Appion III Workshop: Fiducial-less Cryo-ET with Appion-Protomo

Alex Noble

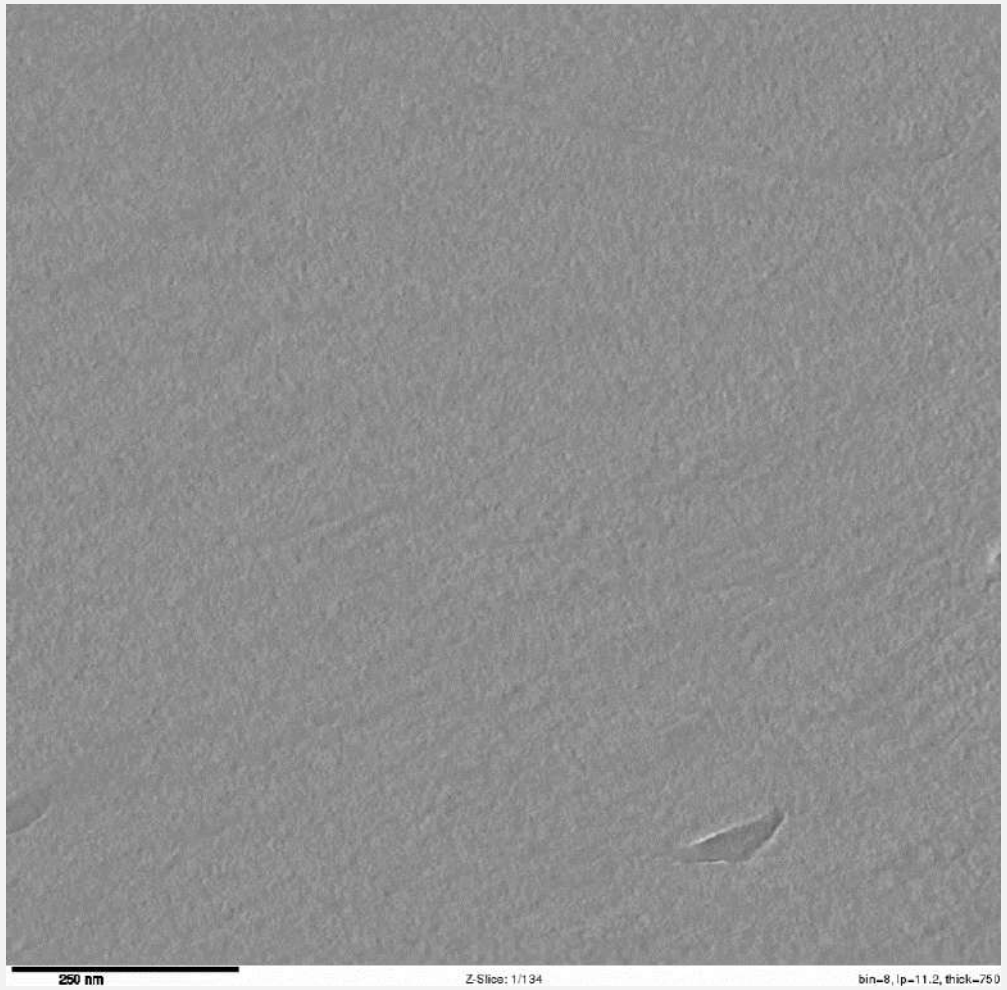
March 2, 2022

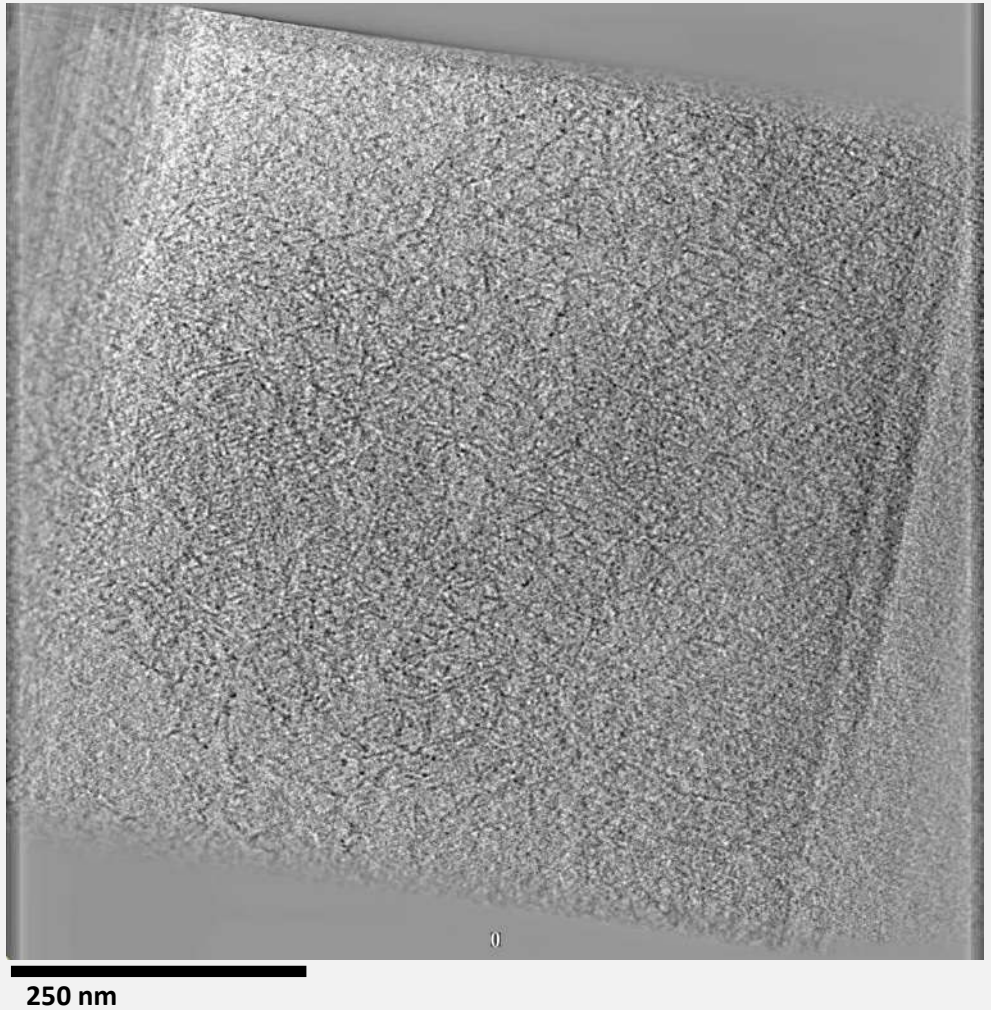
Simons Resource for Automated Molecular Microscopy
National Center for In-situ Tomographic Ultramicroscopy
Simons Electron Microscopy Center
New York Structural Biology Center

What is CryoET?

(cryo-electron tomography)

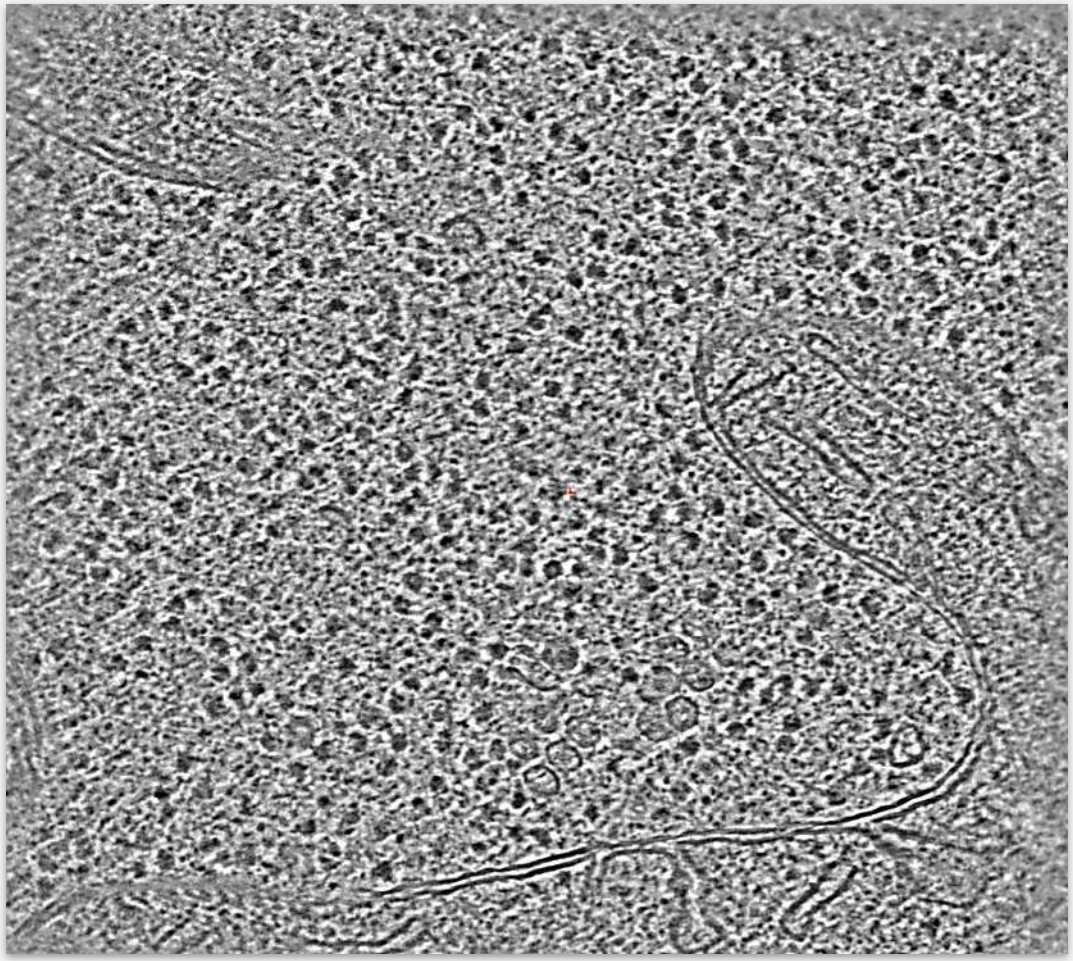
- Cells or complex reconstituted environments

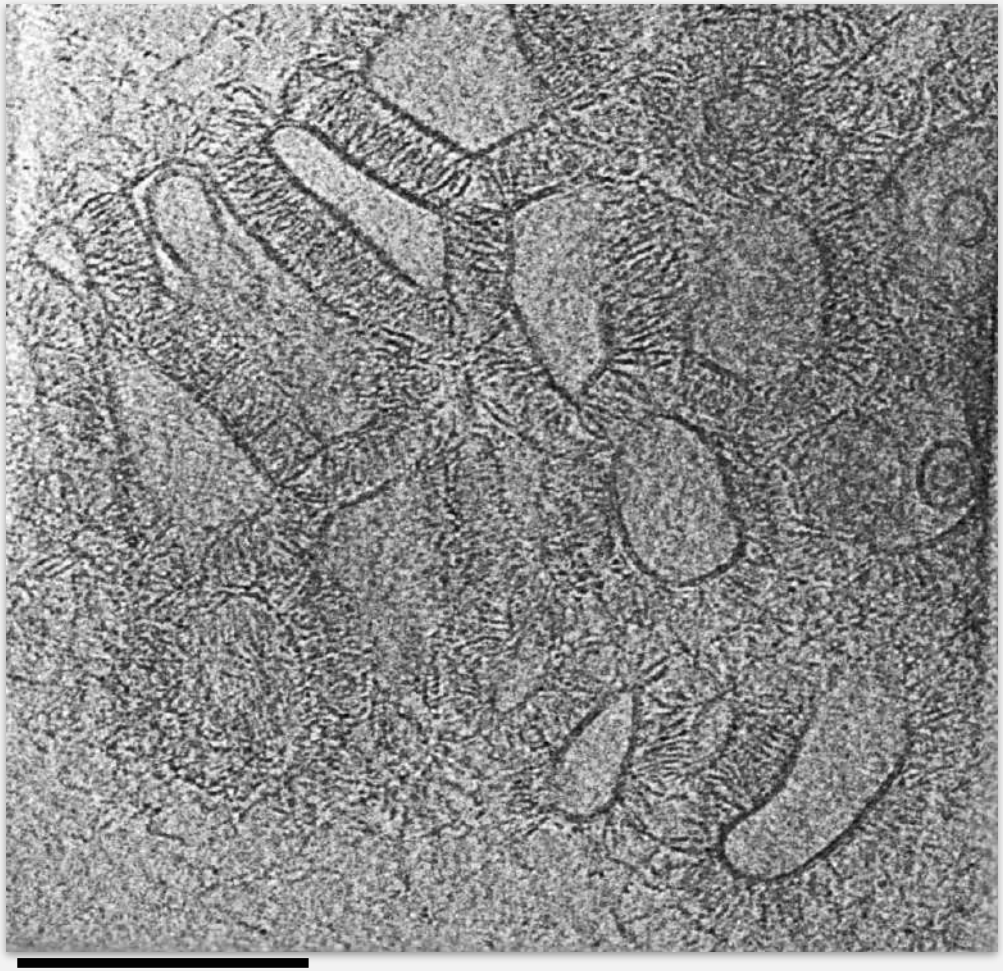




What is CryoET?

(cryo-electron tomography)





>> CryoET is the **highest resolution method** for **native specimen**

What are we doing today?

Cryo-ET Overview

Why CryoET?

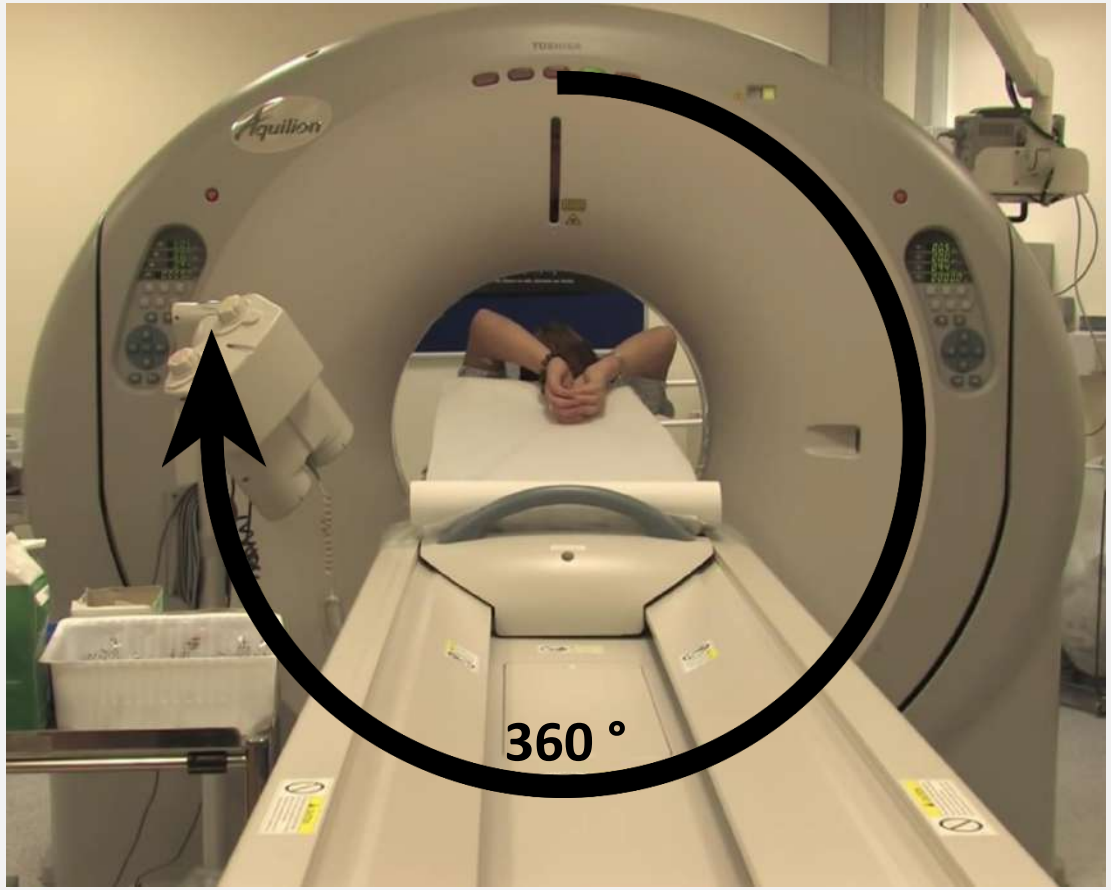
- For morphological **heterogeneity** and **unique** objects

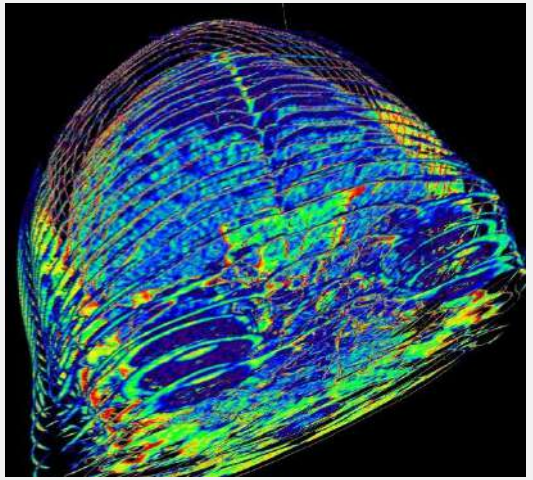
Rough order of events

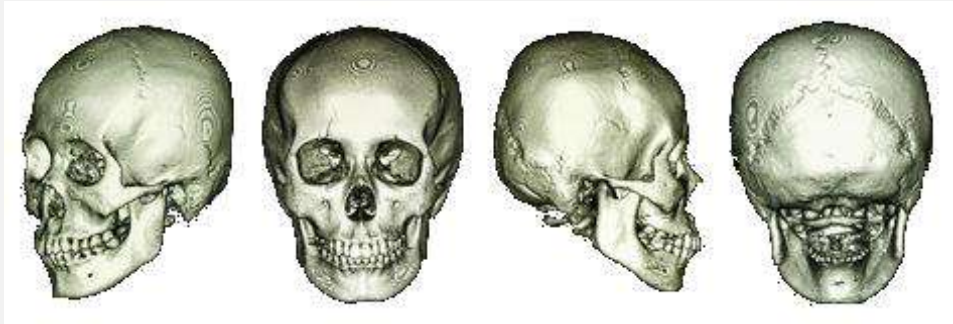
- **Tilt-series alignment** in general
- Segmentation
- Sub-tomogram processing for **repeating objects**

What is tomography in general?

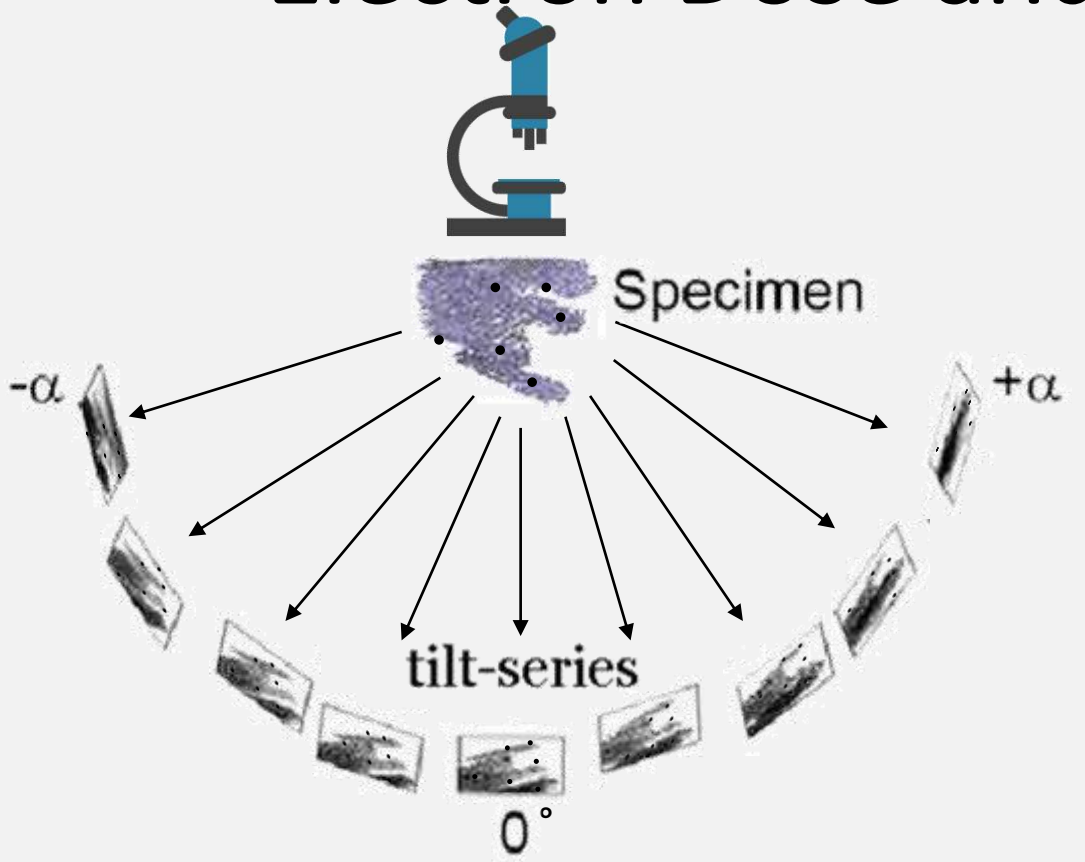
Tomography Overview – Consider How a CT Scan Works

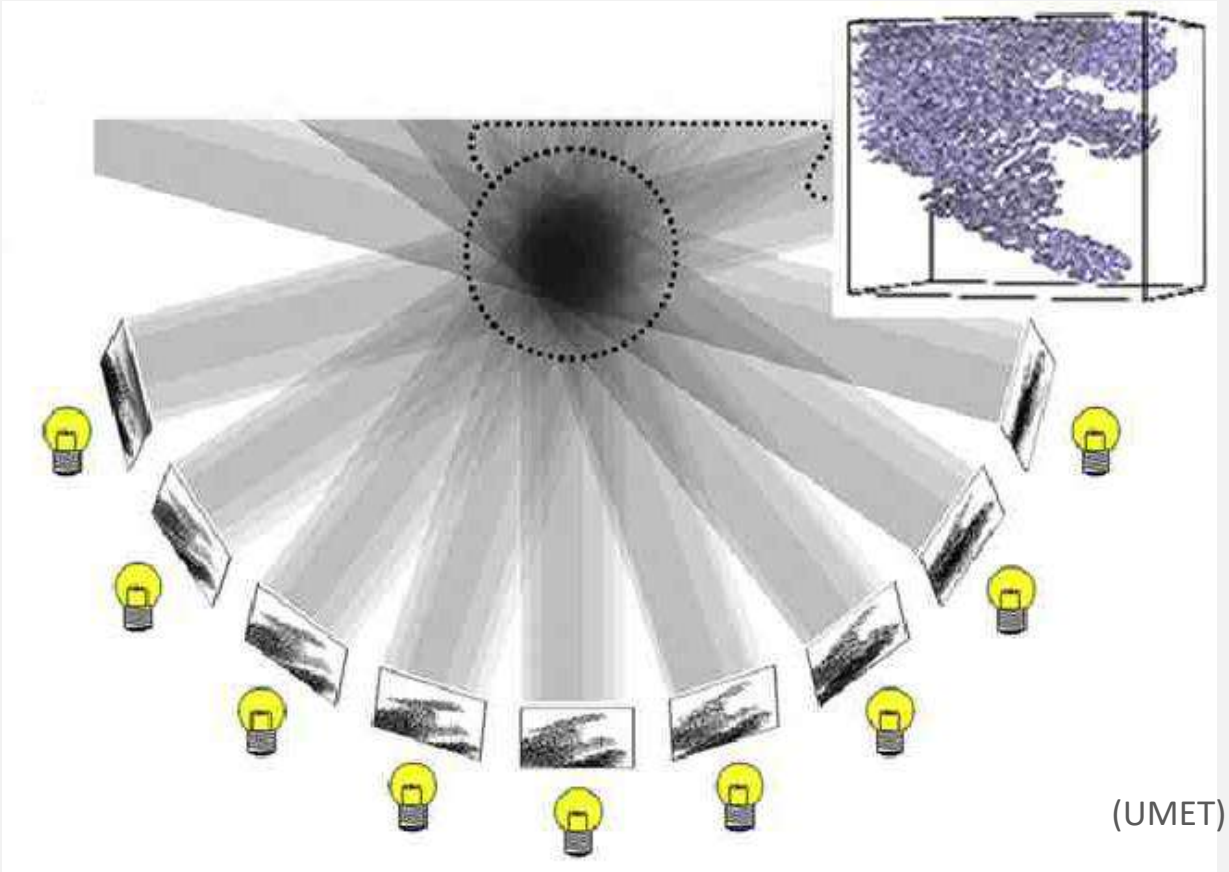






Tomogram Accuracy is Primarily Limited by Electron Dose and Tilt-Series Alignment





Collect → align → reconstruct

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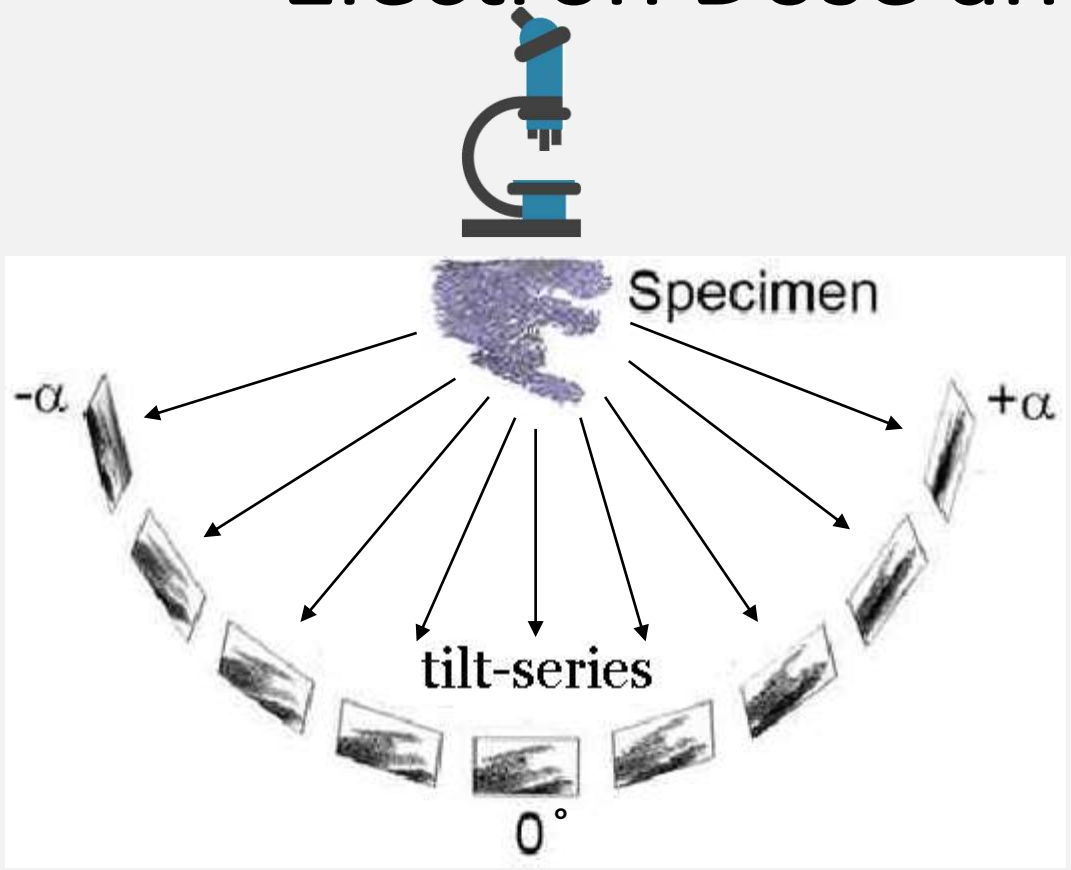
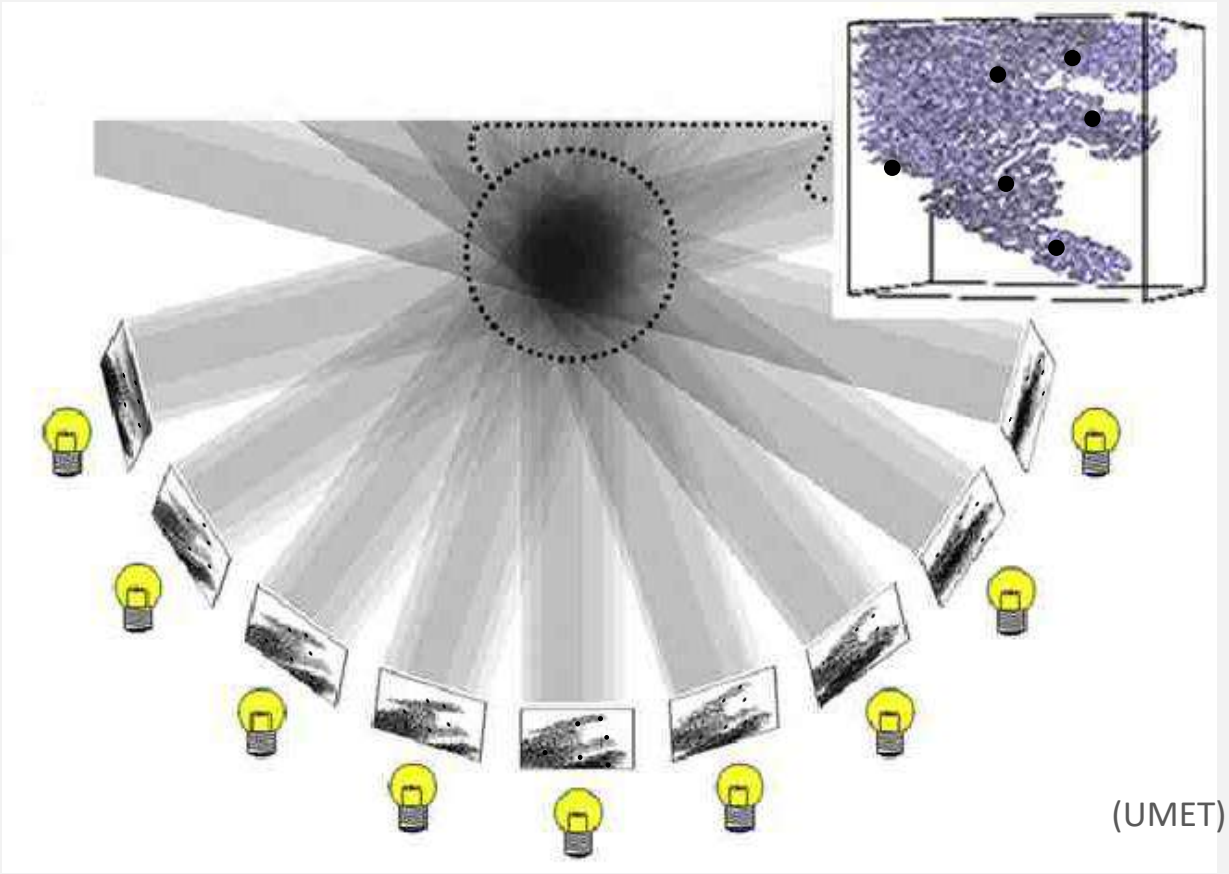
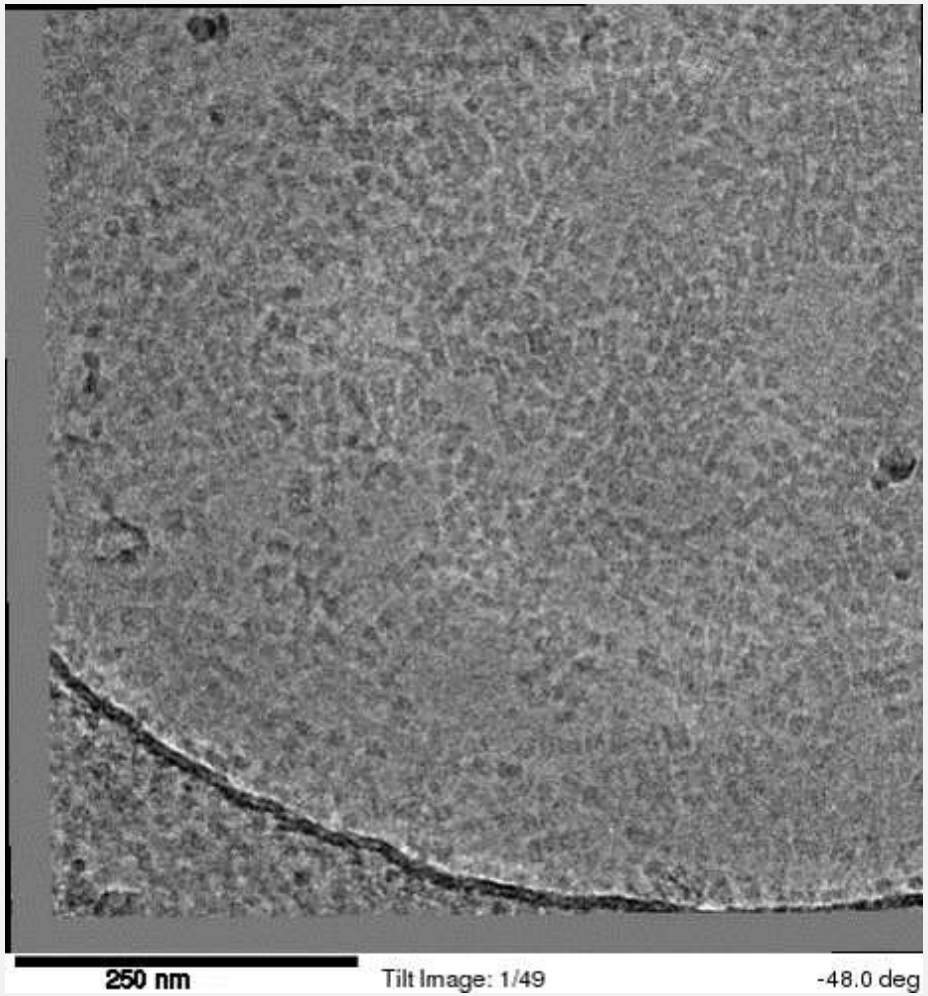




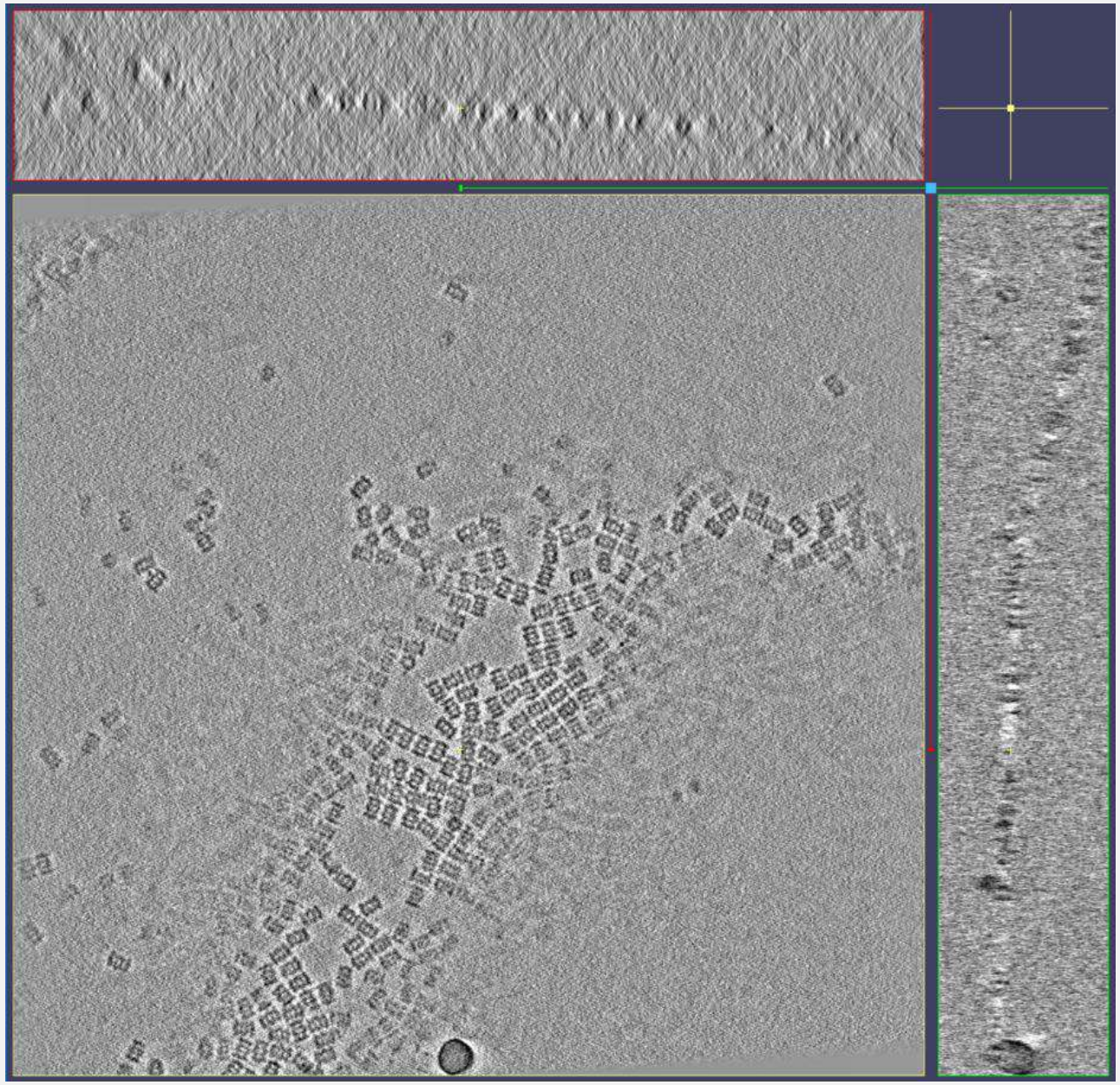
Collect → align → reconstruct

Typical tilt-series
(T20S proteasome single particle)



Anchi Cheng, Radostin Danev, Alex Noble

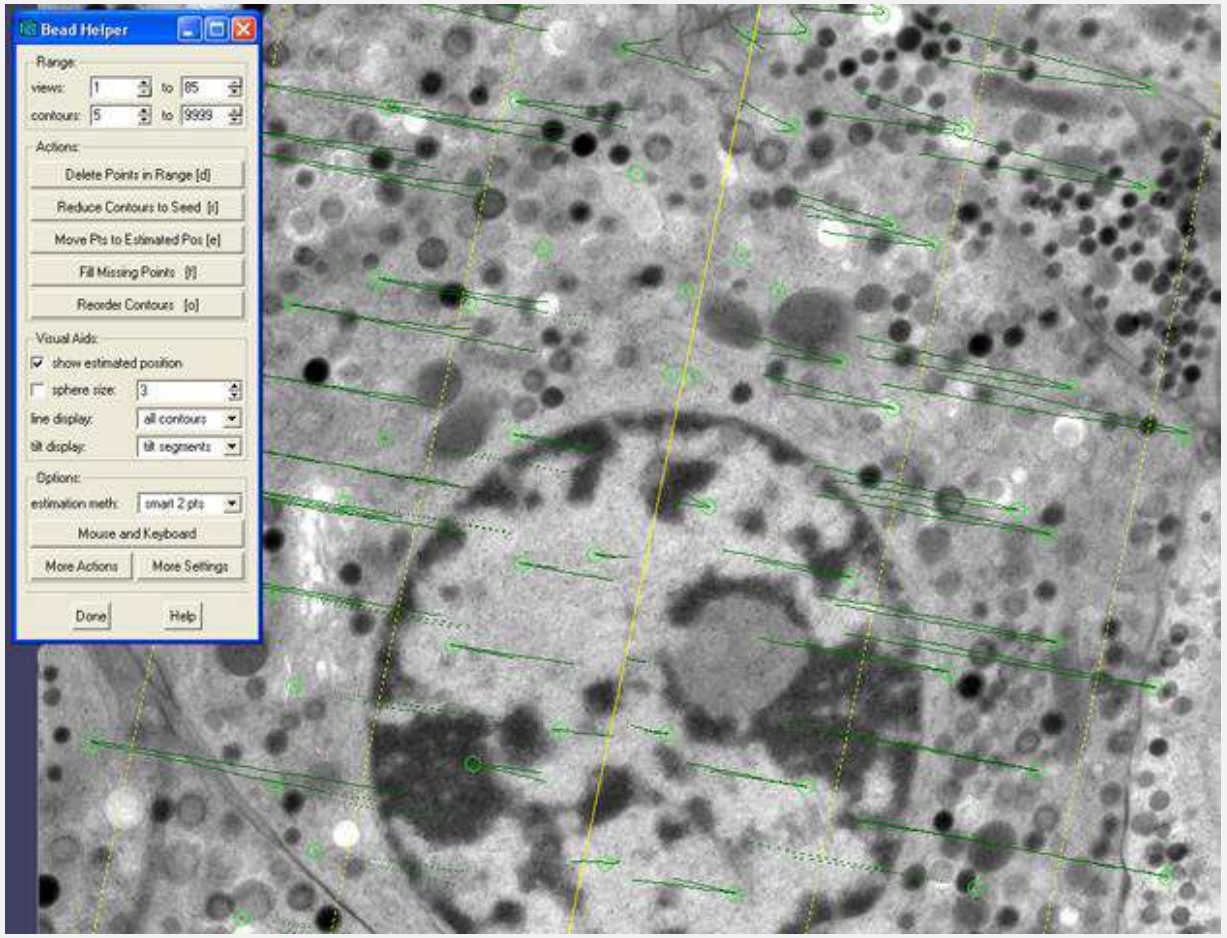
Missing Wedge Effects



Anchi Cheng, Radostin Danev, Alex Noble

Fiducial-based Tilt-Series Alignment

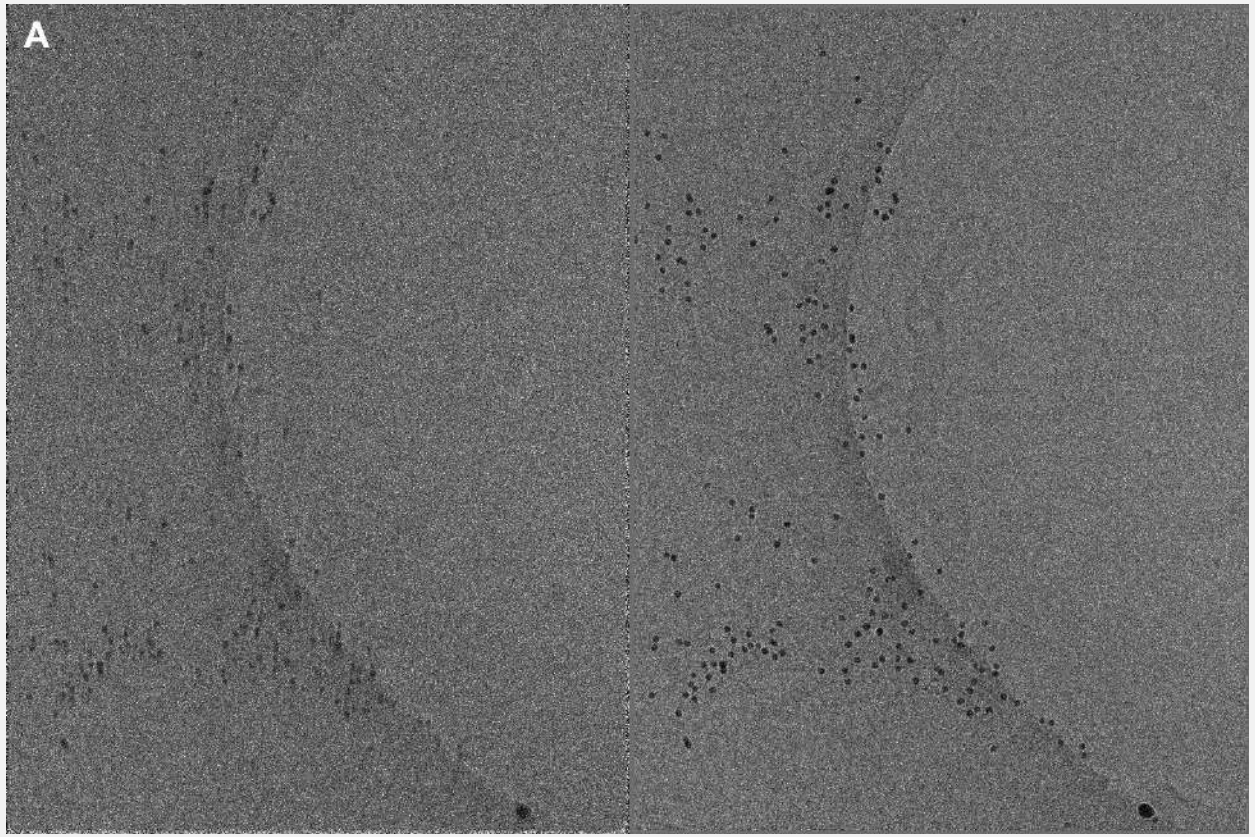
- **Requires** a **sufficient** number of **well-behaved gold beads**
- Gold beads should be **uniformly distributed** around the sample location
- Semi-automated processing



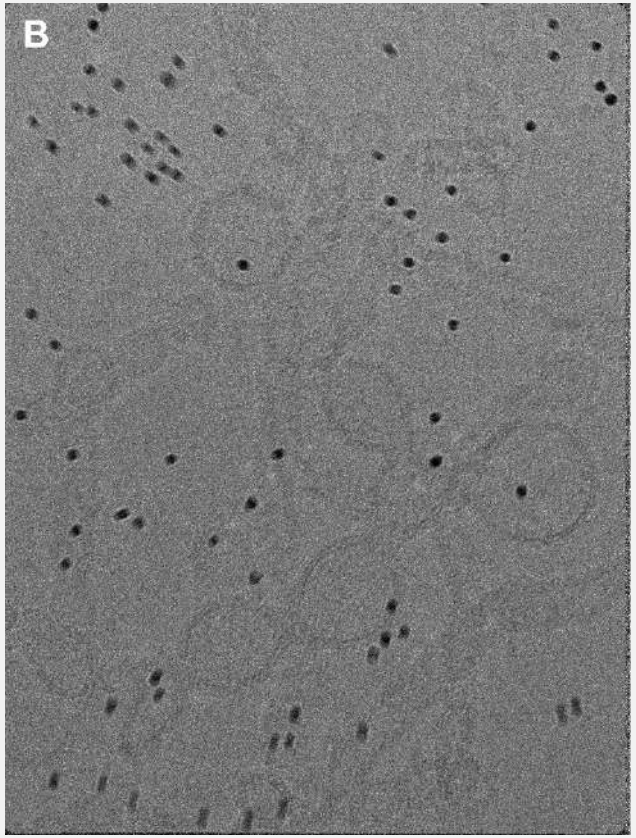
http://bio3d.colorado.edu

Fiducial-based Tilt-Series Alignment Issues

Isotropic and anisotropic fiducial movement relative to the sample (in 3D!)



DE-20 @ 18kx; 51°, 2.34 e^{-}/Å^2 after a cumulative dose of 60 e^{-}/Å^2



DE-20; 57.5 e^{-}/Å^2, 0° exposure

Aggregation

Noble & Stagg, JSB 2015

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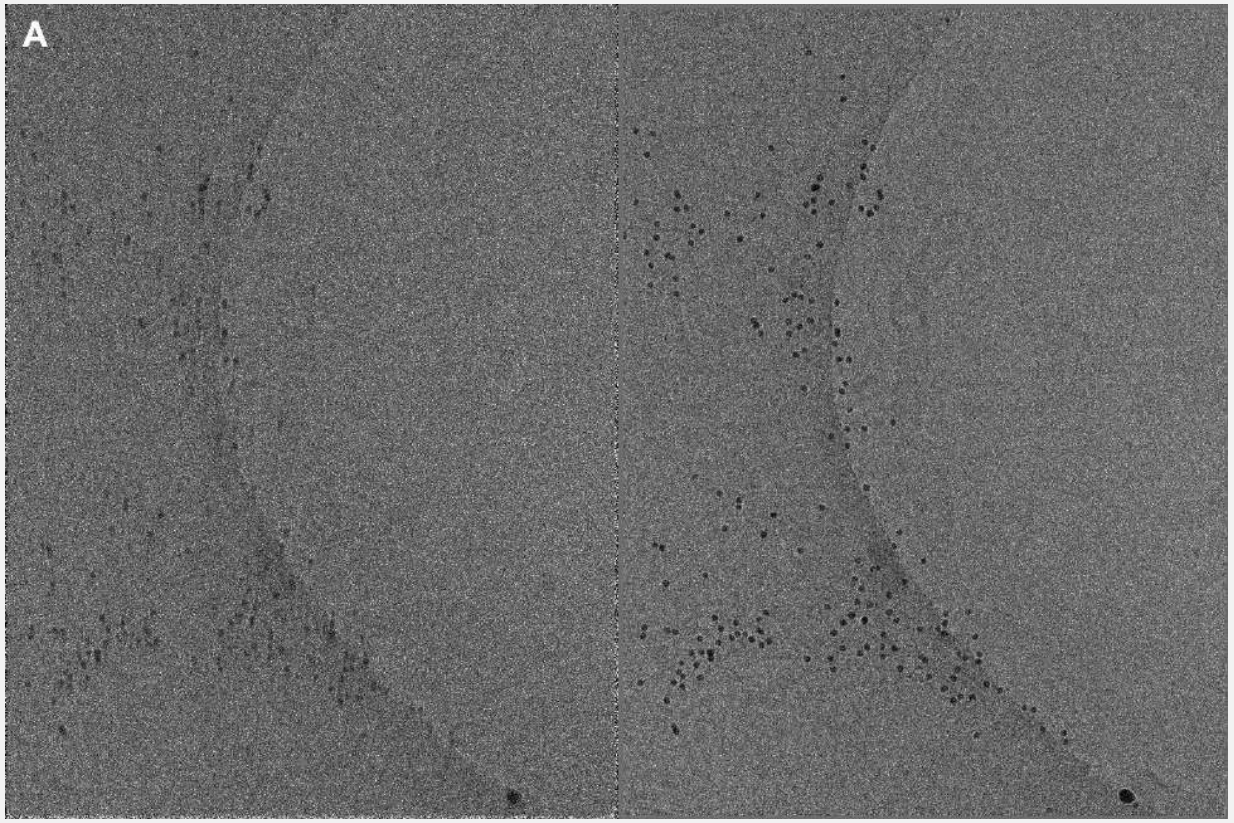

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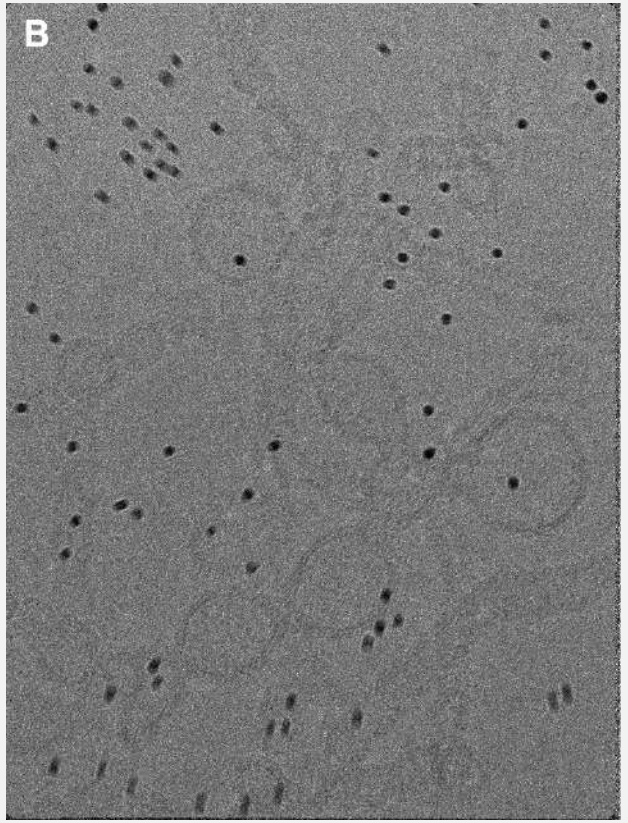

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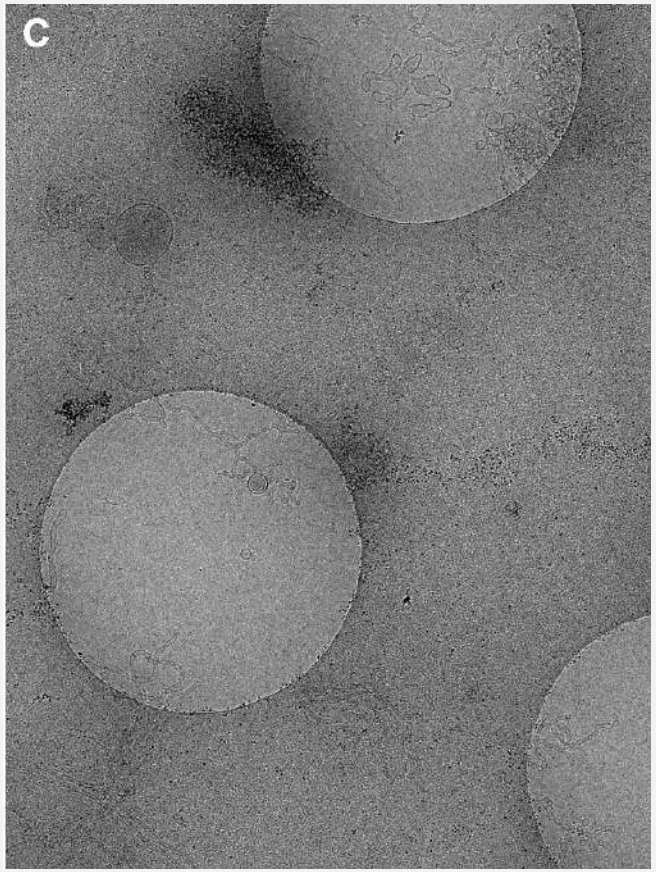

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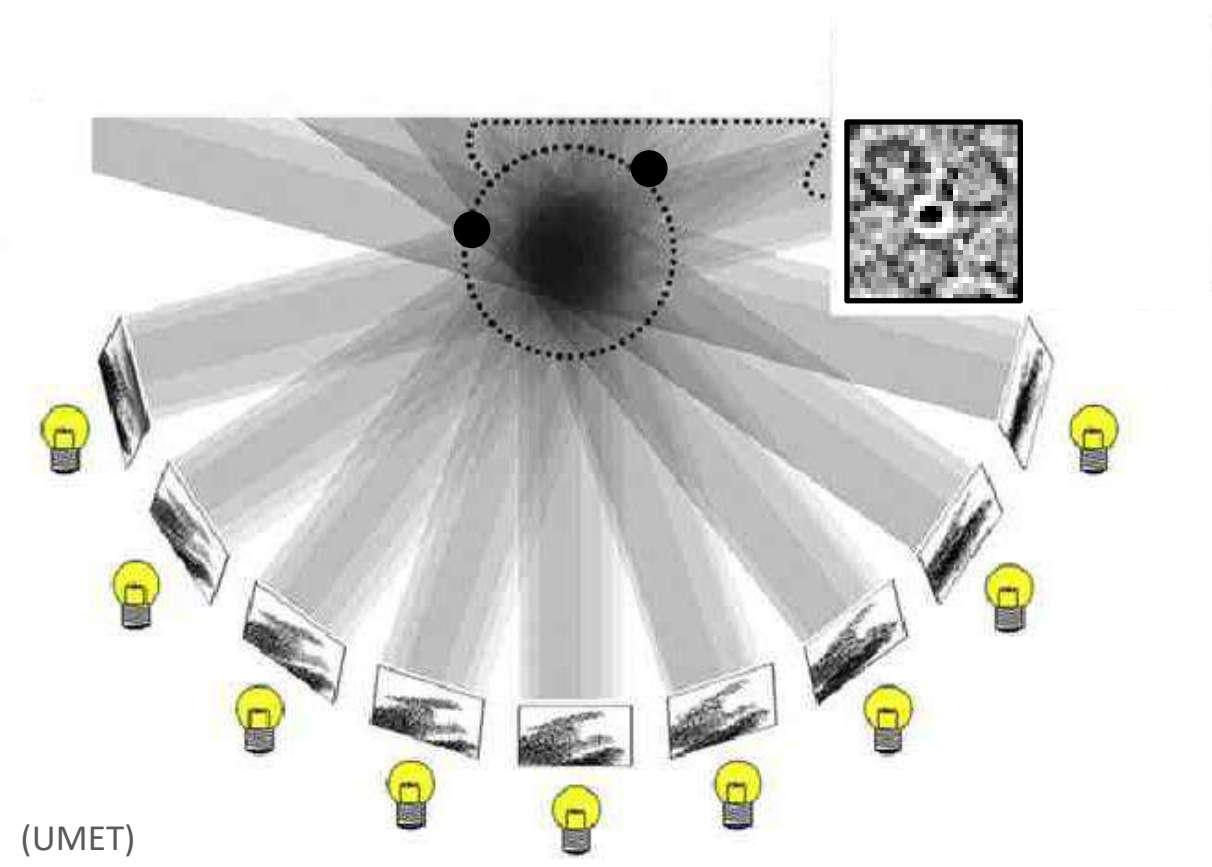
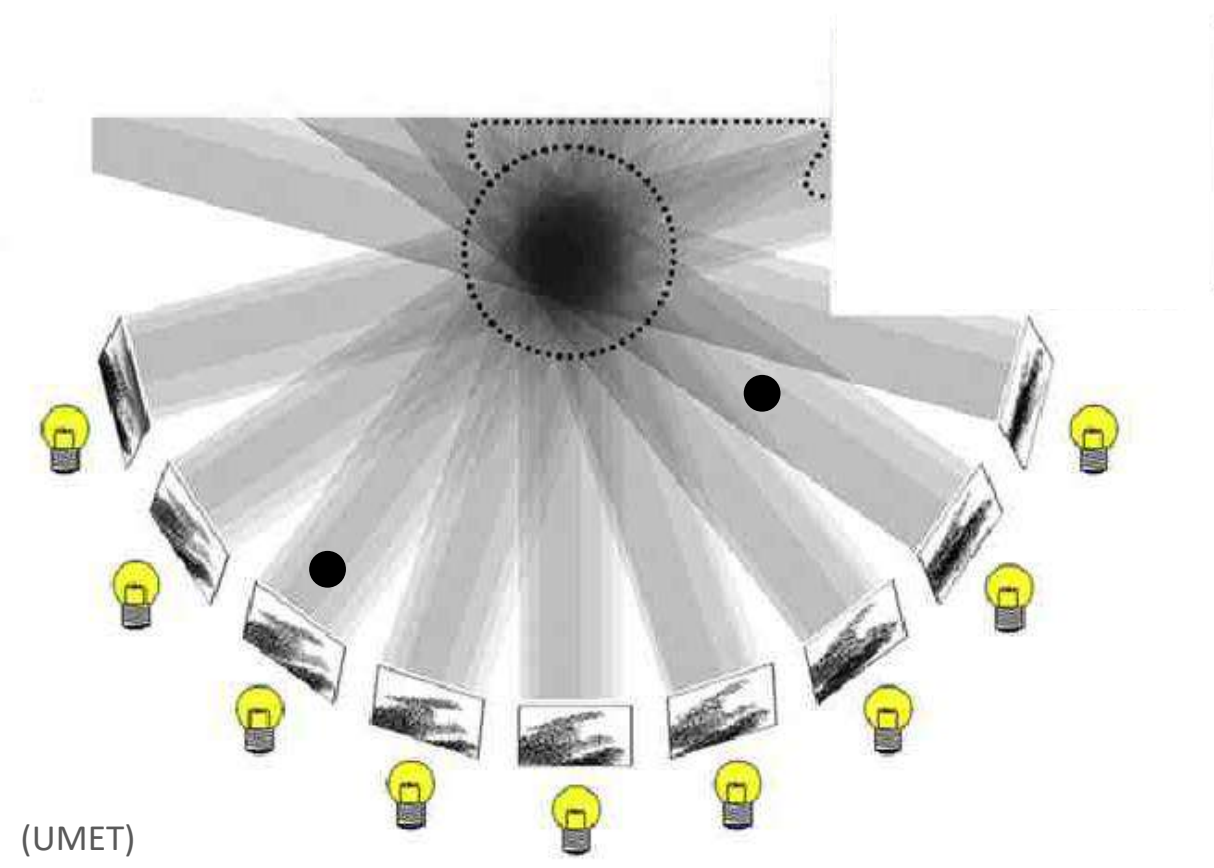


(UMET)

Nearby Fiducials Affect Signal and Contrast

- **Fiducial fringes** change the **power spectrum** of your reconstructed object.

Fiducial-based Tilt-Series Alignment Issues



(UMET)

Fiducials are Present in Much of the Reconstruction, *Even if You Can't See Them!*

- **Distant fiducials** can be in the **projection direction** of your extracted object of interest.
- Erasing fiducials isn't perfect.

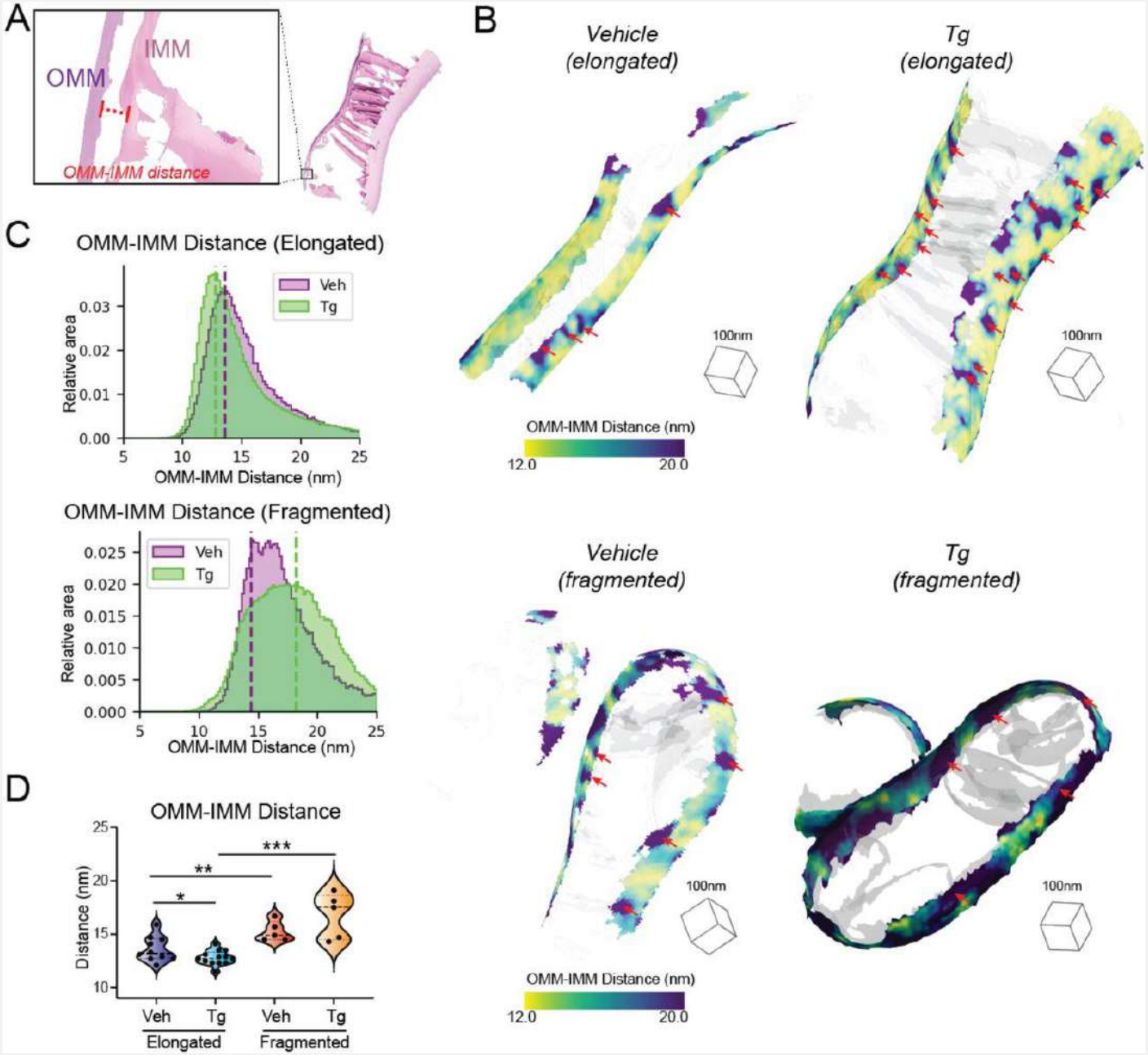
Tilt-series alignment software

- **Software:**
  - ETomo in IMOD – **Fiducial-based** alignment (also **patch tracking**)
  - Markerauto and AuTom – Automated **fiducial-based** alignment
  - Dynamo – **Fiducial-based** alignment
  - Protomo – **Fiducial-less** alignment
  - AreTomo – **Fiducial-less** alignment, GPU-accelerated
- **Must refine** most or all of the following:
  - Tilt image shifts, rotations, defocus changed, & magnification changes
  - Tilt axis location
  - Tilt angles

Annotation and Segmentation Software

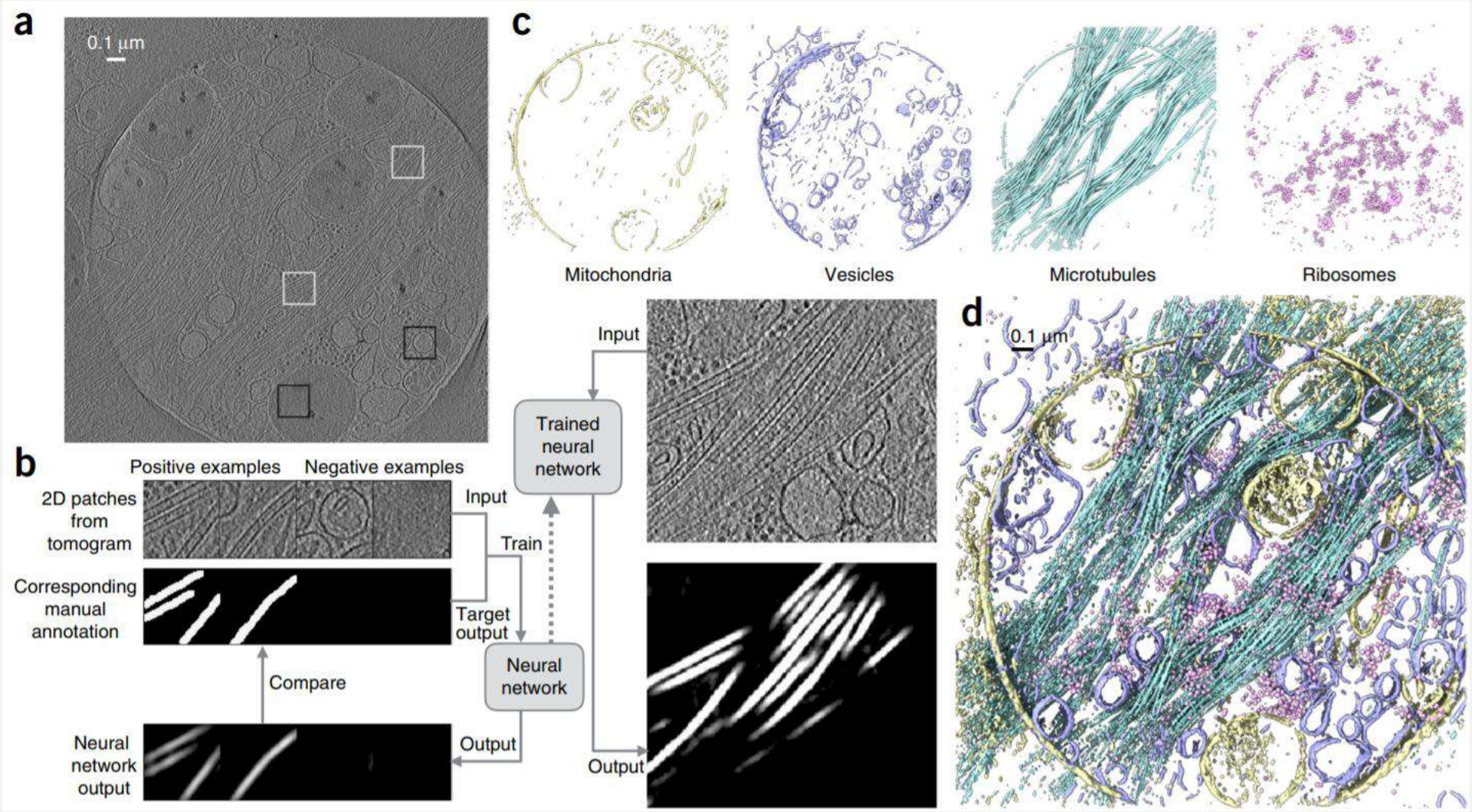
- **Surface morphometrics toolkit** – for mitochondria membrane statistics
- **EMAN2** – Neural network segmentation
- **Dynamo** – Annotate membranes, tubes, helices, crystal structures, vesicles, etc.
- Amira – Interactive segmentation and filtering suite
- Template picking (e.g. MolMatch, Dynamo)
- Difference of Gaussian (DoG) picking

Surface morphometrics toolkit



Barad et al., 2022

Sub-tomogram segmentation with CNNs in EMAN2



Chen et. al., Nat. Meth. 2017

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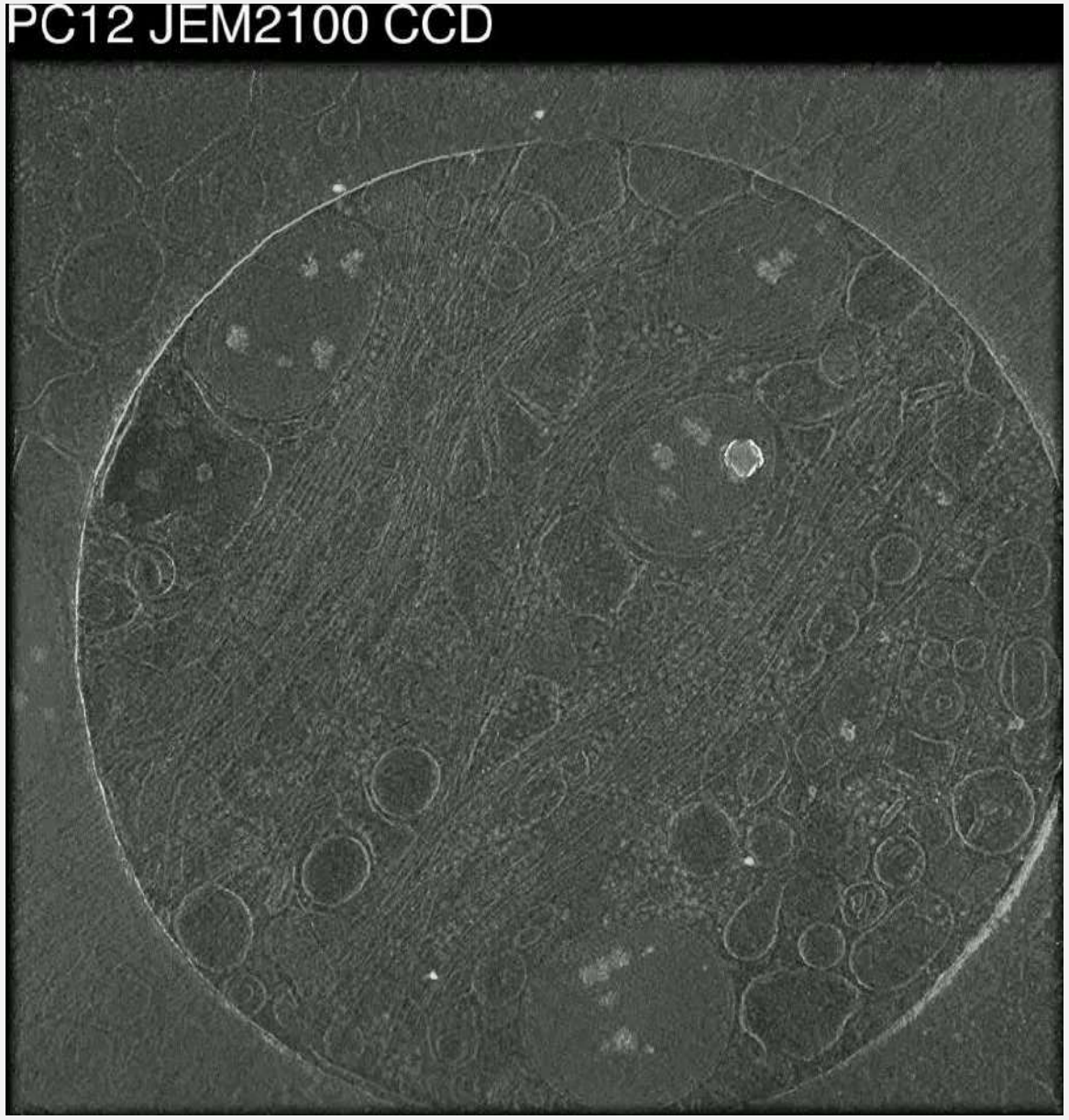
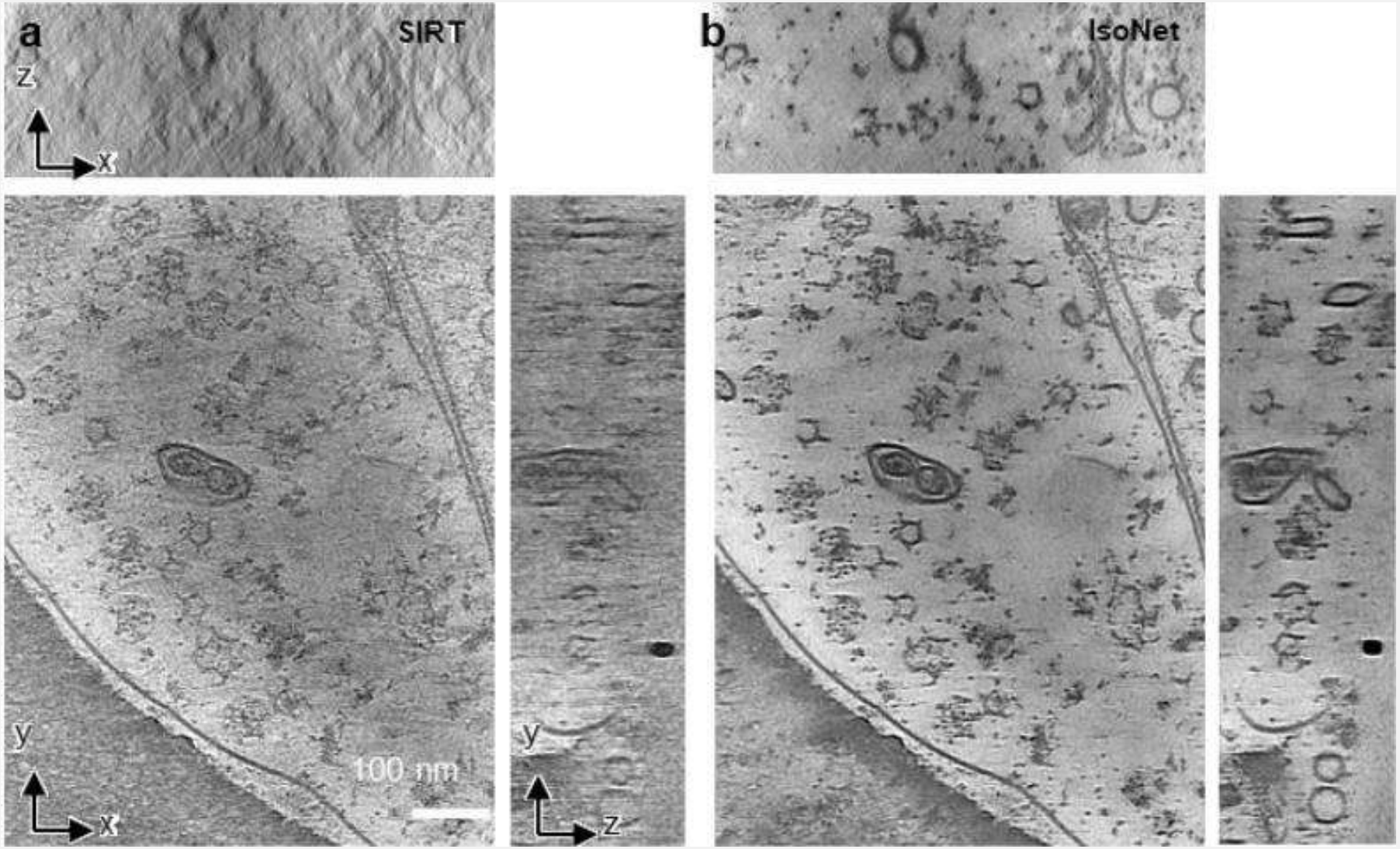


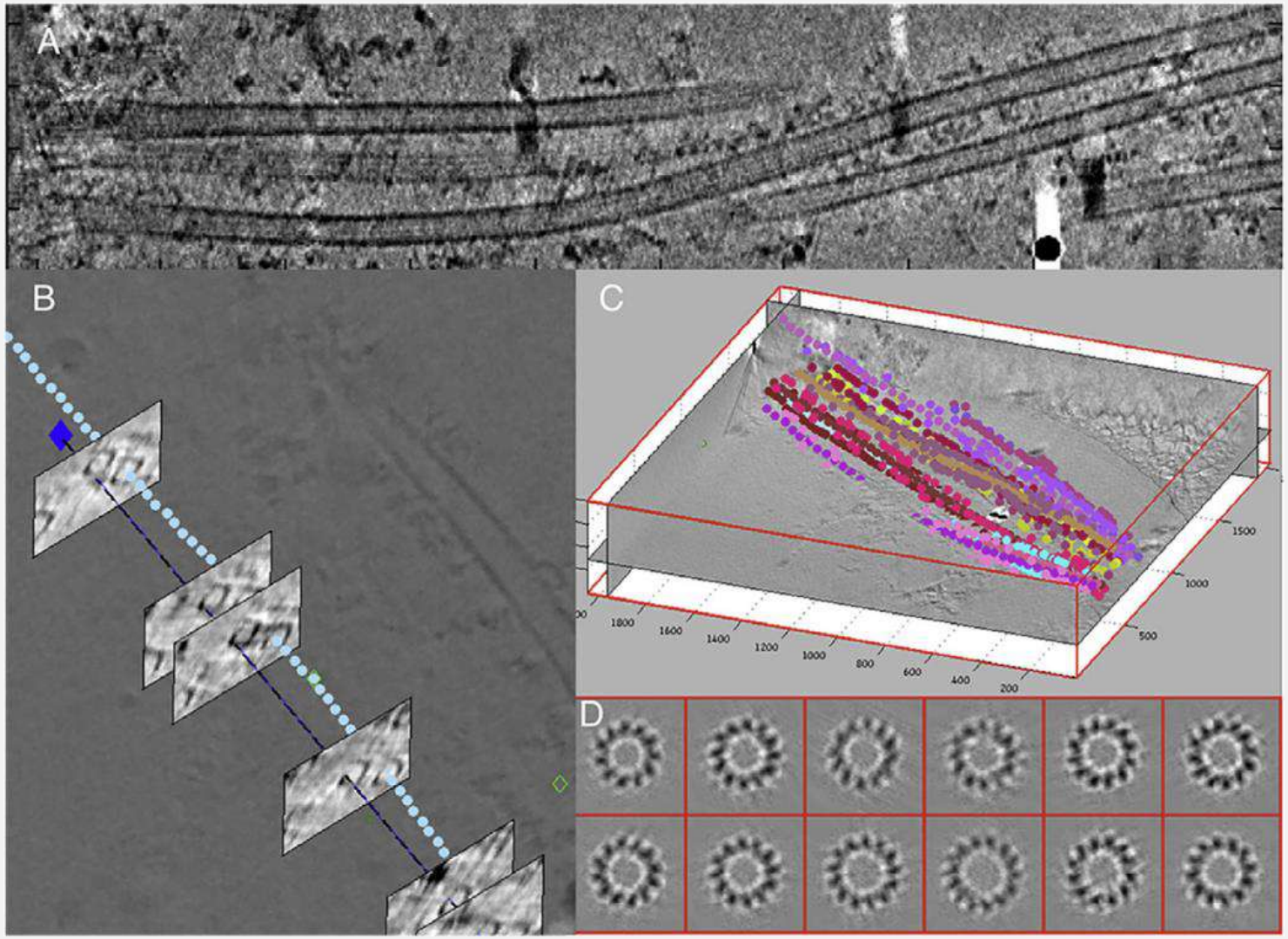
Chen et. al., Nat. Meth. 2017

IsoNet Neural Network missing wedge restoration



Liu et. al., 2021

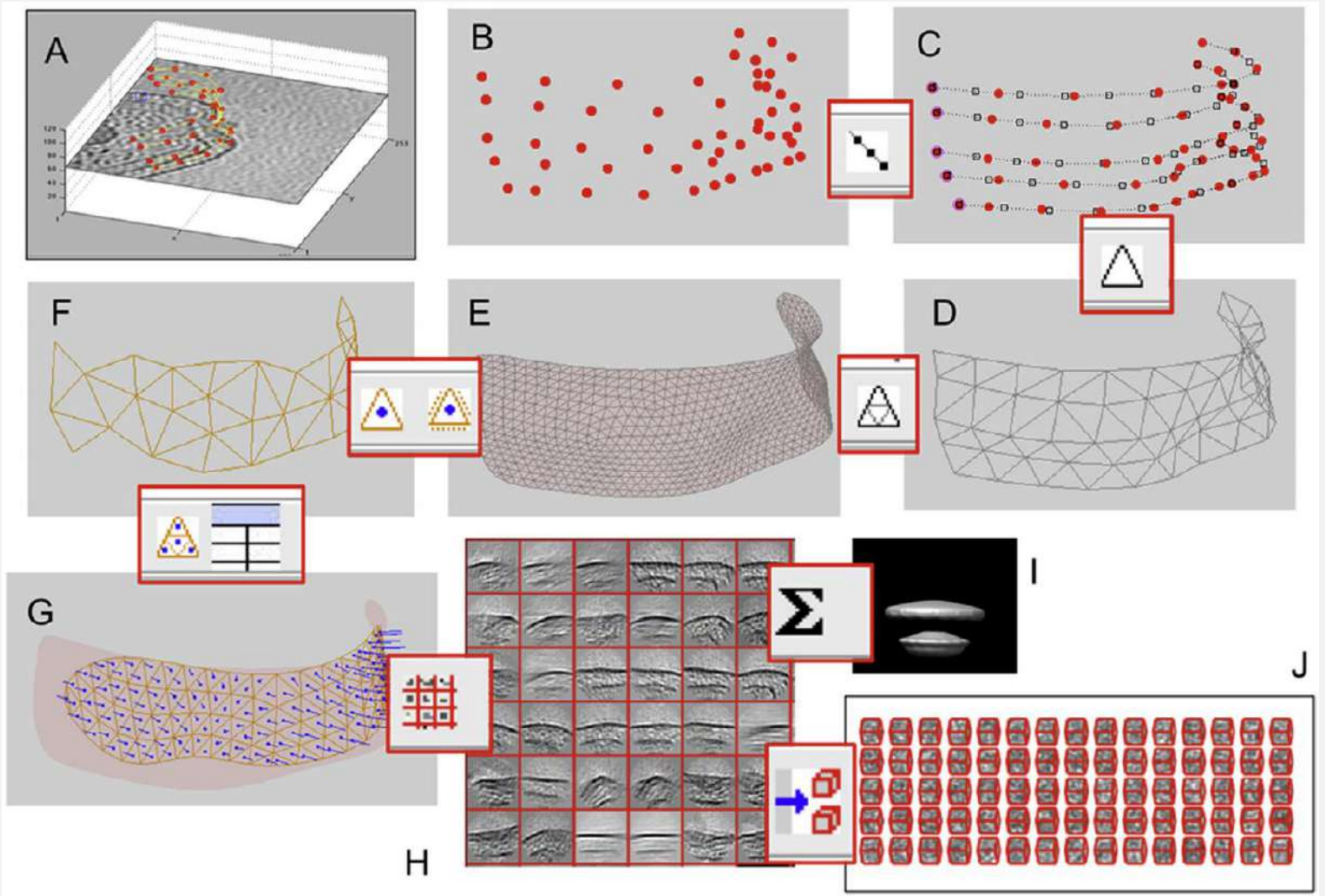
Dynamo Filament Picking



- Backbone, helical, and circumferential picking
- Helical symmetry determination

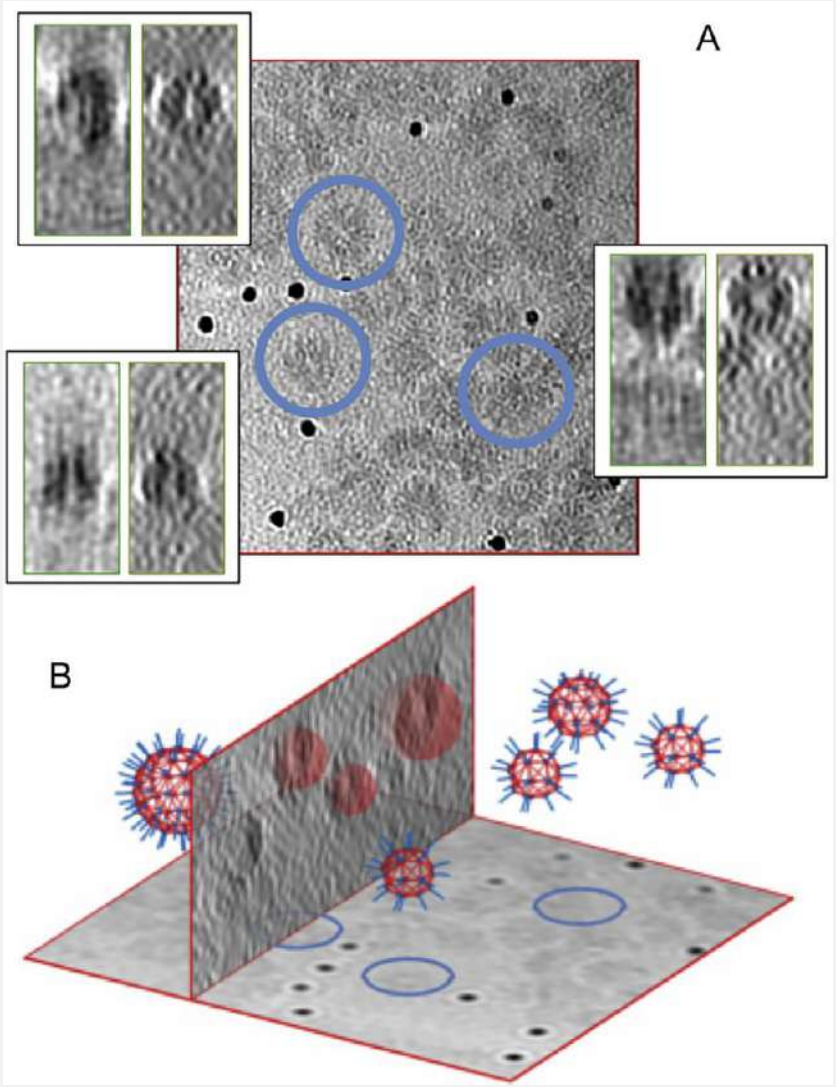
Castaño-Díez et. al., JSB 2012 & 2016

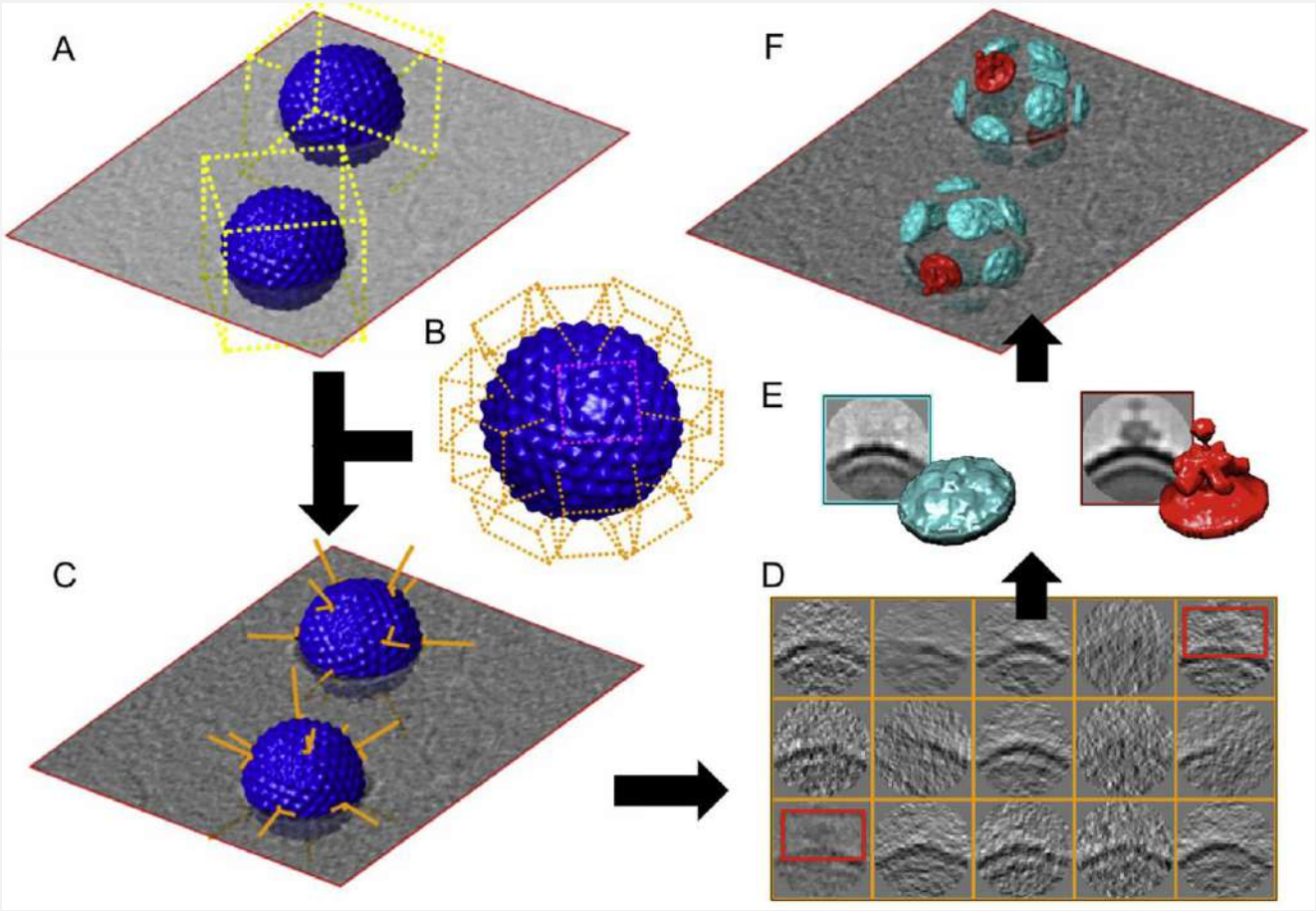
Dynamo Membrane Picking



Castaño-Díez et. al., JSB 2012 & 2016

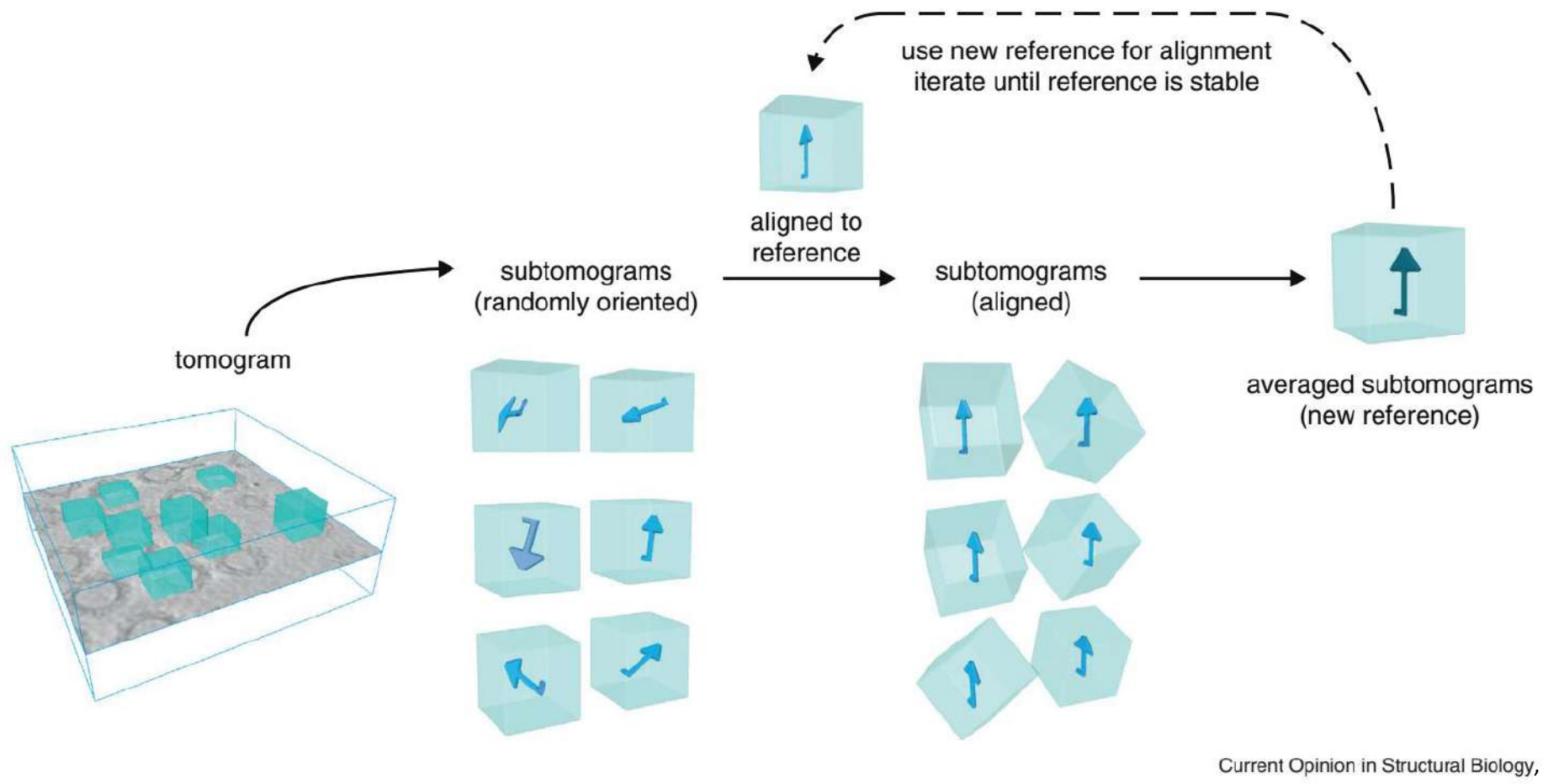
Dynamo Vesicle and Sub-Particle Picking





Castaño-Díez et. al., JSB 2012 & 2016

Sub-tomogram Processing Workflow



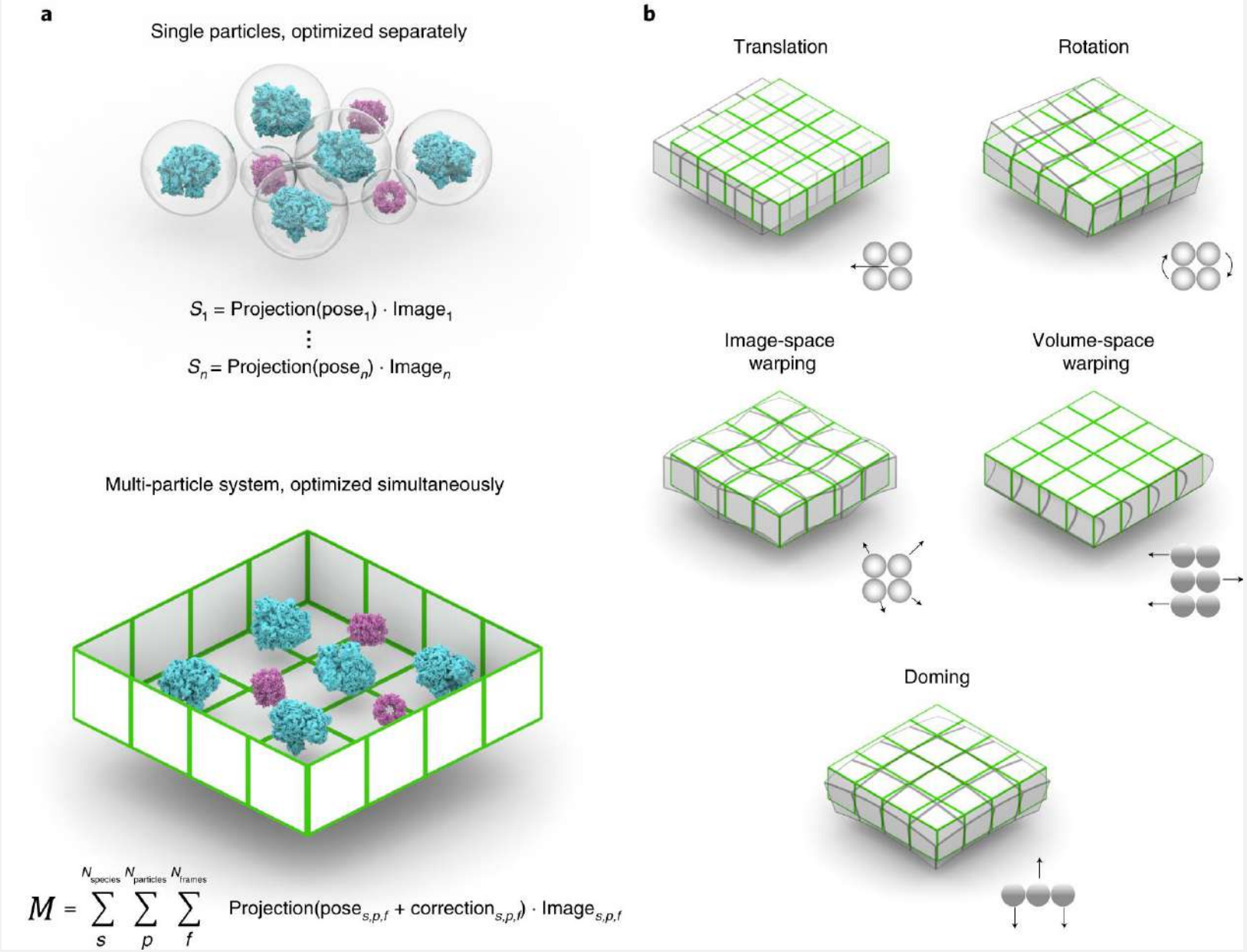
Current Opinion in Structural Biology, Briggs 2013

- Missing wedge must be taken into account for each sub-tomogram

Sub-tomogram Processing Software

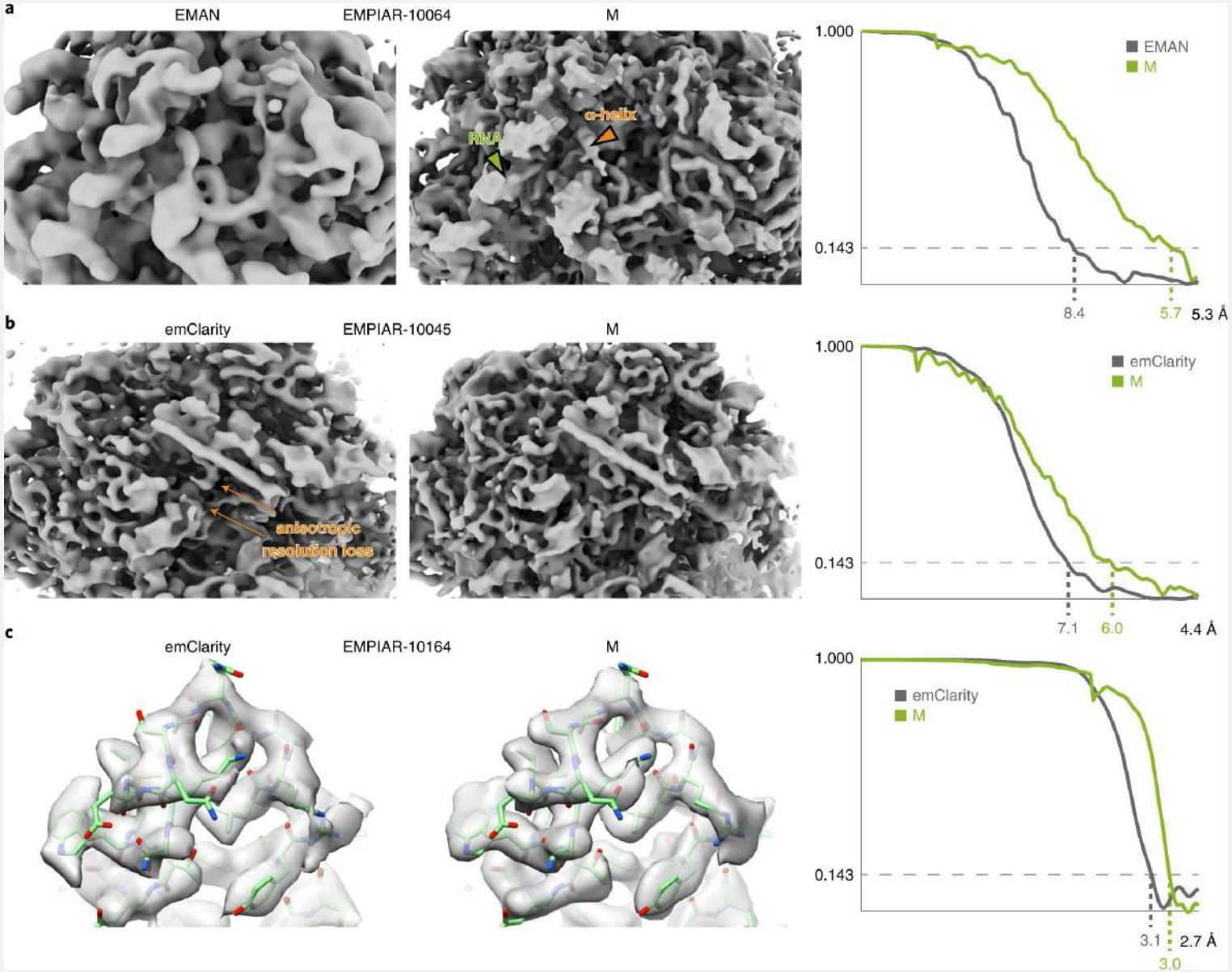
- Warp/M - Sub-tilt-series refinement, multi-species co-refinement
- Relion4 – Sub-tilt-series refinement, Bayesian approach to alignment is used
- EMAN2 – Sub-tilt-series refinement and defocus estimation
- emClarity – Sub-tilt-series refinement and defocus estimation
- Dynamo – GPU accelerated, tomogram database, extensive picking abilities
- PyTom
- PEET
- Jsubtomo
- TOM & AV3
- XMIPP

Warp/M sub-tilt multi-species refinement



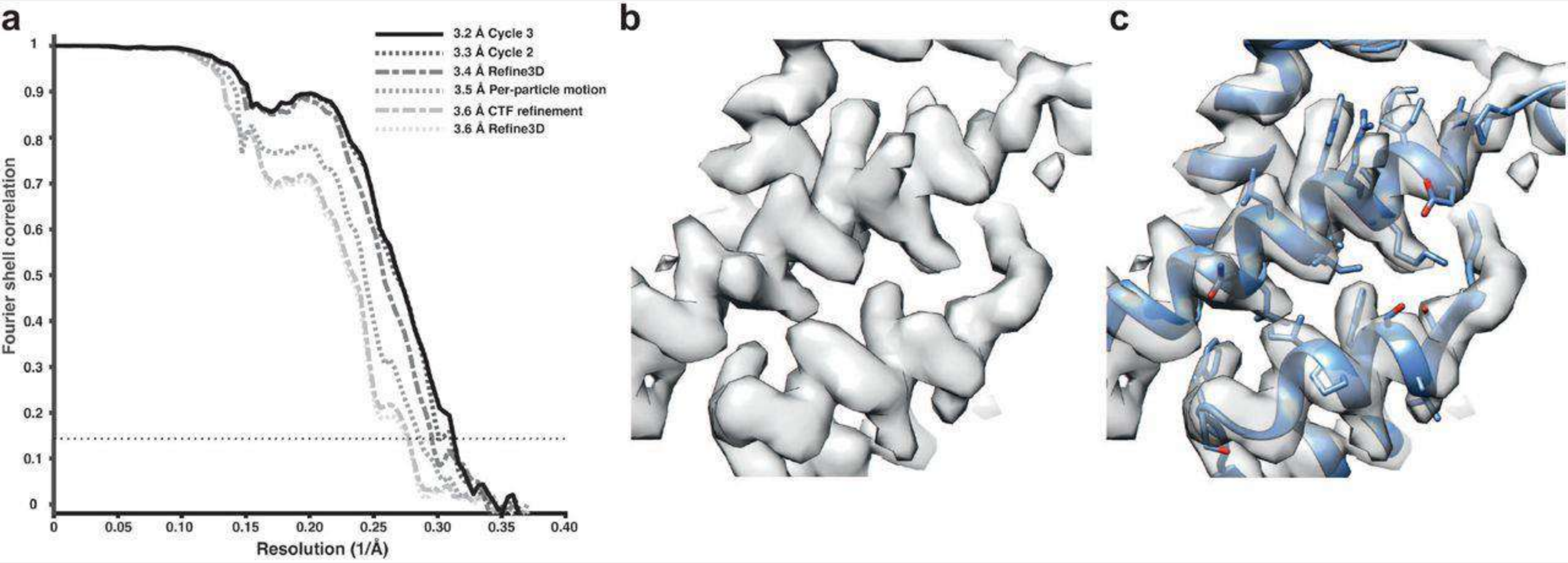
Tegunov, Nat. Meth. 2021

Warp/M sub-tilt multi-species refinement



Tegunov, Nat. Meth. 2021

Relion4 sub-tilt refinement

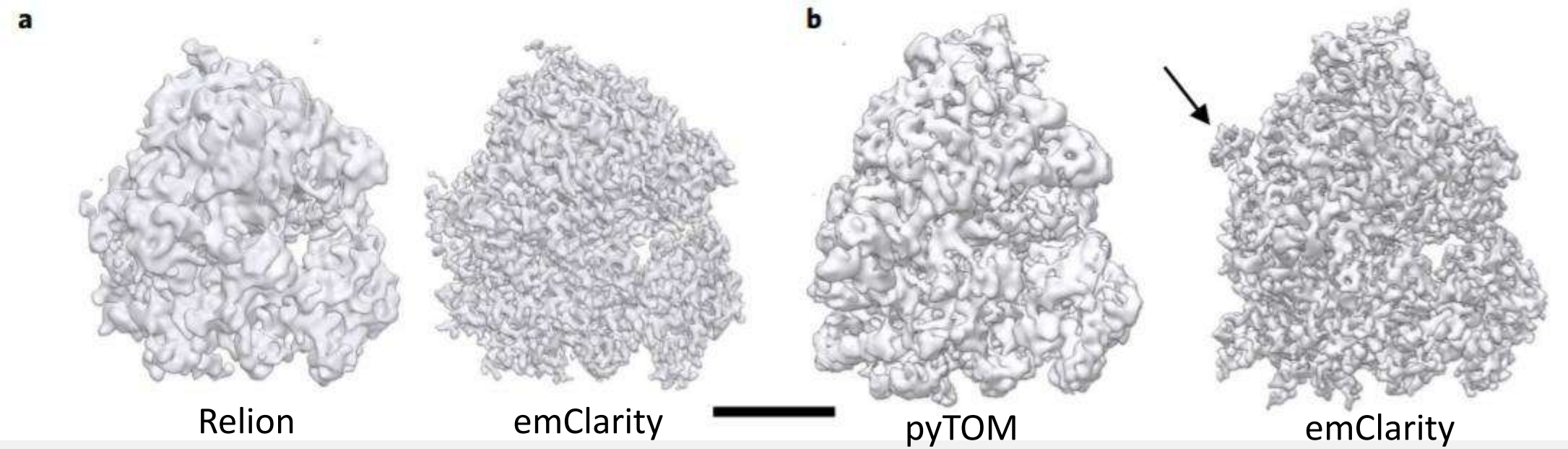


Zivanov et al., 2022

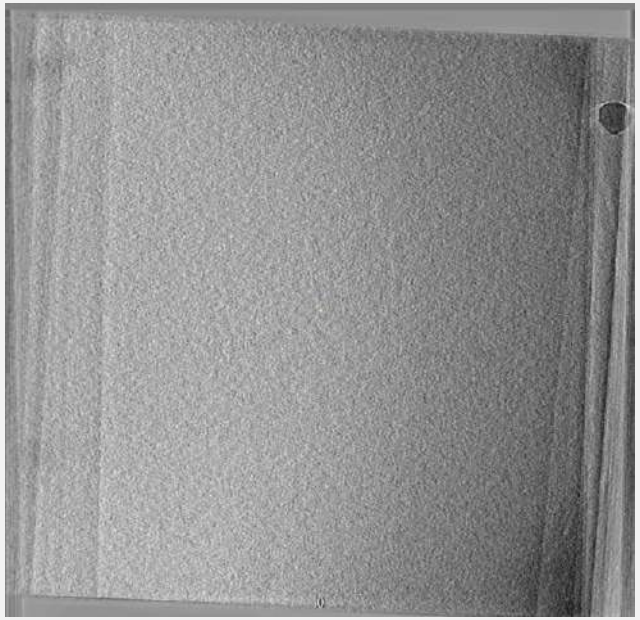
emClarity sub-tilt series refinement

Yeast 80S ribosome

Rabbit 80S ribosome

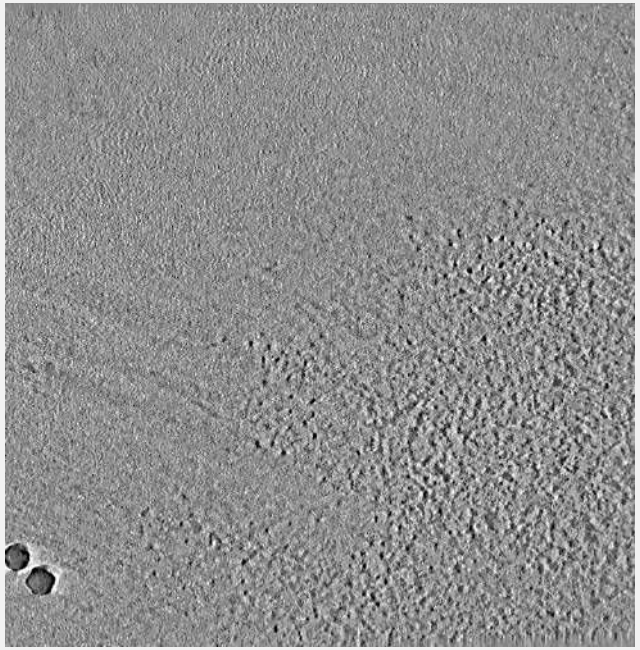


Himes, Nat. Meth. 2018

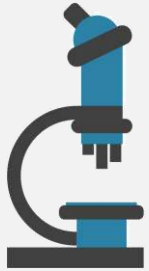


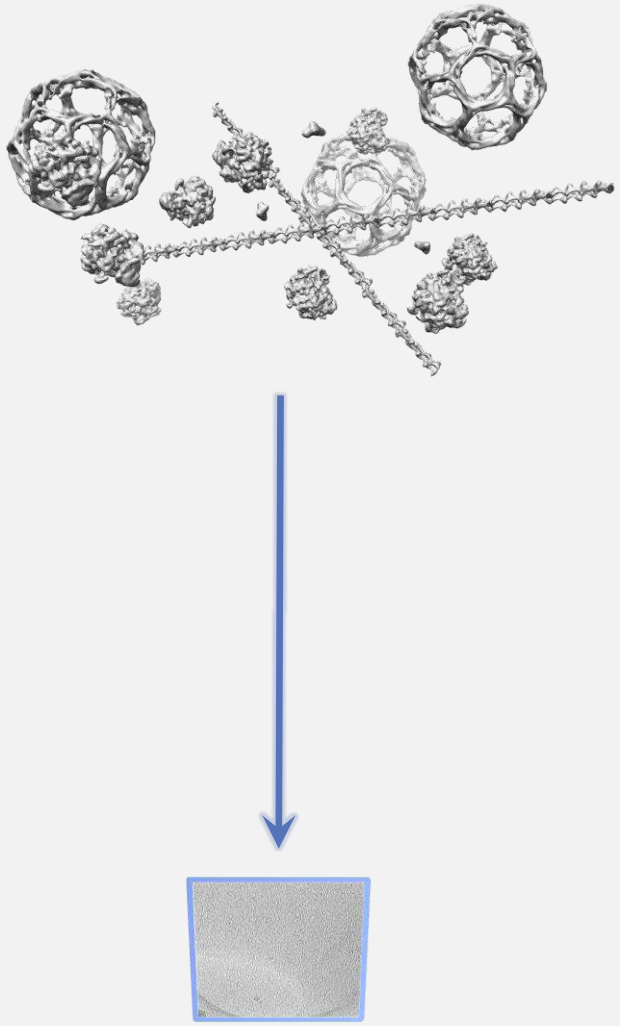
Questions?

Next: Appion-Protomo description



3D specimen movement during collection





(movements are exaggerated)

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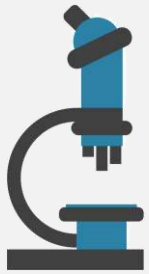

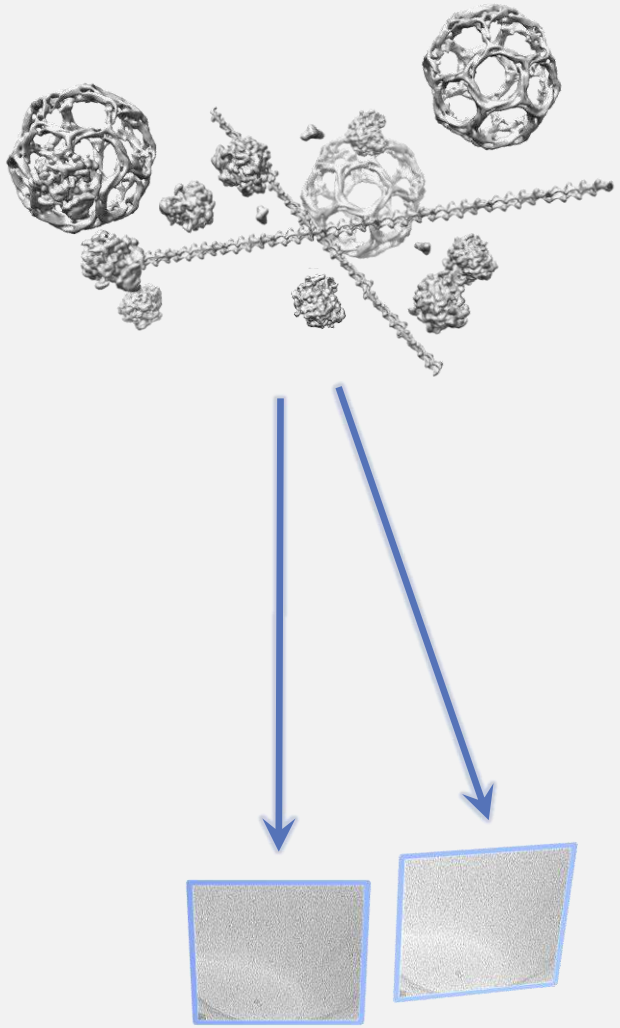

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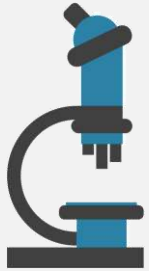

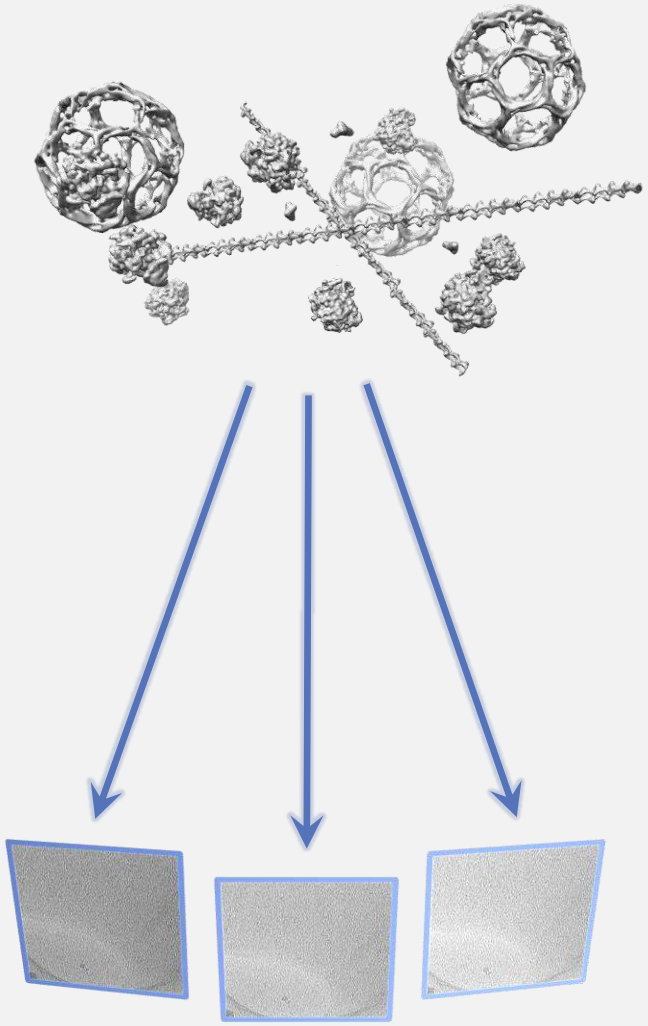

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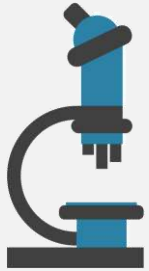

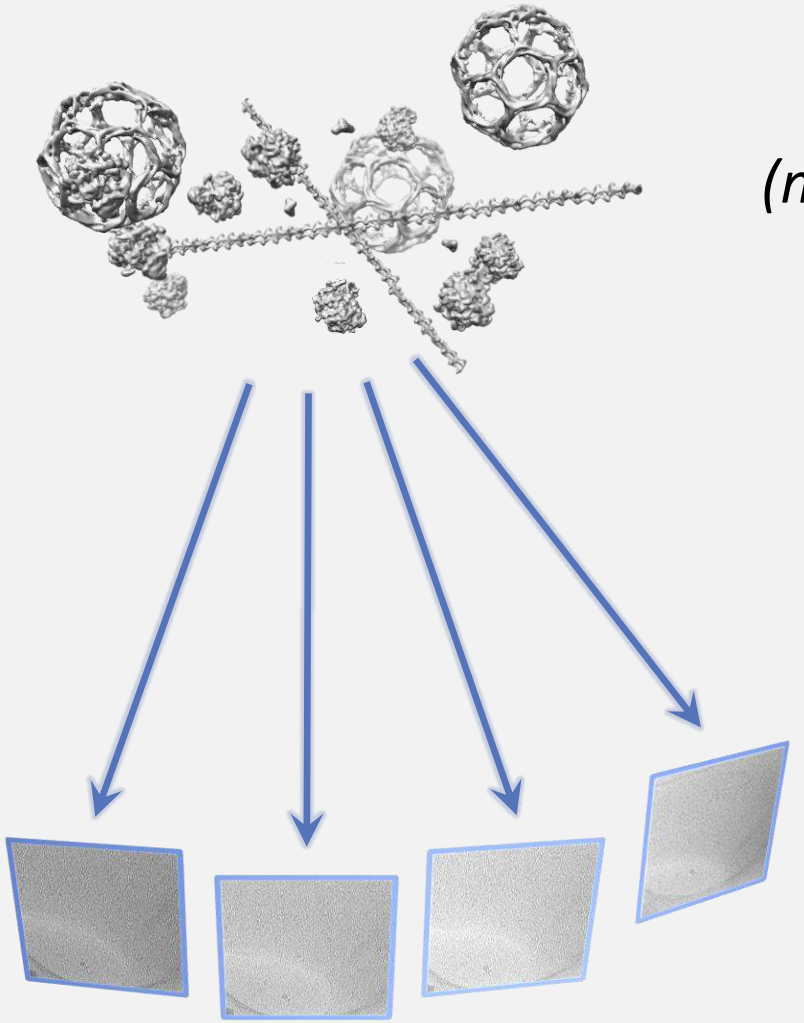

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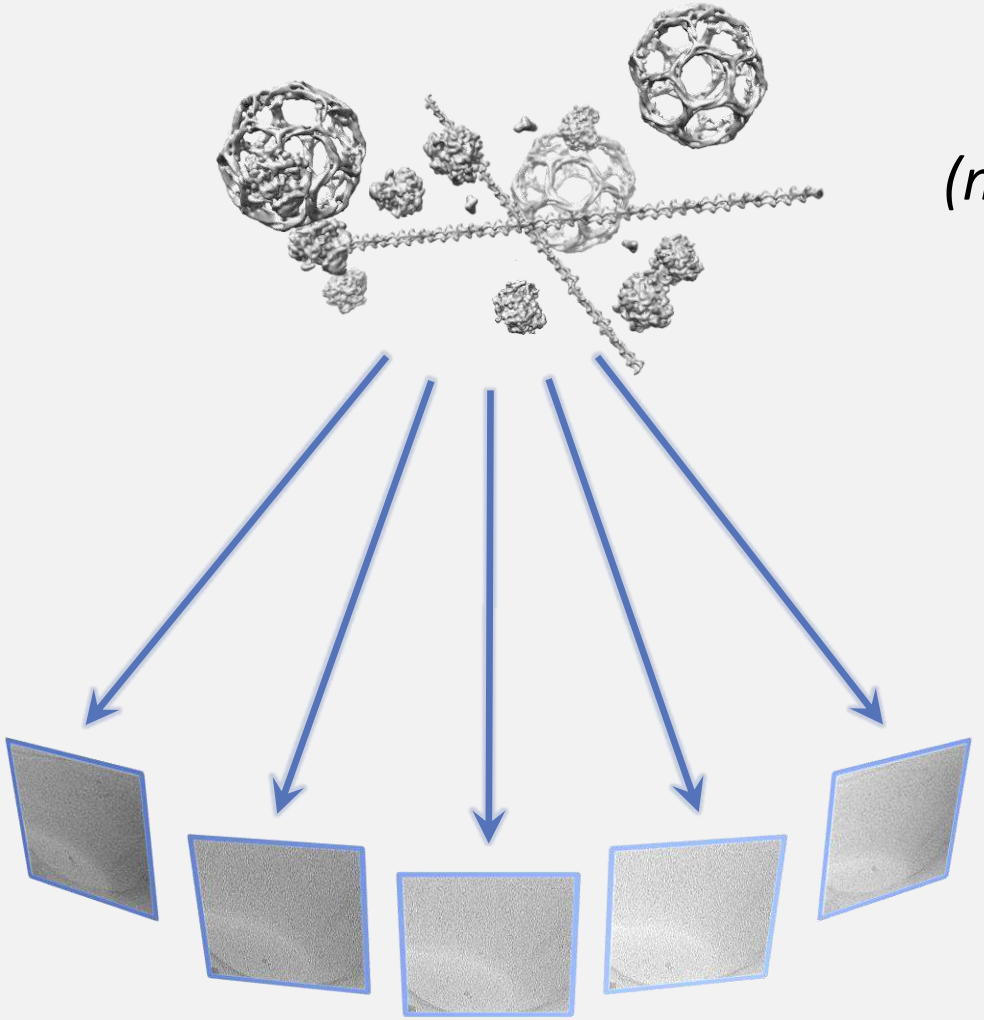

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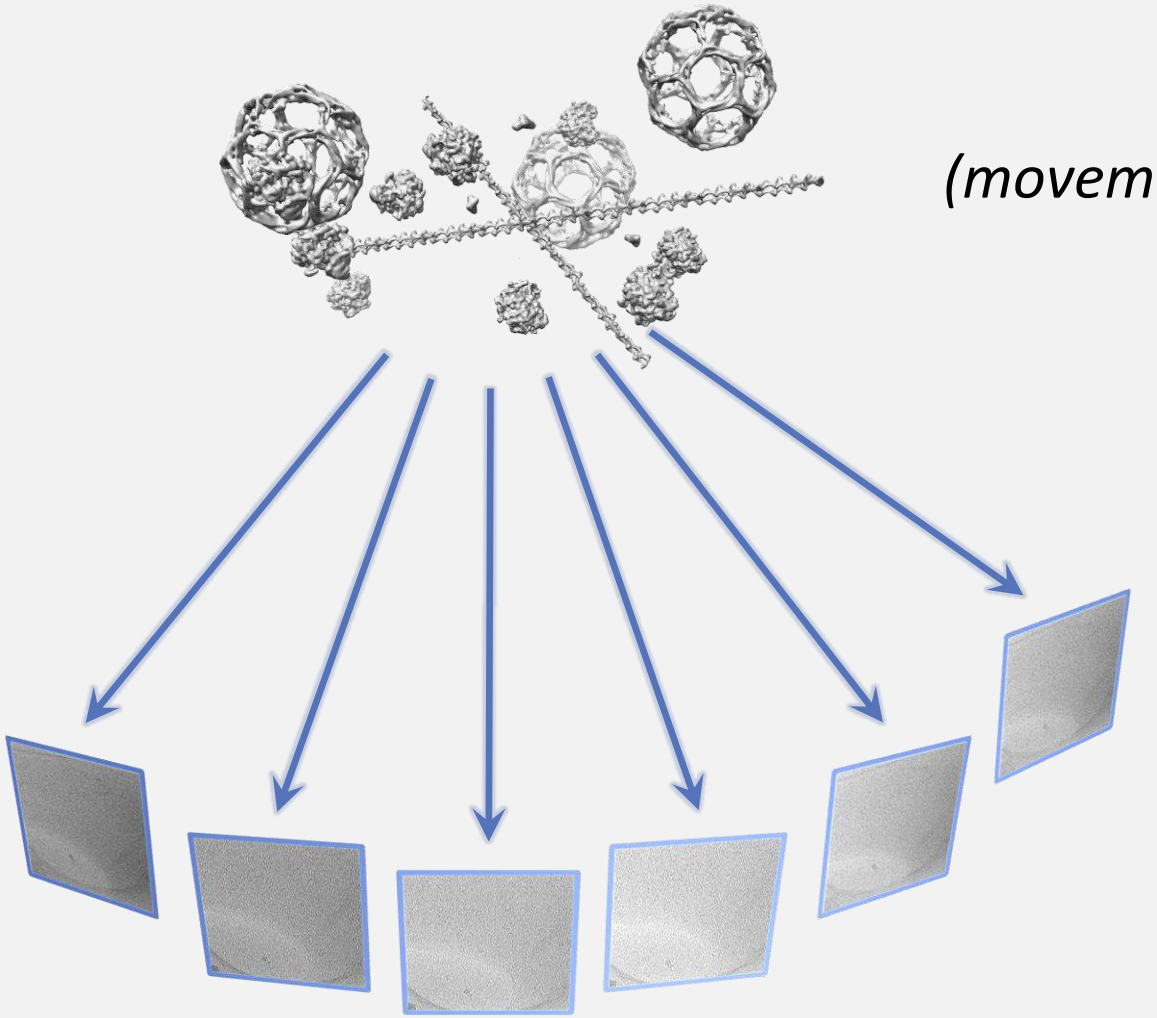
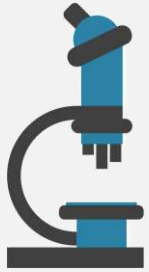
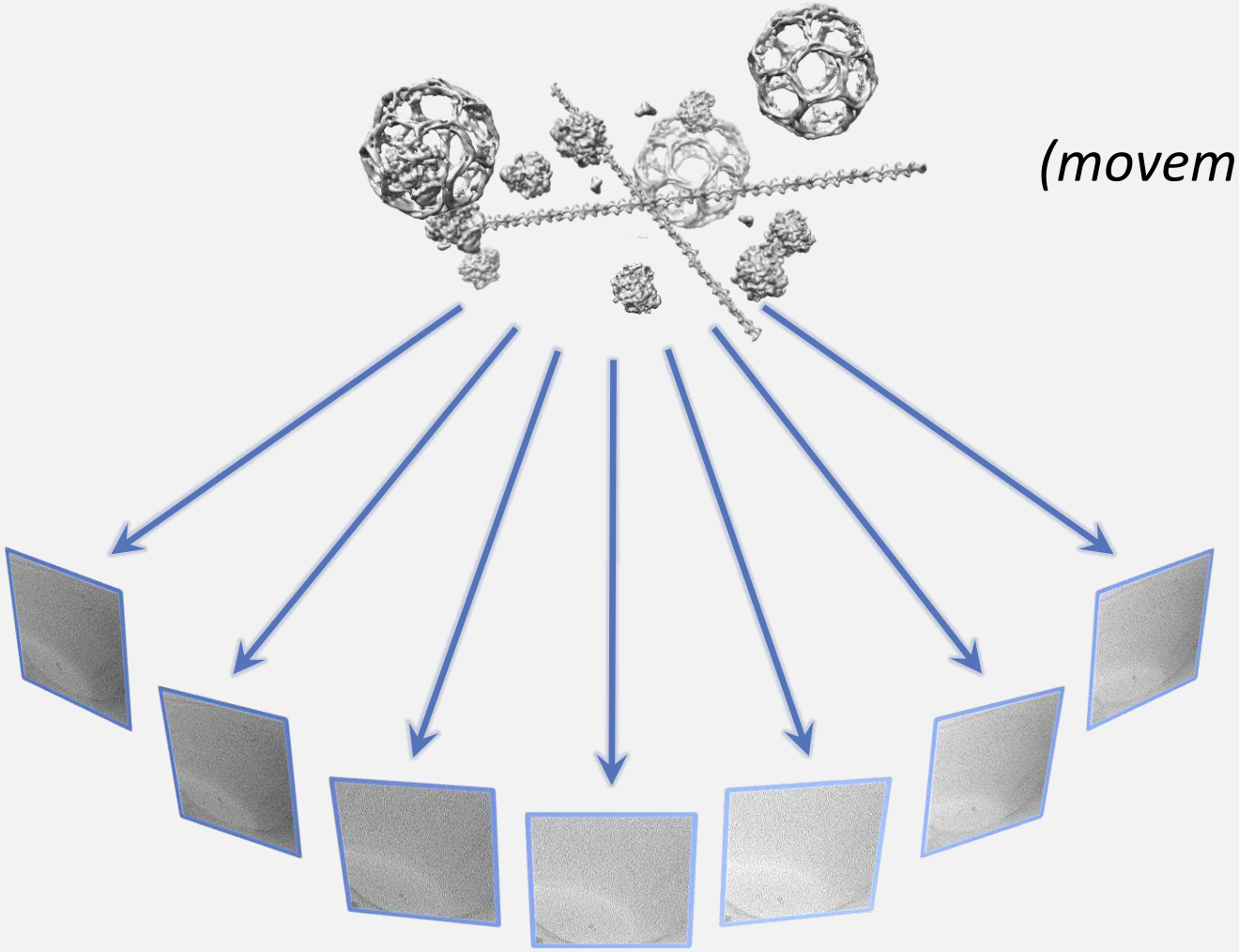


(movements are exaggerated)

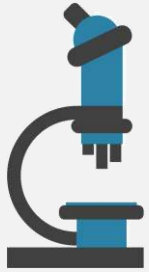
3D specimen movement during collection

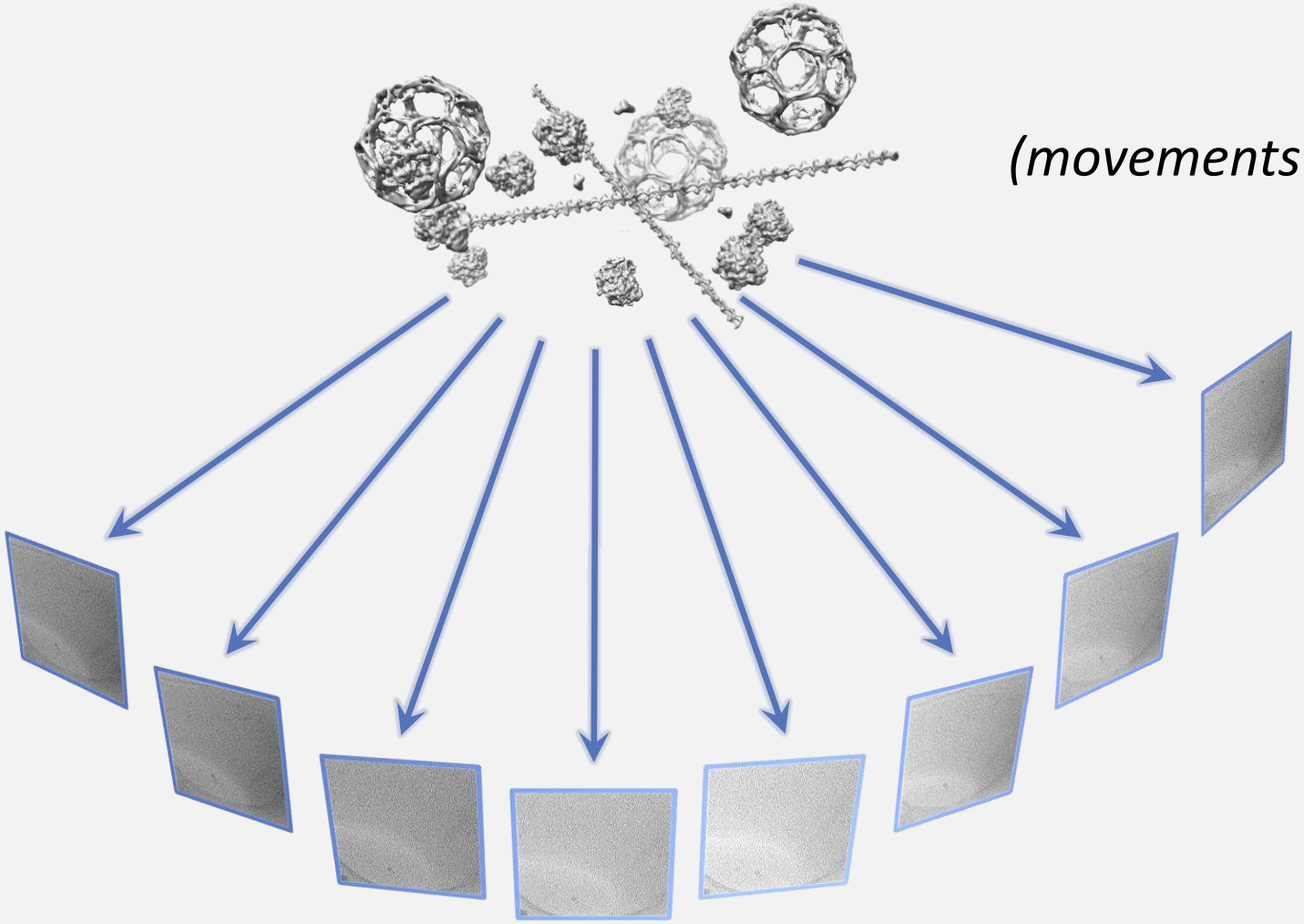




(movements are exaggerated)

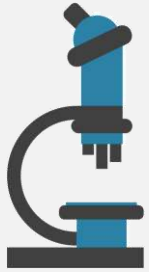
3D specimen movement during collection

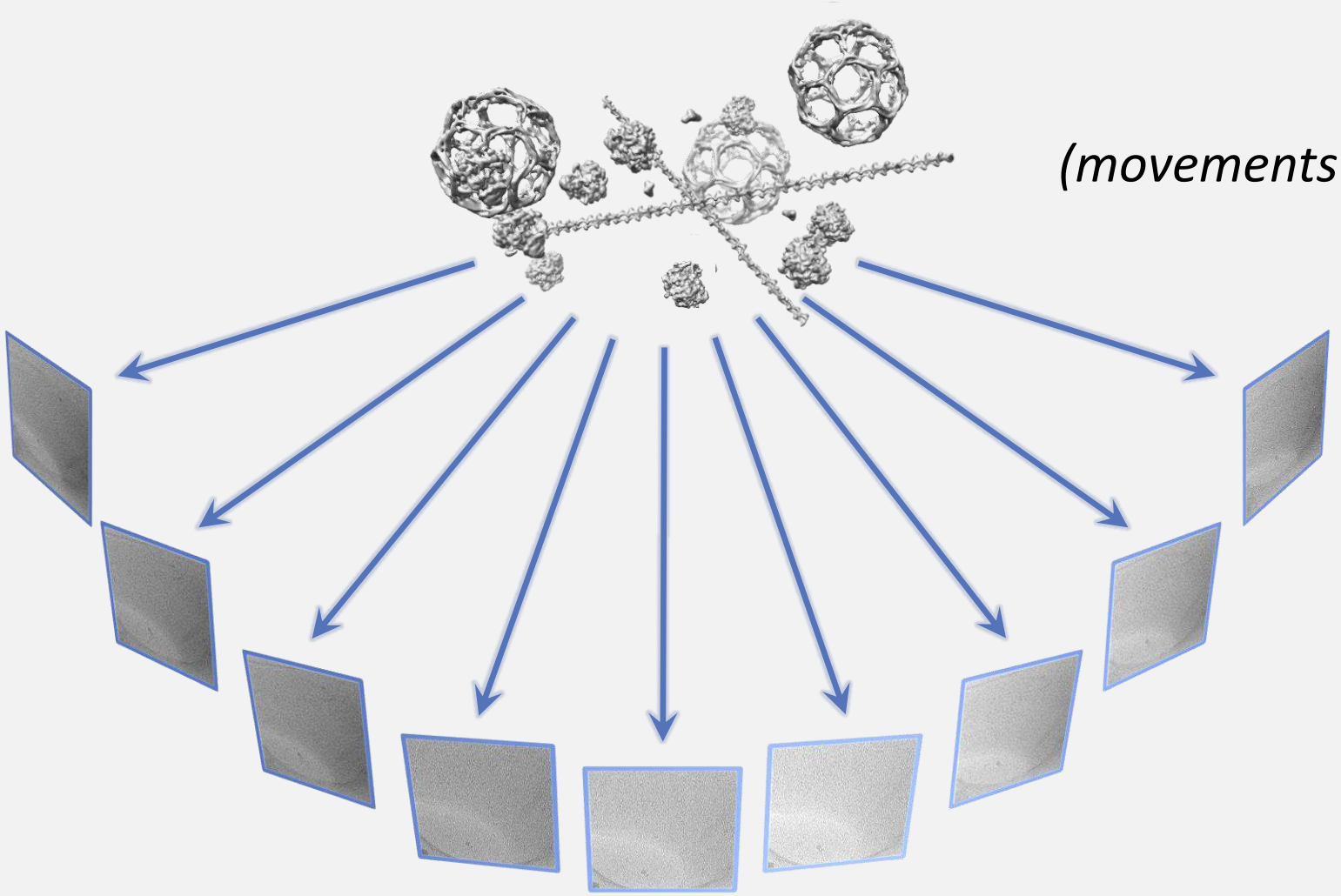




(movements are exaggerated)

3D specimen movement during collection

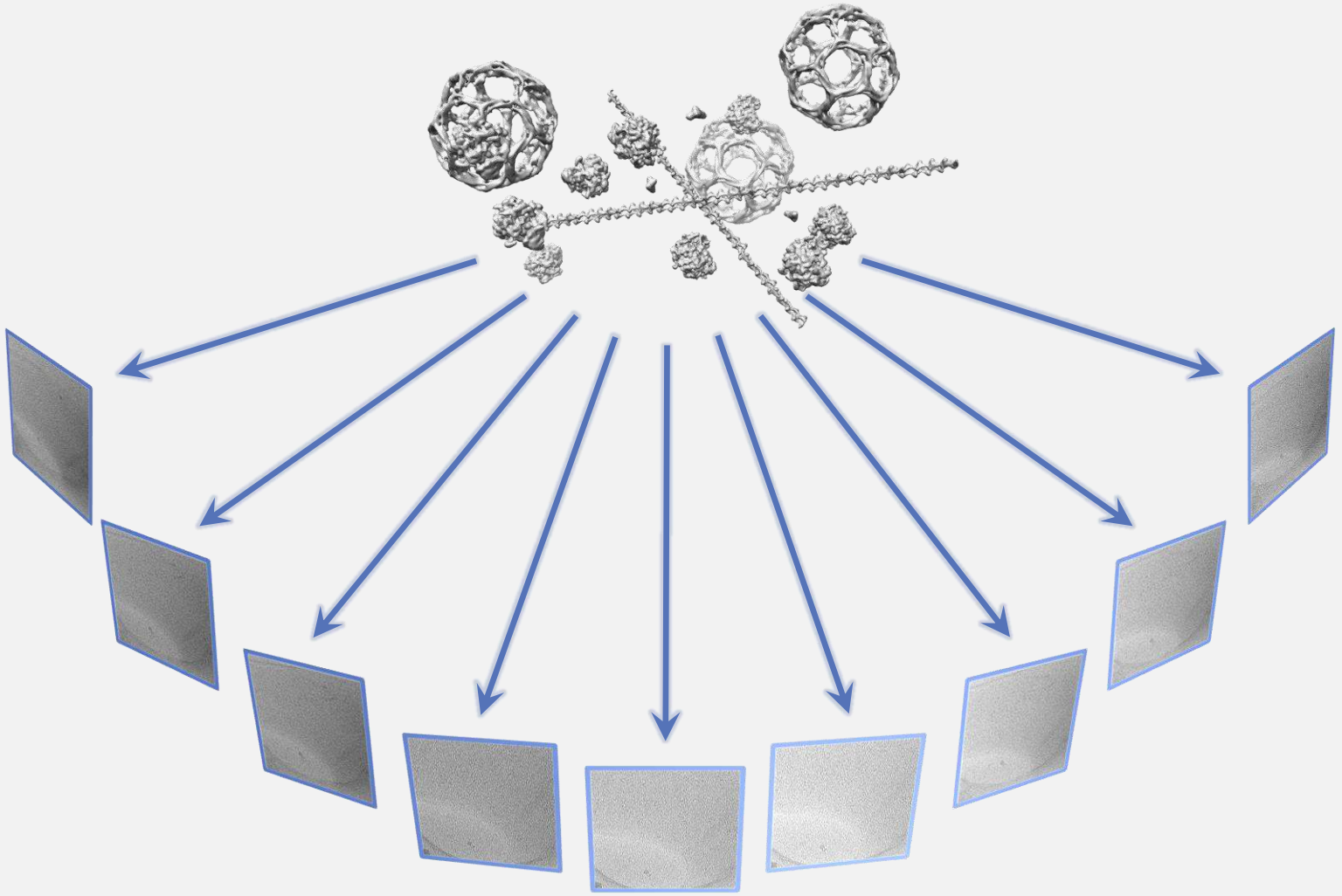


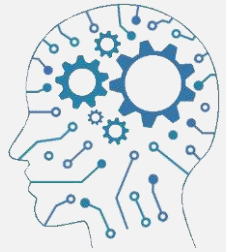


(movements are exaggerated)

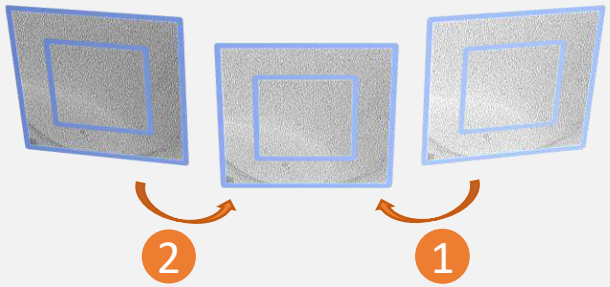
How does alignment in Protomo work?

Collect a tilt-series

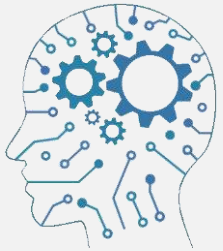




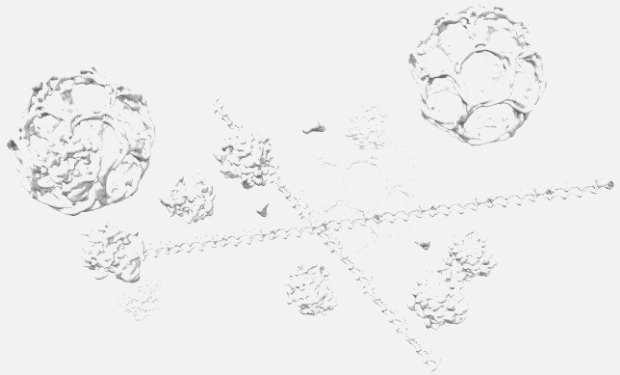
Protomo alignment

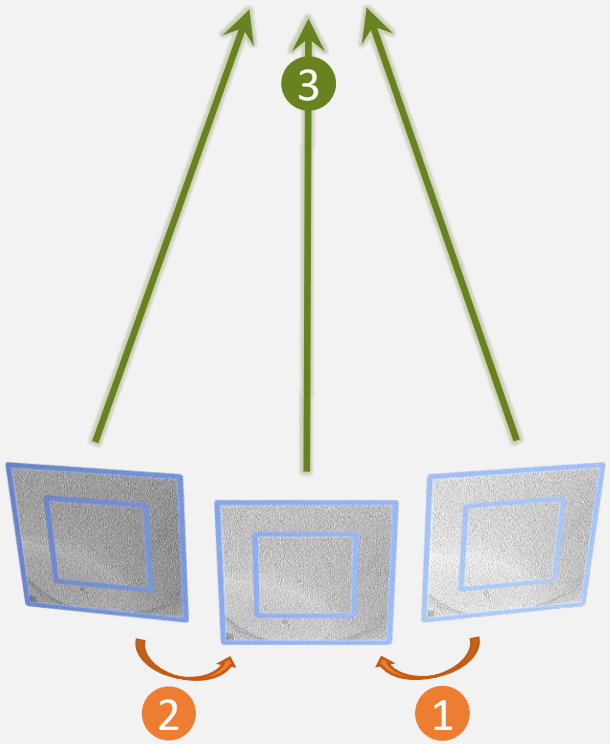


Nearest-neighbor correlation



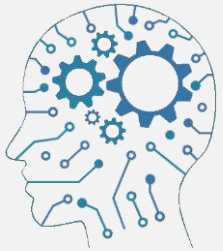
Protomo alignment



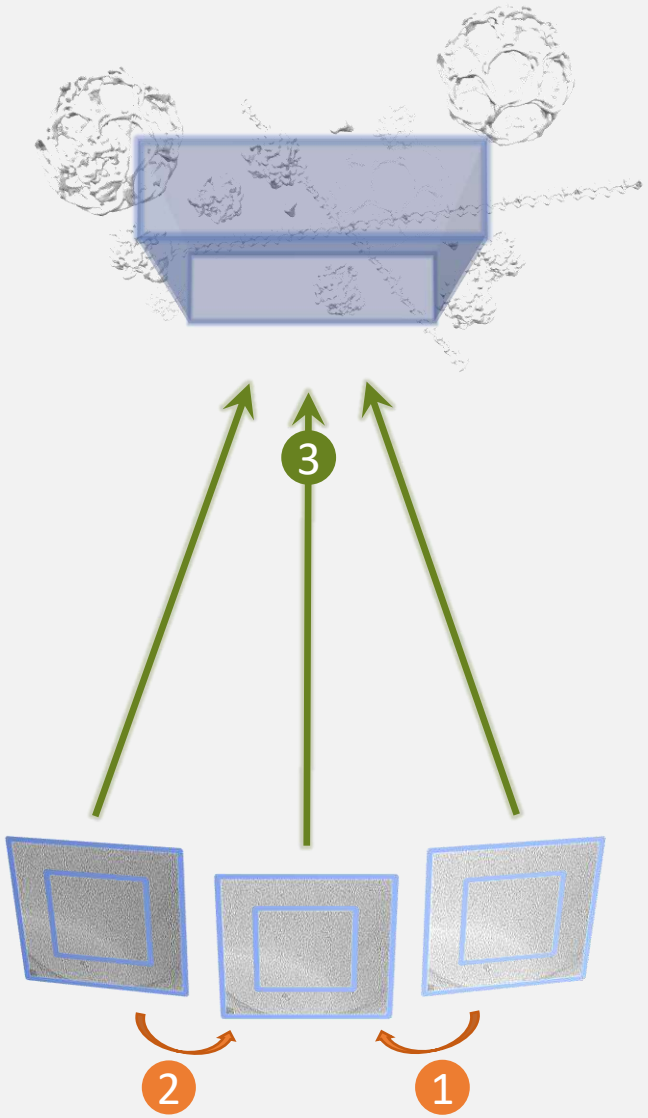


Nearest-neighbor correlation

Weighted back-projection



Protomo alignment



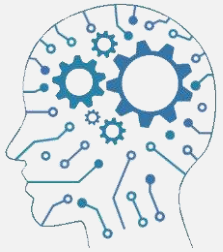
Nearest-neighbor correlation

Weighted back-projection

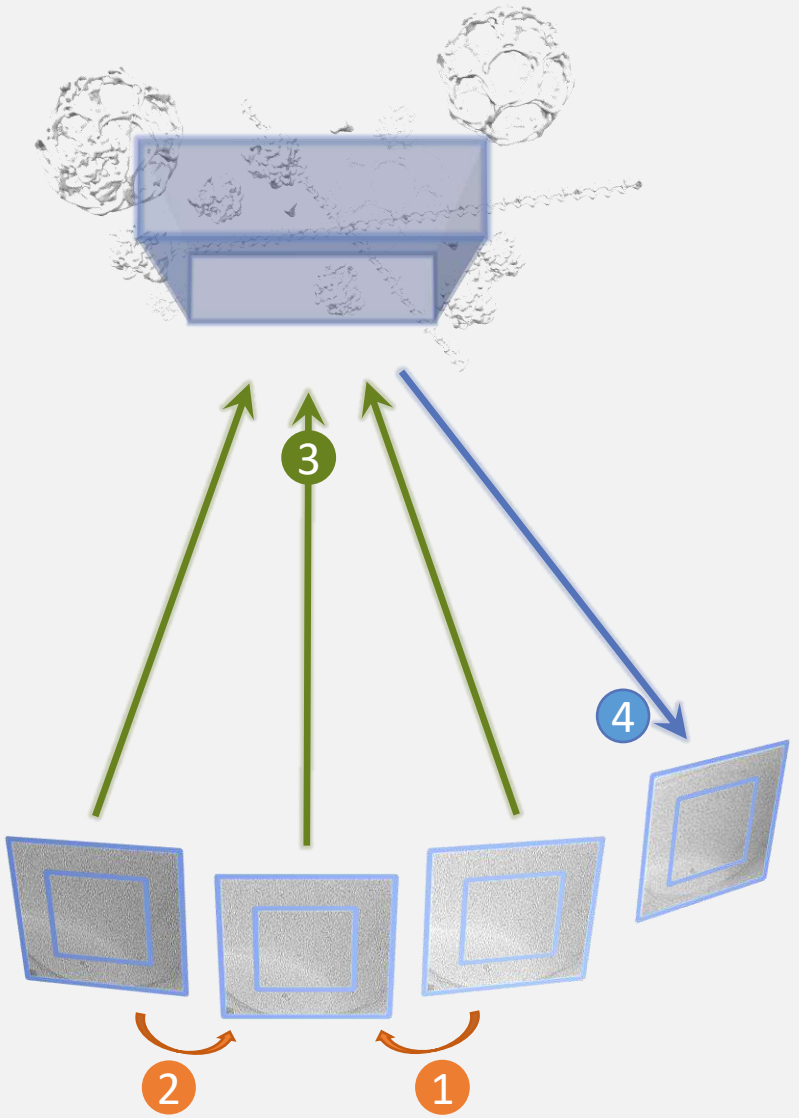
alignment thickness = z

x y

Volume to be re-projected



Protomo alignment

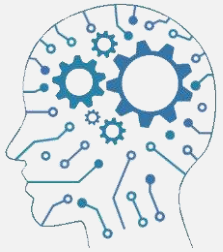


Nearest-neighbor correlation

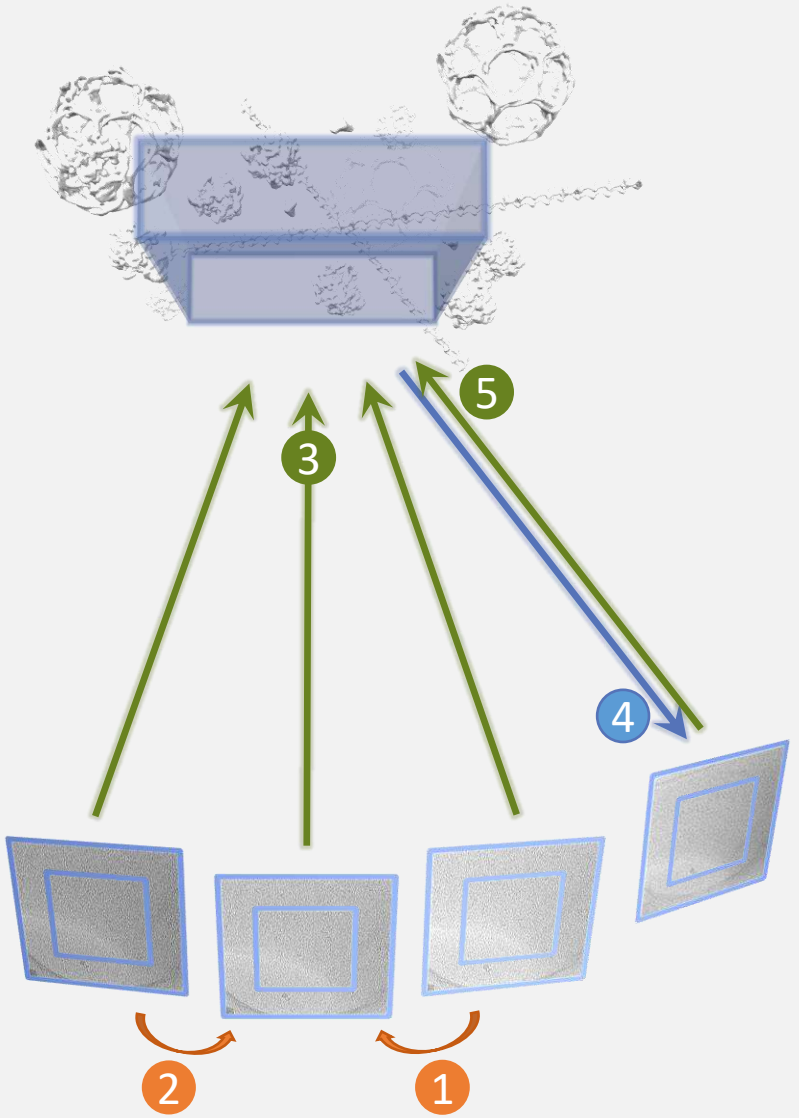
Weighted back-projection

alignment thickness = z
x y
Volume to be re-projected

Re-projection ⟶ correlation



Protomo alignment

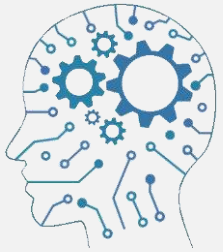


Nearest-neighbor correlation

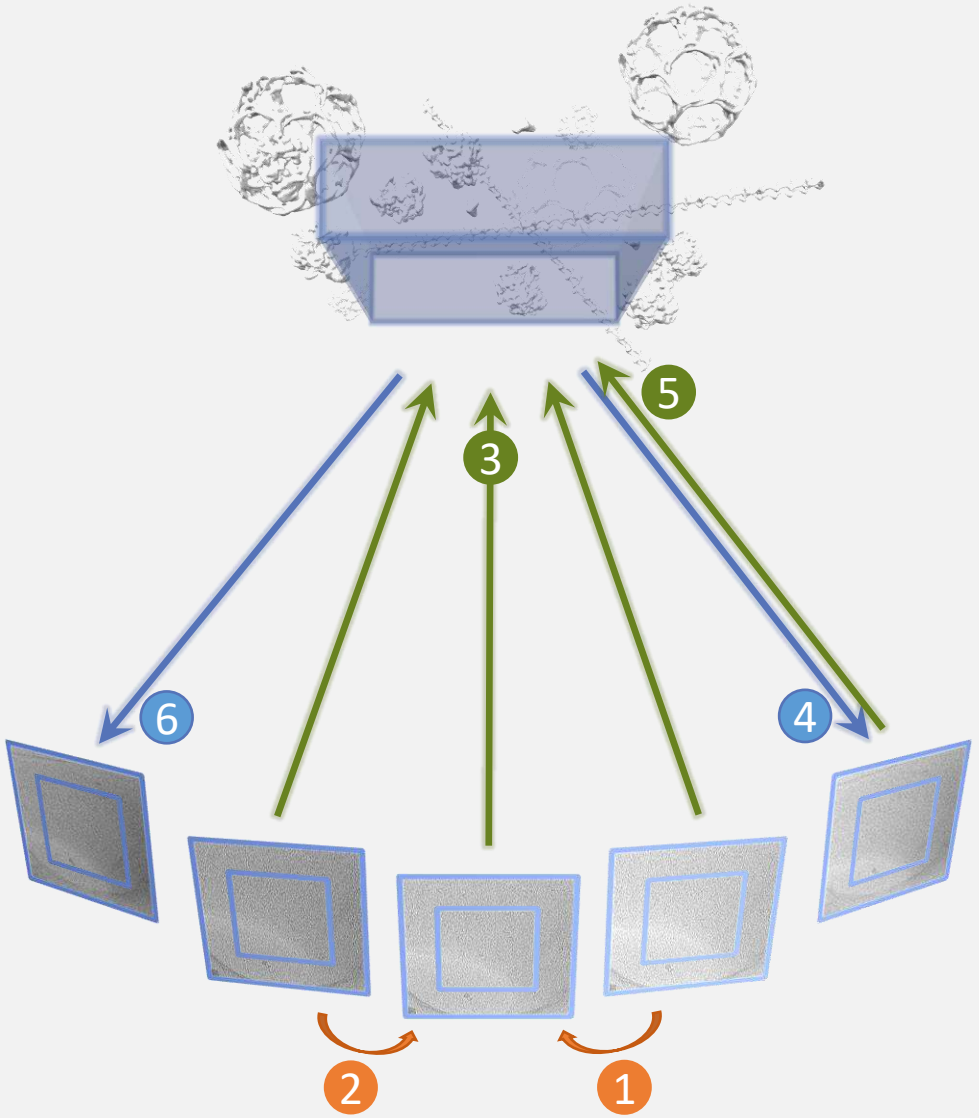
Weighted back-projection

alignment thickness = z
x y
Volume to be re-projected

Re-projection ⟶ correlation



Protomo alignment

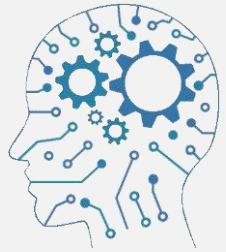


Nearest-neighbor correlation

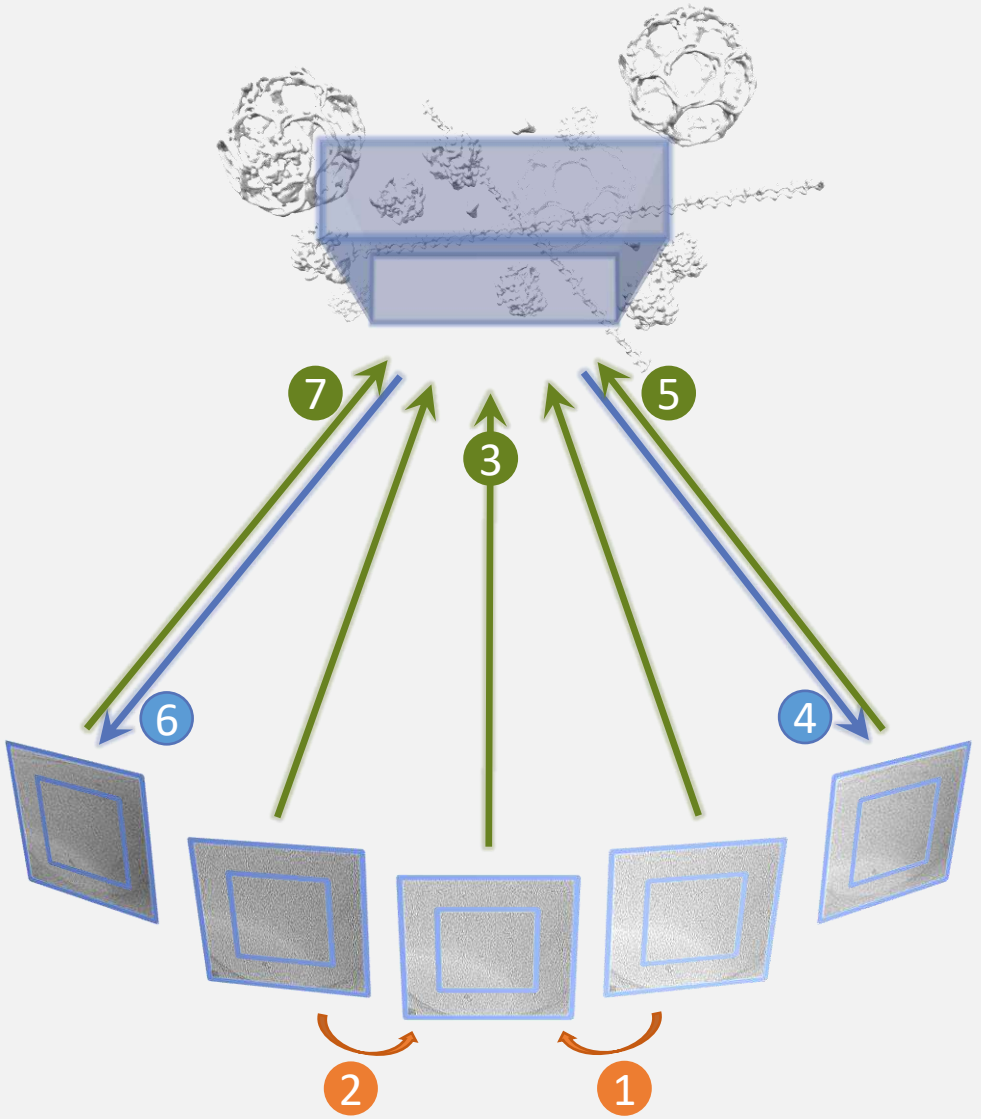
Weighted back-projection

alignment thickness = z
x y
Volume to be re-projected

Re-projection ⟶ correlation



Protomo alignment

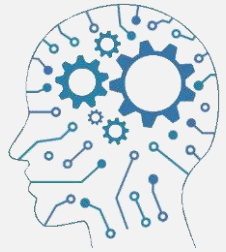


Nearest-neighbor correlation

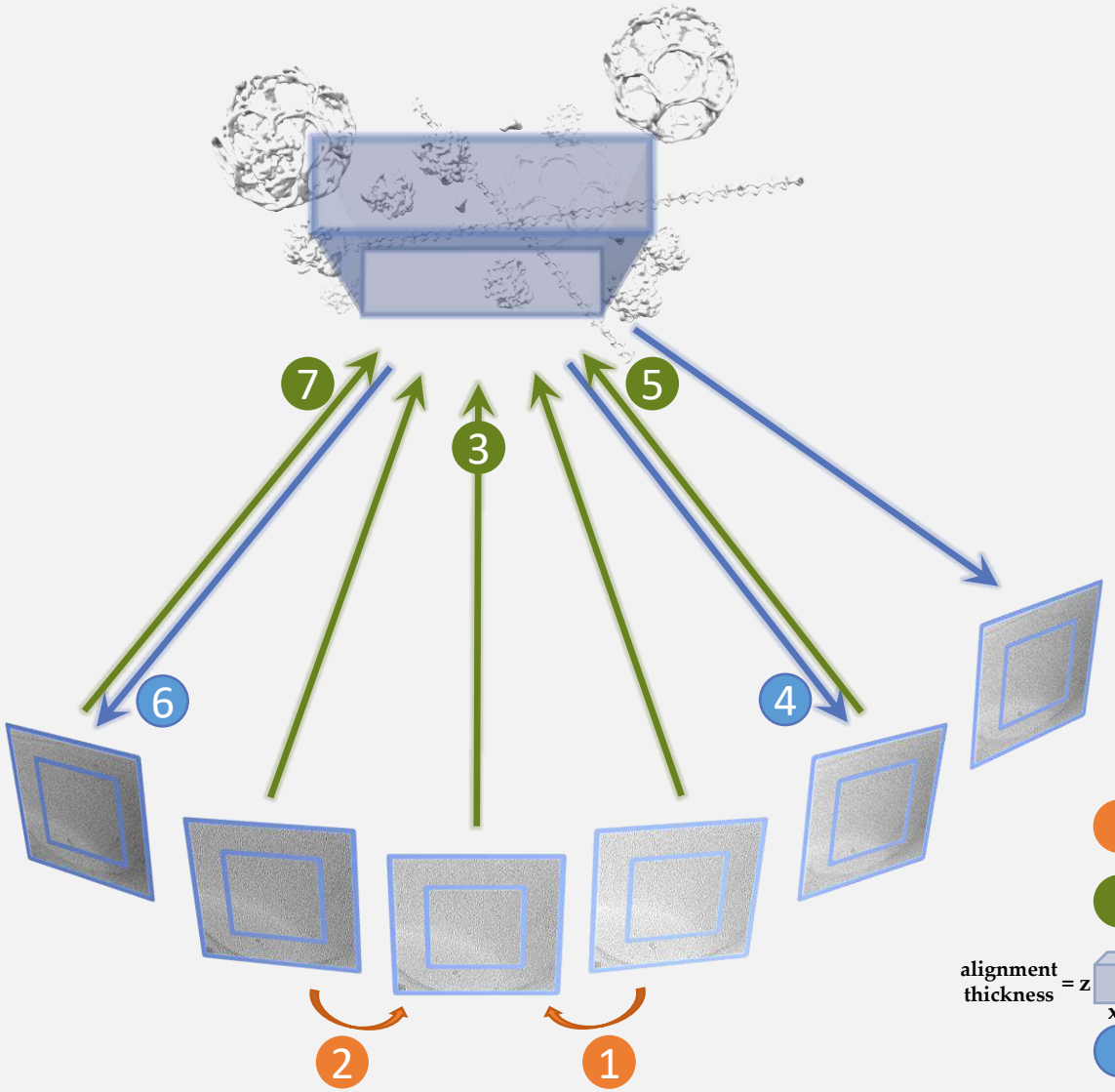
Weighted back-projection

alignment thickness = z, x, y — Volume to be re-projected

Re-projection ⟶ correlation



Protomo alignment

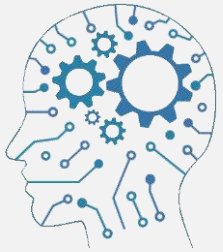


Nearest-neighbor correlation

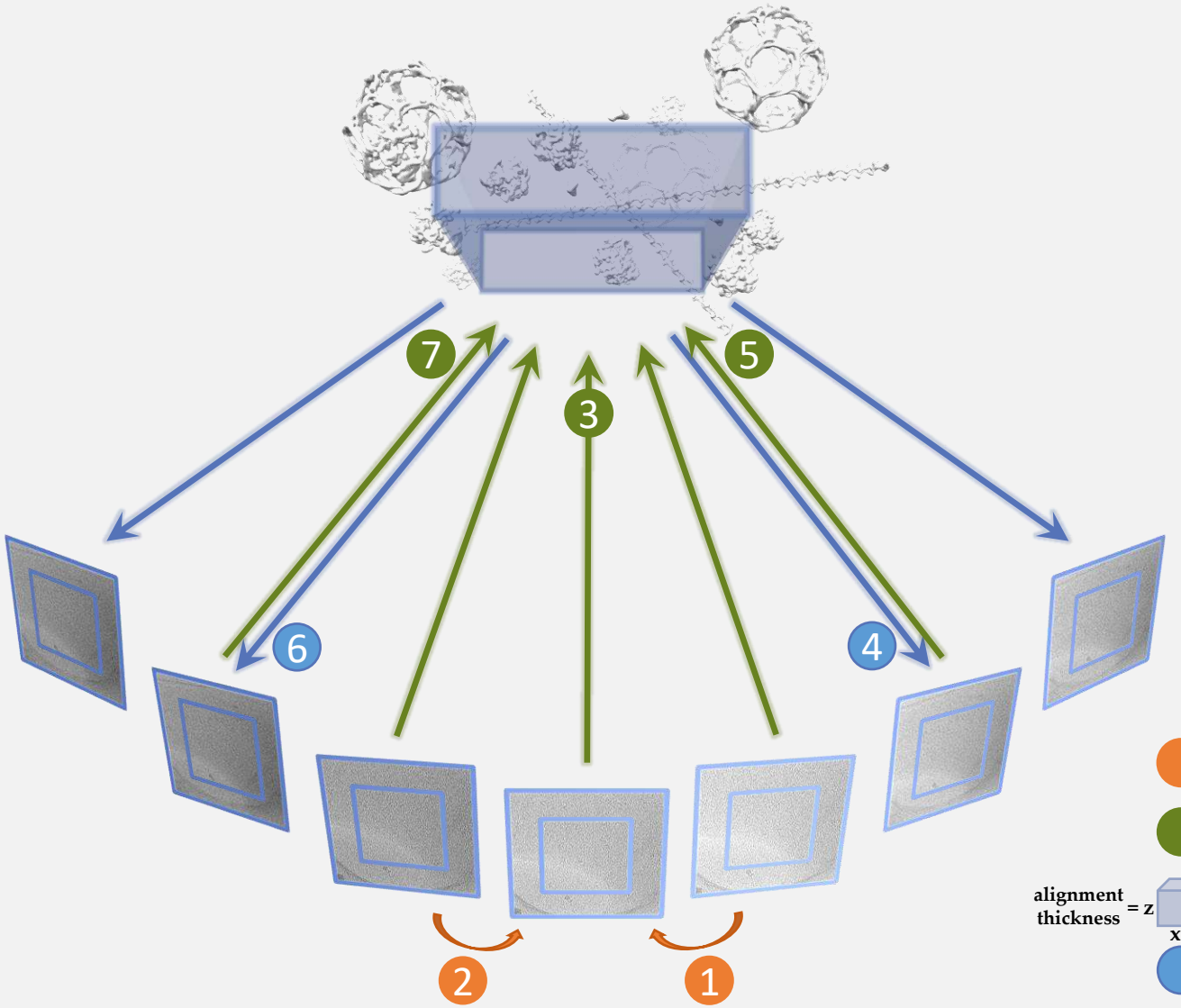
Weighted back-projection

alignment thickness = z, x, y — Volume to be re-projected

Re-projection ⟶ correlation



Protomo alignment

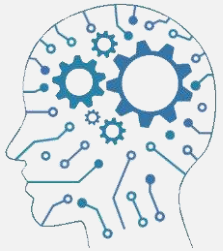


Nearest-neighbor correlation

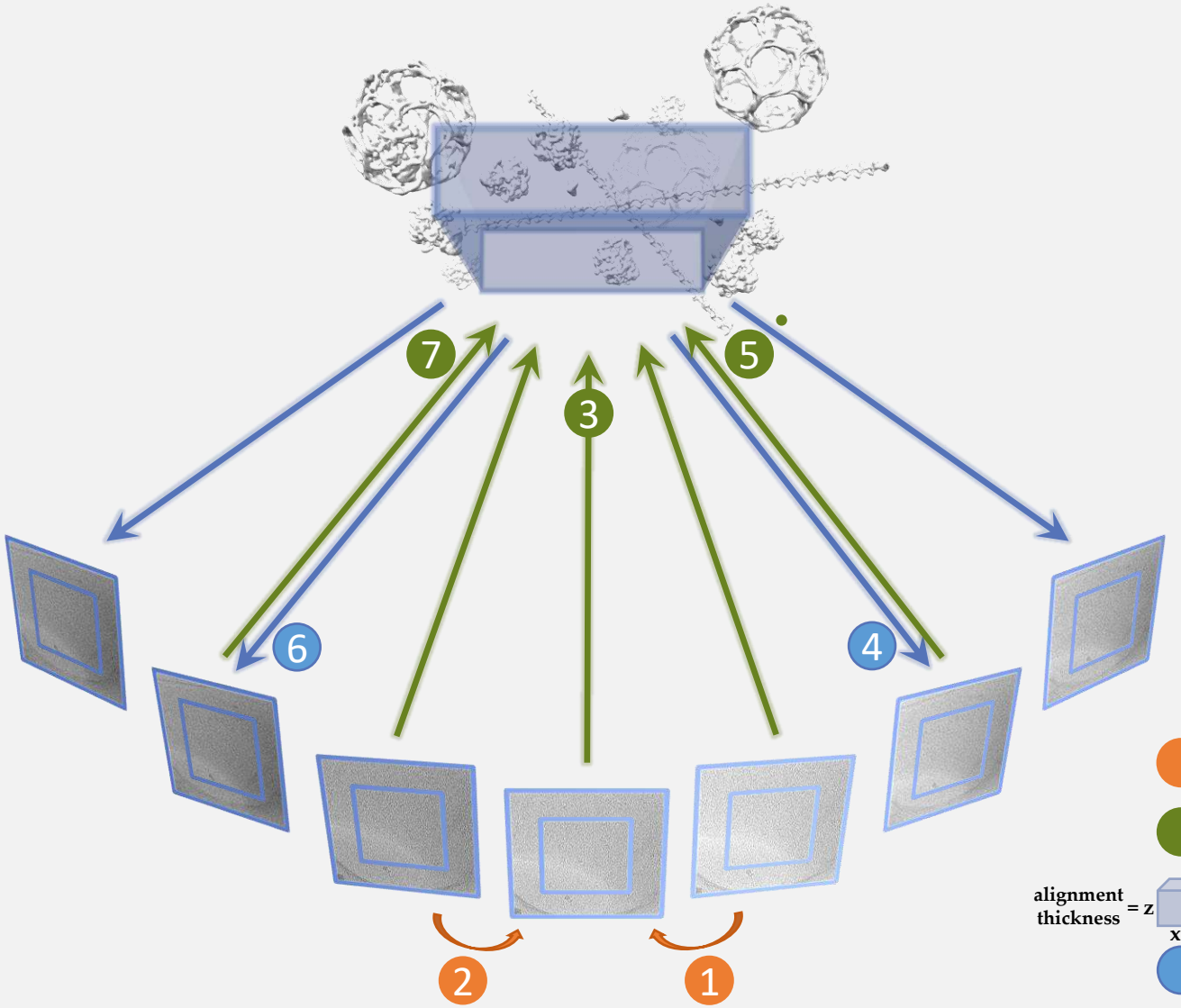
Weighted back-projection

alignment thickness = z
x y
Volume to be re-projected

Re-projection ⟶ correlation



Protomo alignment

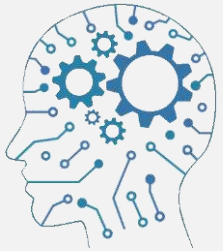


Nearest-neighbor correlation

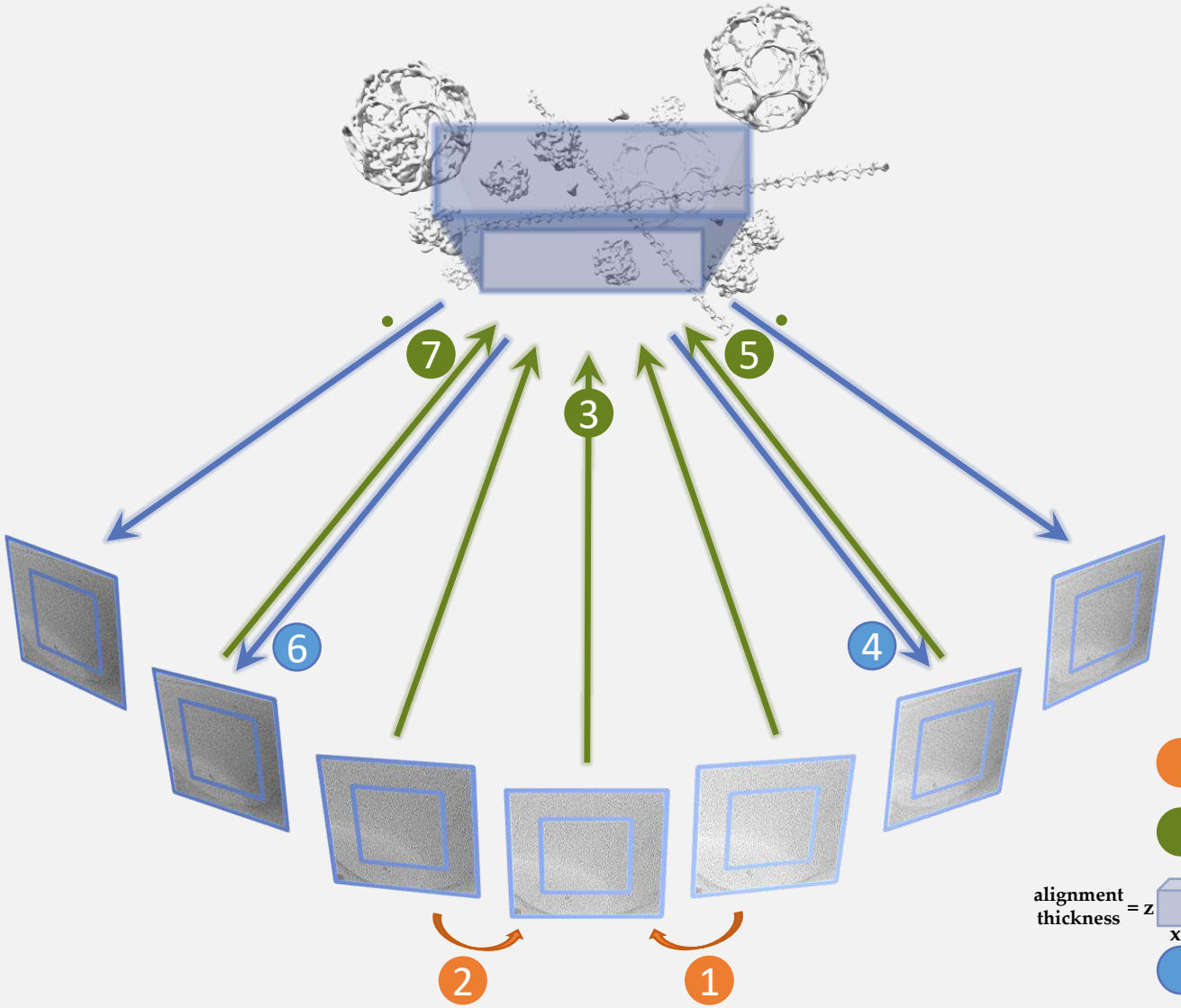
Weighted back-projection

alignment thickness = z
x y
Volume to be re-projected

Re-projection ⟶ correlation



Protomo alignment



Nearest-neighbor correlation

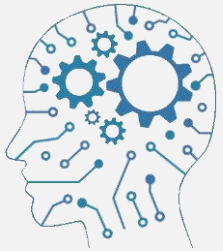
Weighted back-projection

alignment thickness = z

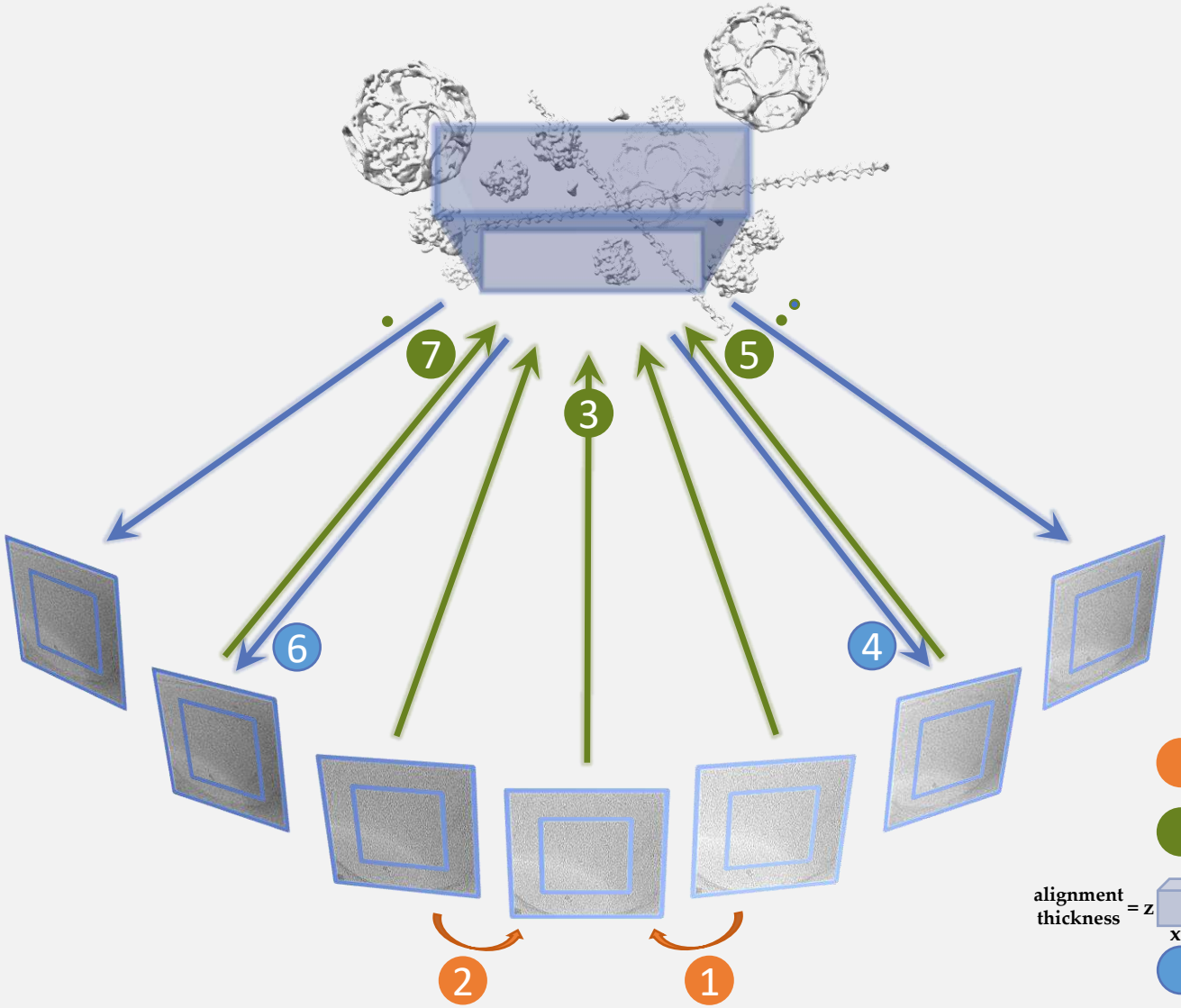
x y

Volume to be re-projected

Re-projection ⟶ correlation



Protomo alignment

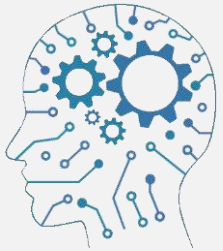


Nearest-neighbor correlation

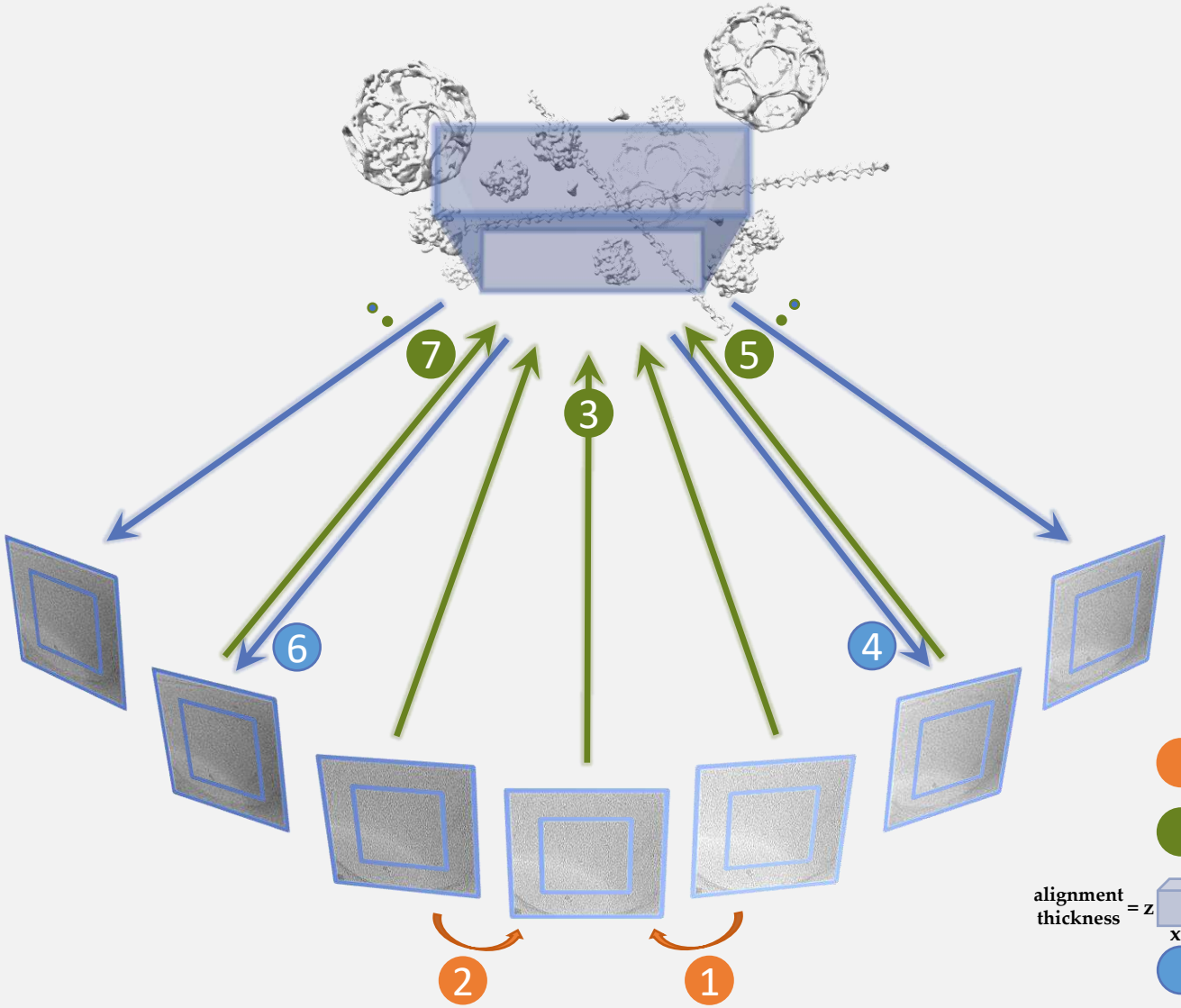
Weighted back-projection

alignment thickness = z
x y
Volume to be re-projected

Re-projection ⟶ correlation



Protomo alignment

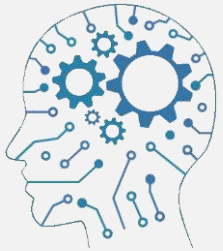


Nearest-neighbor correlation

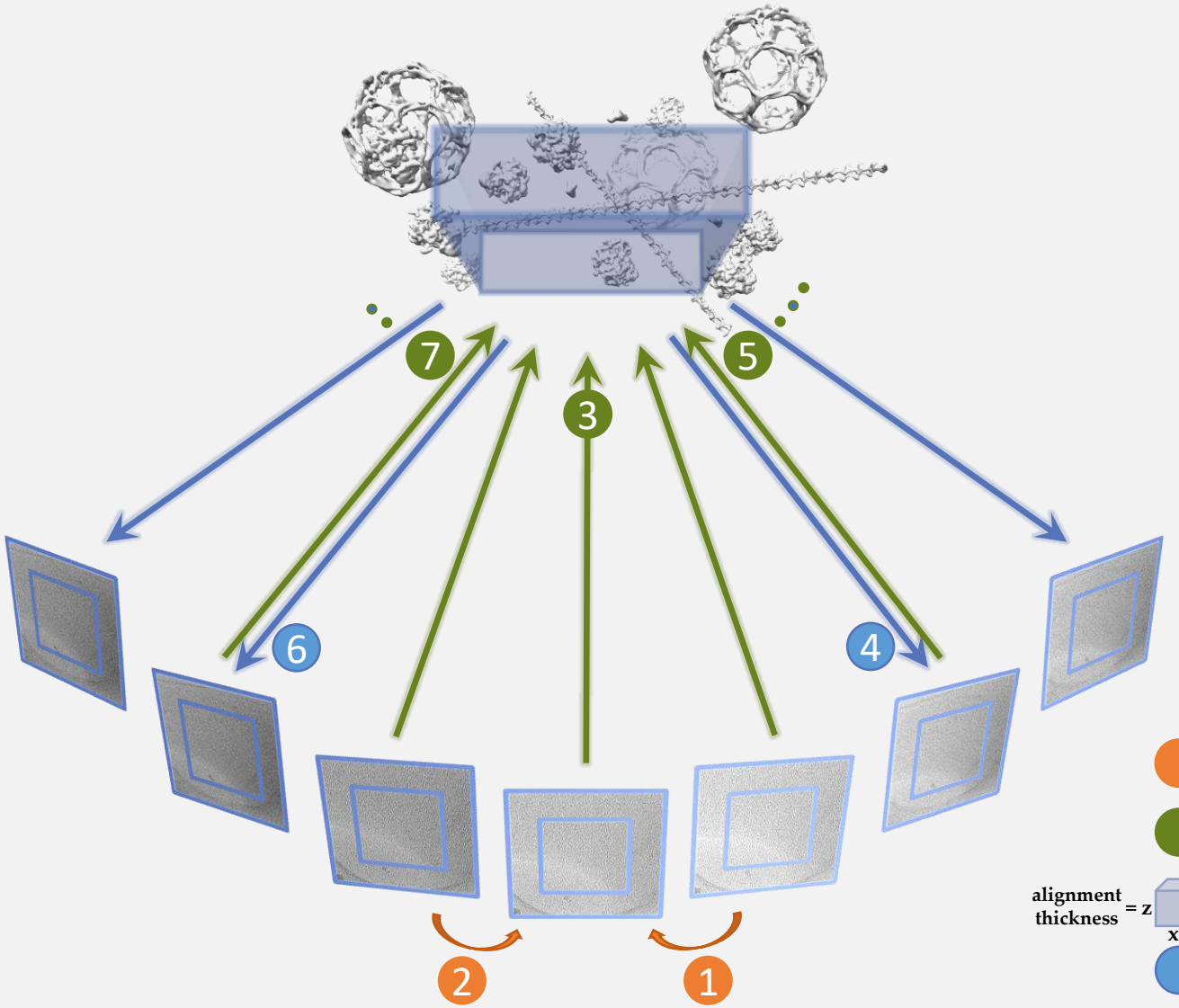
Weighted back-projection

alignment thickness = z
x y
Volume to be re-projected

Re-projection ⟶ correlation



Protomo alignment

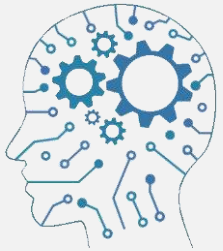


Nearest-neighbor correlation

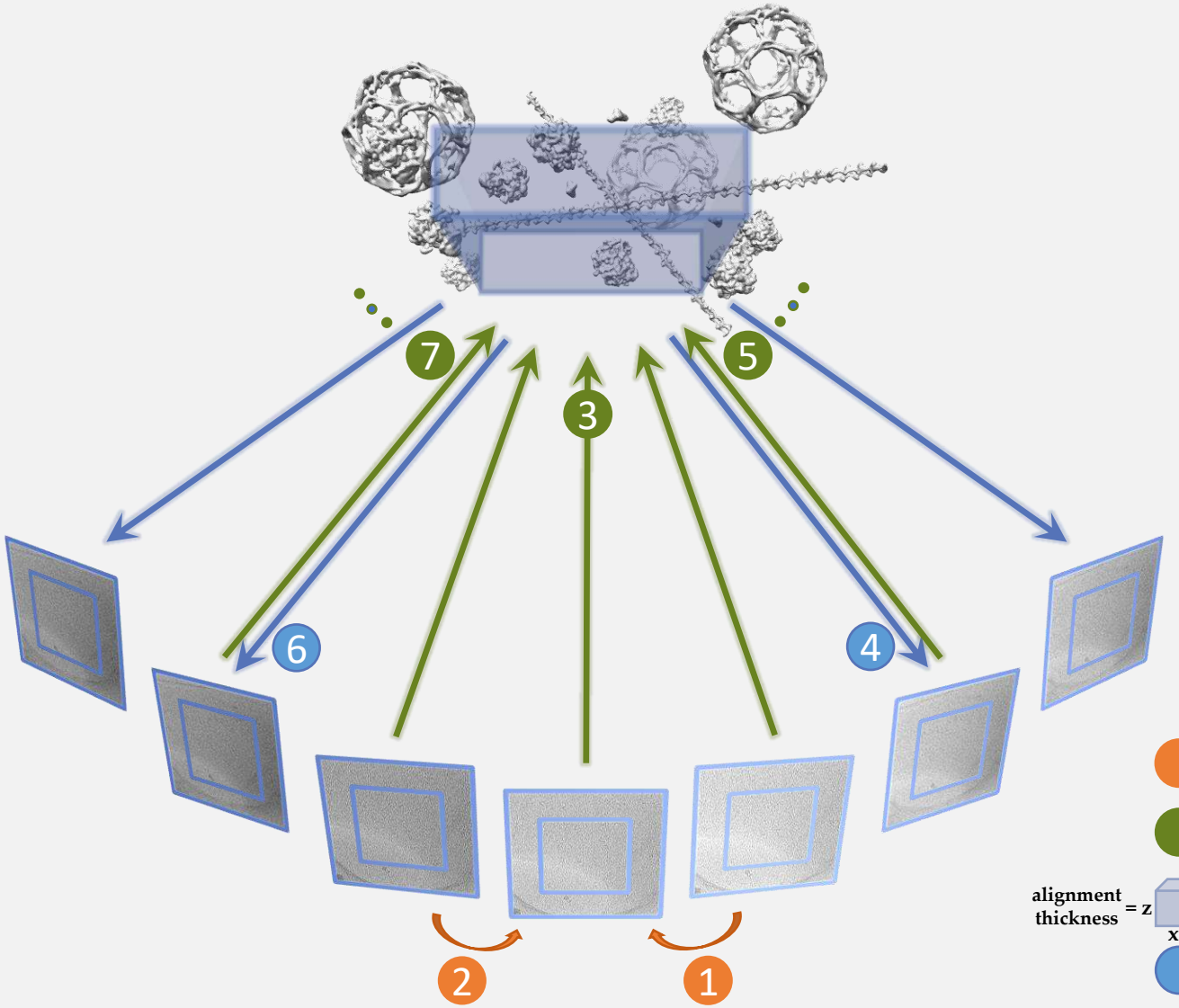
Weighted back-projection

alignment thickness = z
x y
Volume to be re-projected

Re-projection ⟶ correlation



Protomo alignment



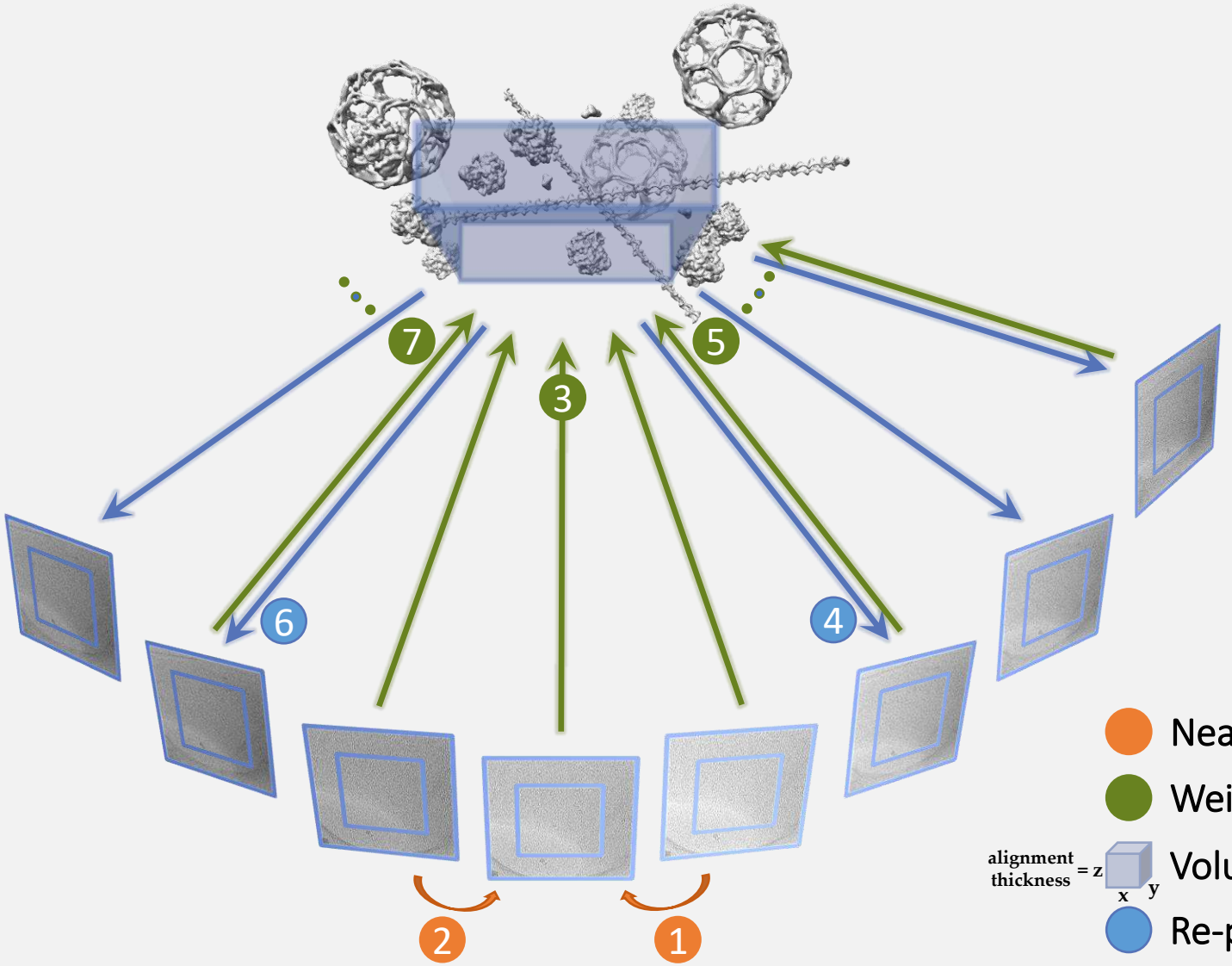
Nearest-neighbor correlation

Weighted back-projection

alignment thickness = z, x, y — Volume to be re-projected

Re-projection ⟶ correlation

Protomo alignment



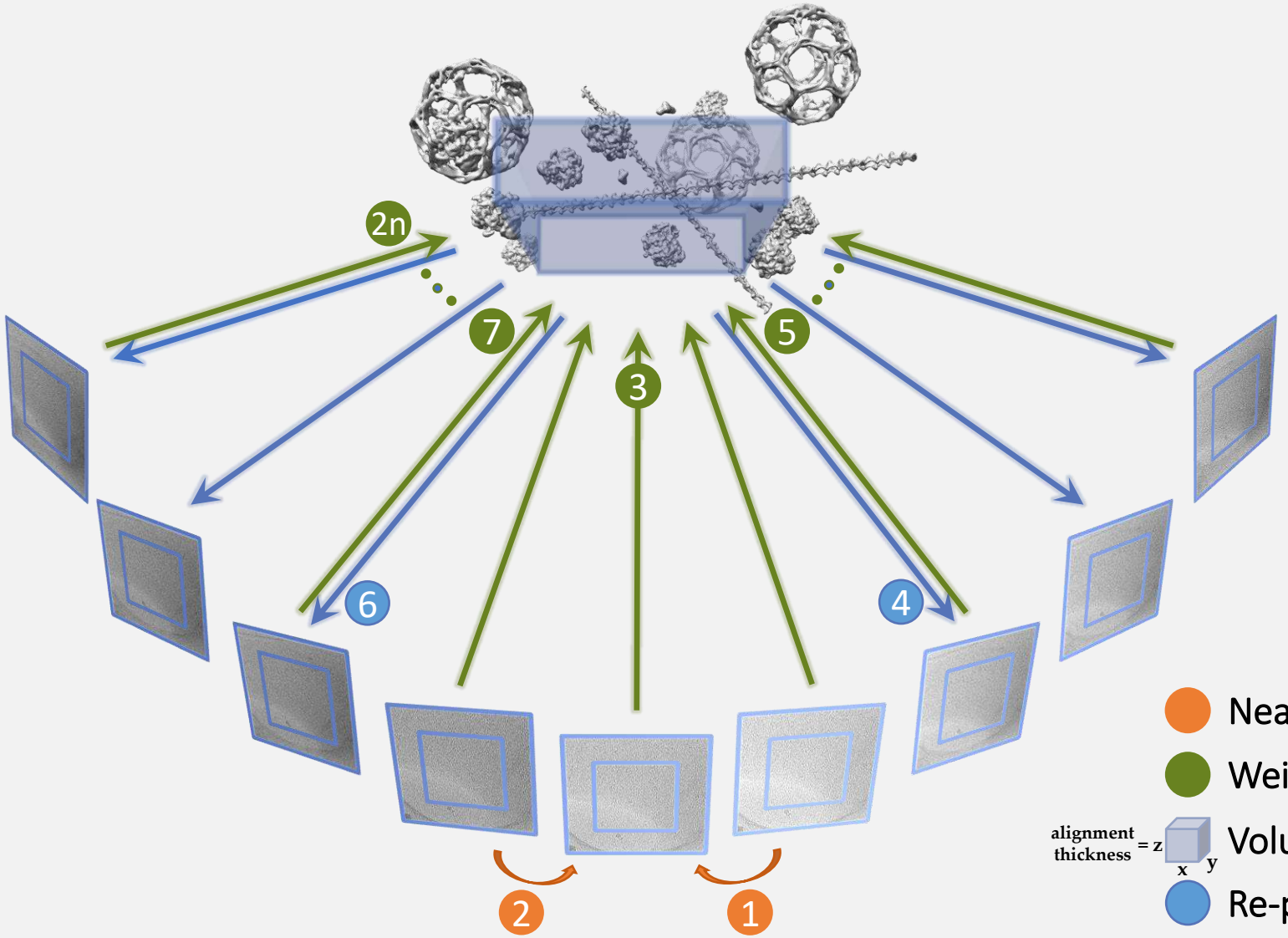
Nearest-neighbor correlation

Weighted back-projection

alignment thickness = z
x y
Volume to be re-projected

Re-projection ⟶ correlation

Protomo alignment

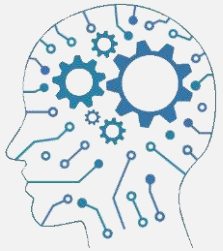


Nearest-neighbor correlation

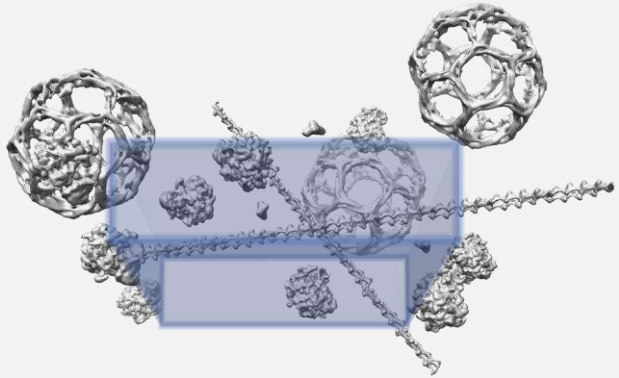
Weighted back-projection

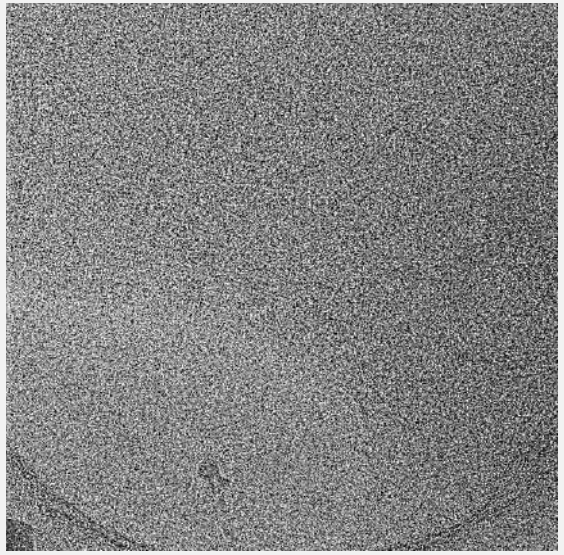
alignment thickness = z; x; y — Volume to be re-projected

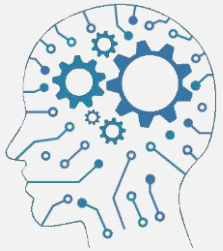
Re-projection ⟶ correlation



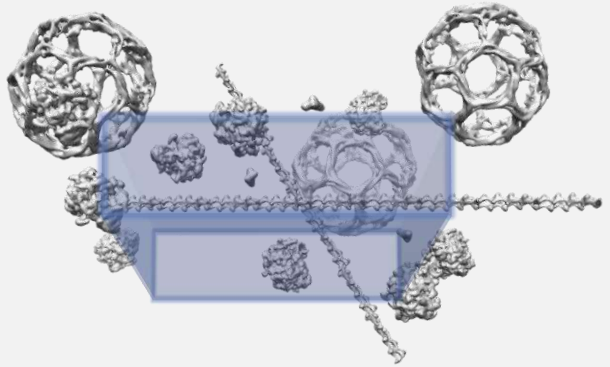
Protomo alignment

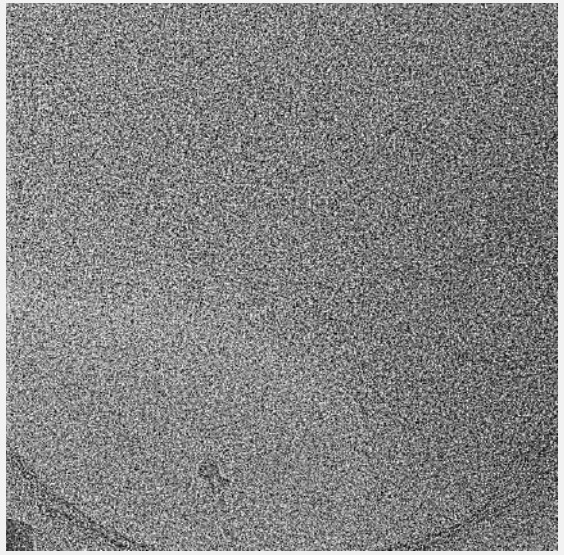




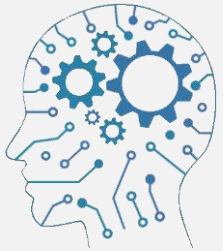


Protomo alignment

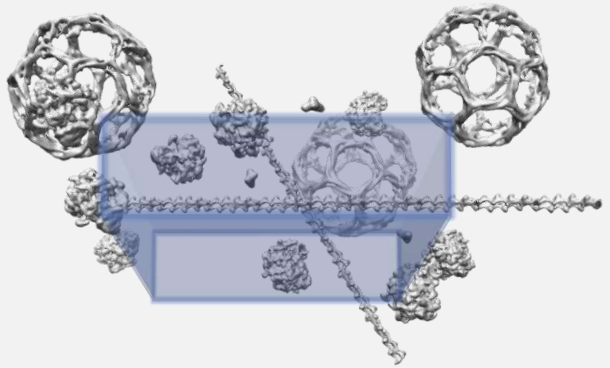


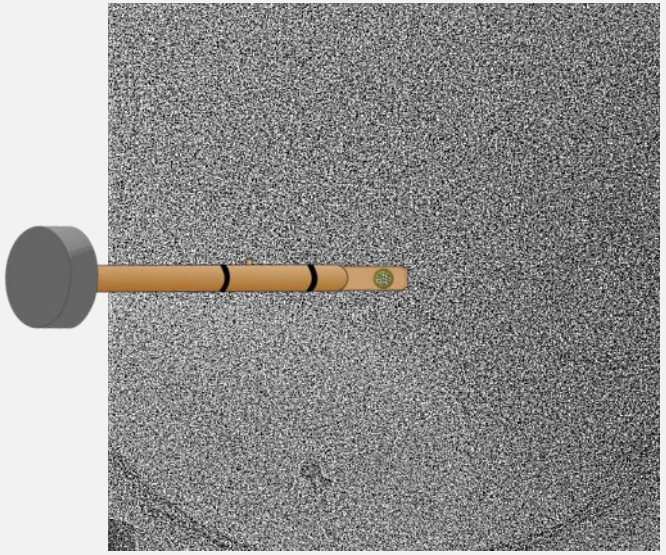


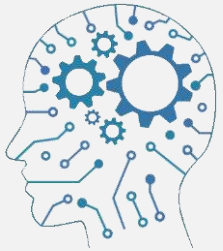
Refine orientations of objects



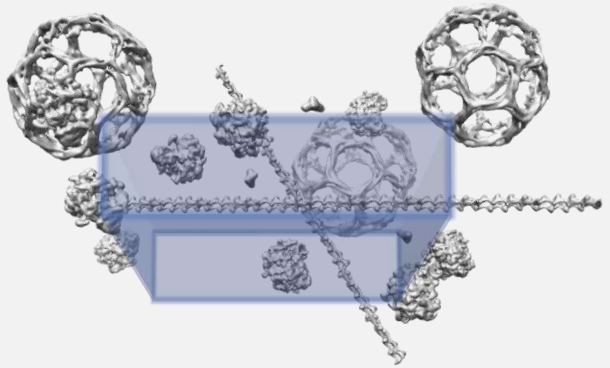
Protomo alignment

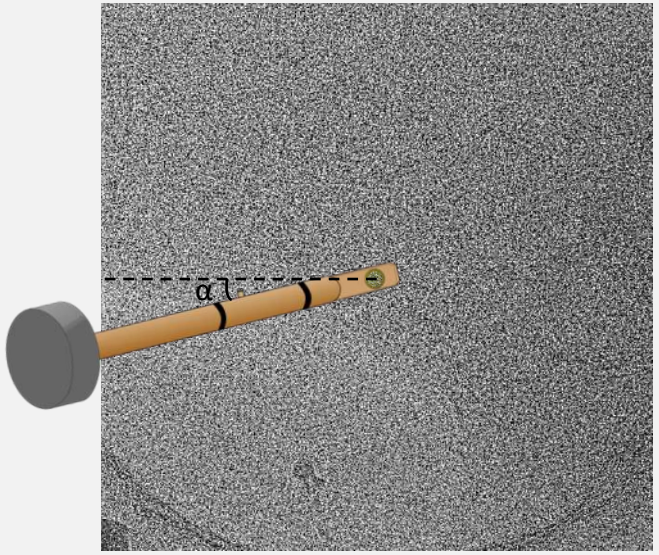






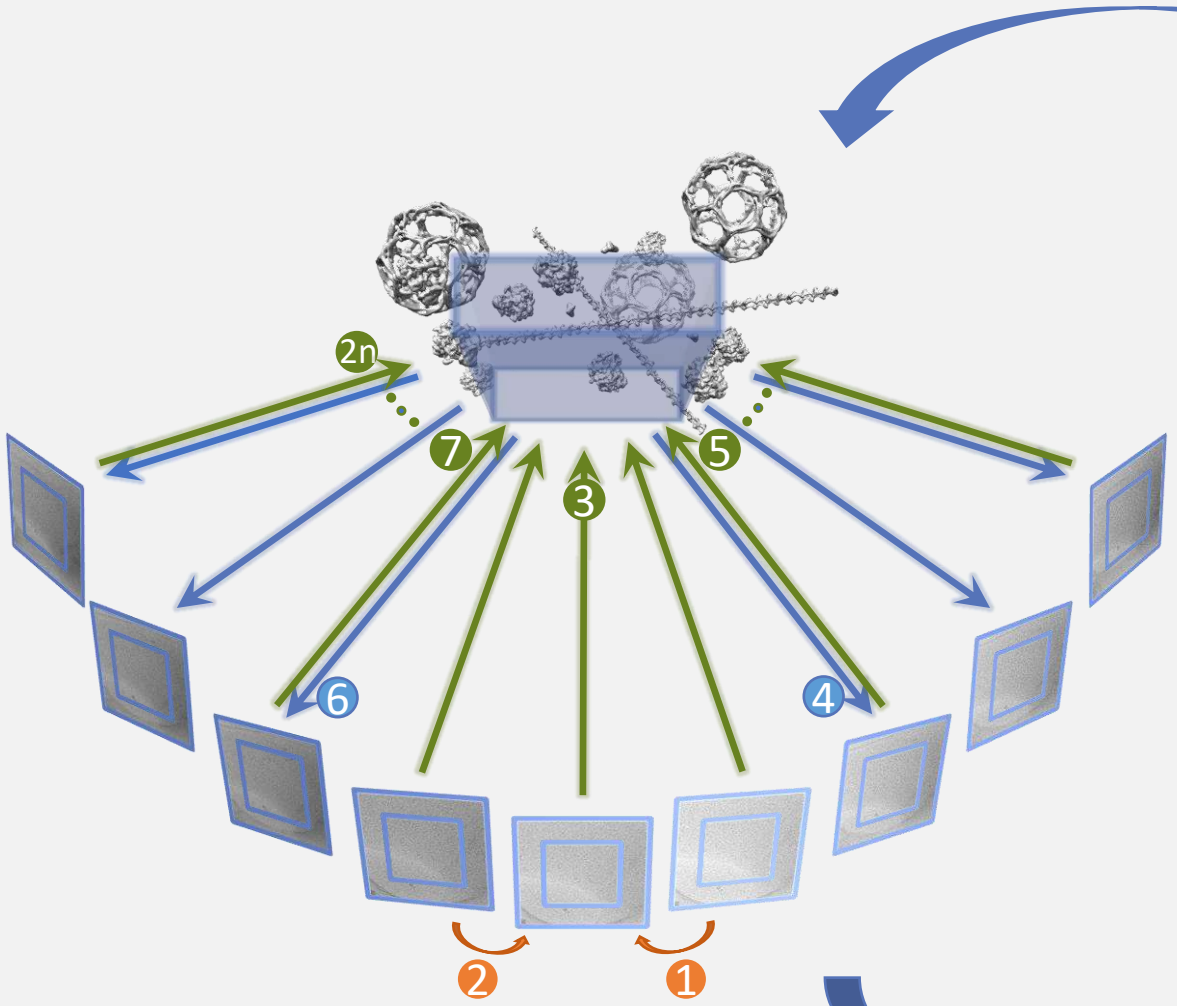
Protomo alignment

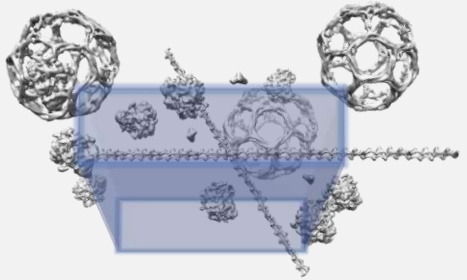


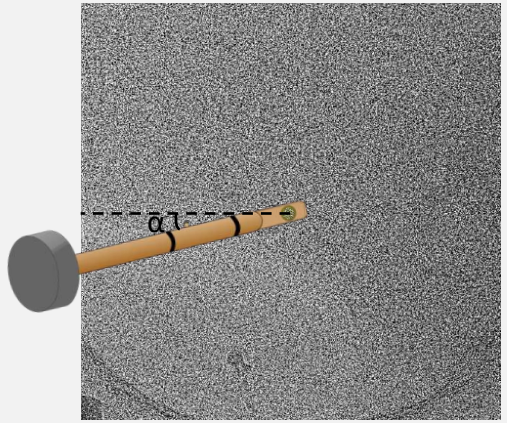


Refine tilt azimuth

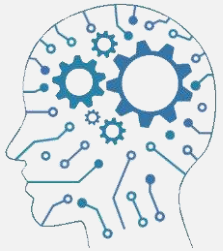
Appion-Protomo refinement



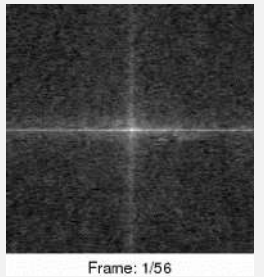




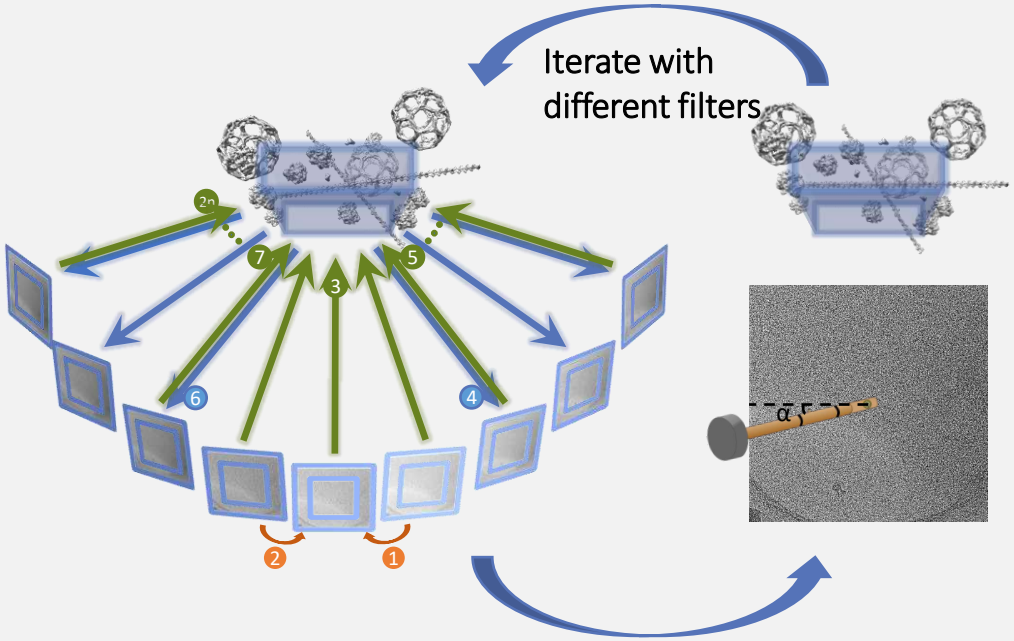
Iterate with different filters



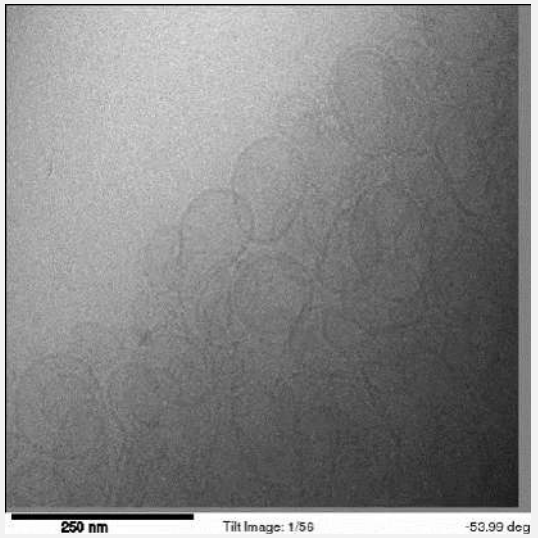
Standard nearest-neighbor alignment

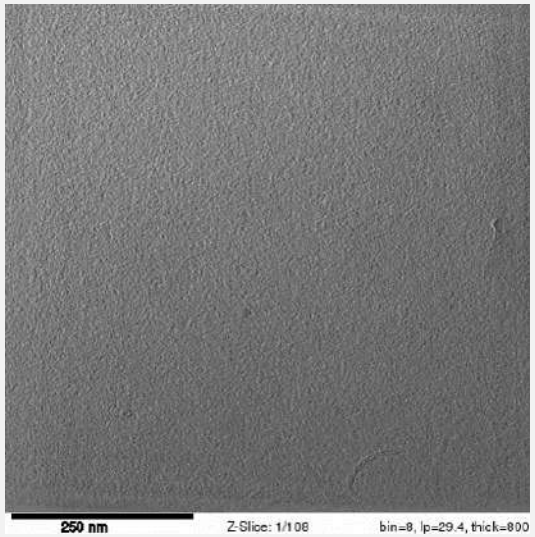


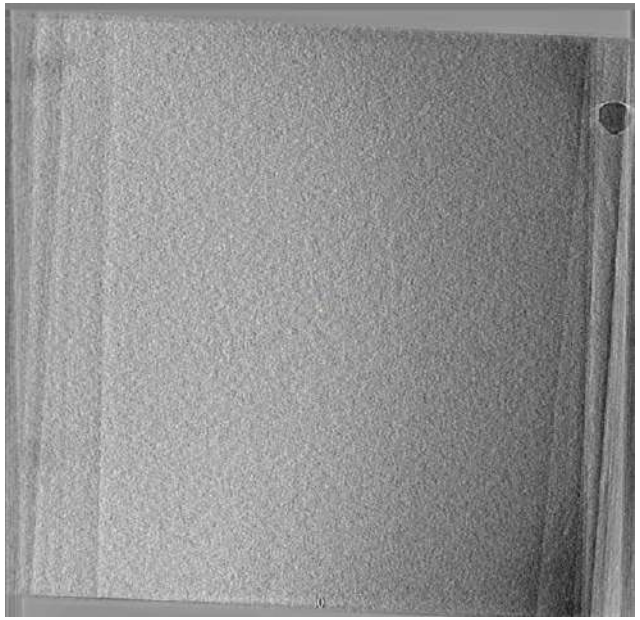
Why is this important?



After Protomo refinement







Questions?

Next: Appion-Protomo hands-on