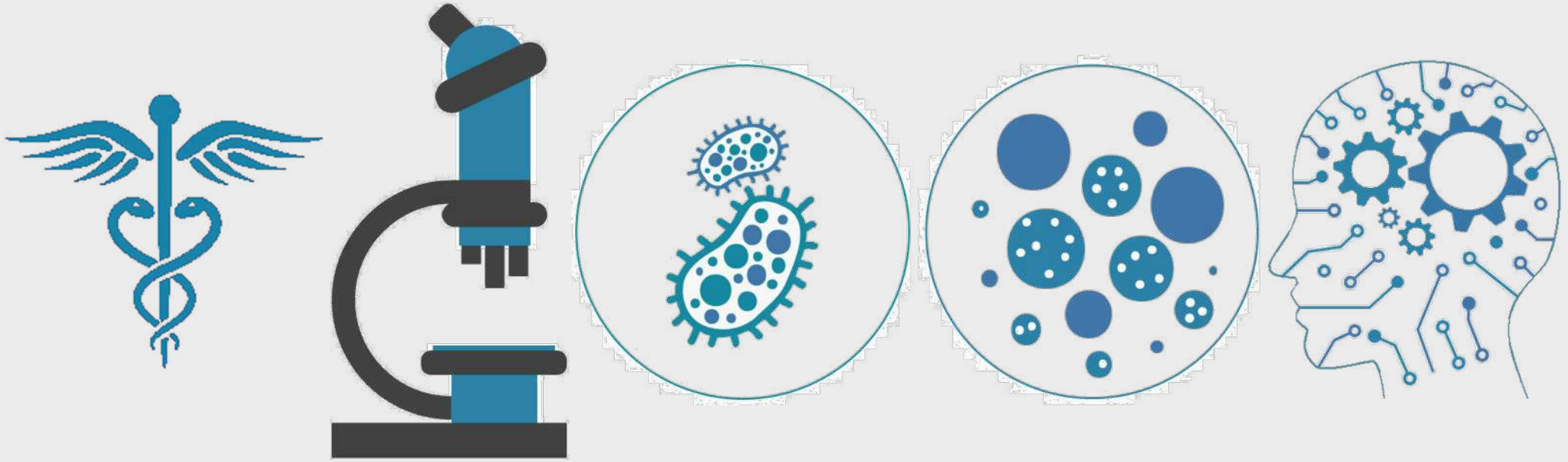
Tomography roundtable
Theory



Tomography Short Course!

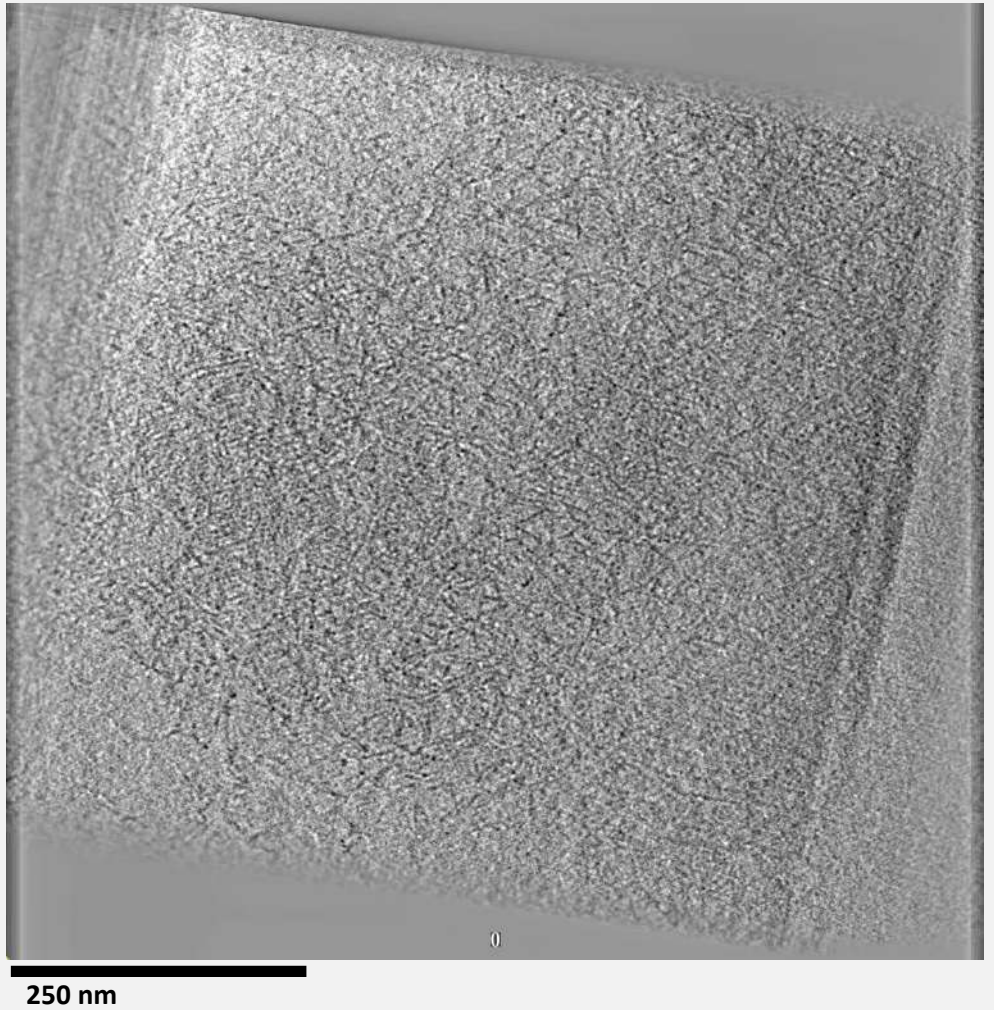
4-12-21

Alex Noble
anoble@nysbc.org

What is CryoET?

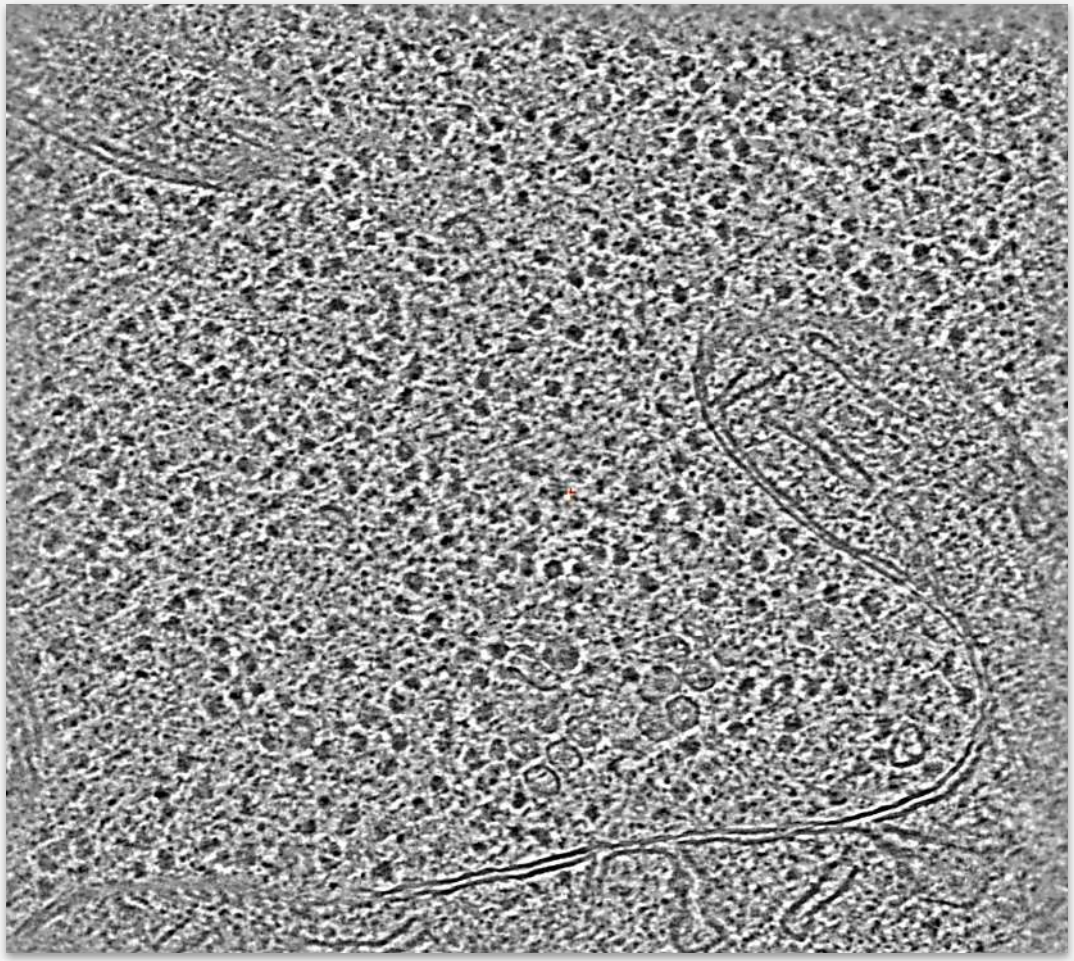
(cryo-electron tomography)

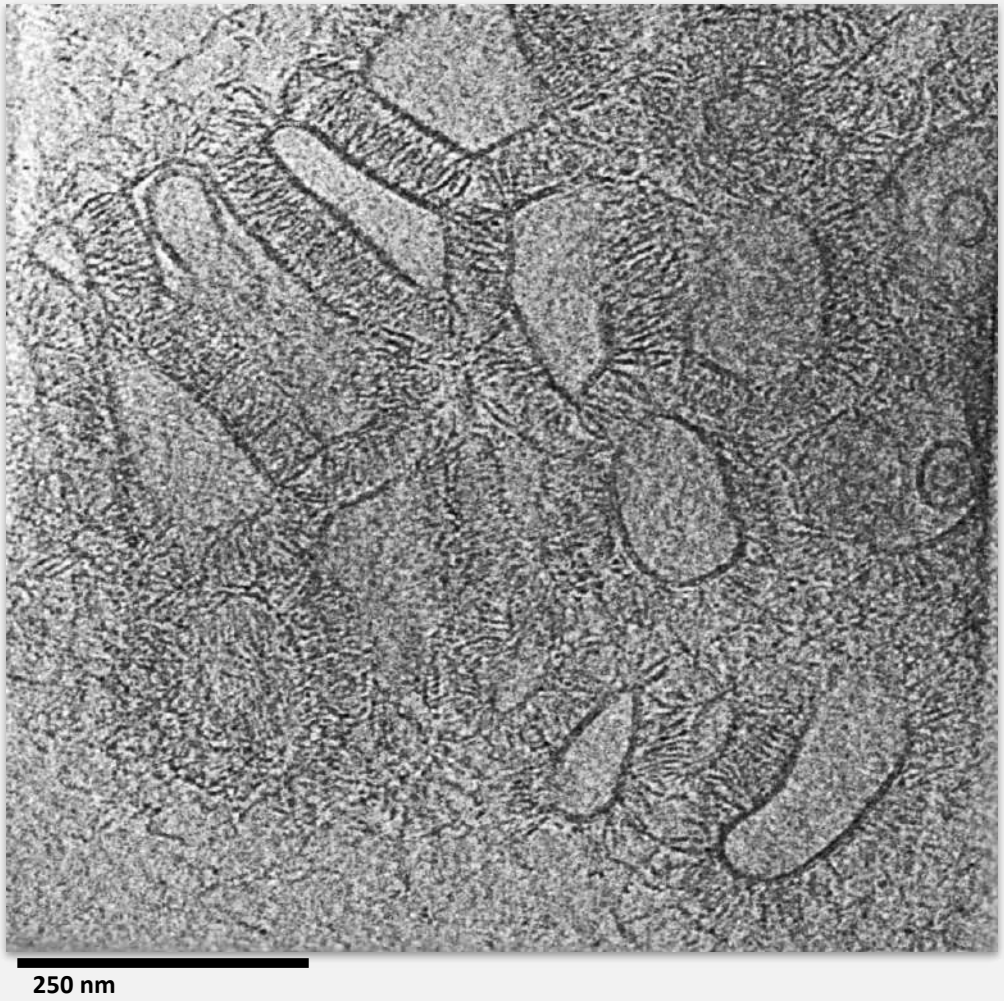
- Cells or complex reconstituted environments



What is CryoET?

(cryo-electron tomography)





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

Overview – Why CryoET?

Why **cryo**?

- Specimen preservation in native or near-native environments.

Why **electrons**?

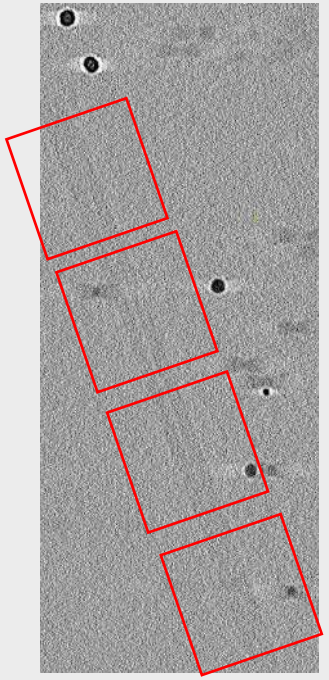
- **+**Small wavelengths (high res), **+**Can be focused, **–**Damage sample

Why **tomography**?

- Some combination of:
  - Sample is **unique**; e.g. cells,
  - Sample is too **heterogeneous** (structurally or morphologically); e.g. viruses with variable # of receptors, or viruses of different non-symmetric shapes,
  - **Domain-stoichiometry** and/or orientation is required,
  - **Sub-nanometer** information is usually **not** required, but may be possible.

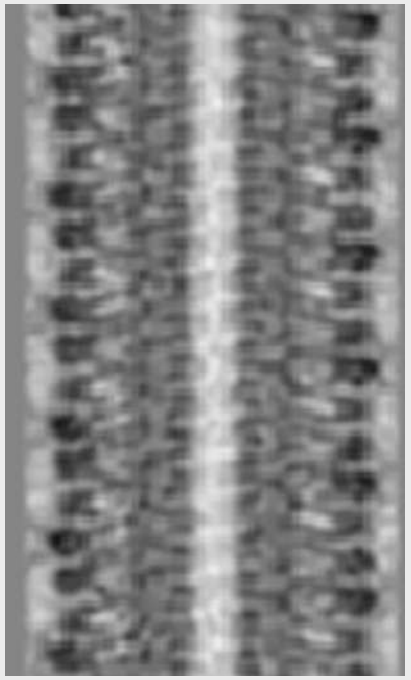
Overview – Why subtomogram averaging?

- Some amount of structural **repetition**,
- Repeating subunit preferred **orientation** overcome by **tilt range**



particles

align

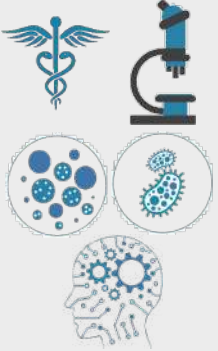


reconstruction

Courtesy of
Misha Kudyashev

Overview

- CryoET limitations
- Tilt-series collection
- Tilt-series alignment
- Defocus estimation and CTF correction
- Sub-tomogram localization
- Sub-tomogram alignment and averaging
- Examples
- Processing limitations
- Future directions and improvements

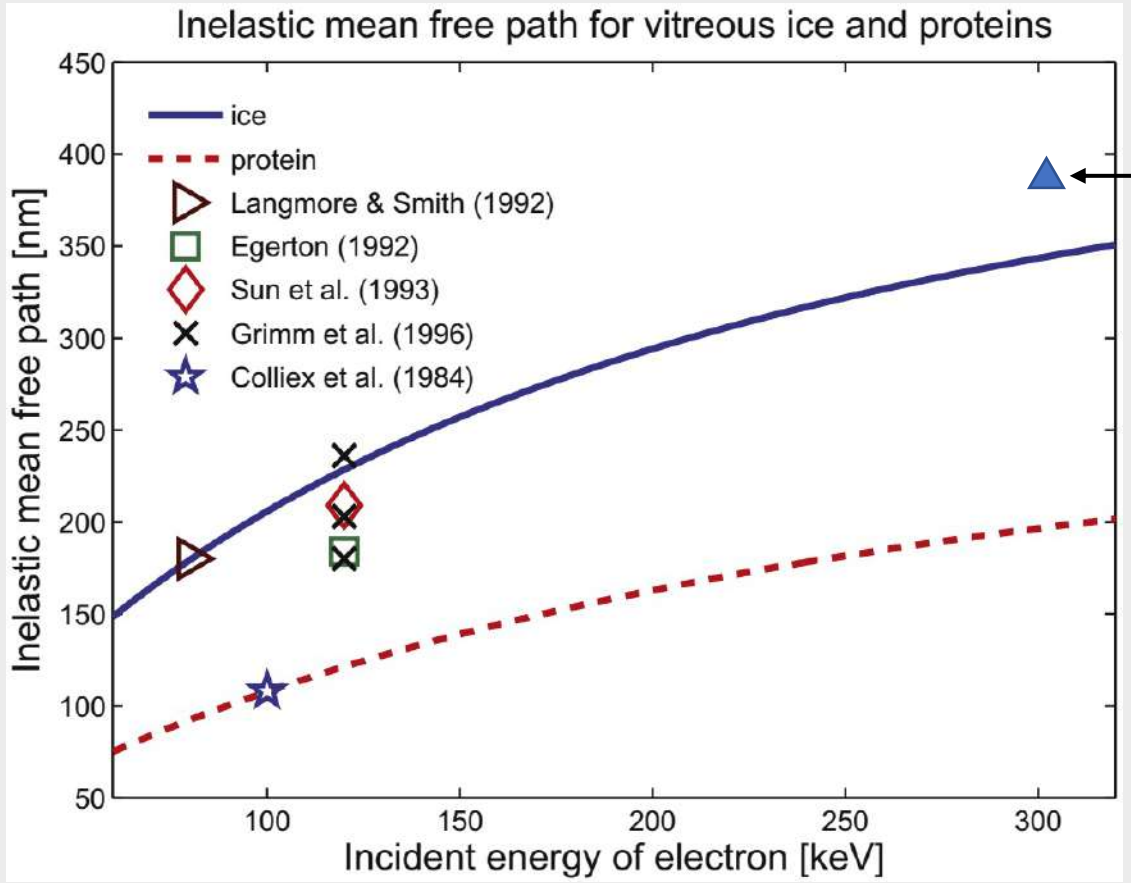


CryoET Limitations

Overview – Limitations

Limitation: Specimen/Ice **thickness**

- At **300keV** in a TEM (e.g. Krios), electrons cannot penetrate more than **0.5-1 µm**



Vulović, 2013

William J. Rice, NYSBC, 2017
300 keV Krios

Overview – Limitations

Limitation: Specimen/Ice **thickness**

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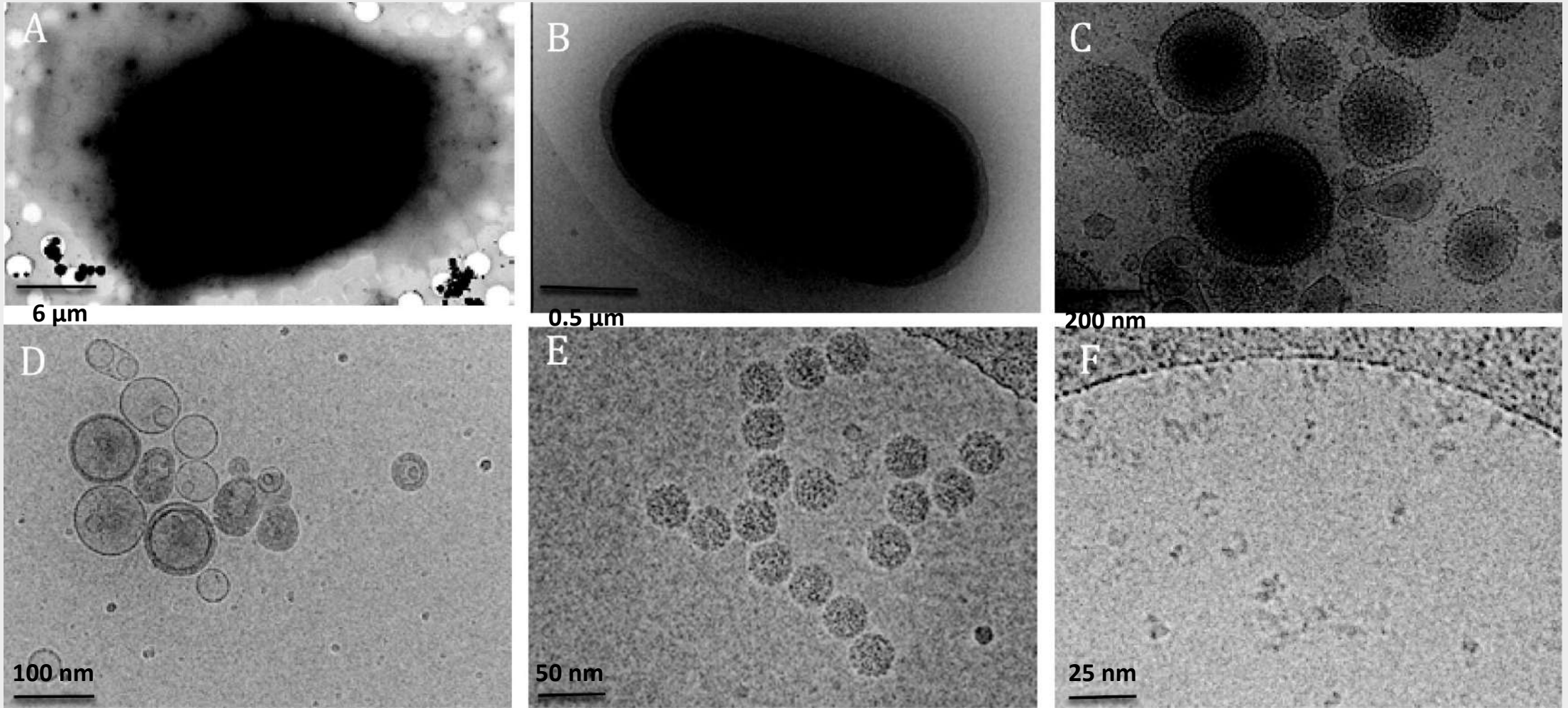
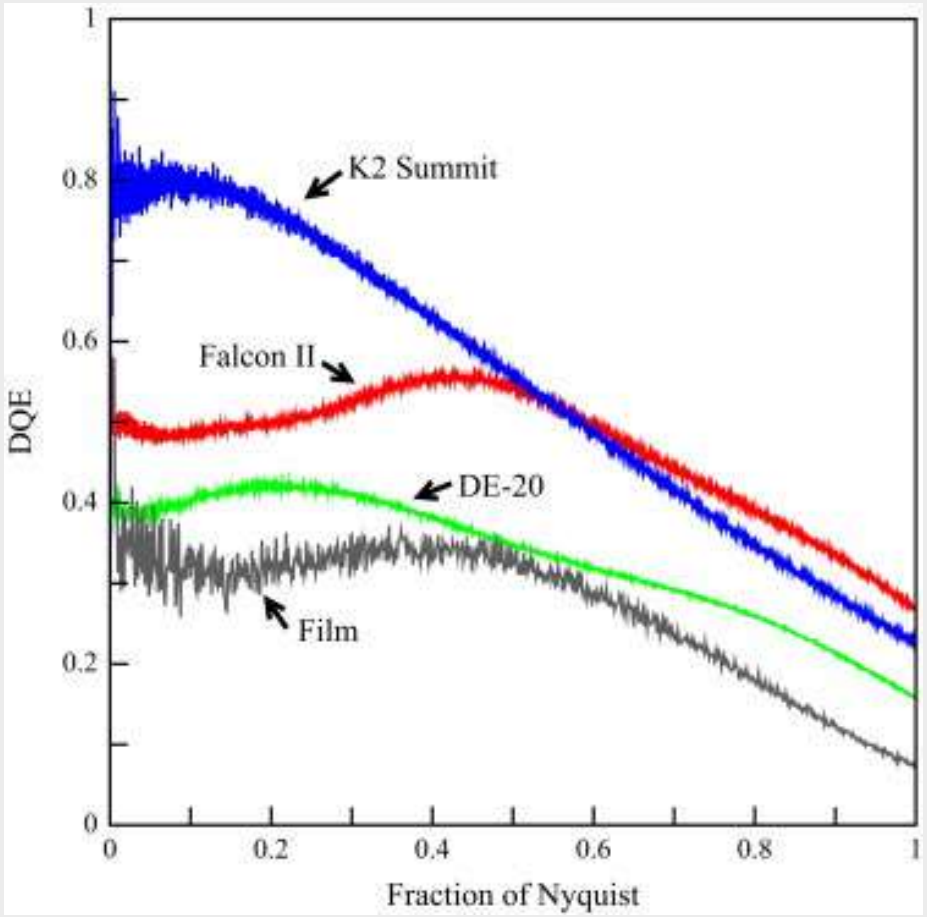


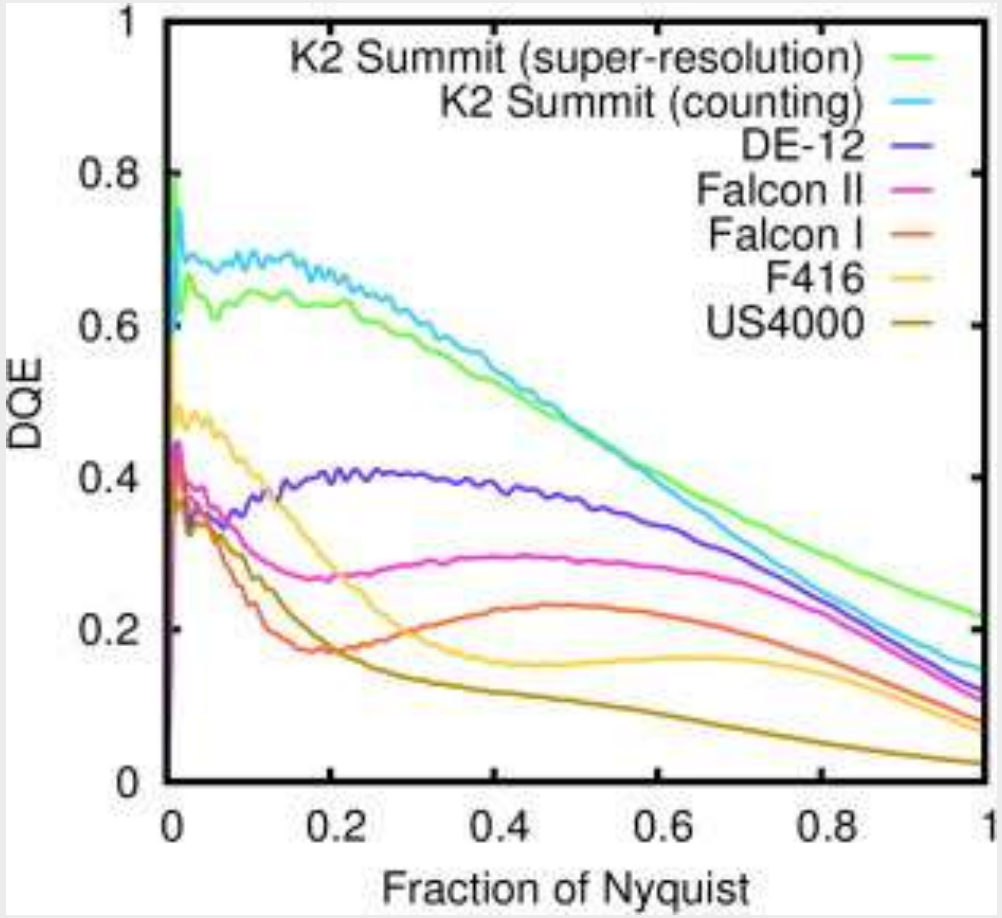
Thompson et. al., 2016

Overview – Limitations

Limitation: Camera fidelity at **localizing electrons**

- Cameras **do not** transfer information **perfectly or equally across frequencies**.



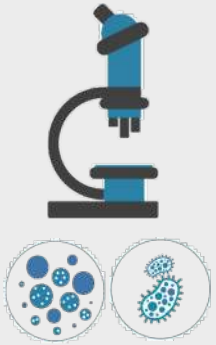


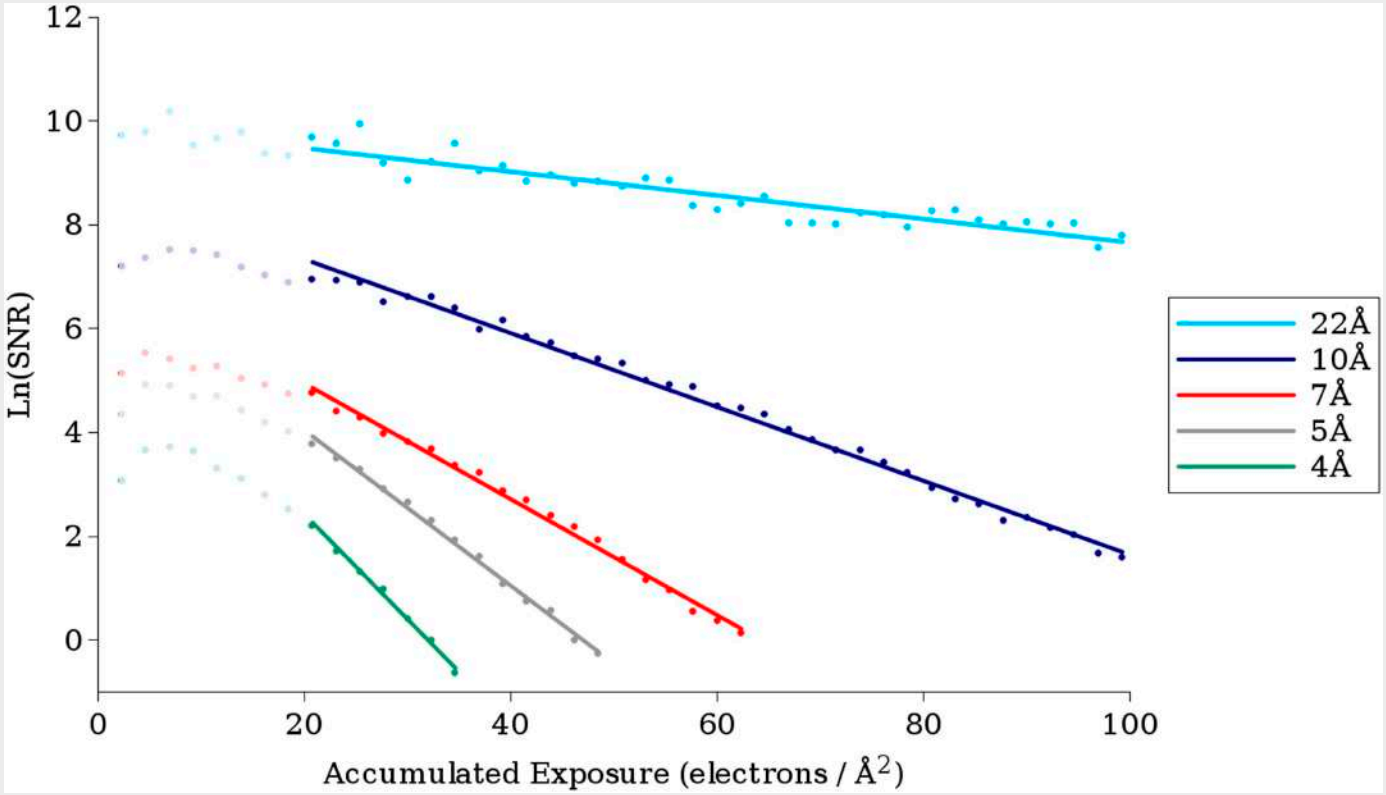
McMullin, 2014 &
Ruskin, 2013

Overview – Limitations

Limitation: **Electron damage** of the specimen

- High voltage electrons damage biological specimen.
  - **High resolution information is lost first** followed by lower resolution info.





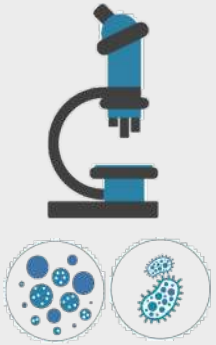
Grant & Grigorieff, 2015

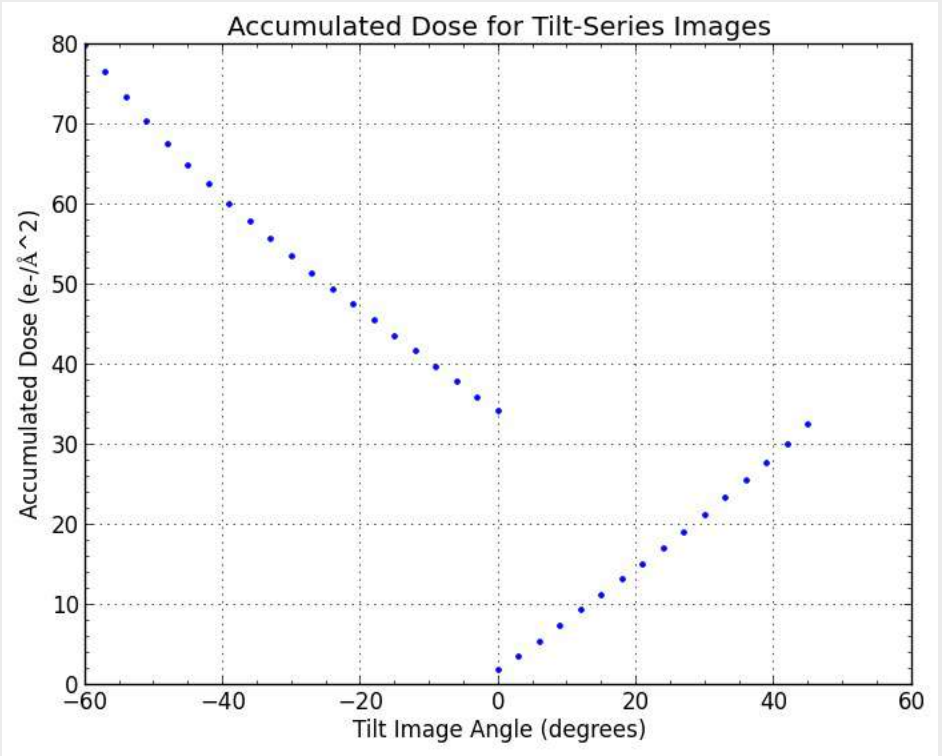
Solution:
Remove damaged information from image frames

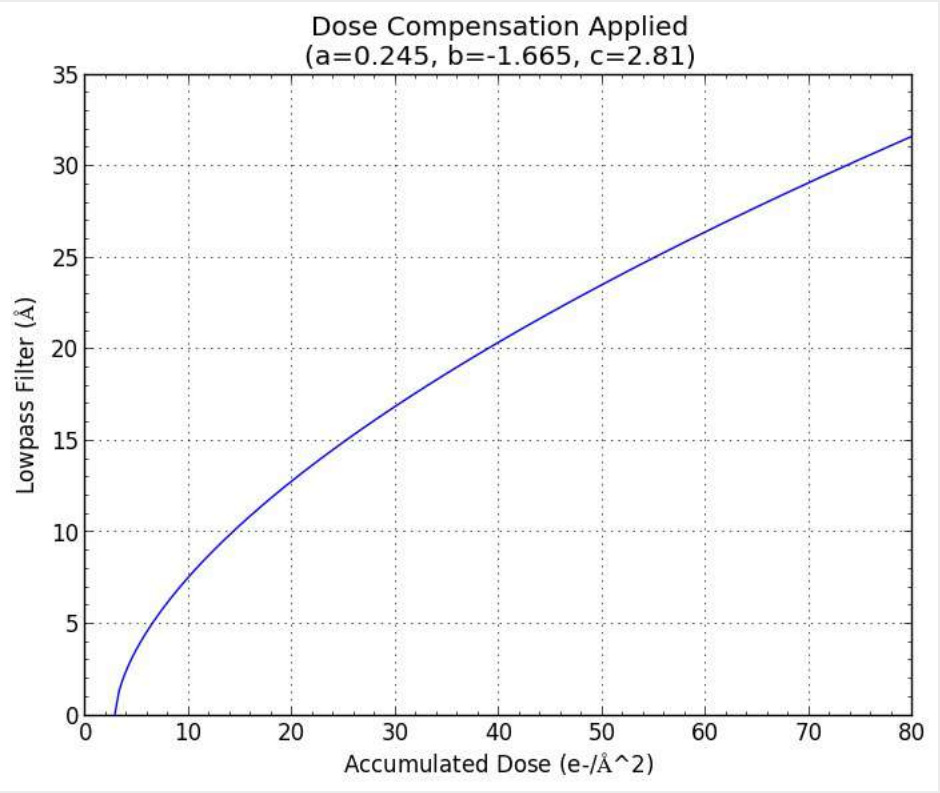
Overview – Limitations

Limitation: **Electron damage** of the specimen

- **Solution:** Remove damaged information from image frames (single particle) or tilt images (tomography):





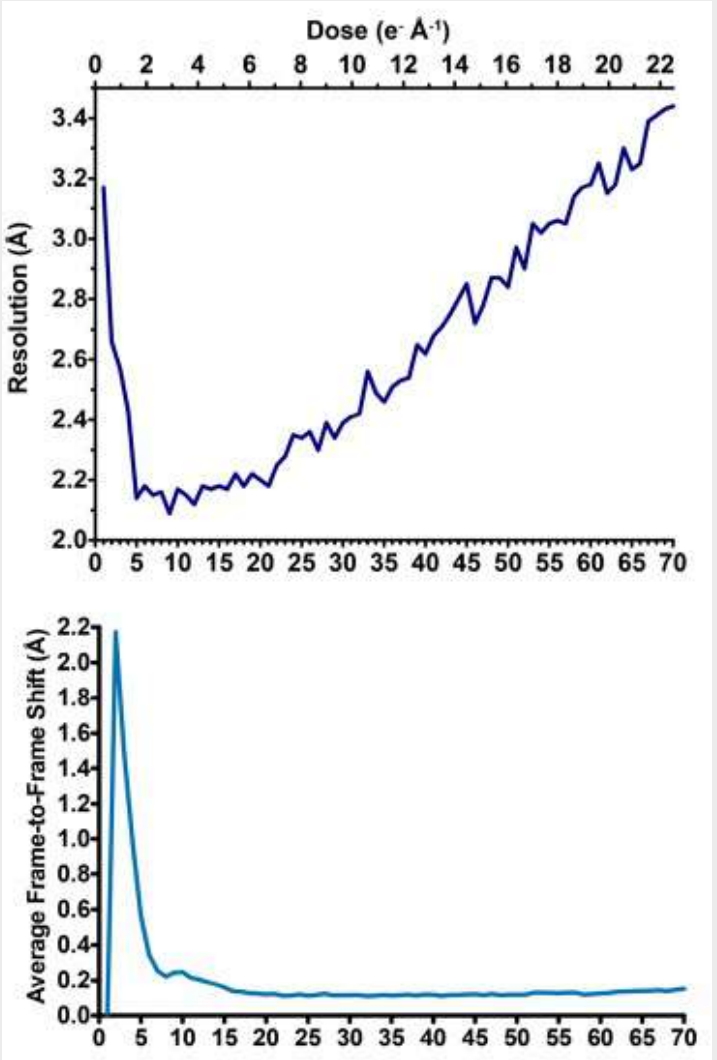


Noble & Stagg, 2015

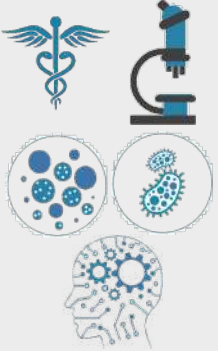
Overview – Limitations

But be careful! There **might be more information** than you think:

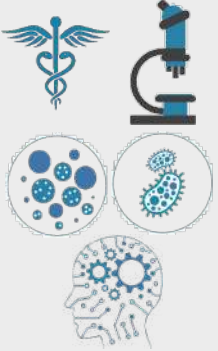
Tan et al., Nat. Comm. 2018



Supplementary Figure 3 | Resolution of individual frame reconstructions. **(a)** Using the best Euler angles and shifts, reconstructions were computed separately for each of the 70 frames. The resulting resolution shows two trends: the first 4 frames (3.17-2.43 Å) suffered from the initial effects of beam-induced motion; after frame 22, the resolution gradually worsens owing to the cumulative effects of radiation damage. **(b)** Frame-to-frame shifts in Ångstroms for all 70 frames are shown in blue. Frame-to-frame shifts were calculated using MotionCor2 global frame alignment mode.



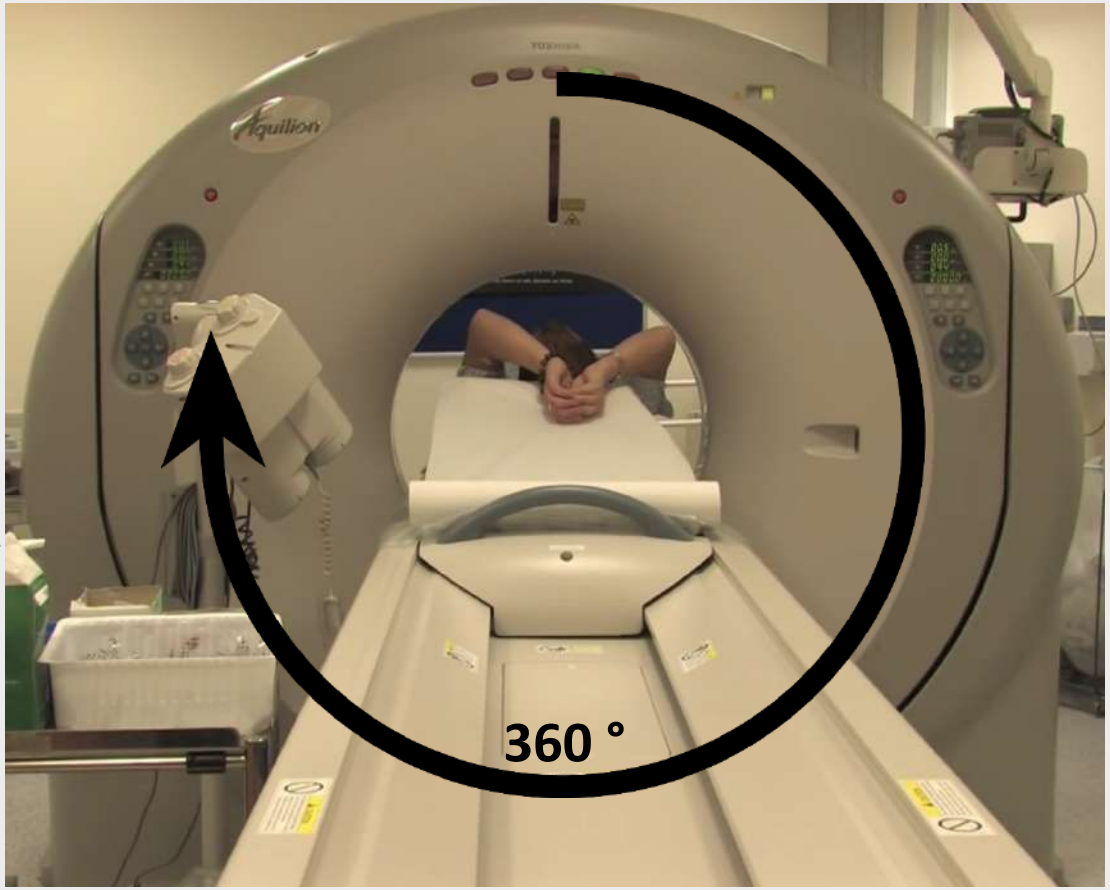
Tomography overview

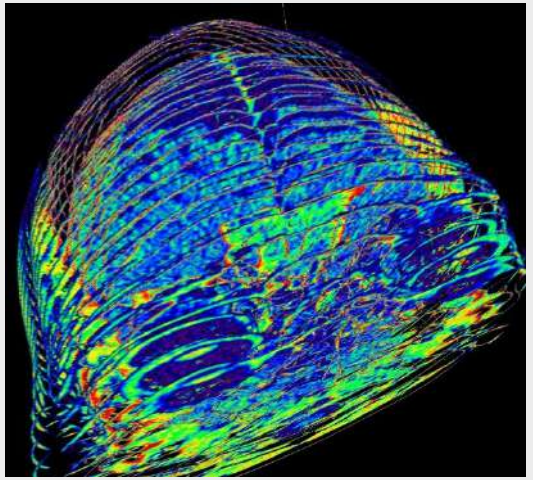


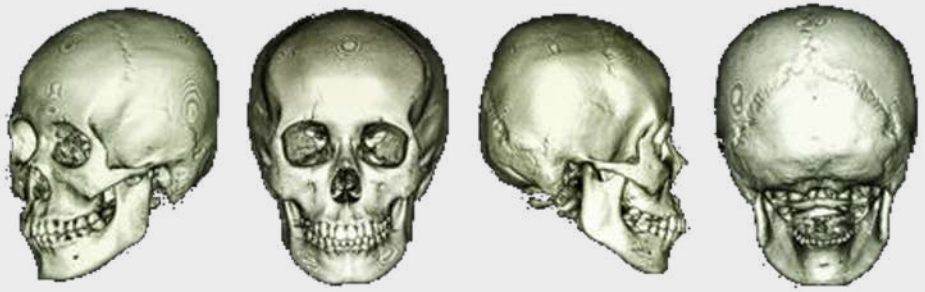
Tomography overview

Tilt-series Collection

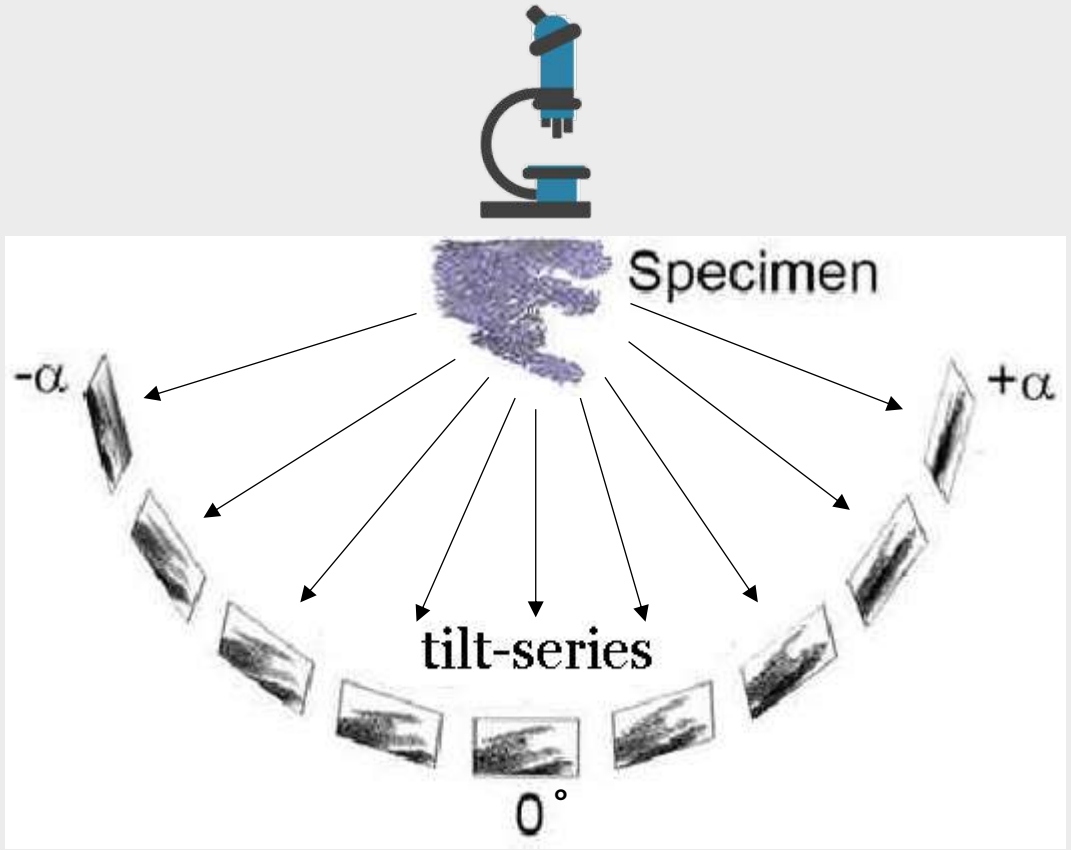
Tomography overview

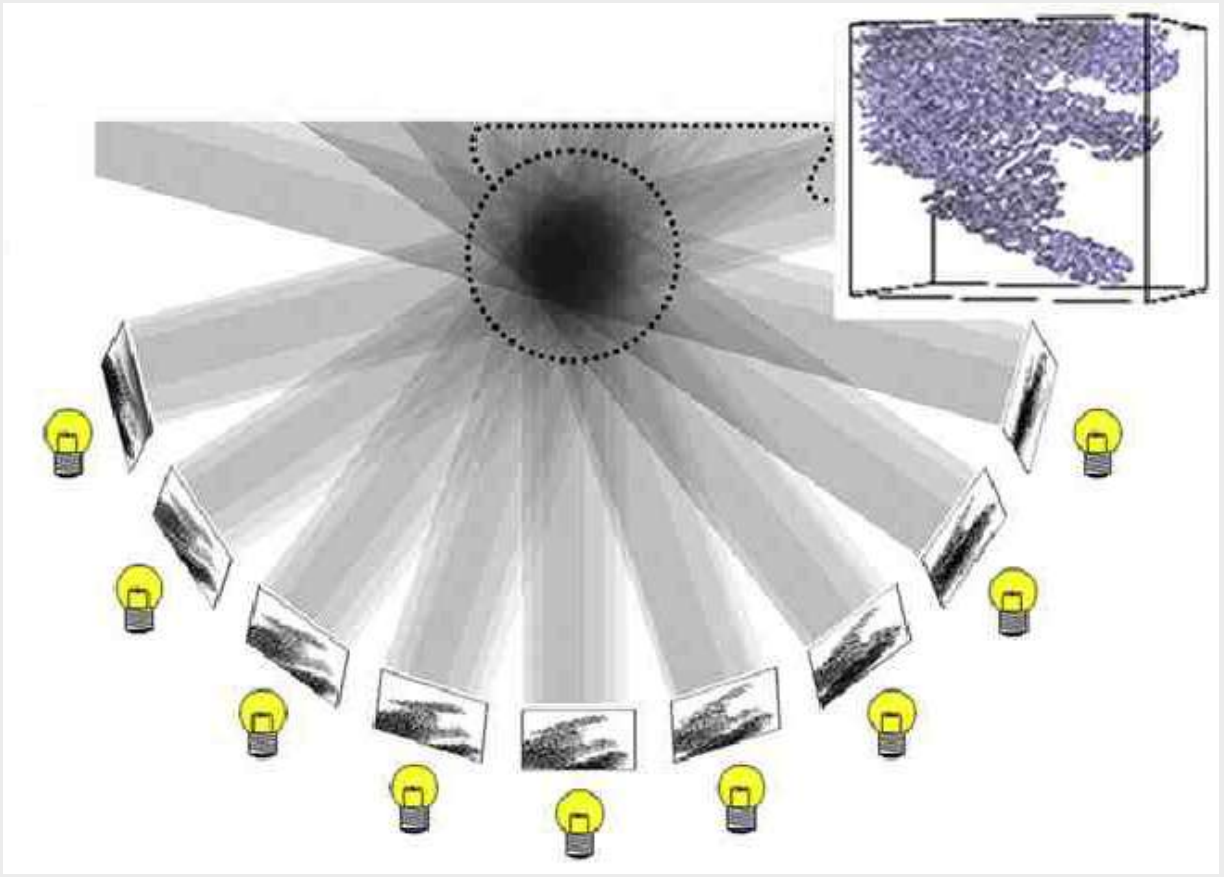






ET/CryoET collection and processing overview

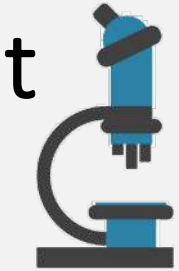


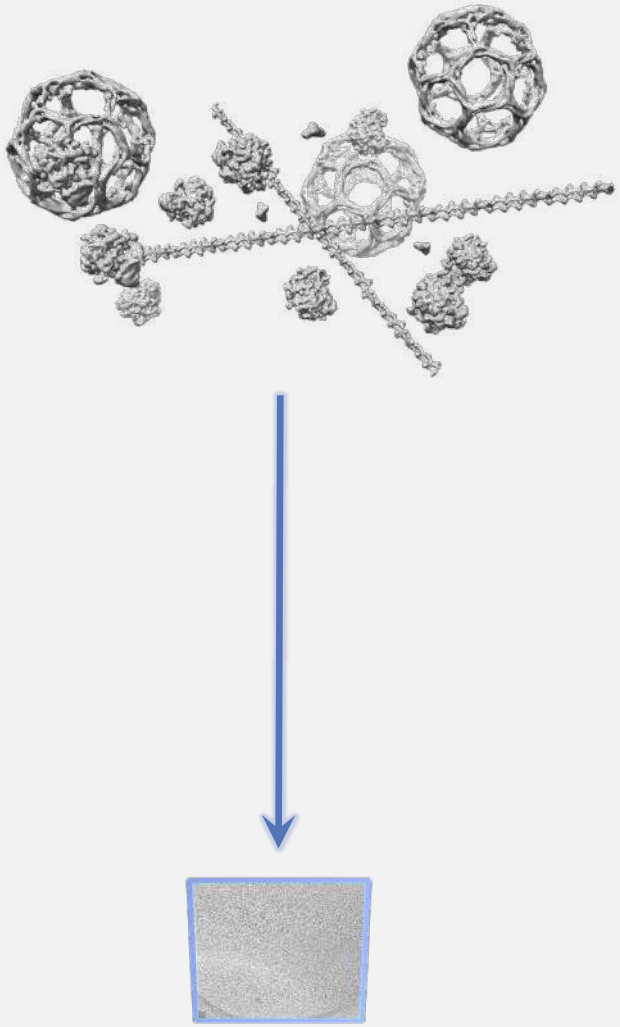


(UMET)

Collect → align → reconstruct

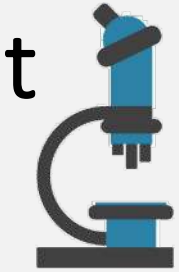
3D specimen movement during collection

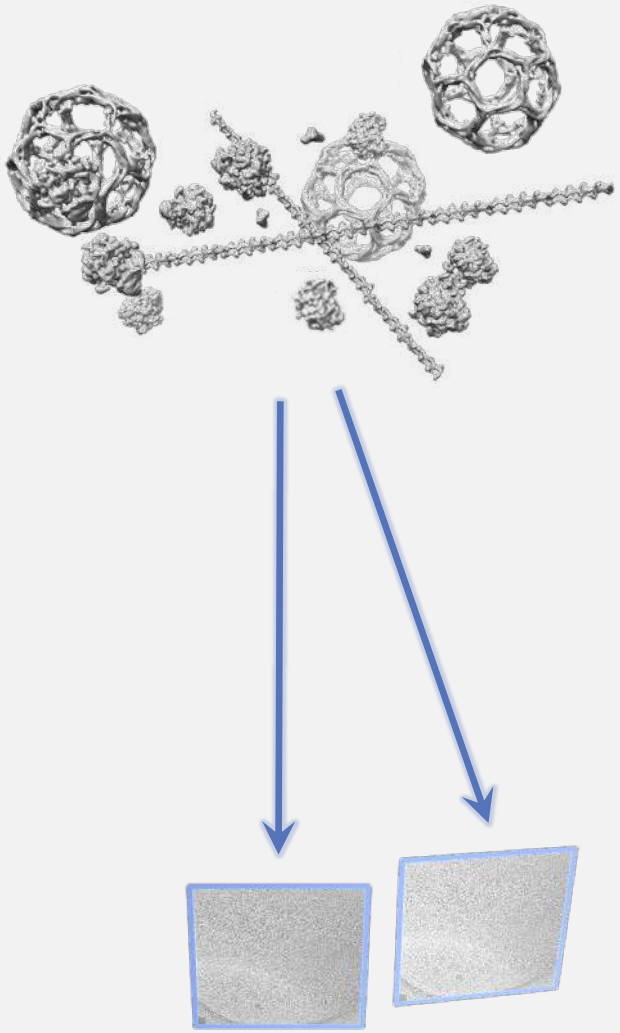




(movements are exaggerated)

3D specimen movement during collection





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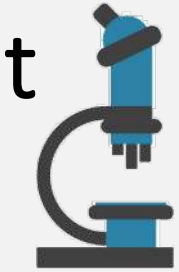

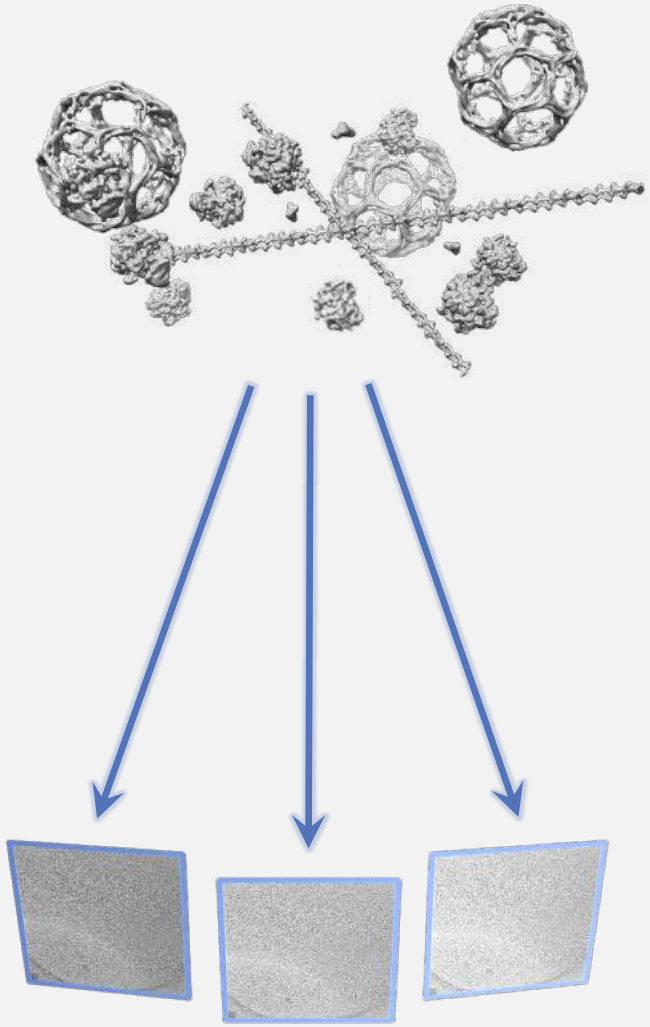

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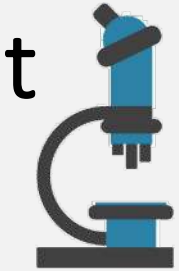
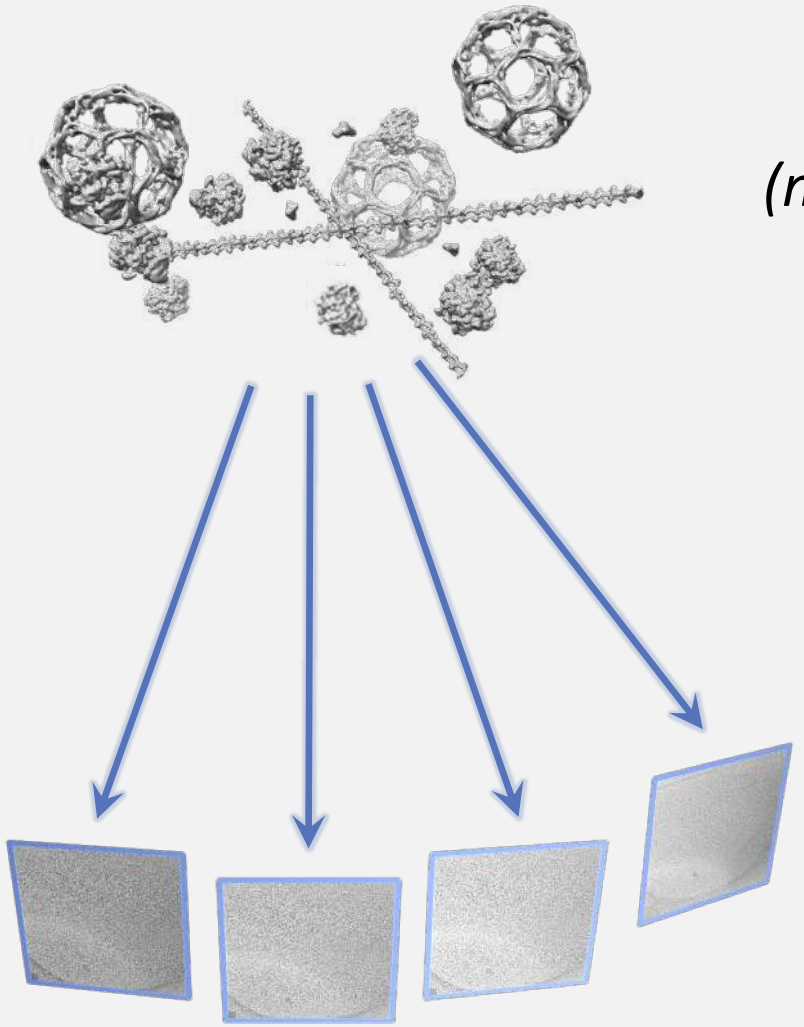
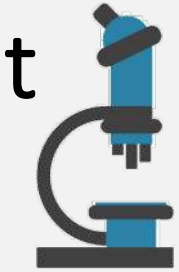
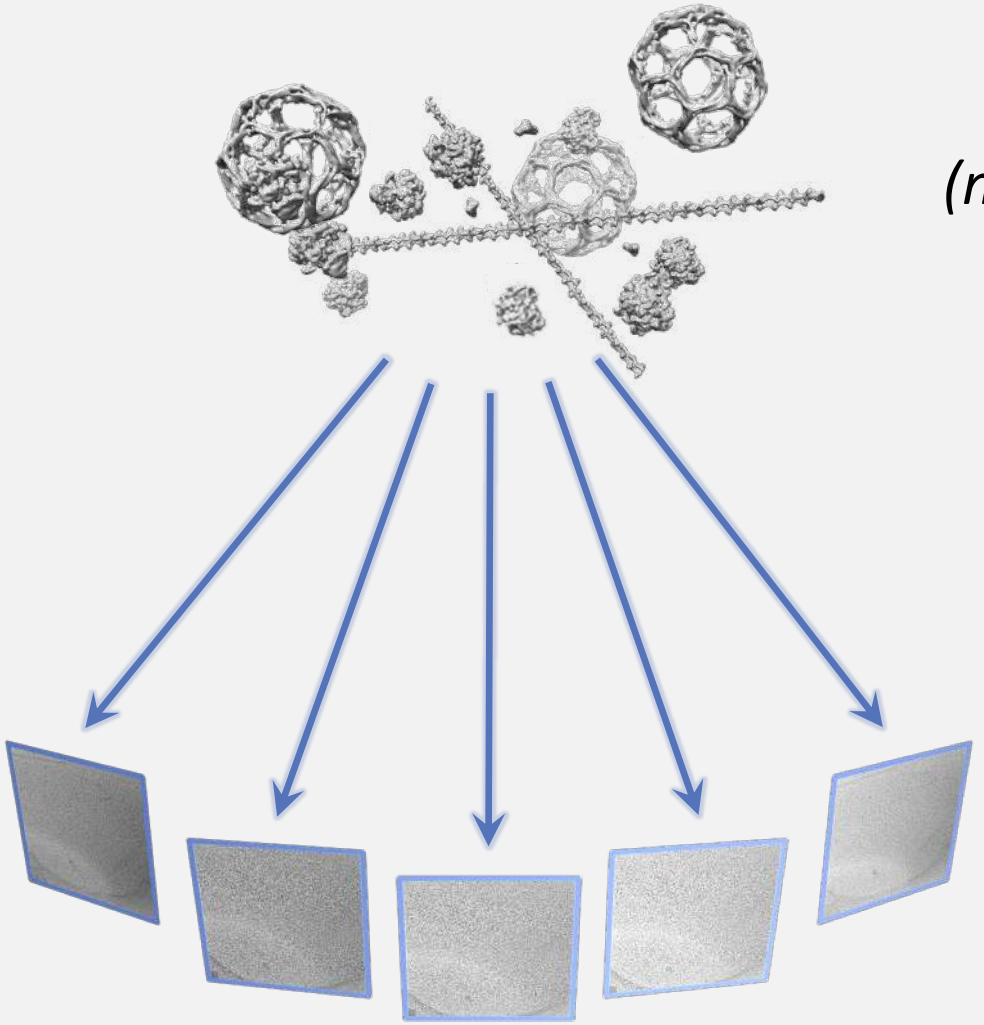




(movements are exaggerated)

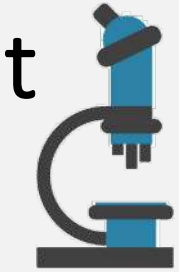
3D specimen movement during collection

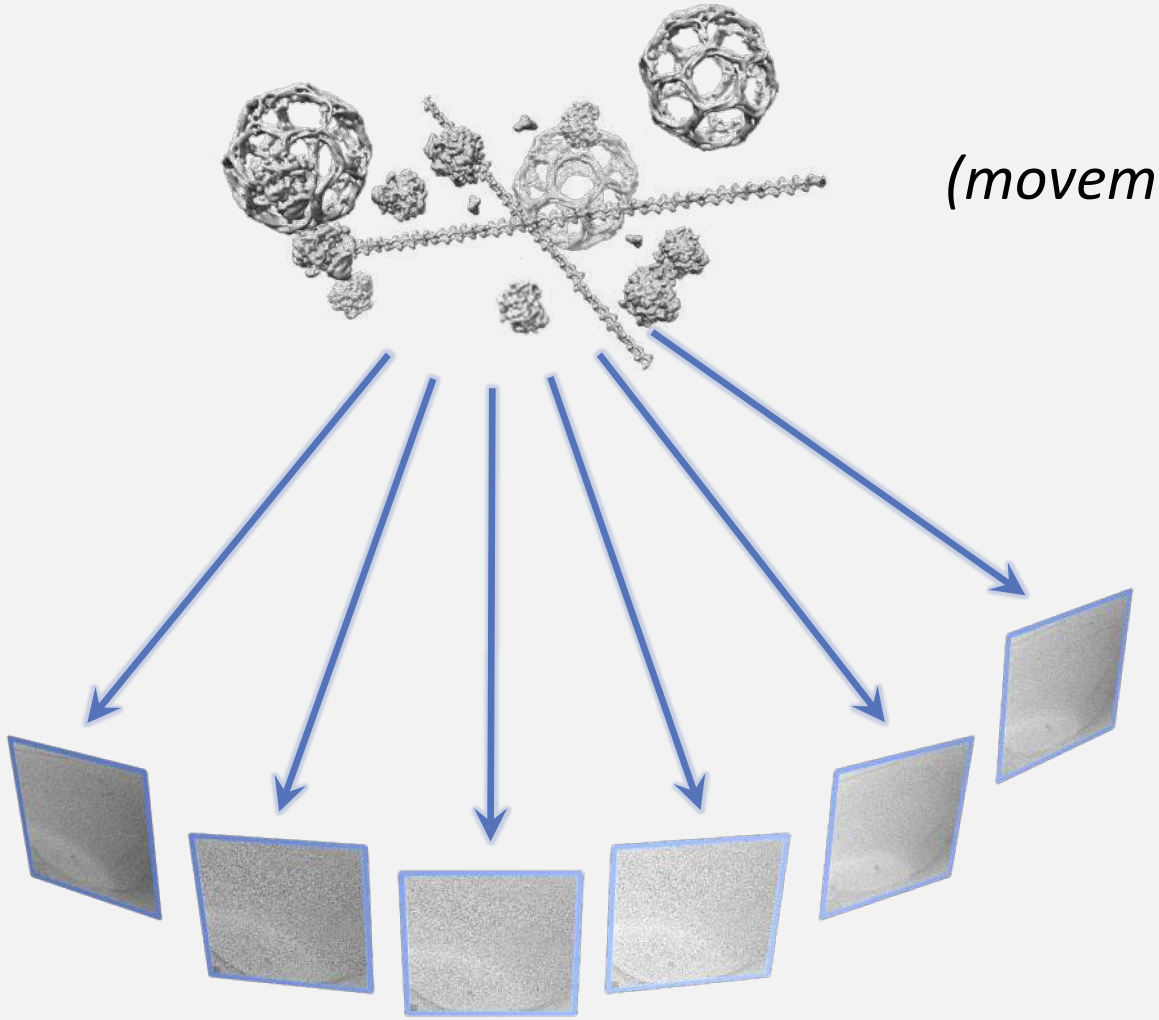




(movements are exaggerated)

3D specimen movement during collection





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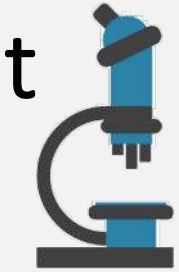
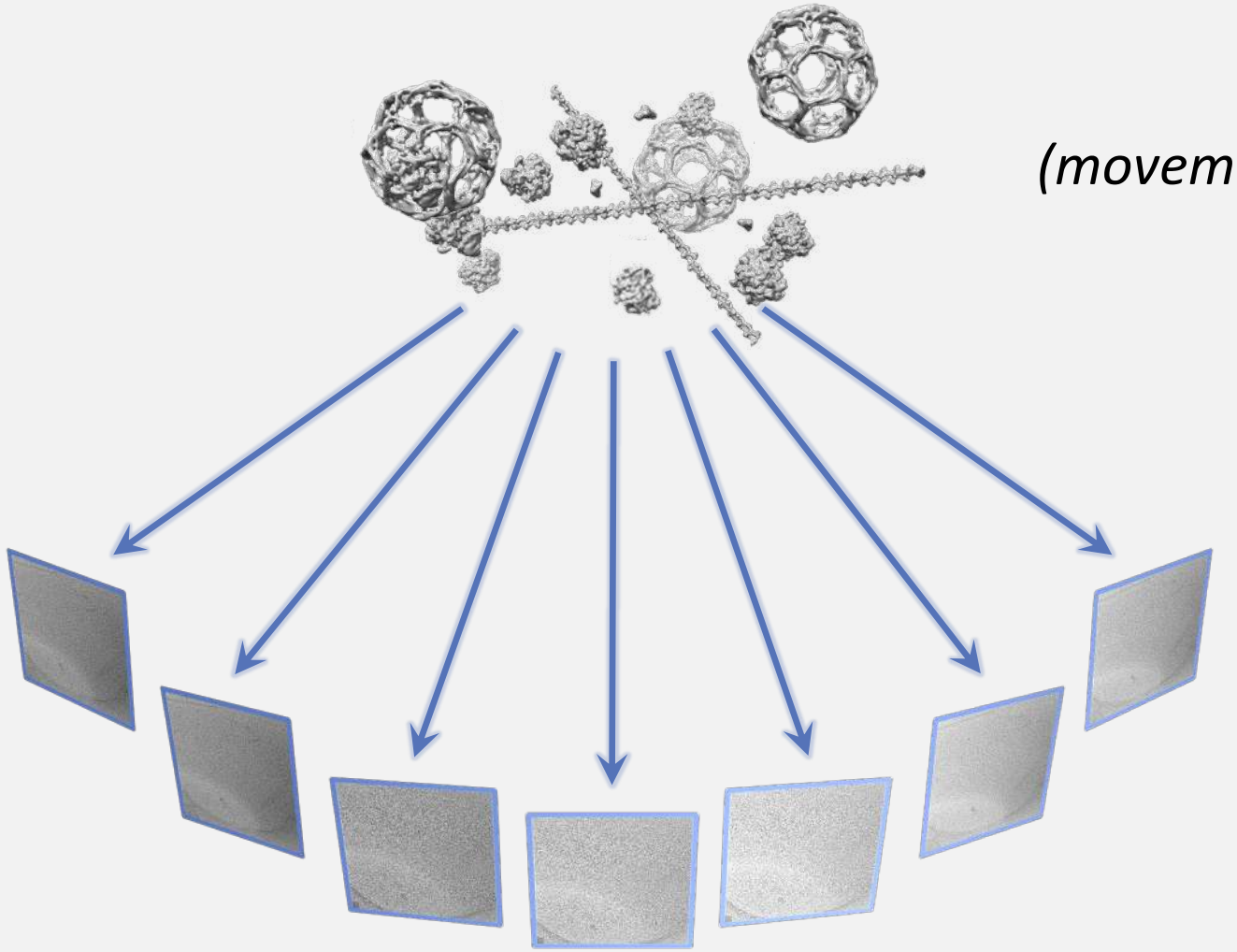
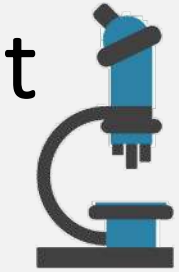
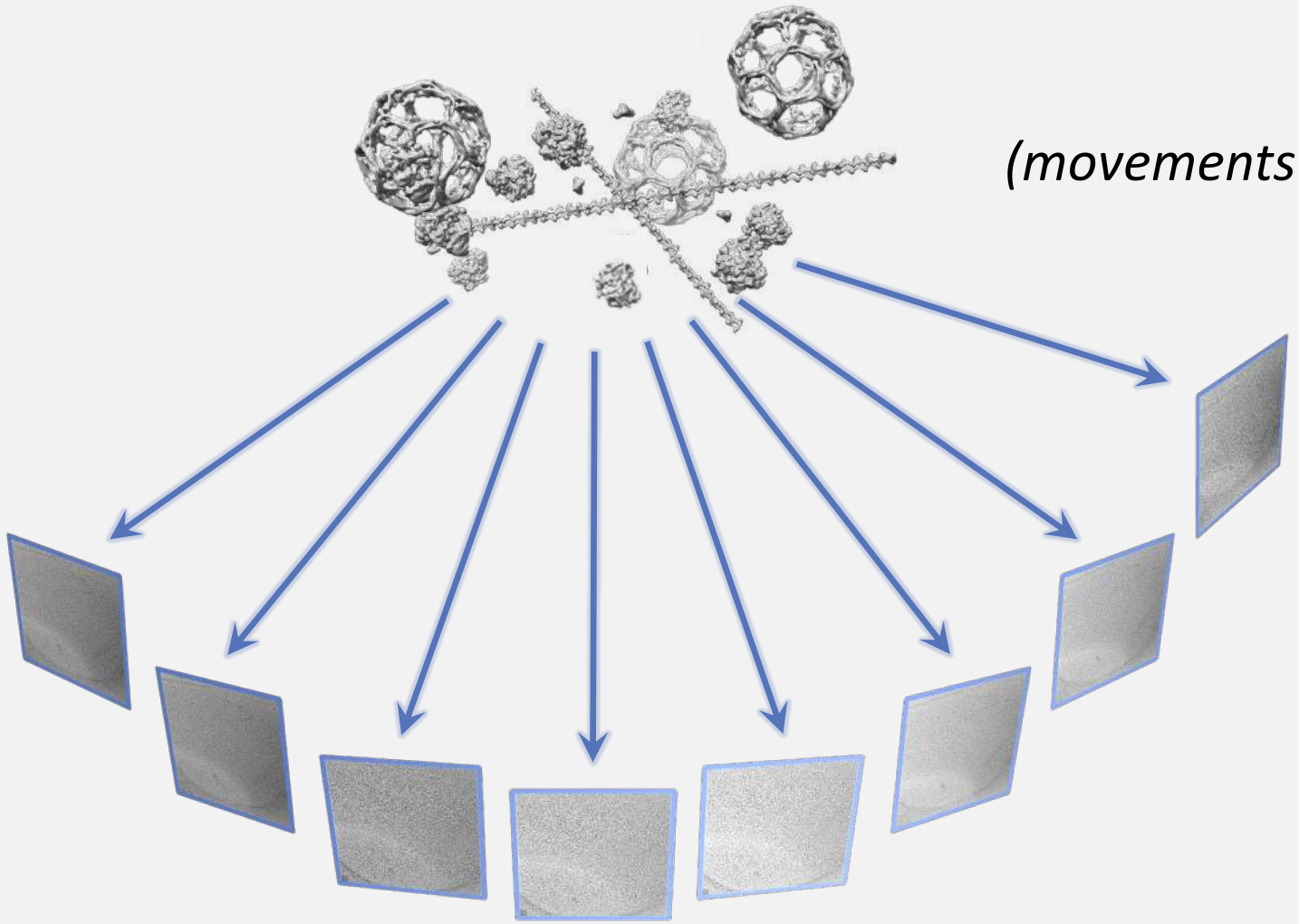




(movements are exaggerated)

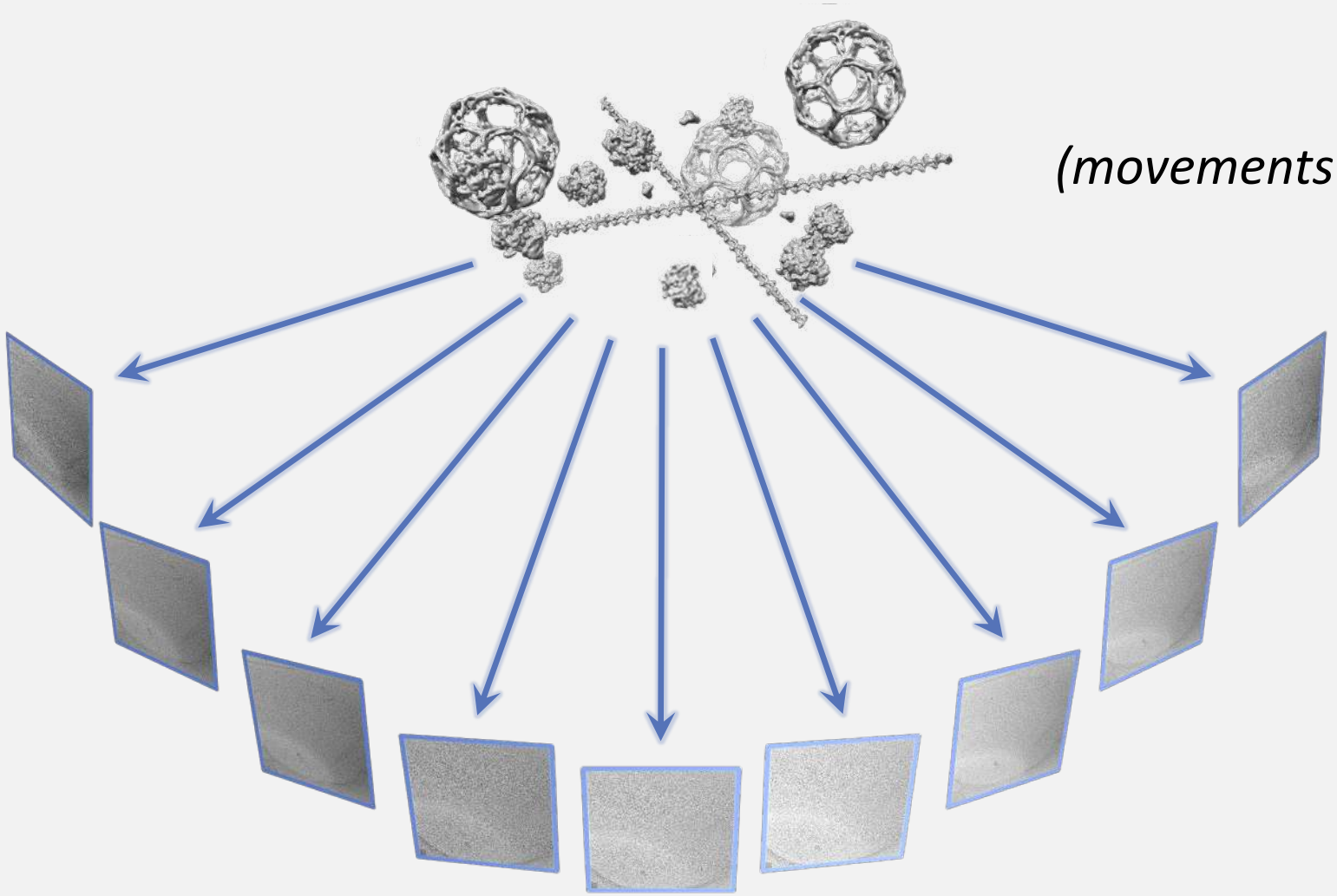
3D specimen movement during collection





(movements are exaggerated)

3D specimen movement during collection



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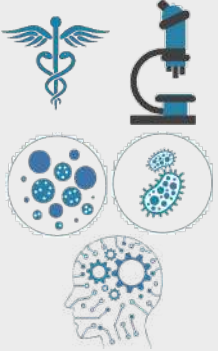
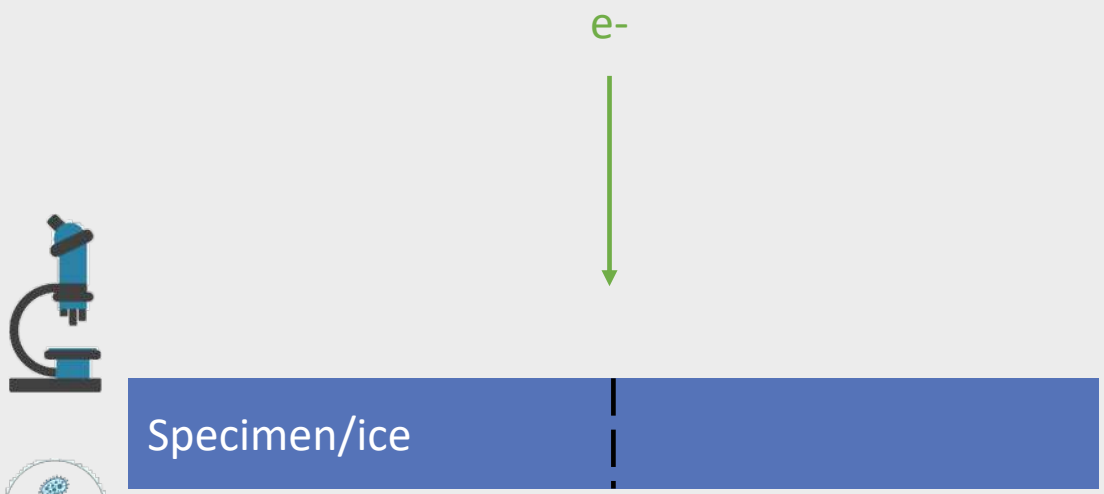
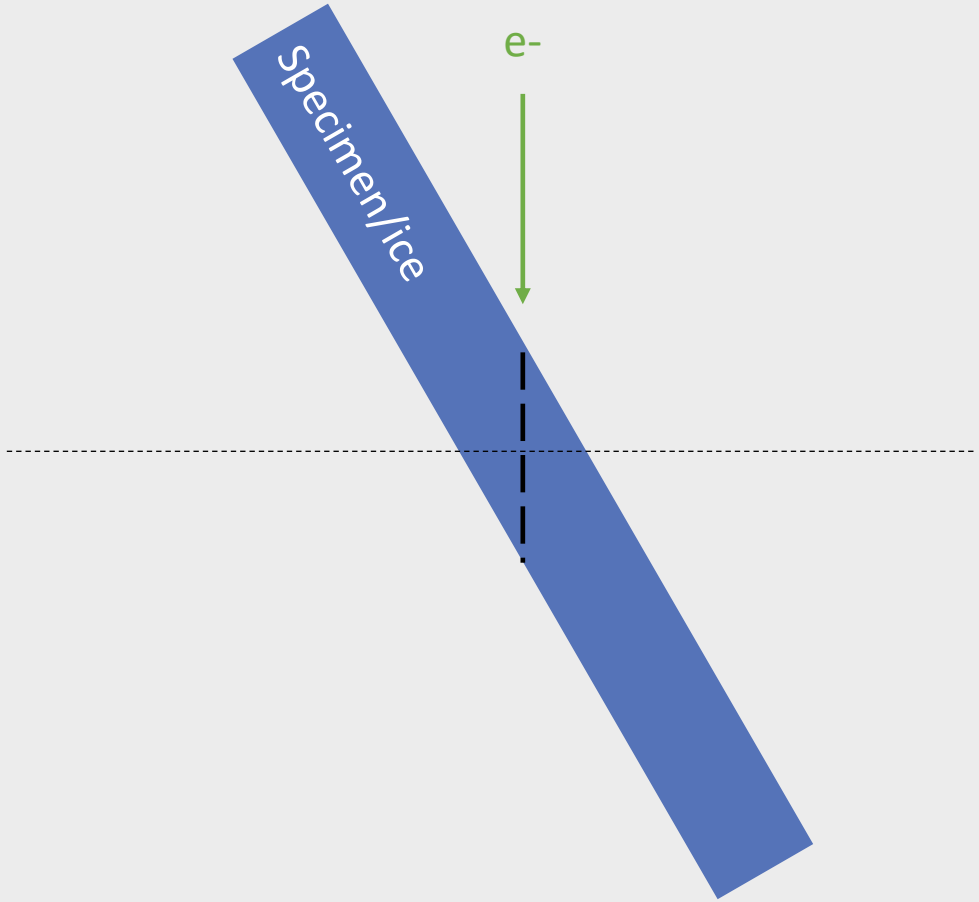


Some more CryoET Limitations

Grid tilting increases thickness

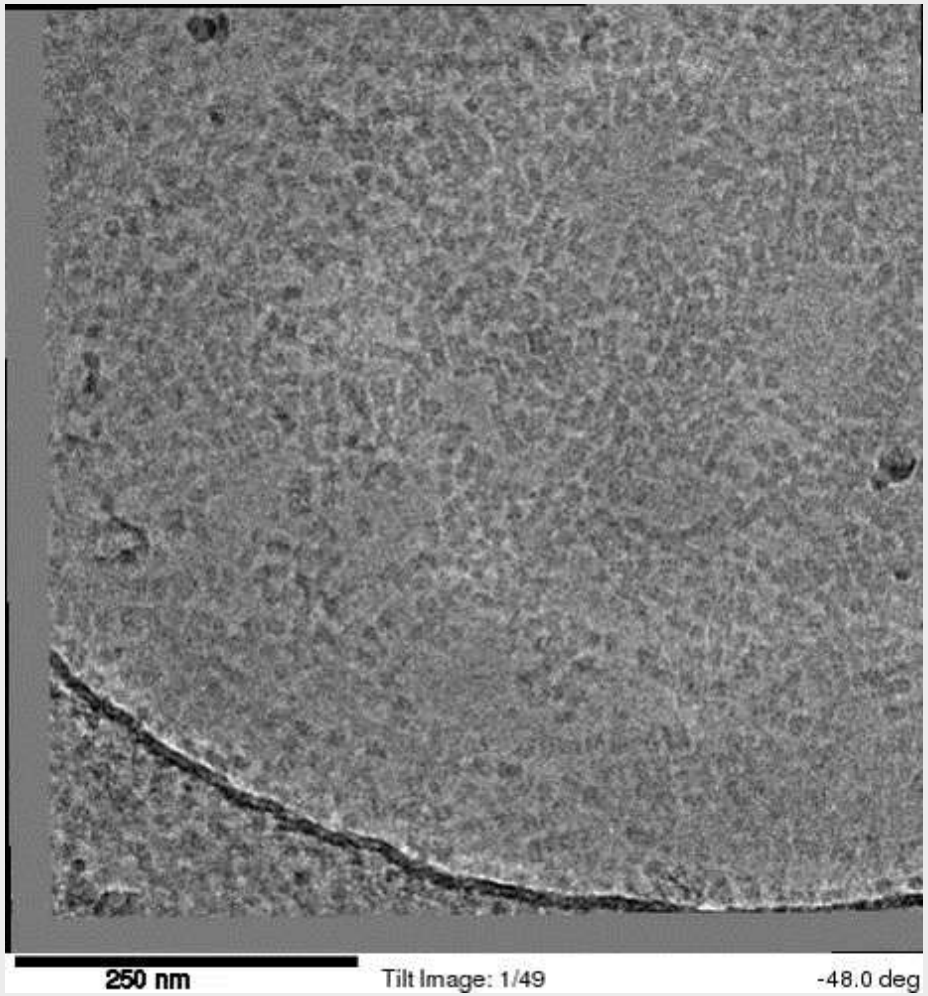


untilted grid



grid tilted 60° = 2x thickness

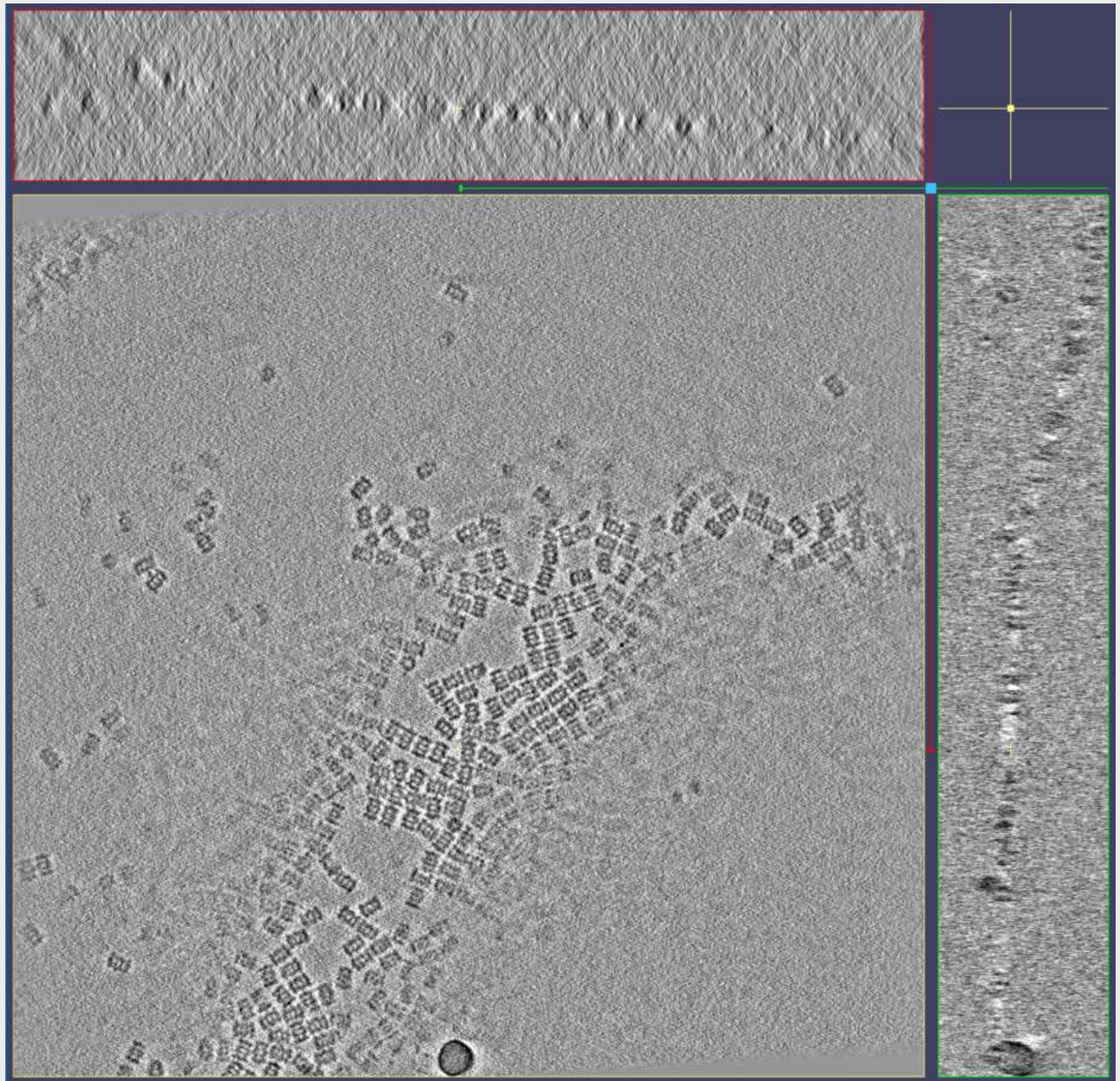
Grid tilting thickness increase limits tilting



Noble et al., 2018

- Phase plate tilt-series of T20S Proteasome
- Tilt axis is **horizontal**

Grid tilting limit results in missing information



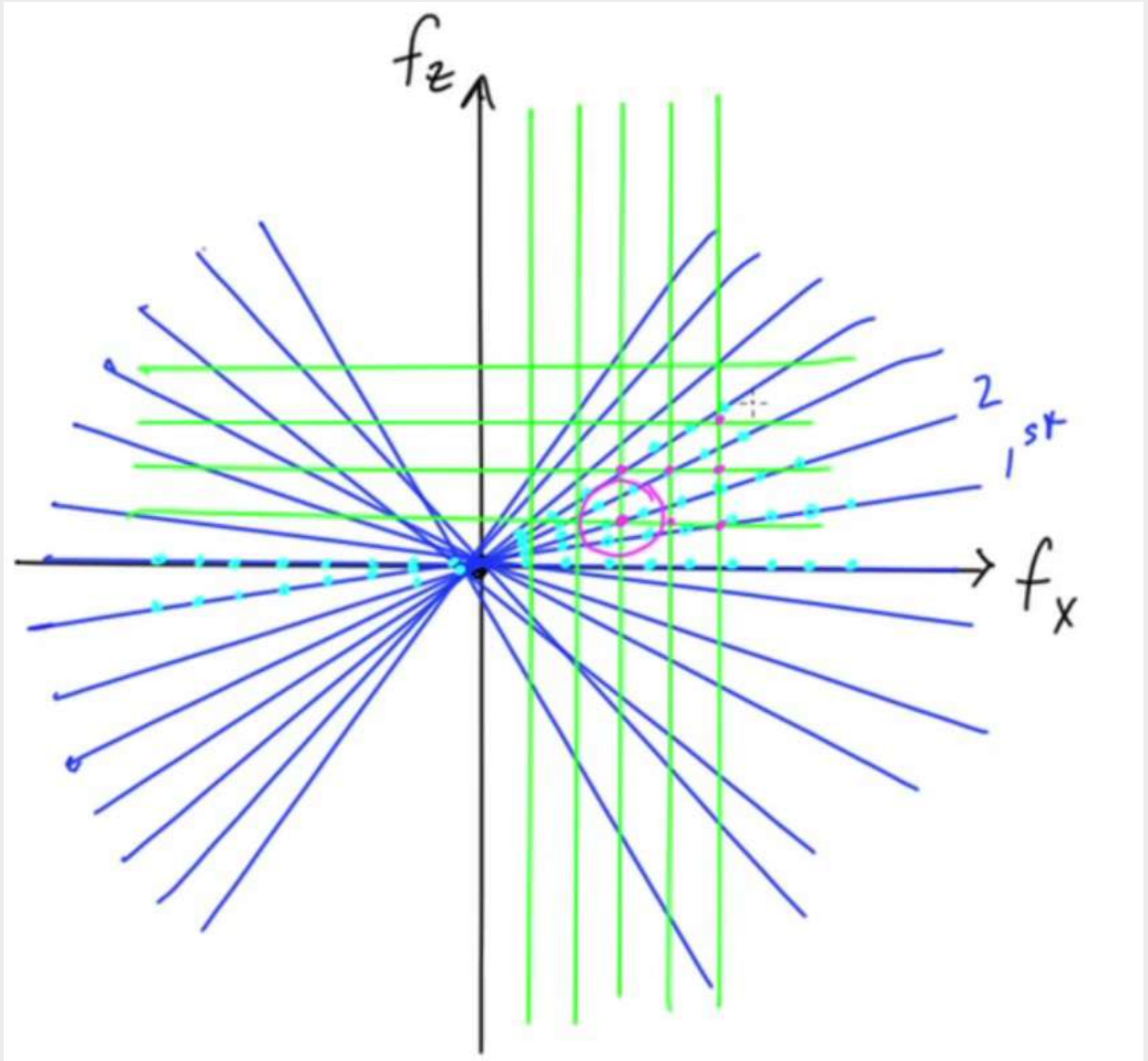
Phase plate tilt-series of T20S Proteasome.

Tilt axis is **vertical**

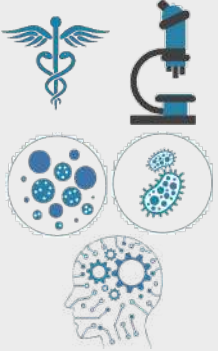
Noble et al., 2018

Reconstruction Implies Interpolation

- Tomographic reconstruction on a **3D grid** requires **interpolation**
- Larger tilt increment = more **missing information** at higher tilt angles



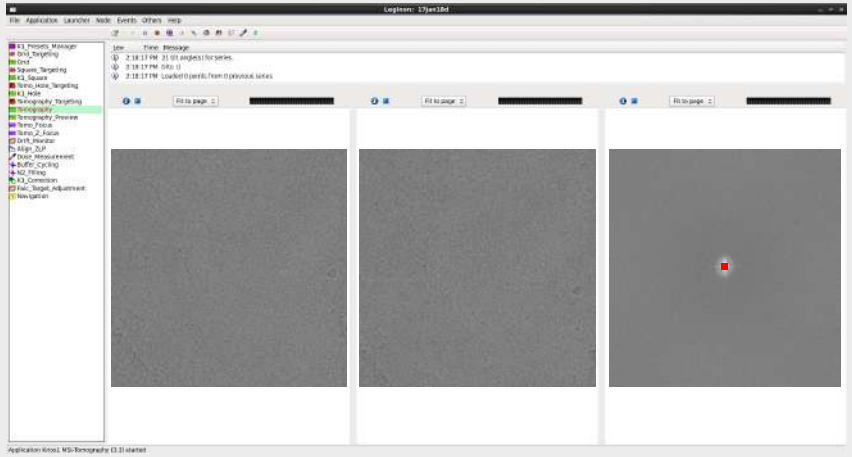
Grant Jensen, YouTube

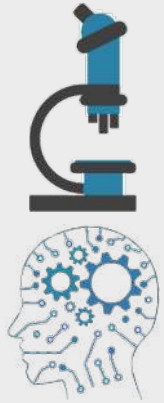


Tilt-series collection

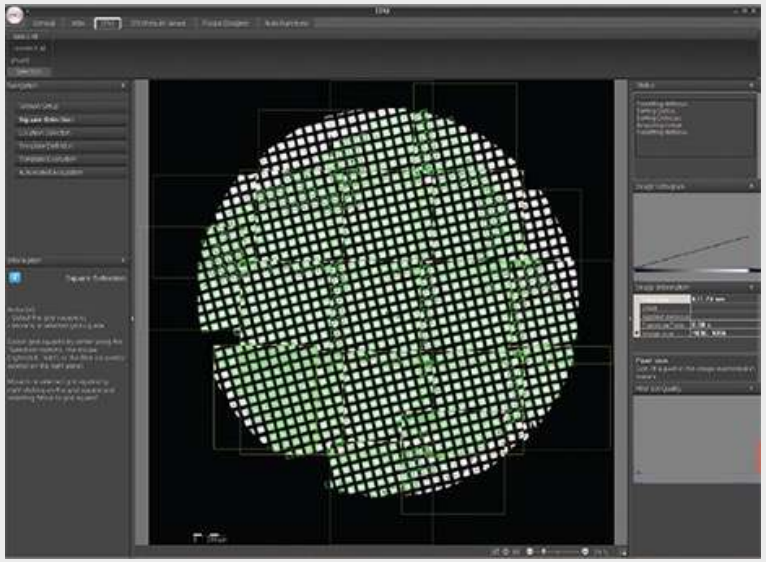
Tilt-series collection software

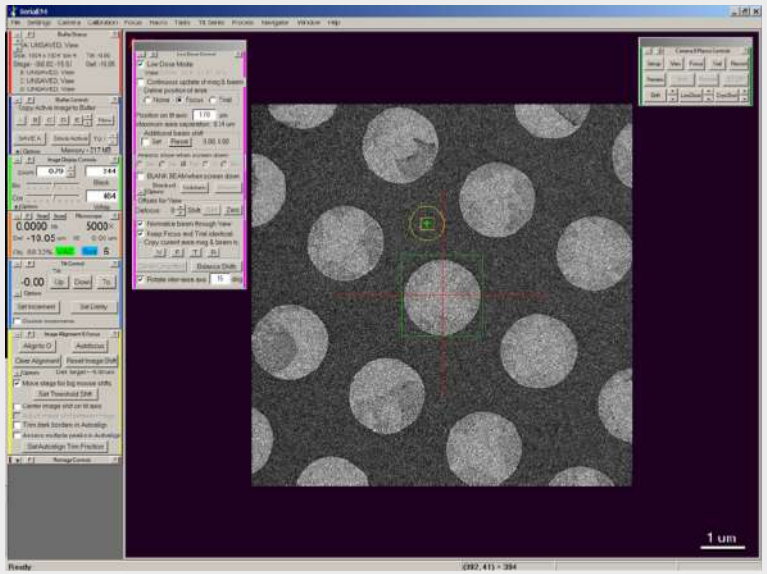
Leginon



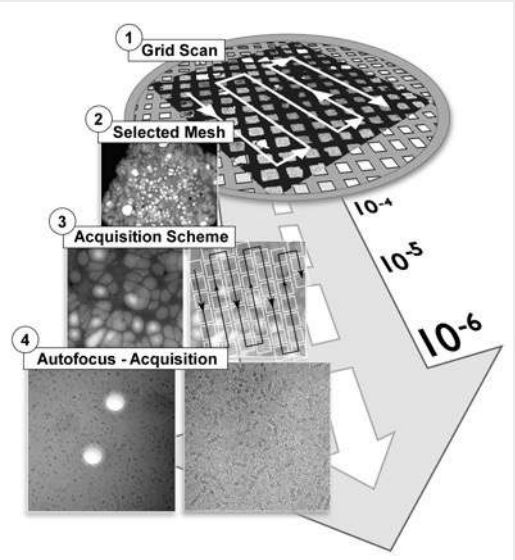


EPU

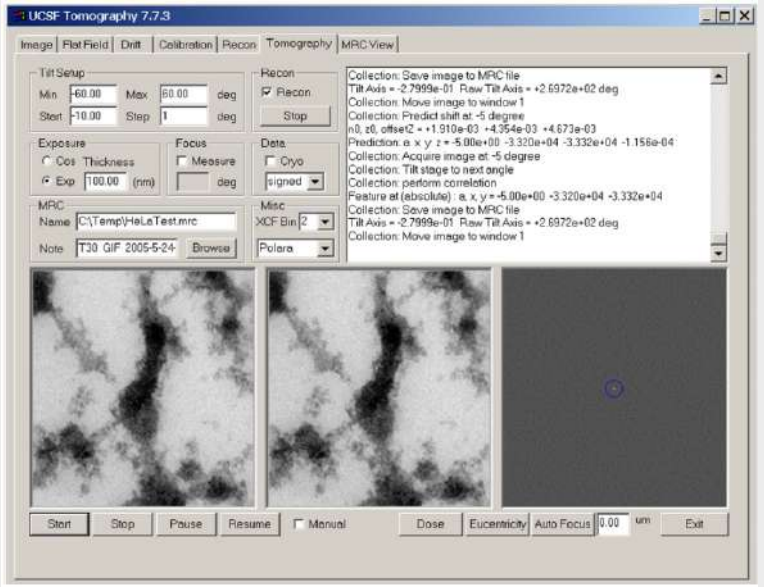




SerialEM



TOM Toolbox



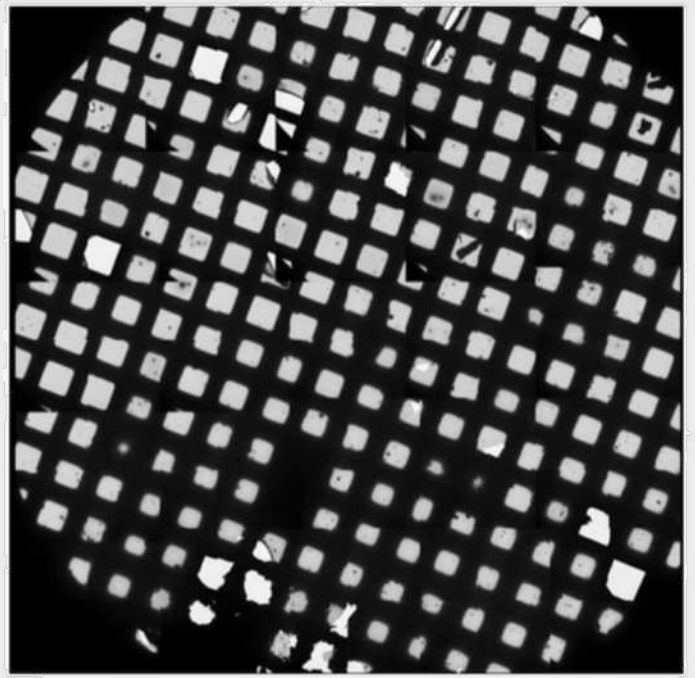
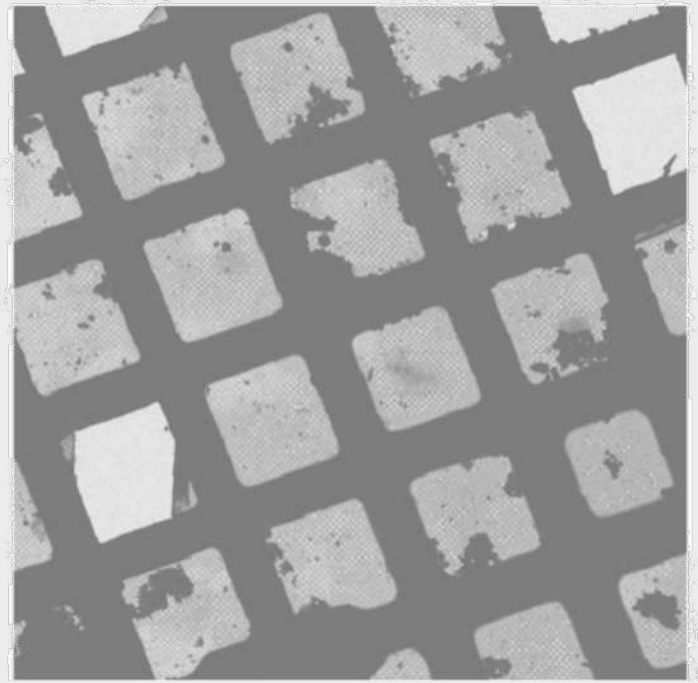
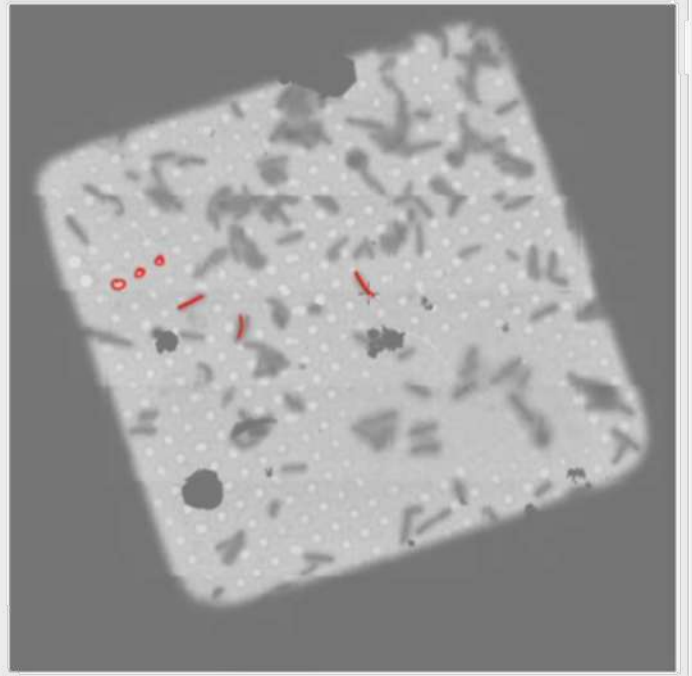
UCSF Tomography

Tilt-series tracking

- **Problem:** You **cannot trust the goniometer** to move where you tell it
- **Problem:** You **cannot use the area of interest to refine** your tracking because you will over-expose your sample
- **Problem:** You need to **refine x, y, and usually z to within 10-100 nm** for a high-mag tilt-series collection.

- **Solution 1:** **Predictive tracking** – Use previous tilt images, previous tilt-series, and possibly known goniometer instabilities.
- **Solution 2:** **Focus position method** – Identify one or two locations along the tilt axis the software will go to re-focus and re-track.
- **Solution 3:** **Pre-calibrated tracking** – Make a model of your goniometer before collecting to predict movements.

Automated tilt-series collection

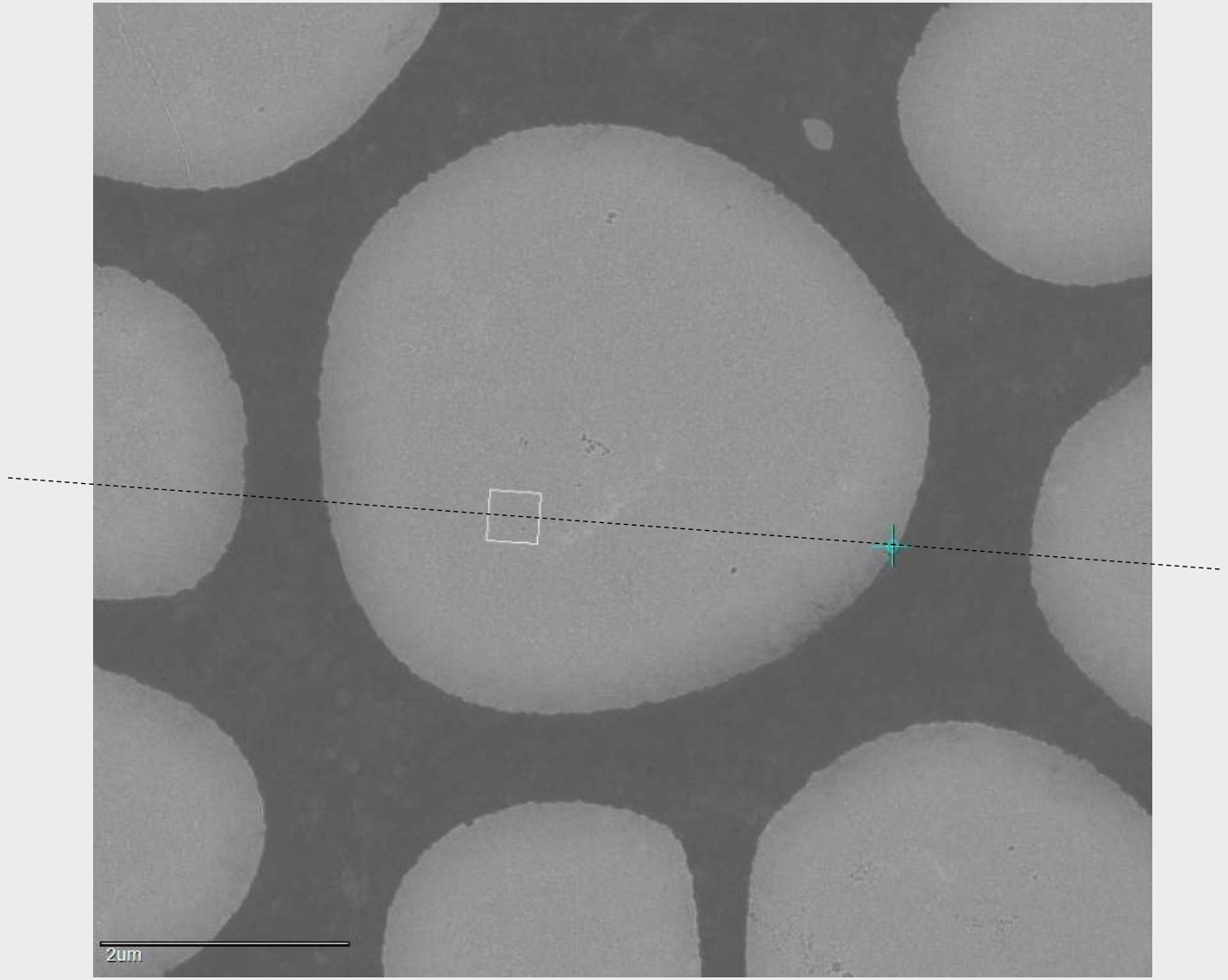
Automated tilt-series collection is currently **routine**

- From an atlas, select multiple squares, and from each square select holes,
- For each hole place an exposure target along with one or more focus targets,
- Set up dose, defocus range, tilt model, etc. appropriately,
- Collect!

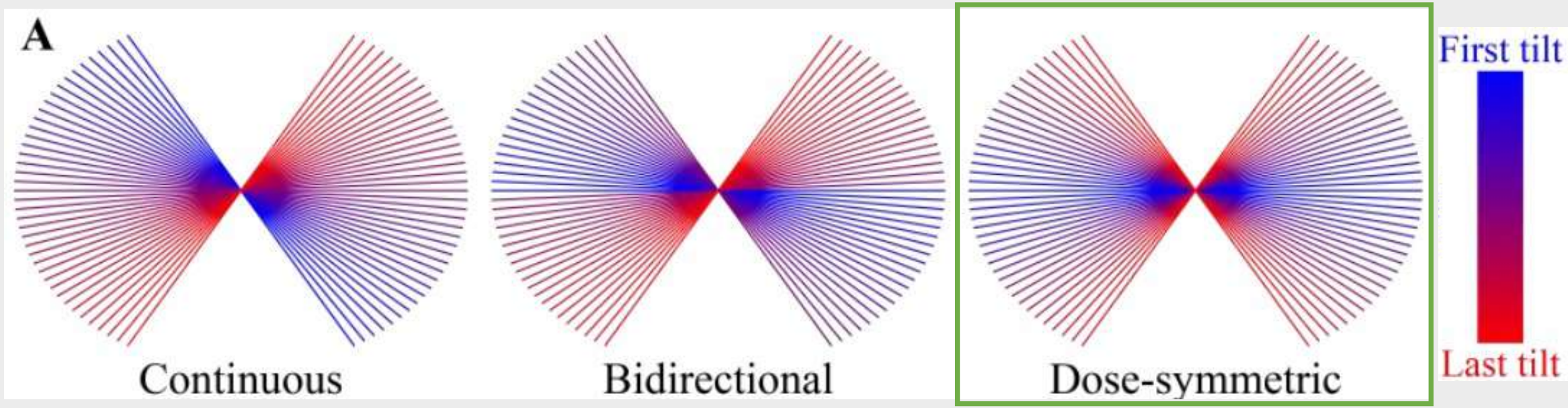
Automated tilt-series collection

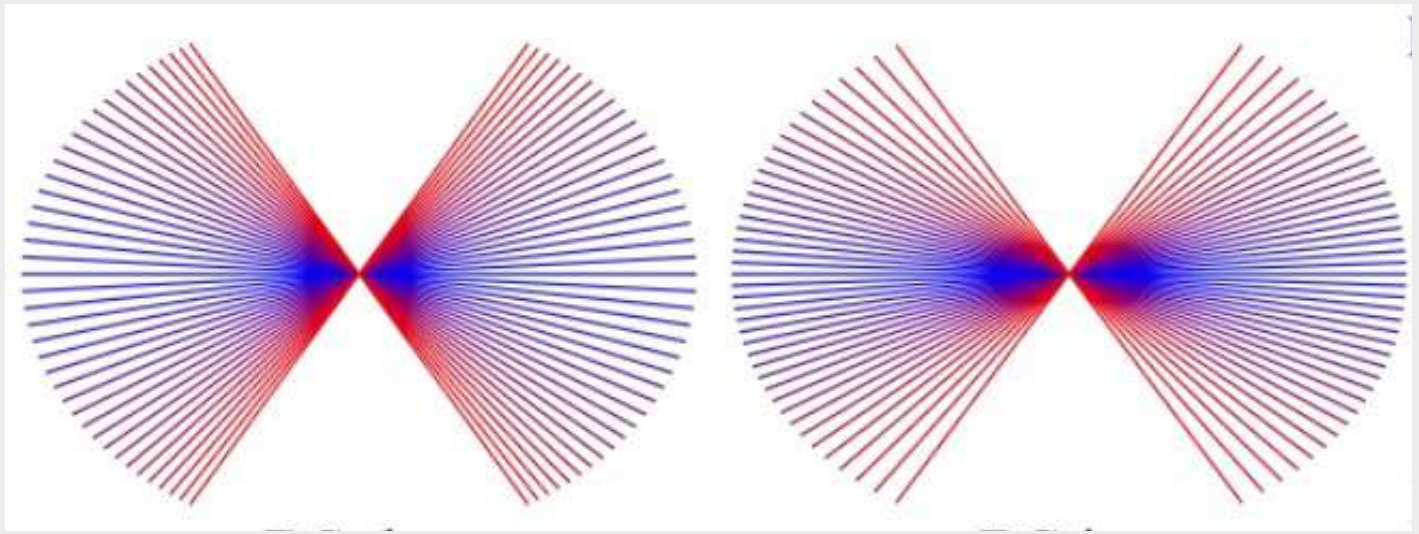
Focus on the tilt axis!

- You want to minimize the amount of tracking error
  - ➢Tilting should not change the x,y,z target location
- This is called getting **eucentric height**.



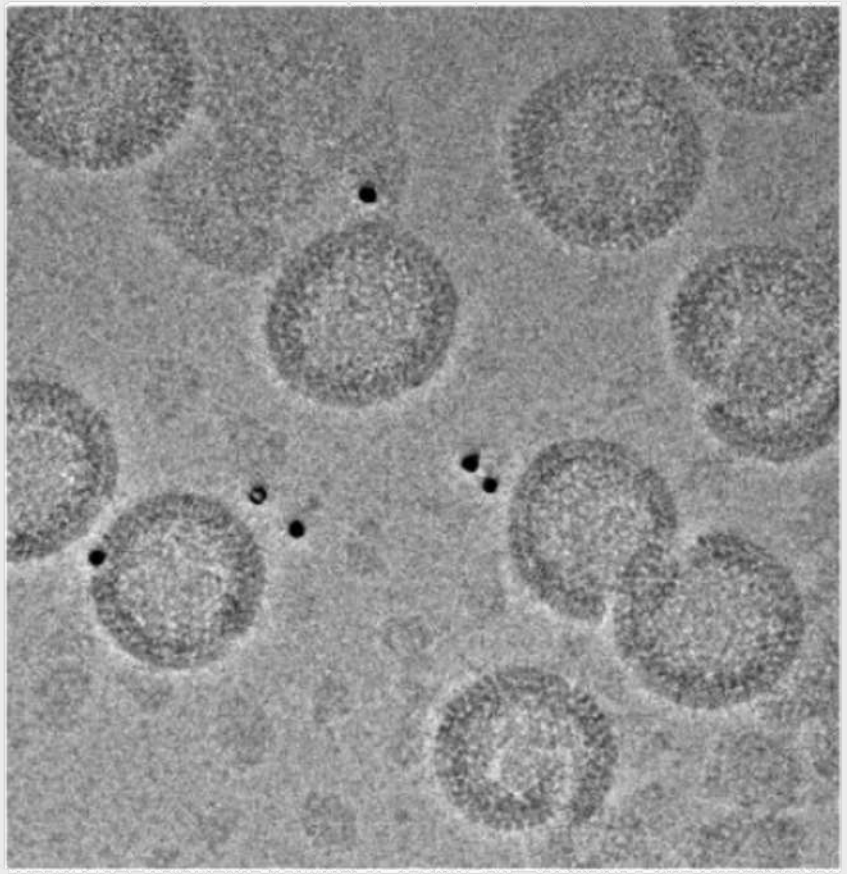
Some Collection Schemes

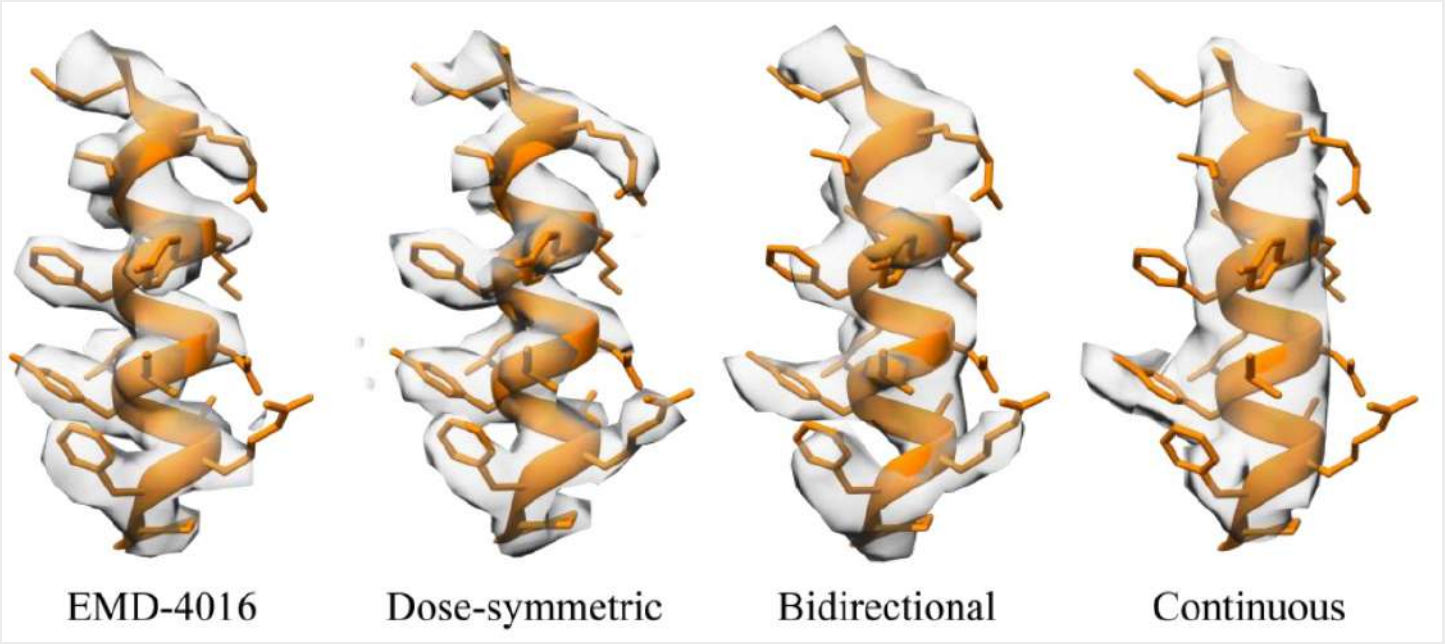




Turoňová et al., 2019 & 2020

Some Collection Schemes on an *Isotropic* Sample





Turoňová et al., 2019

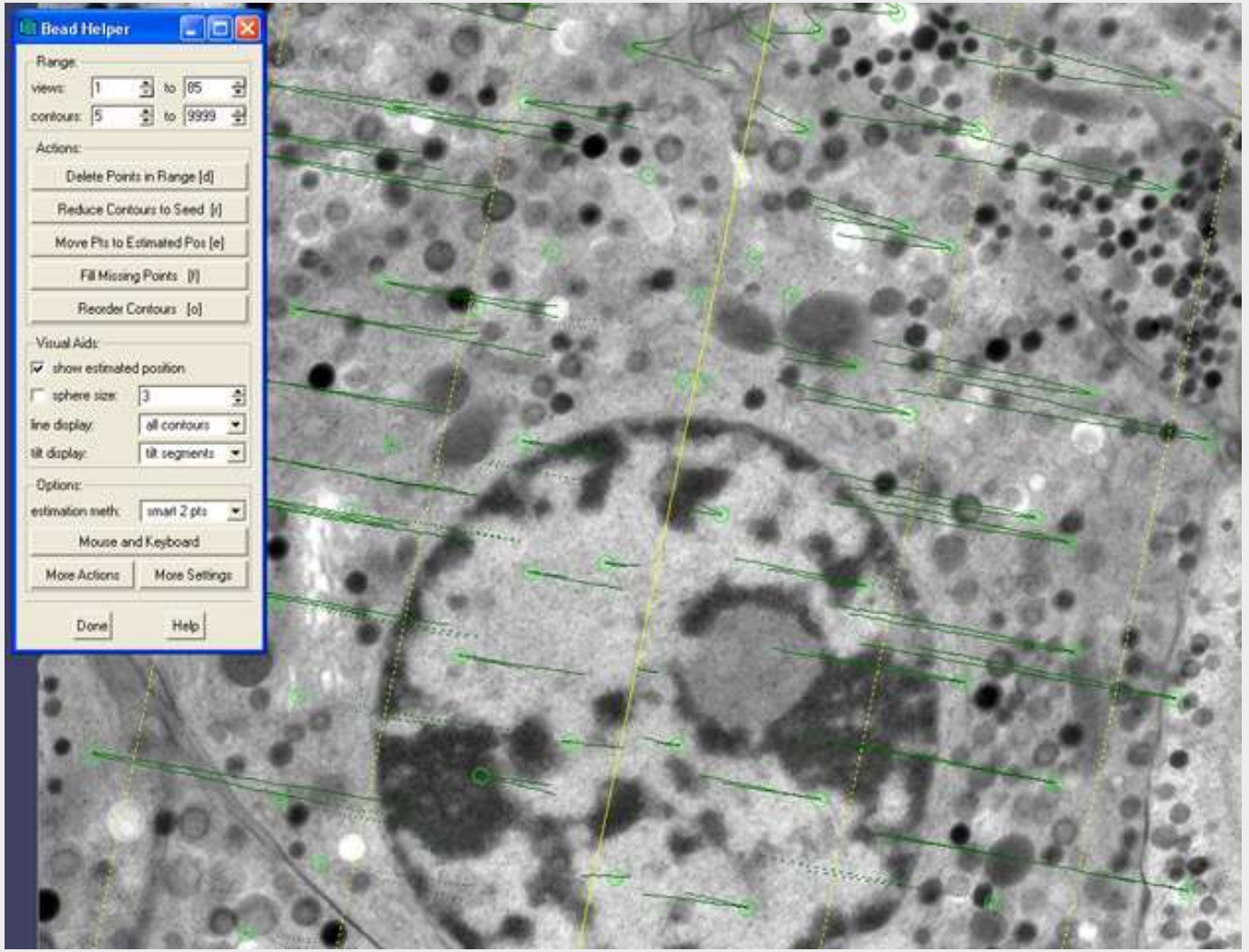
Tilt-series alignment

Tilt-series alignment

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

Fiducial-based tilt-series alignment

- Requires a **sufficient number of well-behaved gold beads**
- Semi-automated (IMOD, Dynamo) or automated (AuTom/markerauto, IMOD) processing



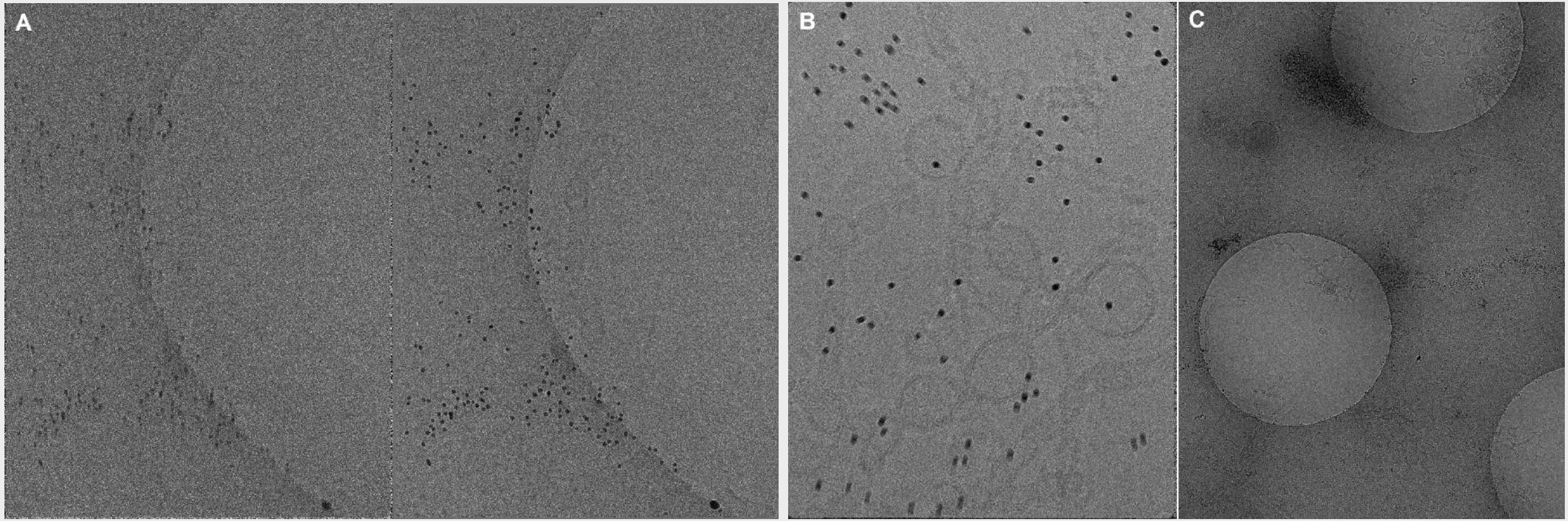
http://bio3d.colorado.edu

Observed fiducial & sample motion in 2D

Fiducial Movement

Anisotropic Bead Motion

Bead Aggregation

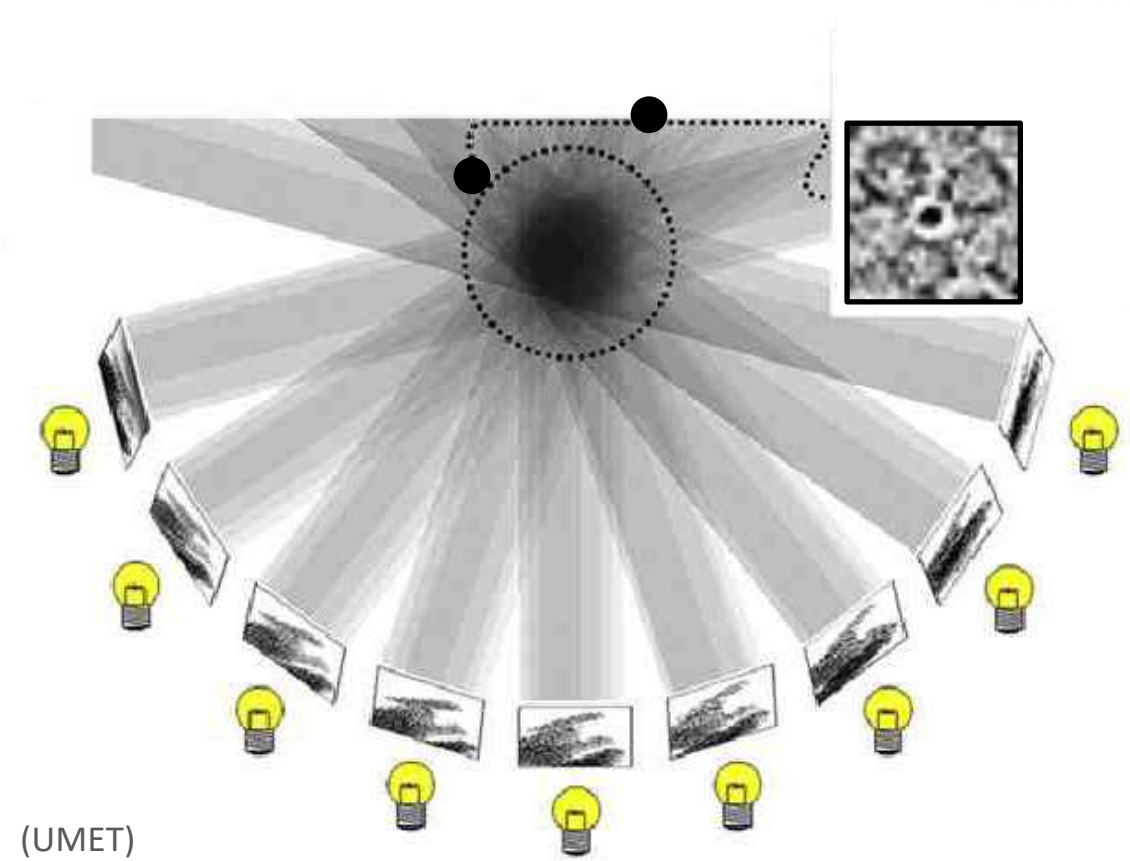


DE-20 @ 18kx; 51°, 2.34 $e^-/Å^2$ after a cumulative dose of 60 $e^-/Å^2$

DE-20; 57.5 $e^-/Å^2$, 0° exposure

Noble & Stagg, JSB 2015

Fiducial-based tilt-series alignment issues

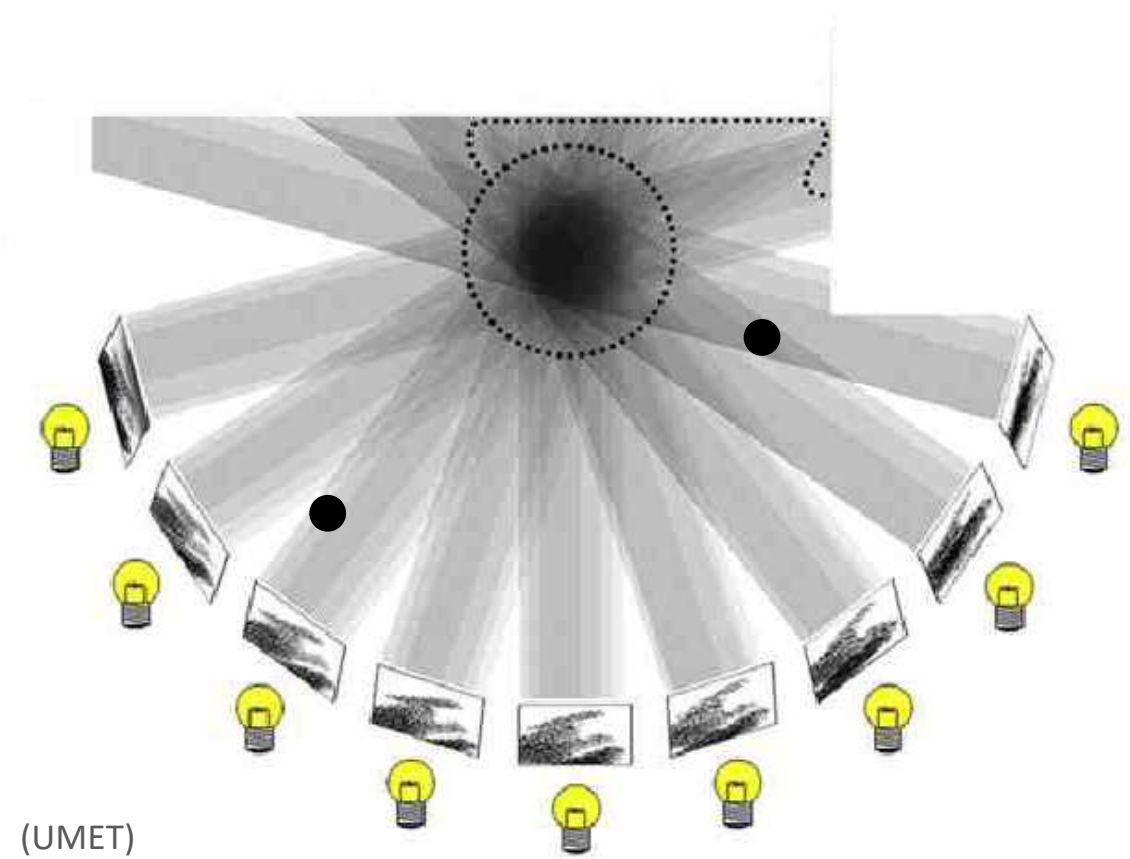


(UMET)

Nearby Fiducials Affect Signal and Contrast

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

Fiducial-based tilt-series alignment issues

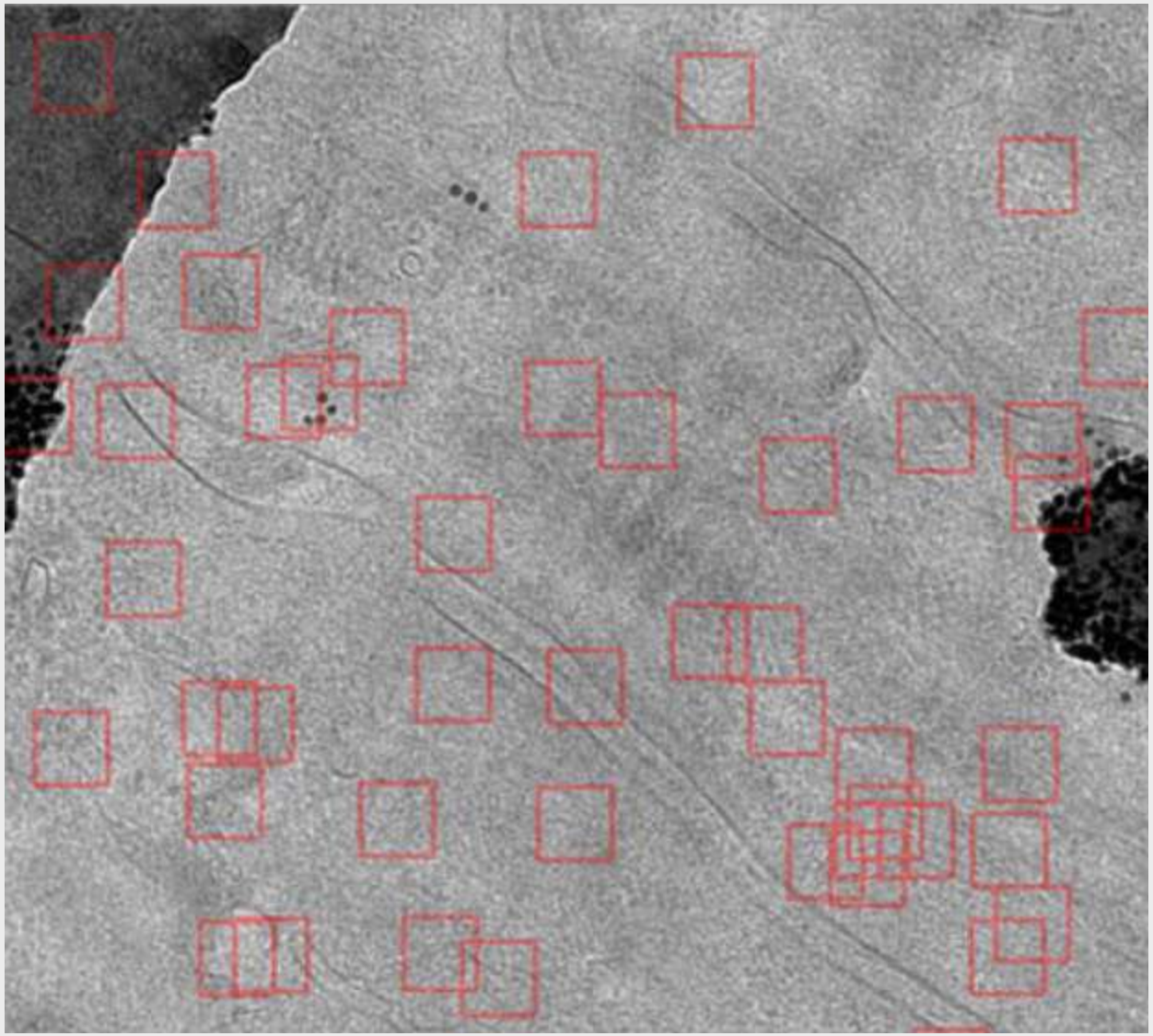


(UMET)

Fiducials are in 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.

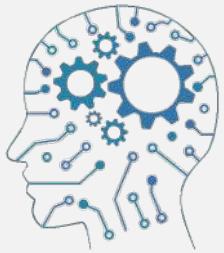
Patch tracking tilt-series alignment



Identify featureful objects with contrast in all tilt images and track them.

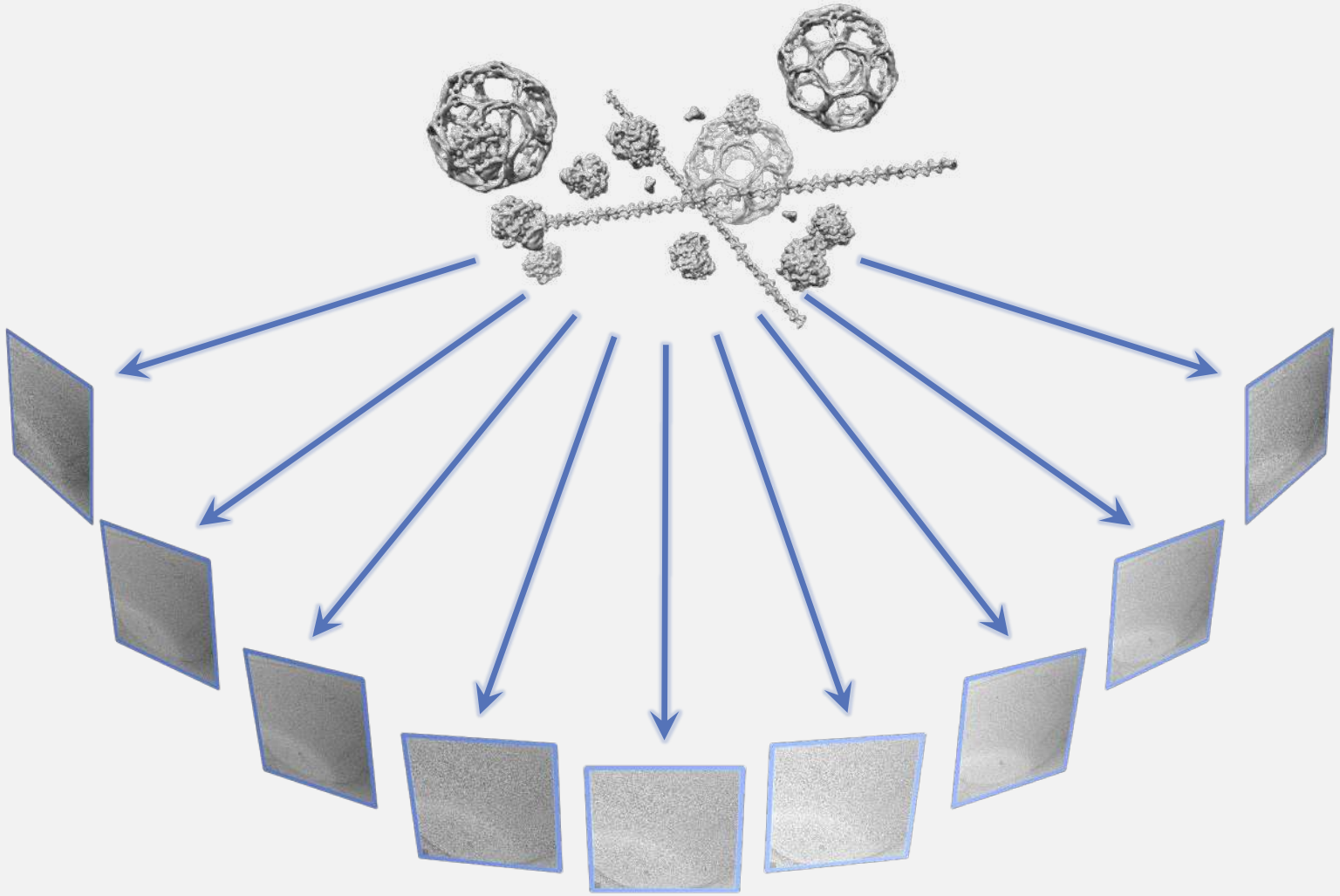
- Semi-automated (IMOD, Alignator)

Castaño-Díez, 2010

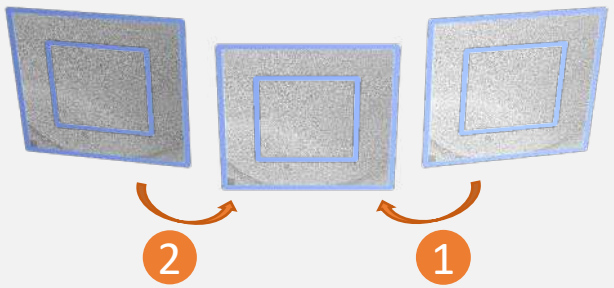


How does fiducial-less alignment in Protomo work?

Collect a tilt-series

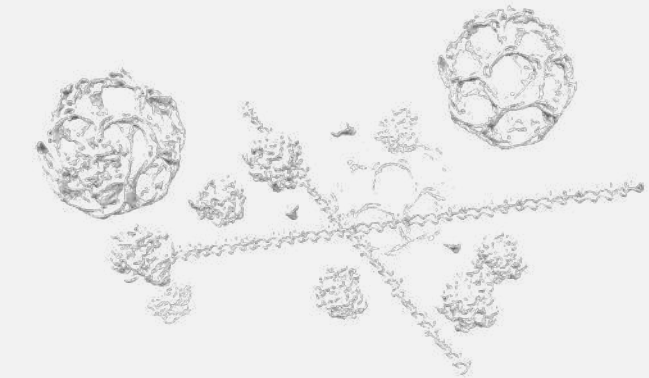


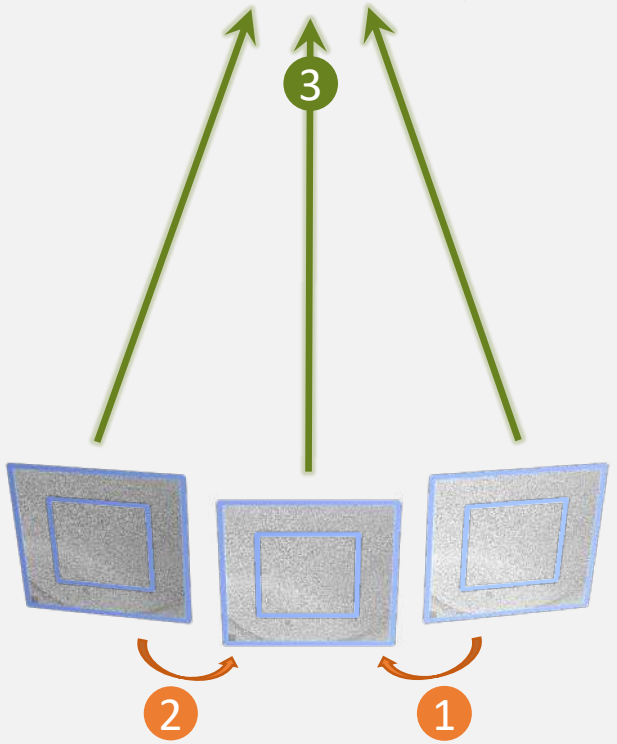
Protomo alignment



Nearest-neighbor correlation

Protomo alignment

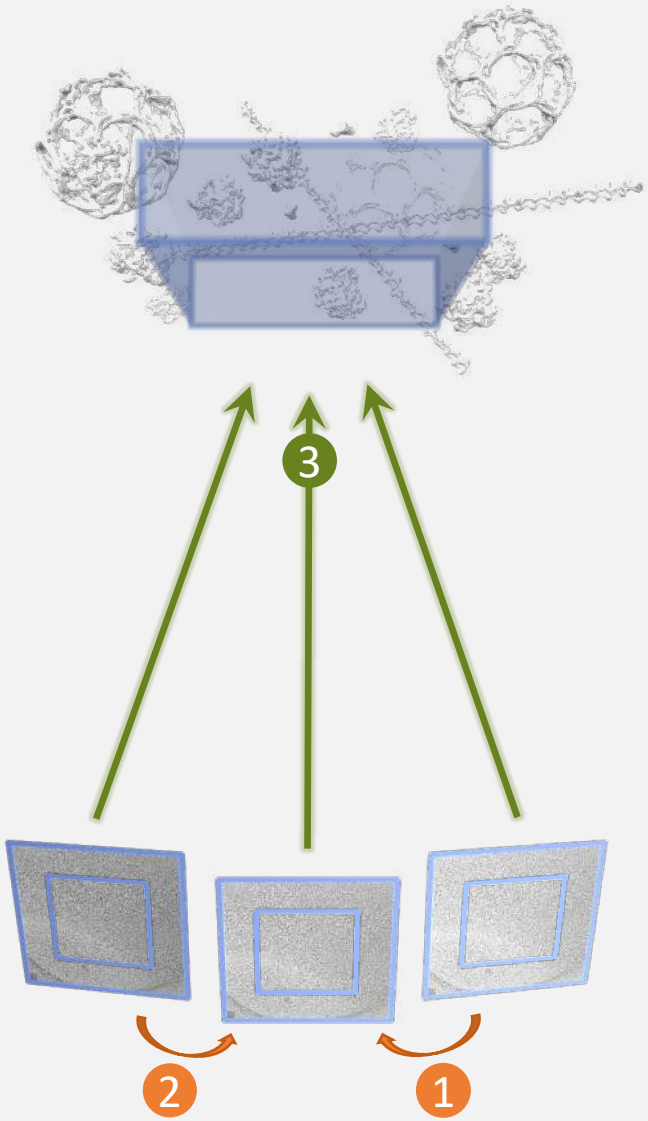




Nearest-neighbor correlation

Weighted back-projection

Protomo alignment



Nearest-neighbor correlation

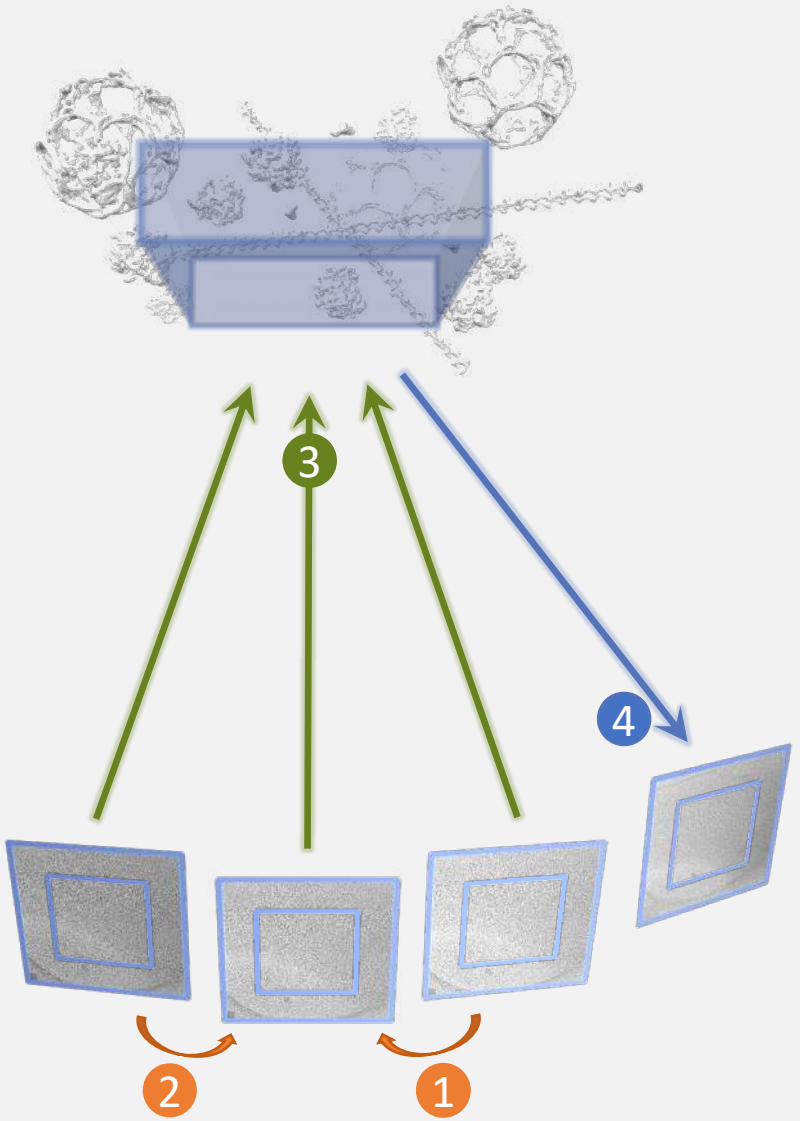
Weighted back-projection

alignment thickness = z

x y

Volume to be re-projected

Protomo alignment



Nearest-neighbor correlation

Weighted back-projection

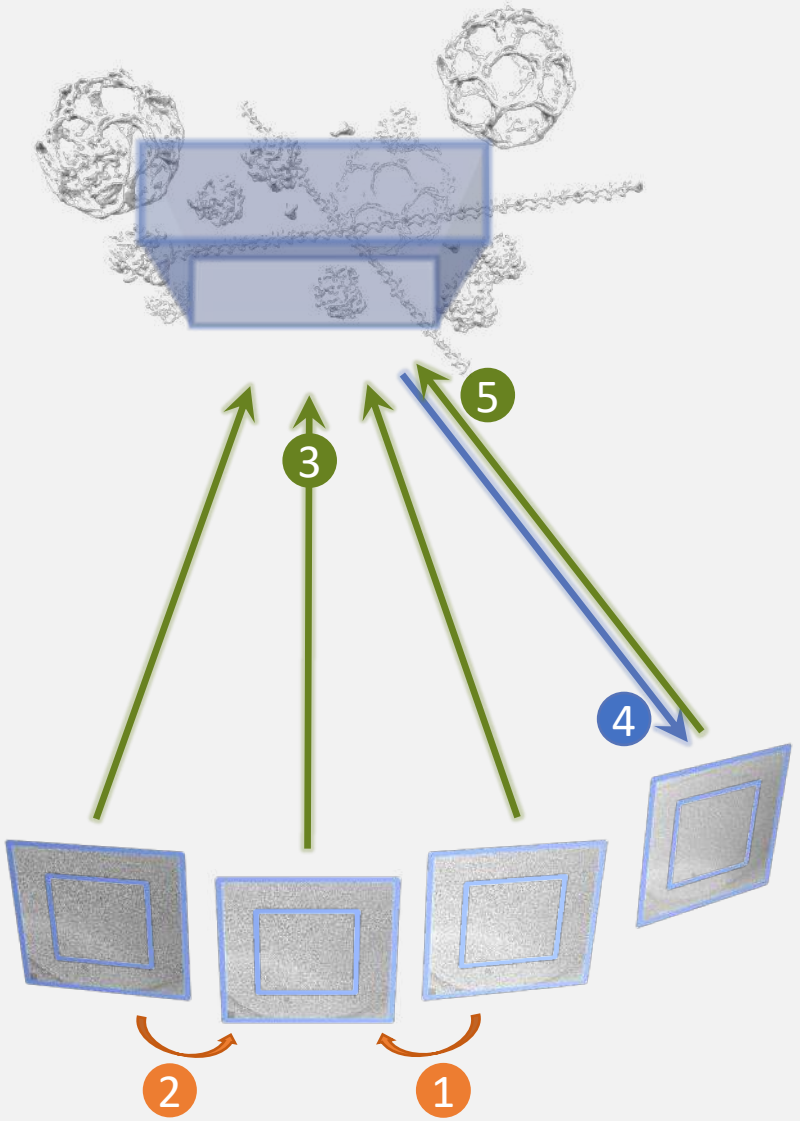
alignment thickness = z

x y

Volume to be re-projected

Re-projection ⟶ correlation

Protomo alignment



Nearest-neighbor correlation

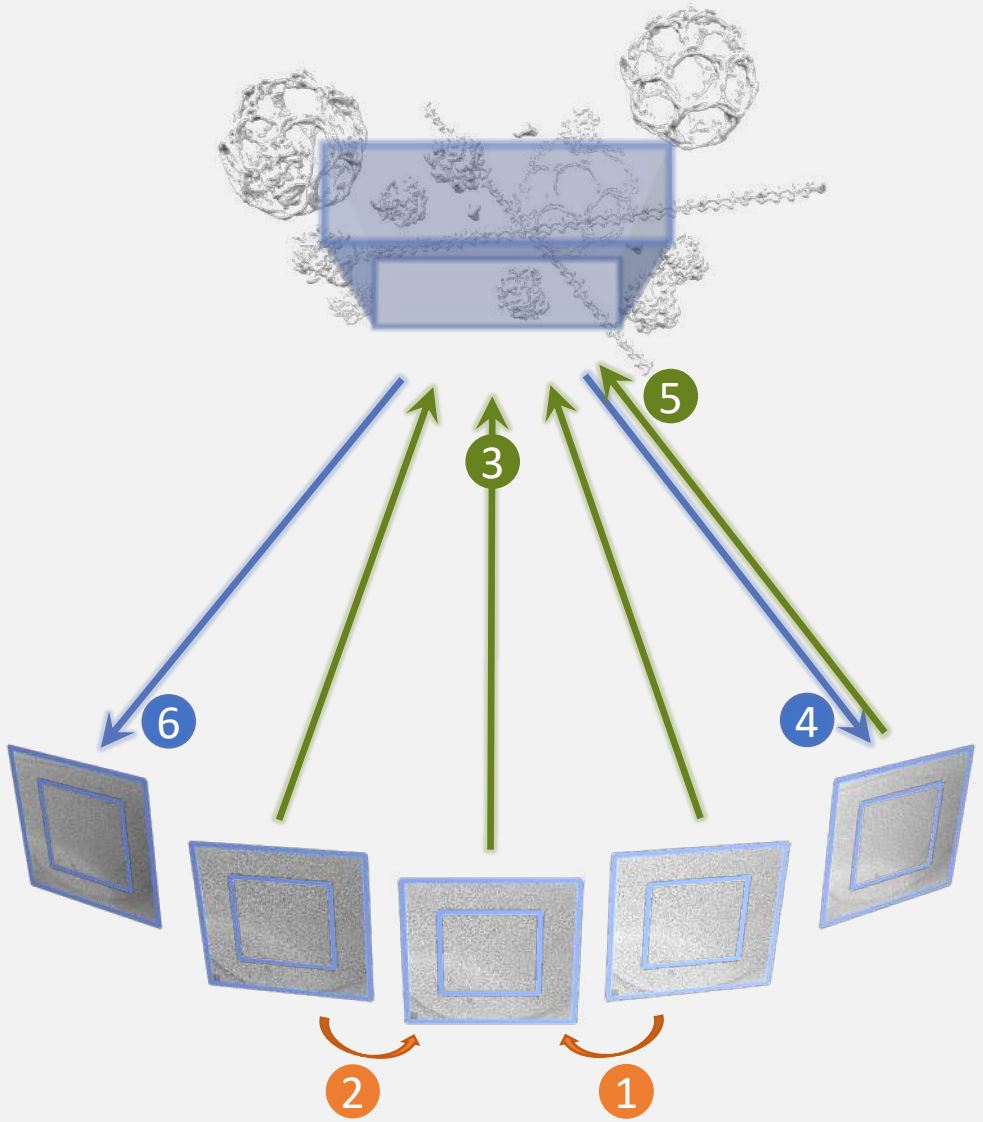
Weighted back-projection

alignment thickness = z

Volume to be re-projected

Re-projection ⟶ correlation

Protomo alignment



Nearest-neighbor correlation

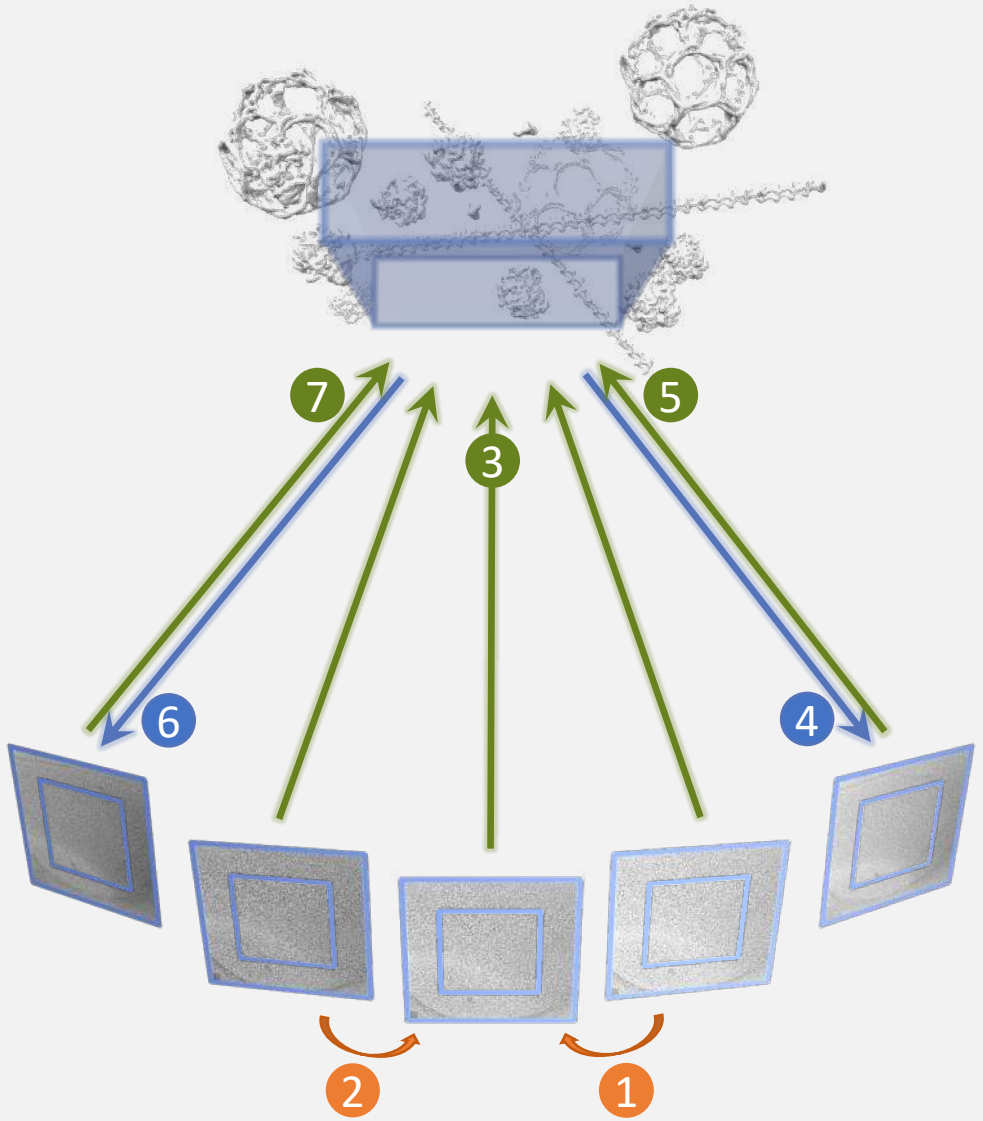
Weighted back-projection

alignment thickness = z, x, y

Volume to be re-projected

Re-projection ⟶ correlation

Protomo alignment



Nearest-neighbor correlation

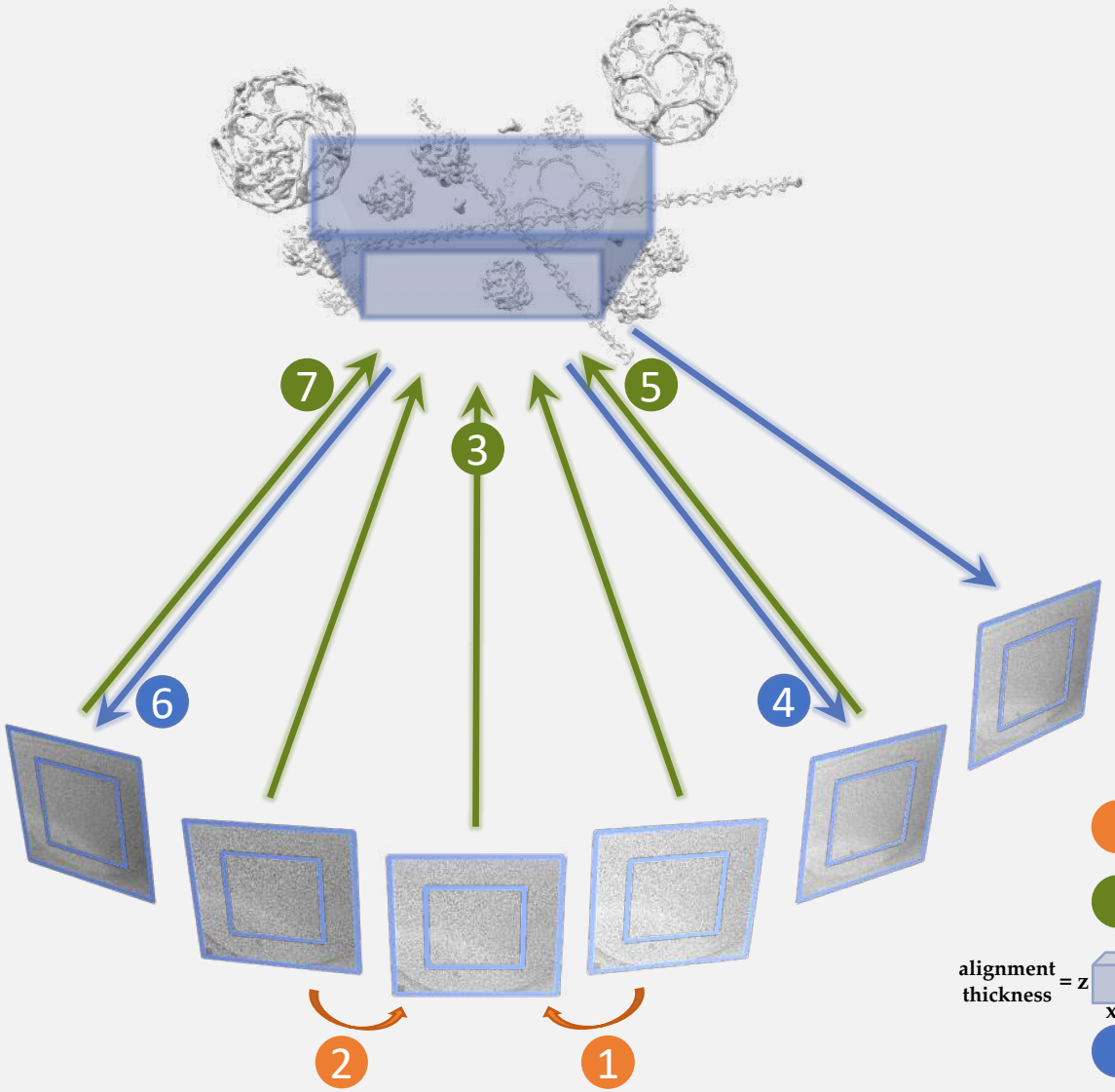
Weighted back-projection

alignment thickness = z, x, y

Volume to be re-projected

Re-projection ⟶ correlation

Protomo alignment



Nearest-neighbor correlation

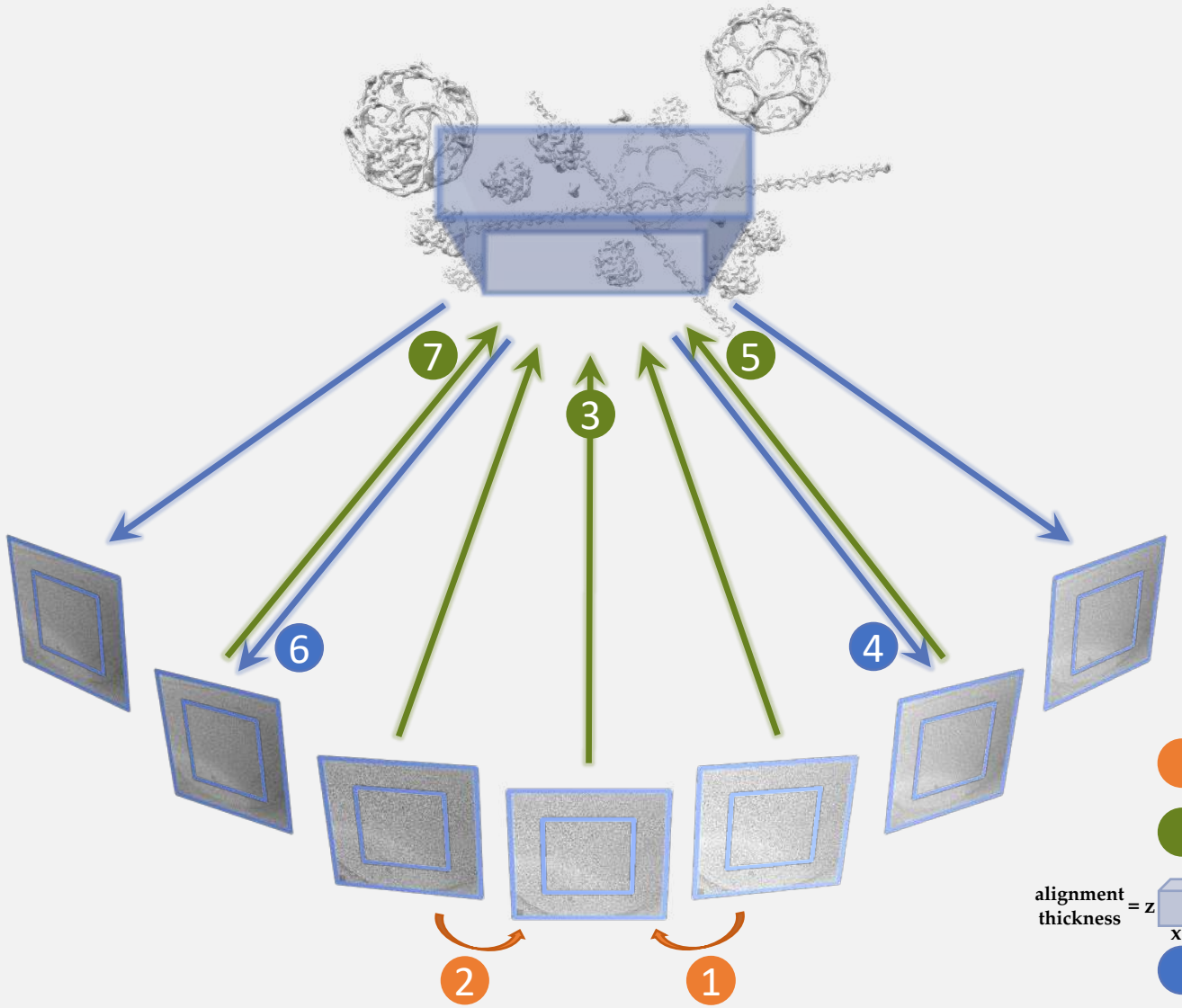
Weighted back-projection

alignment thickness = z, x, y

Volume to be re-projected

Re-projection ⟶ correlation

Protomo alignment



Nearest-neighbor correlation

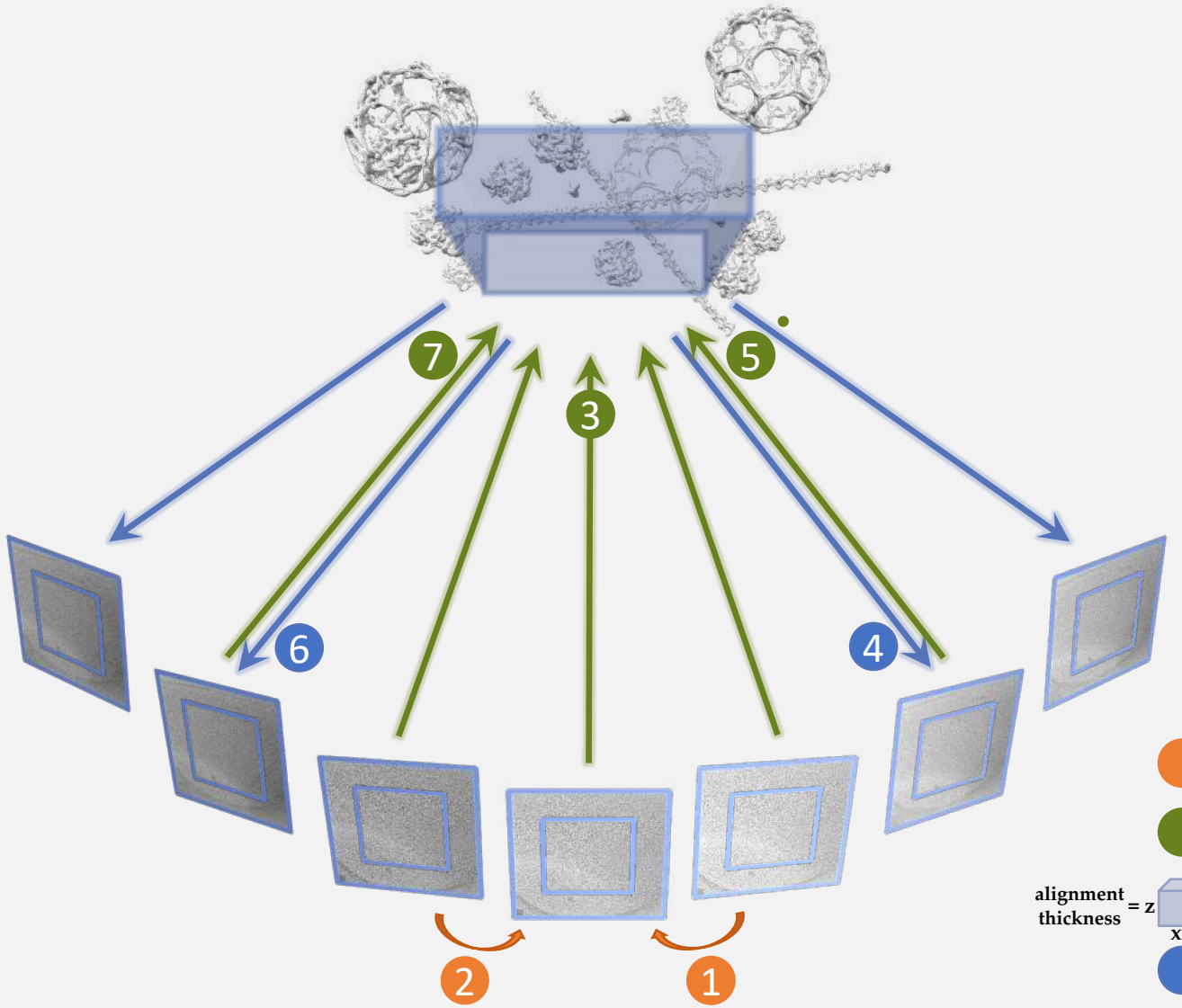
Weighted back-projection

alignment thickness = z, x, y

Volume to be re-projected

Re-projection ⟶ correlation

Protomo alignment



Nearest-neighbor correlation

Weighted back-projection

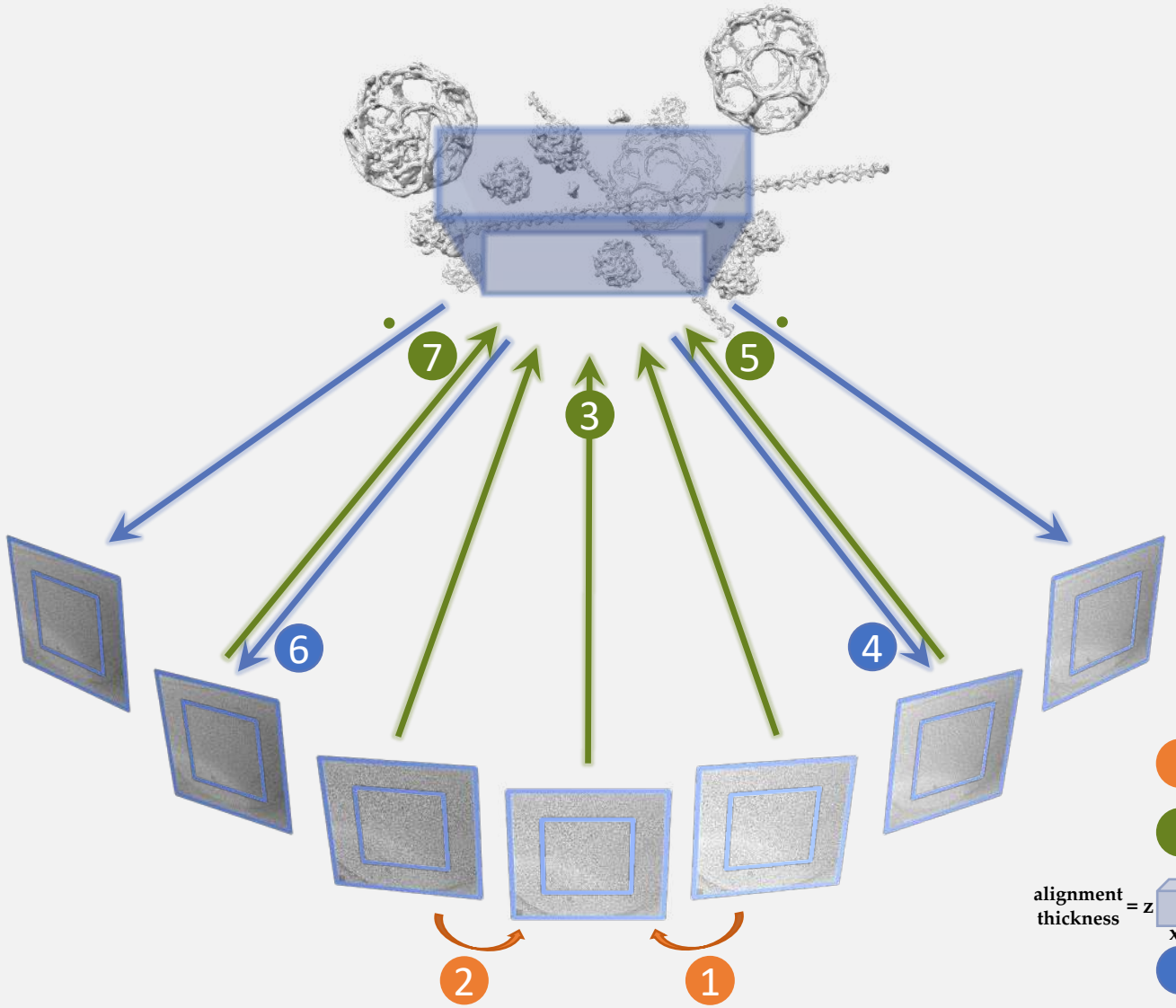
alignment thickness = z

x y

Volume to be re-projected

Re-projection ⟶ correlation

Protomo alignment



Nearest-neighbor correlation

Weighted back-projection

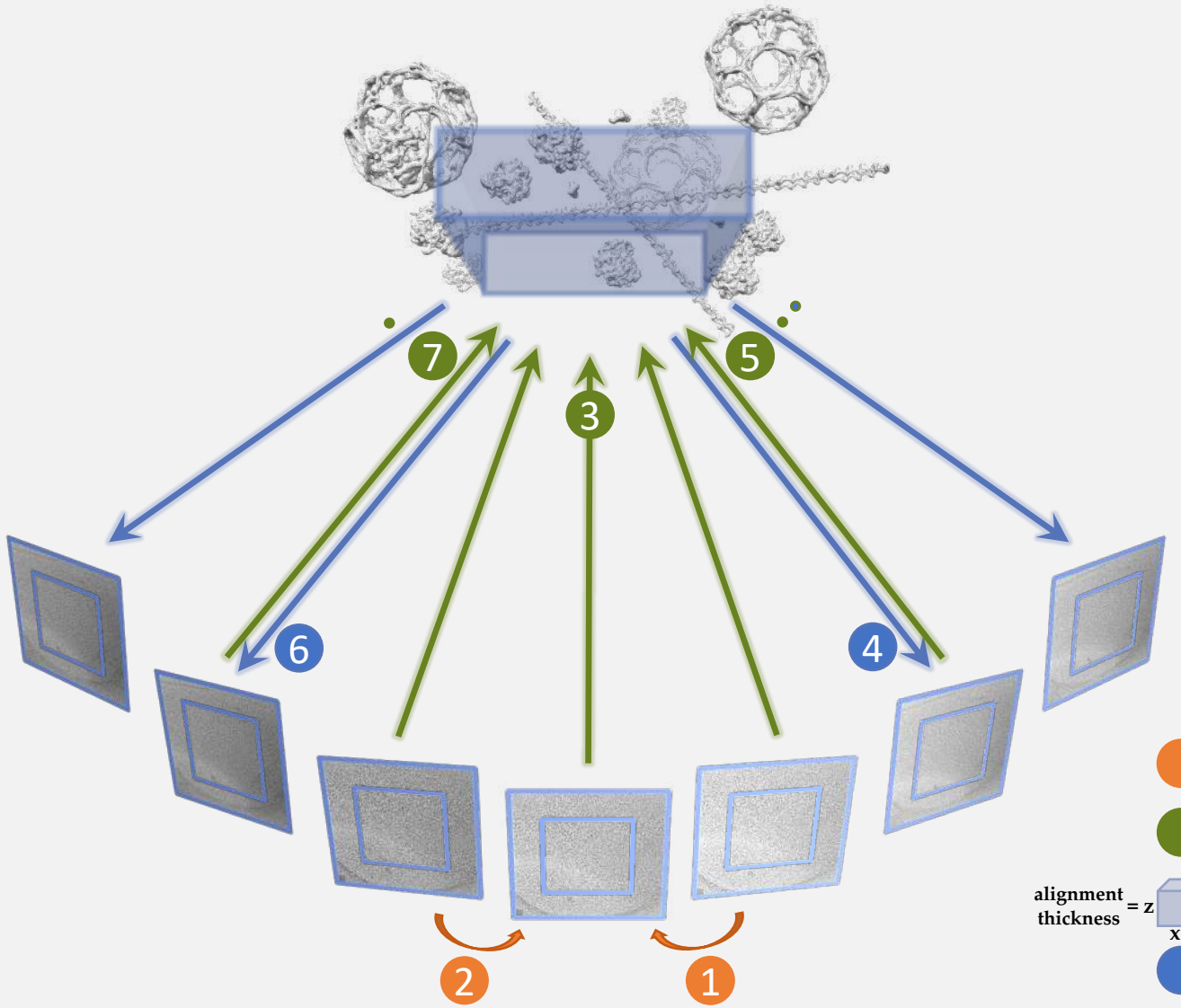
alignment thickness = z

x y

Volume to be re-projected

Re-projection ⟶ correlation

Protomo alignment



Nearest-neighbor correlation

Weighted back-projection

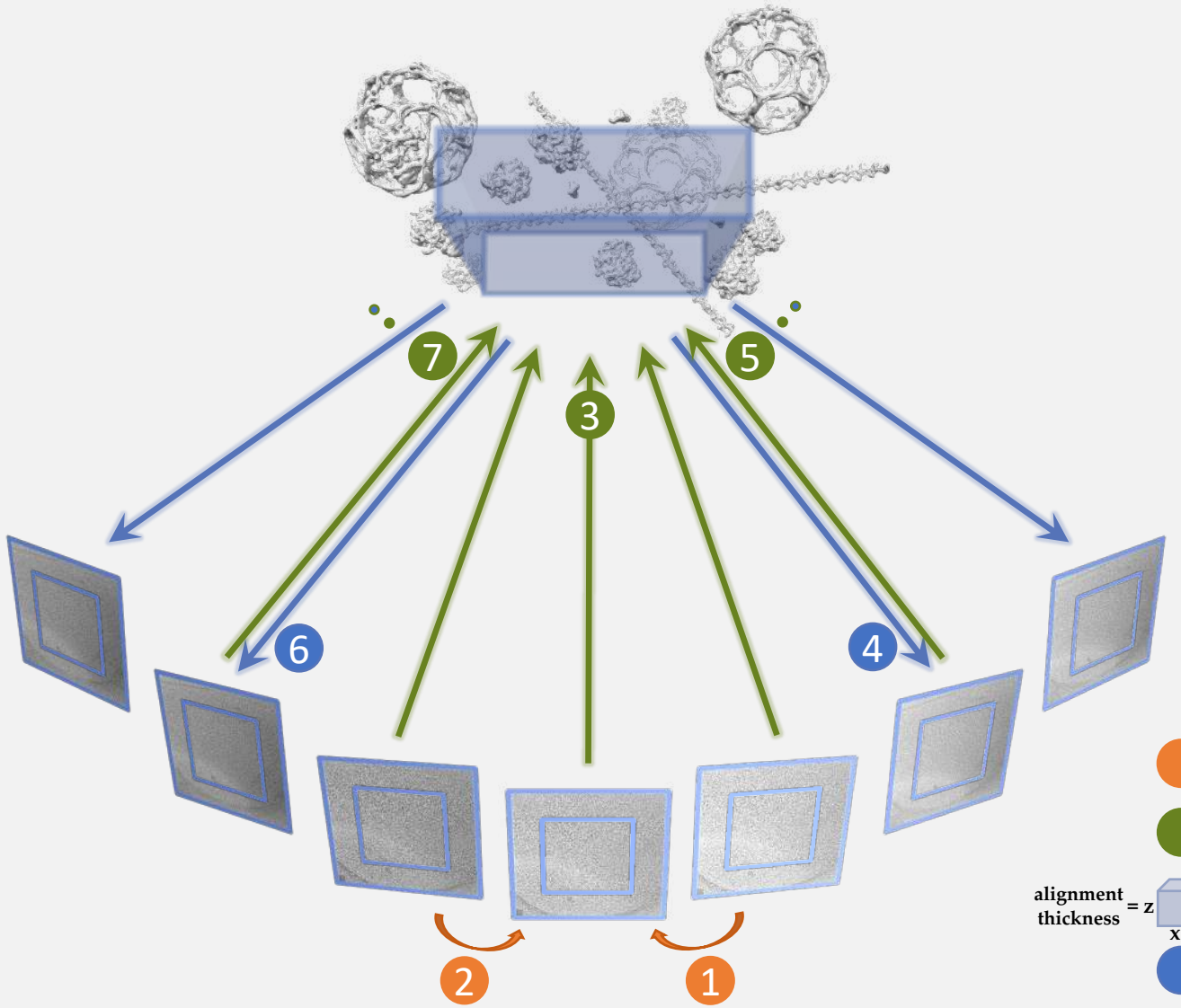
alignment thickness = z

x y

Volume to be re-projected

Re-projection ⟶ correlation

Protomo alignment



Nearest-neighbor correlation

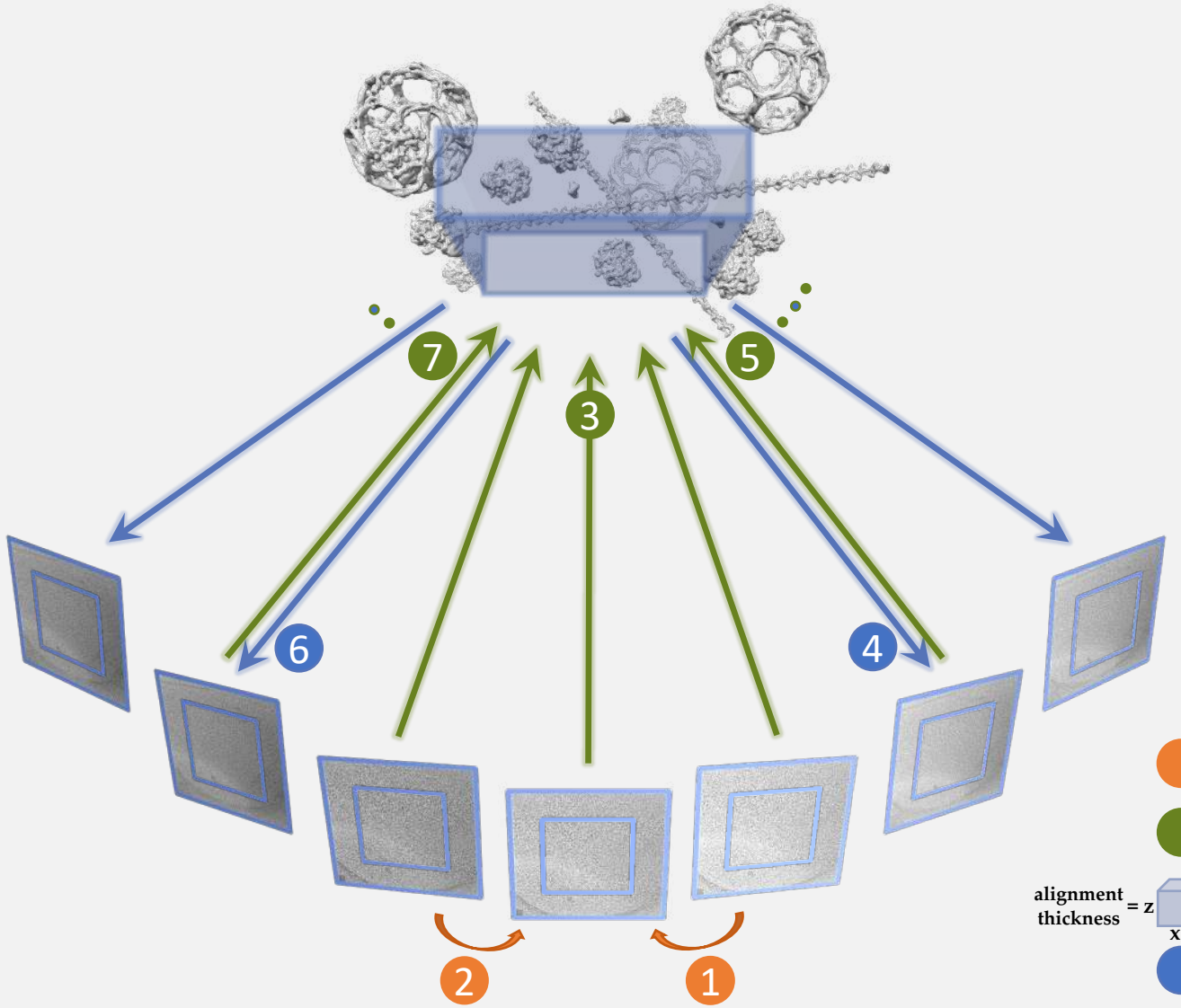
Weighted back-projection

alignment thickness = z

Volume to be re-projected

Re-projection ⟶ correlation

Protomo alignment



Nearest-neighbor correlation

Weighted back-projection

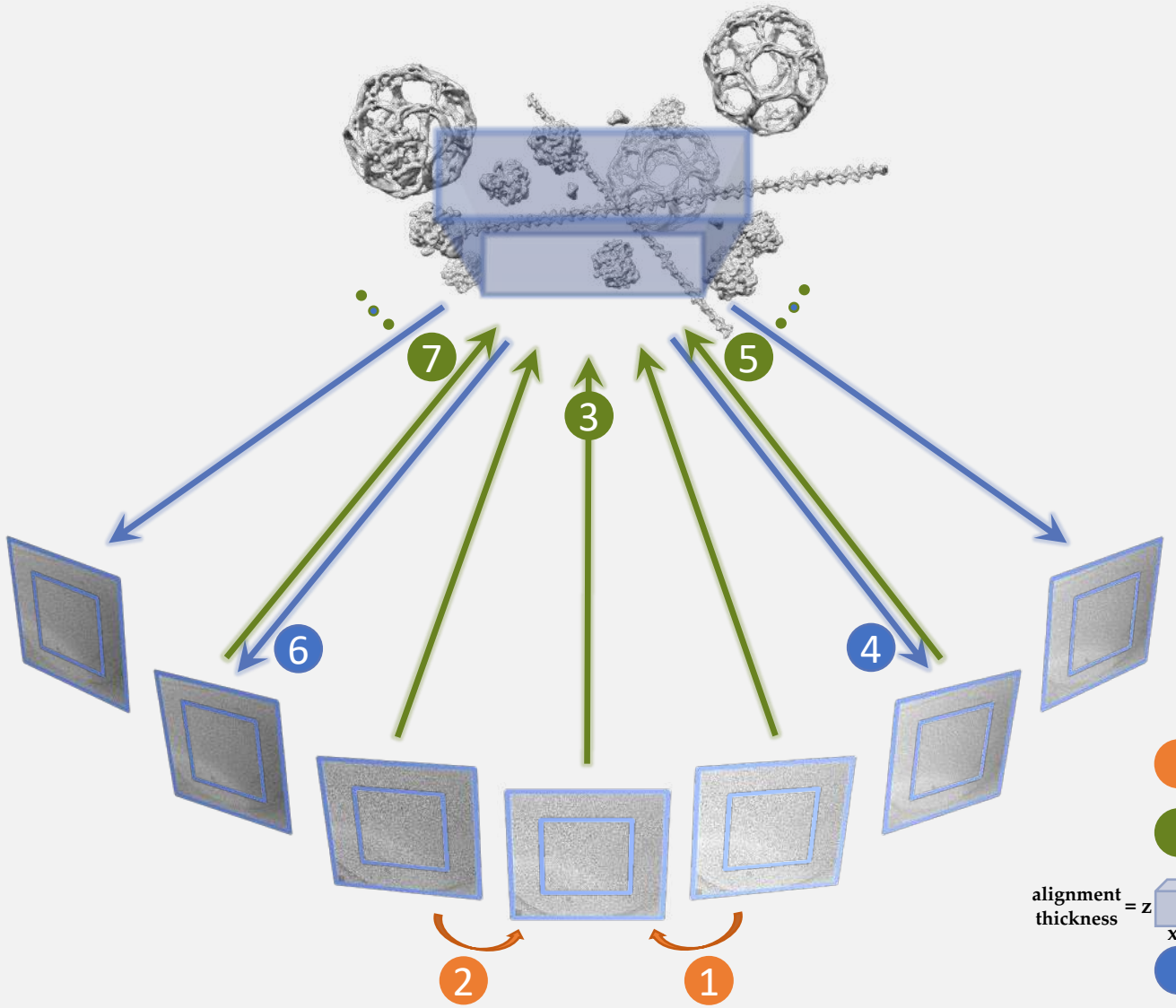
alignment thickness = z

x y

Volume to be re-projected

Re-projection ⟶ correlation

Protomo alignment



Nearest-neighbor correlation

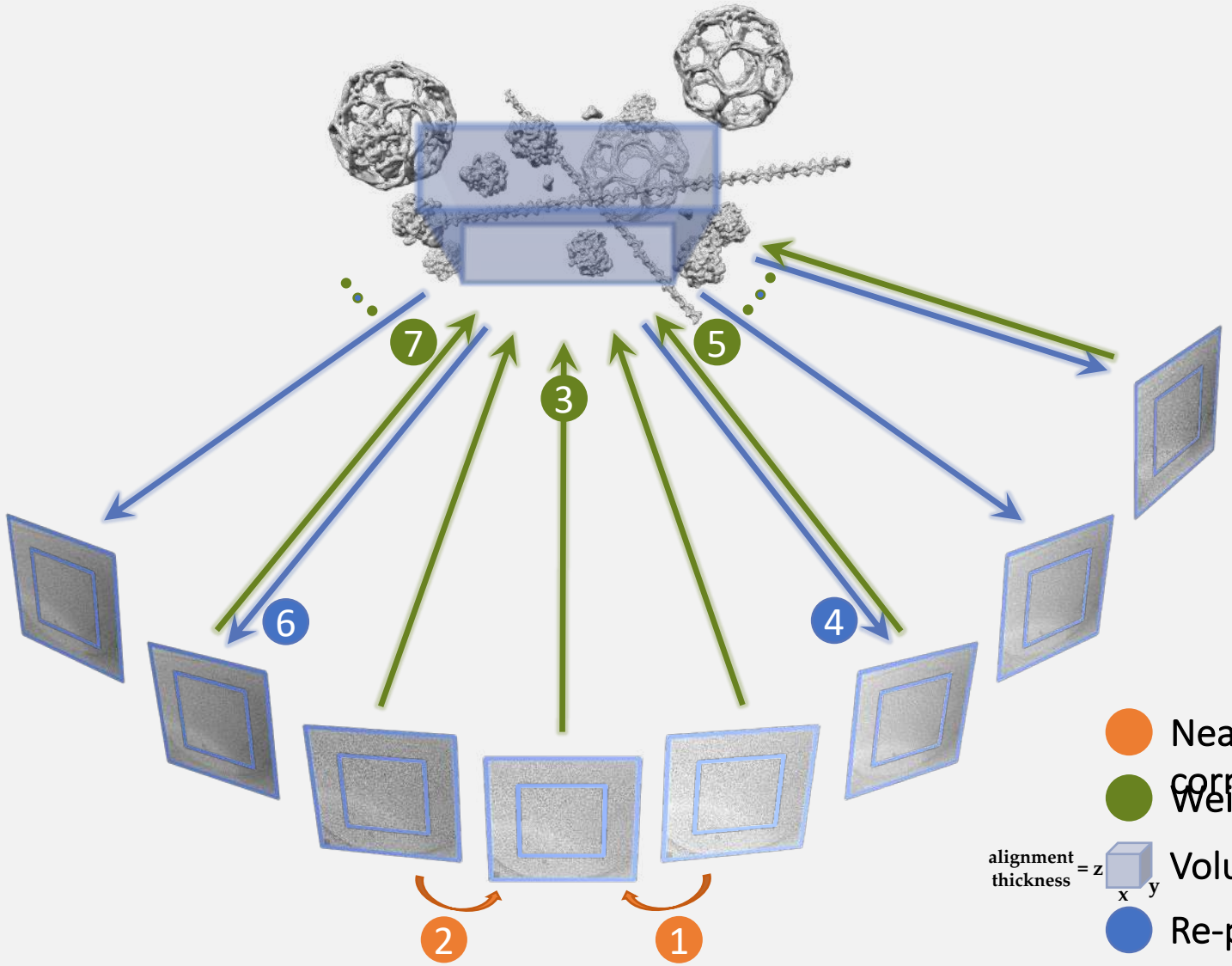
Weighted back-projection

alignment thickness = z

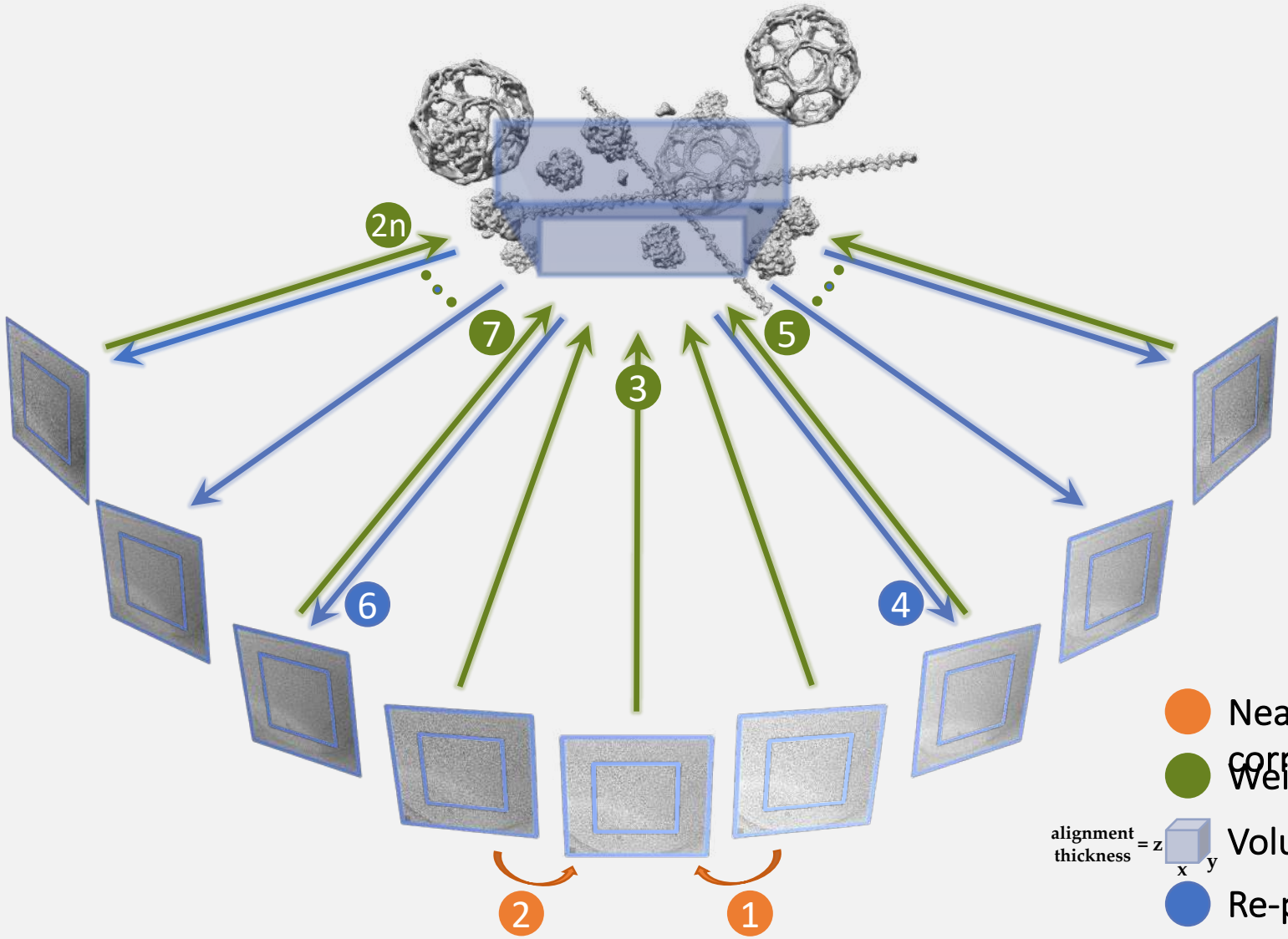
Volume to be re-projected

Re-projection ⟶ correlation

Protomo alignment



Protomo alignment



Nearest-neighbor correlation

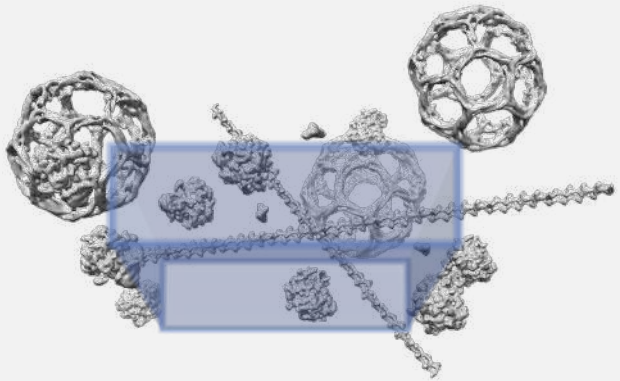
Weighted back-projection

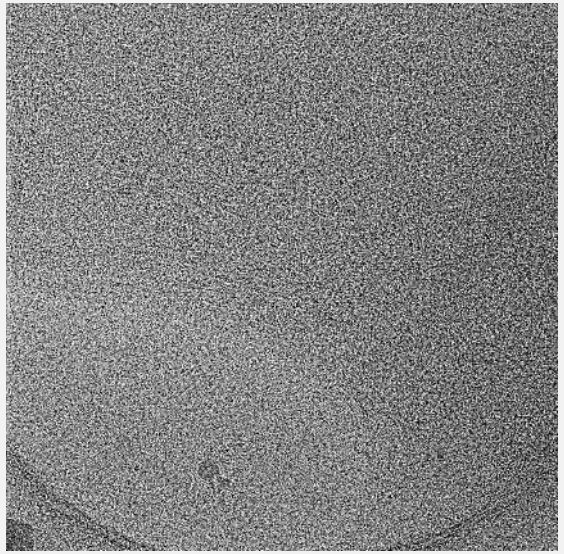
alignment thickness = z

Volume to be re-projected

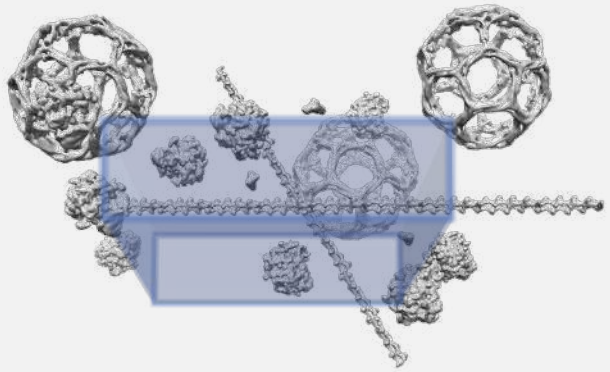
Re-projection ⟶ correlation

Protomo alignment

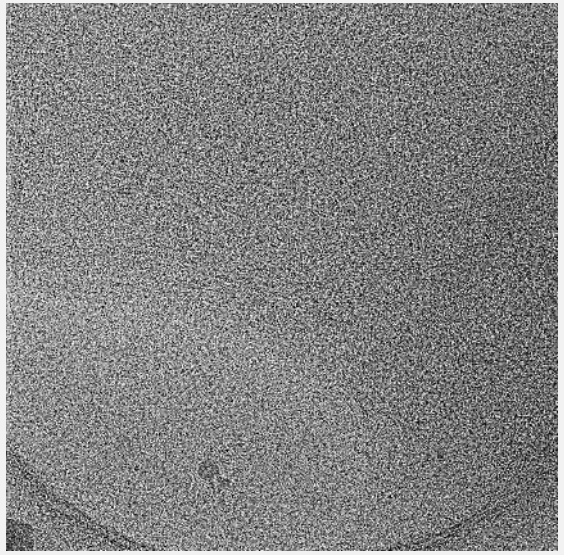




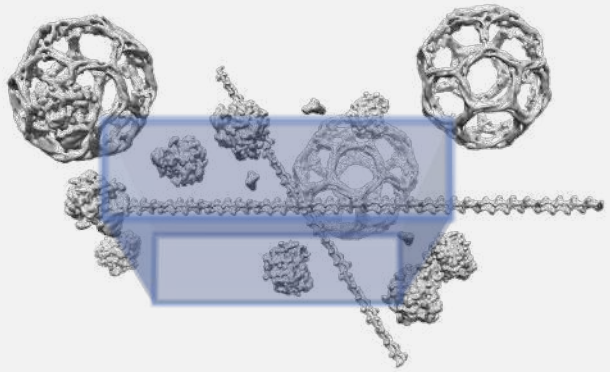
Protomo alignment

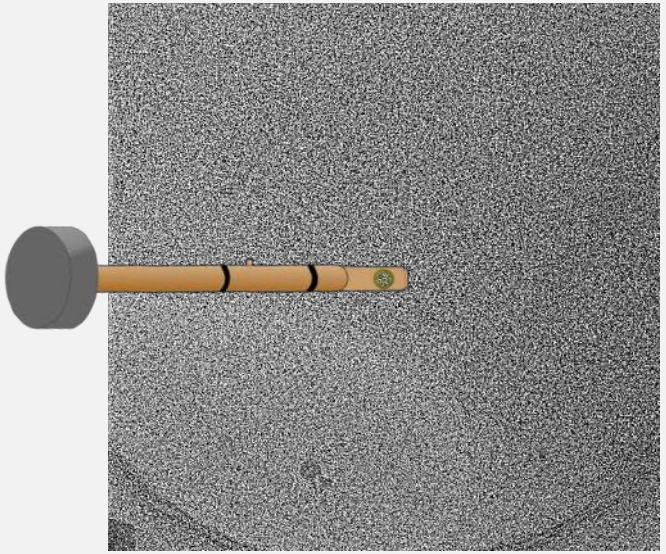


Refine orientations of objects

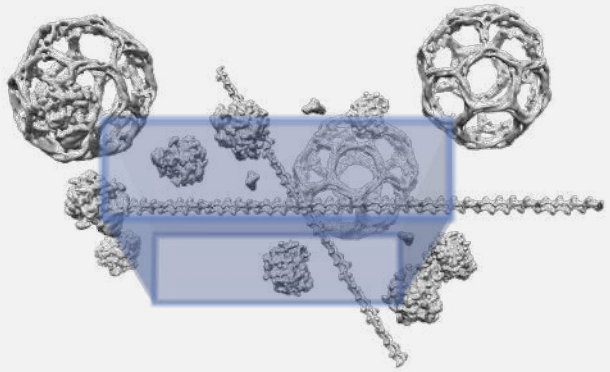


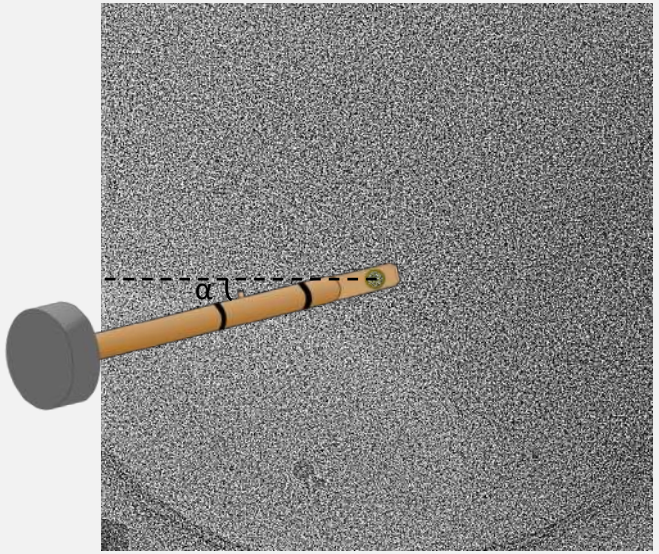
Protomo alignment





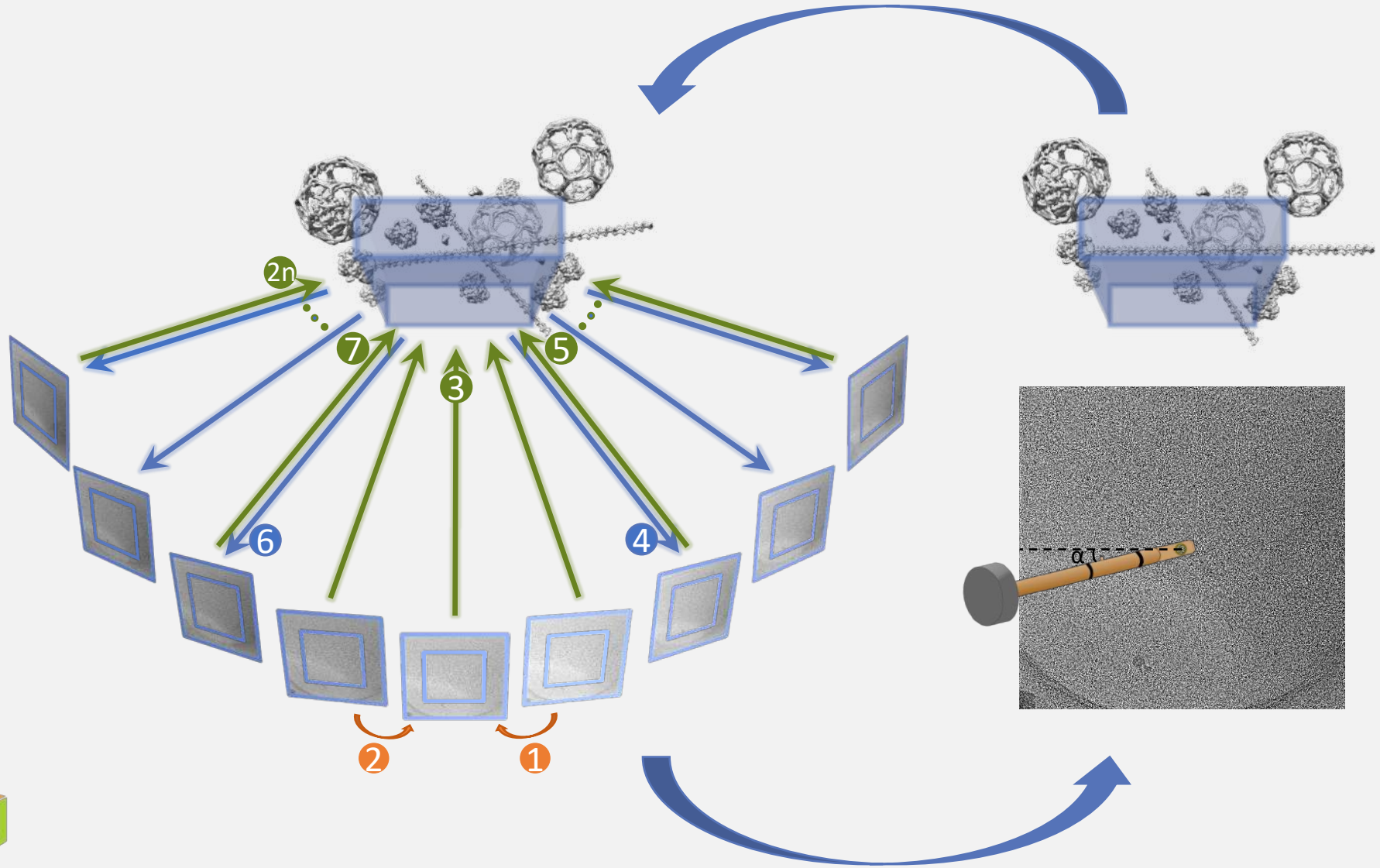
Protomo alignment





Refine tilt azimuth

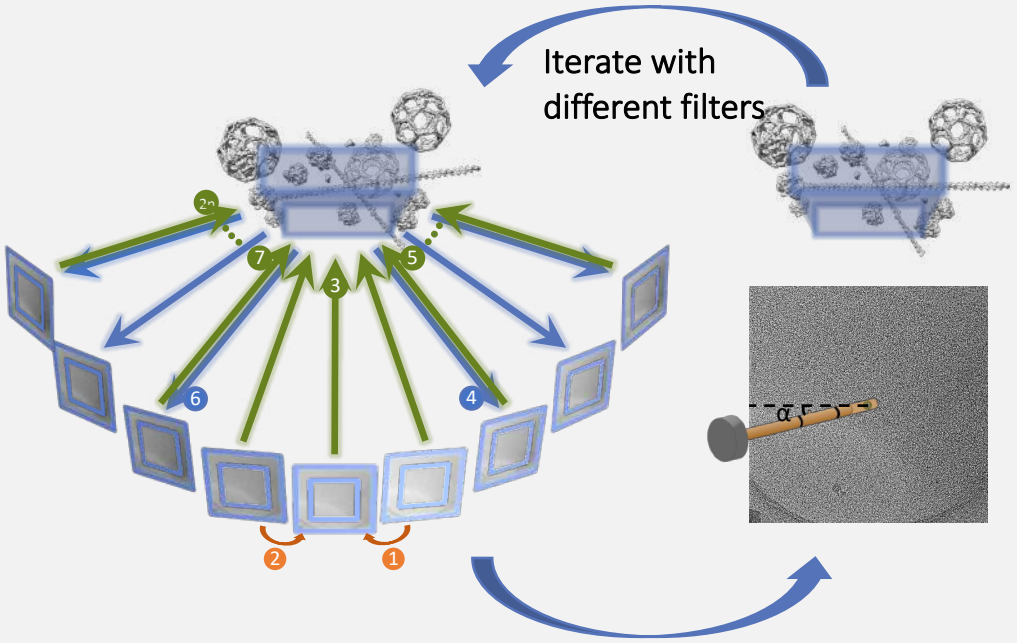
Appion-Protomo refinement



Iterate with
different filters

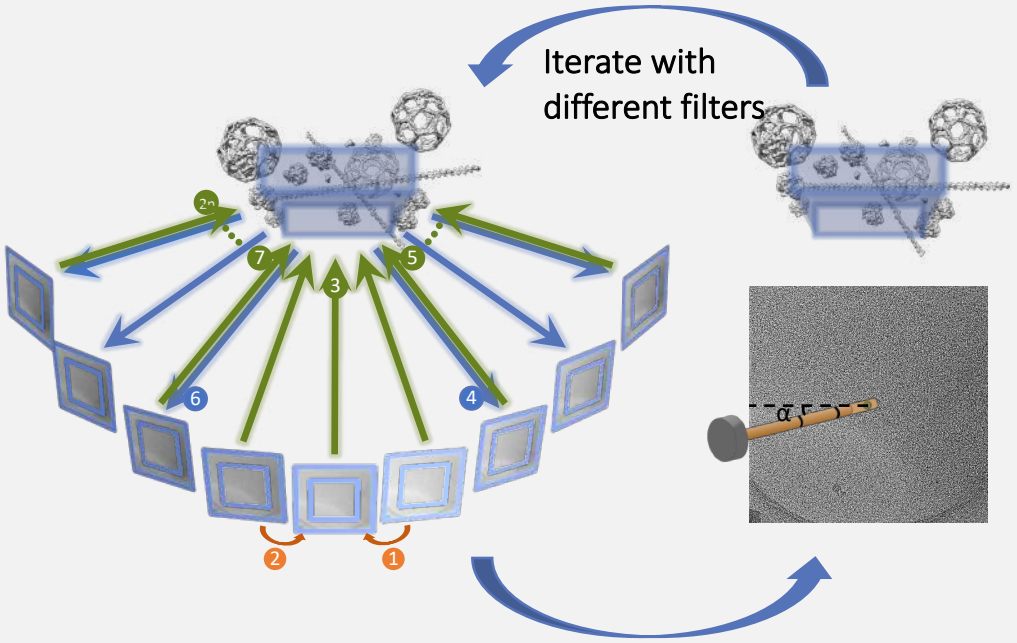
Appion-Protomo refinement

Why is this important?

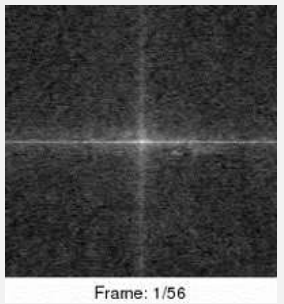


Appion-Protomo refinement

Why is this important?

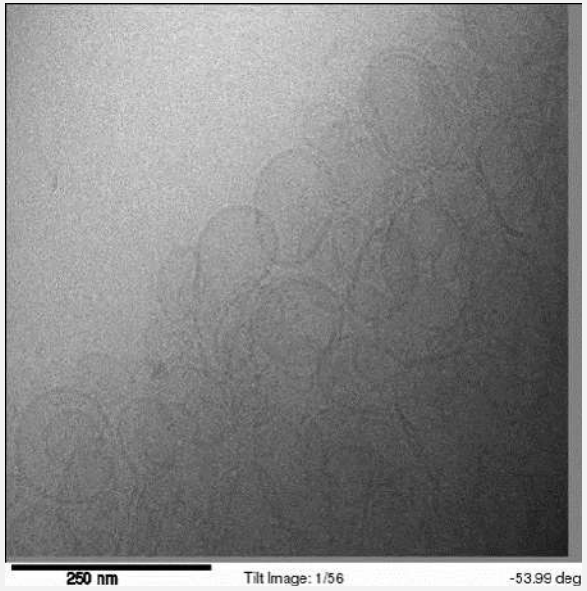


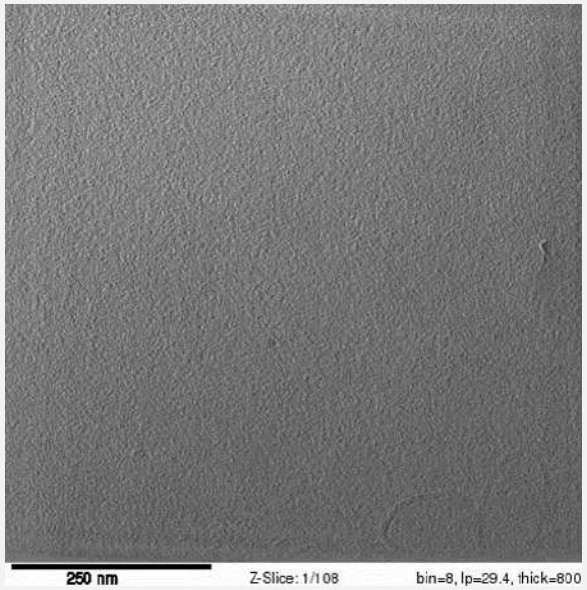
Nearest-neighbor alignment



After refinement

After refinement

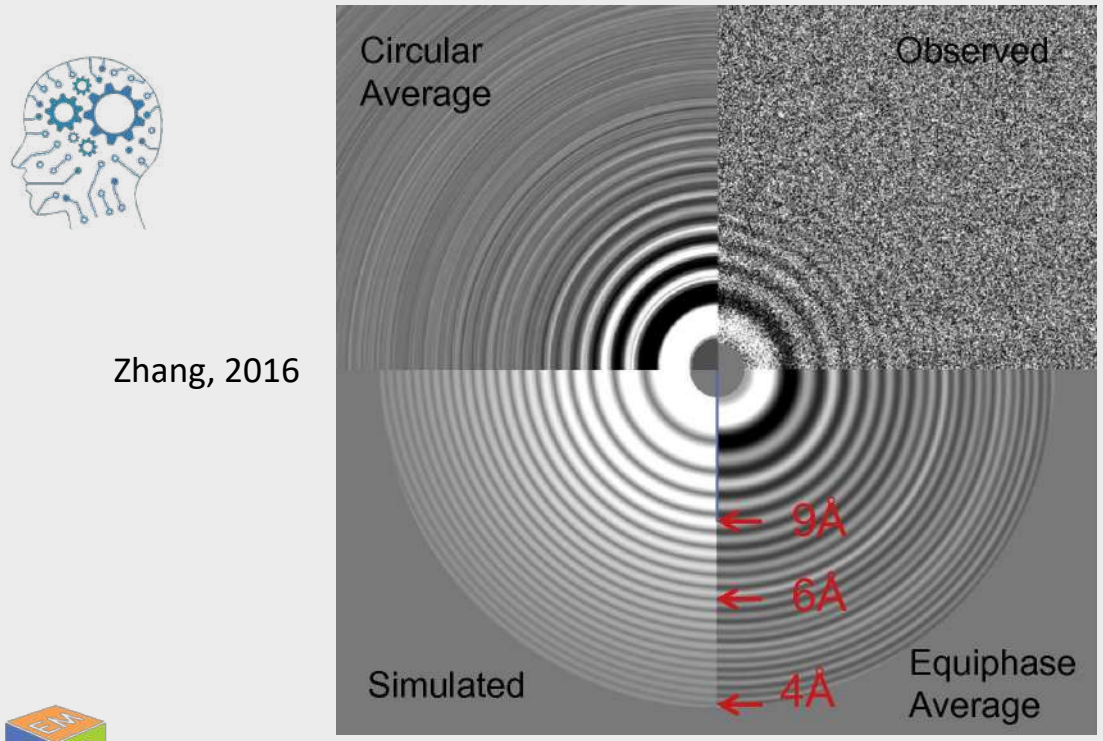




Defocus estimation

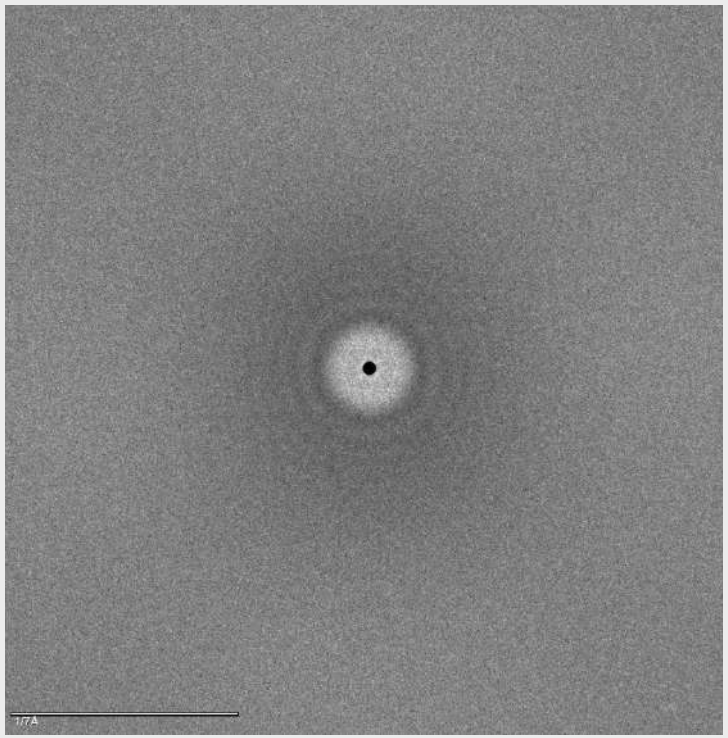
Goal: Find the **height of your objects** of interest to correct for microscope aberrations (CTF)

Problem: **Low** per-image **SNR** and potential poor tracking



Zhang, 2016

High dose single particle image



3 e^{-}/Å^2 single particle tilt image

CTF estimation and correction for tilt-series or tomograms

Defocus estimation methods

Methods ordered approximately **worst-to-best** (depends on sample):

- **Per-image** defocus estimation accounting for tilts (CTFFIND4, GCTF, etc.)
- Per-tomogram post-hoc estimation by using **SPT FSC to locate the first CTF zero**
- **Image tiling** to estimate the **defocus of the untilted plane** (TomoCTF)

- Defocus estimation and **interpolation using two focus locations** on the tilt axis (Eibauer, 2012)
- Per-particle tilt image fine estimation and correction that accounts for the **3D location of each particle**
- Per-particle tilt image fine estimation and correction that takes into **account overlapping objects** in each tilt image of each particle and accounts for the 3D location of each particle – can use all particles in each tilt image to refine!

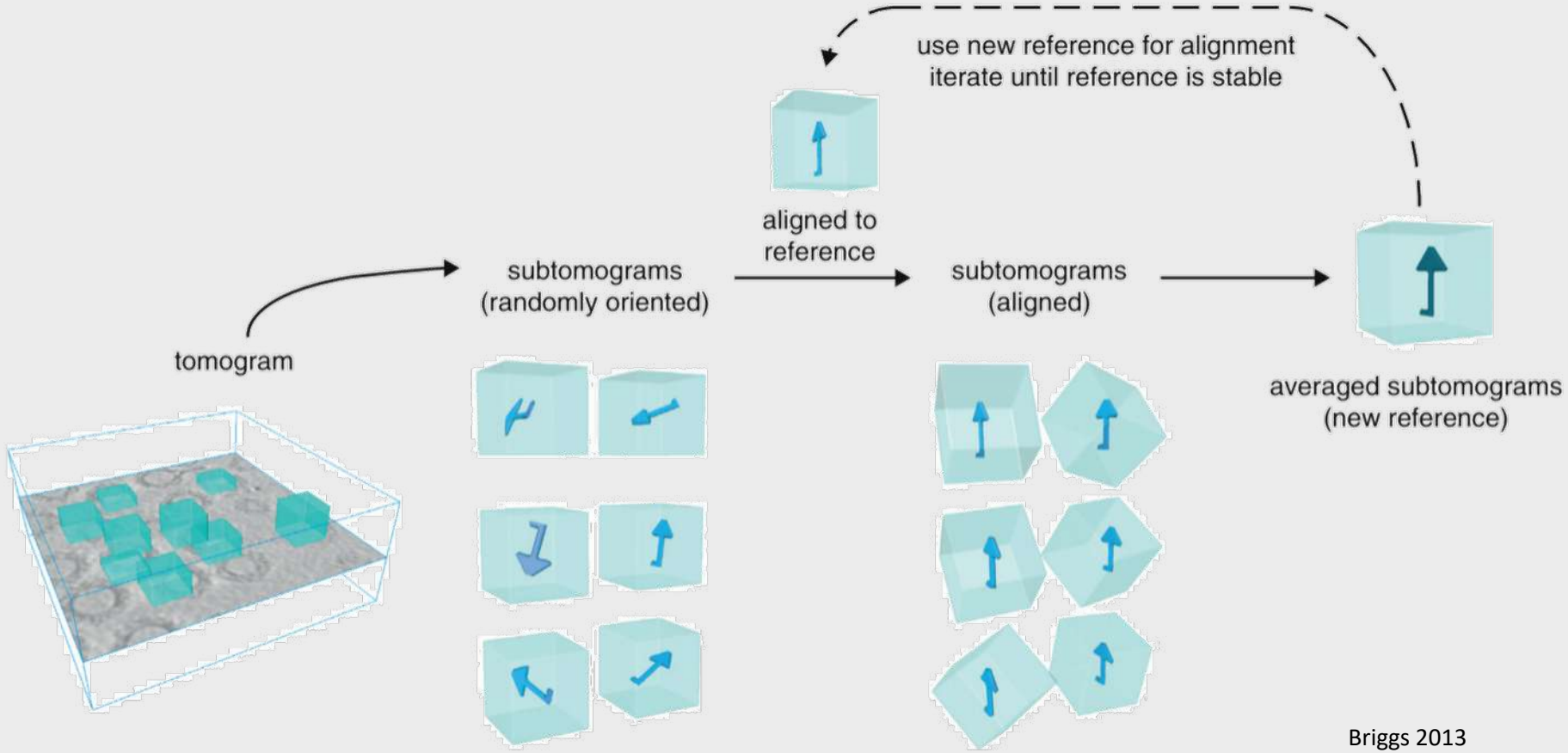
CTF correction methods

Methods ordered approximately **worst-to-best** (depends on sample):

- **Per-image** correction
- Strip-based correction with TomoCTF or IMOD ctfphaseflip
  - Flips phases and optionally corrects amplitudes (TomoCTF) on a strip-by-strip basis.
  - Error will depend on the amount of non-eucentricity
- 3D CTF model (Relion) takes into account x,y,z particle locations
- Per-particle/tiling CTF correction (EMAN2)
- During tomographic reconstruction (EmSART, NovaCTF)

Sub-tomogram processing

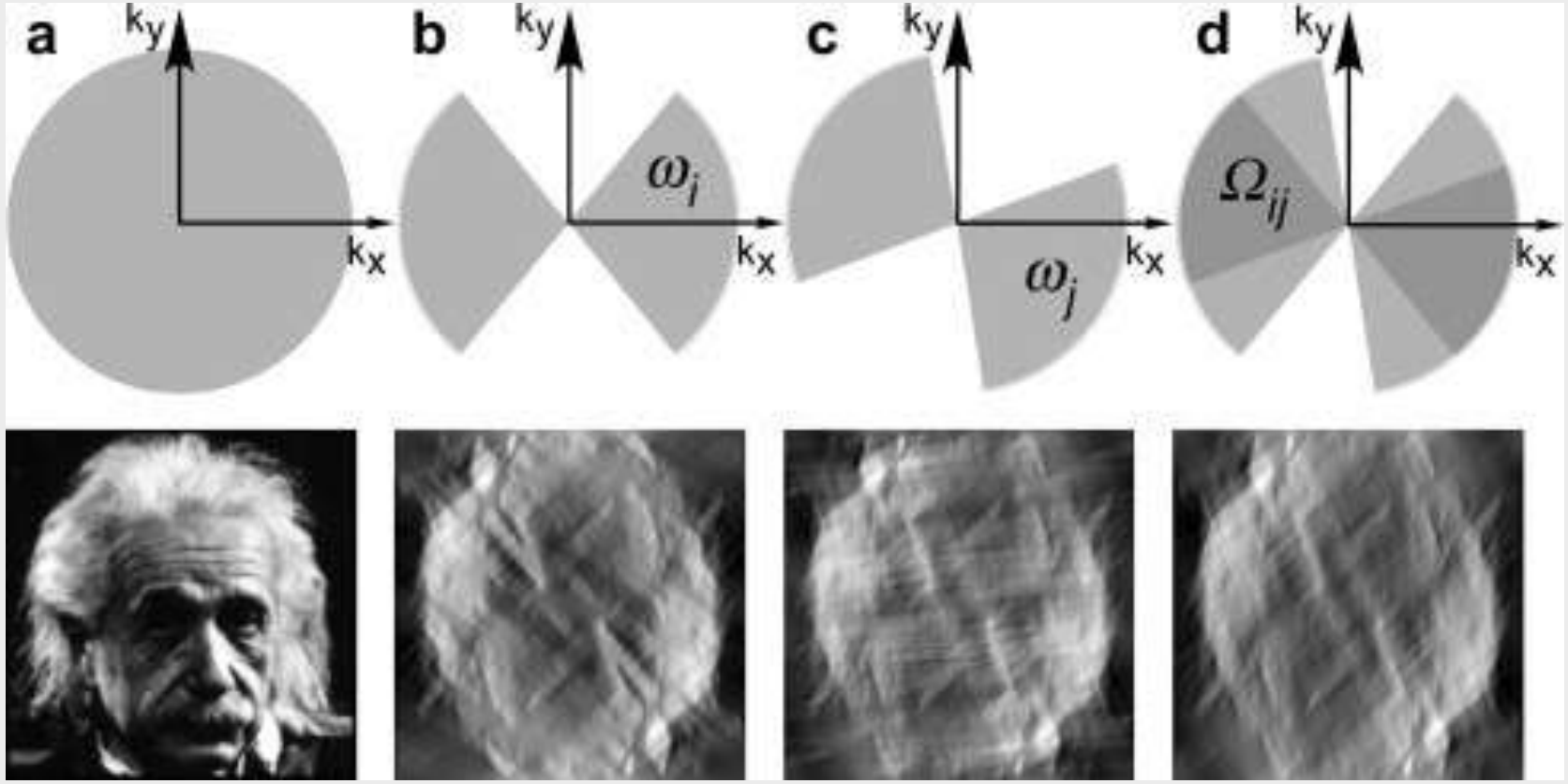
Sub-tomogram processing workflow



Briggs 2013

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

Must take into account subtomogram missing wedges



Forster et al, J. Struct. Biol, 2008

- Effectively align volume in common in Fourier space

Classification (in Dynamo)

- PCA + K-Means
  - Calculate **eigenvolumes** to reduce the dimensionality
  - **Separate** particles according to **eigenvalues**

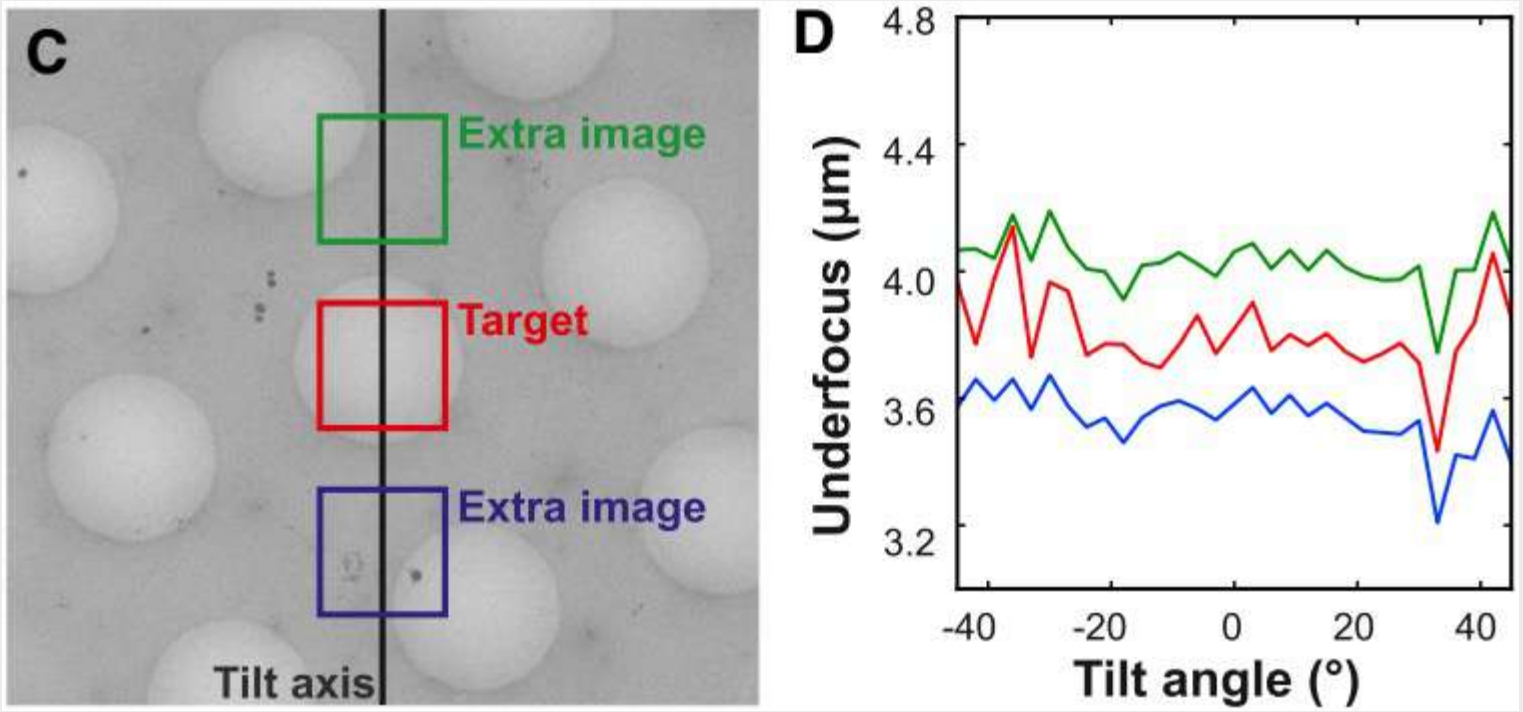
- MRA / ML
  - Generate **M seeds** and **align each particle** to each seed
  - **Iterate** till convergence
- Challenges
  - **May classify** direction of missing wedge, defocus, etc.
  - Computationally expensive

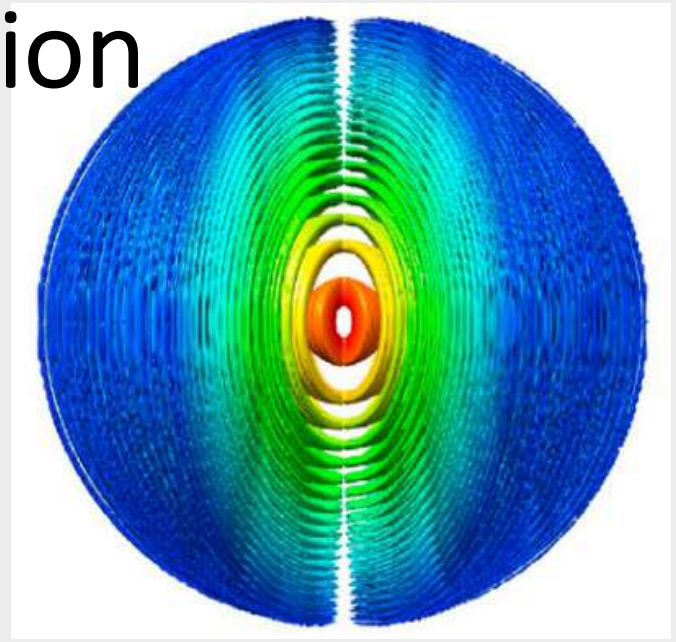
slide courtesy of
Misha Kudyashev

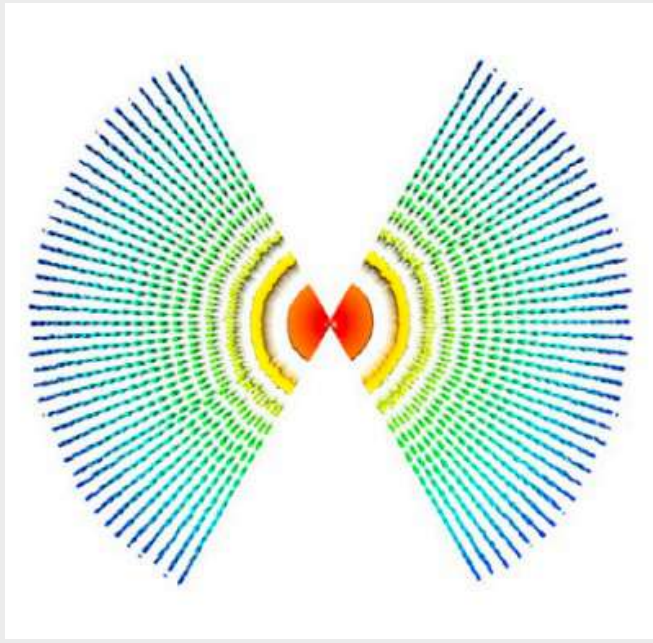
Sub-tomogram processing software

- Dynamo – GPU accelerated, tomogram database, extensive picking abilities
- Relion – 3D CTF model, Bayesian approach to alignment is used
- EMAN2 – Sub-tilt-series refinement and defocus estimation/correction
- emClarity – Sub-tilt-series refinement and defocus estimation/correction
- TYGRESS – Intended for use w/ high dose 0 degree image (Nicastro group)
- PyTom
- PEET
- Jsubtomo
- TOM & AV3
- XMIPP
- Warp

Sub-tomogram processing in Relion



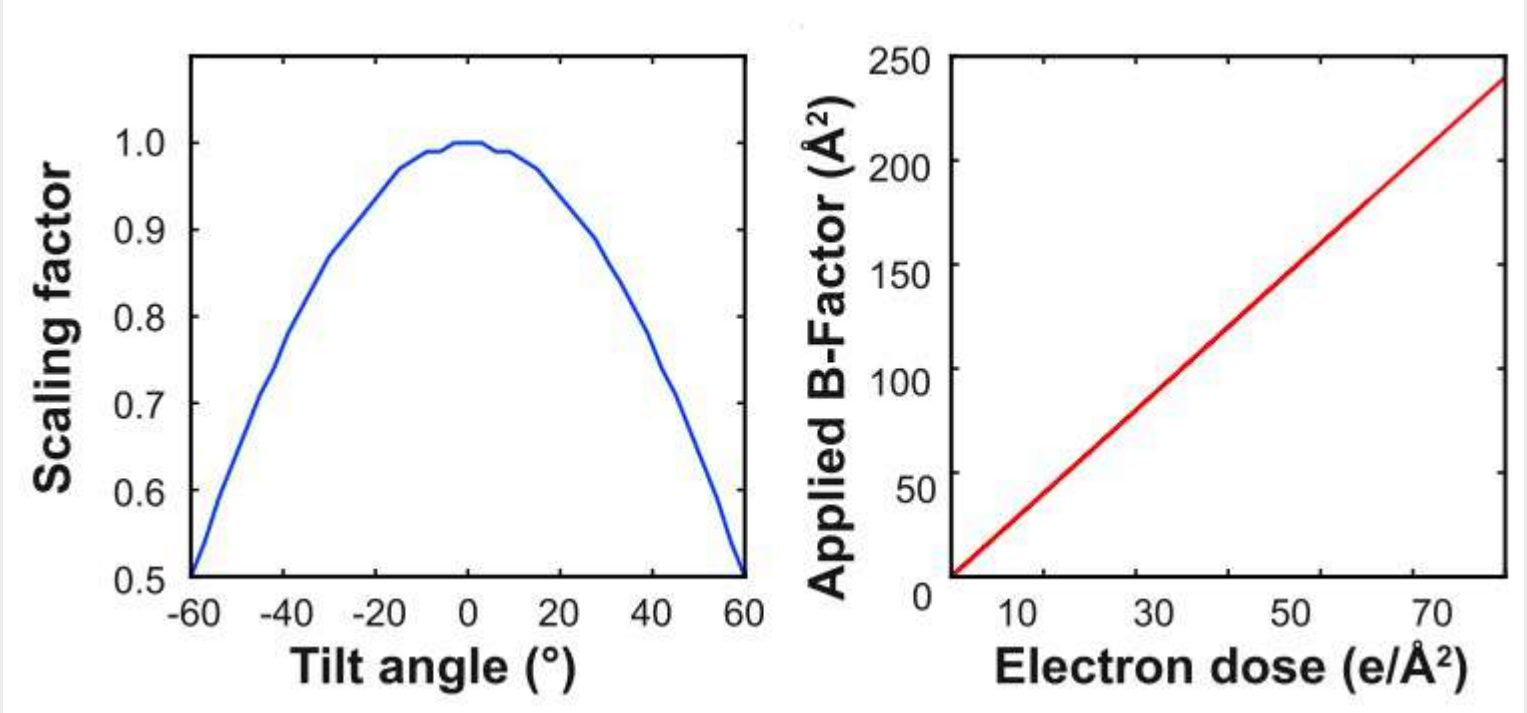


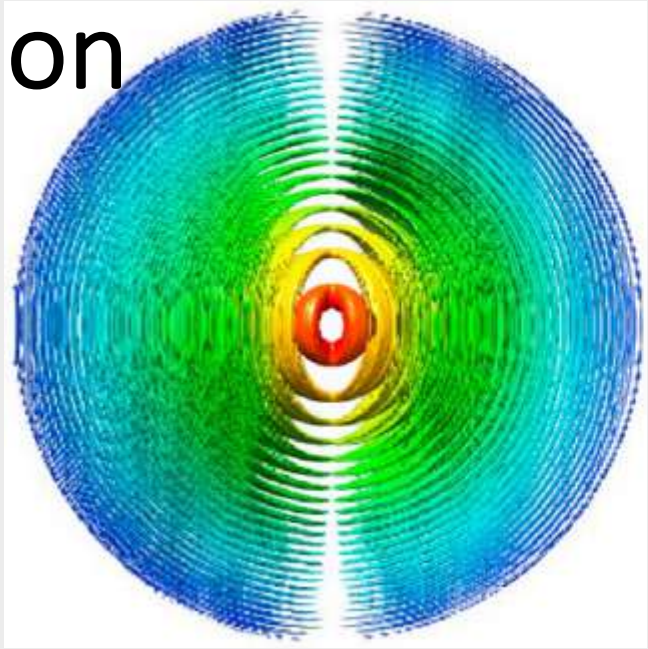


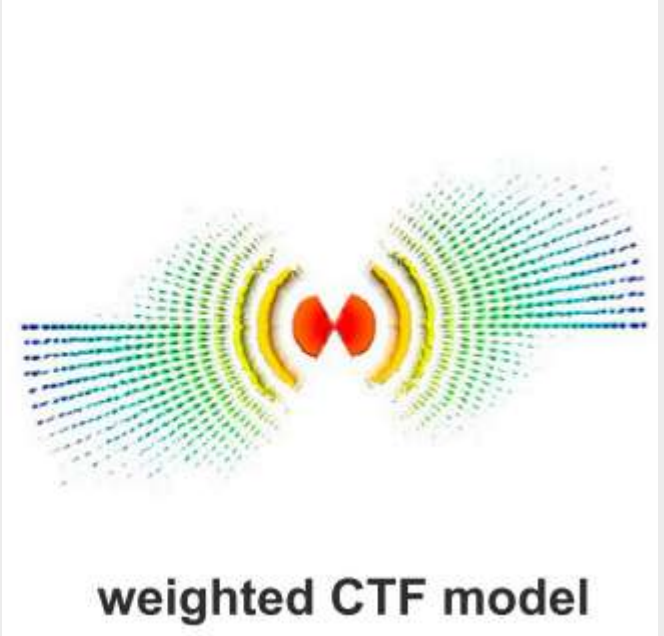
- Uses normal Relion workflow.
- Potential issues:
  - Extra images are likely not at the same focus as the Target
  - 3D FSC may eliminate properly interpolated values due to sampling

Bharat et. al., Structure 2015

Sub-tomogram processing in Relion

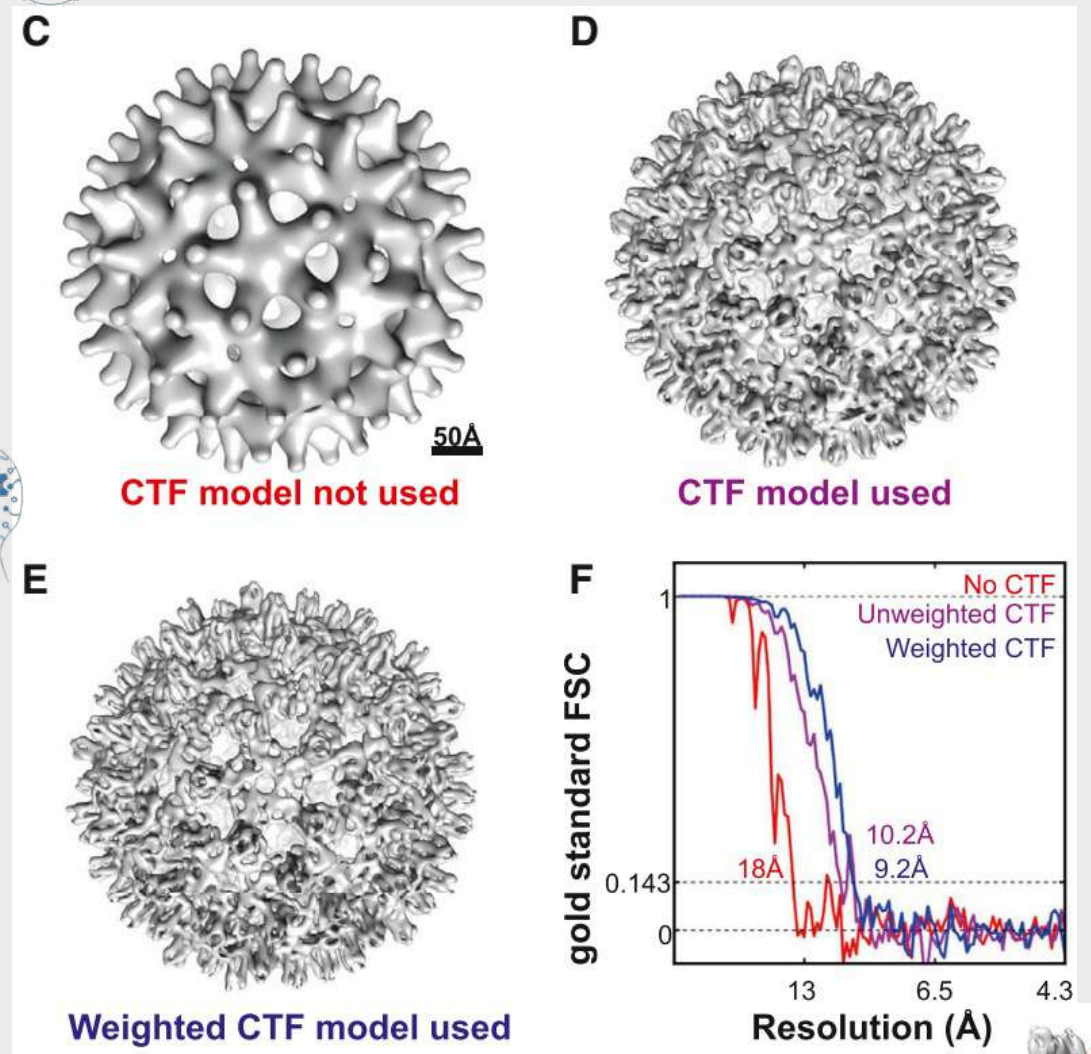


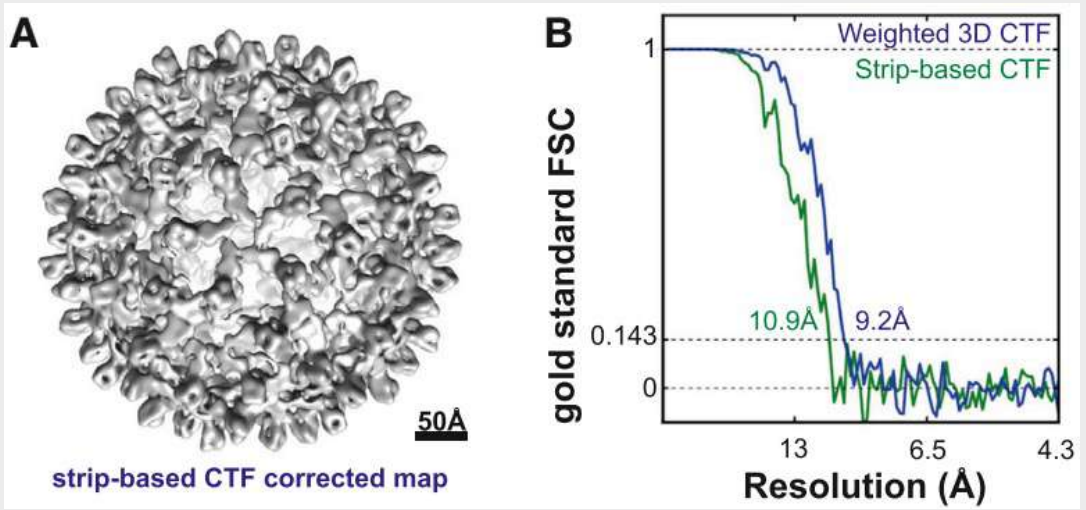


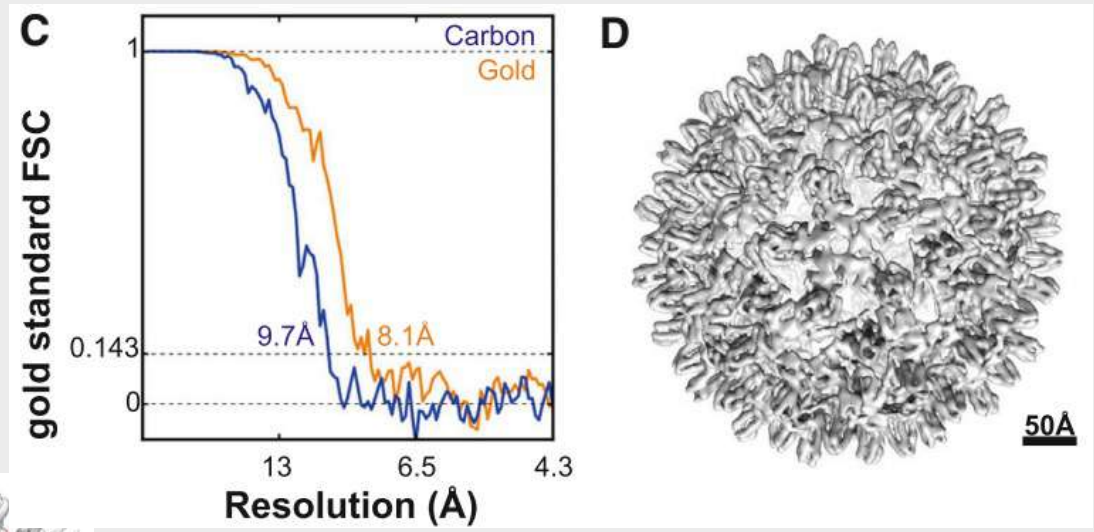


Bharat et. al., Structure 2015

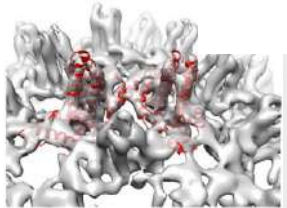
Sub-tomogram processing in Relion





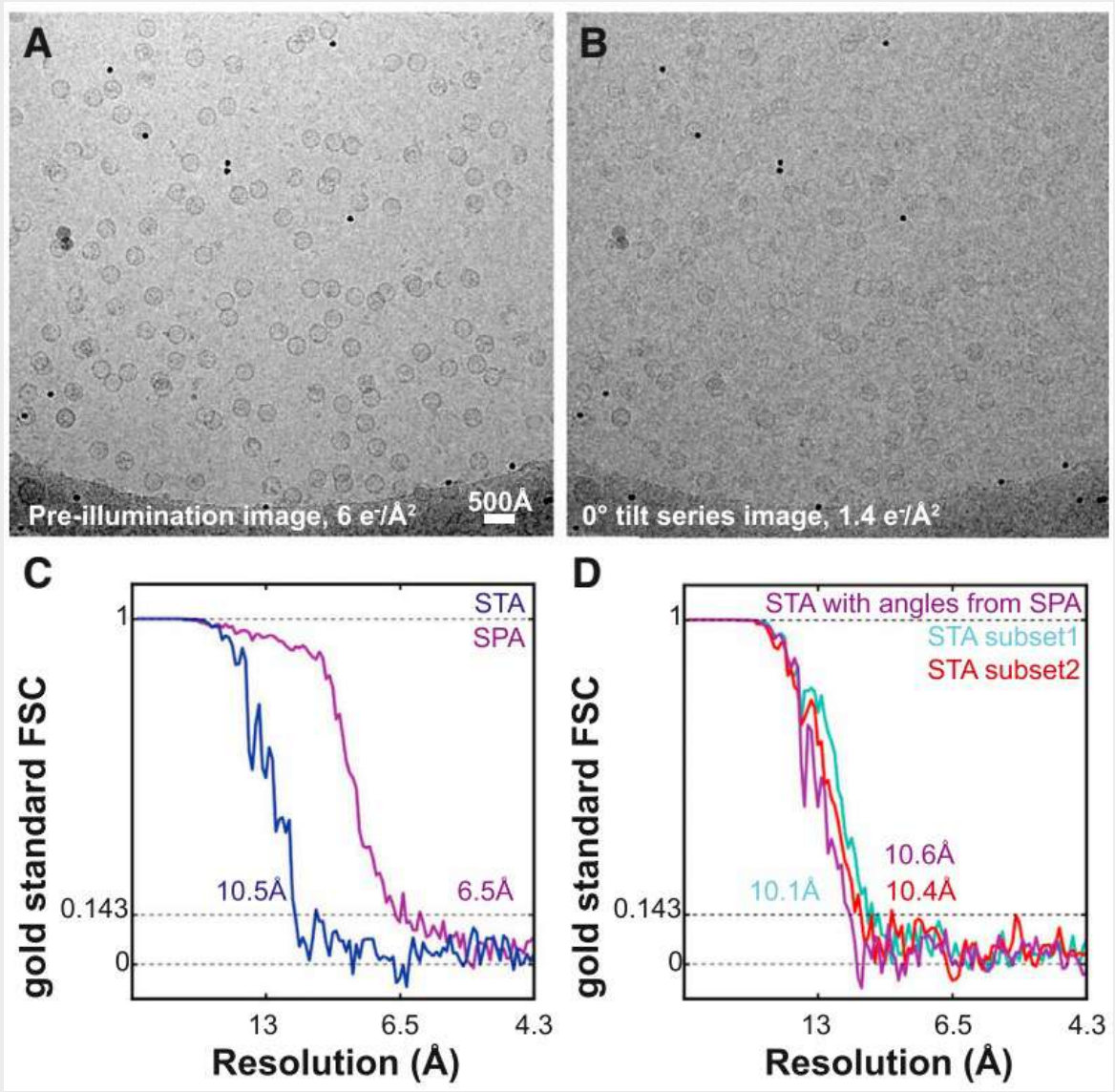


- Test case: Hepatitis B capsid



Bharat et. al., Structure 2015

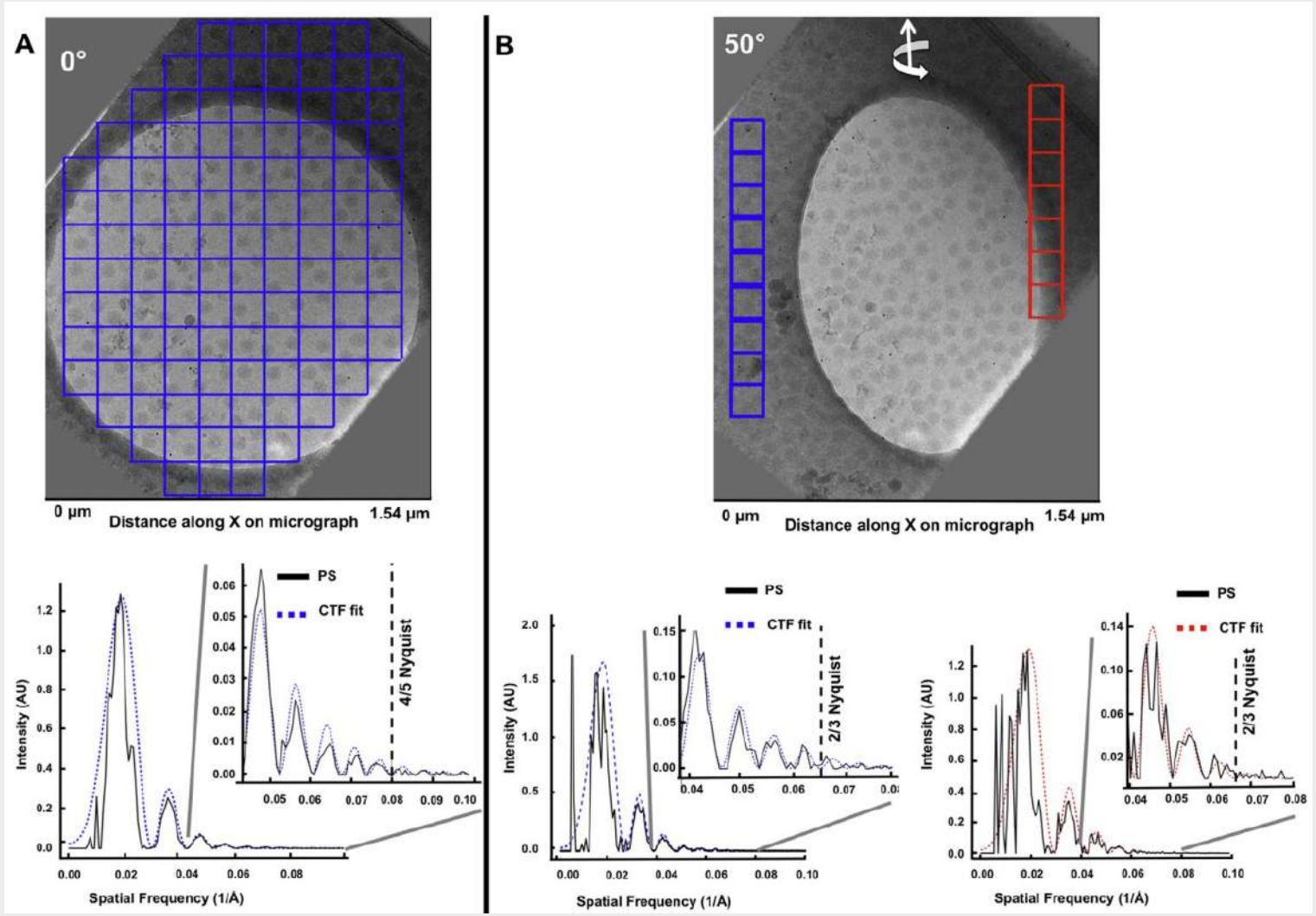
Sub-tomogram processing in Relion



- 6e-/A^2 pre-exposures prior to tilt-series collected were collected and analyzed with single particle

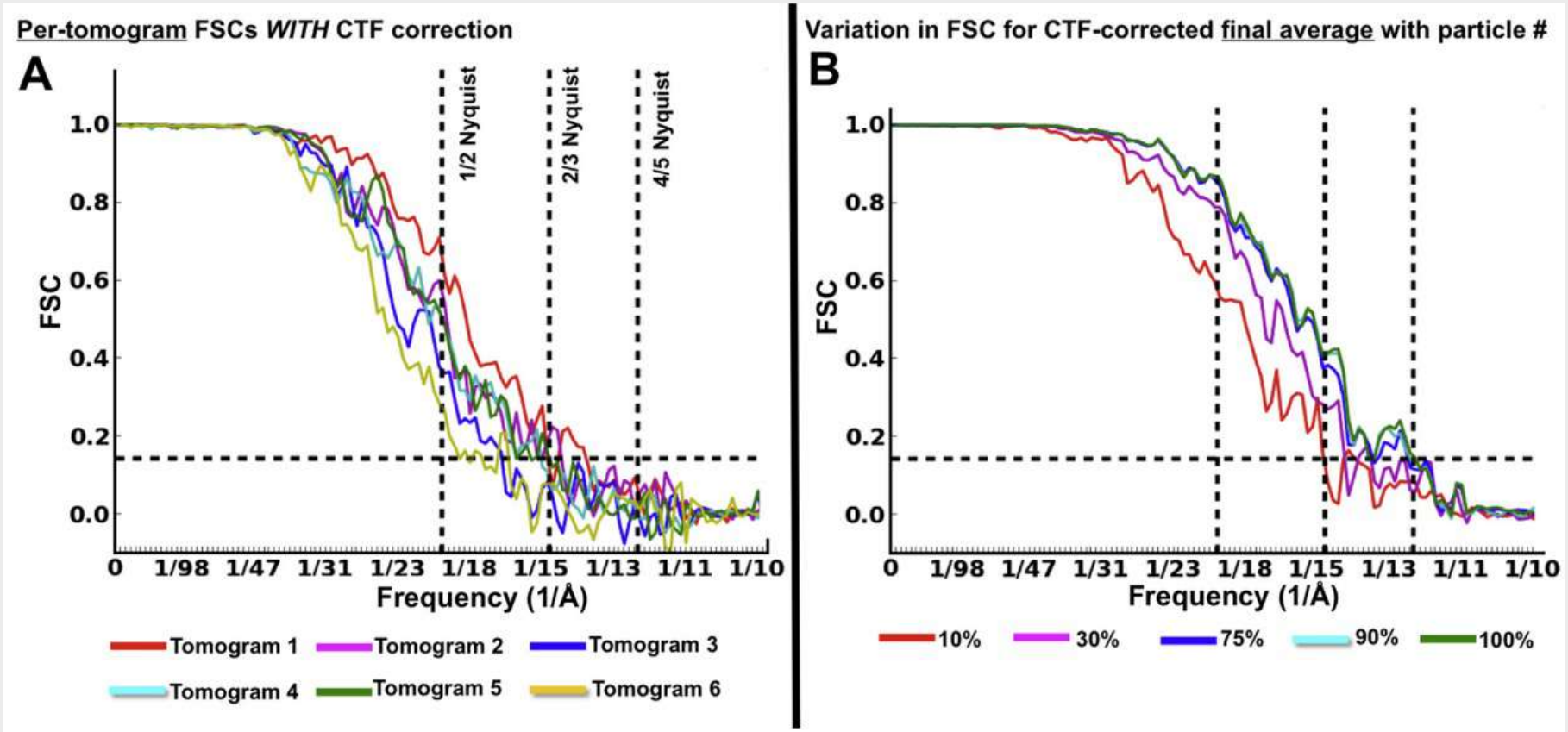
Bharat et. al., Structure 2015

Sub-tomogram processing in EMAN2



Galaz-Montoya, JSB 2016

Sub-tomogram processing in EMAN2



- Better than 2/3 Nyquist

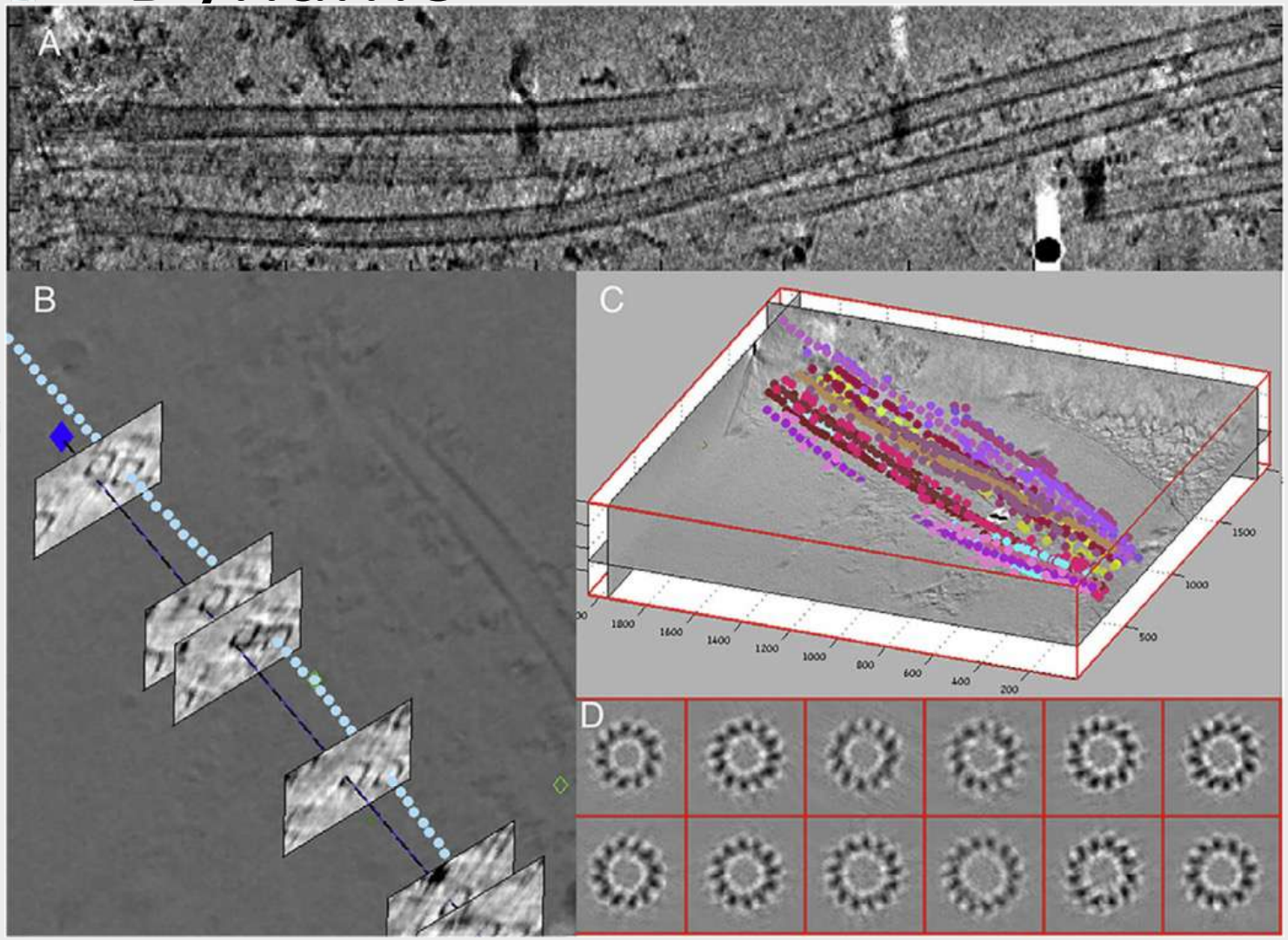
Galaz-Montoya, JSB 2016

Tomogram annotation

Tomogram/sub-tomogram annotation and segmentation software

- Dynamo – Annotate membranes, tubes, helices, crystal structures, vesicles, etc.
- EMAN2 – Shallow learning neural network
- Amira – Interactive segmentation and filtering suite
- UCSF Chimera w/ Segger - Interactive segmentation
- Template picking – MolMatch, Dynamo
- Various deep learning picking and segmentation softwares (search biorxiv)

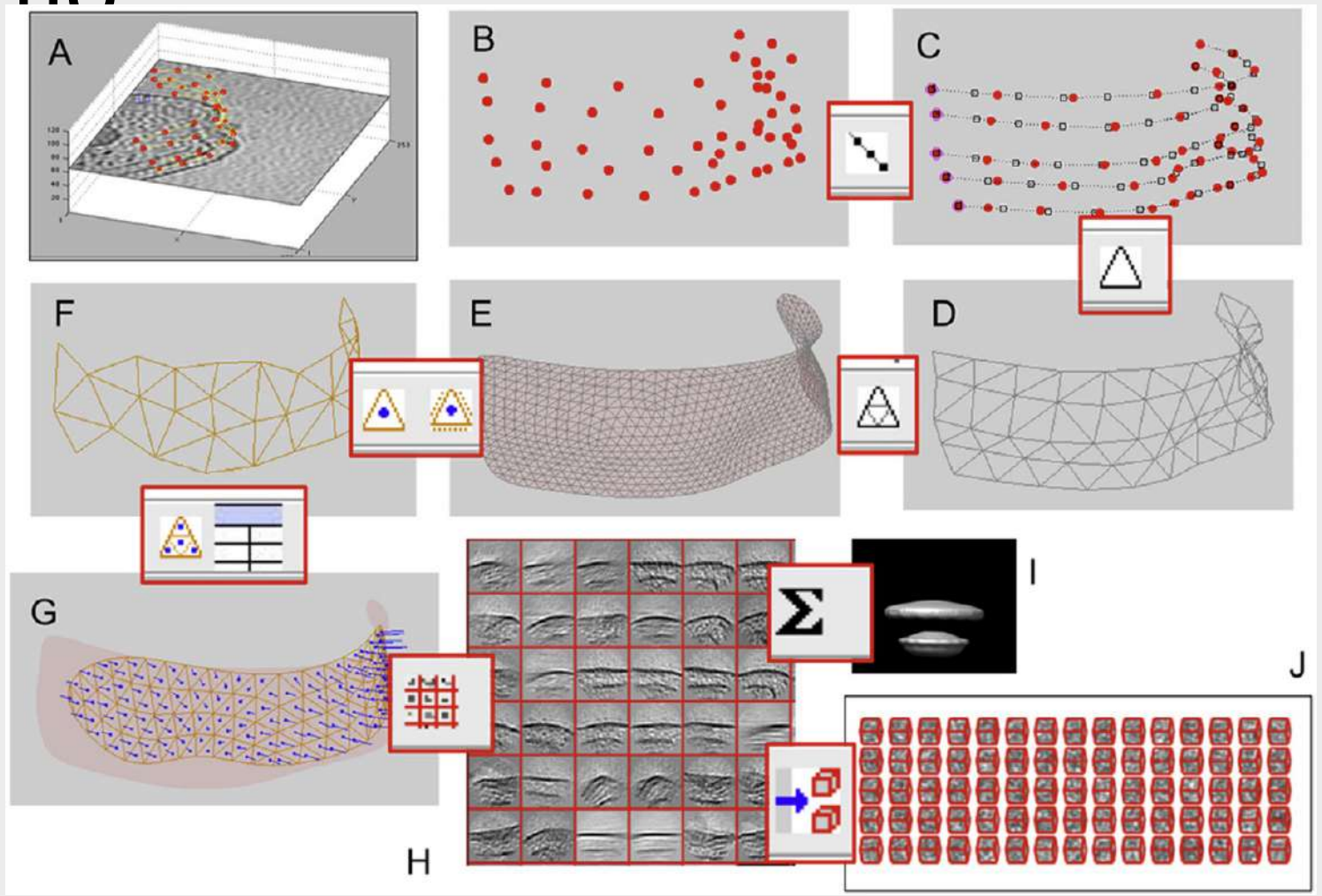
Sub-tomogram annotation processing in Dynamo



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

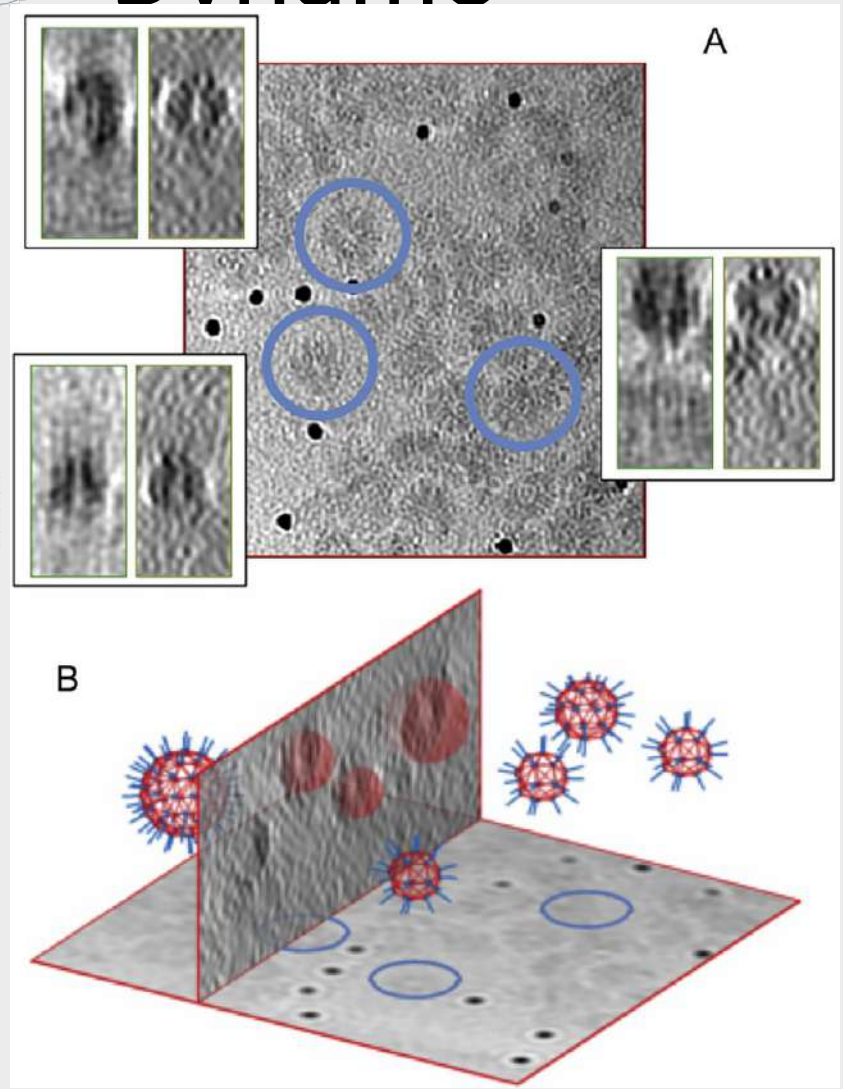
Castaño-Díez et. al., JSB 2012 & 2016

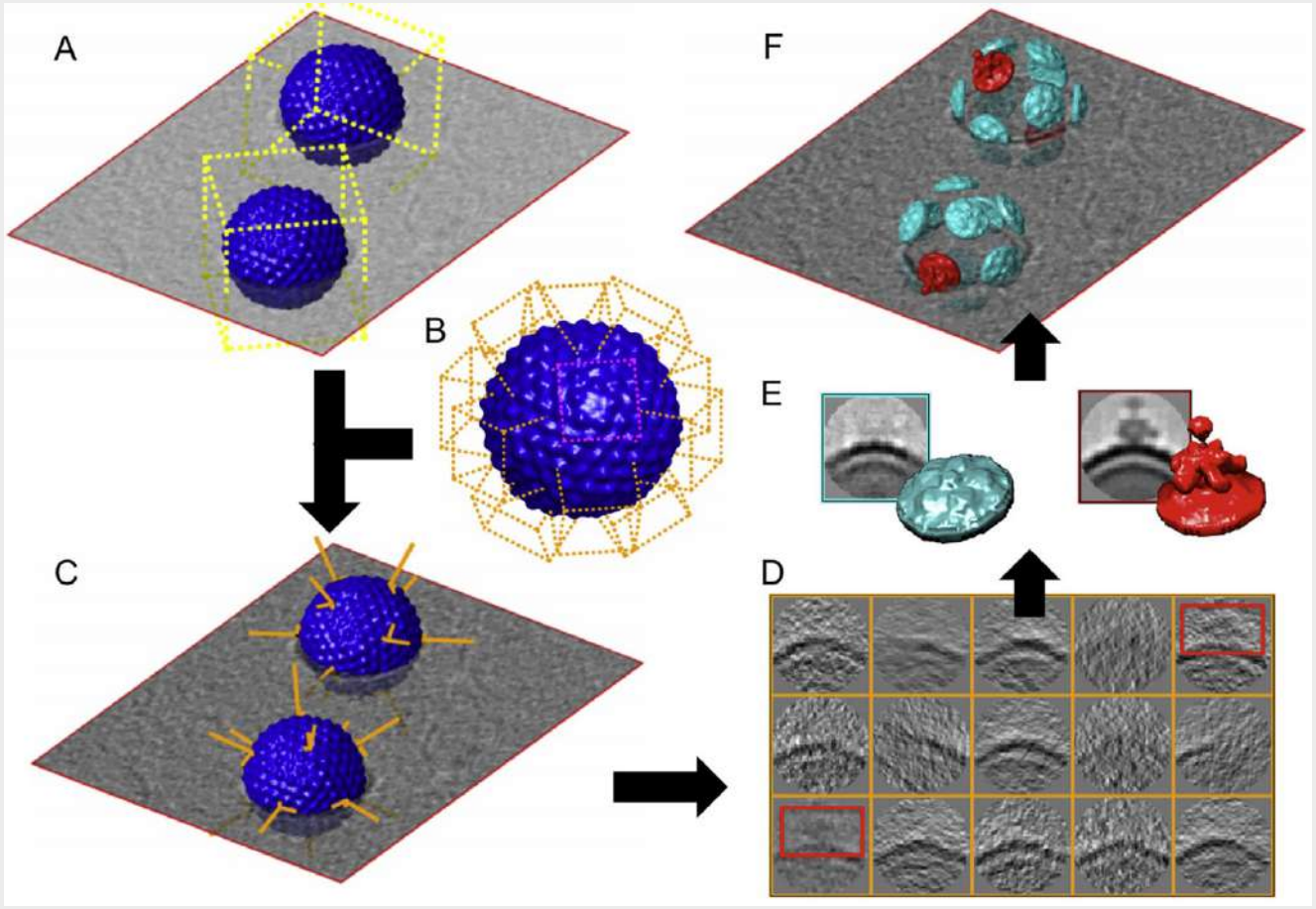
Sub-tomogram annotation processing in Dynamo



Castaño-Díez et. al., JSB 2012 & 2016

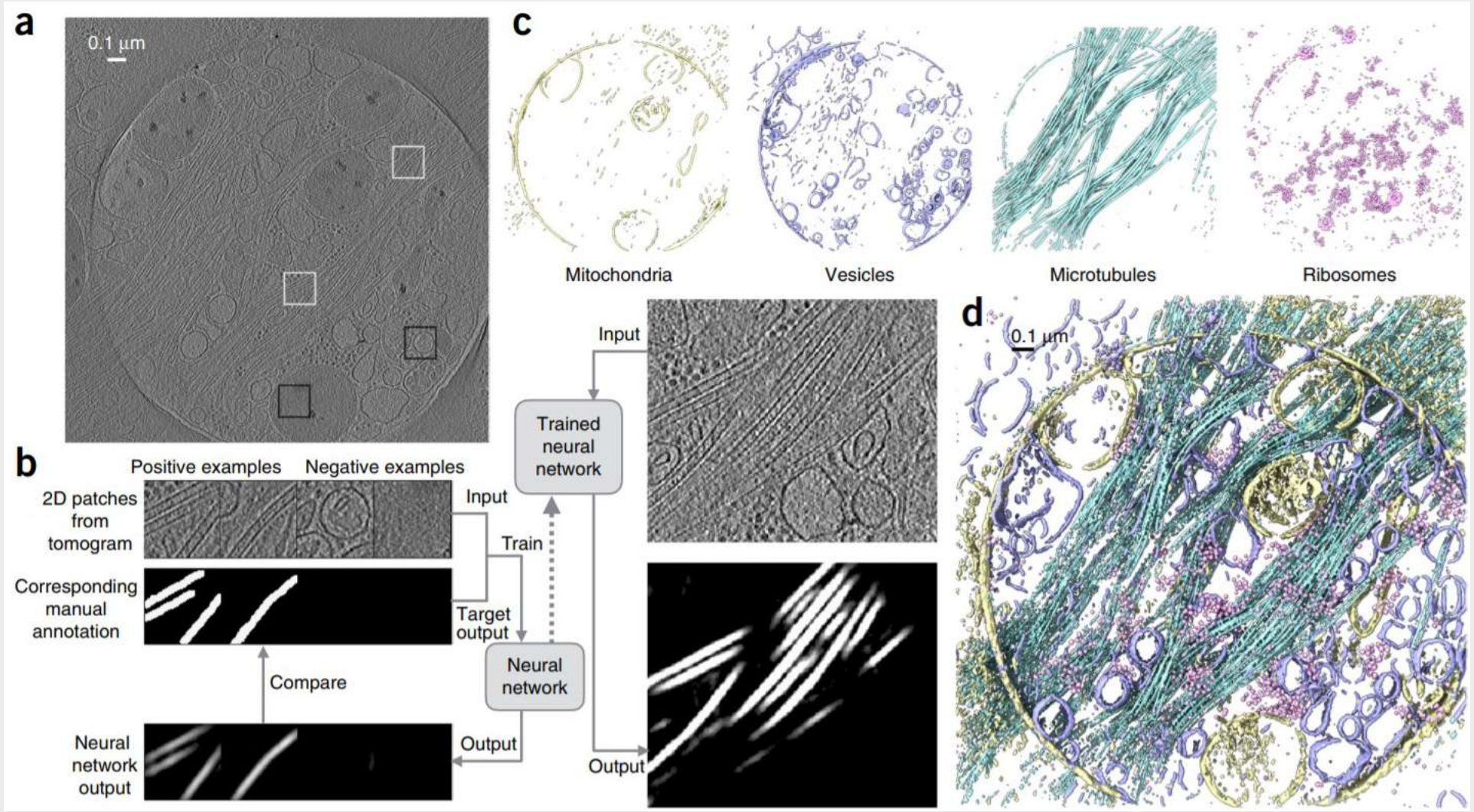
Sub-tomogram annotation processing in Dynamo





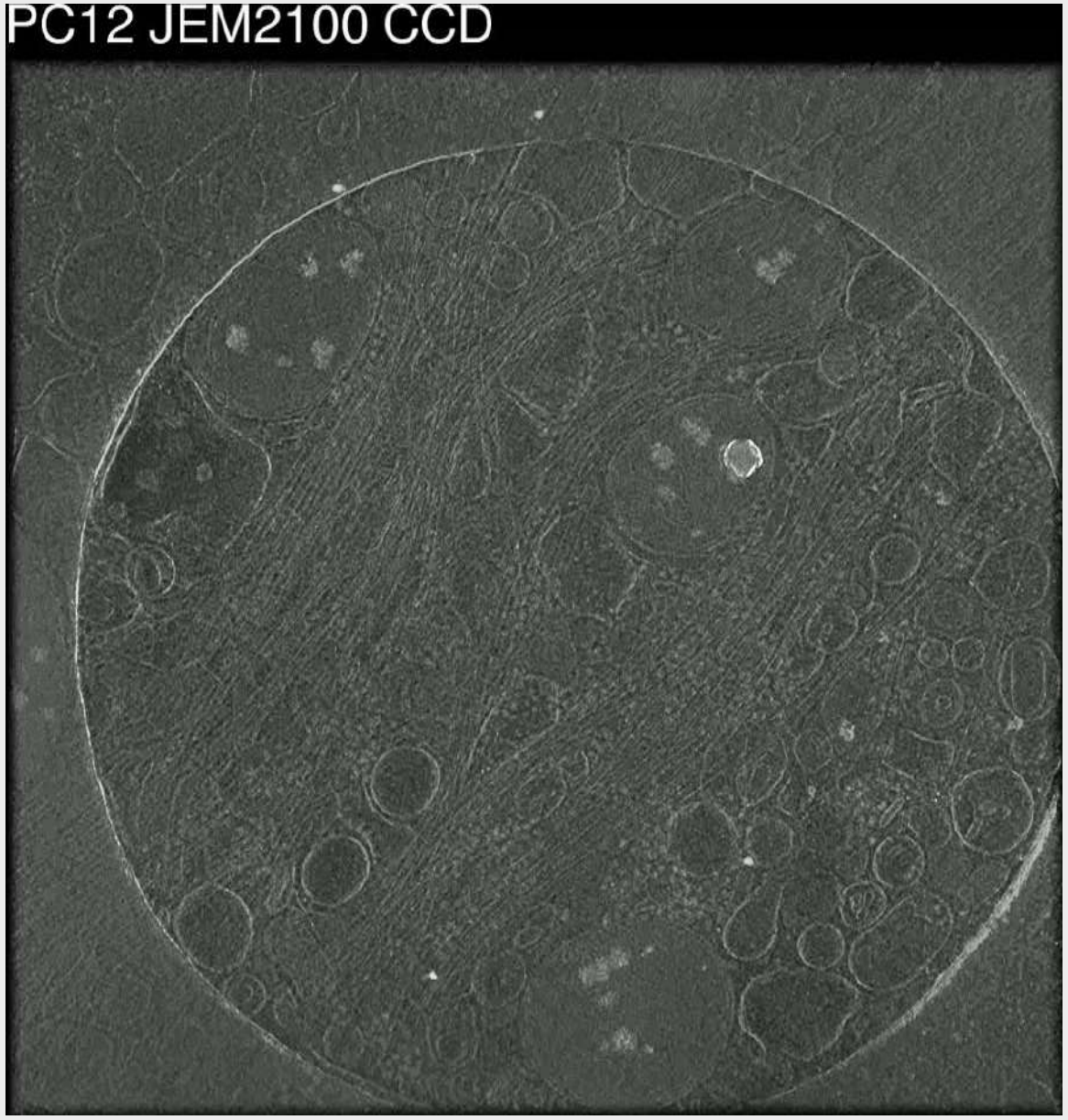
Castaño-Díez et. al., JSB 2012 & 2016

Sub-tomogram segmentation with CNNs in EMAN2



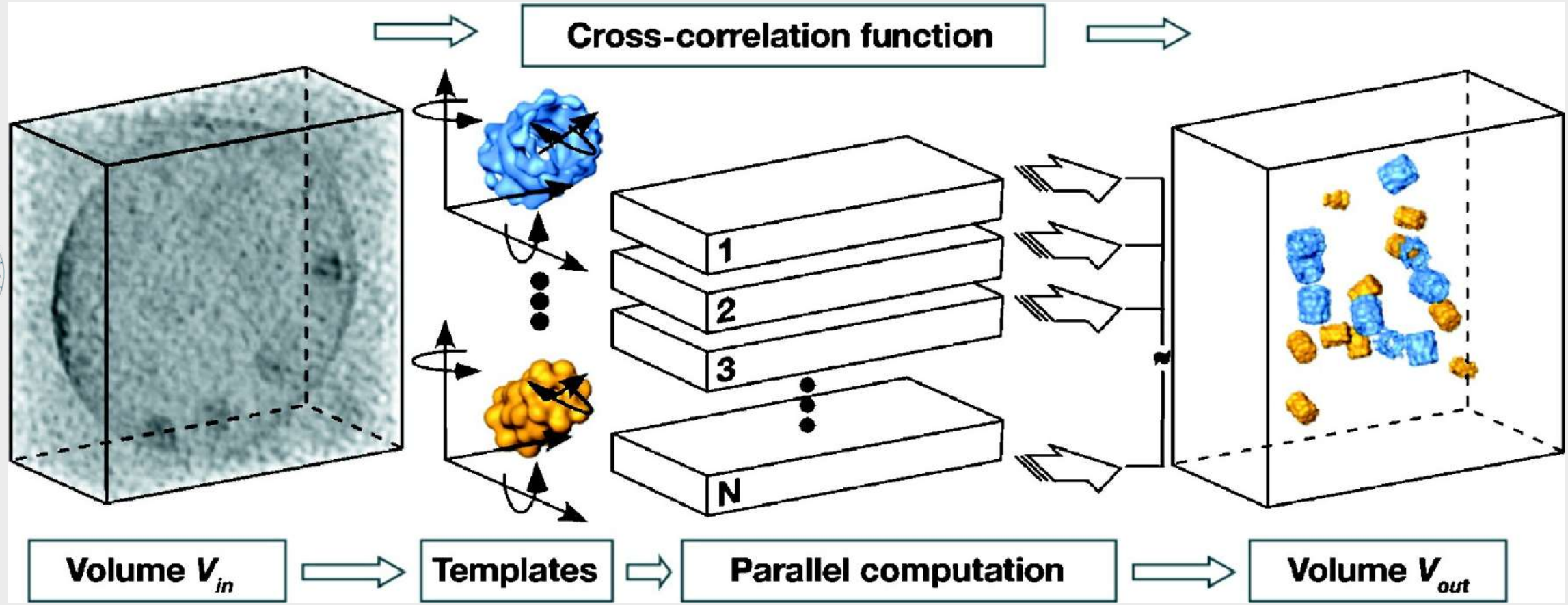
Chen et. al., Nat. Meth. 2017

Sub-tomogram segmentation with CNNs in EMAN2



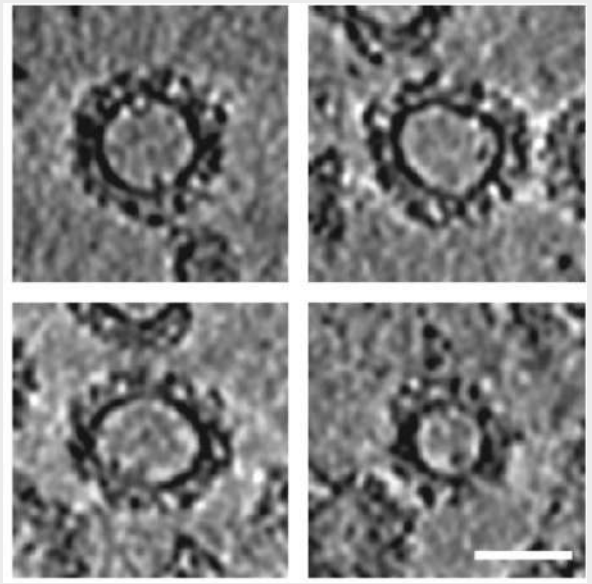
Chen et. al., Nat. Meth. 2017

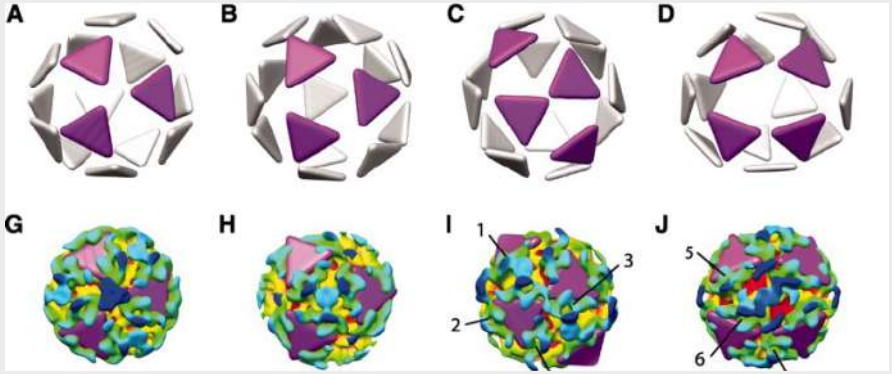
Template matching



Lucic et al, 2005, Annu. Rev. Biochem

Example: STA followed by placing averages to the tomograms



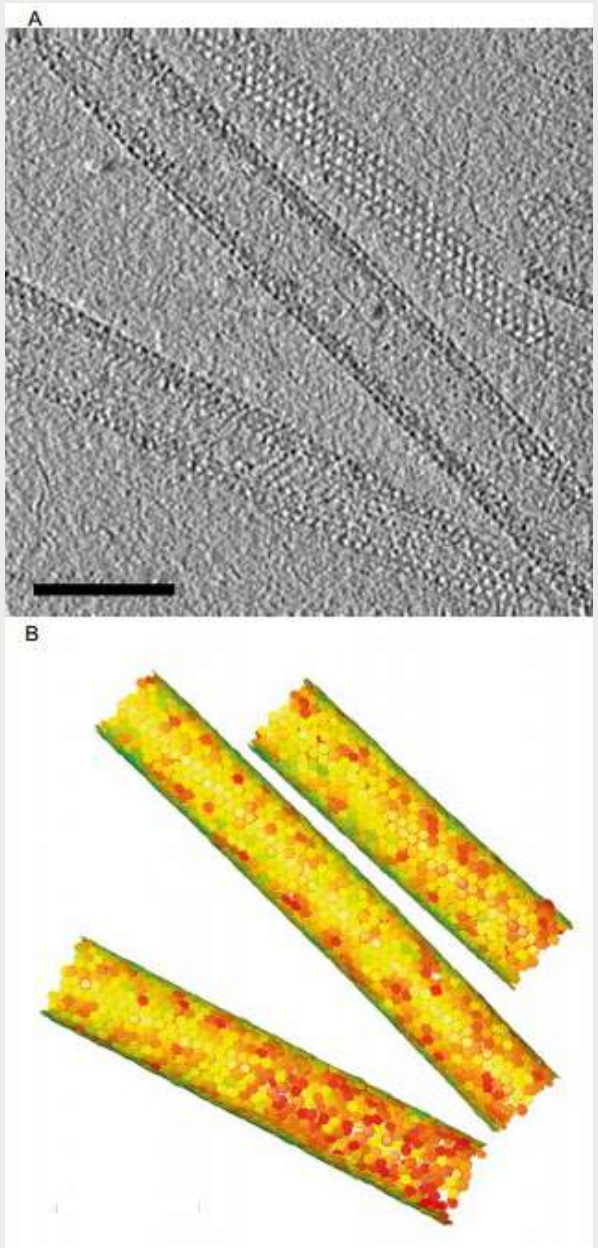


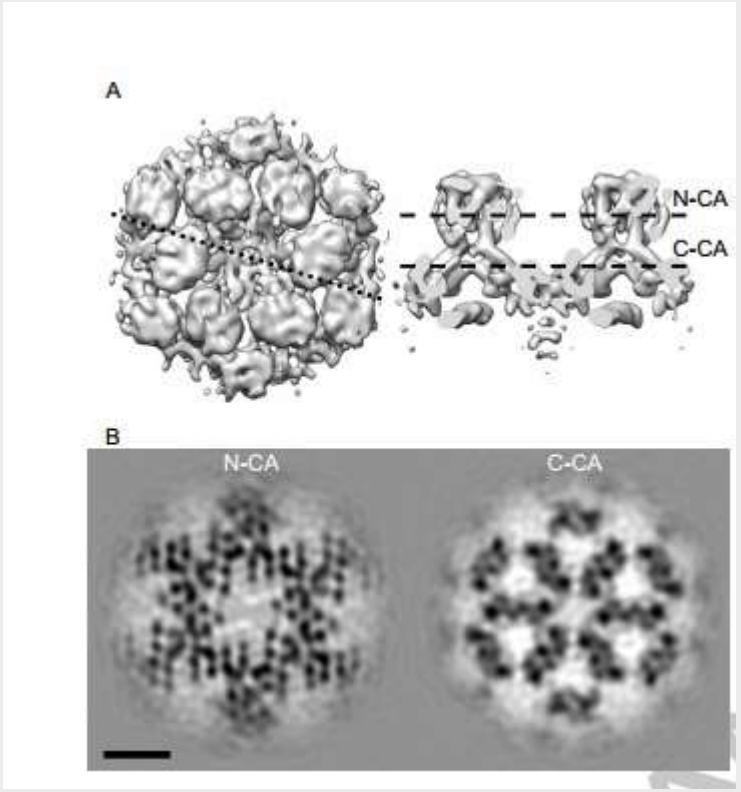
COP-I coated vesicles From: Faini et al, Science, 2012

Example: Mason-Pfizer monkey virus Gag protein

Over-picking to find

repeating units



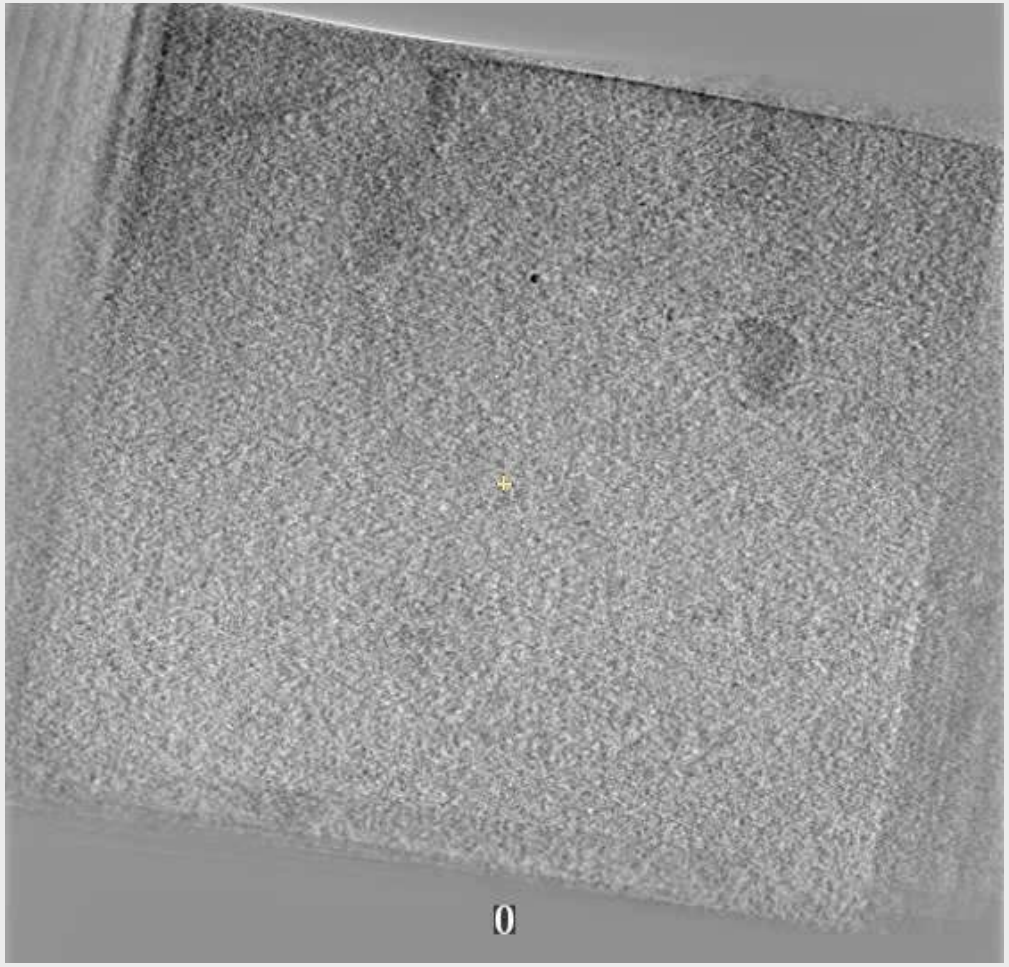


Schur et al, J. Struct Biol, 2013

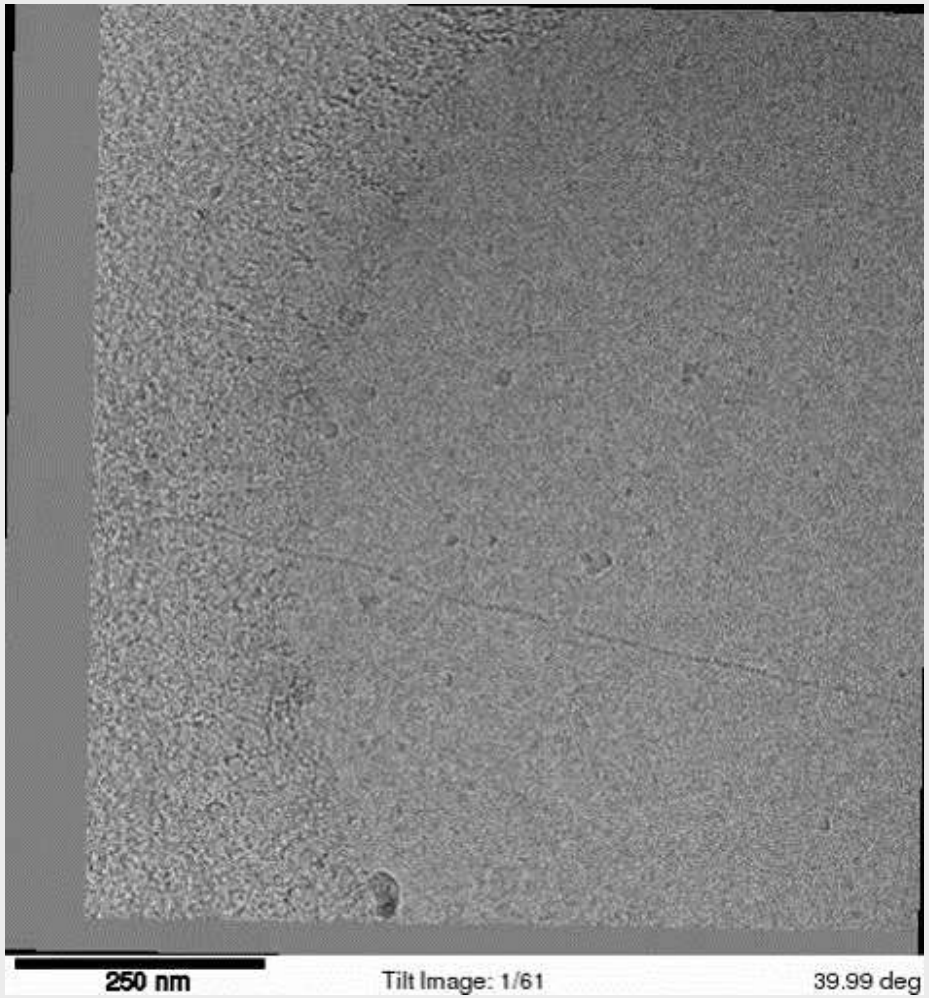
Schur et al, Nature , 2015

Various examples

Example: Liposomes and VLPs

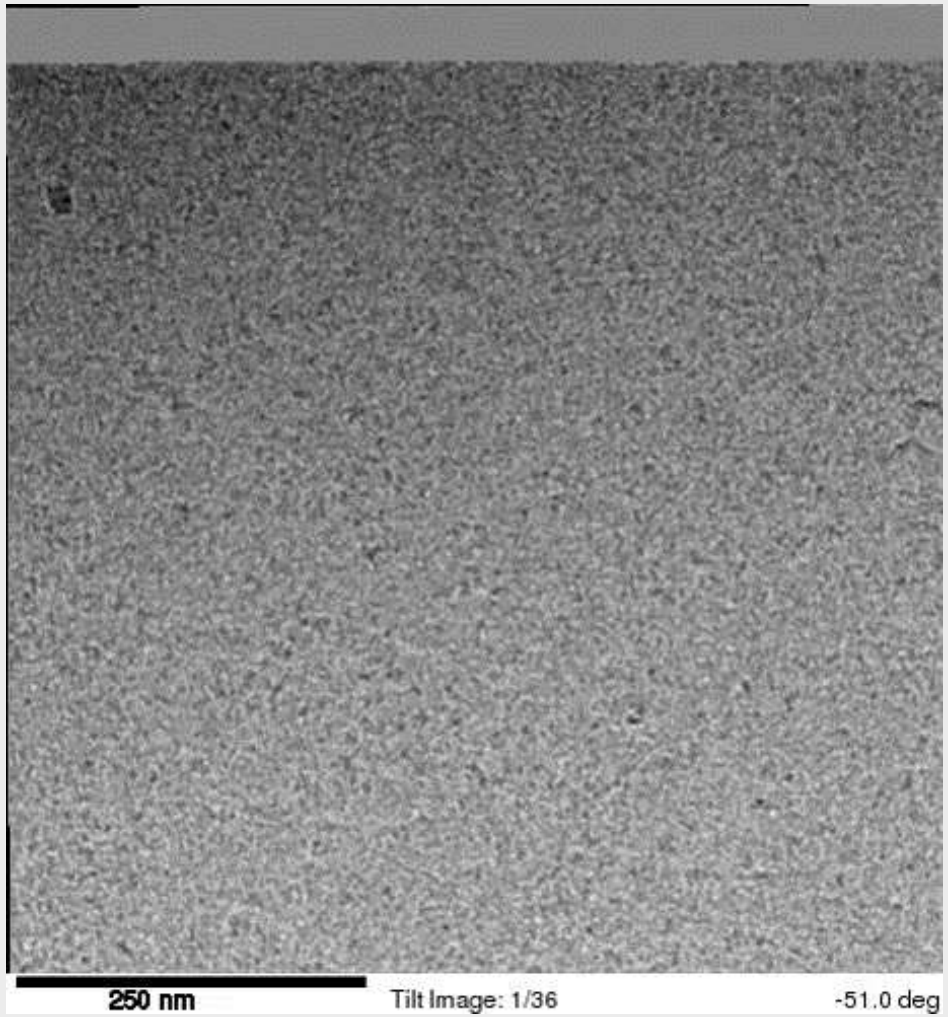


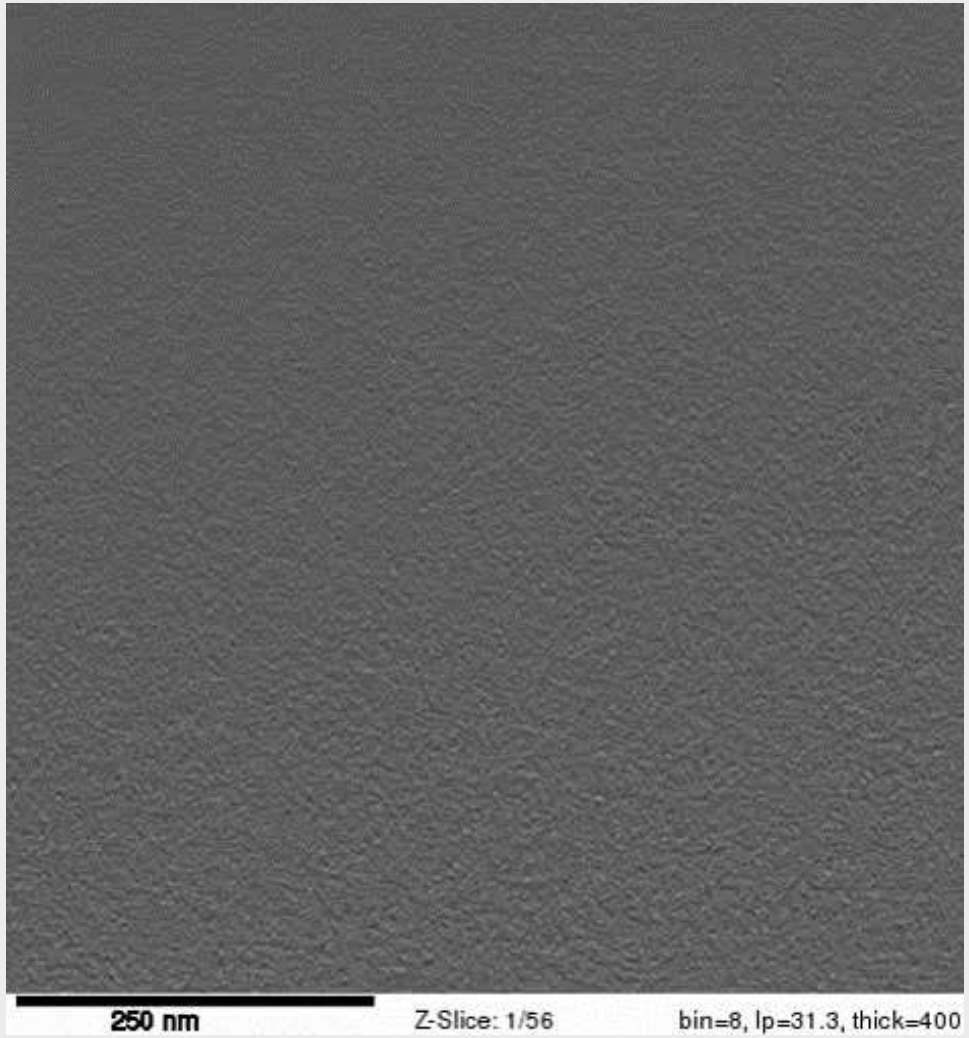
Example: Actin Filaments



Pinar Gural, Greg Alushin, Alex Noble
unpublished

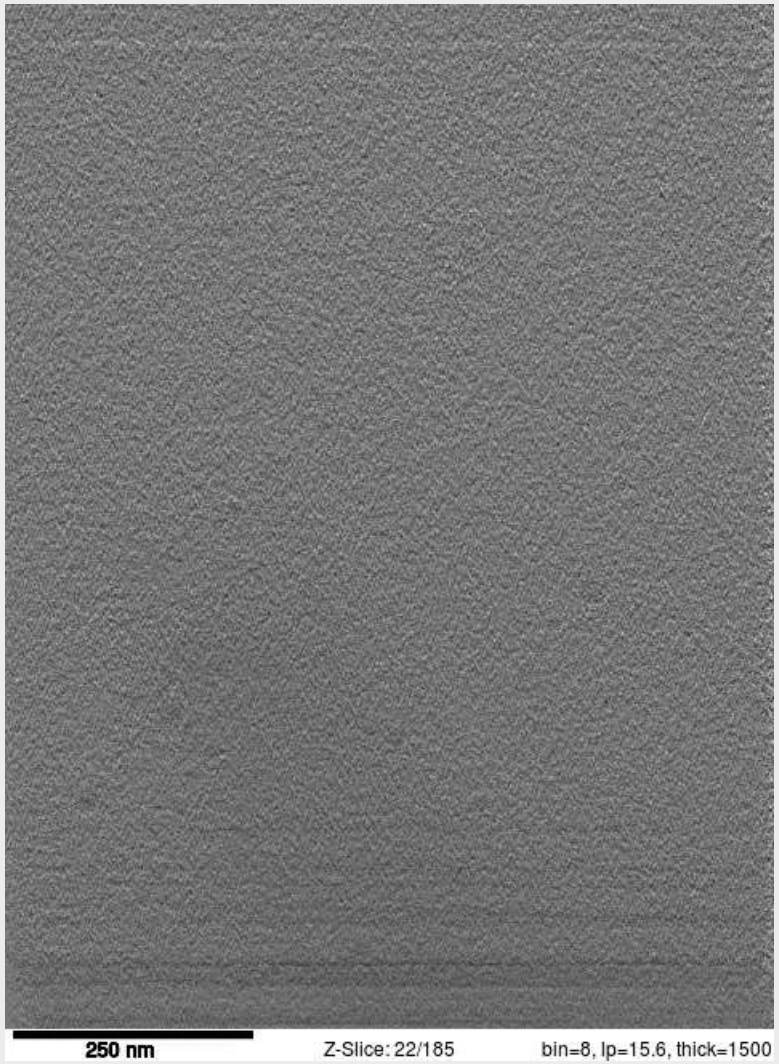
Example: HIV-1 trimer single particle





Priyamvada Acharya & Alex Noble
eLife, 2018

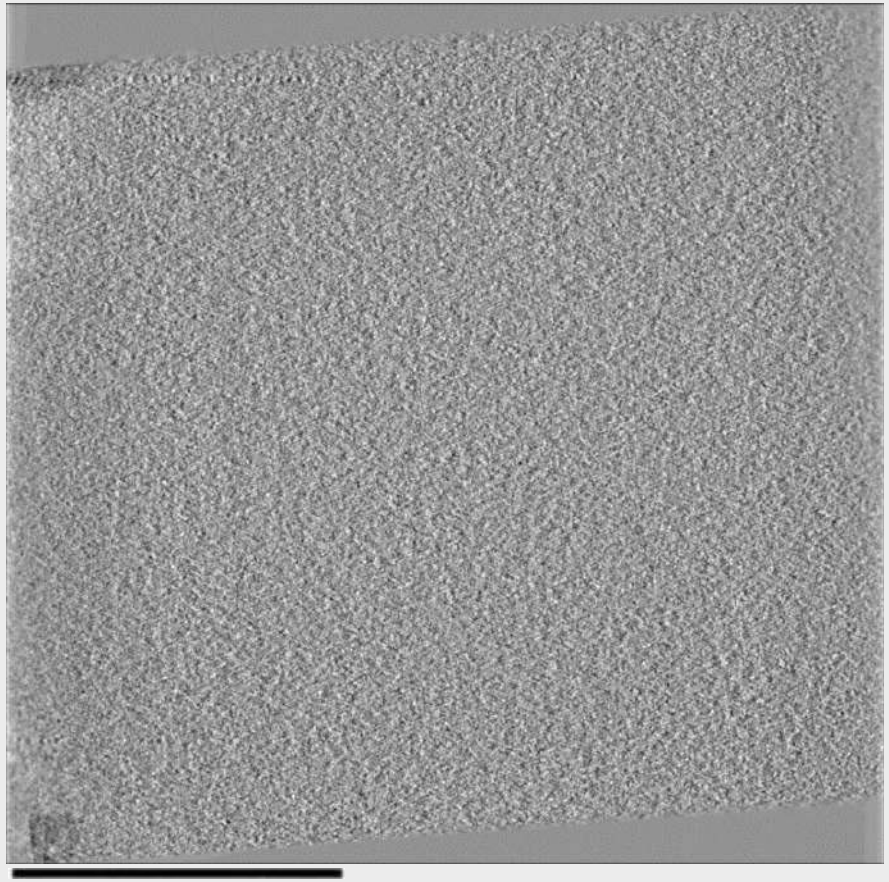
Example: Exotically Shaped Samples



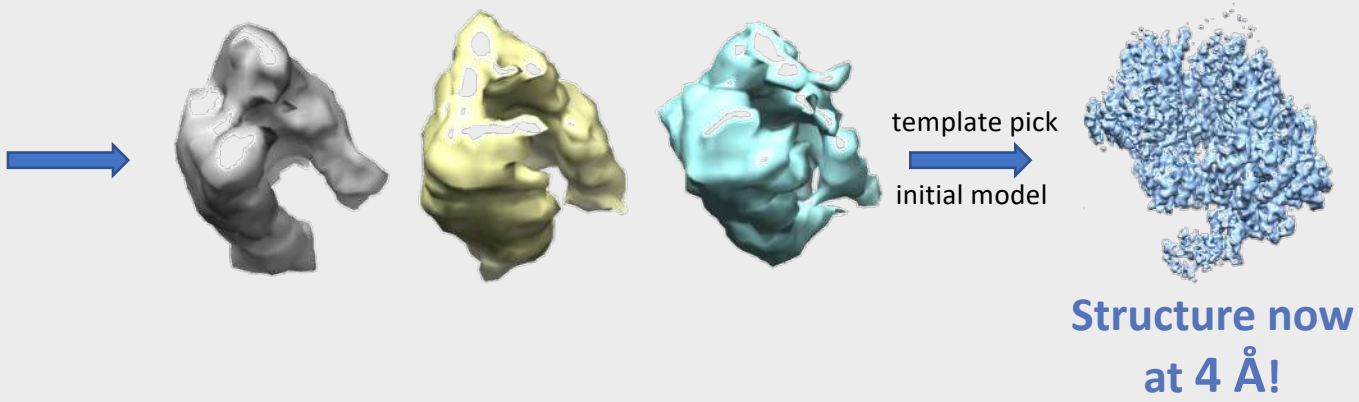
ZmNDPK1: GTP complex

Mykhailo Kopylov & Beth Stroupe
unpublished

Example: Tomography for single particle initial model



Z-slices through tomogram/ice

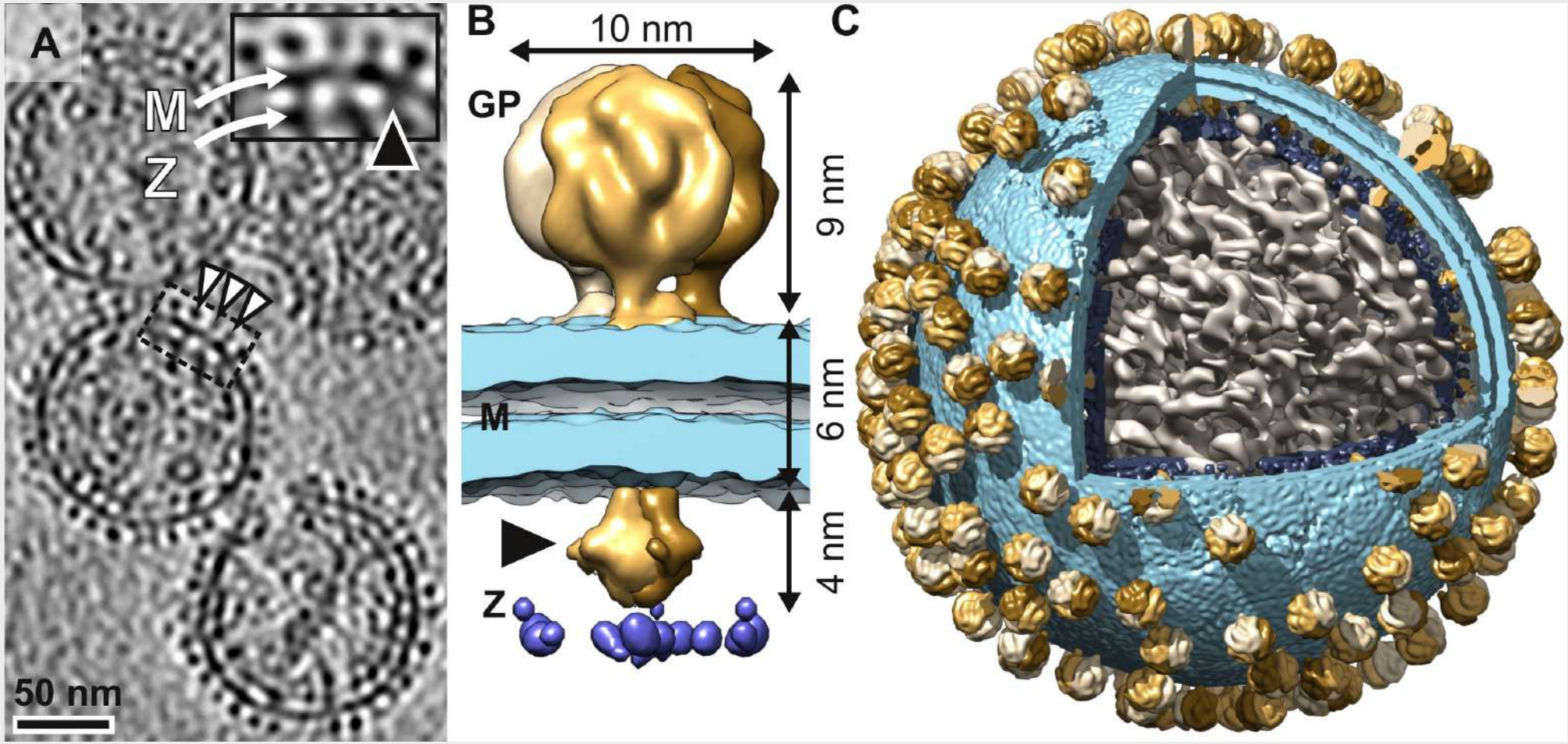


Structure now at 4 Å!

- 5 tomograms were collected
- ~1,000 particles picked, aligned, and classified
- Classes used as templates for picking single particle micrographs
- Single particle now at 4 Å without anisotropy.

Jillian Chase and Alex Noble
eLife, 2018 and 2019

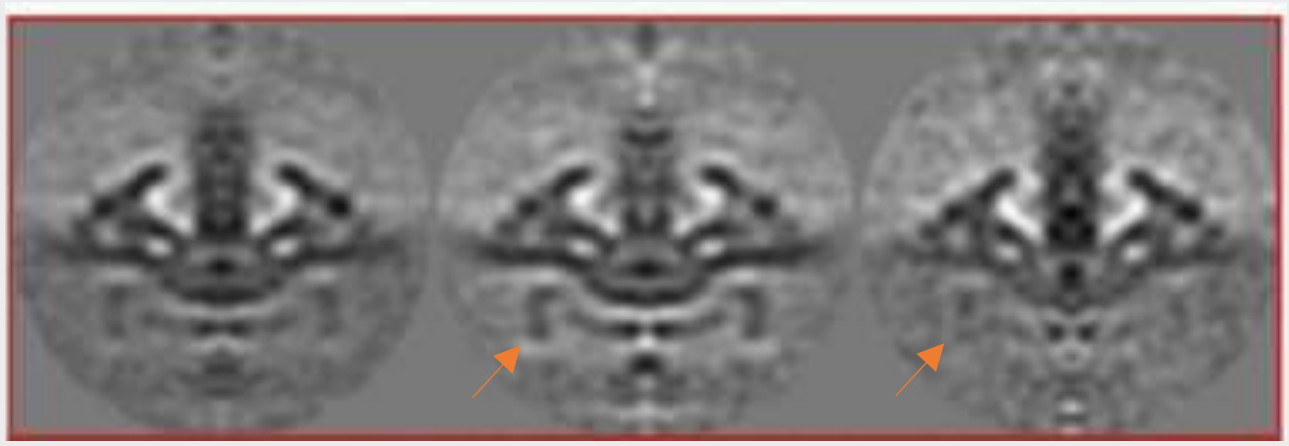
Example: Lassa virus glycoprotein spike



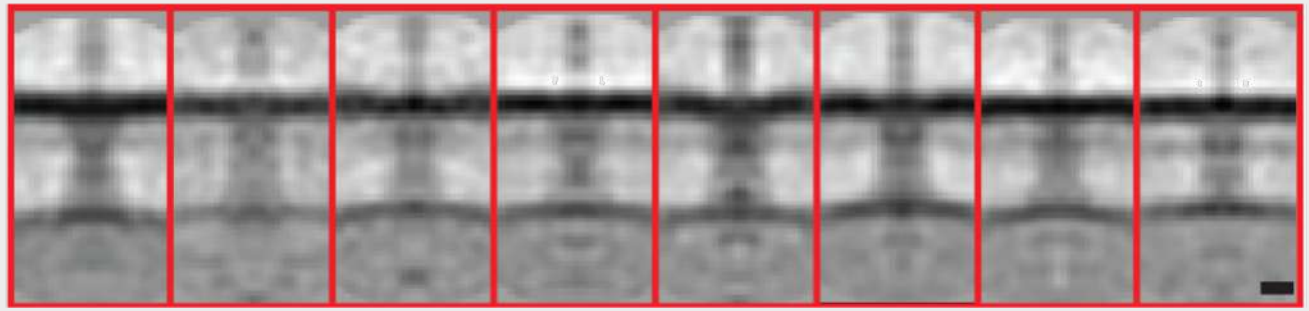
- **Heterogeneous shape** makes single particle difficult/impossible
- Sub-tomogram processing on spiked allows for **13.6 Å spike structure**
- Can **re-map spikes** onto all particles in the tomogram

Li et. al., PLOS 2016

Example: Bacterial flagella motor and type III secretion injectisome



Kudryashev et al, JSB, 2010 and
Castaño-Díez, et al, JSB, 2012

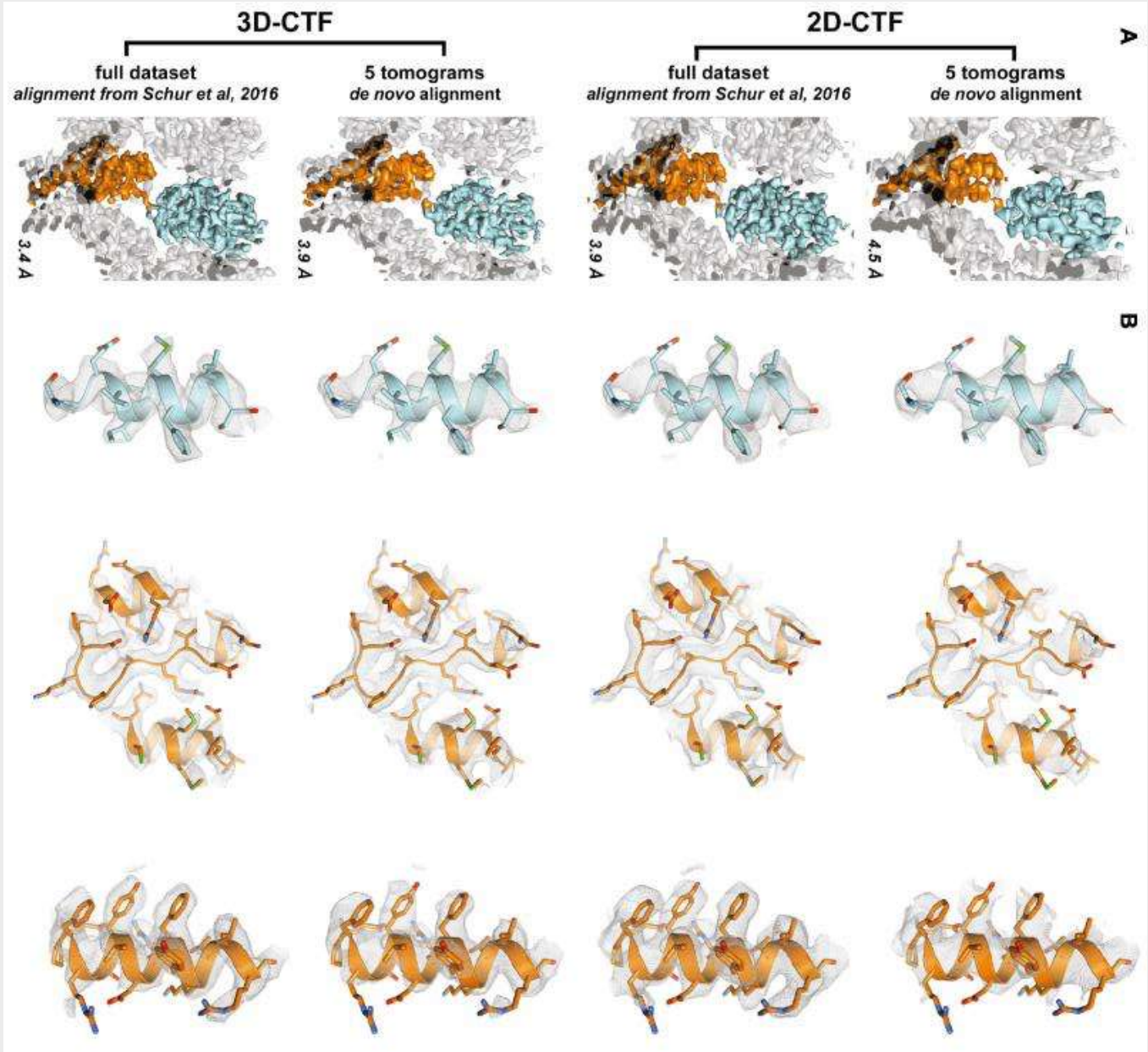


Kudryashev et al, eLife, 2013

- Conformational states studied *in situ*
- Presence and absence of C-ring
- Elongation of injectisome

Example: HIV-1 Capsid-SP1 at 3.9/3.4/3.2 Å

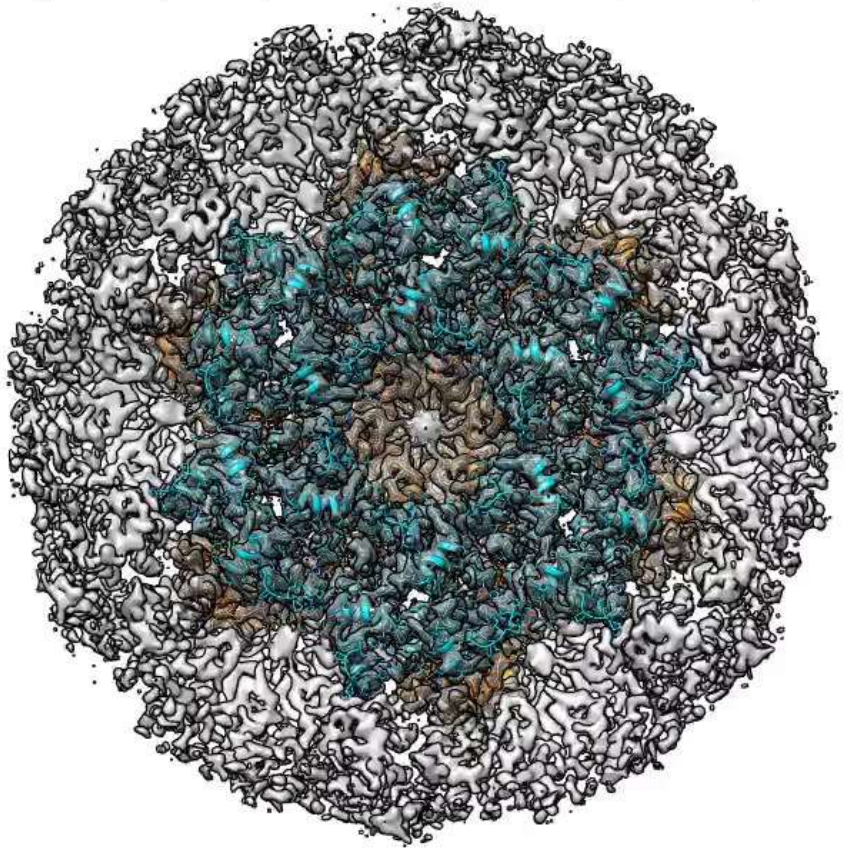
- Krios + Super-res K2 + Gatan Energy Filter
- Fiducial tilt-series alignment
- 1.5 – 5 micron defocus
- Strip-based CTF correction
- ~750,000 sub-particles used
- TOM, AV3, Dynamo, and in-house scripts were used
- NovaCTF 3D CTF pushed it to 3.4 Å
- emClarity pushed to 3.2 Å



Schur, 2016
Turoňová, 2017

Example: HIV-1 Capsid-SP1 at 3.9 Å

An atomic model of HIV-1 capsid-SP1 reveals structures regulating assembly and maturation
Schur F.K.M, Obr M., Hagen W.J.H, Wan W., Jakobi A.J., Kirkpatrick J.M., Sachse C., Kräusslich H-G., Briggs J.A.G



Schur et al., 2016

Example: HIV-1 Capsid-SP1 at 3.9 Å

Sample		HIV-1 ΔMACANCSP2 VLPs	HIV-1 ΔMACANCSP2 VLPs + 100 µg/ml Bevirimat	Immature HIV-1 (D25A) virus
Acquisition settings	**Microscope**	FEI Titan Krios	FEI Titan Krios	FEI Titan Krios
	Voltage (keV)	300	300	300
	Detector	Gatan Quantum K2	Gatan Quantum K2	Gatan Quantum K2
	Energy-filter	Yes	Yes	Yes
	Slit width (eV)	20	20	20
	Super-resolution mode	Yes	Yes	Yes
	Å/pixel	1.35	1.35	1.35
	Defocus range (microns)	-1.5 to -4.5	-1.5 to -5.0	-1.5 to 5.0
	Defocus step (microns)	0.25	0.25	0.25
	Acquisition scheme	-60/60°, 3°, Serial EM	-60/60°, 3°, Serial EM	-60/60°, 3°, Serial EM
	Total Dose (electrons/Å^2)	~90 - 270	~120 - 145	~120-221
	Dose rate (electrons/Å^2/sec)	~3 - 8	~3 - 3.8	~1.5 – 5.5
	Frame number	6 – 10	8 – 10	10 – 12
	Tomogram number	93	43	74
Processing settings	**VLPs/Viruses**	285	383	484
	Asymmetric units Set A	265,506	386,040	301,302
	Asymmetric units Set B	263,910	386,598	301,920
	Final resolution (0.143 FSC) in Å	4.5	3.9	4.2

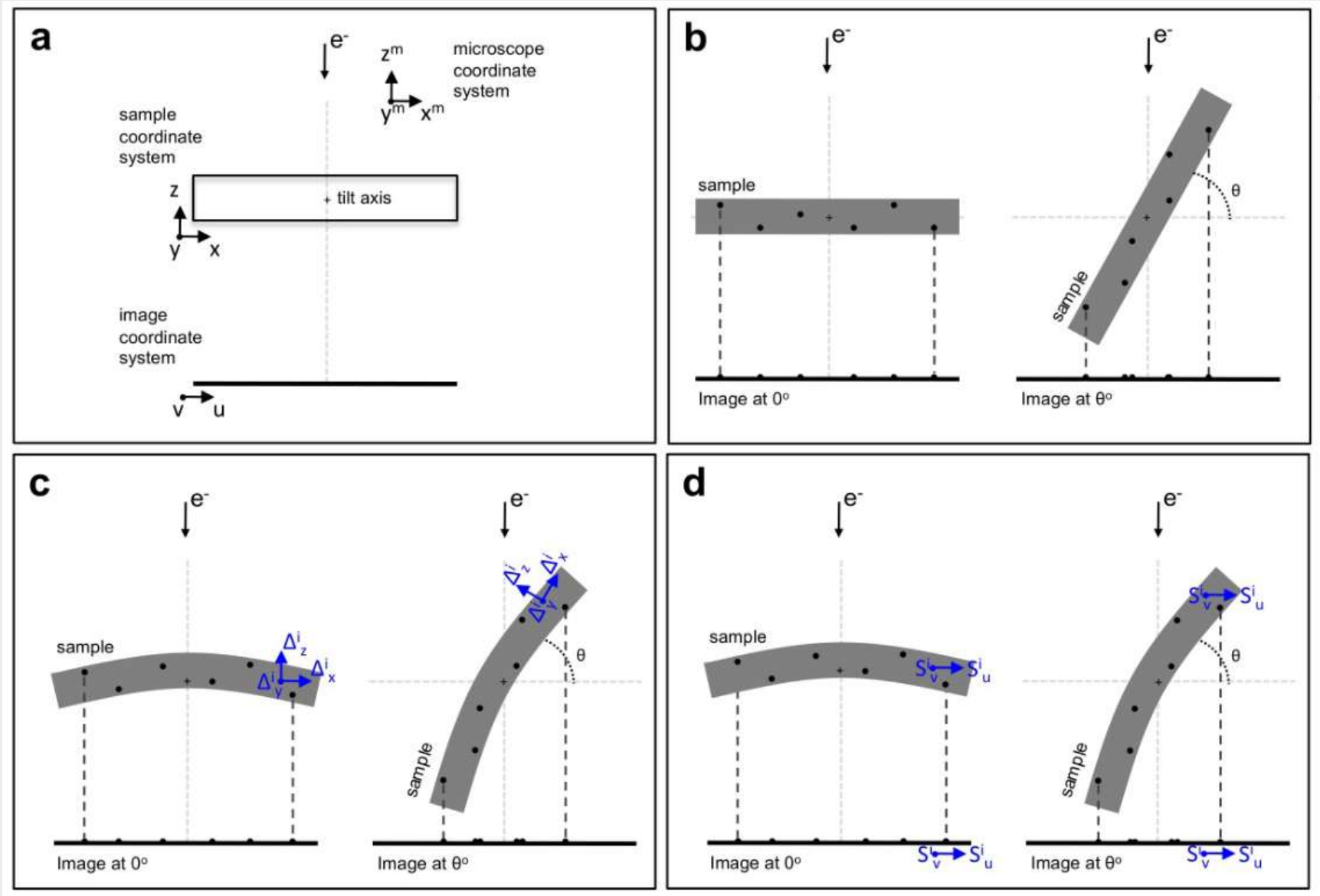
Schur et. al., Science 2016

Processing/Resolution limits

- Pixelsize (highest resolution = 2 x pixelsize = **Nyquist**)
- Isotropic motion (monitor your **drift** before full collection)
- Inherent specimen **flexibility**
- **Ice warping** in 3D during collection (doming)
- Beam-induced **motion of objects** of interest **in 3D** (particularly anisotropic)

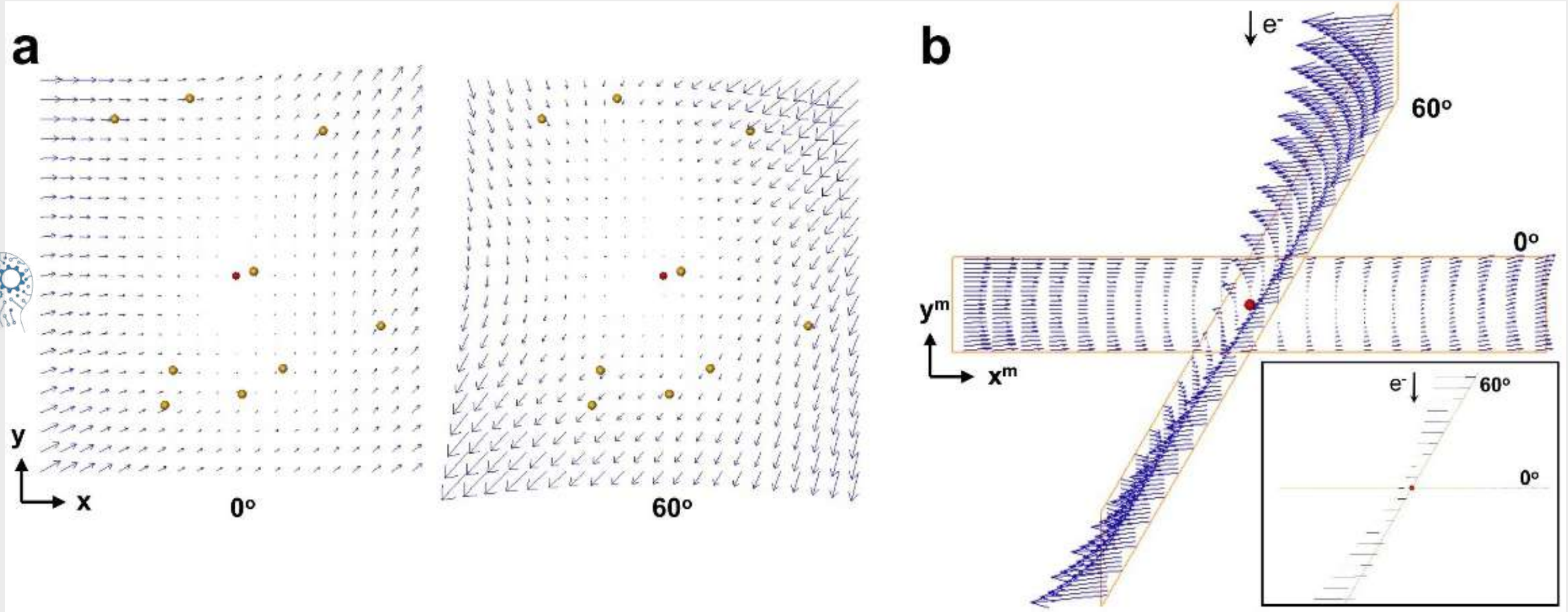
Already discussed: Sample thickness, camera accuracy, and specimen damage

Refining tilt-series alignment by tracking beads in 3D



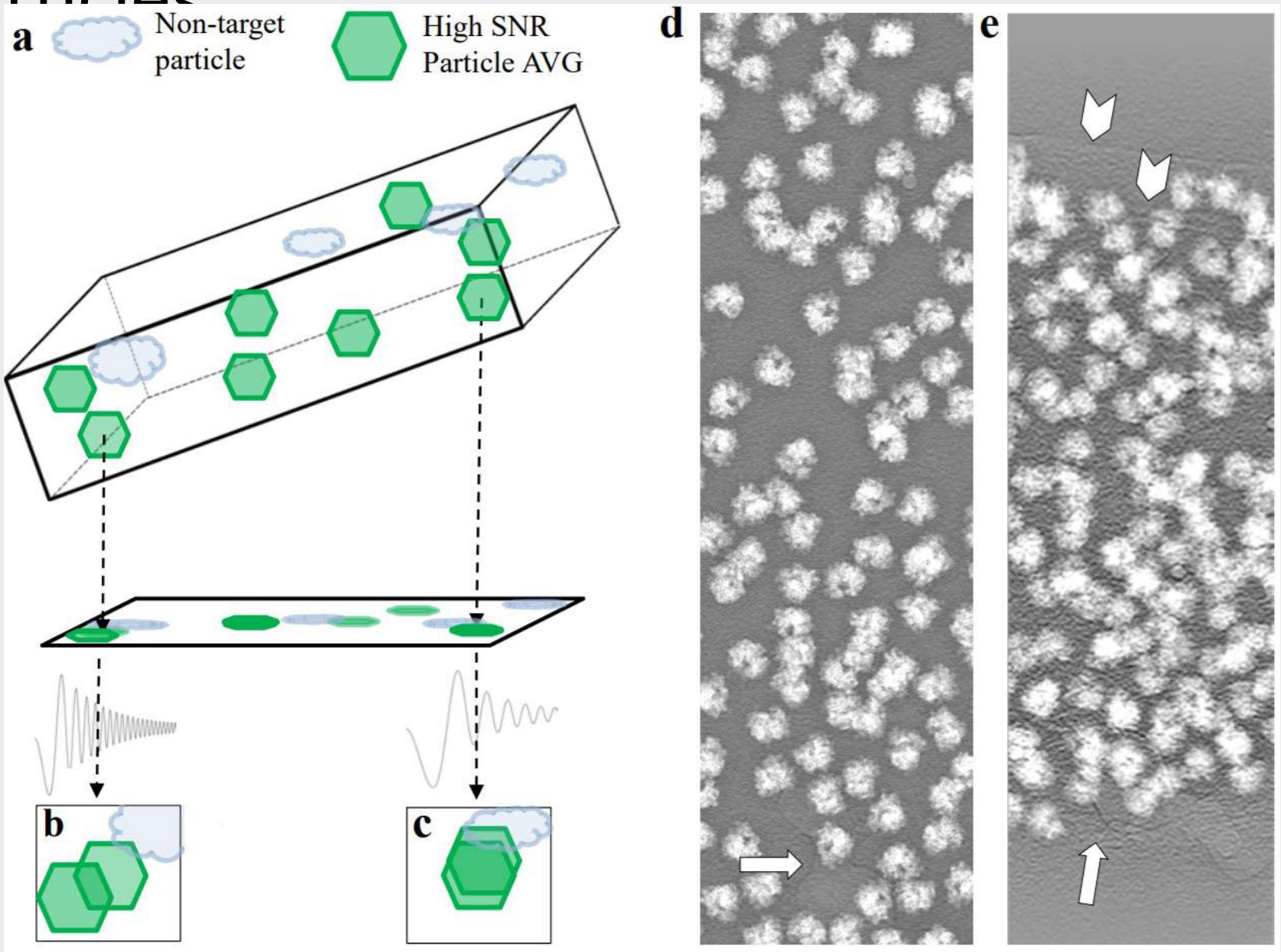
Fernandez et al., 2018

Refining tilt-series alignment by tracking beads in 3D



Fernandez et al., 2018

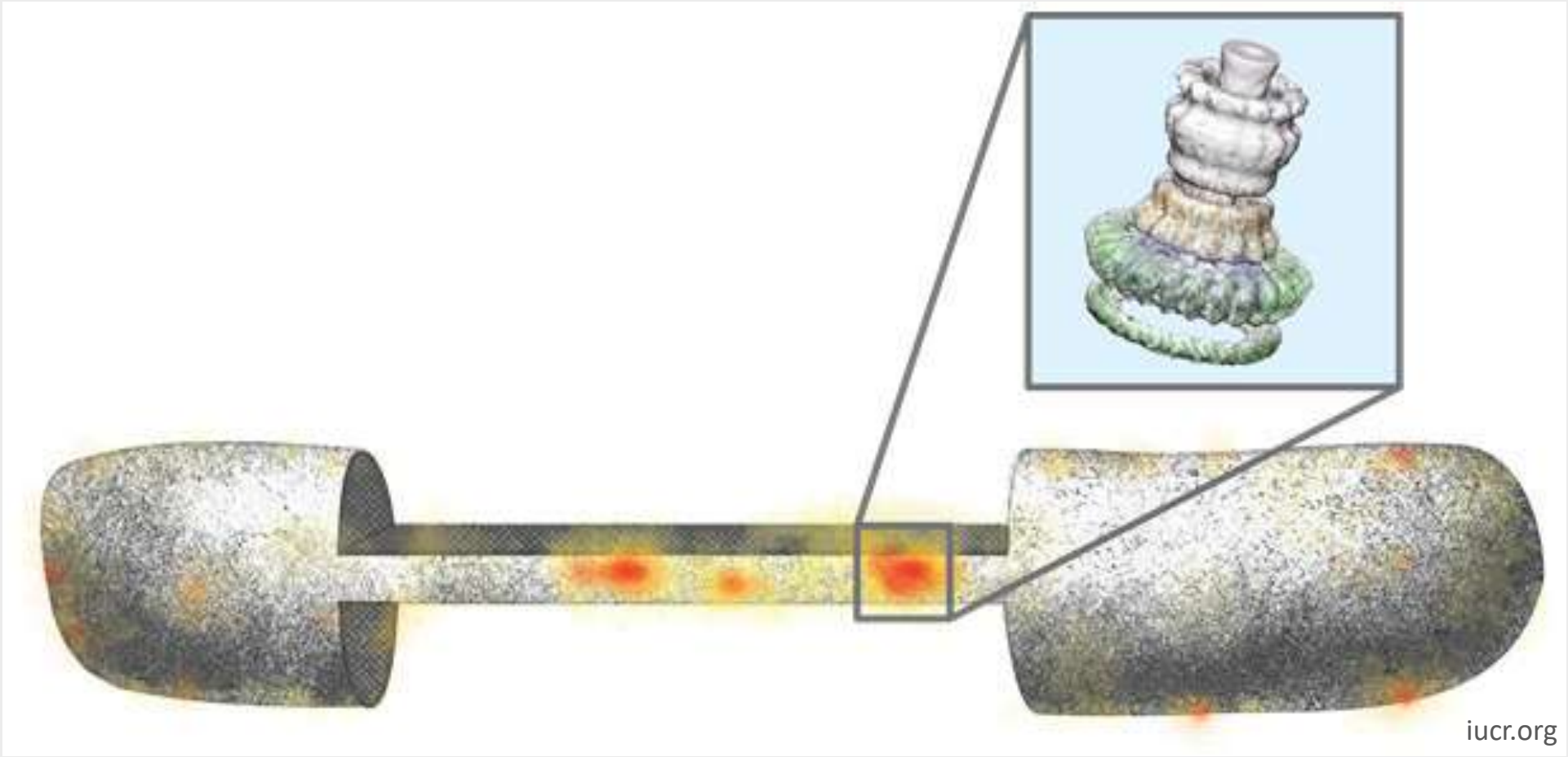
Refining tilt-series alignment by tracking just particles



Himes et al., 2017

Current/future directions in tomography

Future hardware improvements in the field: 3D cryo-CLEM

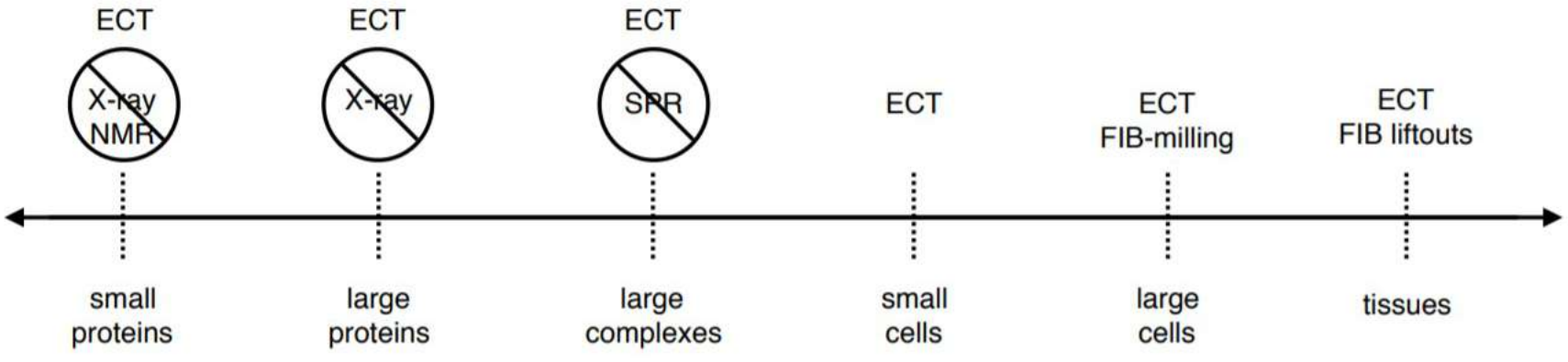


Hardware improvement – Rapid tilting

Nominal magnification	Pixel size (Å)	Exposure time (s)	Total frames	Total time per tilt-series (min)
33kx	4.32	126	5040 or less	9.7
53kx	2.74	50	2000 or less	7.6
81kx	1.78	20	800	6.7
130kx	1.09	12	480	5.0

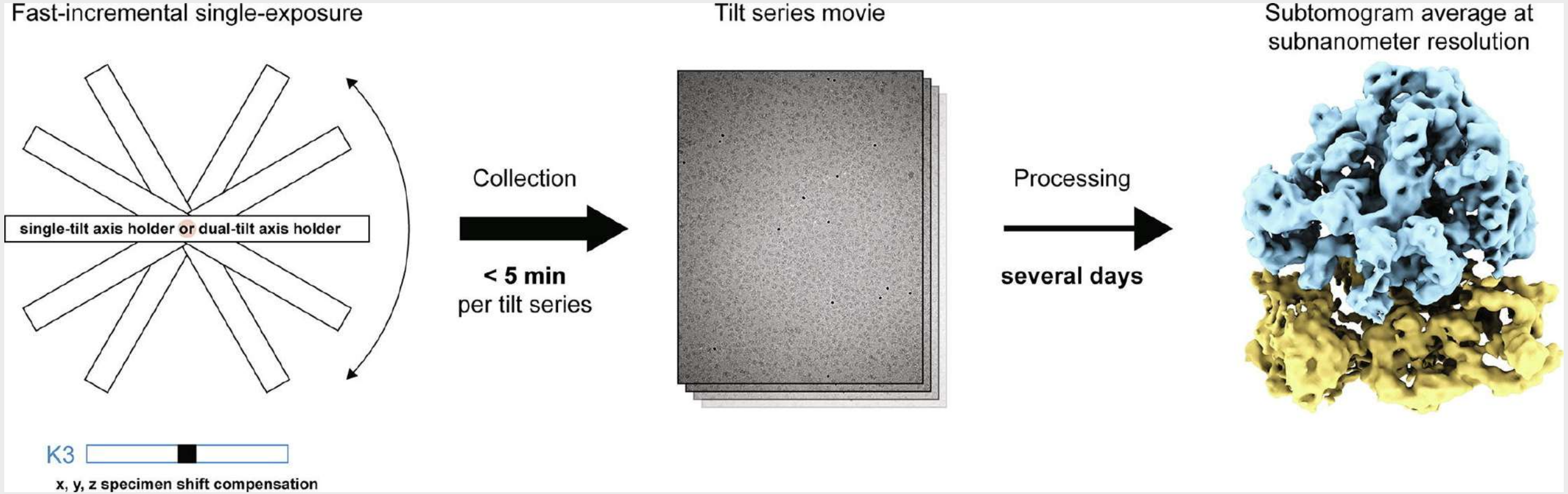
MOSTLY *MOST*

~~ALL~~ cryotomography, ~~ALL~~ the time



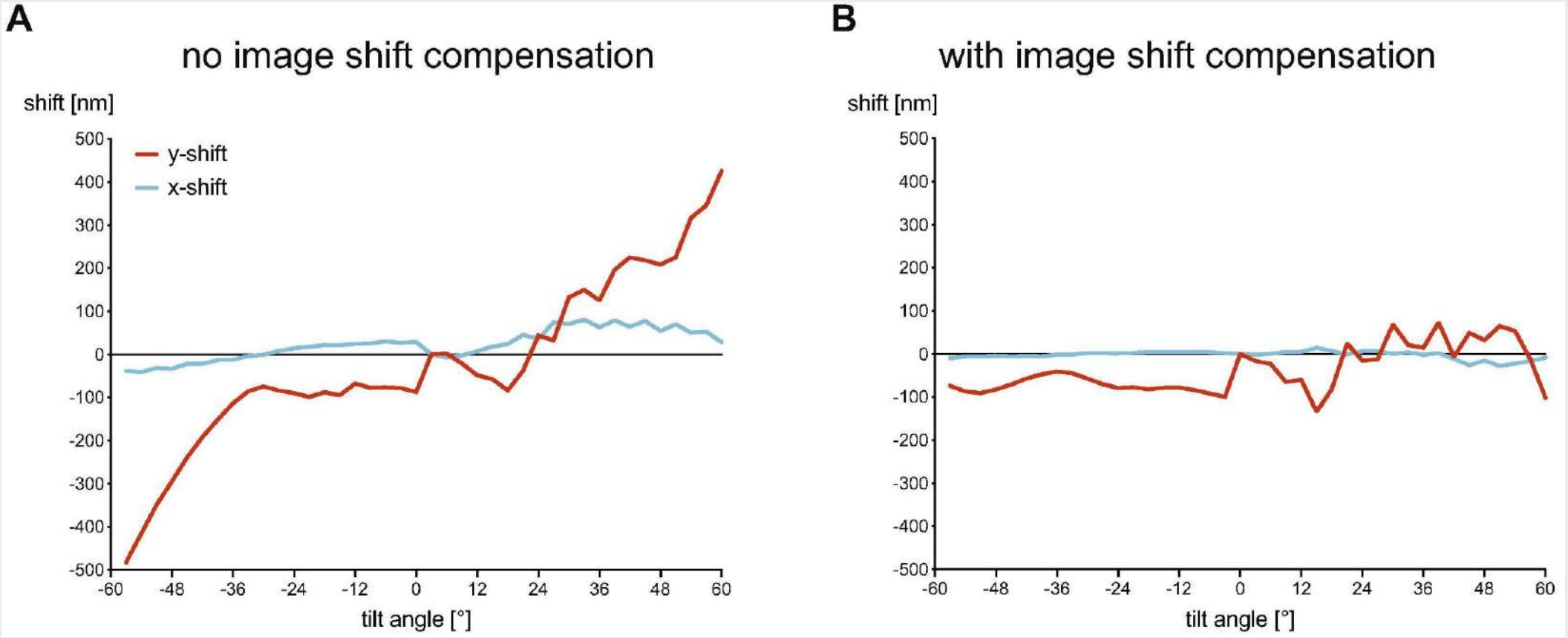
Chreifi et al., 2019

Hardware/software improvement
Pre-calibrated rapid tilting!



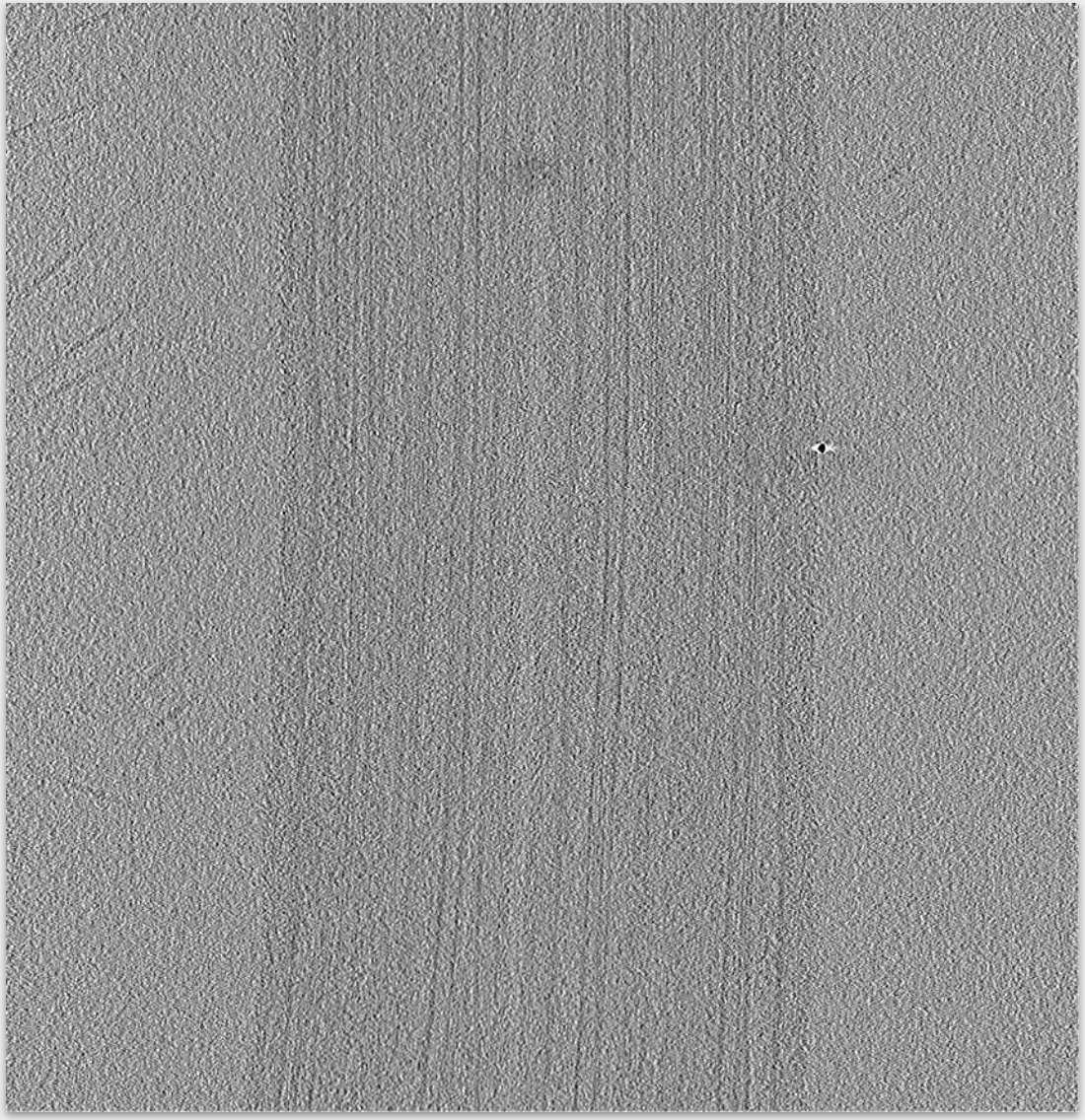
Eisenstein et al., 2019

Software improvements in the field
Pre-calibrated Rapid tilting

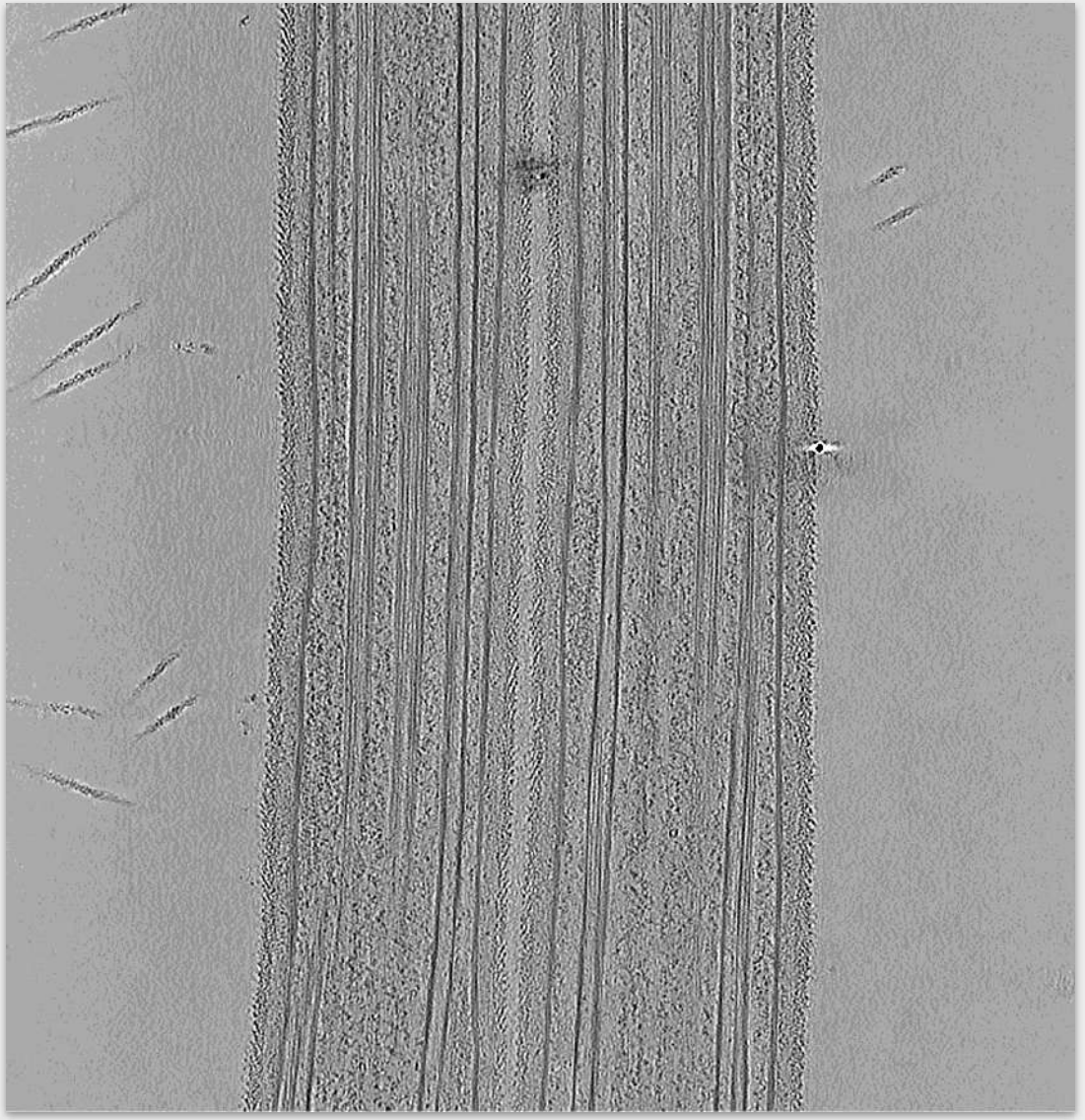


Eisenstein et al. 2019

Post-processing improvement - *Denoising* Cryo-CARE (3D Noise2Noise):



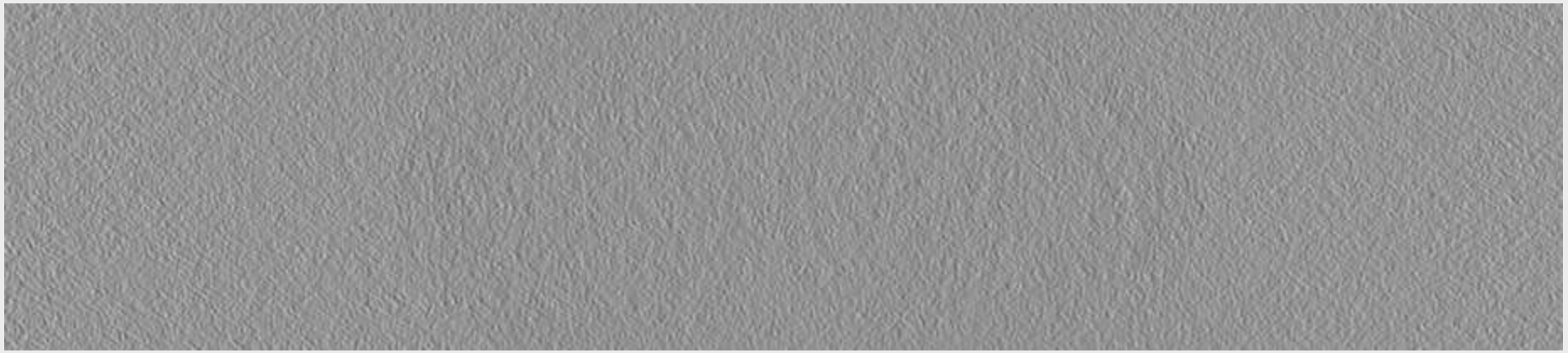
Before



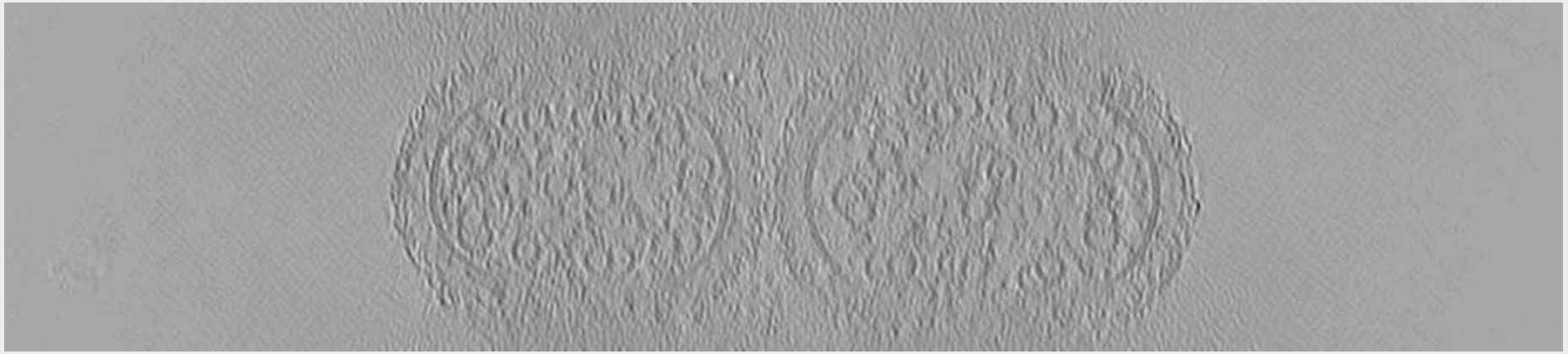
After!

Buchholz et al., 2019

Post-processing improvement - *Denoising* Cryo-CARE (3D Noise2Noise):



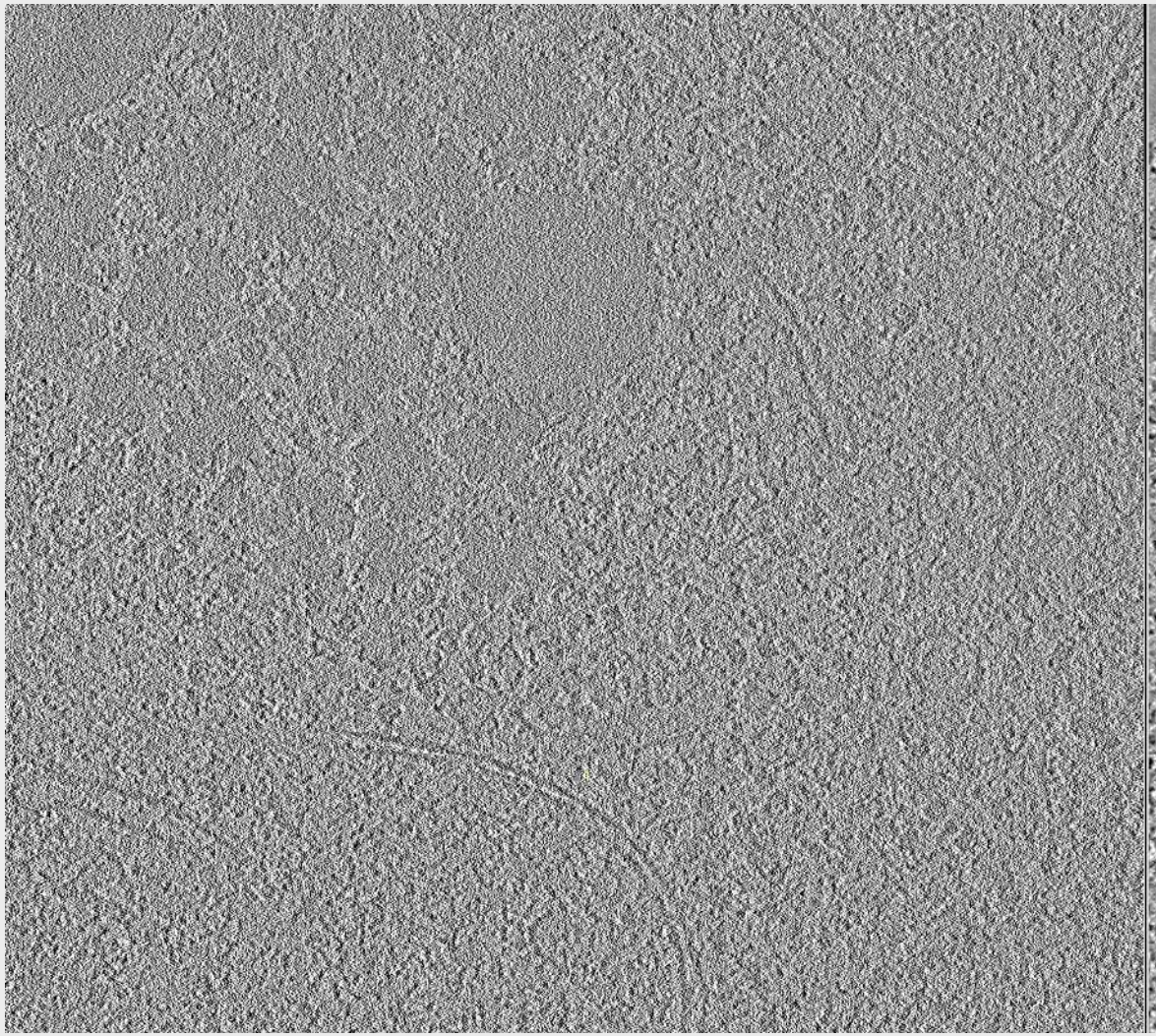
Before

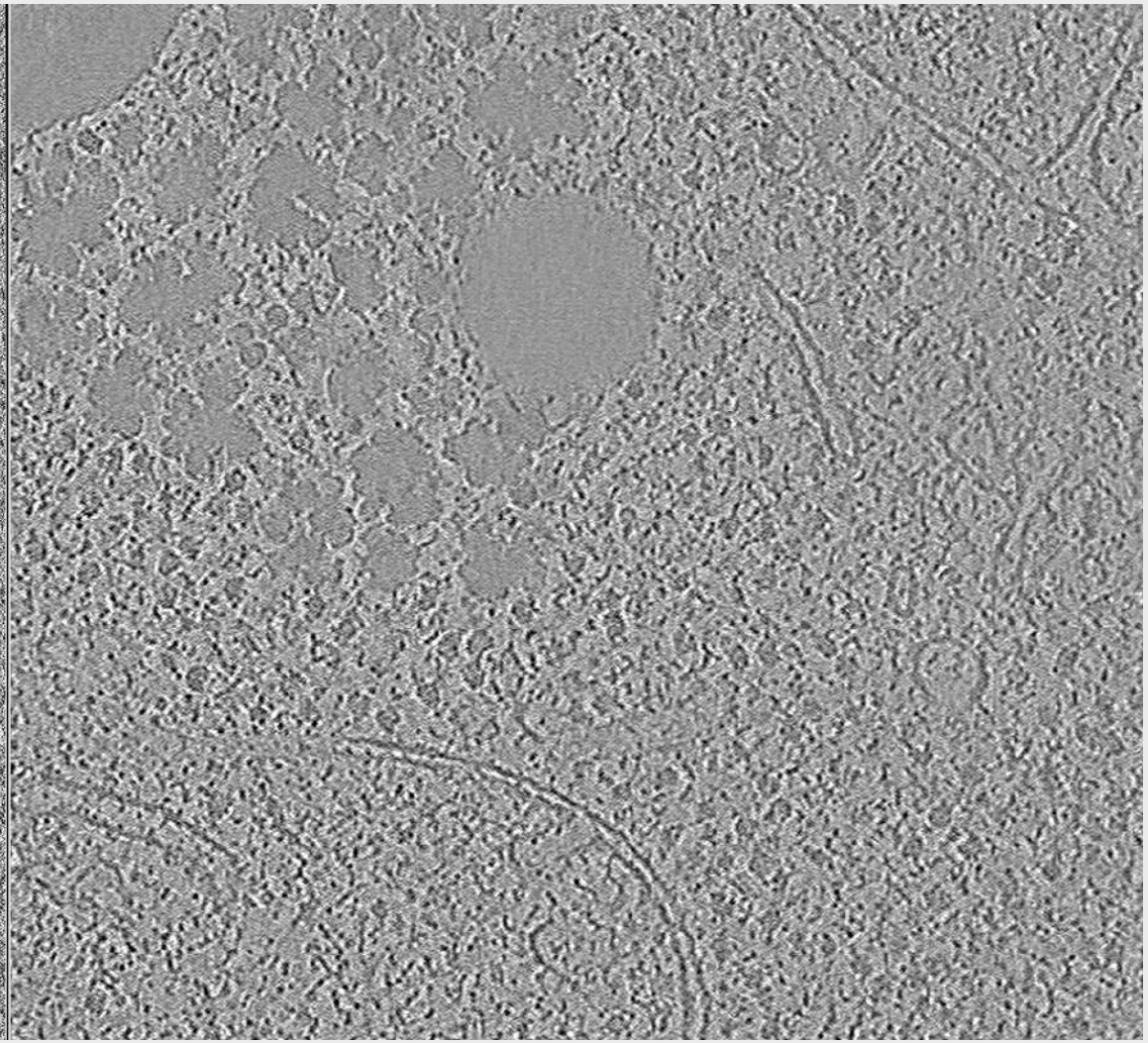


After!

Buchholz et al., 2019

Post-processing improvement - *Denoising*
Topaz (3D Noise2Noise):

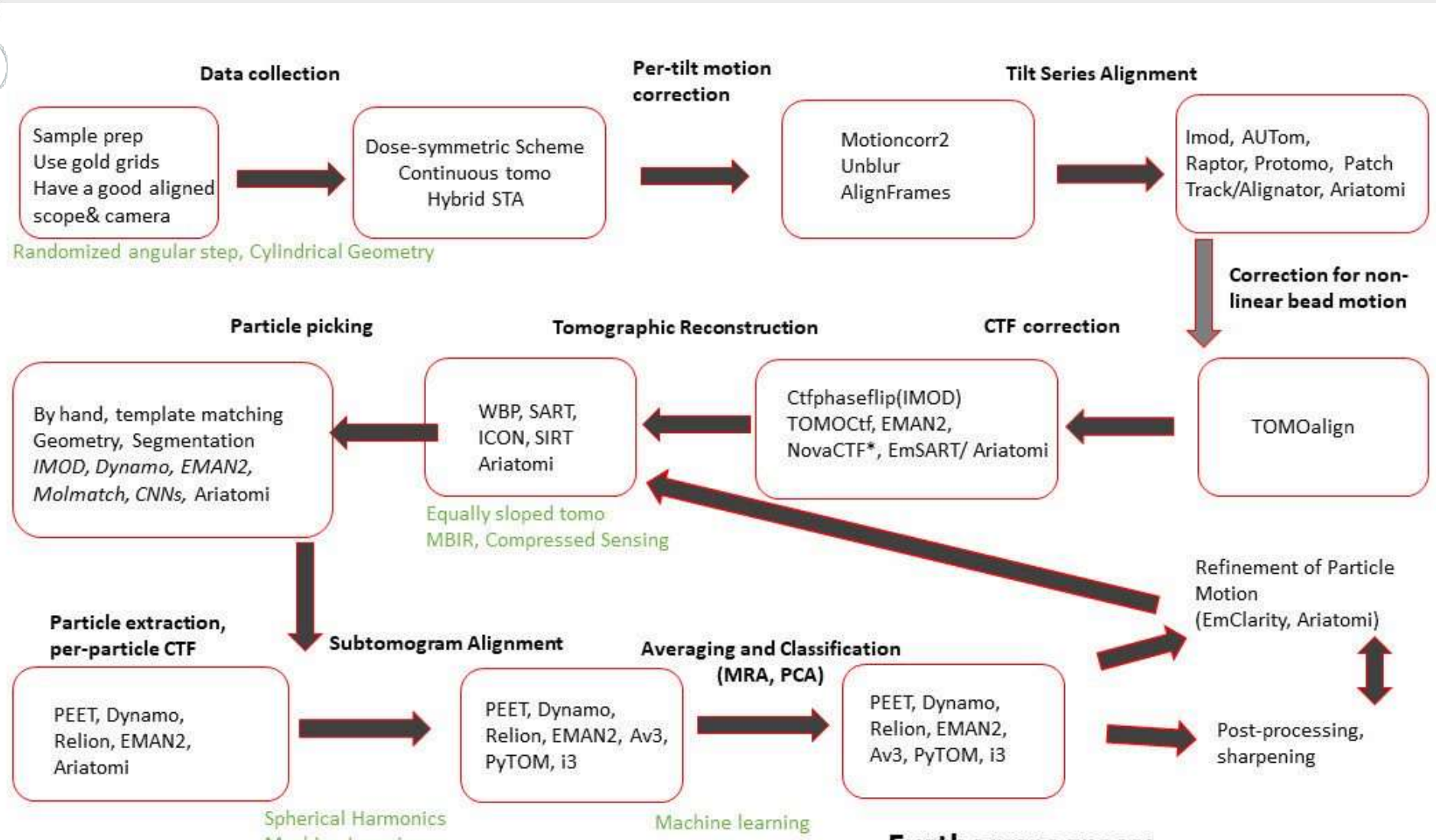




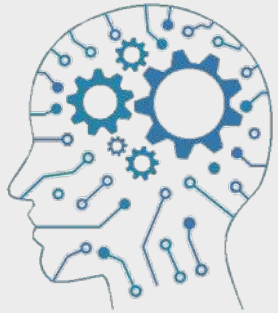
Original

Topaz denoised

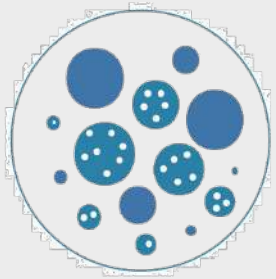
manuscript on bioRxiv



Leigh et al., 2019



Thank you!
Questions?



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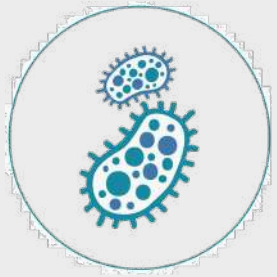

National Resource for Automated Molecular Microscopy
Simons Electron Microscopy Center
New York Structural Biology Center