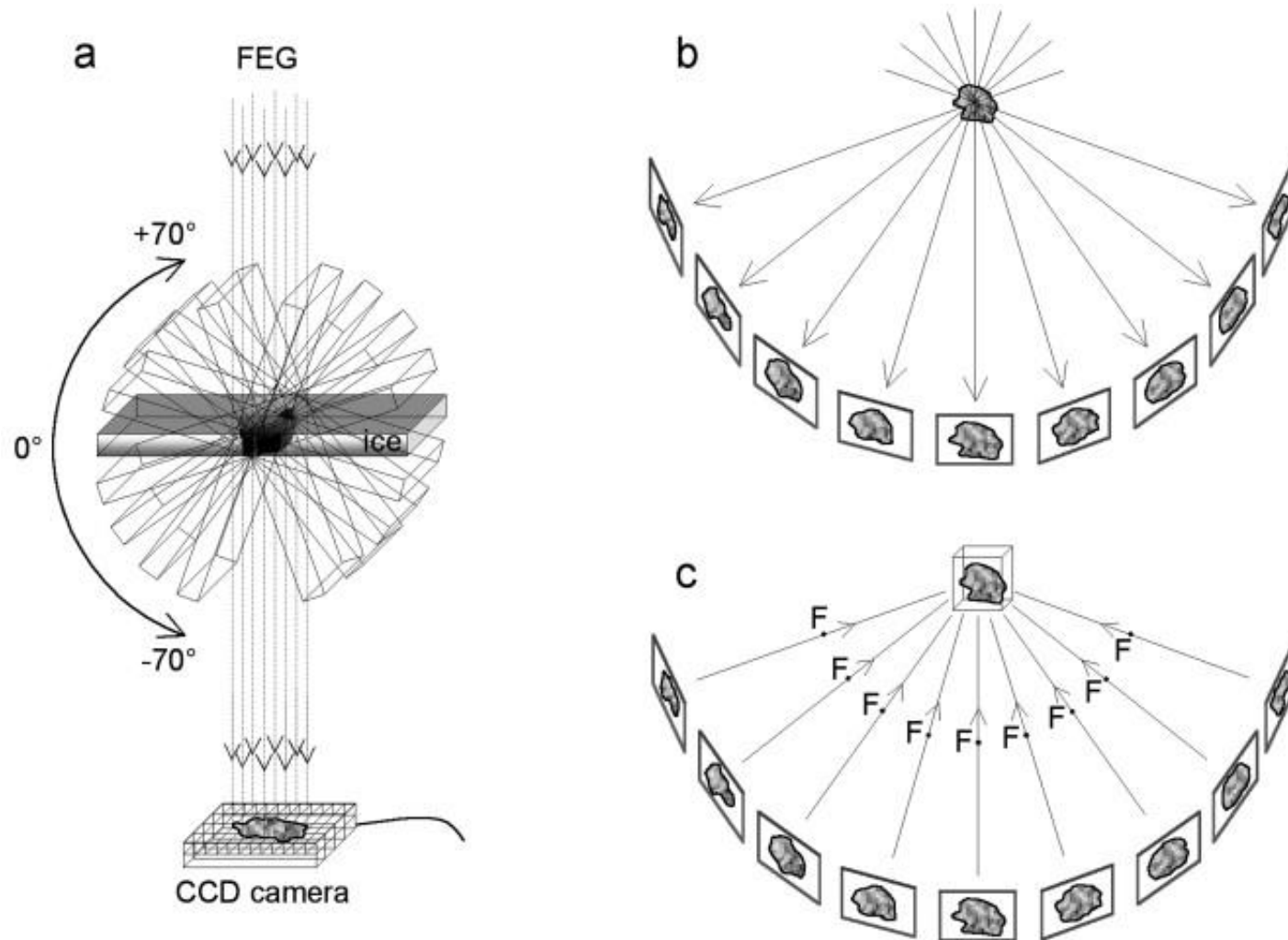# Cryo-Electron Tomography:
Image Processing & Interpretation

Nadav Elad

26 Nov 2020
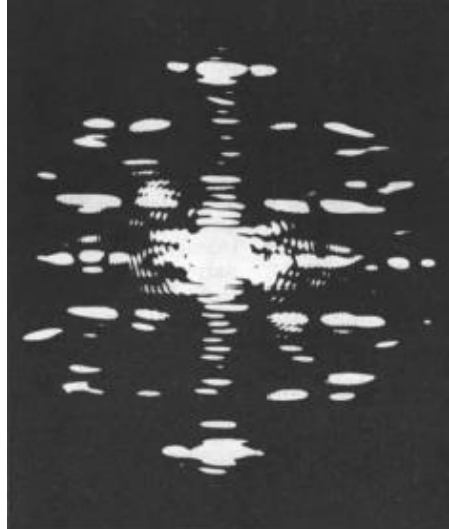
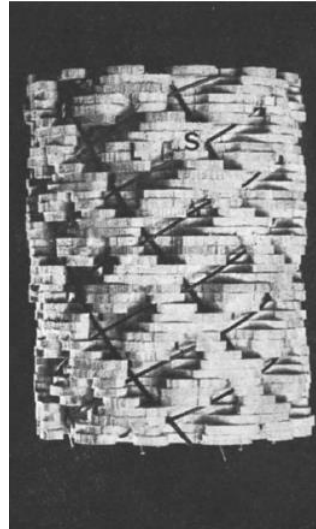**IMpaCT online tomography lecture course**

Tomography
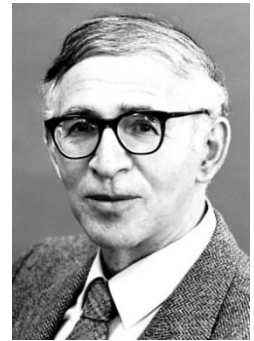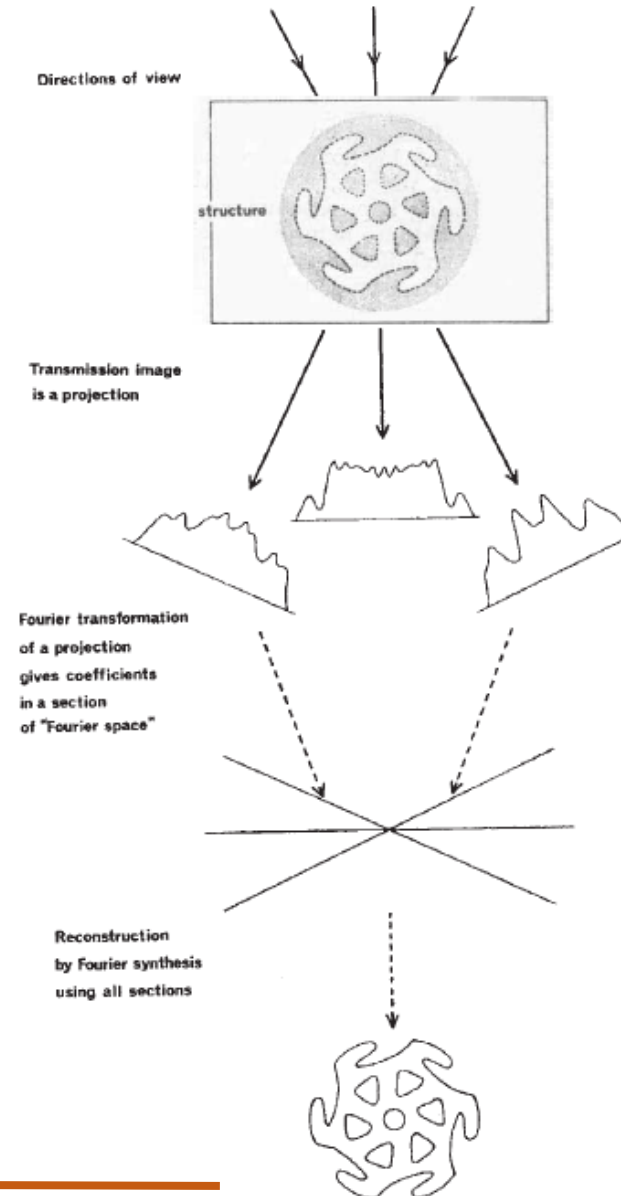
Principle of 3D reconstruction from EM micrographs

TEM projection

Fourier transform

3D map

Directions of view

structure

Transmission image is a projection

Fourier transformation of a projection gives coefficients in a section of "Fourier space"

Reconstruction by Fourier synthesis using all sections

DeRosier and Klug (1968) Nature

Tomogram reconstruction from tilt series

- Alignment by cross correlation
- Fiducial model
- Accounting for distortions
- Weighted back projection
- The projection theorem
- SIRT

Raw tilt series

- Titan Krios @WIS
- 300 kV
- K3 direct detector
- Energy filter with 20eV slit
- SerialEM
- +60° to -60°, 2° increment
- Dose symmetric tilt scheme
- 81k magnification, 1.1 Å/pixel
- Dose/tilt: 2.46 e/Å
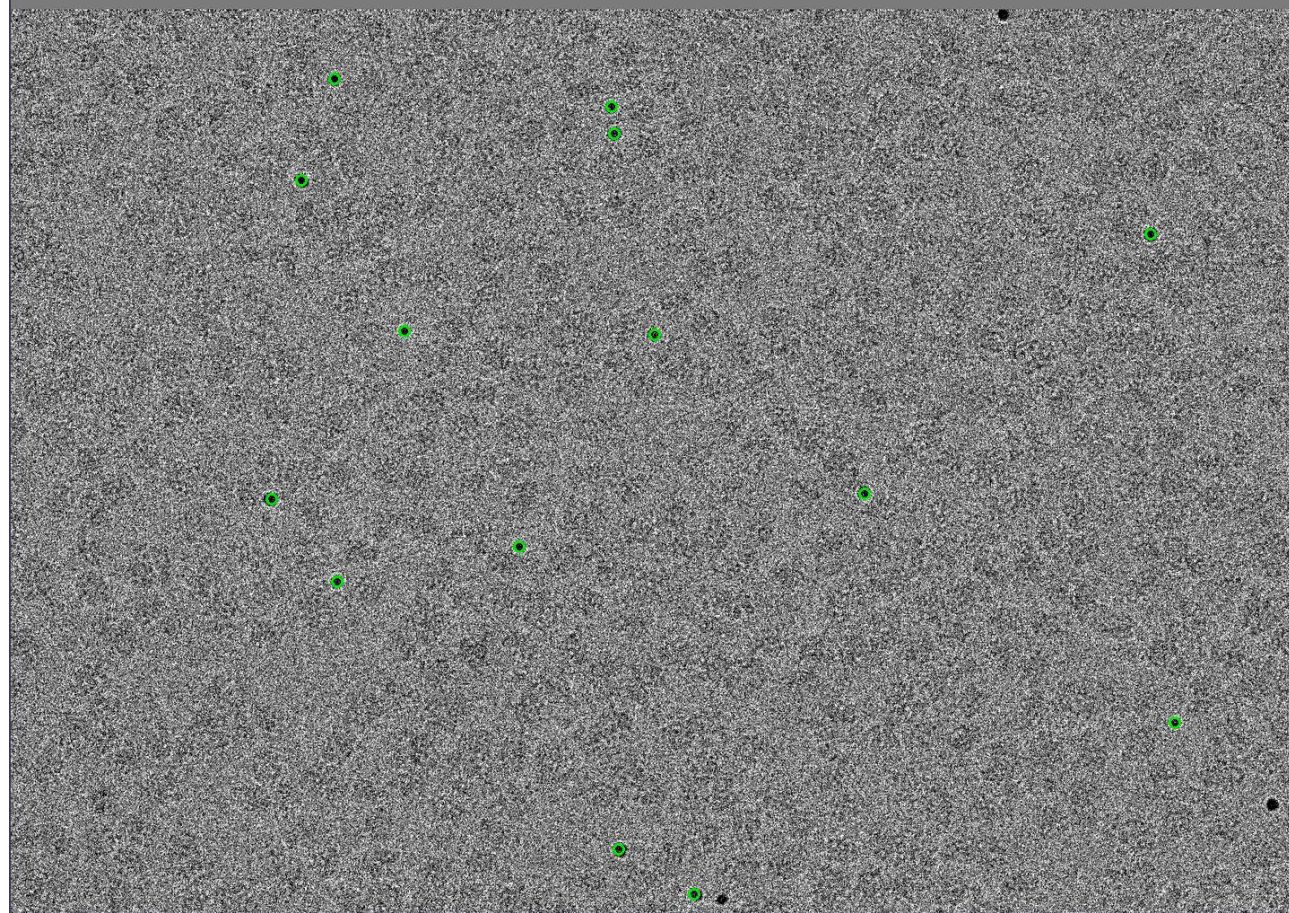- Total dose: 150 e/Å
- 1.8 µm defocus
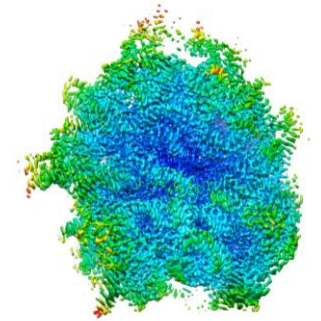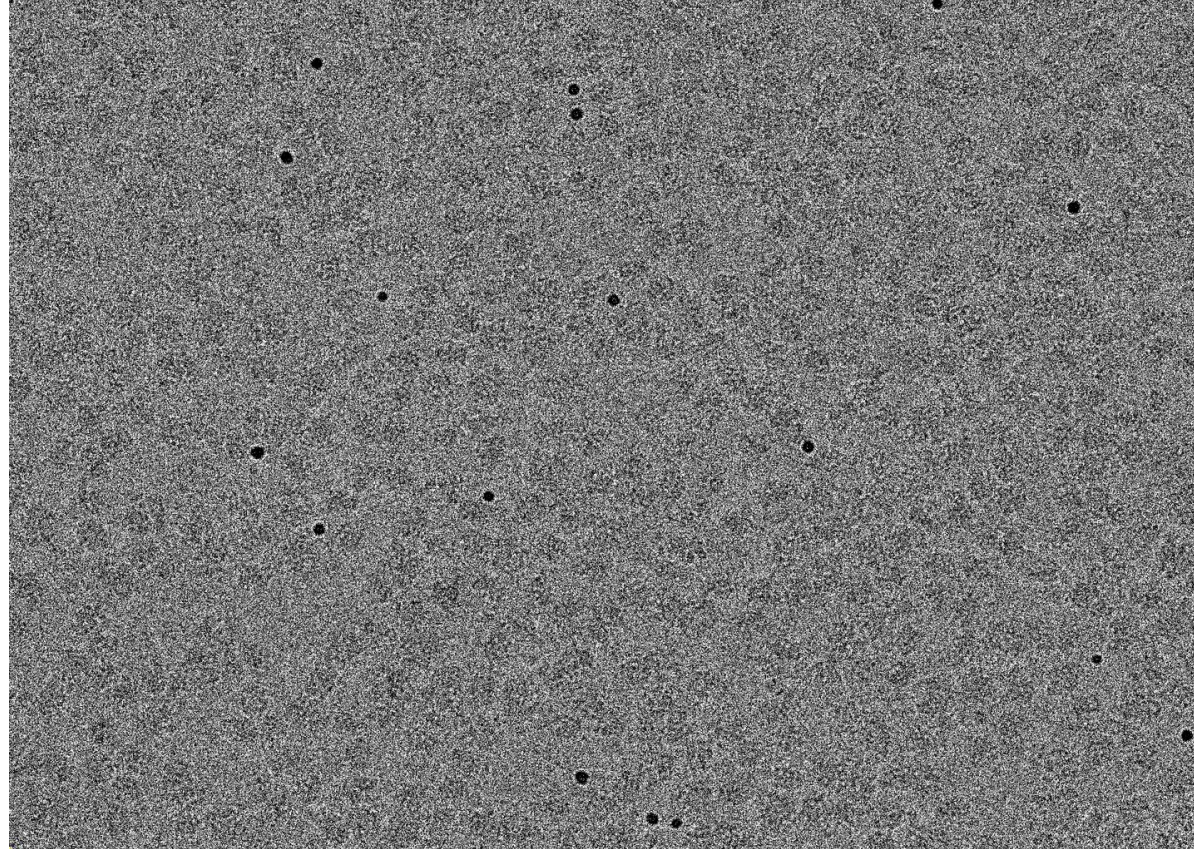
S. Aureus 70S ribosome from
Elinor Breiner-Goldstein
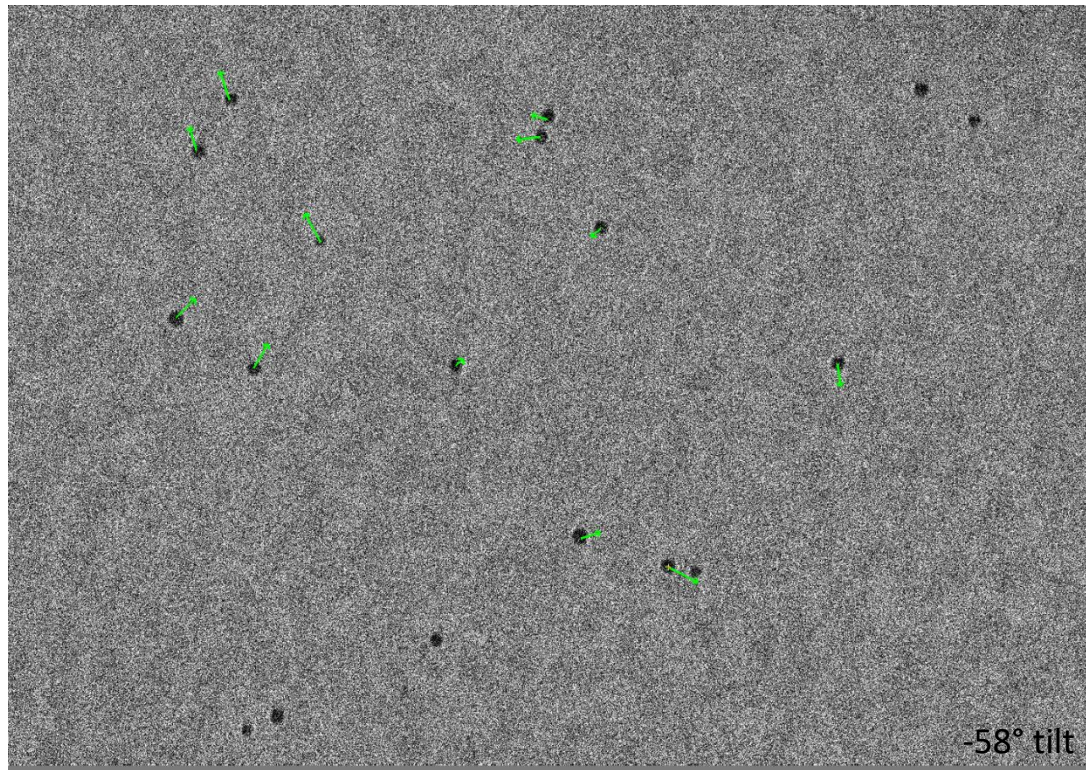*Yonath group*

Alignment of tilt series by cross correlation

IMOD software https://bio3d.colorado.edu/imod/
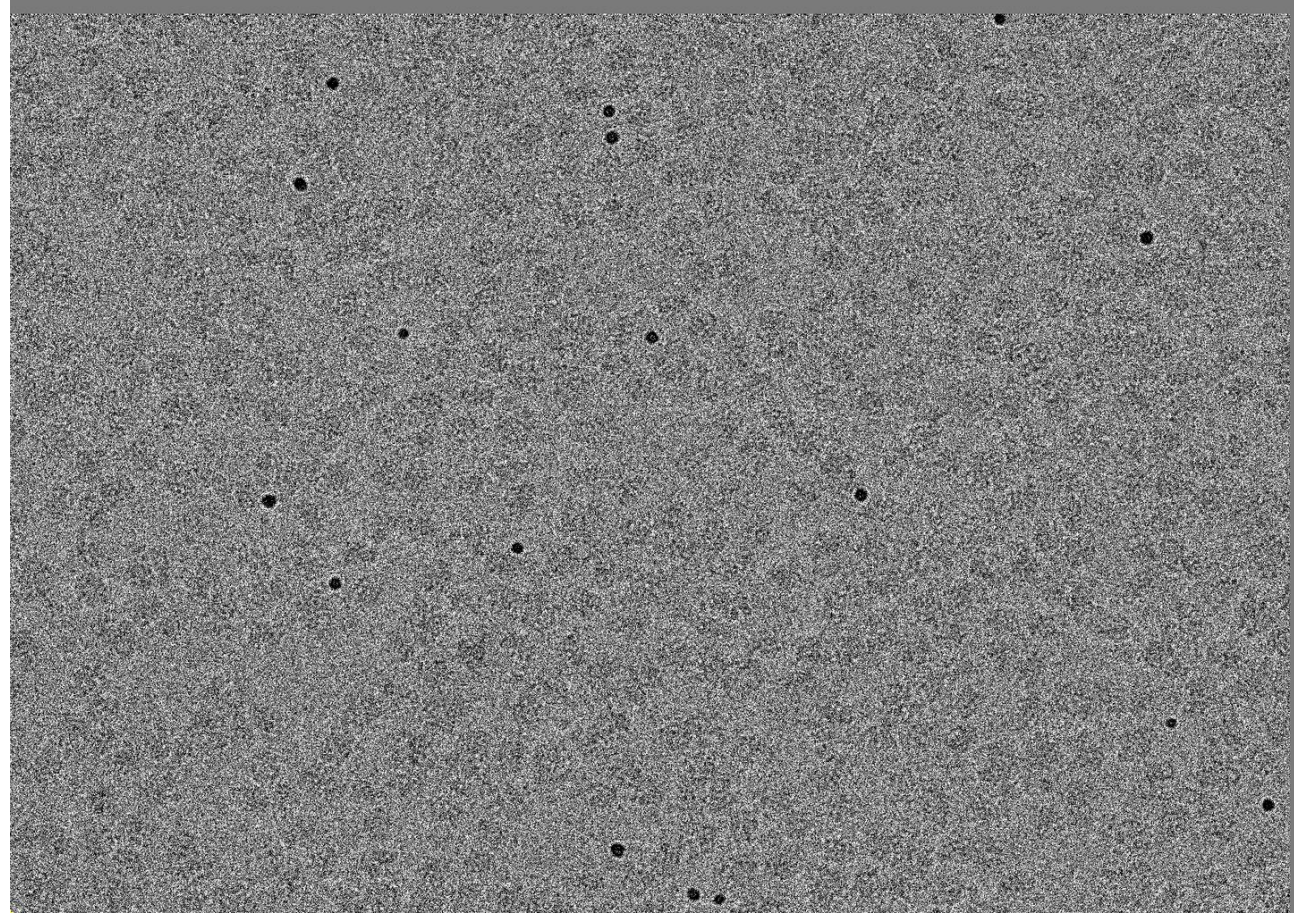
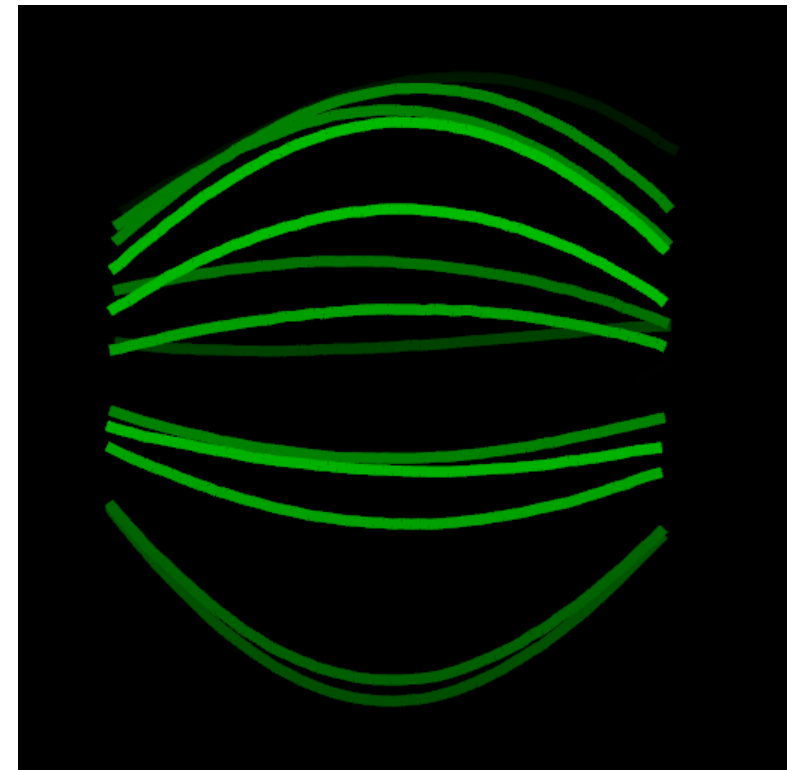# Alignment of tilt series with fiducial markers

Fiducial model

Residual vectors

-58° tilt

mean residual error 0.479, sd 0.324 nm
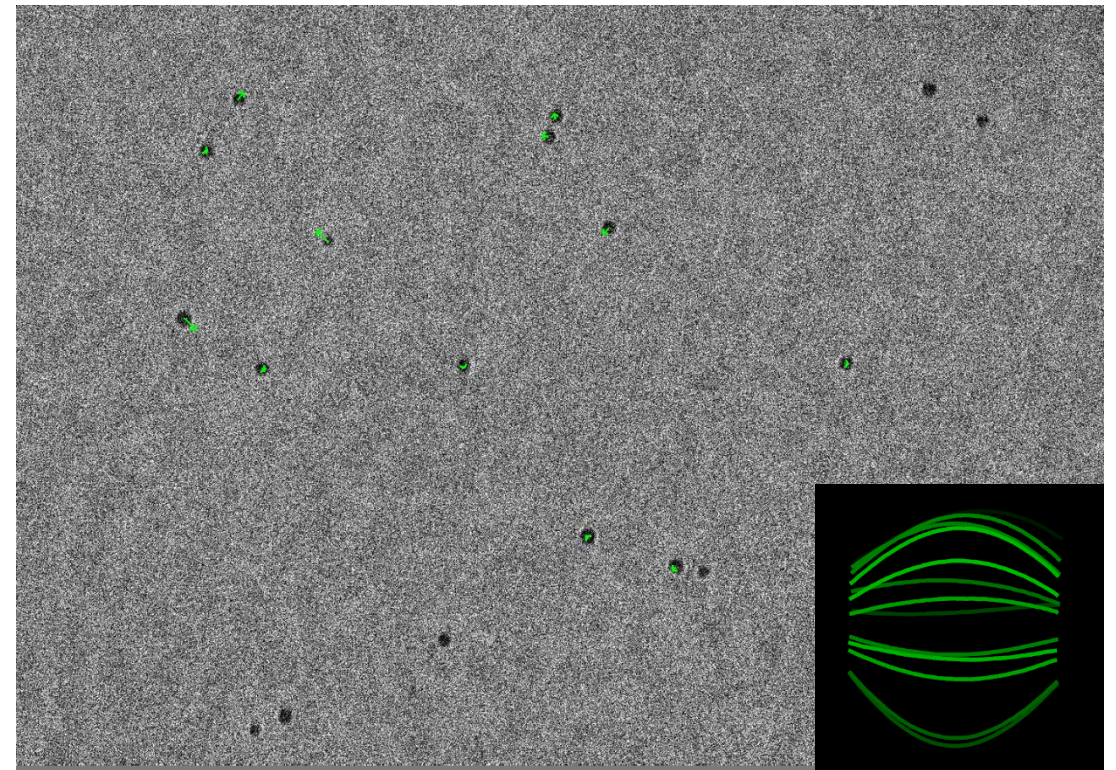
Fiducial model

Fiducial model - accounting for distortions

No corrections
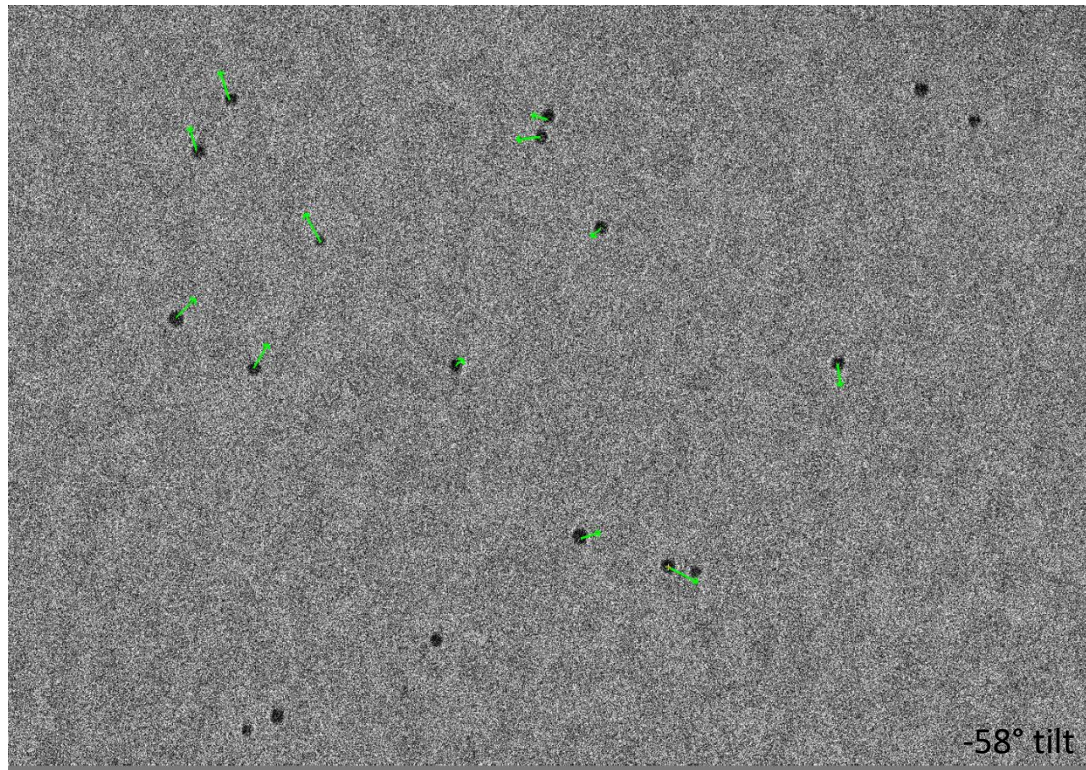
With correction:
Rotation, magnification, tilt angle, image distortions

-58° tilt

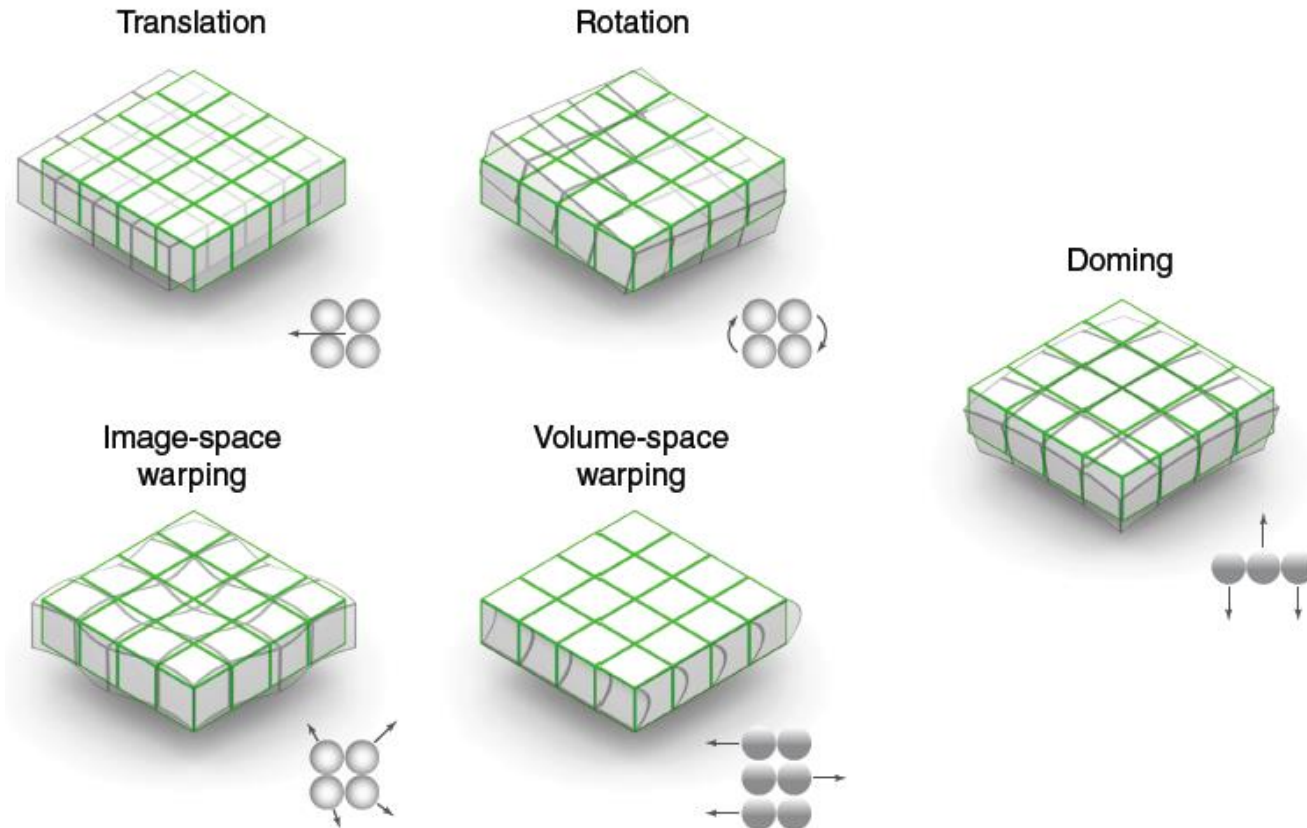Mean residual error 0.479, sd 0.324 nm

Mean residual error 0.294, sd 0.164 nm

Accounting for distortions

Fernandez et al (2018) *JSB*. Cryo-tomography tilt-series alignment with consideration of the beam-induced sample motion
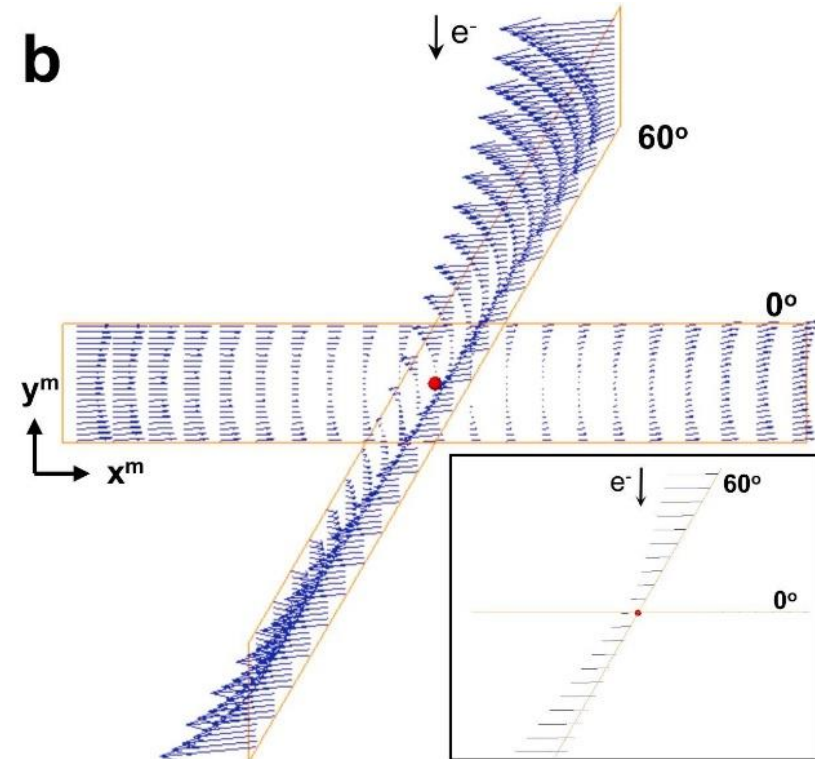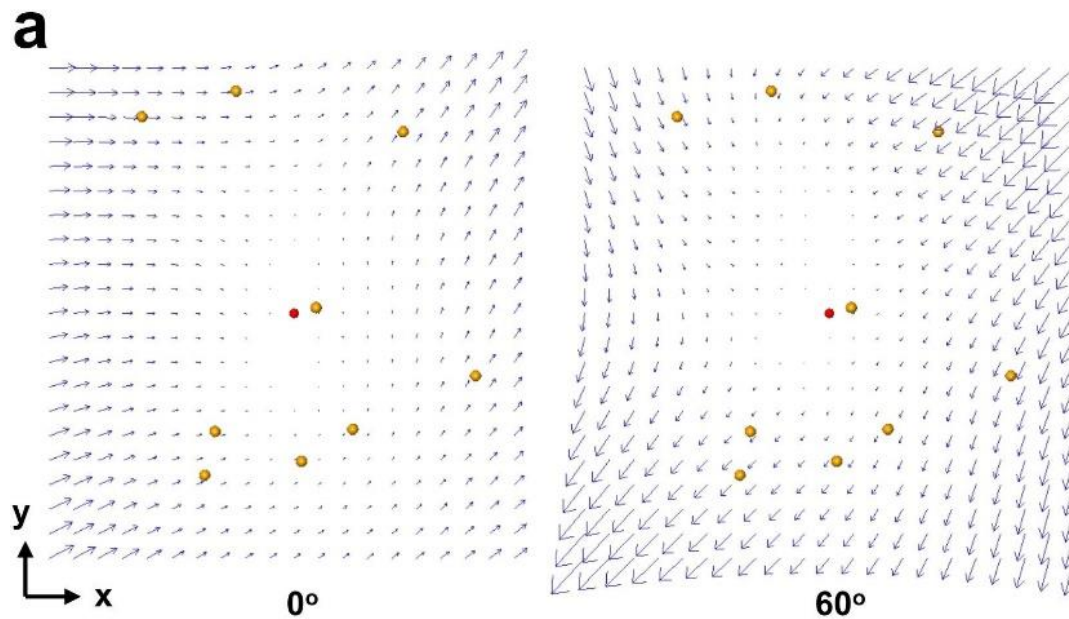
TomoAlign software
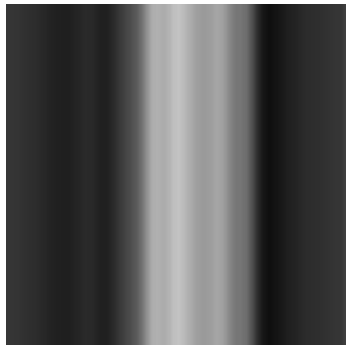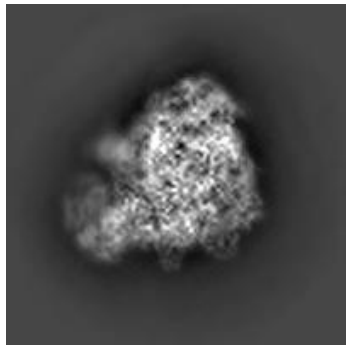
Accounting for distortions

Tegunov et al (2020) *BioRxiv.* Multi-particle cryo-EM refinement with M visualizes ribosome-antibiotic complex at 3.7 Å inside cells
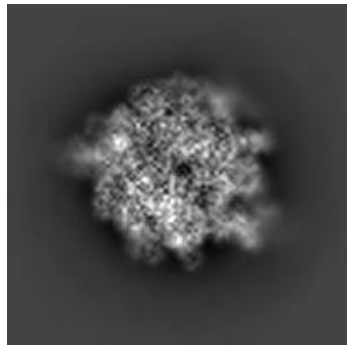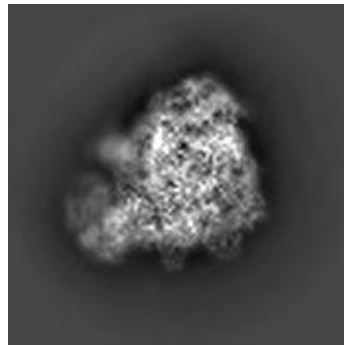
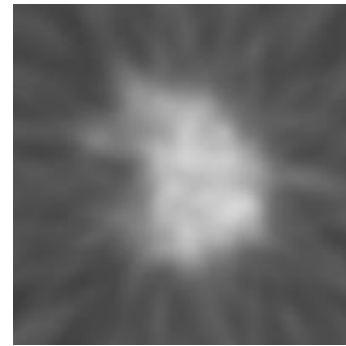# Back-projection

(b)
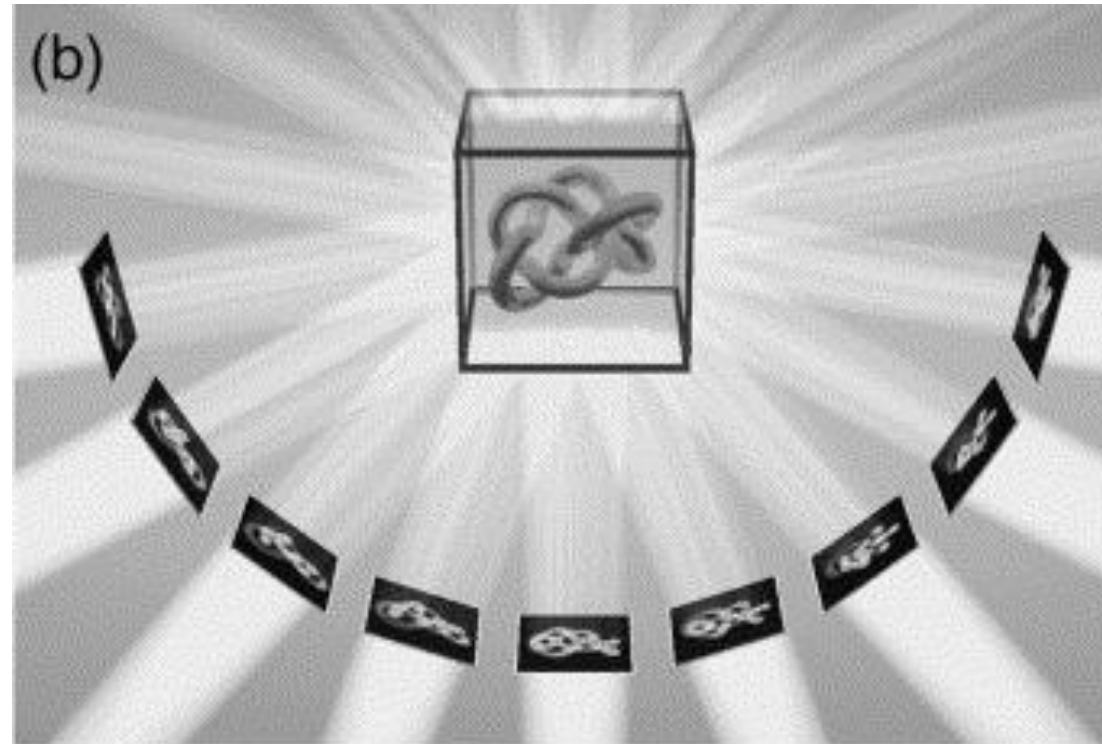
Back-projection

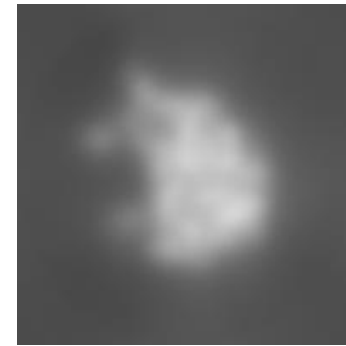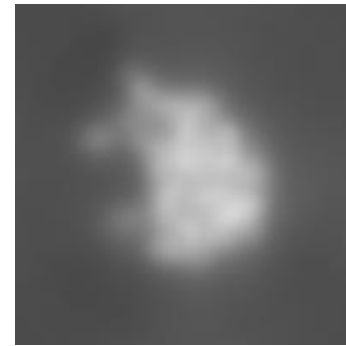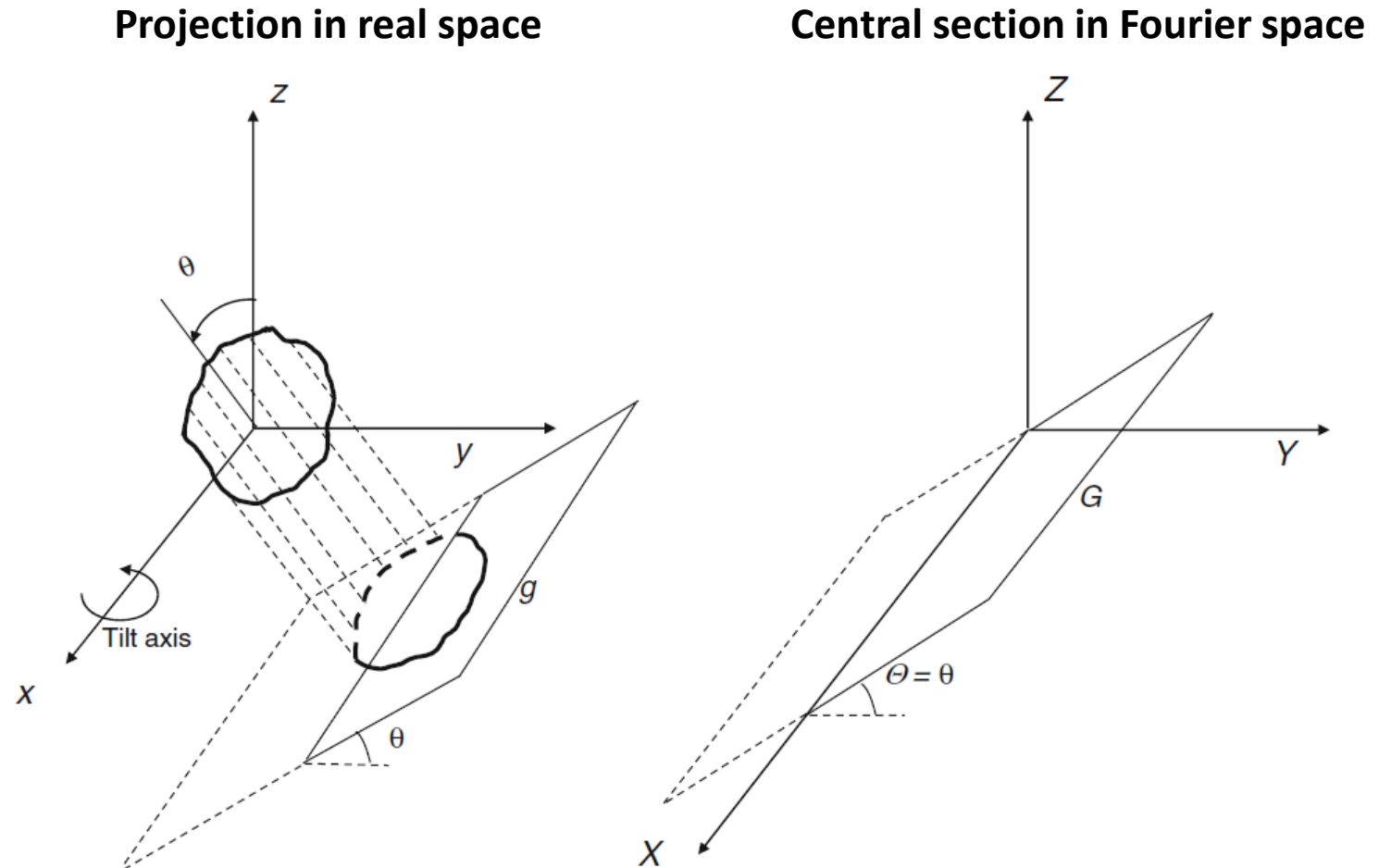1 angle   2 angles   9 angles   36 angles   180 angles
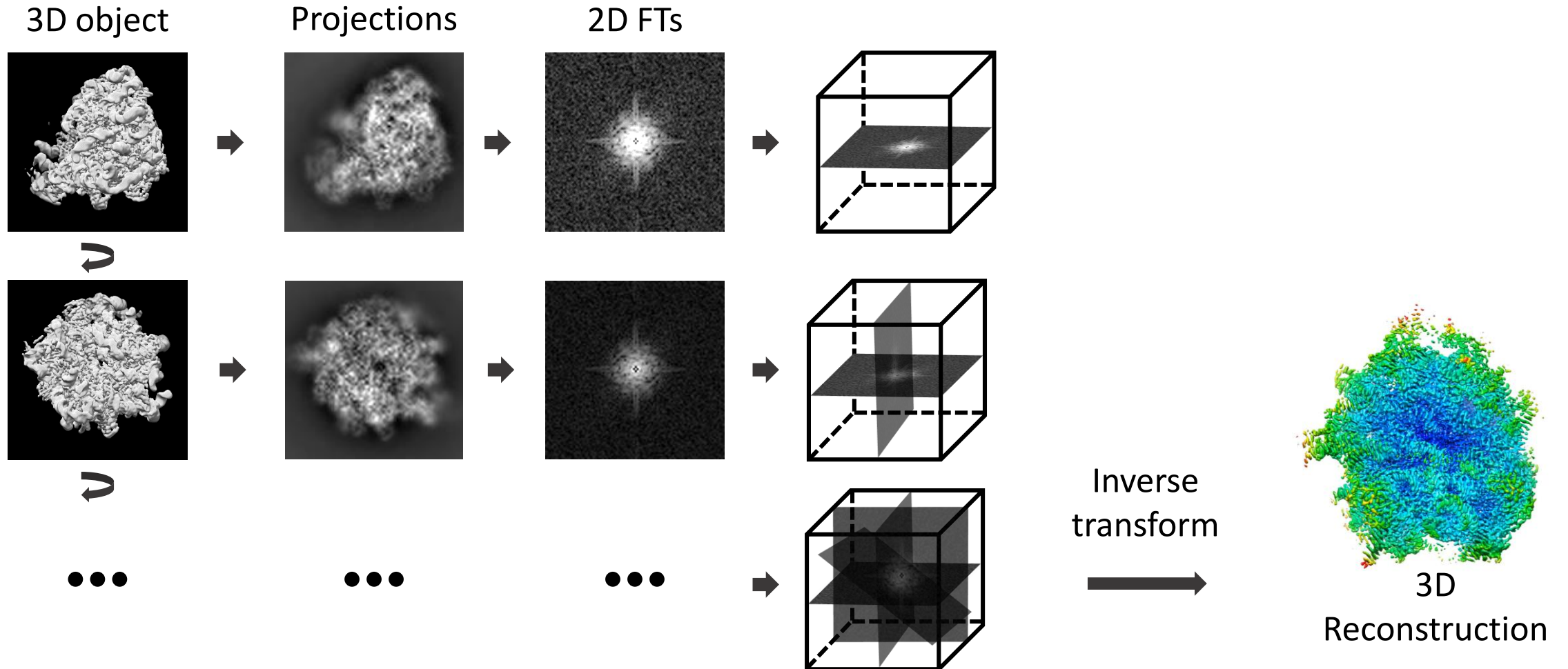
The central section theorem

**The 2D FT of an object's projection forms a central plane through the 3D FT of the object**

Projection in real space

Central section in Fourier space

The central section theorem

The 2D FT of an object's projection forms a central plane through the 3D FT of the object

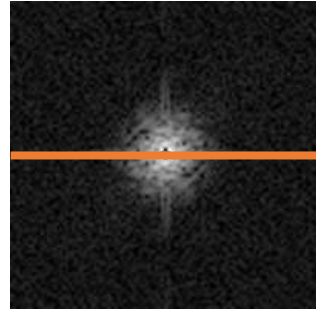3D object   Projections   2D FTs

Inverse transform

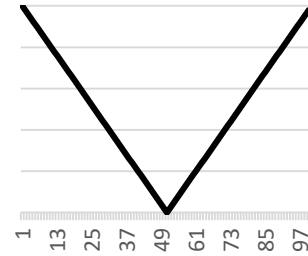3D Reconstruction

Over sampling of low frequencies in Fourier space
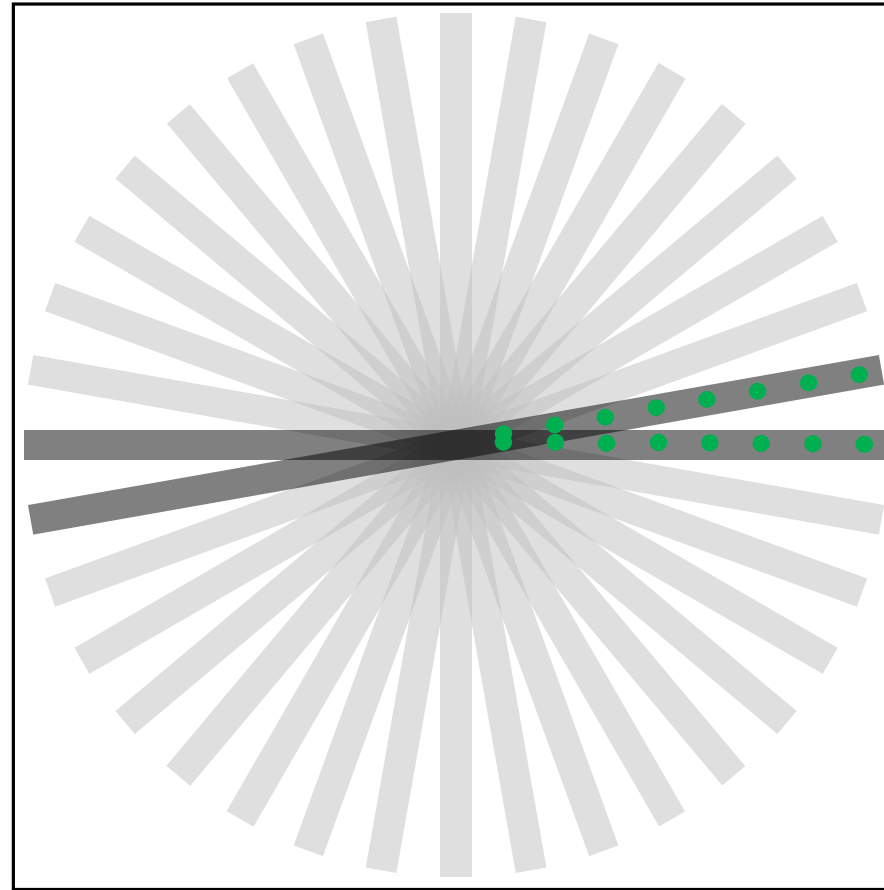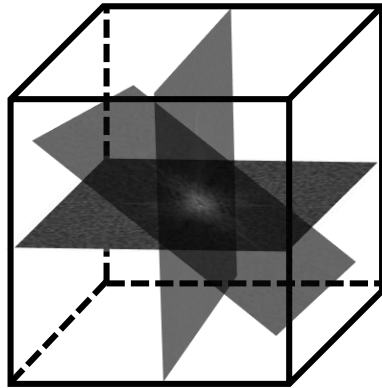
Weighted back-projection

FT of tilt

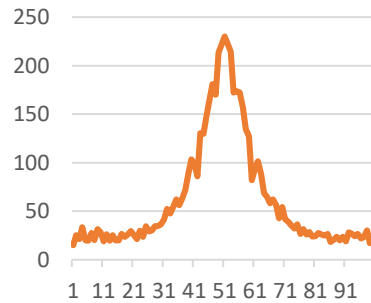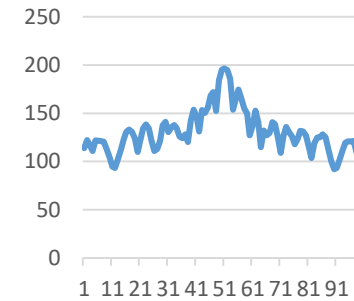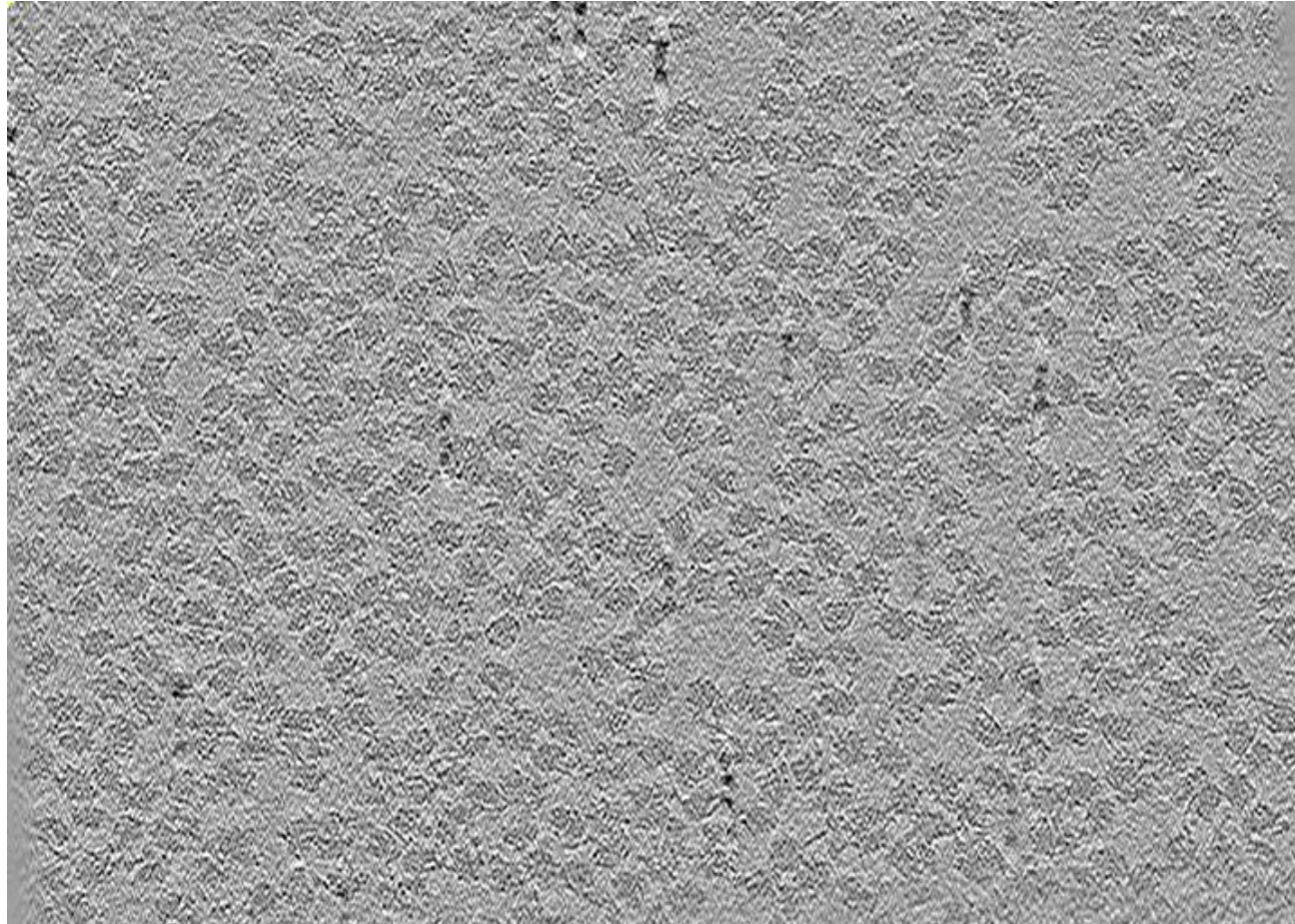×

R-weighting of tilts

=
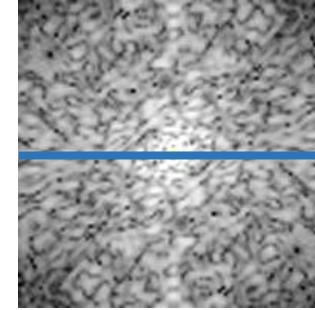
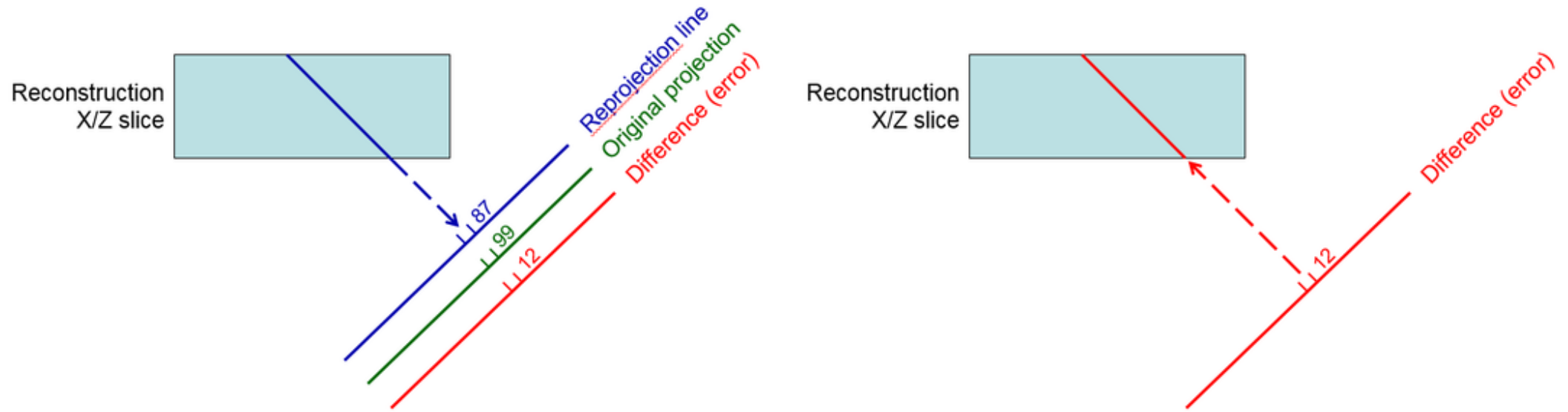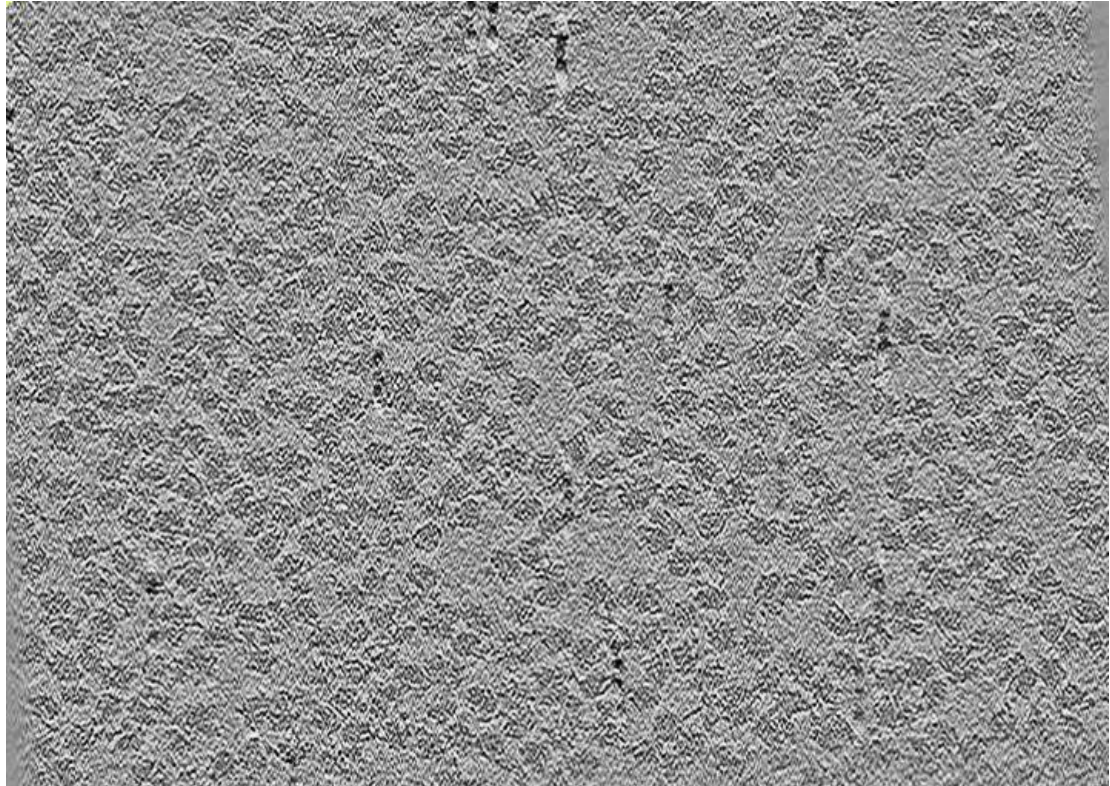Cross section of FT

Slice through reconstruction
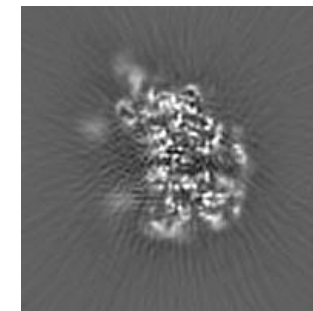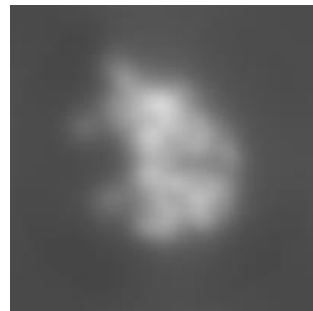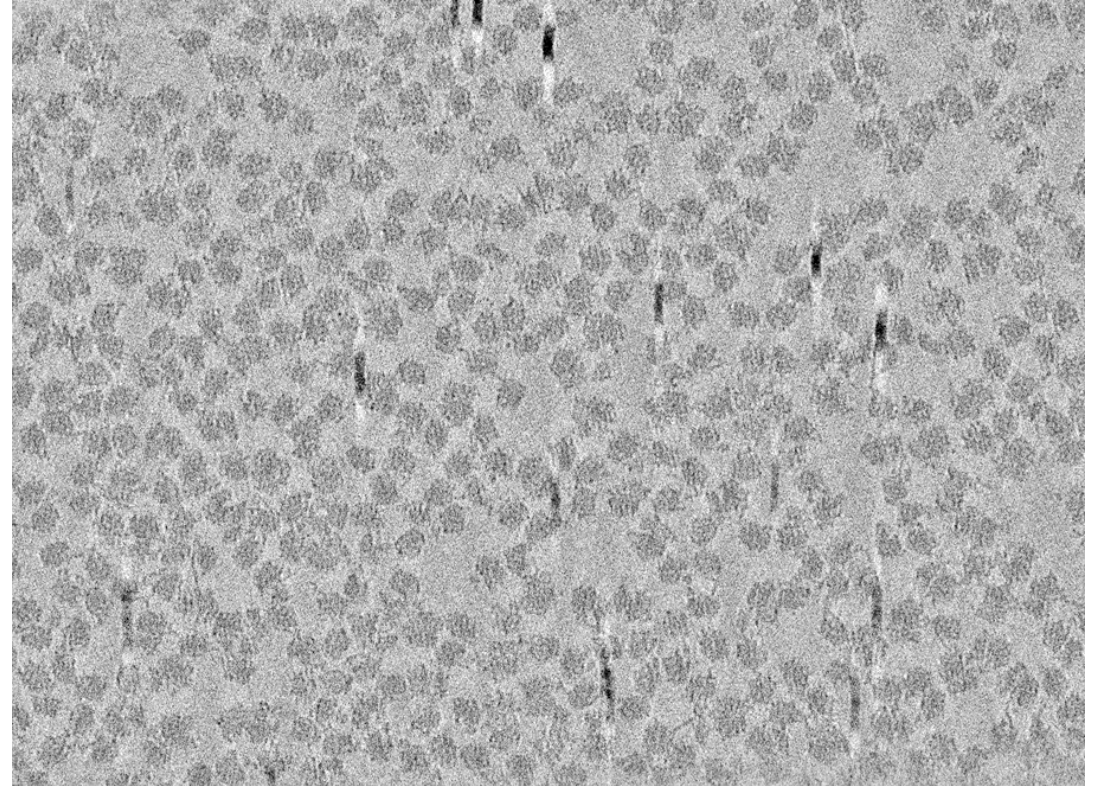
Reconstruction

SIRT (Simultaneous Iterative Reconstruction Technique)

SIRT (Simultaneous Iterative Reconstruction Technique)

WBP

+ SIRT

Missing information in cryo-ET

- Missing wedge
- Discrete sampling
- Electron dose
- Defocus gradient (CTF)

Missing wedge

3D power spectrum

XY

XZ

Palmer et al. (2020) Nature Nanotechnology
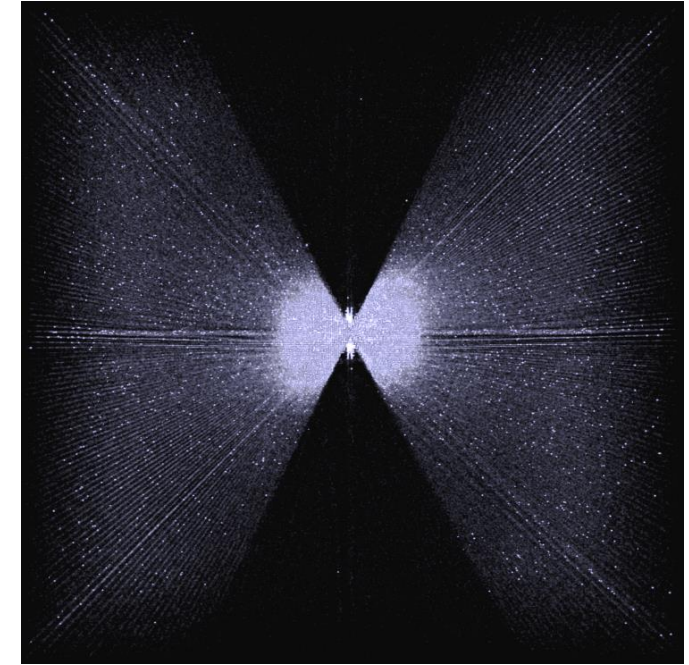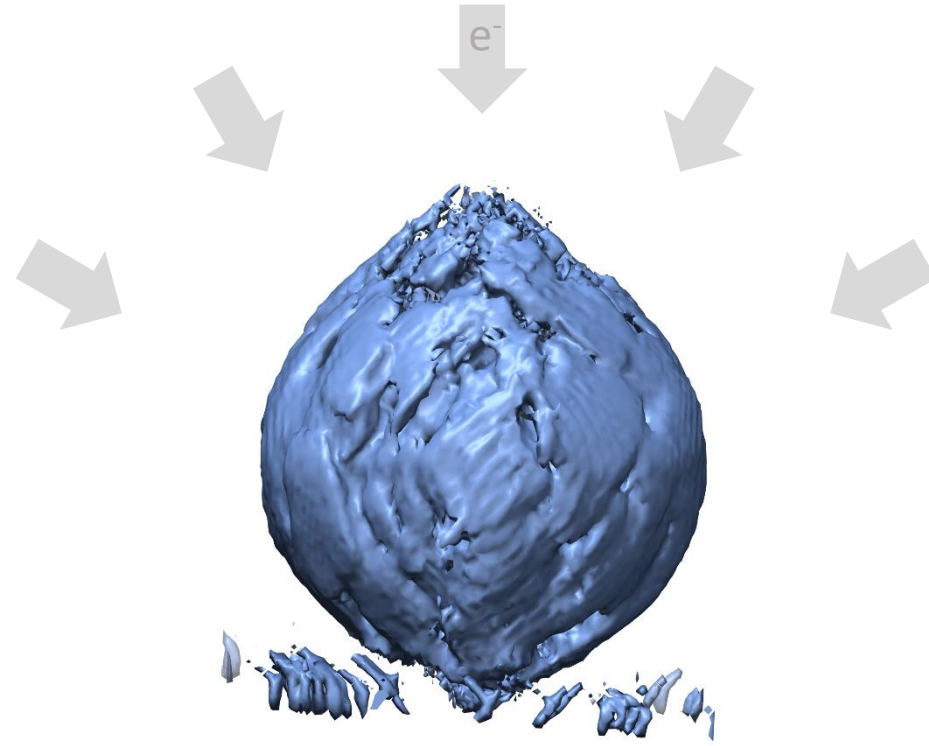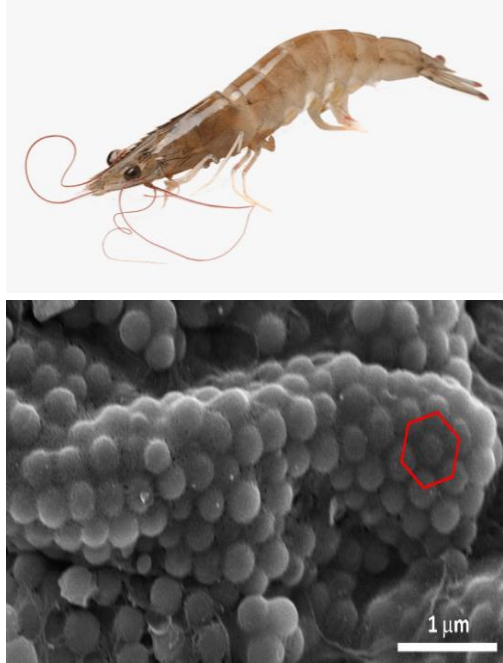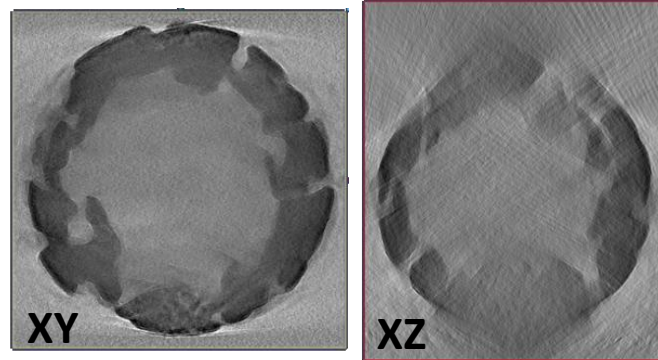
Missing information due to discrete sampling

Crowther Criterion

The minimum number of views to reconstruct an object of diameter **D** to a resolution of **r** :

N = πD/r

Crowther et al. (1969) Proceedings of the Royal Society of London

Discrete sampling + missing wedge

Leary and Midgley (2019) Electron Tomography in Materials Science
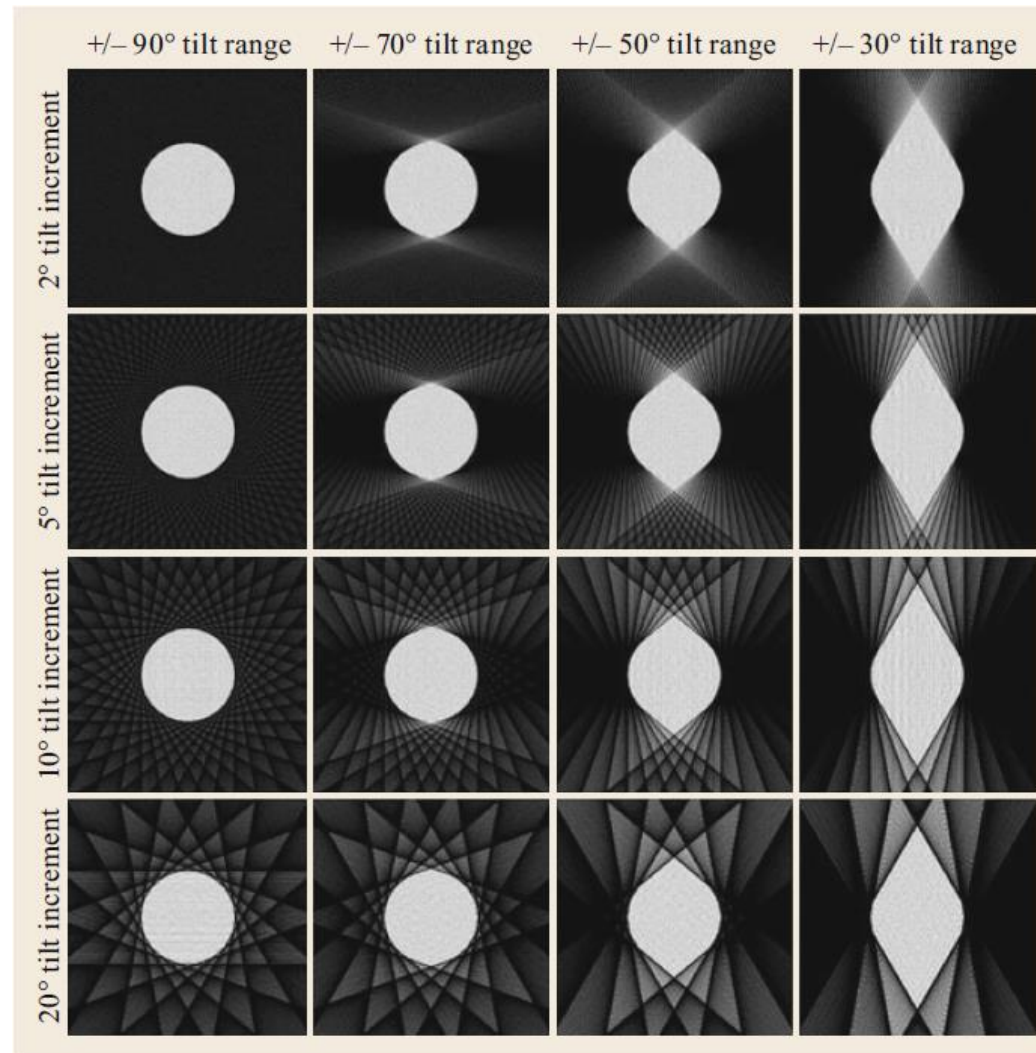
Accumulated beam damage

Frames 4-6
3.1 - 4.6 e⁻/Å²
2.9Å

Frames 25-27
19.2 - 20.8 e⁻/Å²
3.4Å

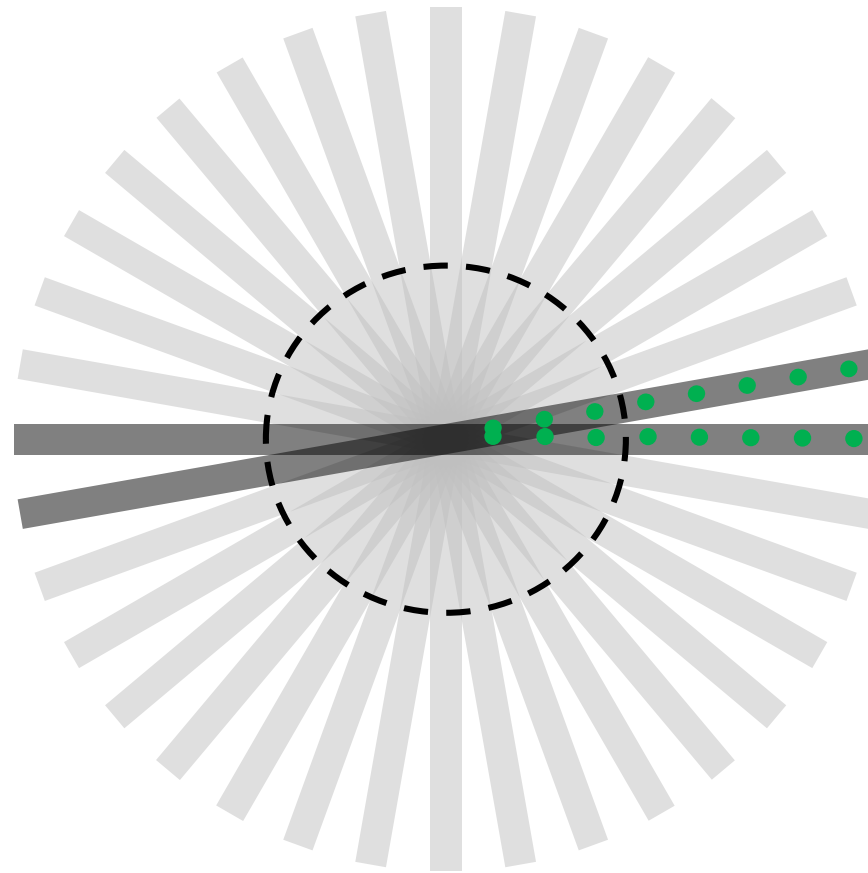Frames 46-48
35.4 - 36.9 e⁻/Å²
4.1Å

Frames 67-69
51.5 - 53.1 e⁻/Å²
5.7Å
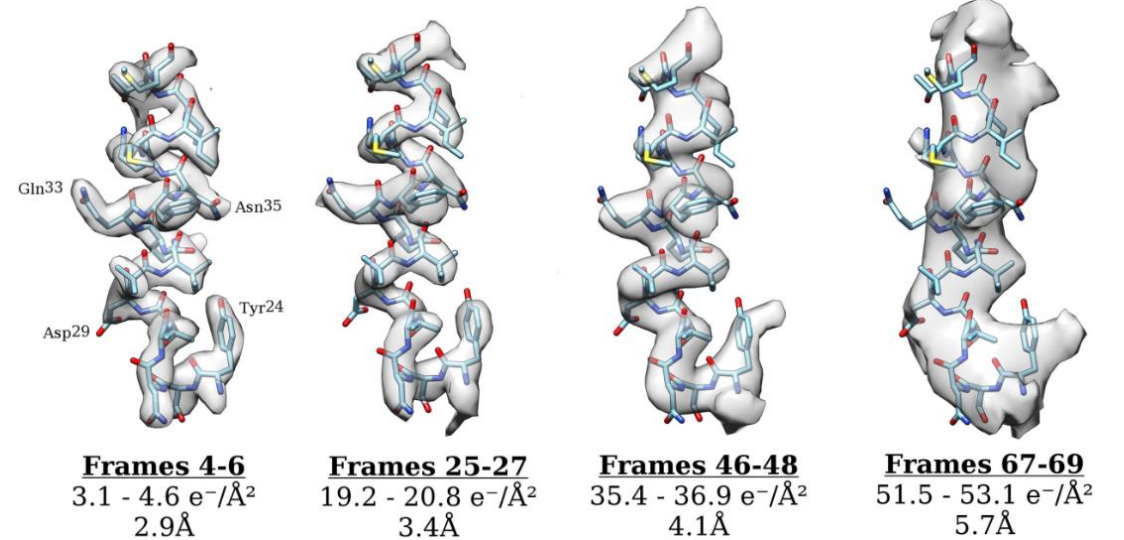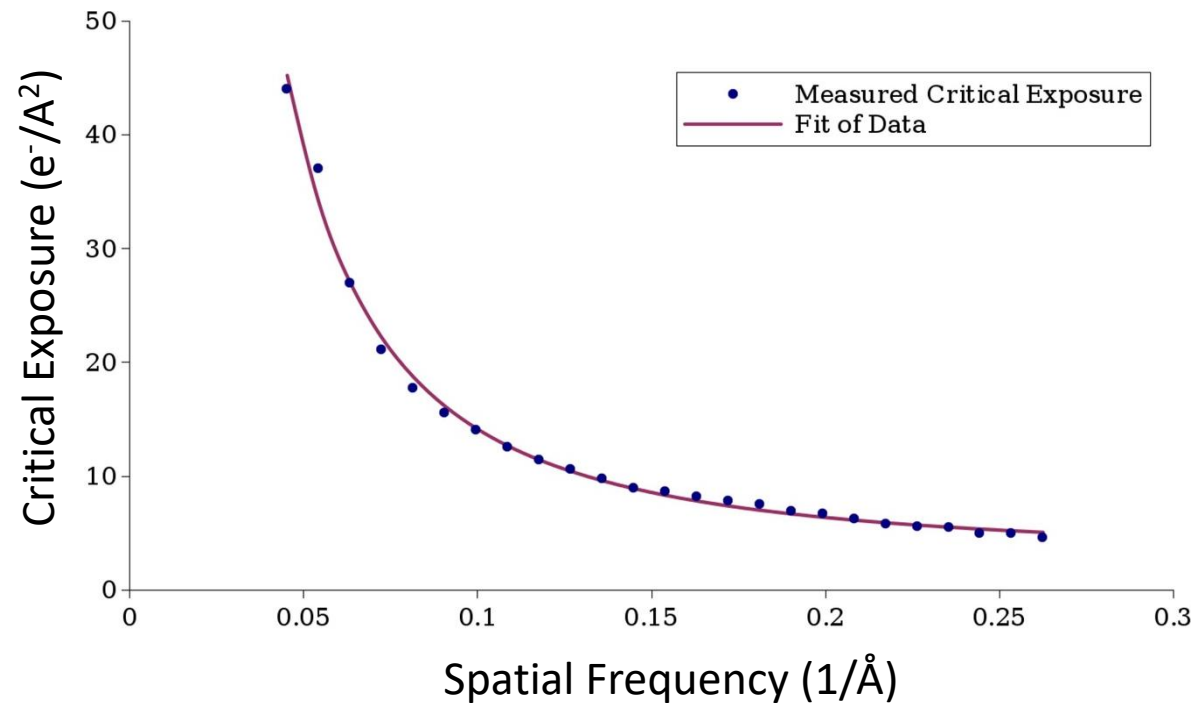
Grant & Grigorieff (2015) *eLife*. Measuring the optimal exposure for single particle cryo-EM using a 2.6 Å reconstruction of rotavirus VP6.

Defocus gradient in tilts
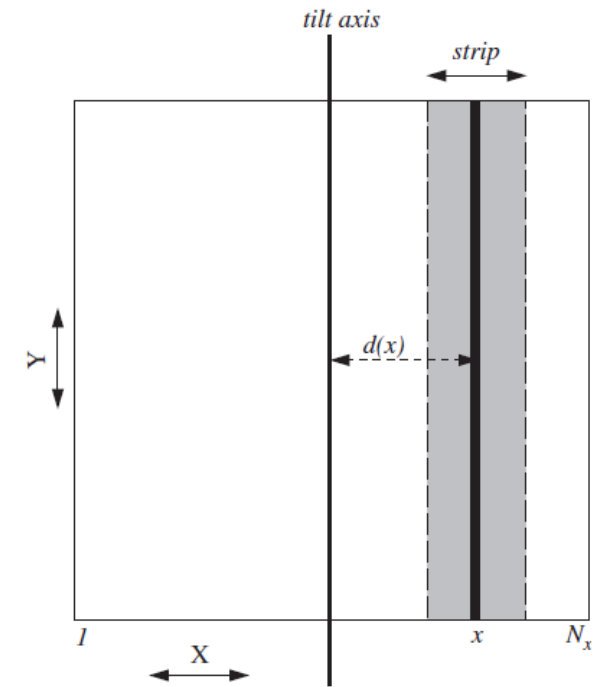
Fernández and Crowther (2006) *Ultramicroscopy*. CTF determination and correction in electron cryotomography.
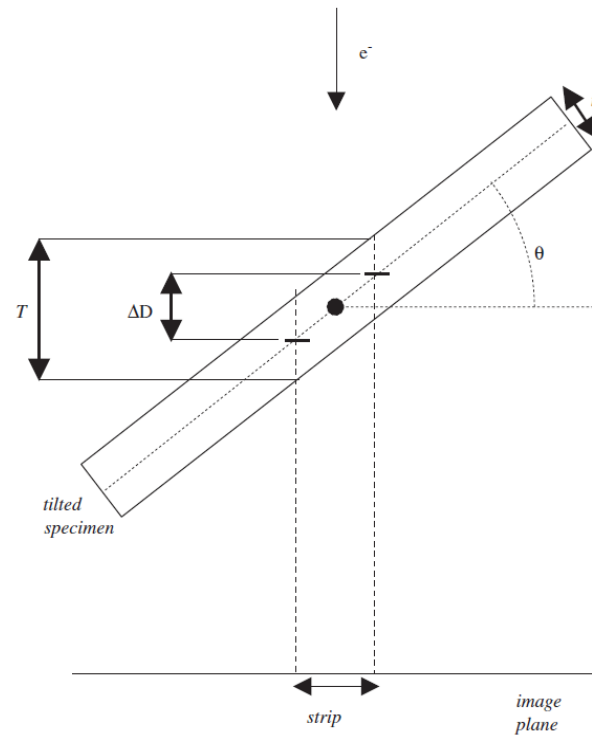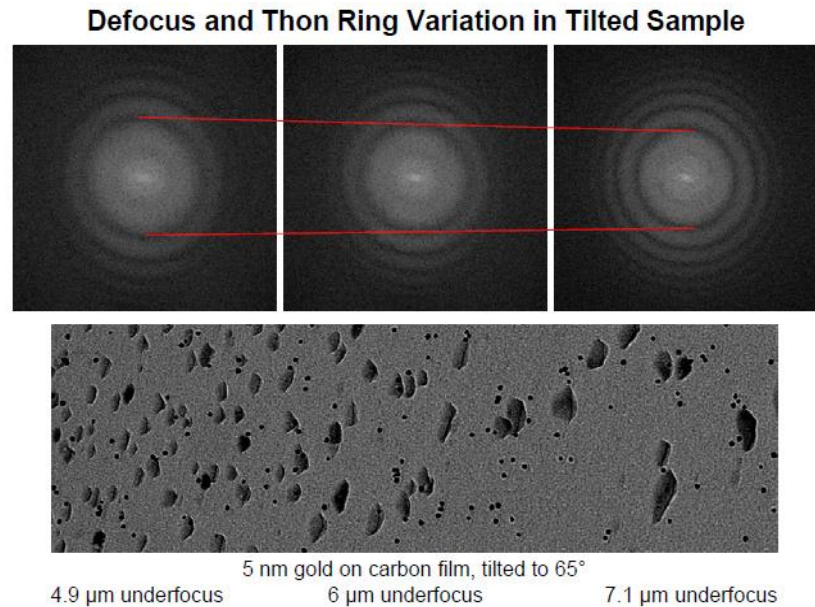https://bio3d.colorado.edu/RML_2017/2017_IMOD_PEET_Workshop/Lectures/CTFcorrInIMOD.pdf

CTF with phase plate
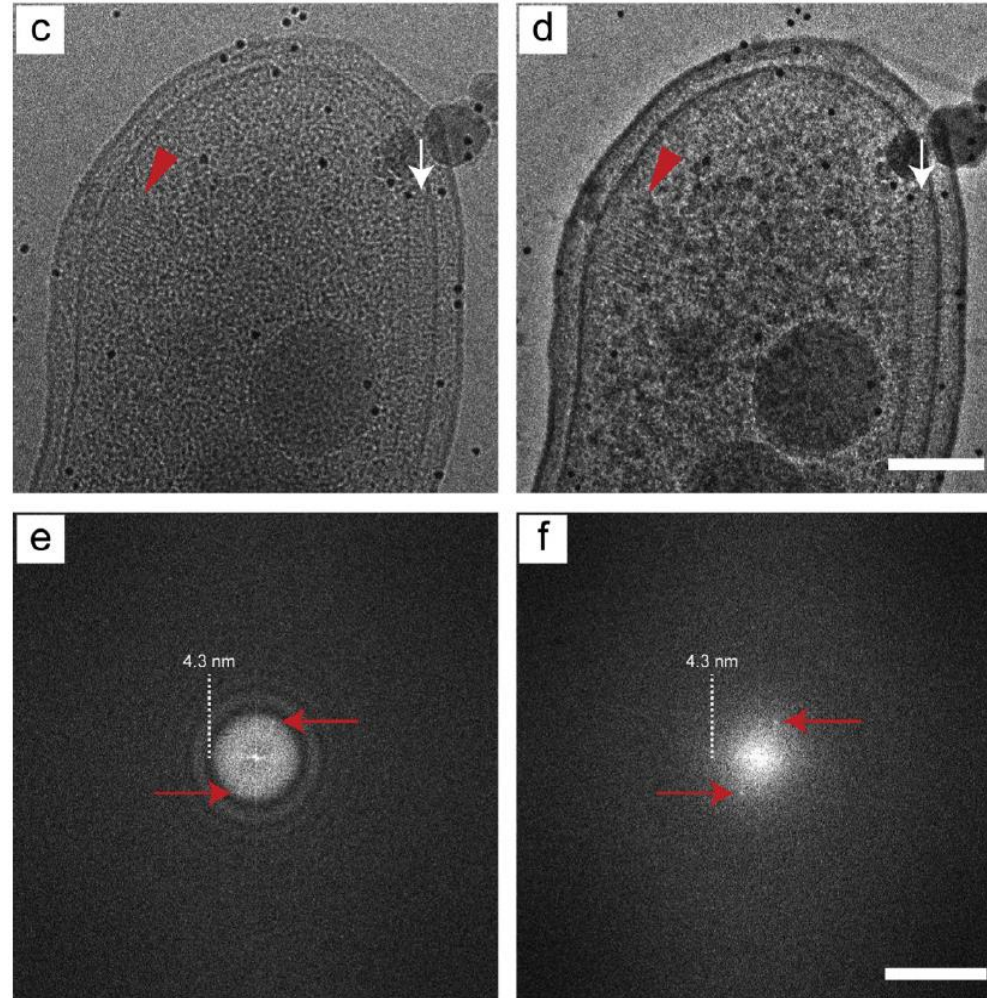
Fukuda et al (2015) *JSB*. Electron cryotomography of vitrified cells with a Volta phase plate

Interpretation of tomograms

- Manual segmentation
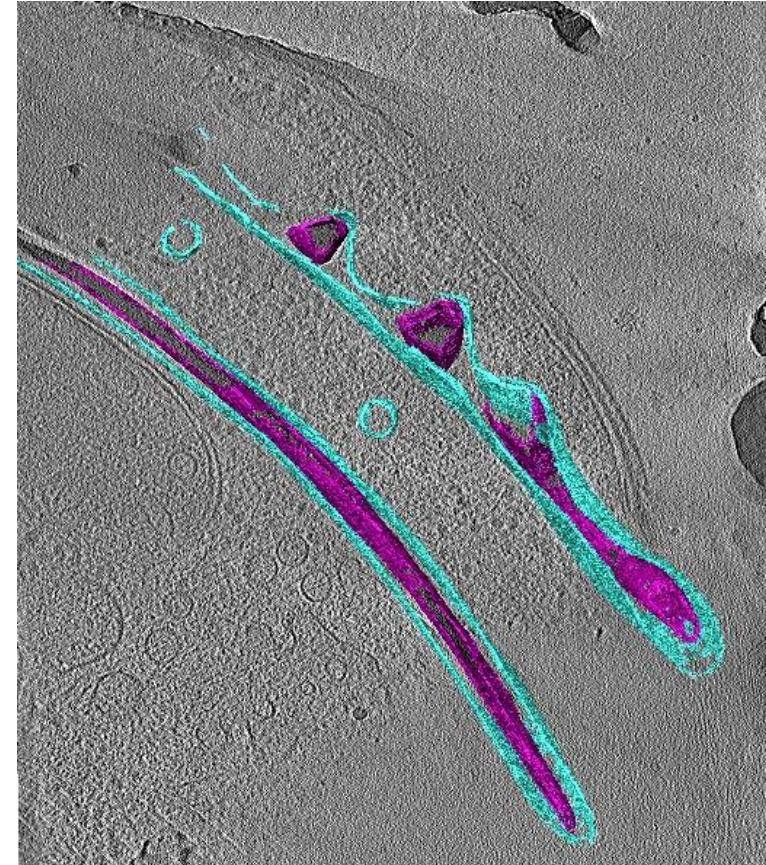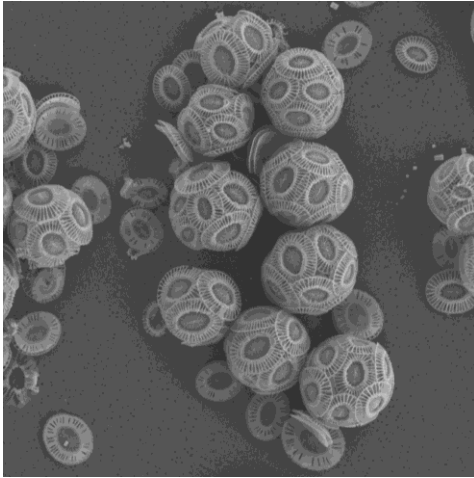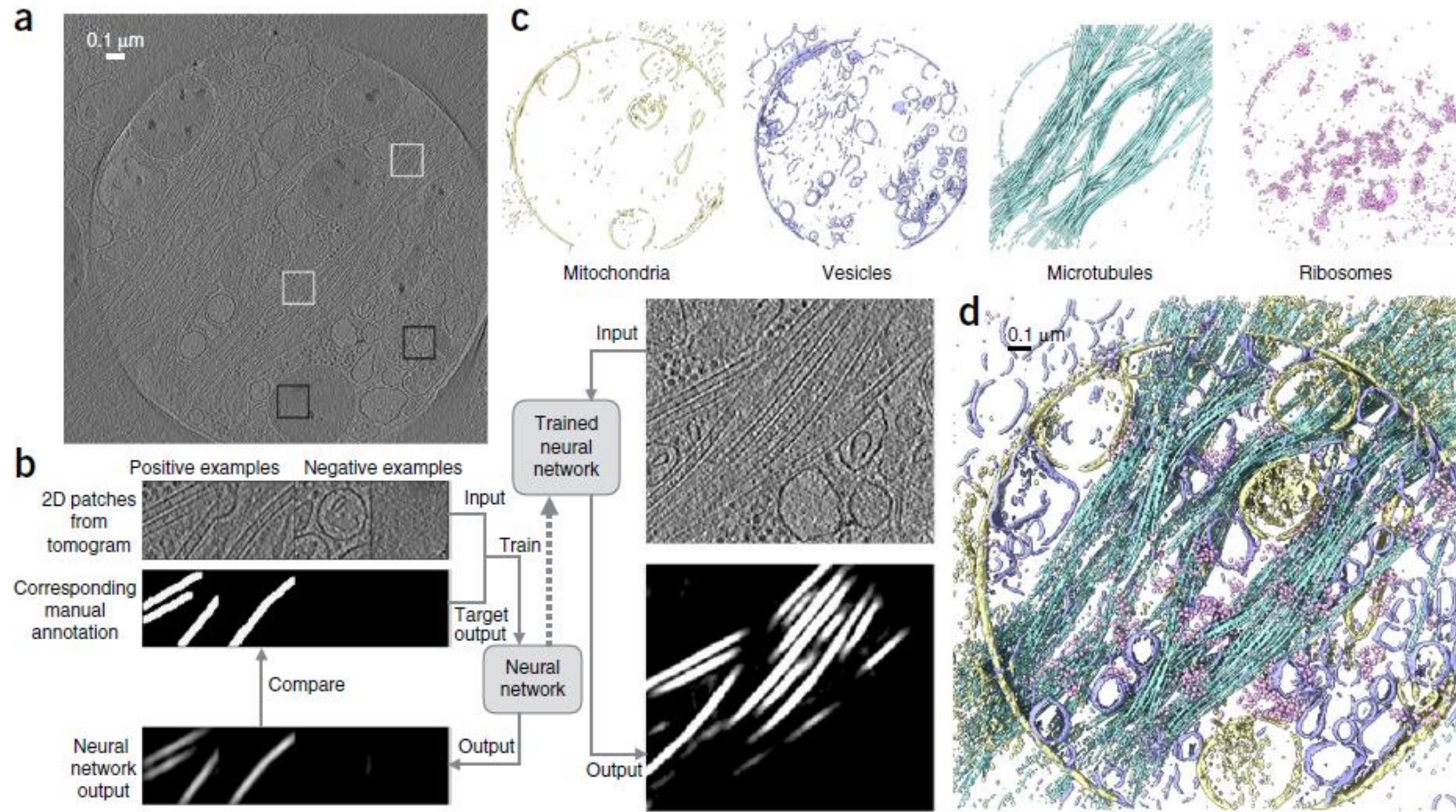- Annotation with neuronal networks
- Template matching
- Sub-tomogram averaging
- Elemental characterization

Manual segmentation

Zohar Eyal, Assaf Gal's group, Department of Plant and Environmental Sciences, WIS
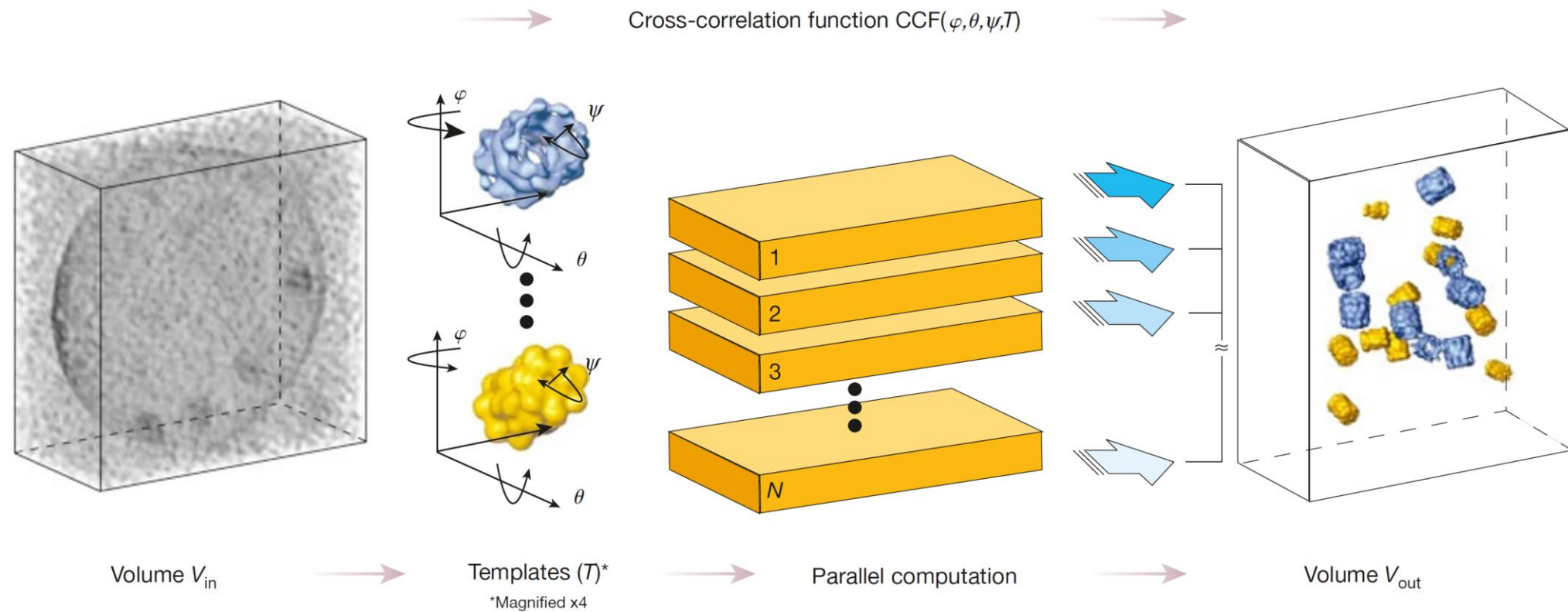
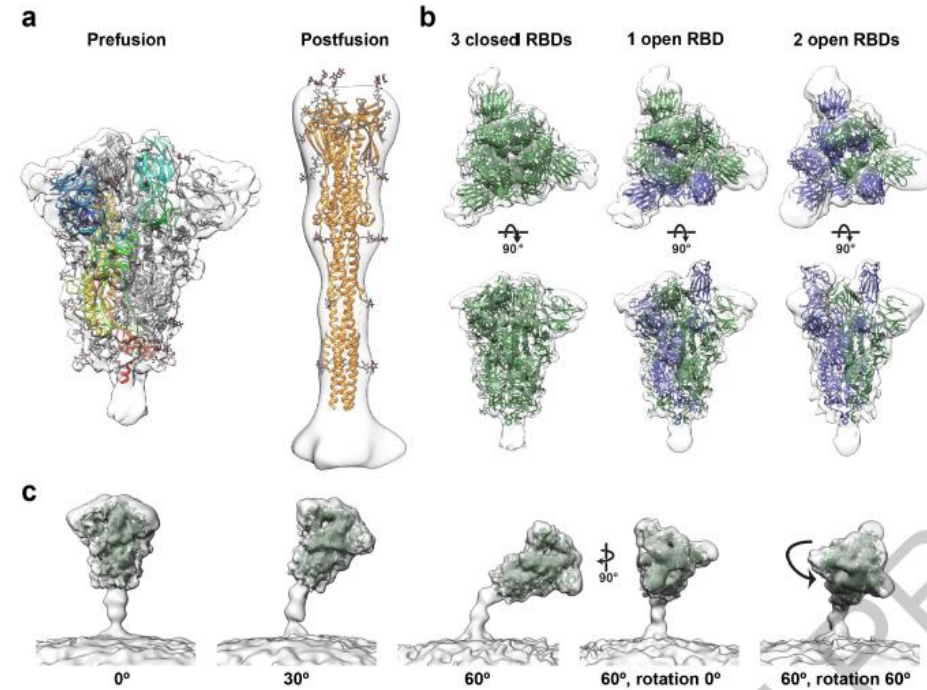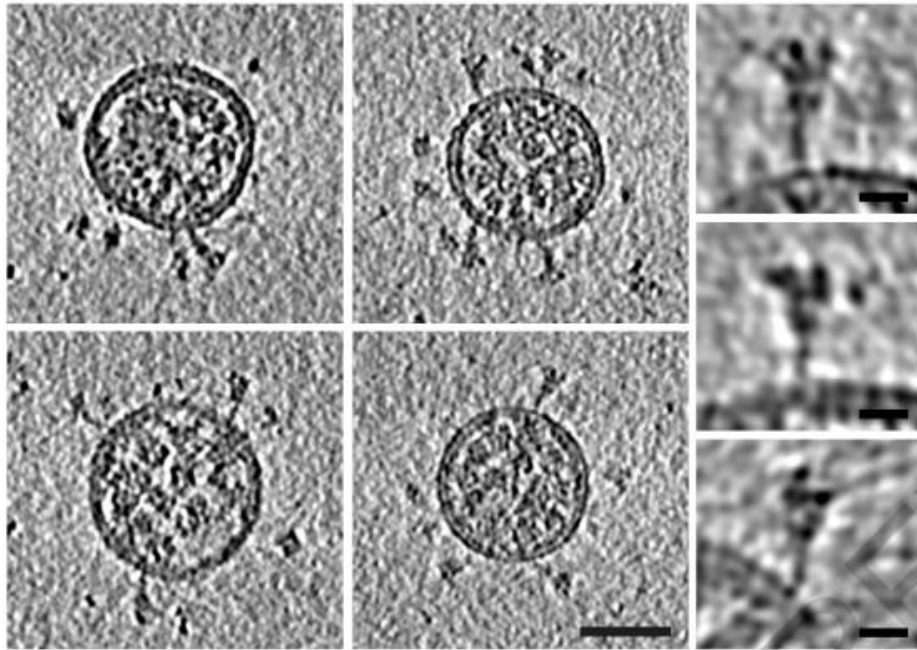# Tomogram annotation using neural networks

EMAN2 software

Chen et al (2017) *Nature Methods.* Convolutional neural networks for automated annotation of cellular cryo-electron tomograms

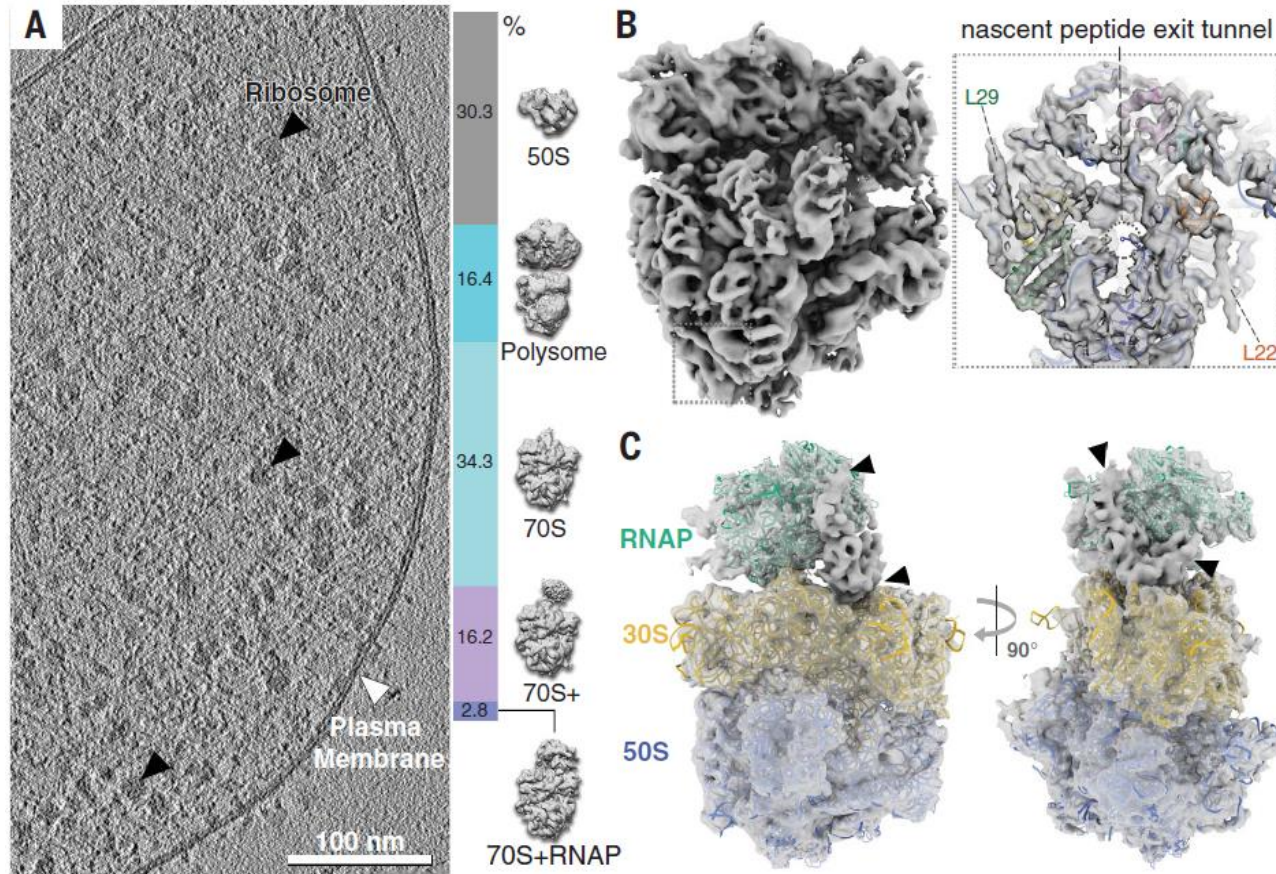# Template matching

Cross-correlation function CCF(φ, θ, ψ, T)

Volume V_{in}

Templates (T)*
*Magnified x4

Parallel computation

Volume V_{out}

Sali et al (2003) *Nature*. From words to literature in structural proteomics

Template matching and sub-tomogram averaging

Ke *et al* (2020) *Nature*. Structures and distributions of SARS-CoV-2 spike proteins on intact virions
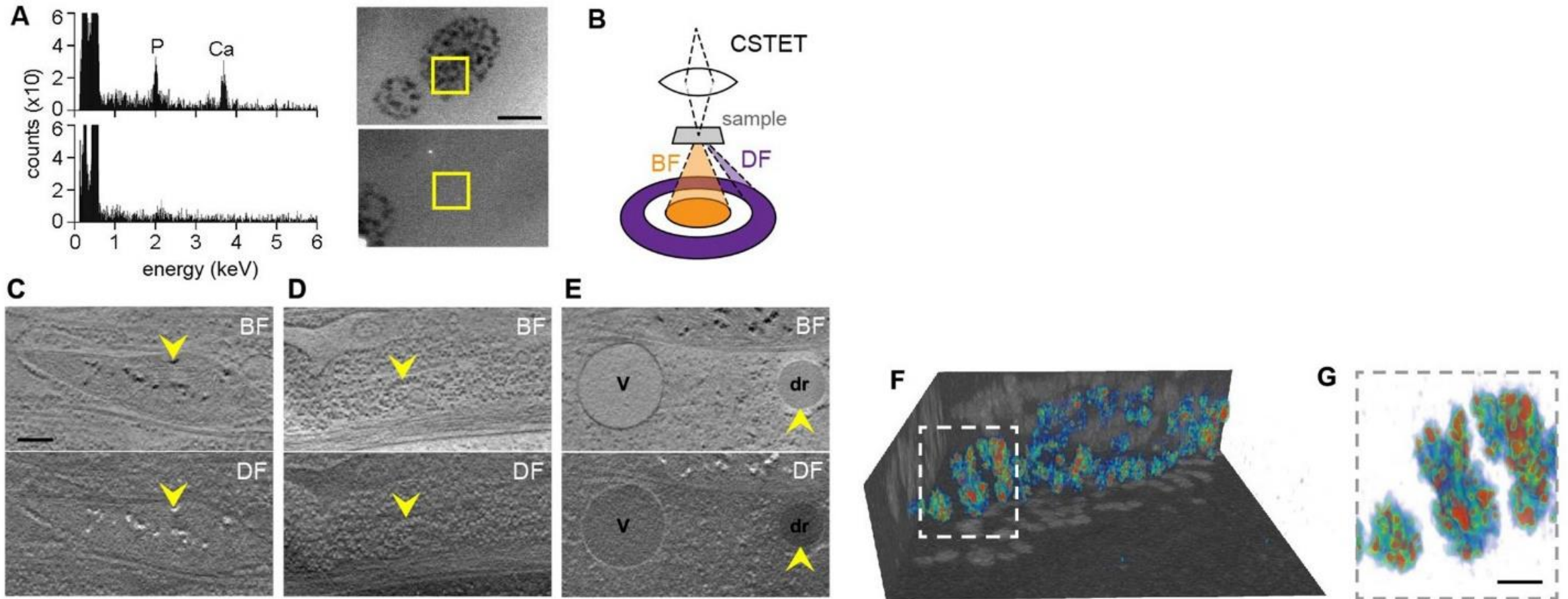
Template matching and sub-tomogram averaging

O'Reilly et al (2020) *Science*. In-cell architecture of an actively transcribing-translating expressome

Elemental characterization by cryo-STEM tomography

Thank you!