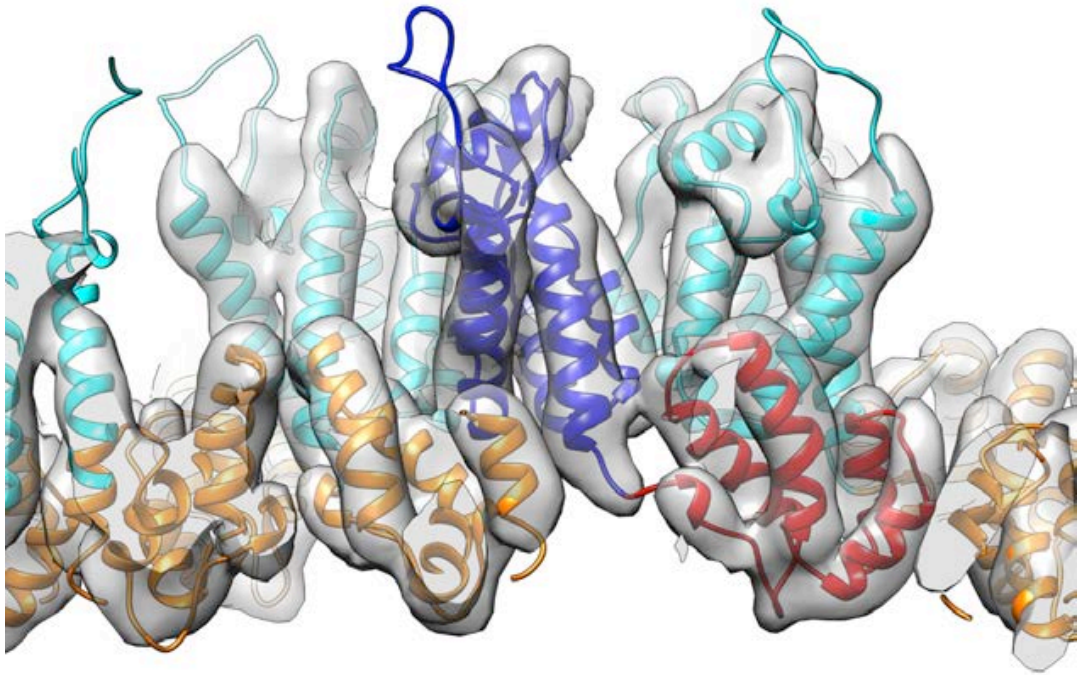
Subtomogram averaging allows structures to be determined when other objects in the path of the electron beam would otherwise prevent alignment.

(and has potential for application in single-particle type projects)

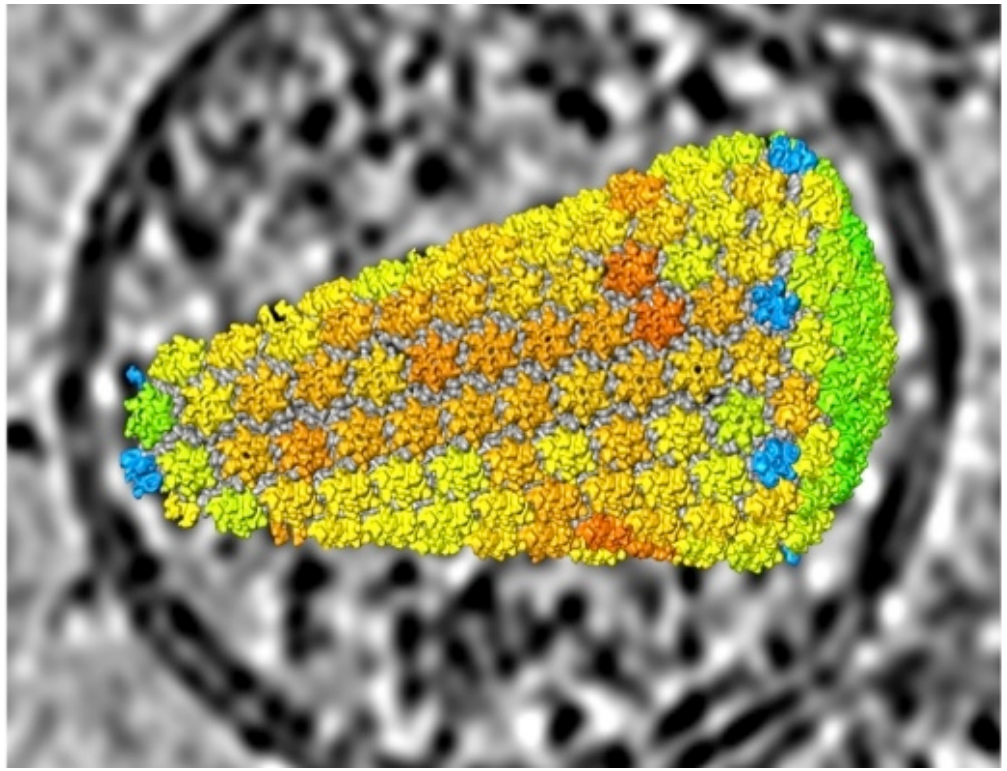
Subtomogram averaging

Can generate two kinds of information

Structure



Position and context



Subtomogram averaging - challenges

Sample flexibility and heterogeneity

Higher apparent sample thickness (especially at tilt)

Two alignment and reconstruction steps

Sample movement/change during data collection

Higher total electron dose

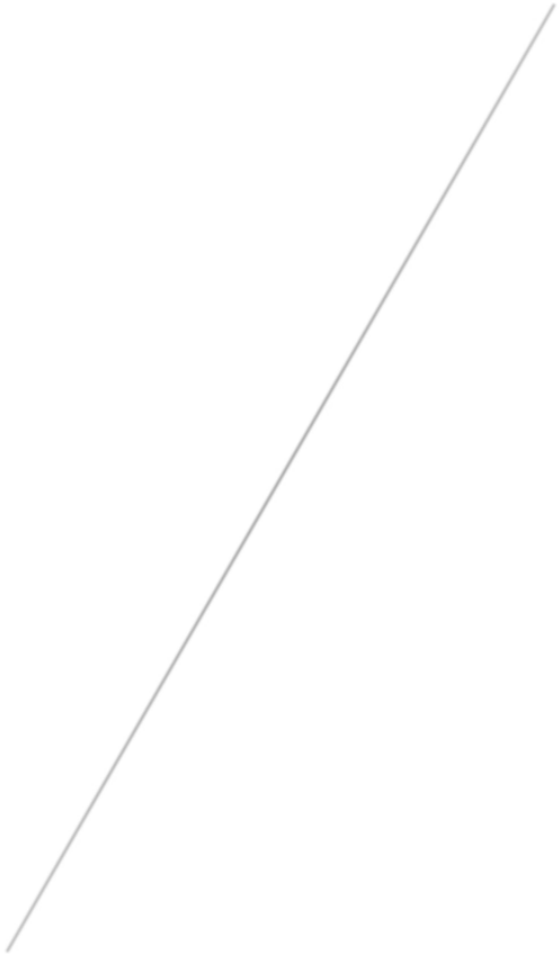
Smaller datasets (due to more time-consuming data collection and processing)

Difficult in determination and correction of CTF

Subtomogram averaging - challenges

In which order should we collect the images?

Continuous tilt scheme



Bidirectional tilt scheme

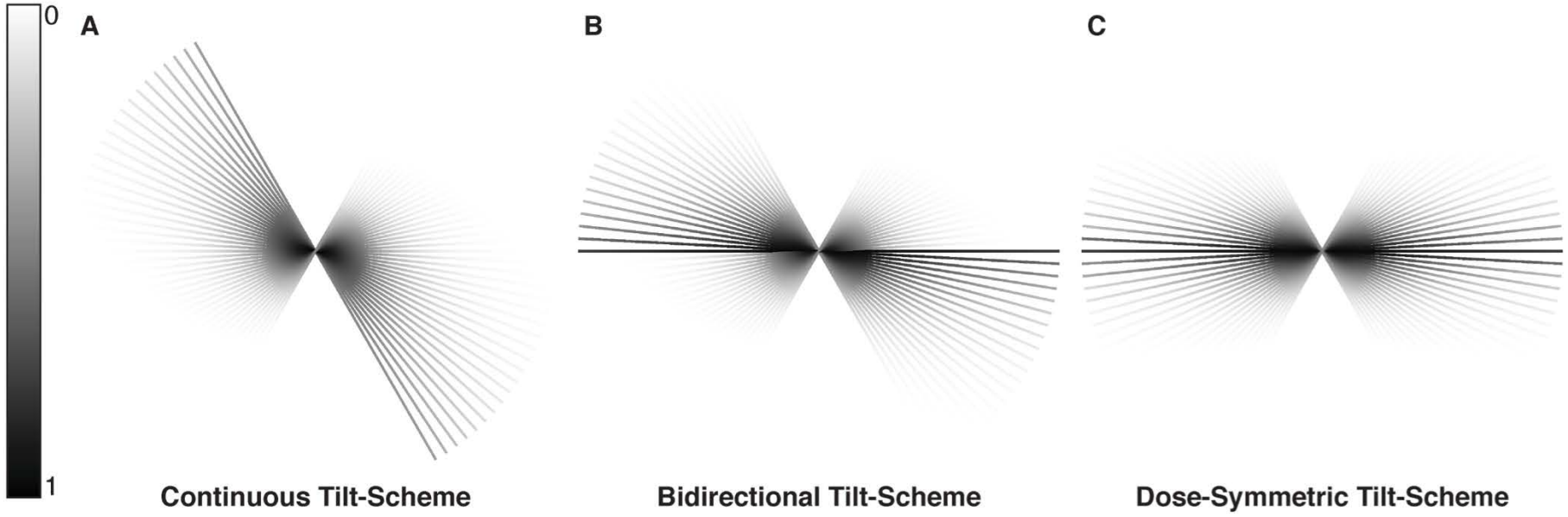
Dose-symmetric tilt scheme

Tilt schemes

Continuous

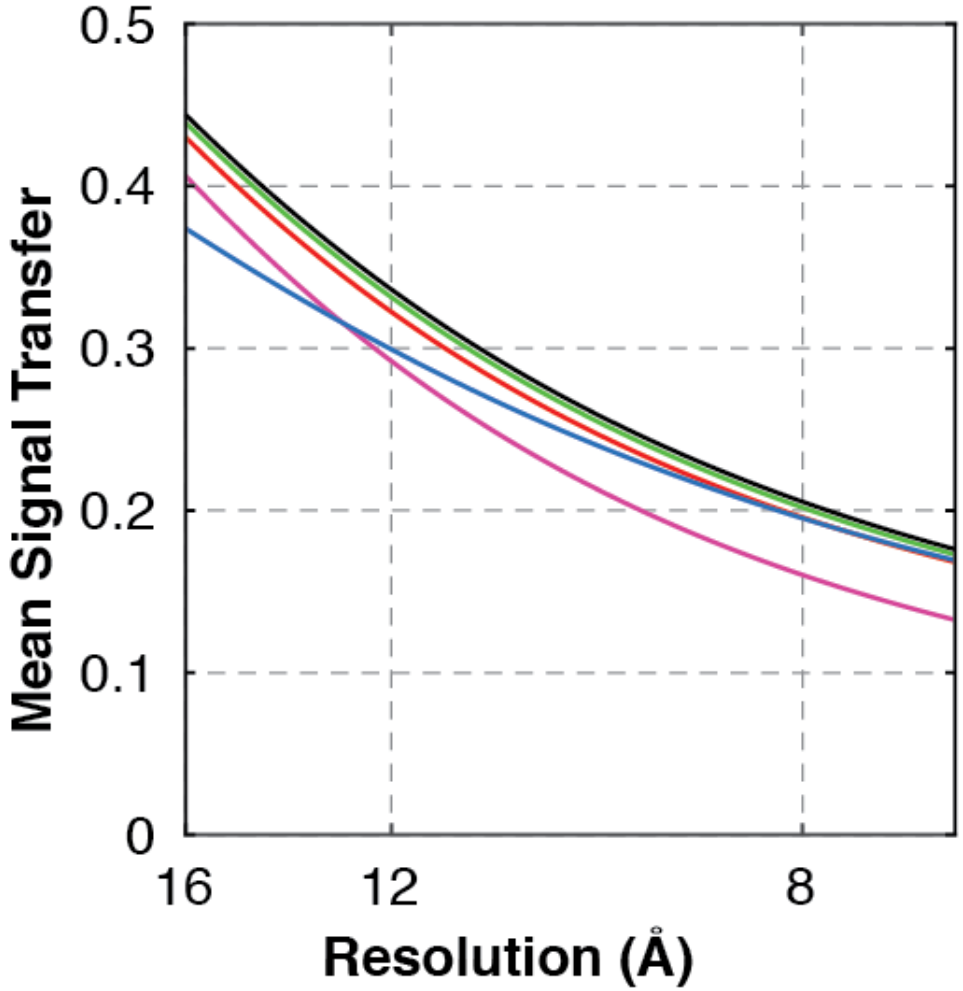
Bidirectional from 0

Dose-symmetric



Hagen et al. JSB 2017

Tilt schemes – signal transfer



Continuous

Bidirectional from 0

Bidirectional from -21

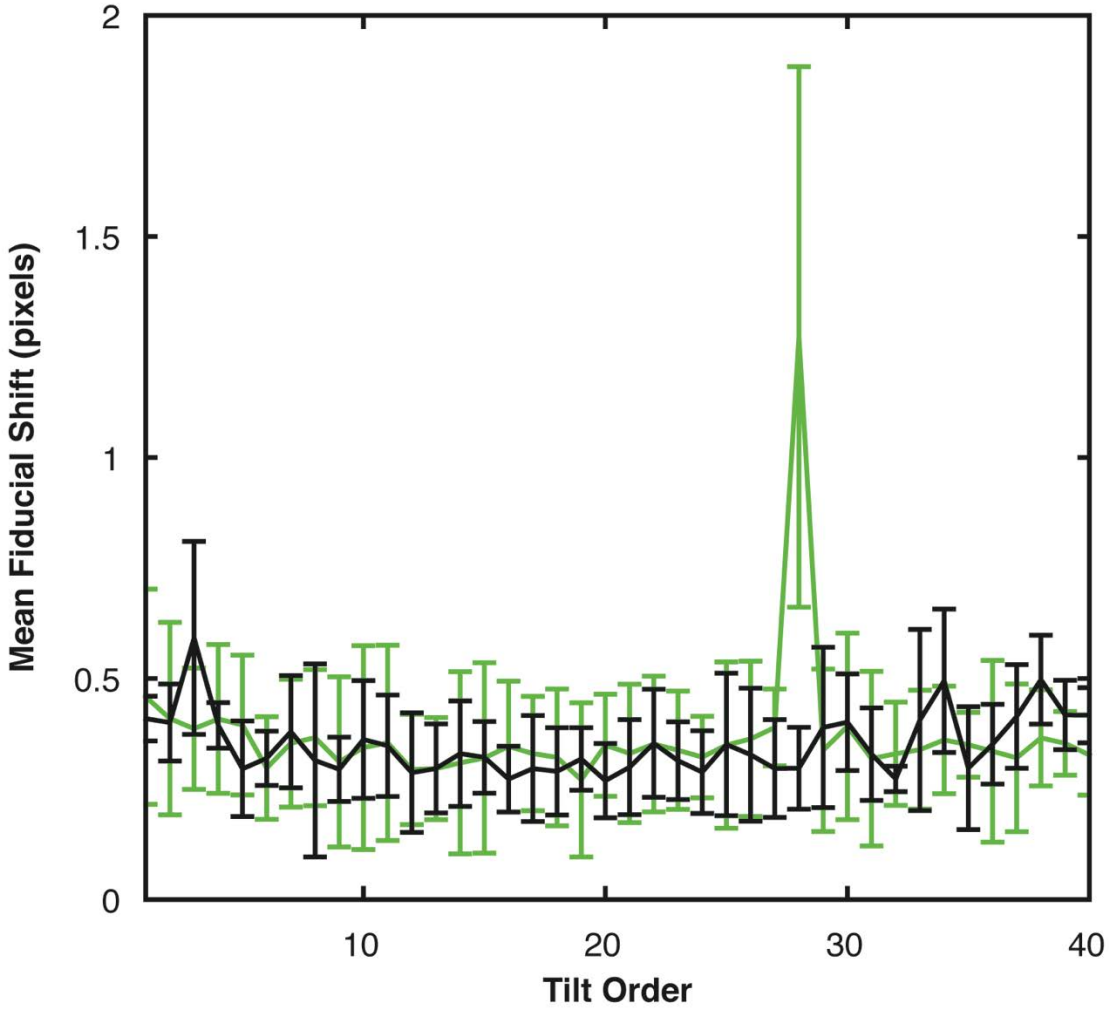
Bidirectional from -21
deleting second branch

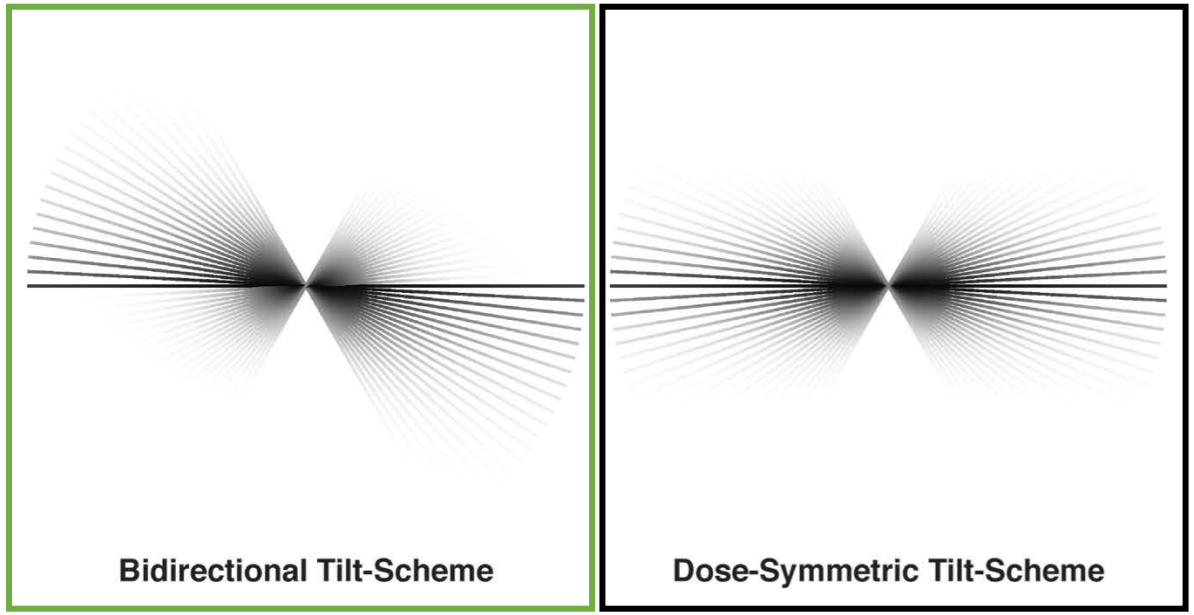
Dose-symmetric

Note – the difference is greater if you also consider increased sample movement at tilt

Hagen et al. JSB 2017

Dose-dependent sample changes



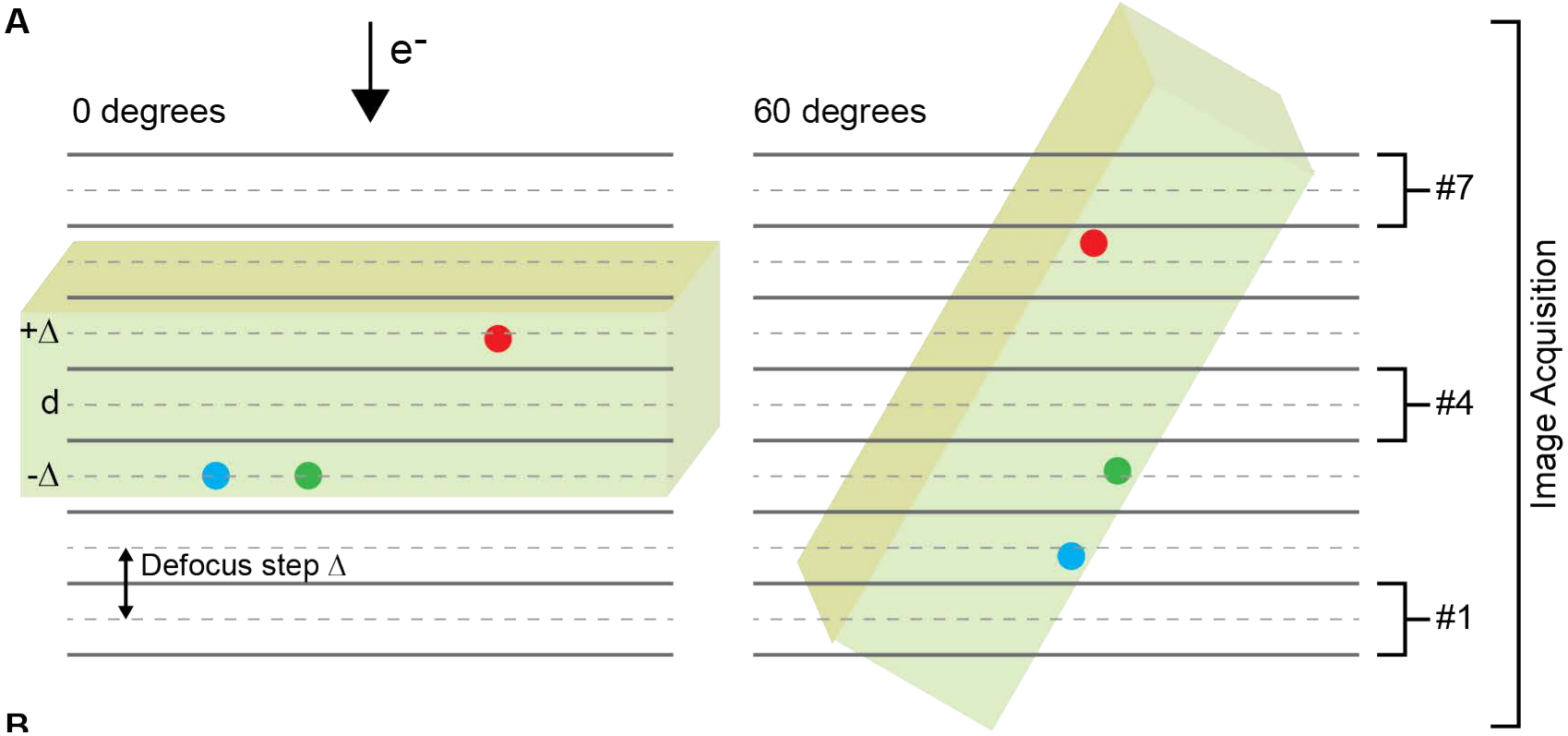


Hagen et al. JSB 2017

Subtomogram averaging - challenges

CTF Correction

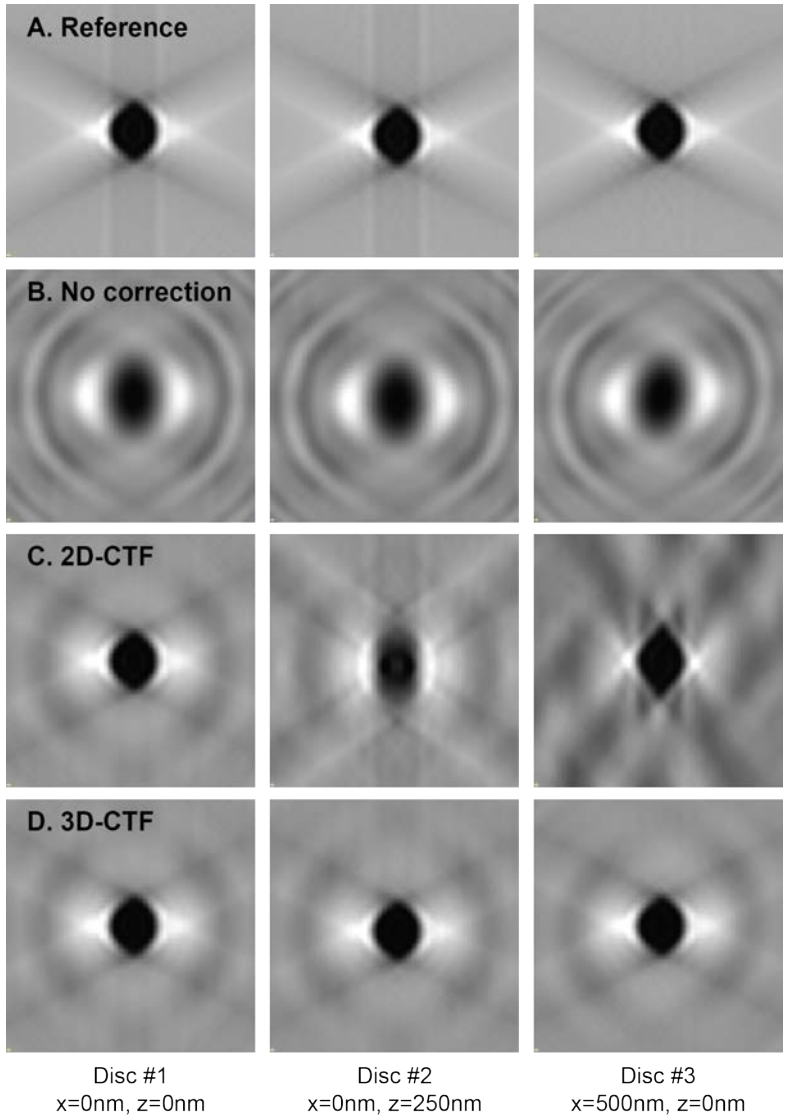
Defocus gradients in the sample



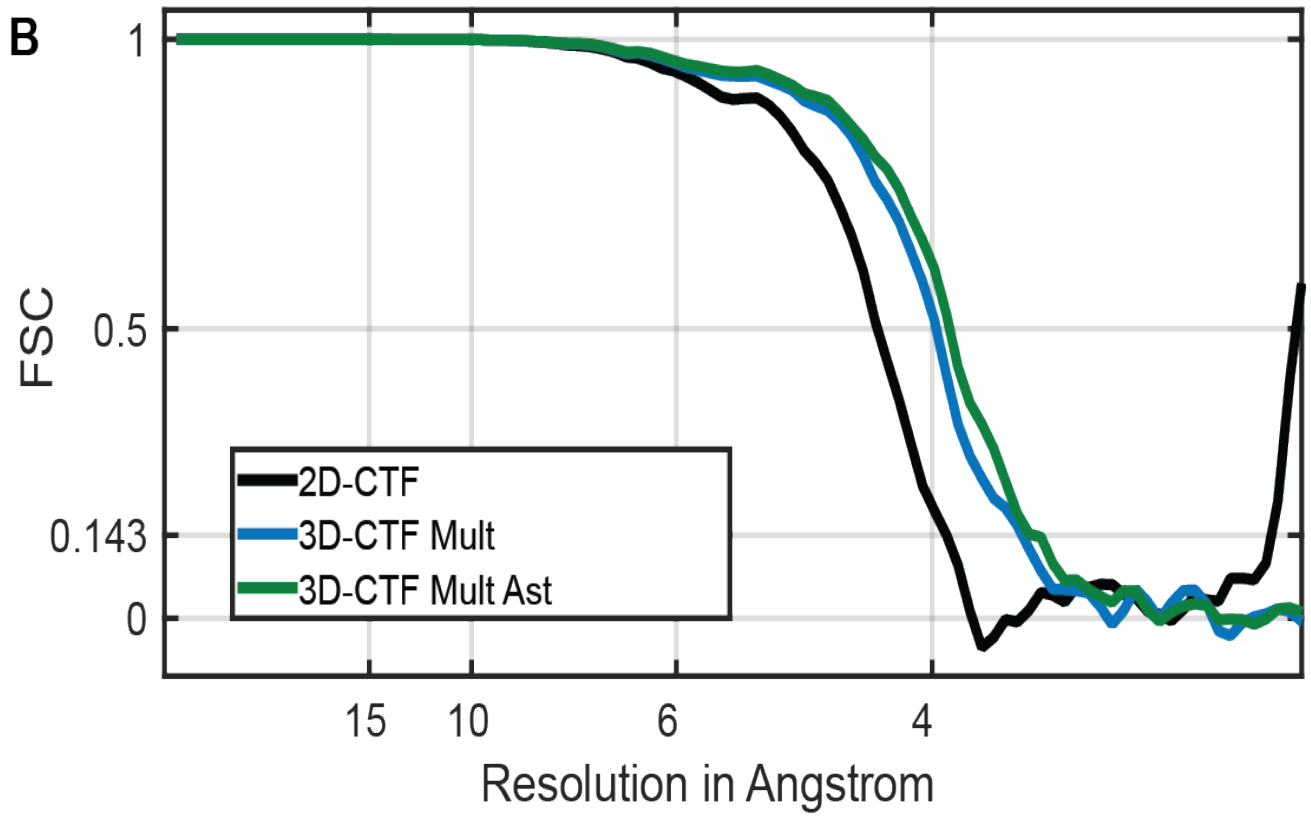
Turonova. et al

2D CTF correction considers only the gradient due to tilt, 3D CTF also considers the gradient through the thick sample

Defocus gradients in the sample



Defocus gradients in the sample



Turonova. et al

Subtomogram averaging - challenges

Sample flexibility and heterogeneity

Higher apparent sample thickness (especially at tilt)

Separate alignment and reconstruction steps

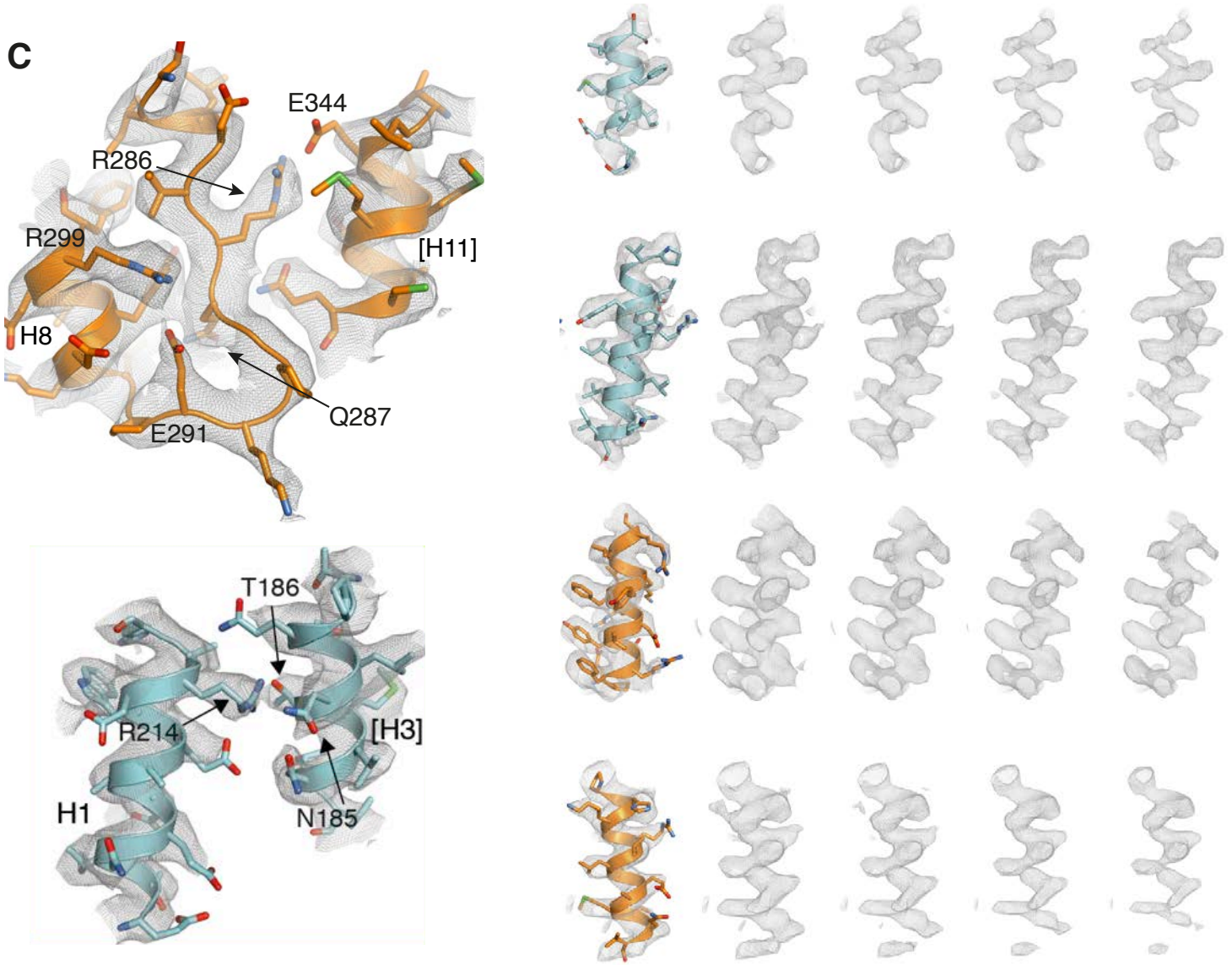
Sample movement/change during data collection

Higher total electron dose

Smaller datasets (due to more time-consuming data collection and processing)

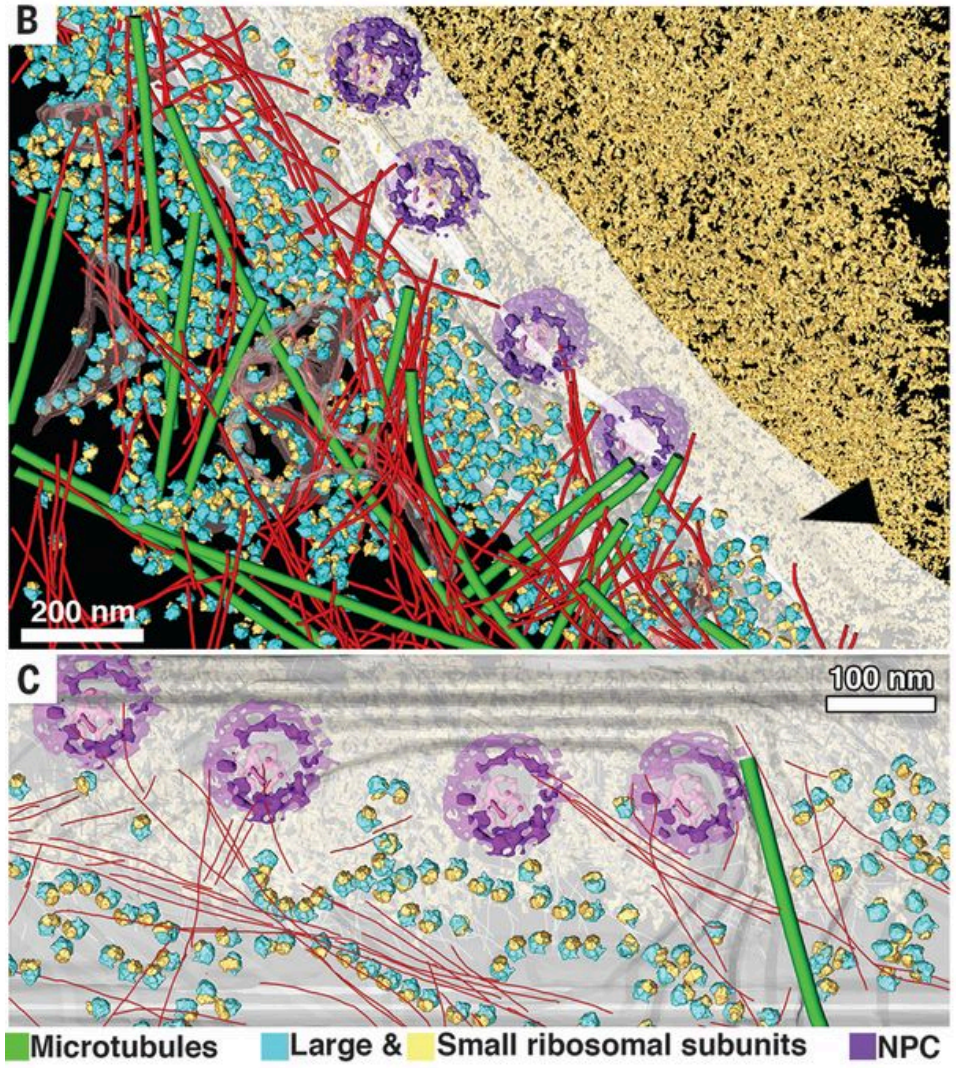
Difficult in determination and correction of CTF

It is possible to obtain <4Å resolution

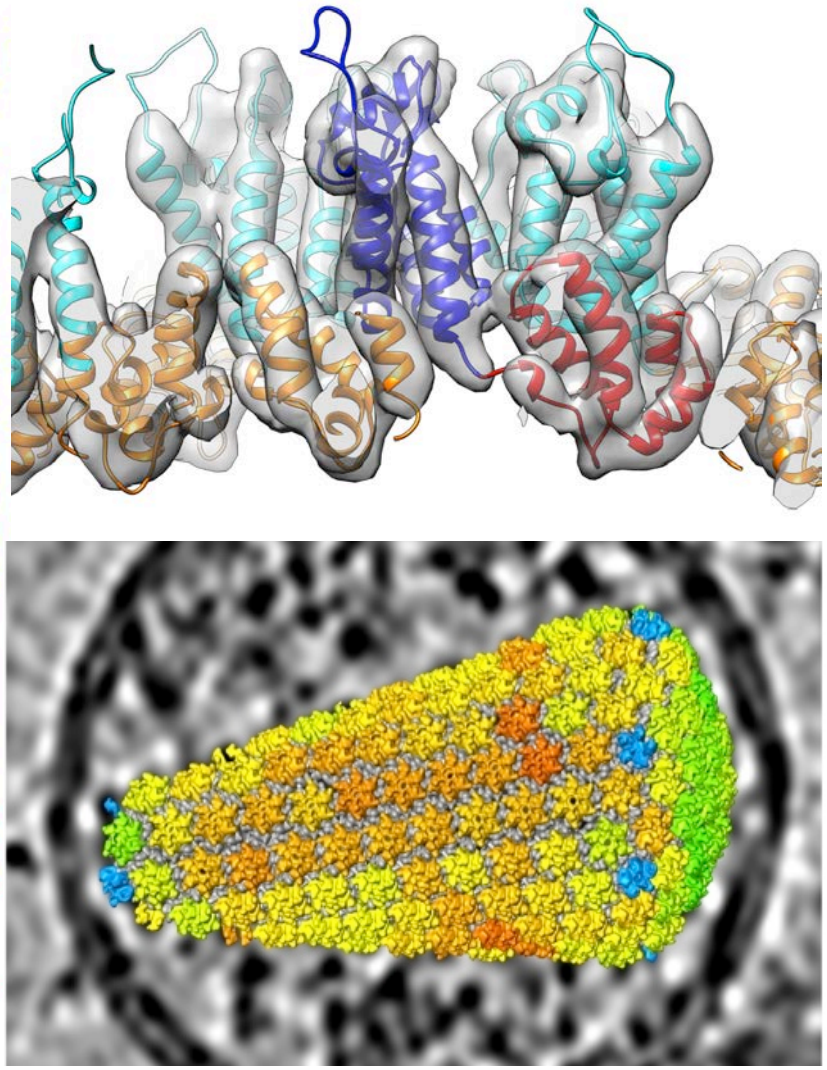


Schur et al. Science 2016

Subtomogram averaging



Mahamid et al, Science 2015



Mattei et al. Science 2016

Structure “in situ”

Cellular context

Structure for “Discovery”

Potential SP applications

Wan, W. and Briggs, J.A.G., “Cryo-electron tomography and subtomogram averaging” (2016) Methods in Enzymology, 579, 329-367

and articles by many authors cited therein