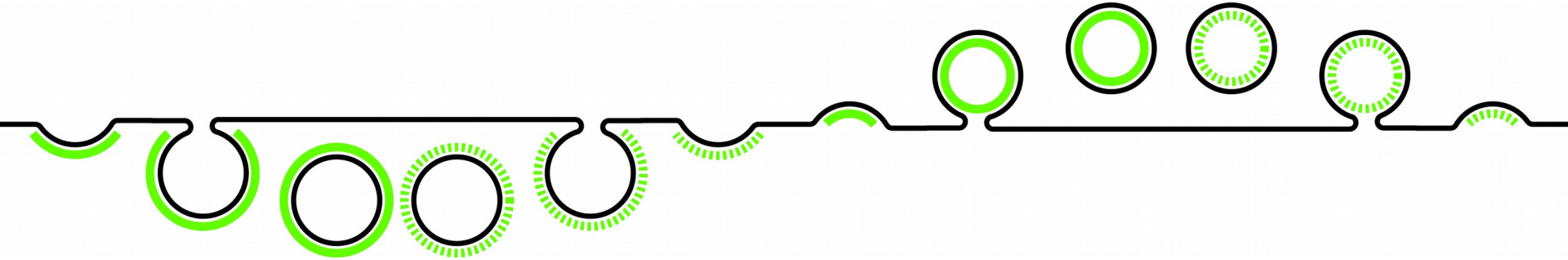


Higher-resolution subtomogram averaging

In situ or in vitro?

Complex systems
Components in situ

Simple systems
Purified components in vitro

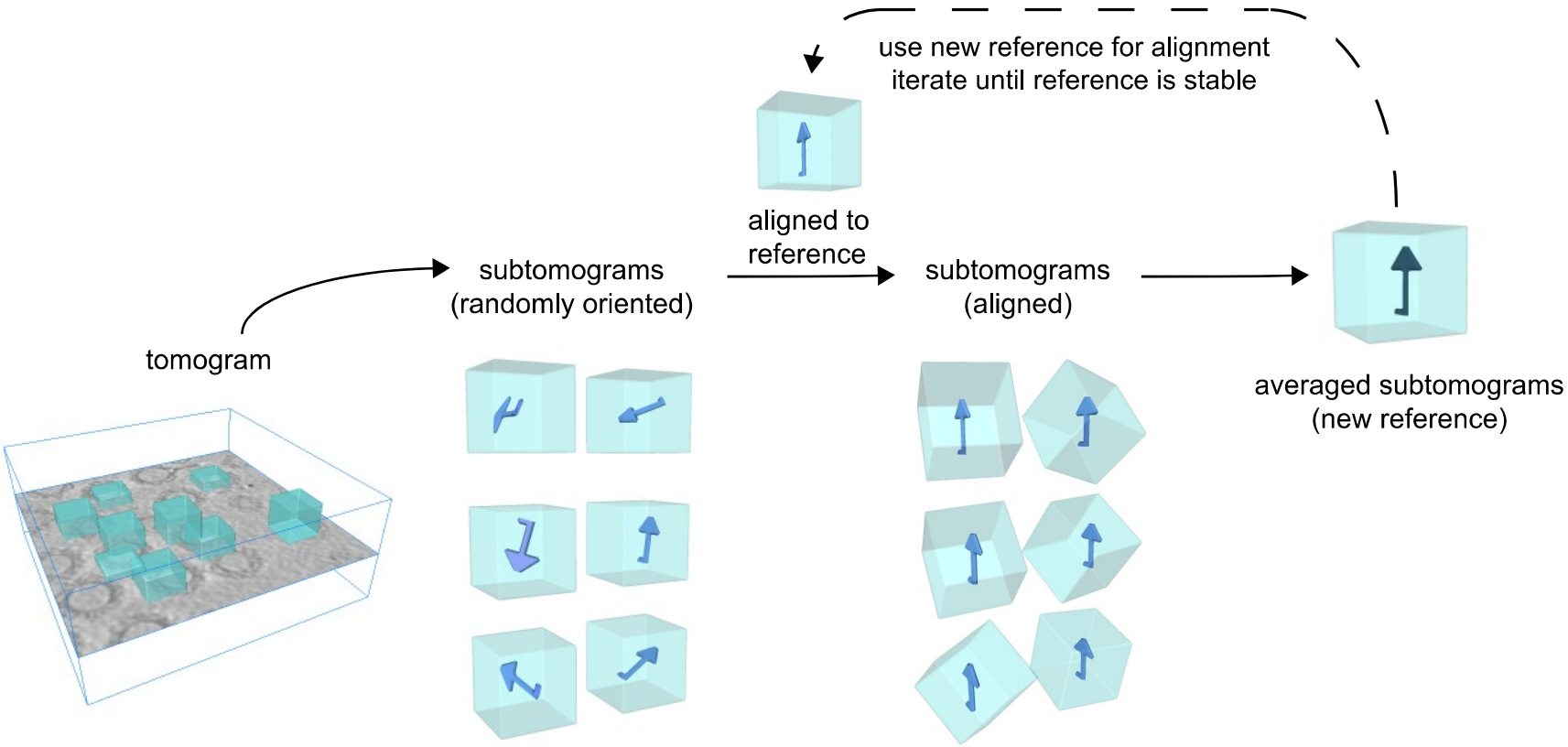
Low-resolution “blobs”

High-resolution detail

Cryo-electron tomography

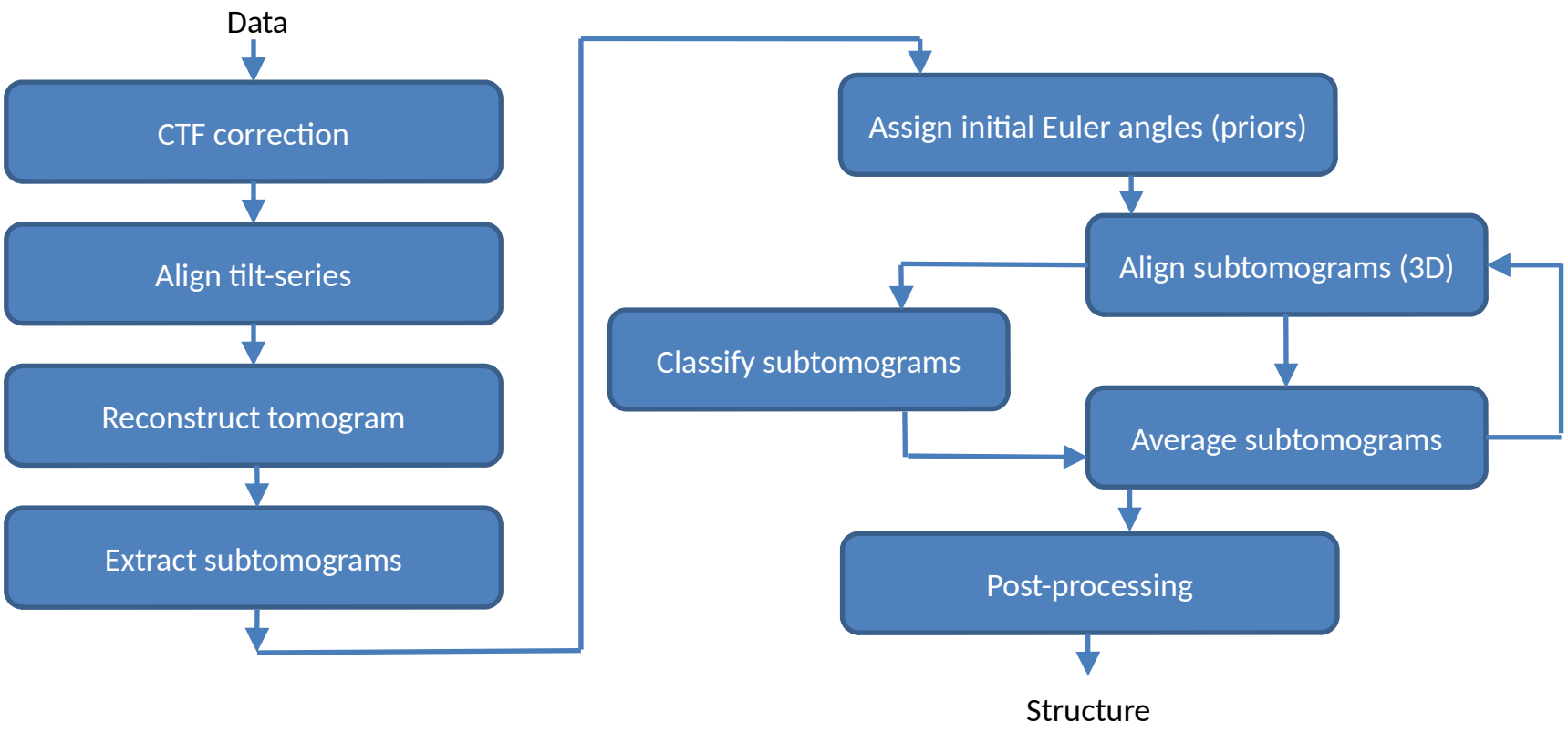
Cryo-electron microscopy

Subtomogram averaging

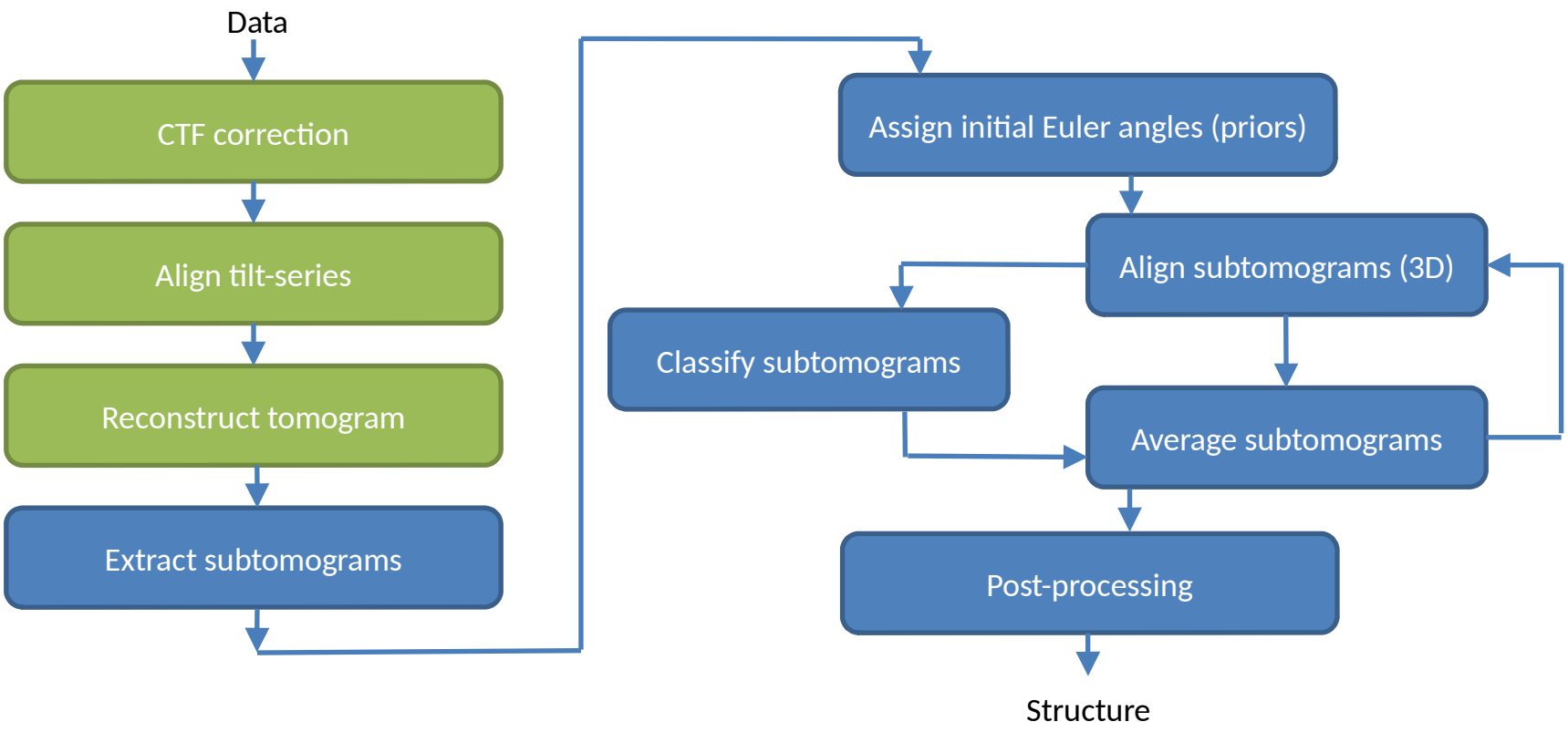


Briggs Curr Opin Struct Biol 2013

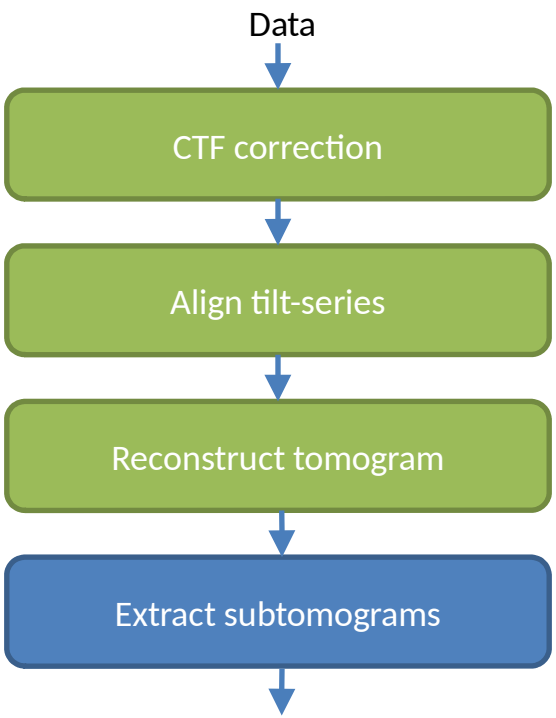
Subtomogram averaging



Subtomogram averaging



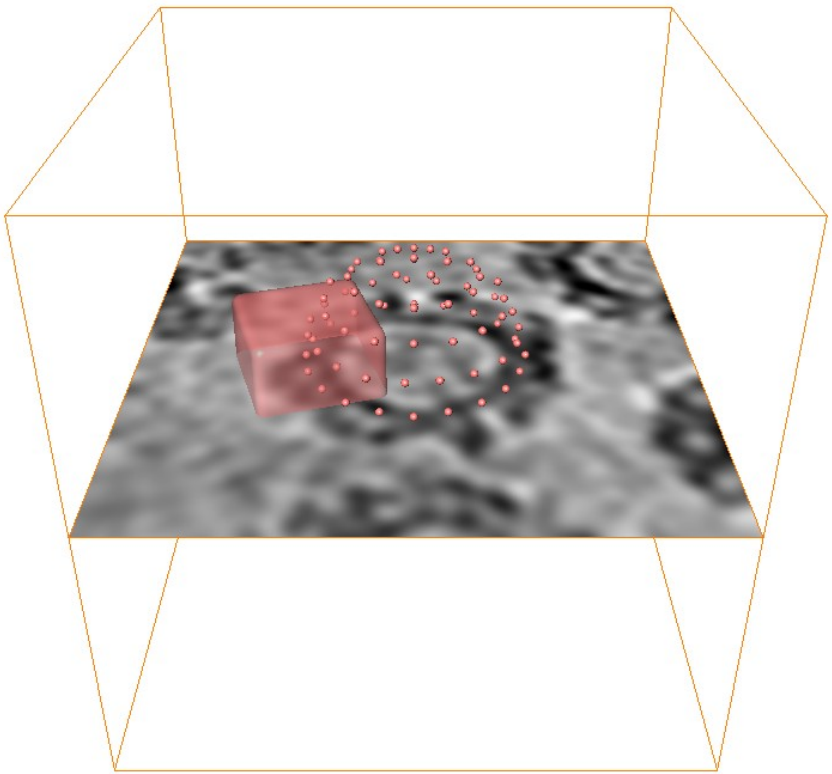
Subtomogram averaging



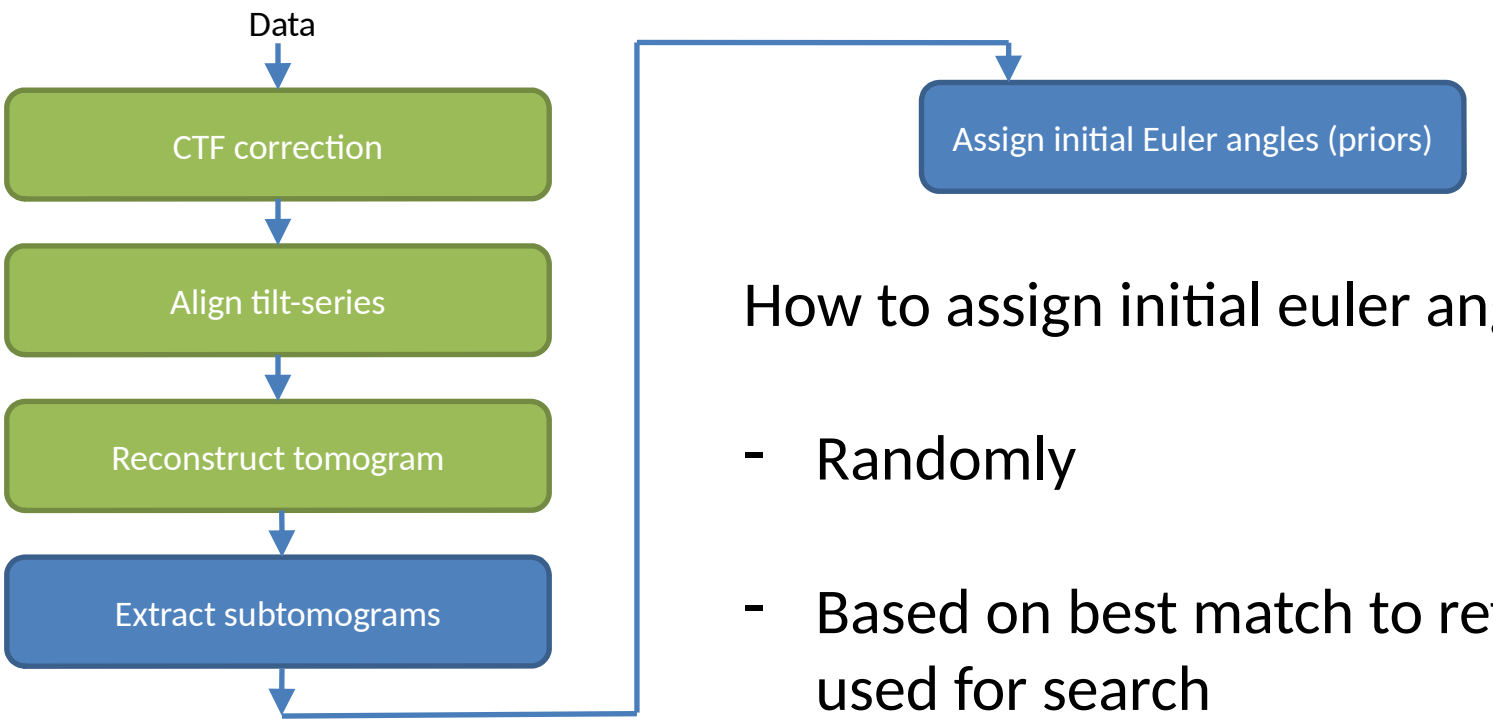
How to decide where to extract your subtomograms?

- Click on them
- Search for them using a reference
- Assign “random” positions based on defined area or shape

Subtomogram averaging



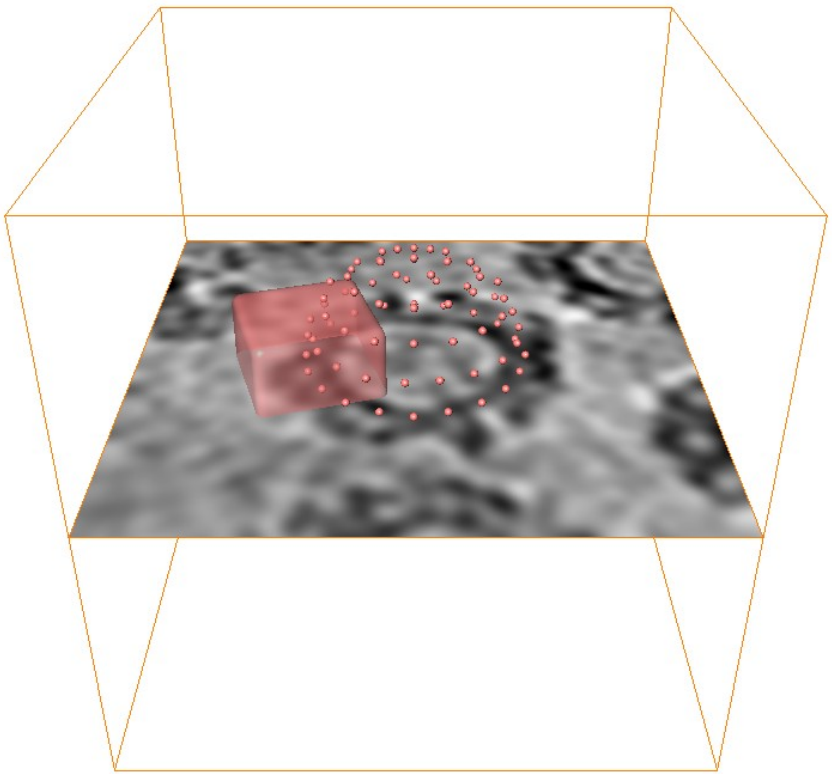
Subtomogram averaging



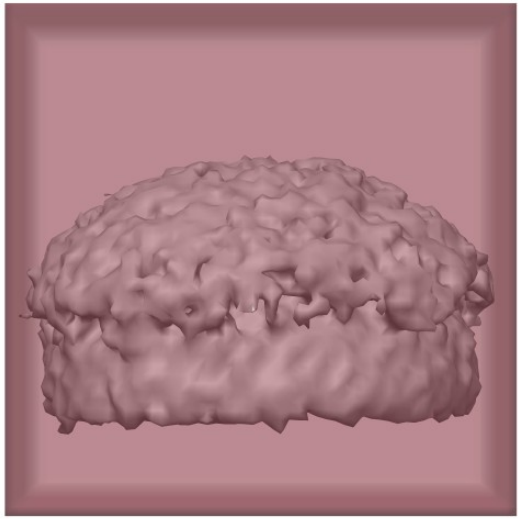
How to assign initial euler angles?

- Randomly
- Based on best match to reference used for search
- Based on prior knowledge of sample geometry

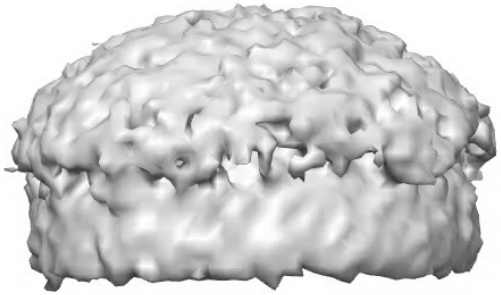
Subtomogram averaging

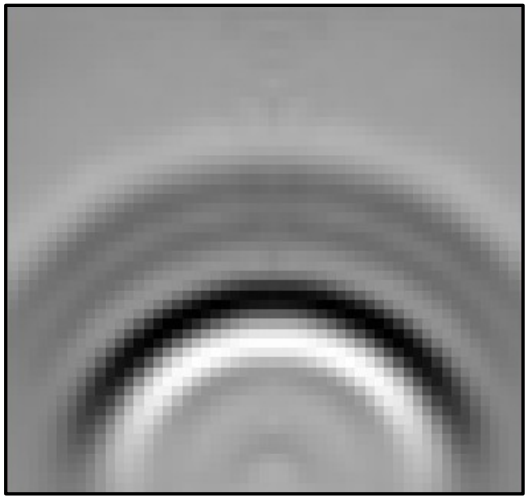


Subtomogram averaging



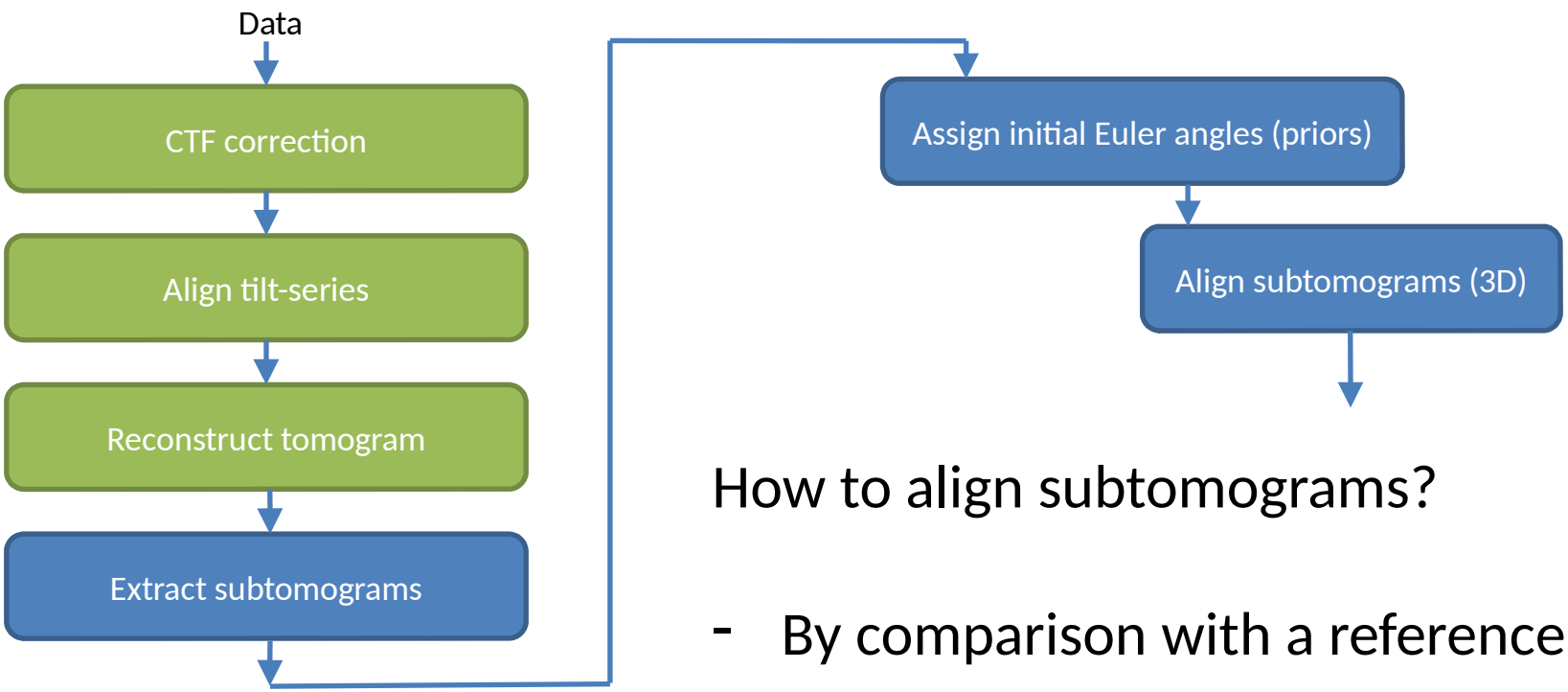
Subtomogram averaging





Radial average

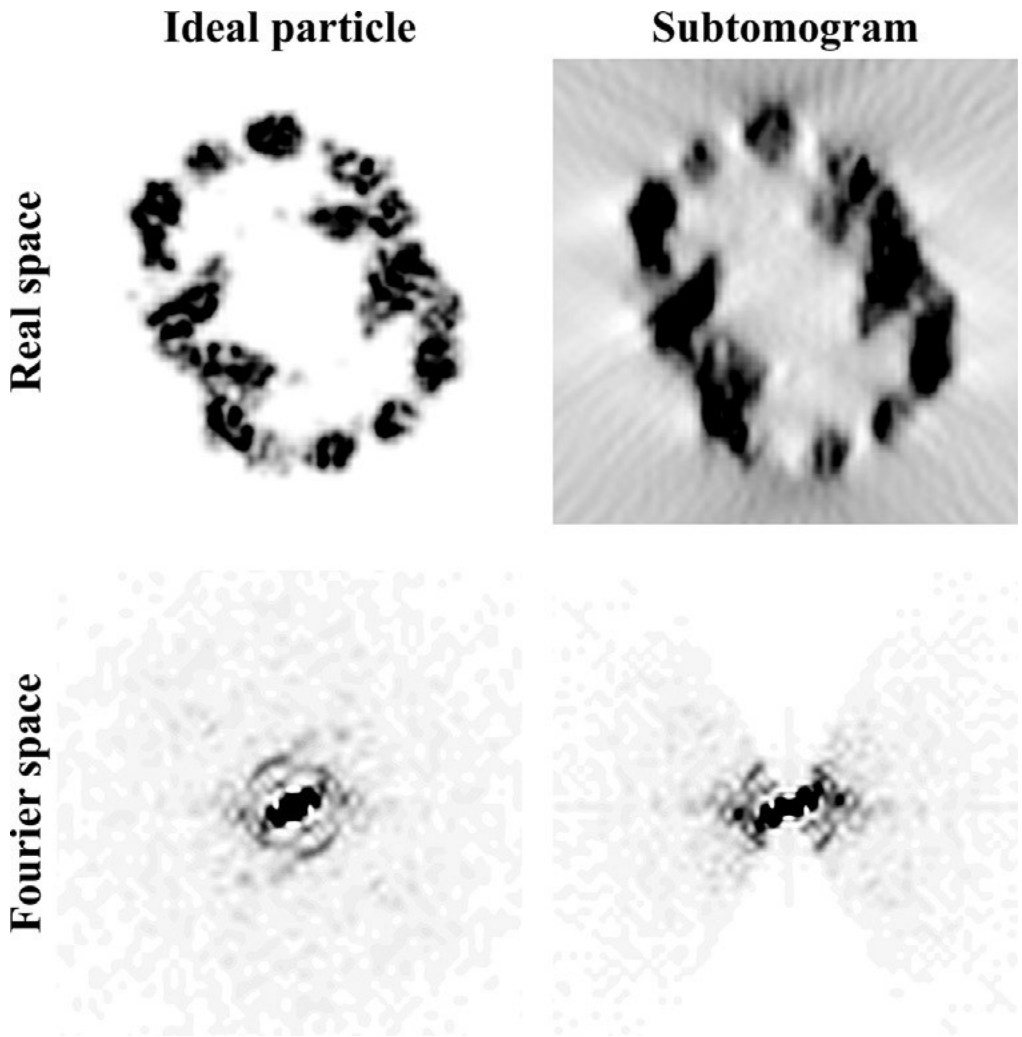
Subtomogram averaging



How to align subtomograms?

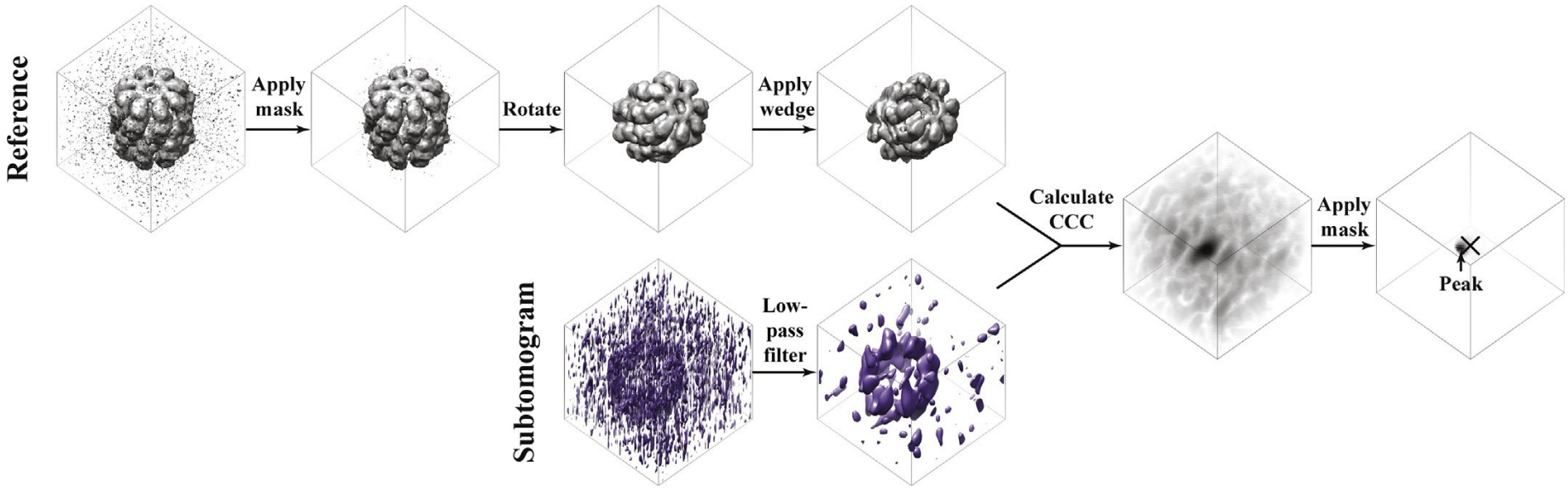
- By comparison with a reference

High resolution subtomogram averaging?



Wan and Briggs 2016

Subtomogram averaging

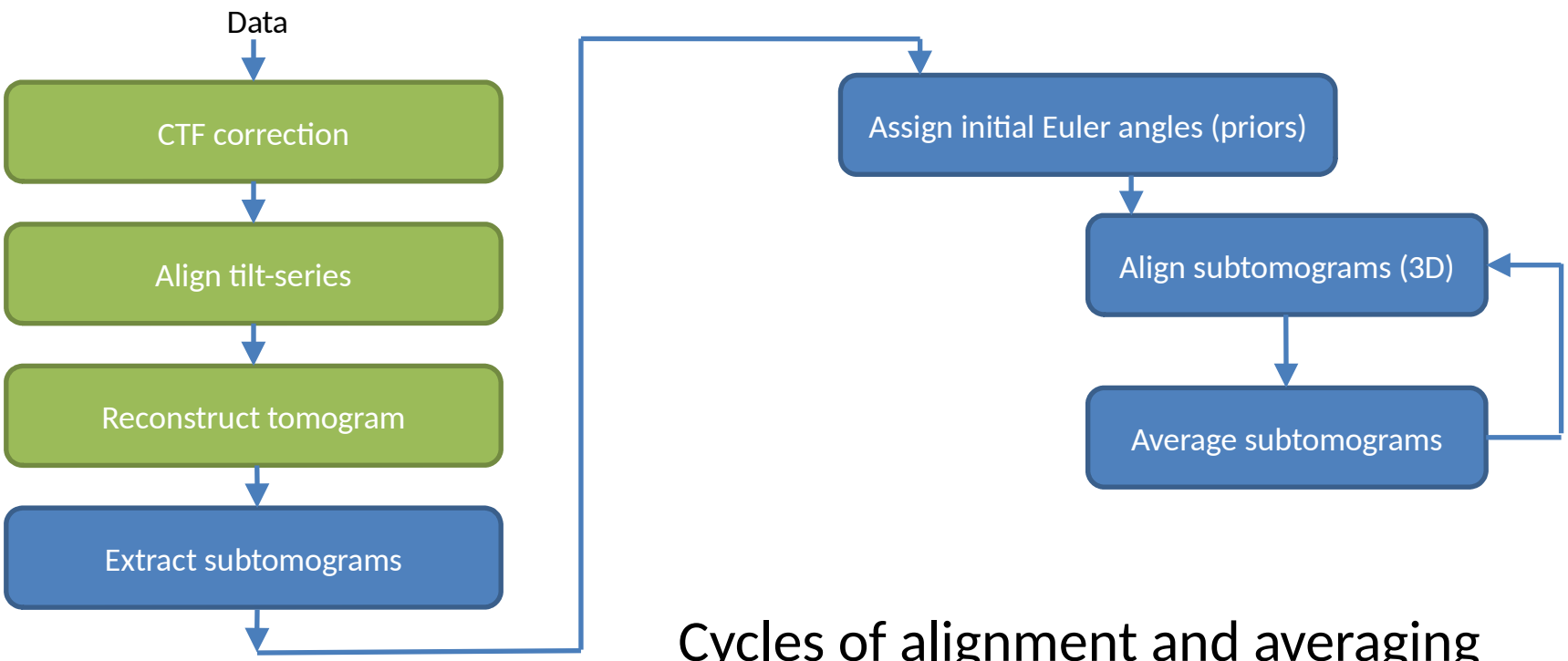


The “classical” missing wedge compensated maximum cross correlation approach

(can replace cross correlation with another metric, and wedge with another wedge)

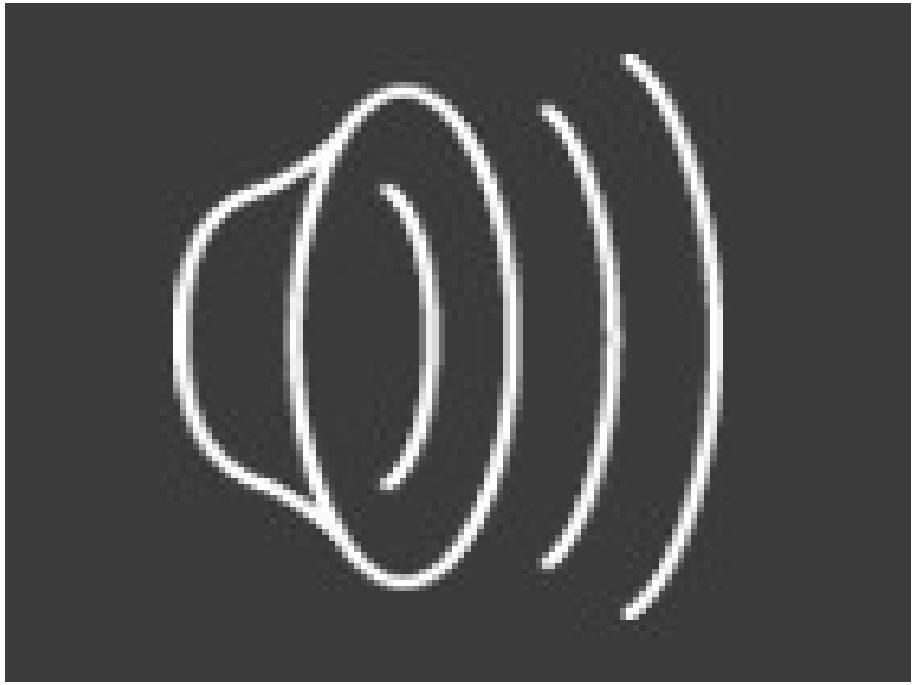
Wan and Briggs 2016

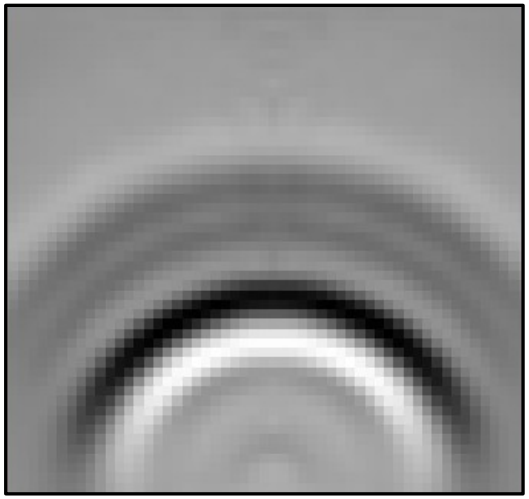
Subtomogram averaging



Cycles of alignment and averaging

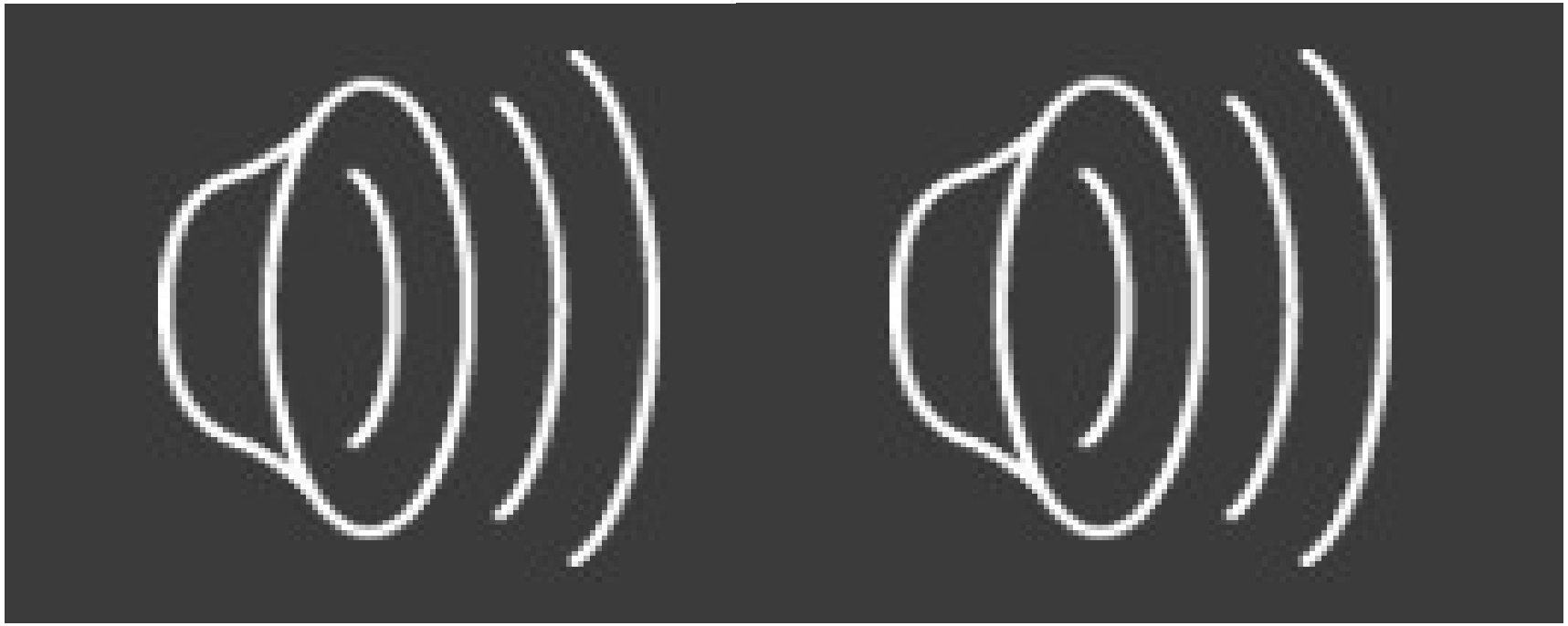
Subtomogram averaging





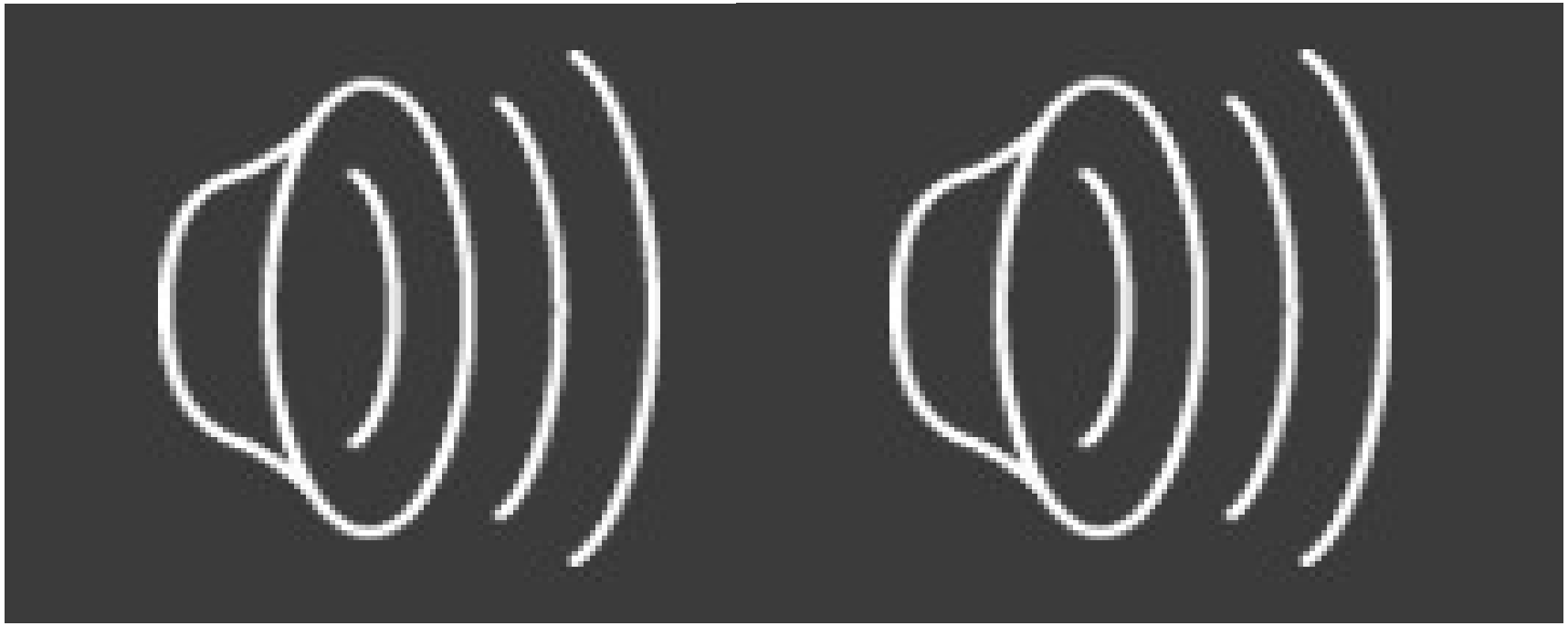
Radial average

Subtomogram averaging

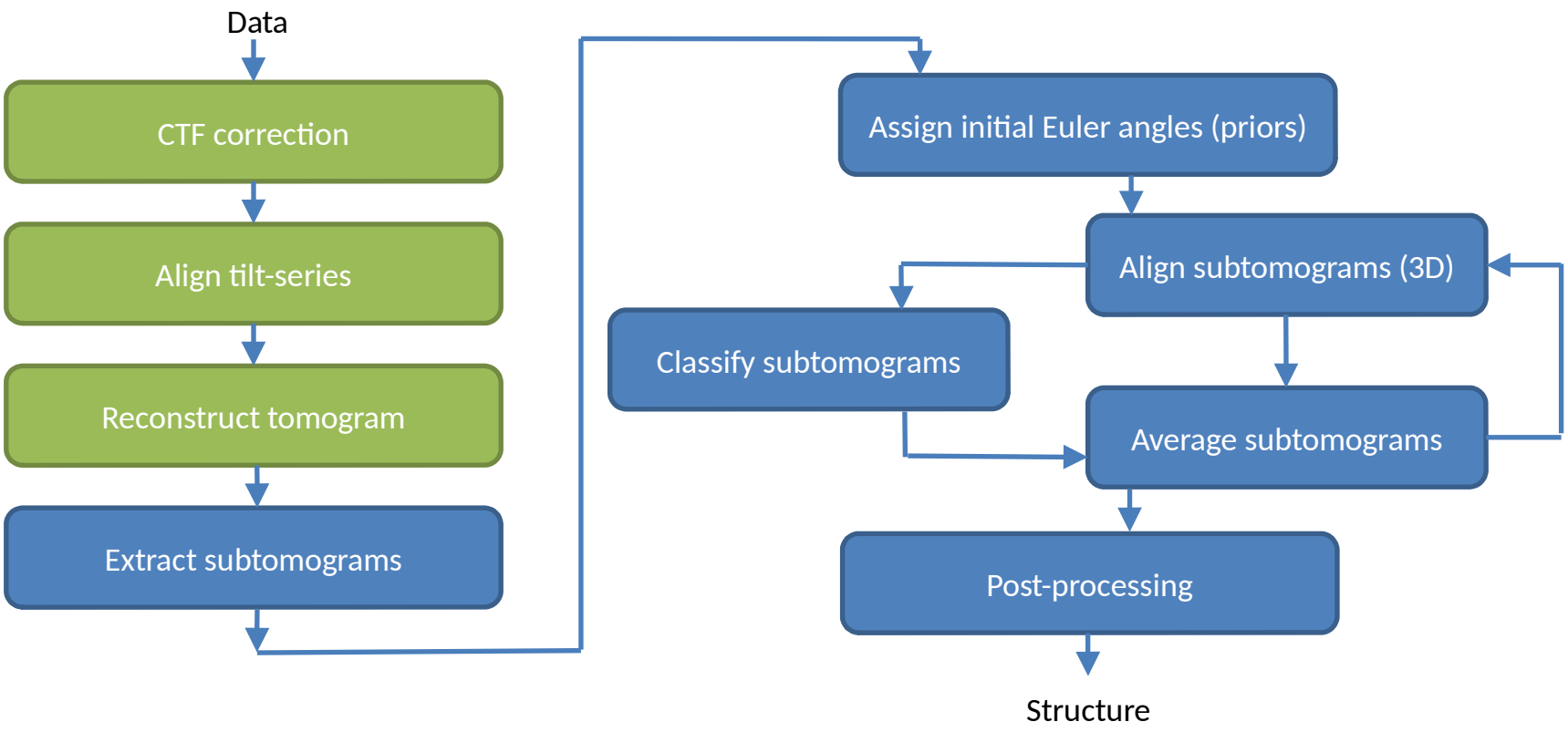


Independent biological replicates

Subtomogram averaging



Subtomogram averaging

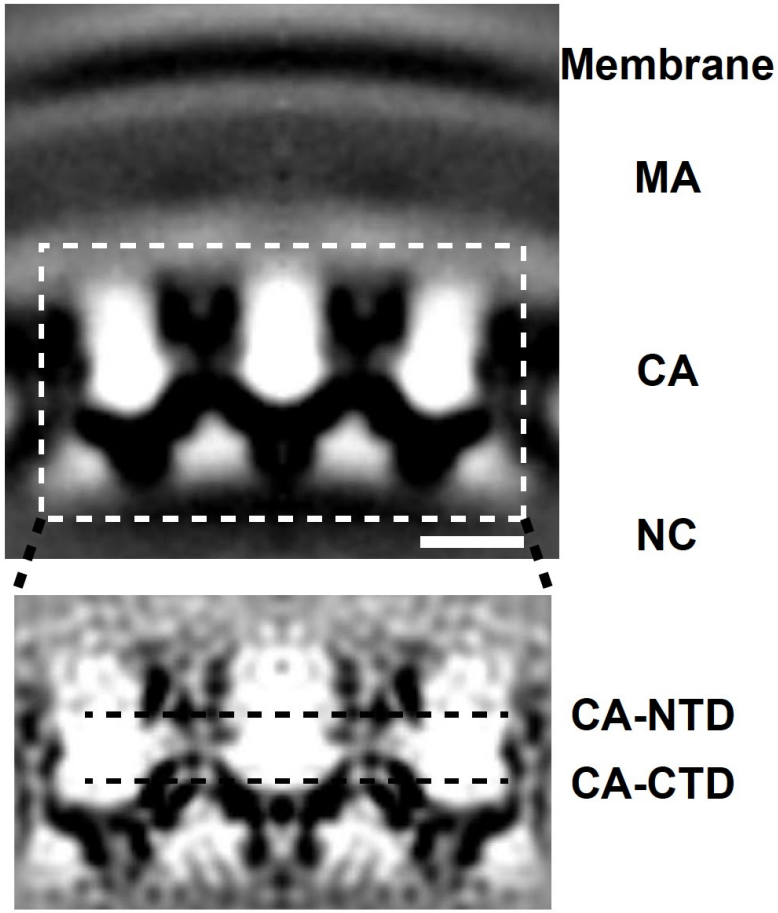


Postprocessing – sharpening, “CTF reweighting”, etc

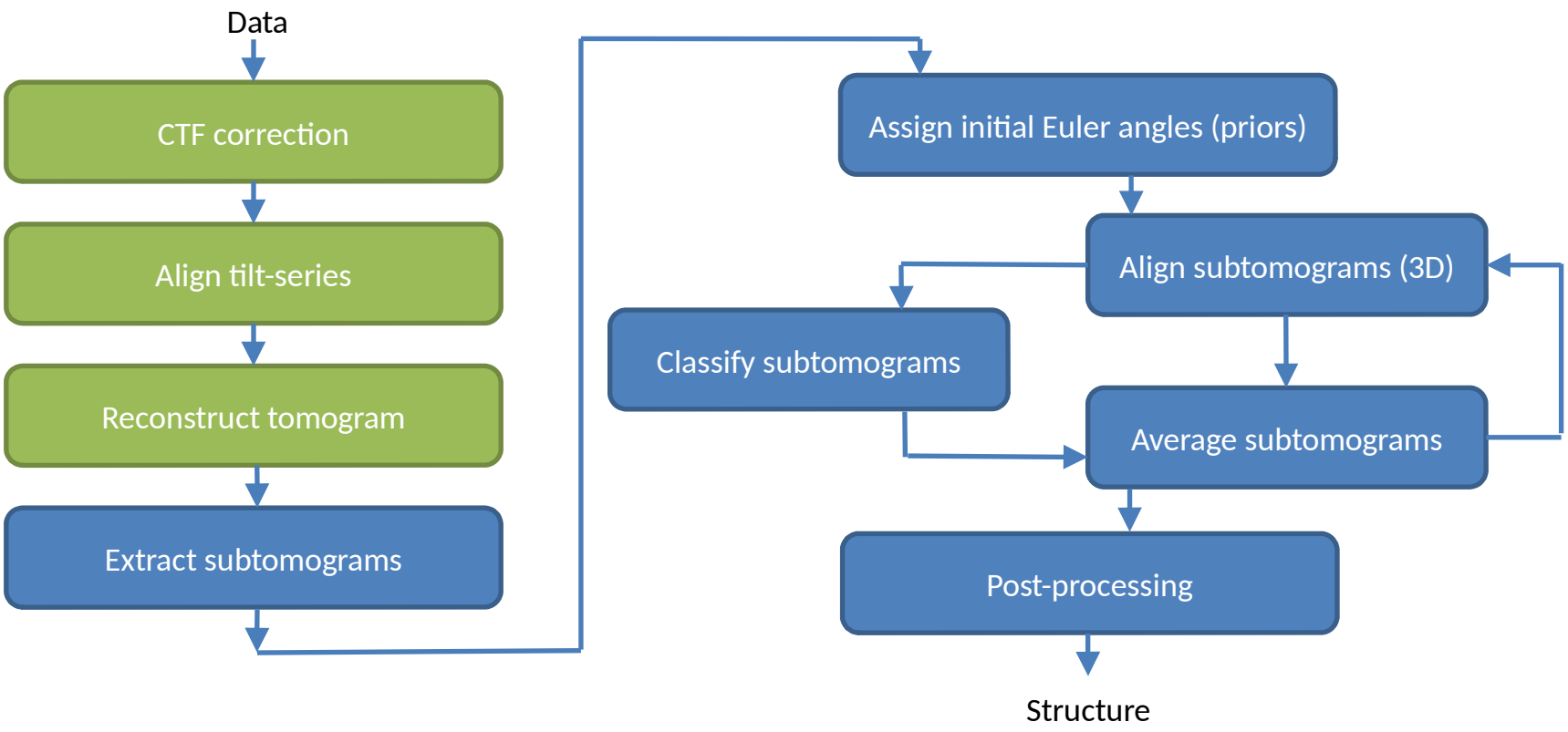
Subtomogram averaging

For example, higher frequencies may be well-measured, but weighted due to the overall transfer function.

Sharpening corrects for that.

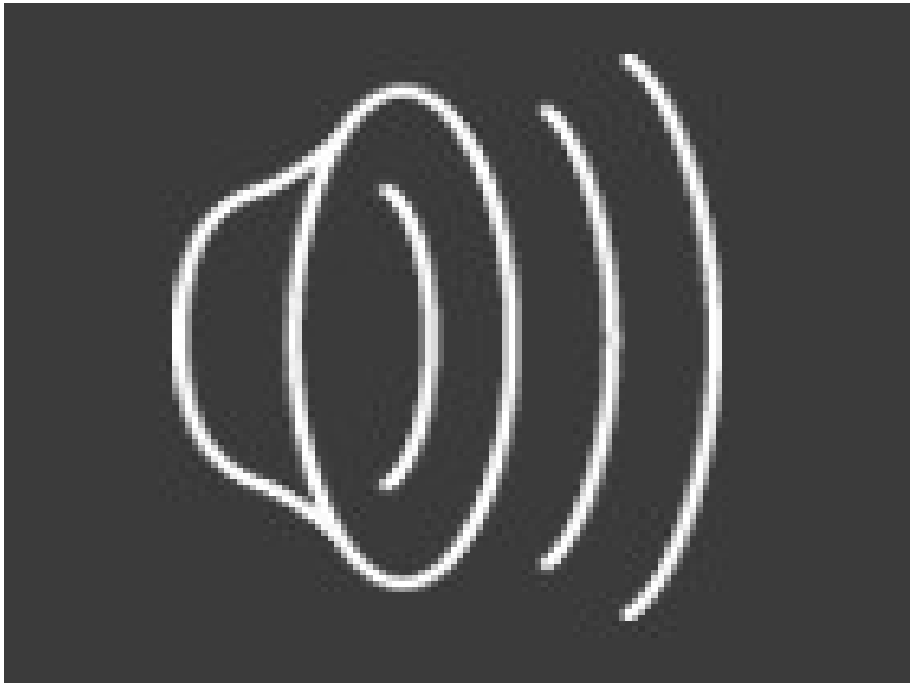


Subtomogram averaging



After subtomogram averaging we have more than just the structure

COPI vesicle coat



We can revisit and interpret the positions of aligned subtomograms

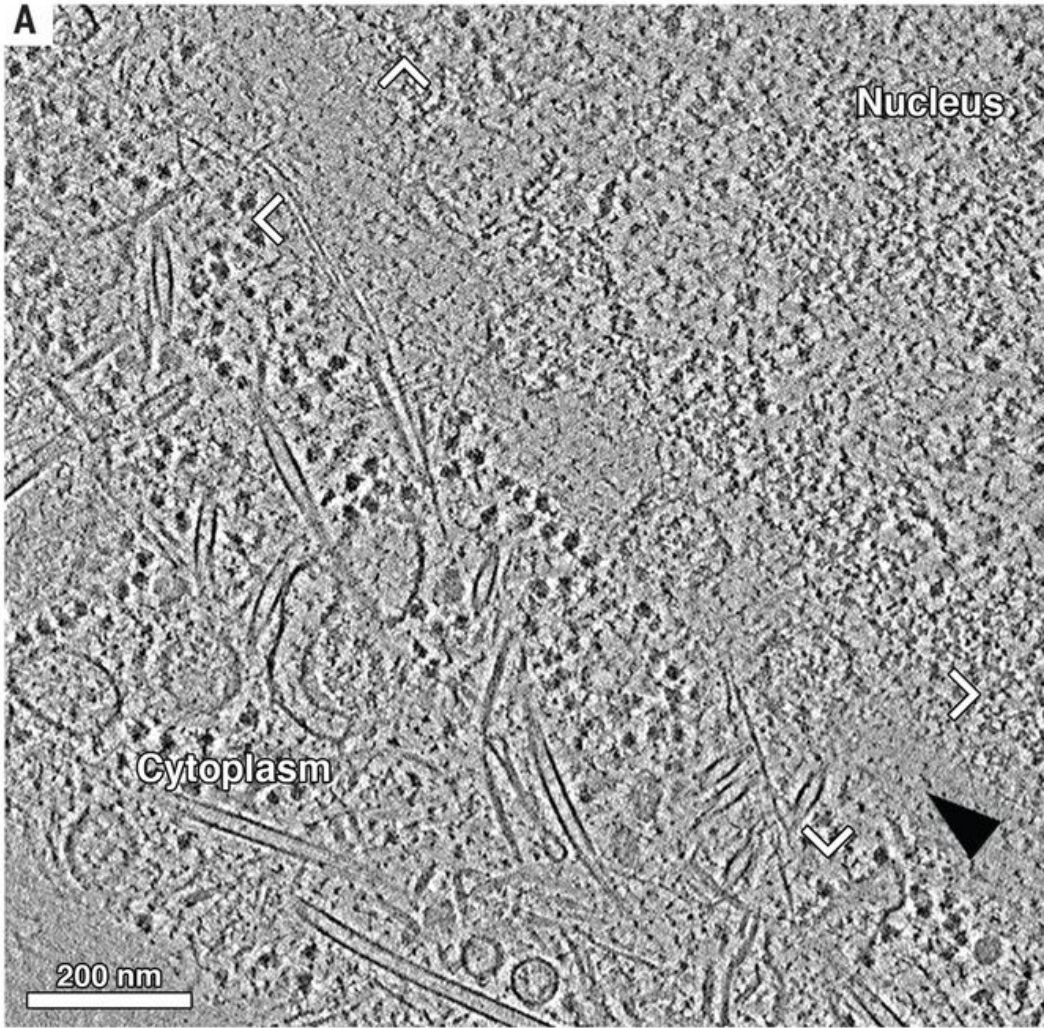
What limits the resolution of subtomo structures?
(Why is it typically lower than that of single particle structures?)

Challenges related to the sample

Sample complexity, heterogeneity, flexibility

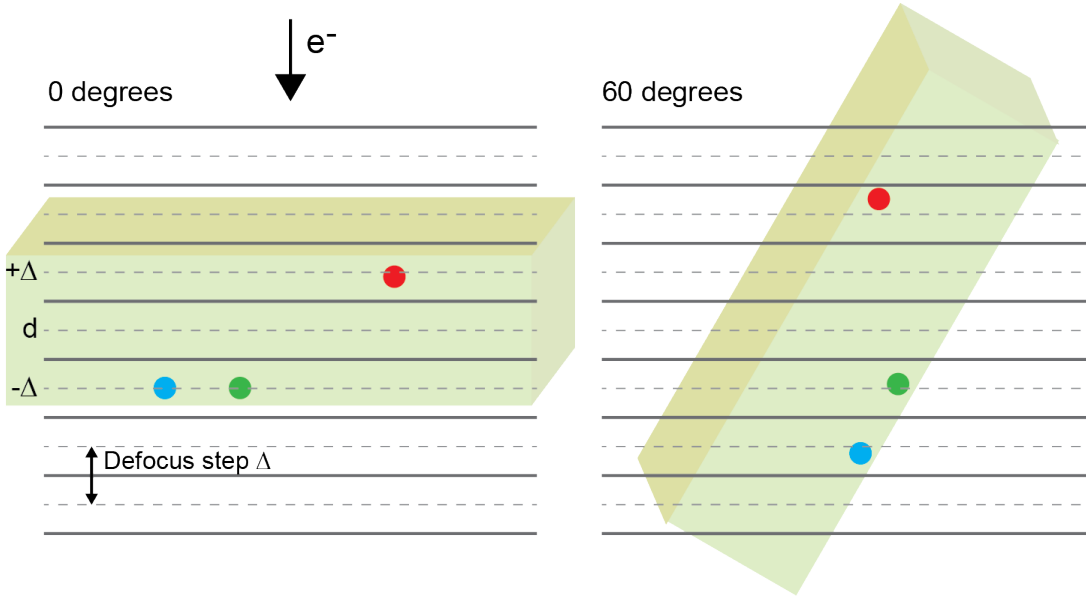
Challenges related to the sample

Sample complexity, heterogeneity, flexibility



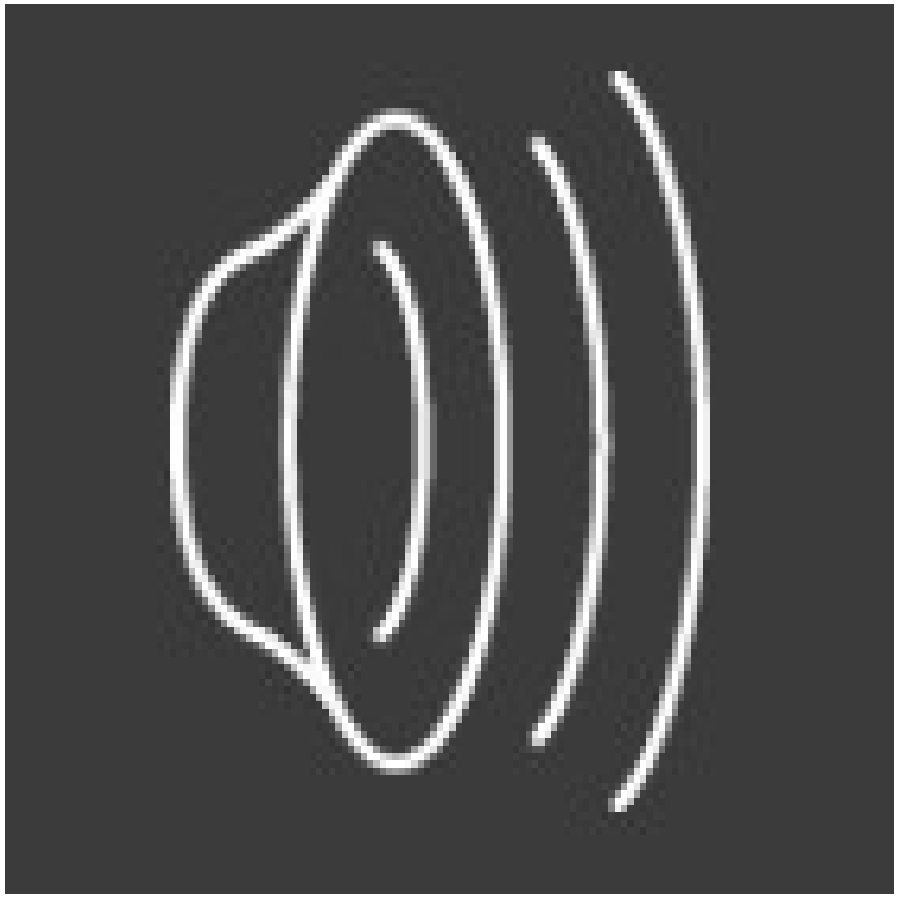
Challenges in data collection

Higher apparent sample thickness (especially at tilt)



Challenges in data collection

Higher apparent sample thickness (especially at tilt)

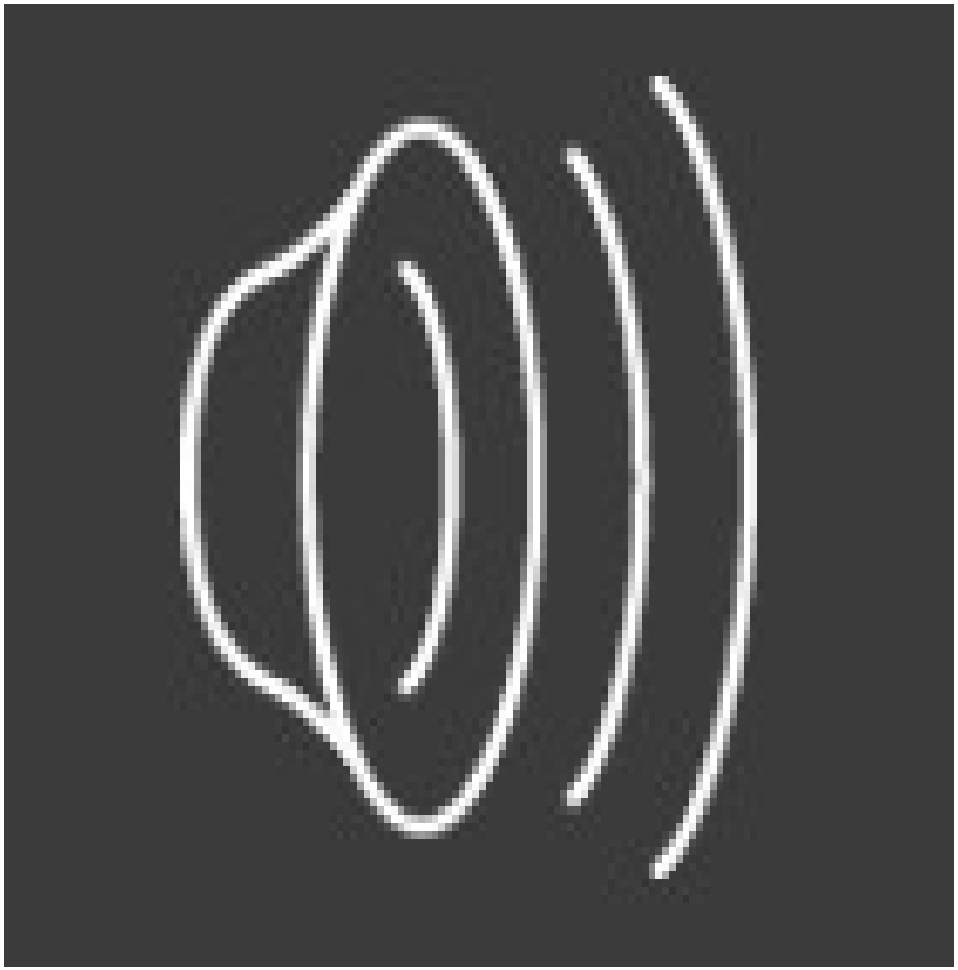


Challenges in data collection

Sample changes during data collection

There are changes in the sample as dose accumulates:

thinning
distortions
charge build-up



Wim Hagen

e.g.
Wright et al. (2006) J. Struct. Biol,. 153, 241-252
McMullan et al. (2015) Ultramicroscopy, 158, 26-32
and many more!

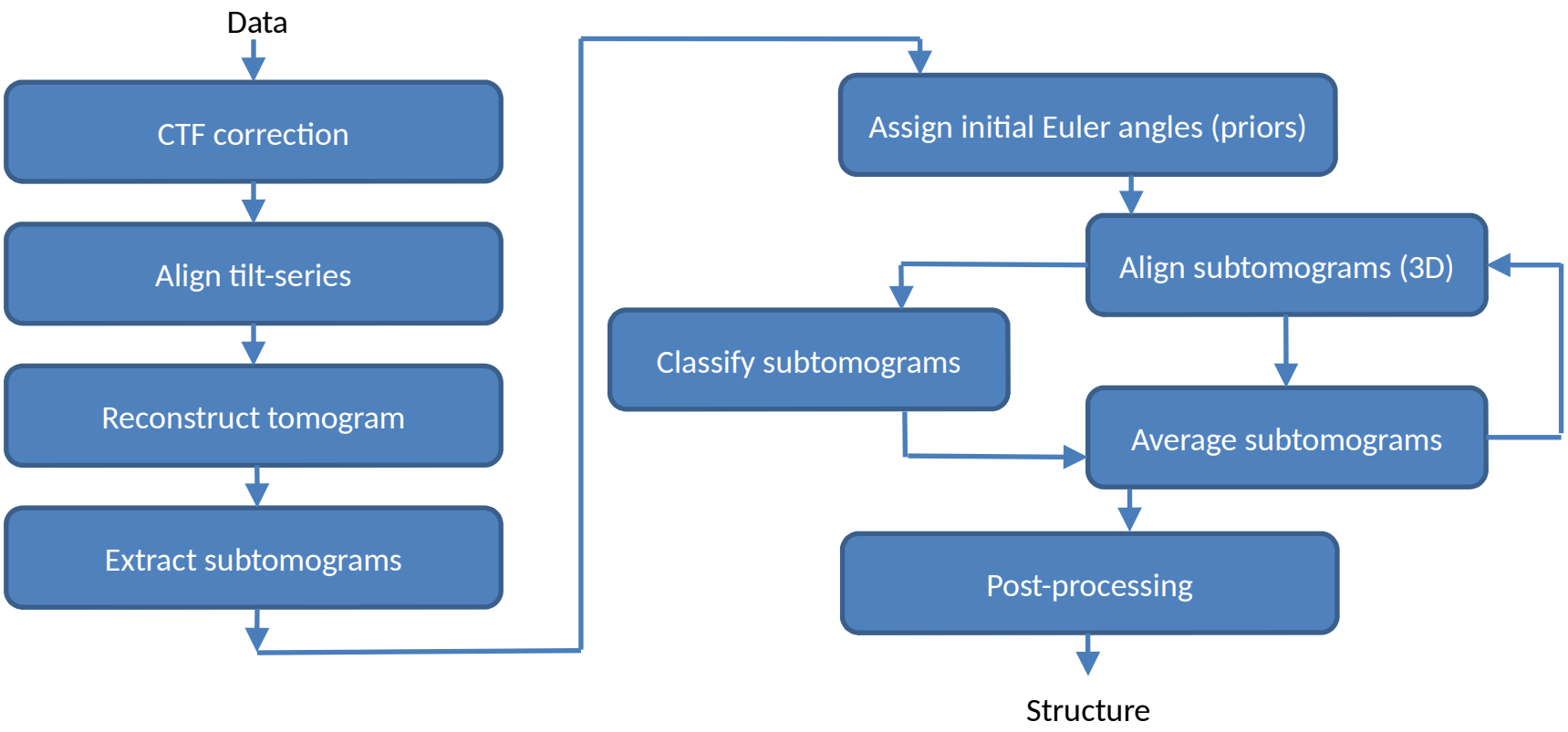
Challenges in data collection

Increased sample movement at tilt

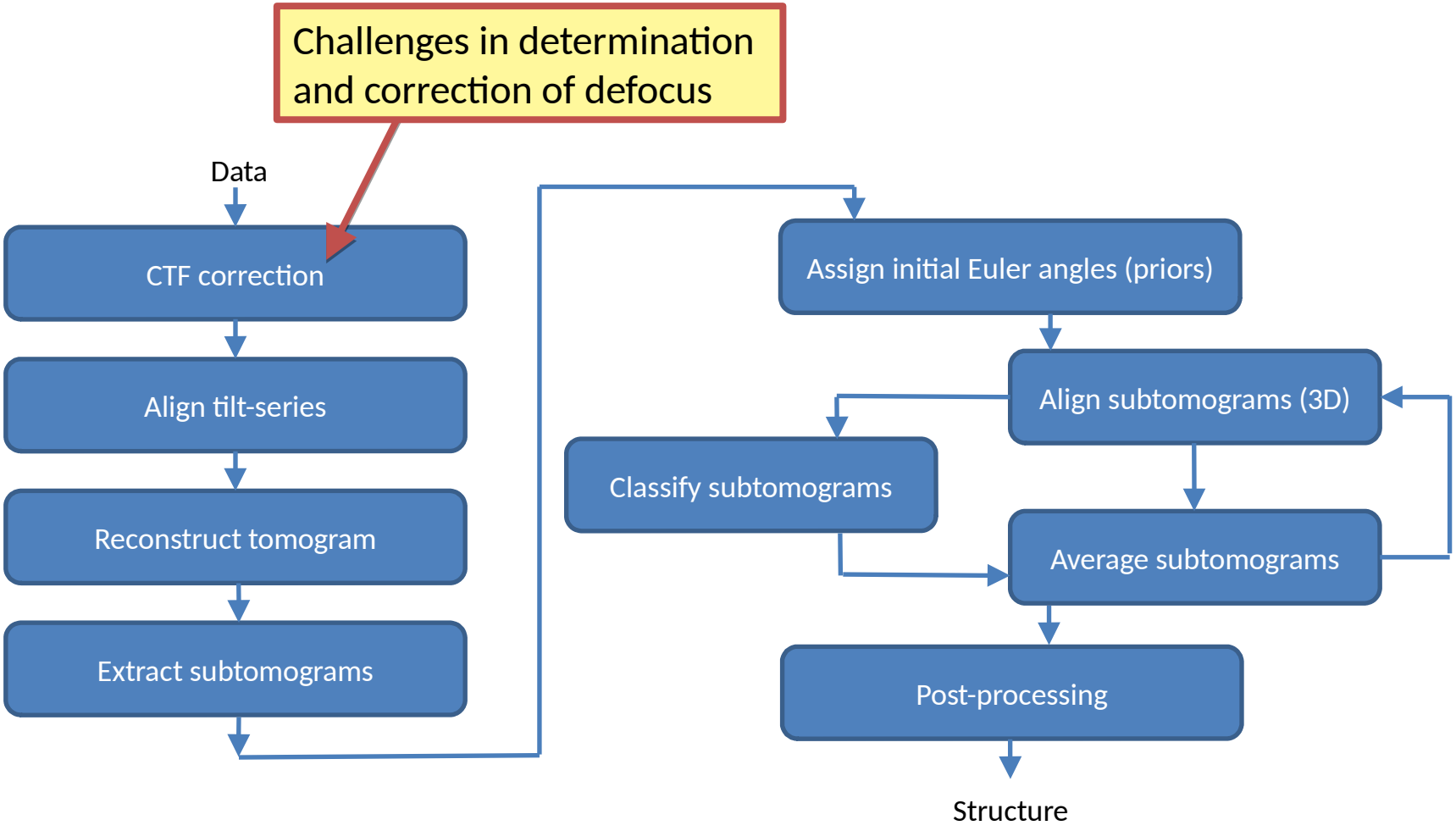
Typically higher total electron dose

Slower data collection leads to smaller datasets

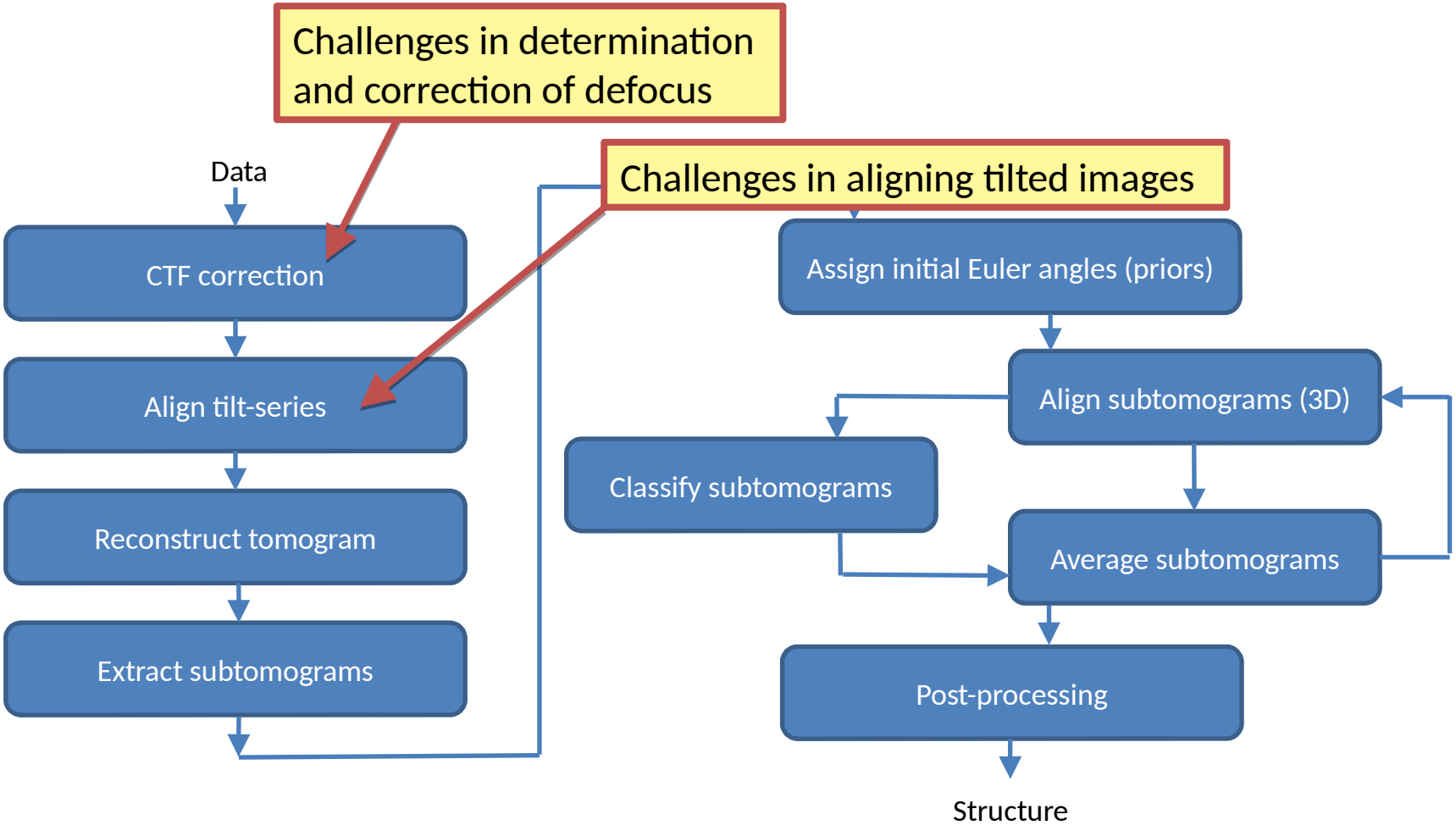
Challenges in data processing



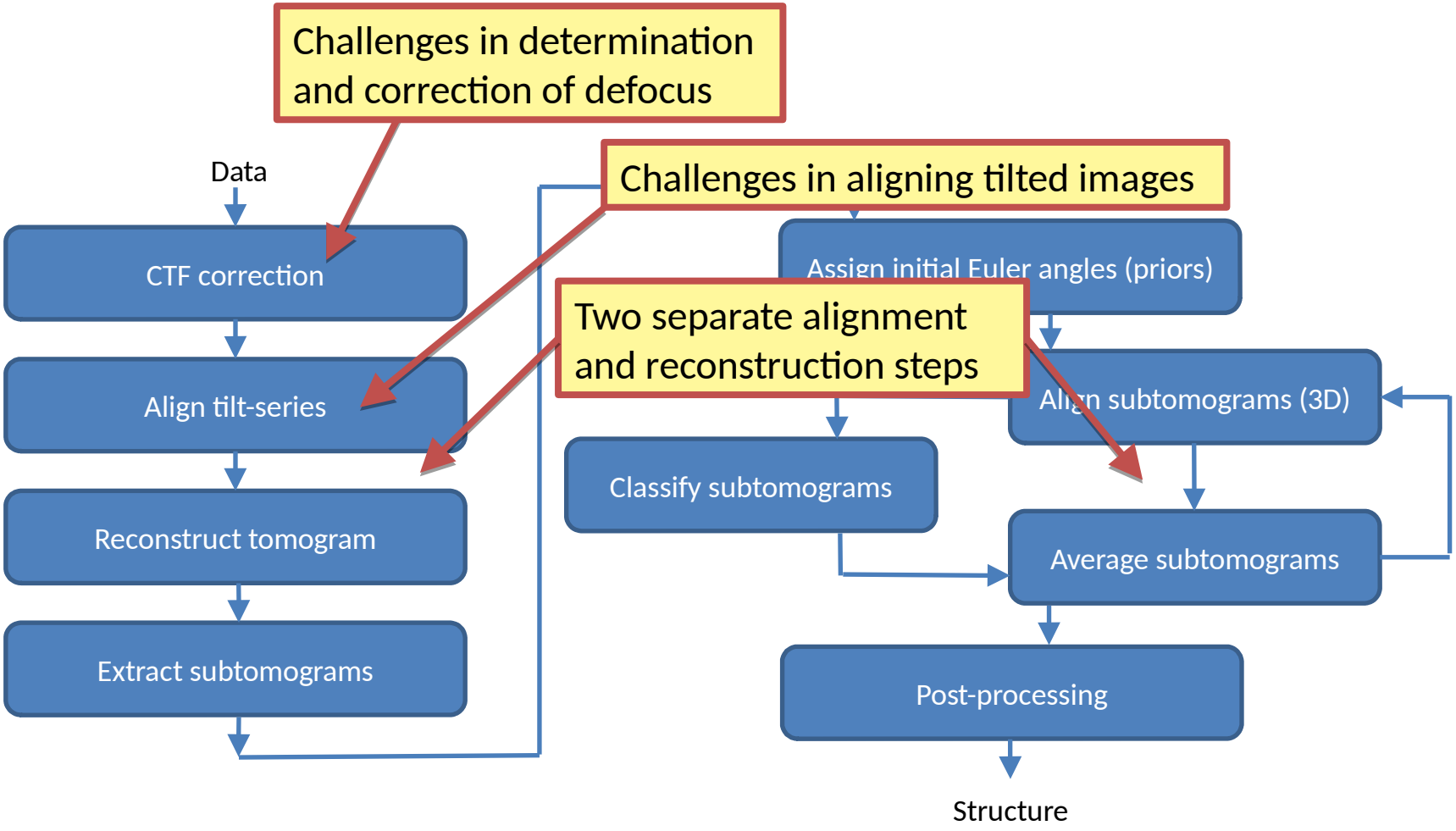
Challenges in data processing



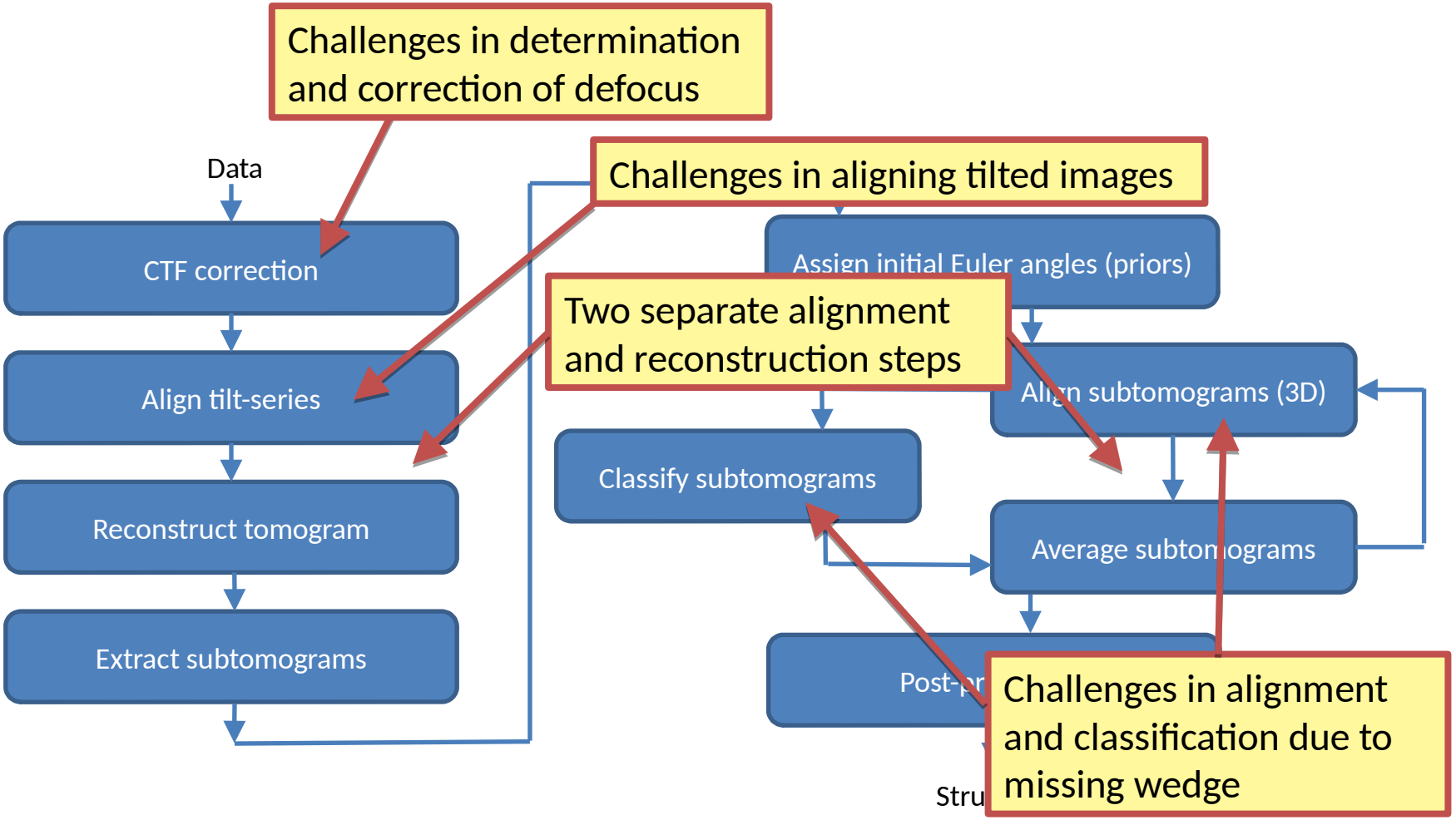
Challenges in data processing



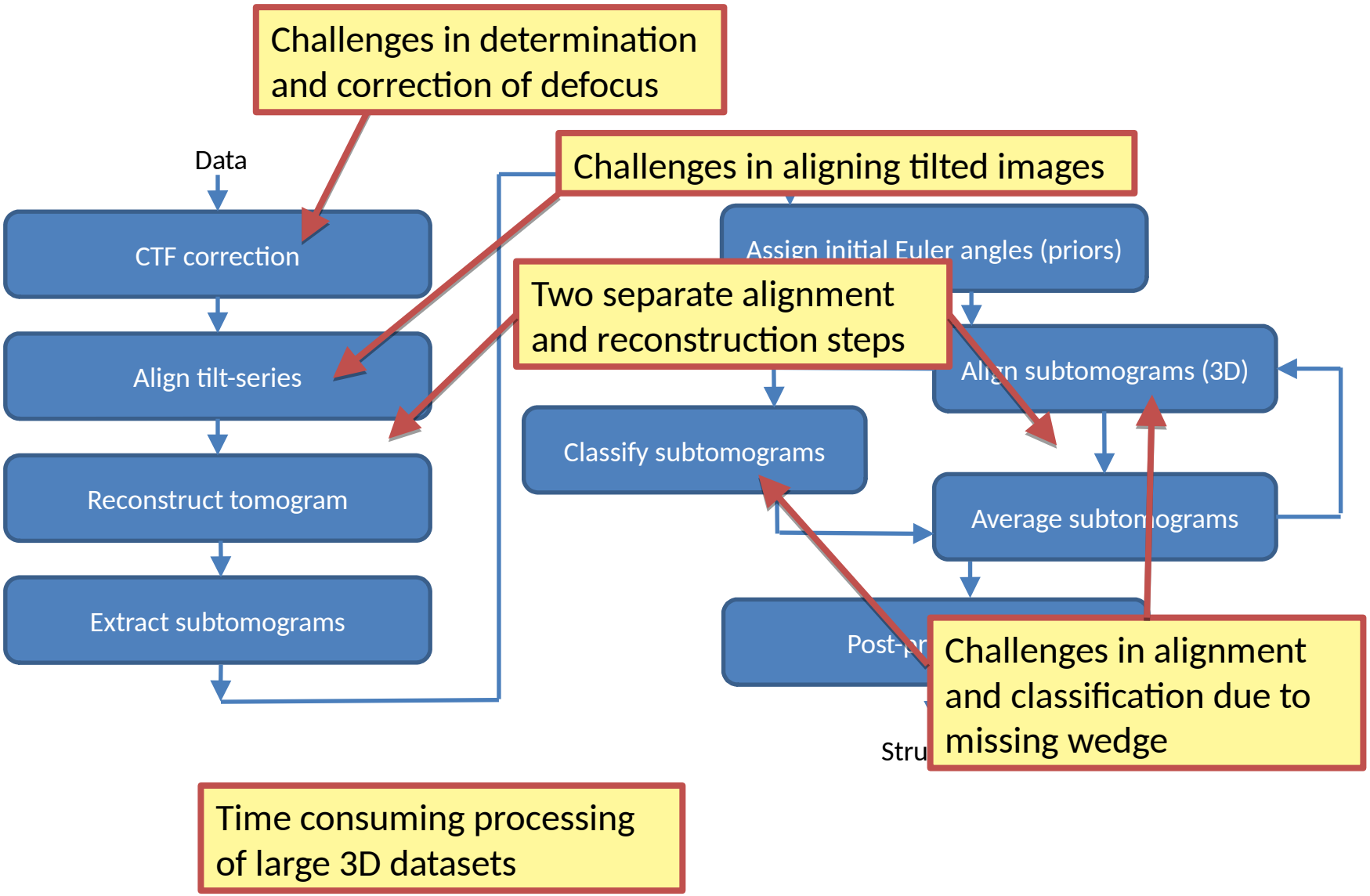
Challenges in data processing



Challenges in data processing



Challenges in data processing



How can we do subtomogram averaging at high-resolution?

Challenges related to the sample

Sample complexity, heterogeneity, flexibility

Higher apparent sample thickness (especially at tilt)

Optimizing the sample

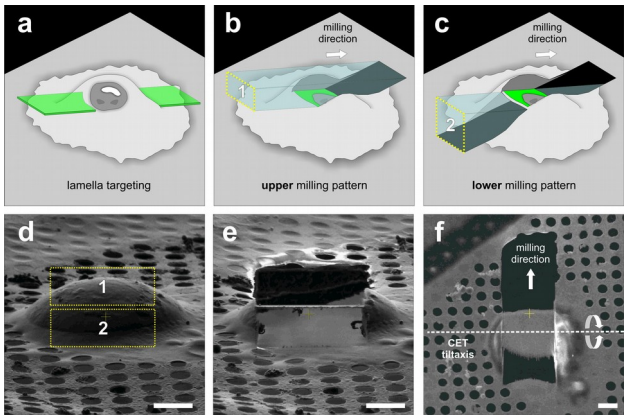
Sample complexity, heterogeneity, flexibility

Higher apparent sample thickness (especially at tilt)

Cell Biology

Biochemistry

In vitro reconstitution



Rigort and Plitzko 2015

Cut thinner lamella (within reason)

… at some point it might be a single particle project

Challenges in data collection

Sample changes during data collection

Increased sample movement at tilt

Typically higher total electron dose

Slow data collection leads to smaller datasets

Optimizing the data collection

Sample changes during data collection

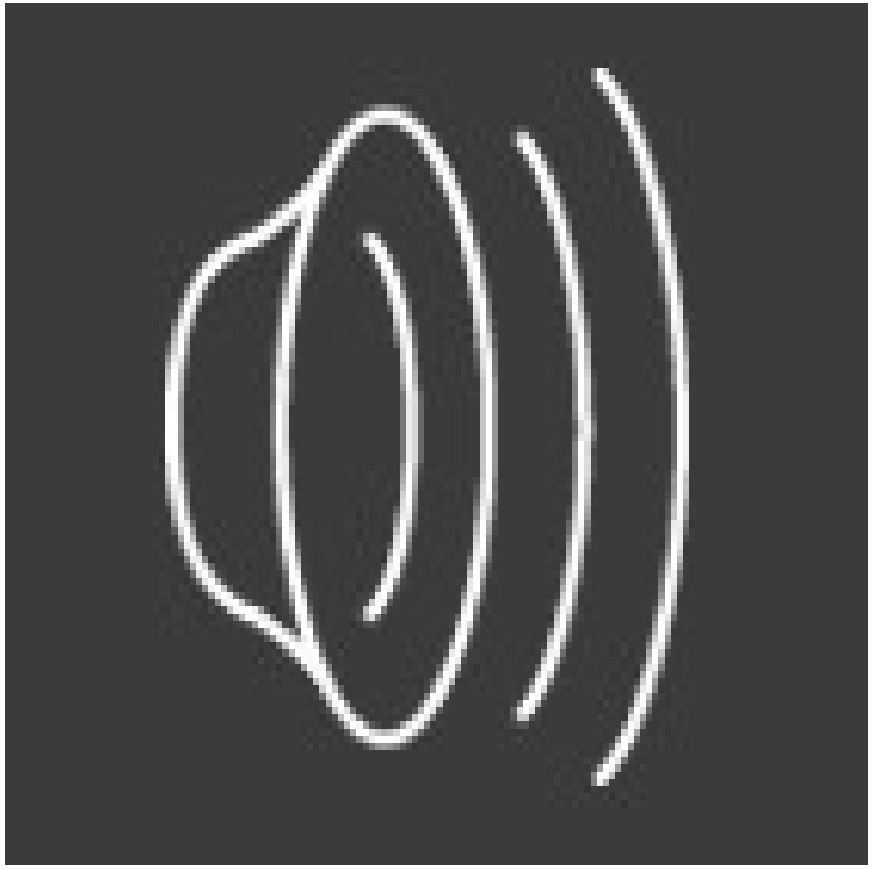
Increased sample movement at tilt

Typically higher total electron dose

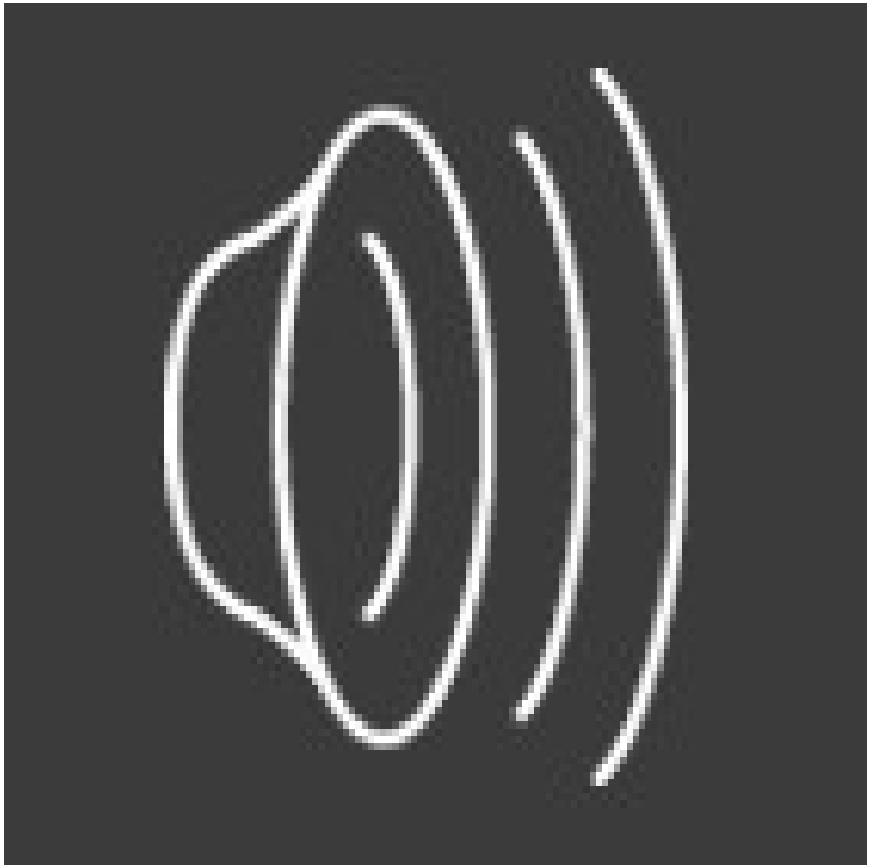
Slow data collection leads to smaller datasets

All of these problems can be minimized (but not eliminated), by modifying your data collection scheme.

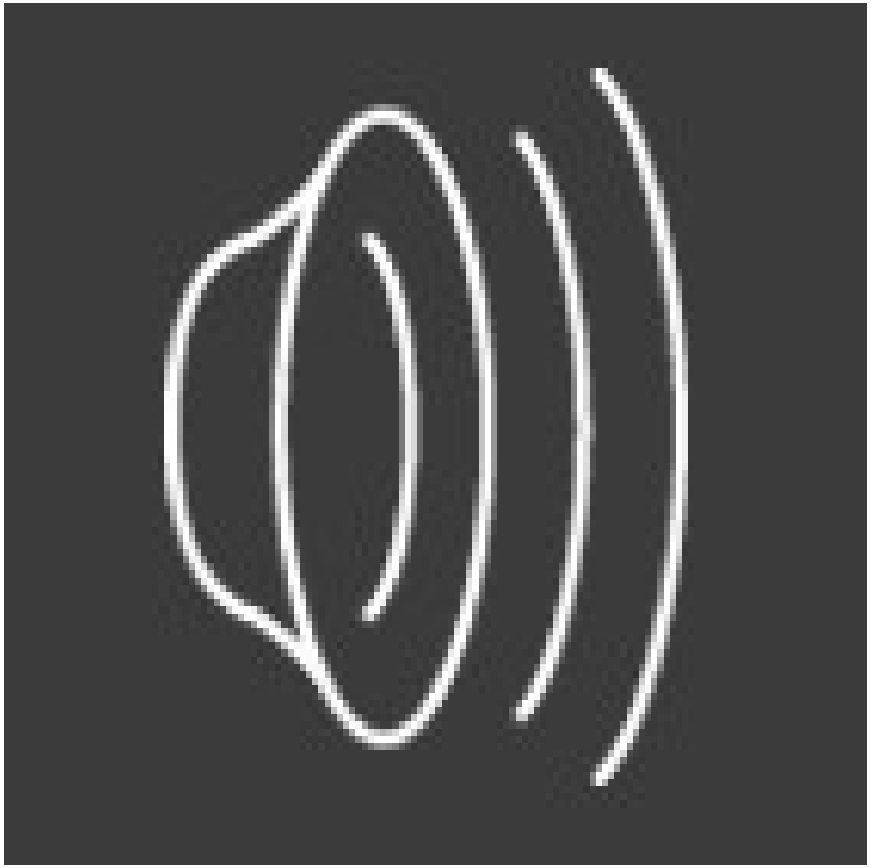
Continuous tilt scheme



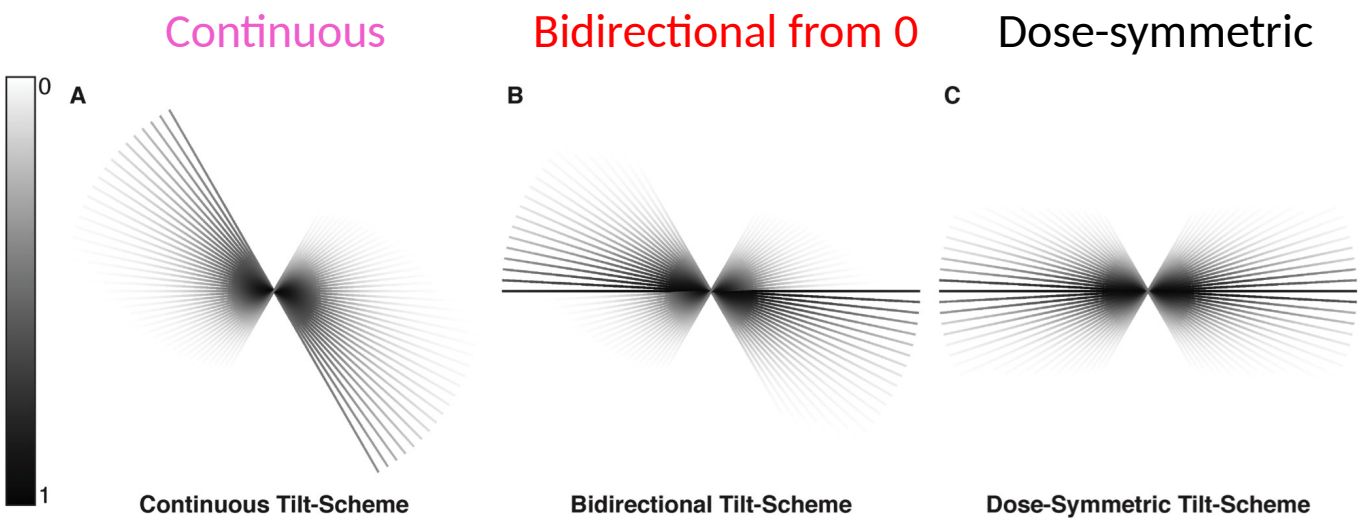
Bidirectional tilt scheme



Dose-symmetric tilt scheme

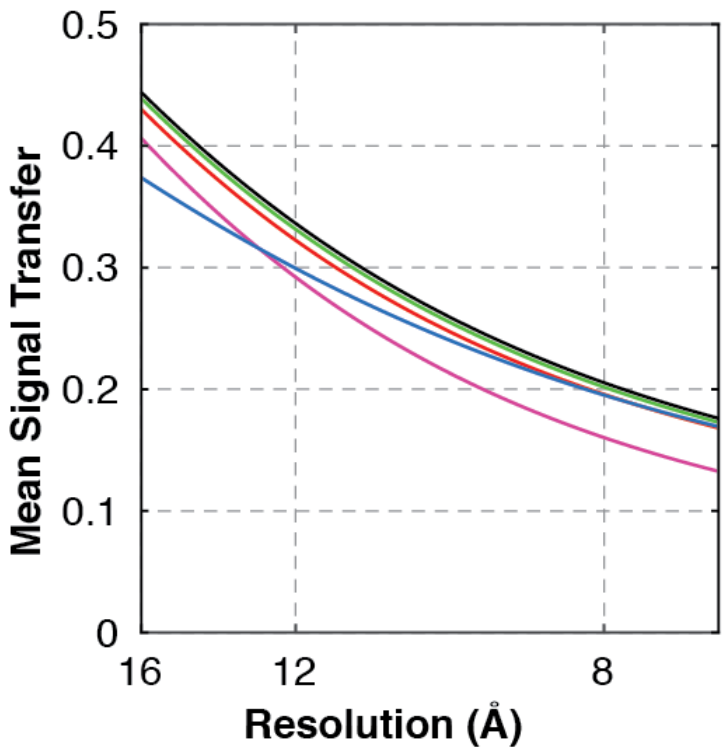


Tilt schemes



Hagen et al. JSB 2017

Tilt schemes- signal transfer



Continuous

Bidirectional from 0

Bidirectional from -21

Bidirectional from -21
deleting second branch

Dose-symmetric

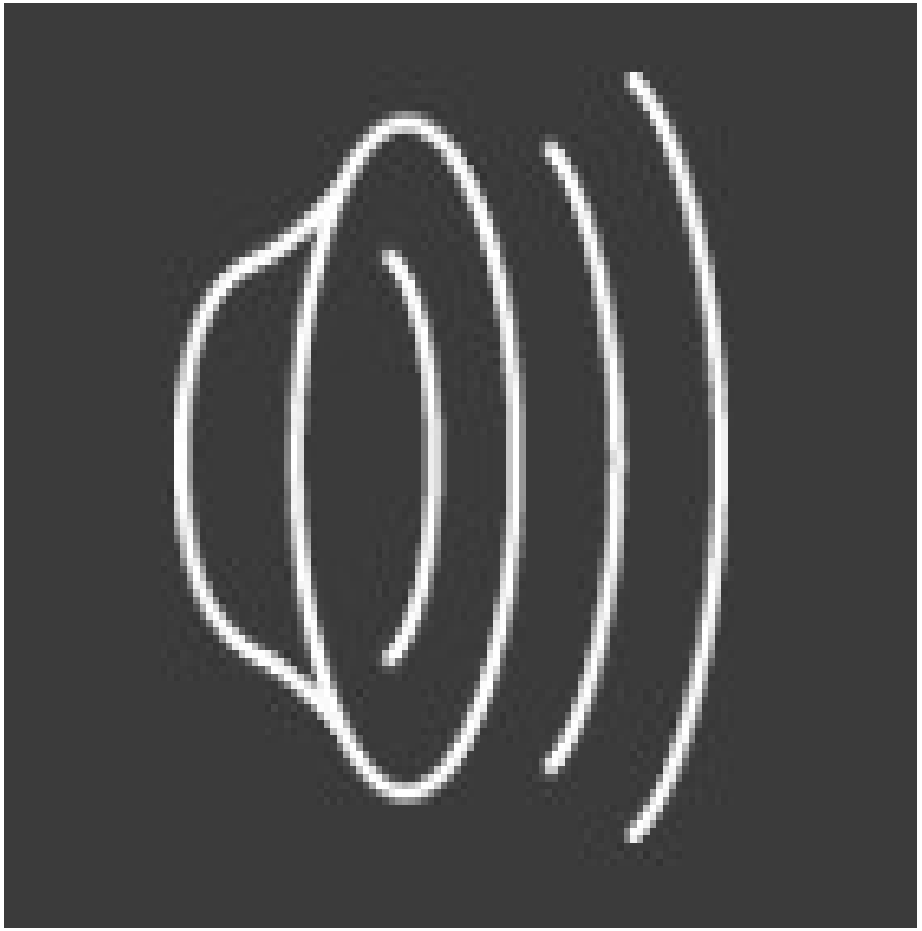
Note – the difference is greater if you also consider increased sample movement and poorer alignment at tilt

Hagen et al. JSB 2017

Challenges in data collection

There are changes in the sample as dose accumulates:

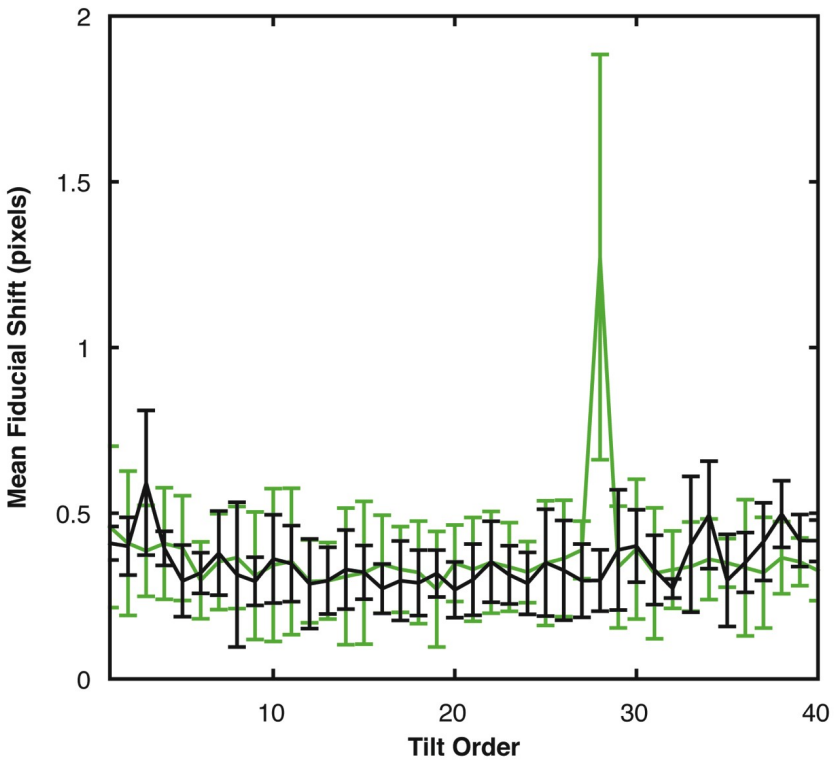
thinning
distortions
charge build-up

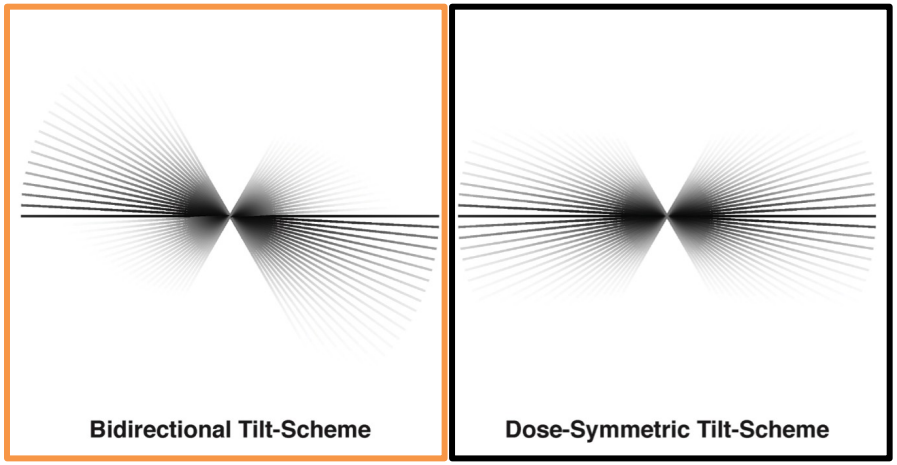


Wim Hagen

e.g.
Wright et al. (2006) J. Struct. Biol,. 153, 241-252
McMullan et al. (2015) Ultramicroscopy, 158, 26-32
and many more!

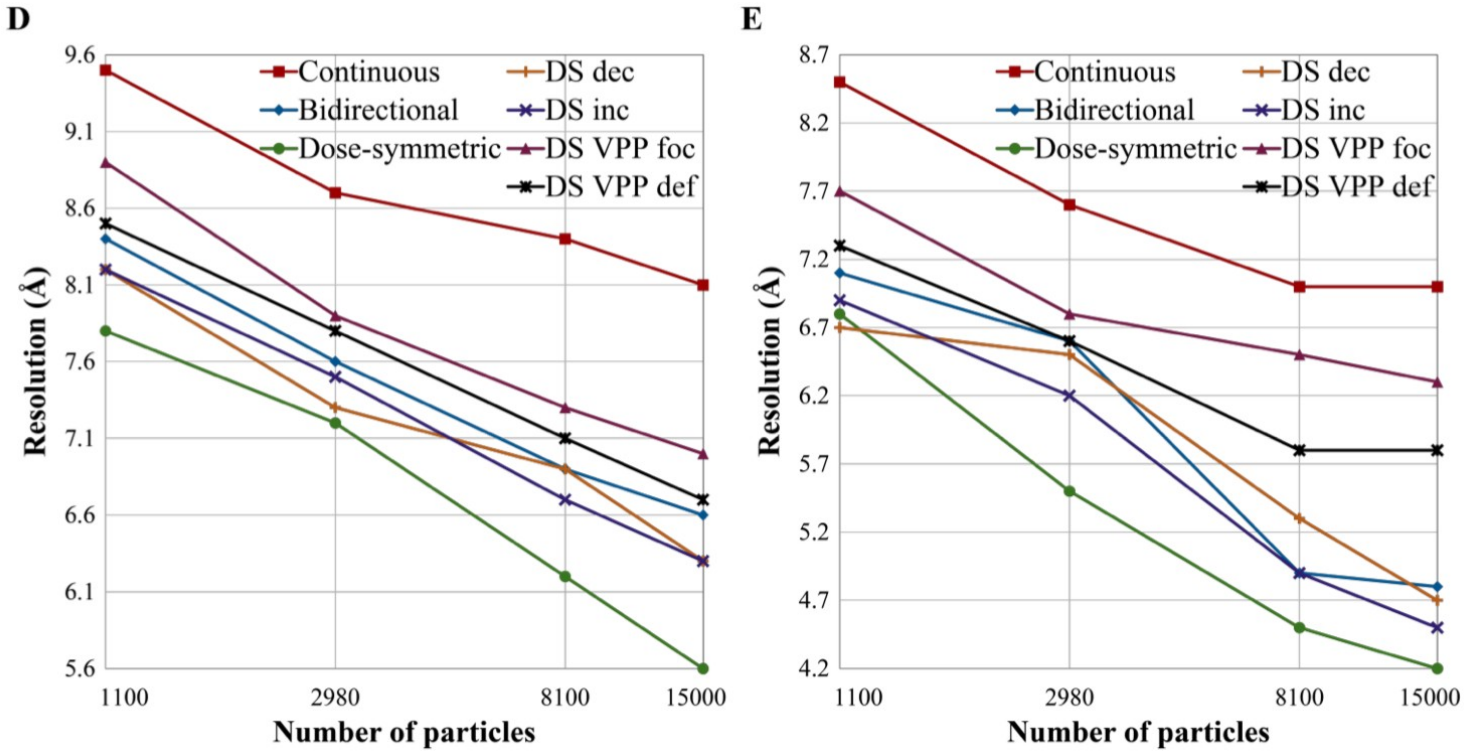
Does-dependent sample changes





Hagen et al. JSB 2017

Does-dependent sample changes



Turonova et al. BioRxiv

Optimizing the data collection

What total dose? Resolution vs signal-to-noise

What tilt range? Completeness of information vs dose and speed

What angular increment? Resolution vs dose and speed

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

What magnification? Resolution and DQE vs field of view

What defocus? High-frequency information vs low frequency information

Optimizing the data collection

Increased sample movement at tilt

Sample changes during data collection

Typically higher total electron dose

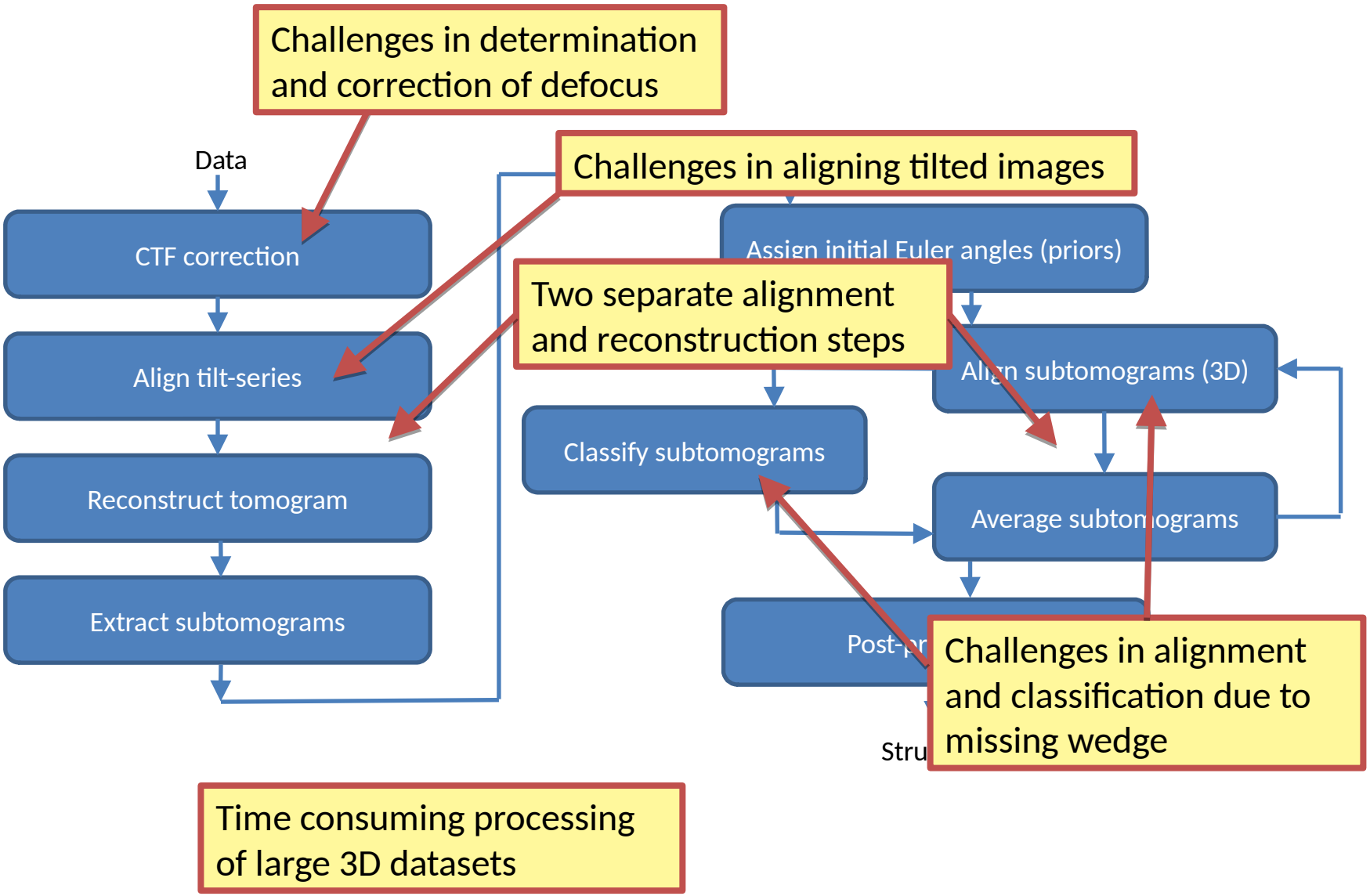
Slow data collection leads to smaller datasets

Minimized data collection schemes (tilts, mag etc)

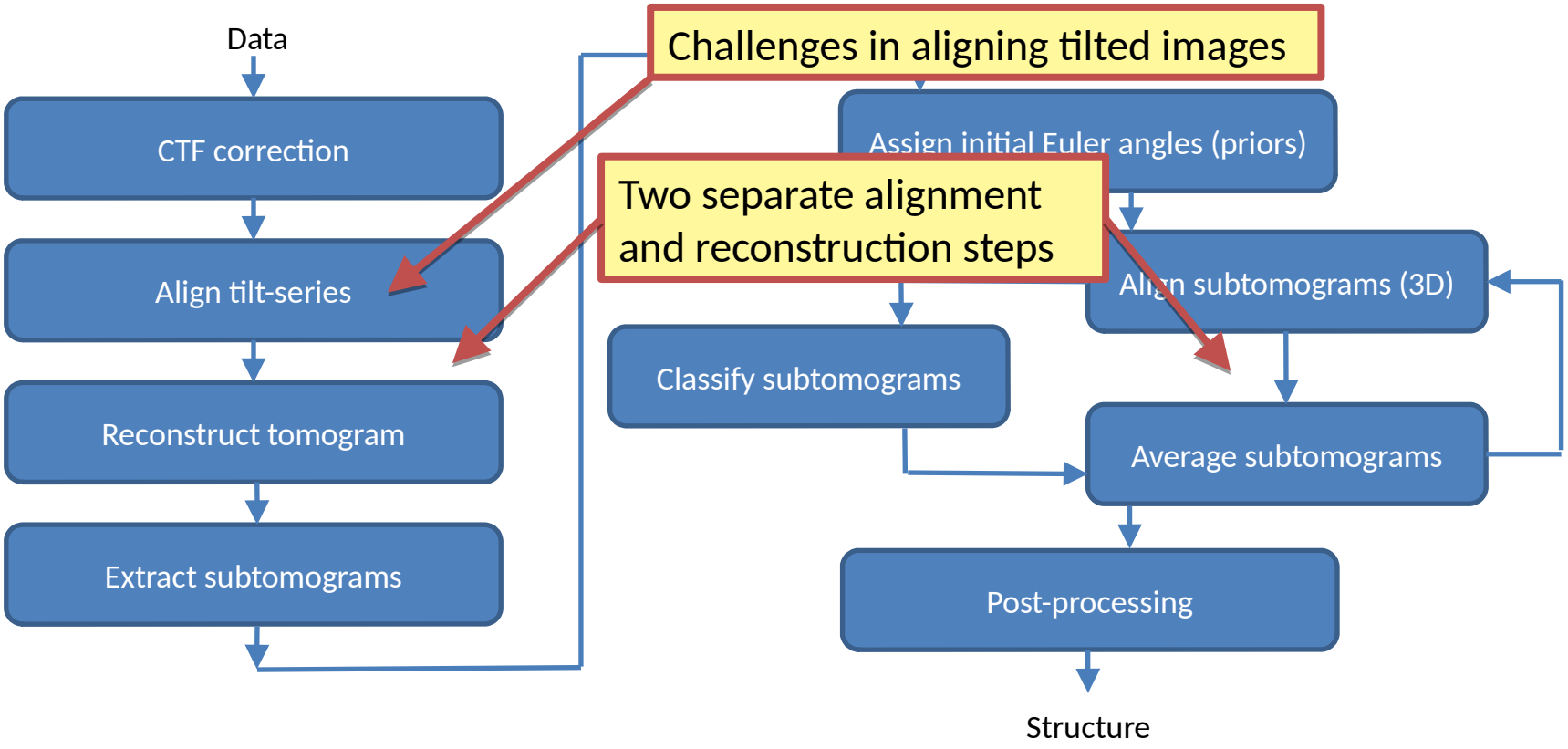
Faster data collection schemes

Improved hardware

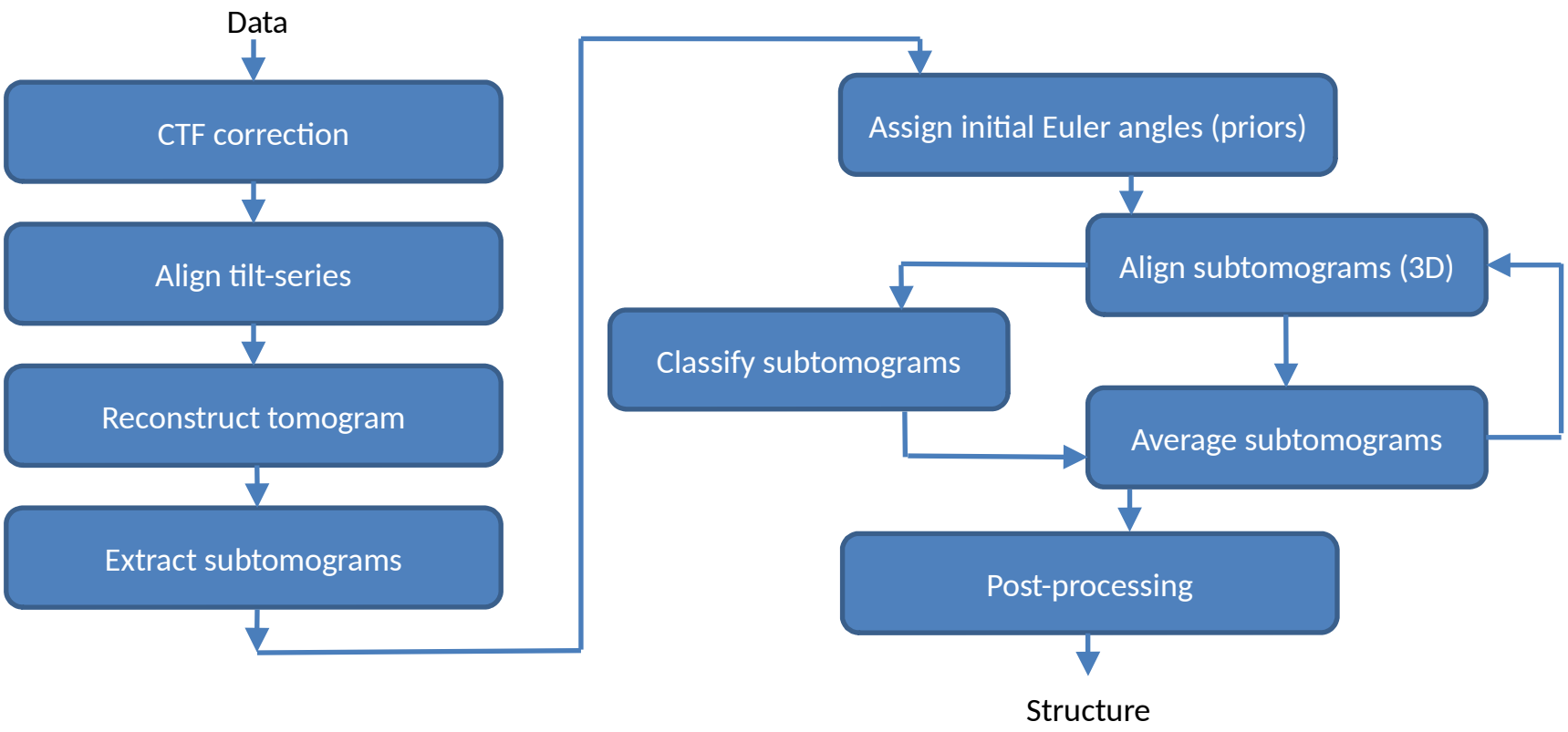
Challenges in data processing



Challenges in data processing

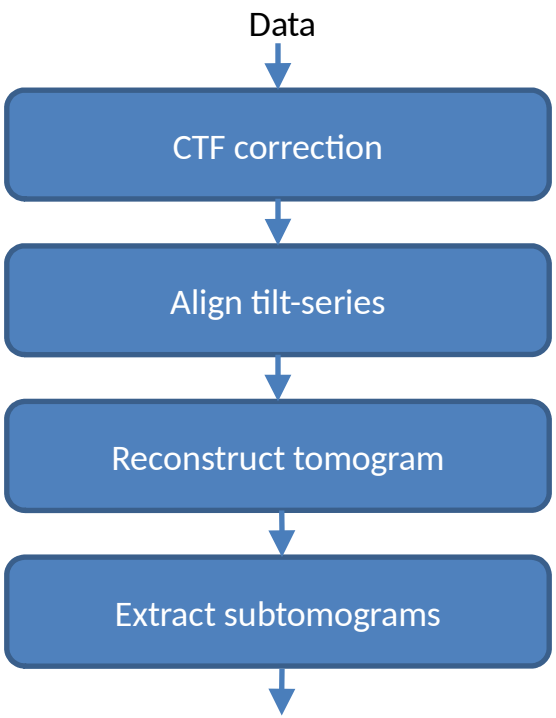


Subtomogram averaging



The quality of the initial tilt-series alignment can become limiting

Subtomogram averaging



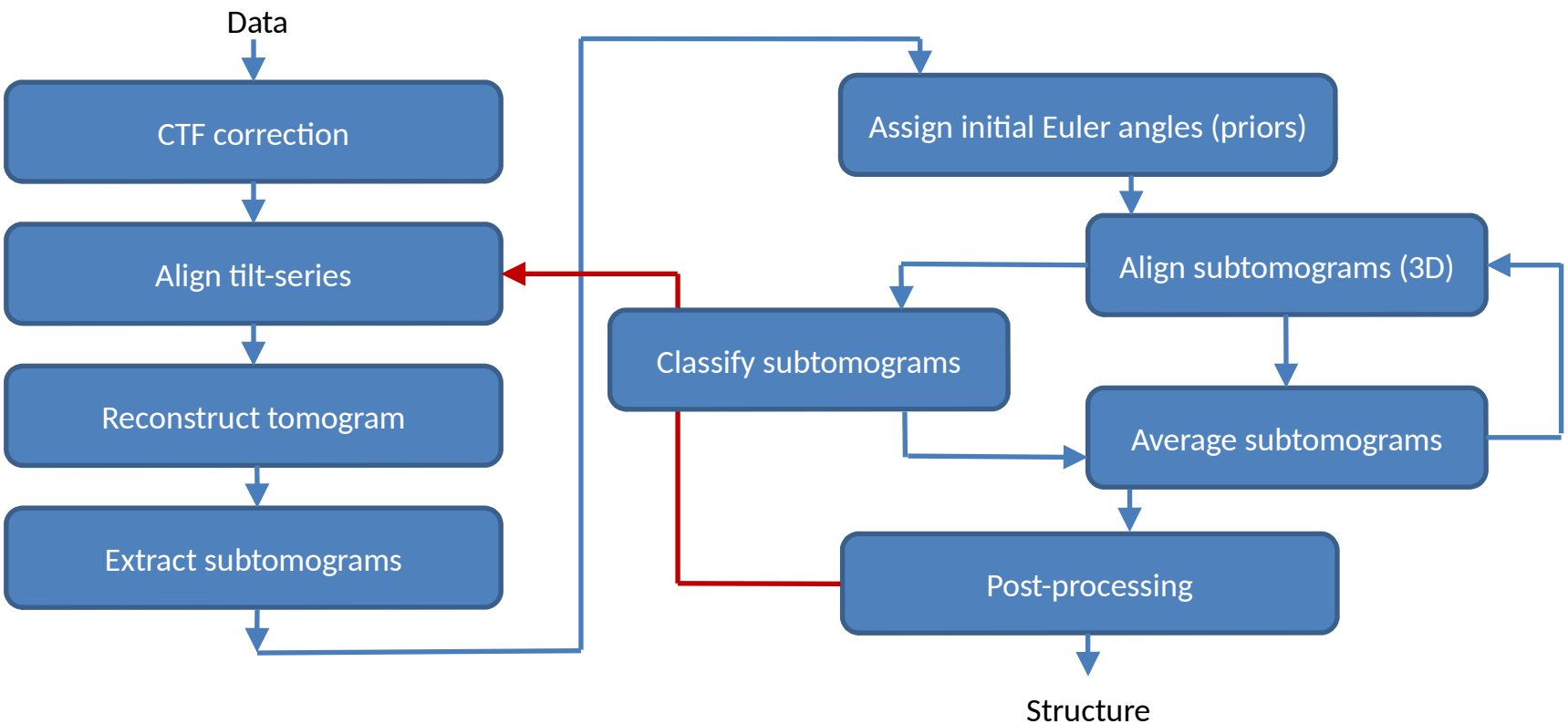
To improve:

Optimize alignment by improving tracking of gold fiducials or sample features

Considering sample distortions that occur during data collection and correcting for them (JJFernandez, Warp)

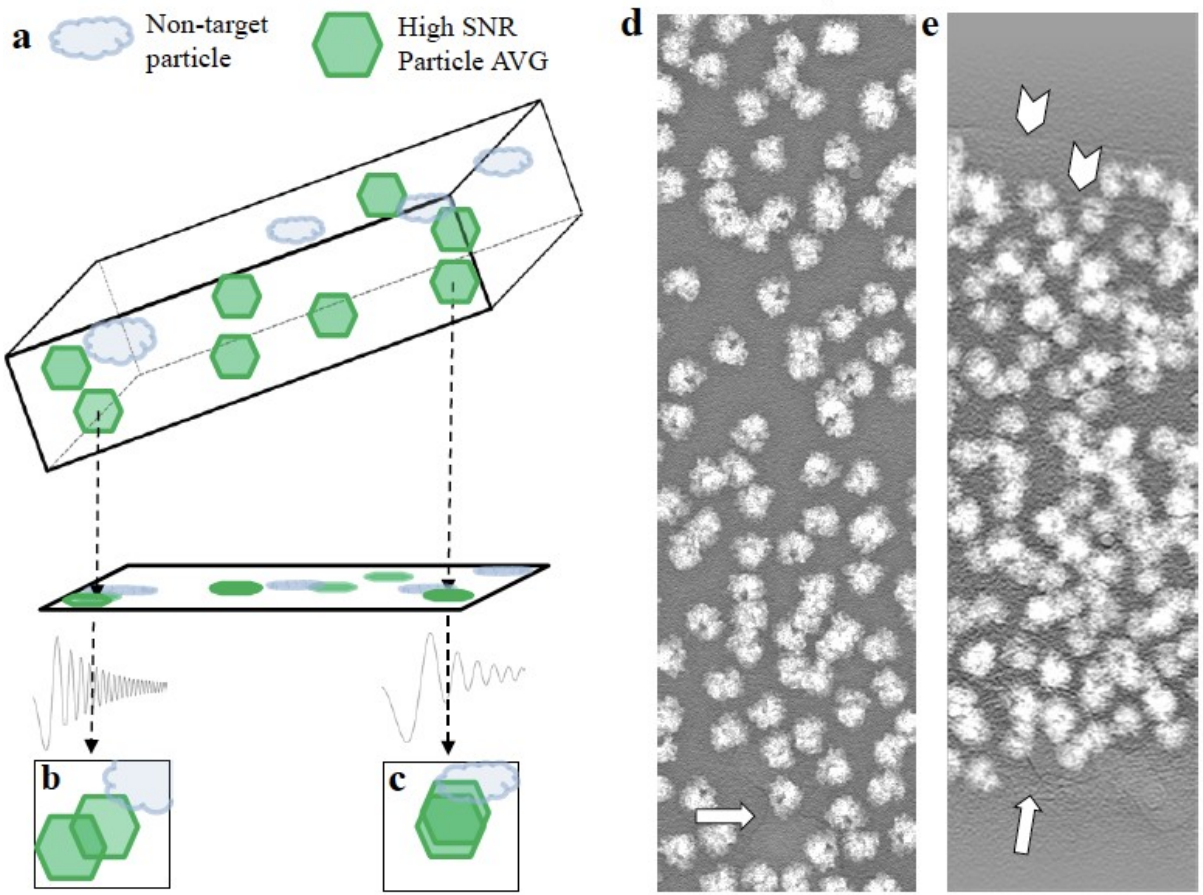
Use the structure determined by subtomogram averaging to improve the alignment

Subtomogram averaging



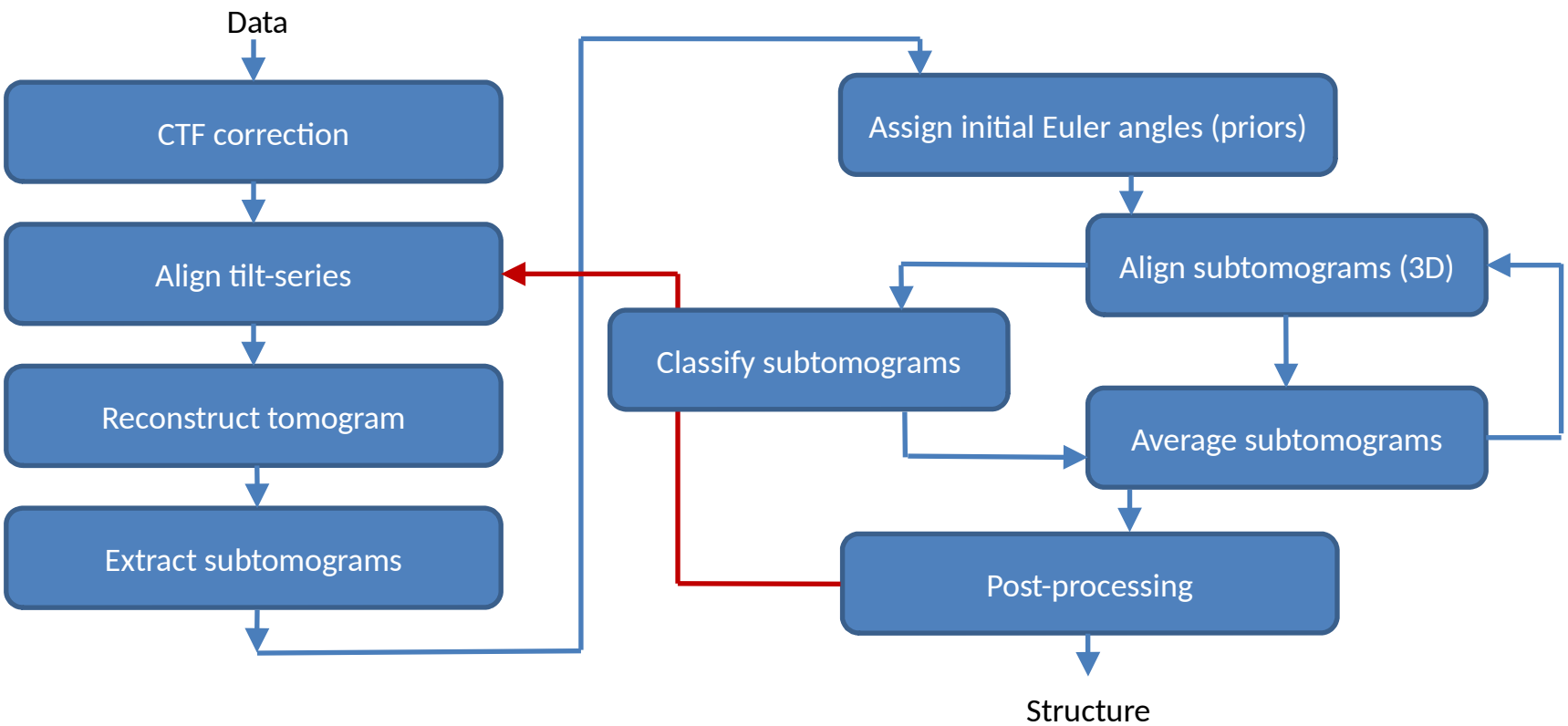
Use the structure determined by subtomogram averaging to improve the alignment (eg emClarity)

Subtomogram averaging



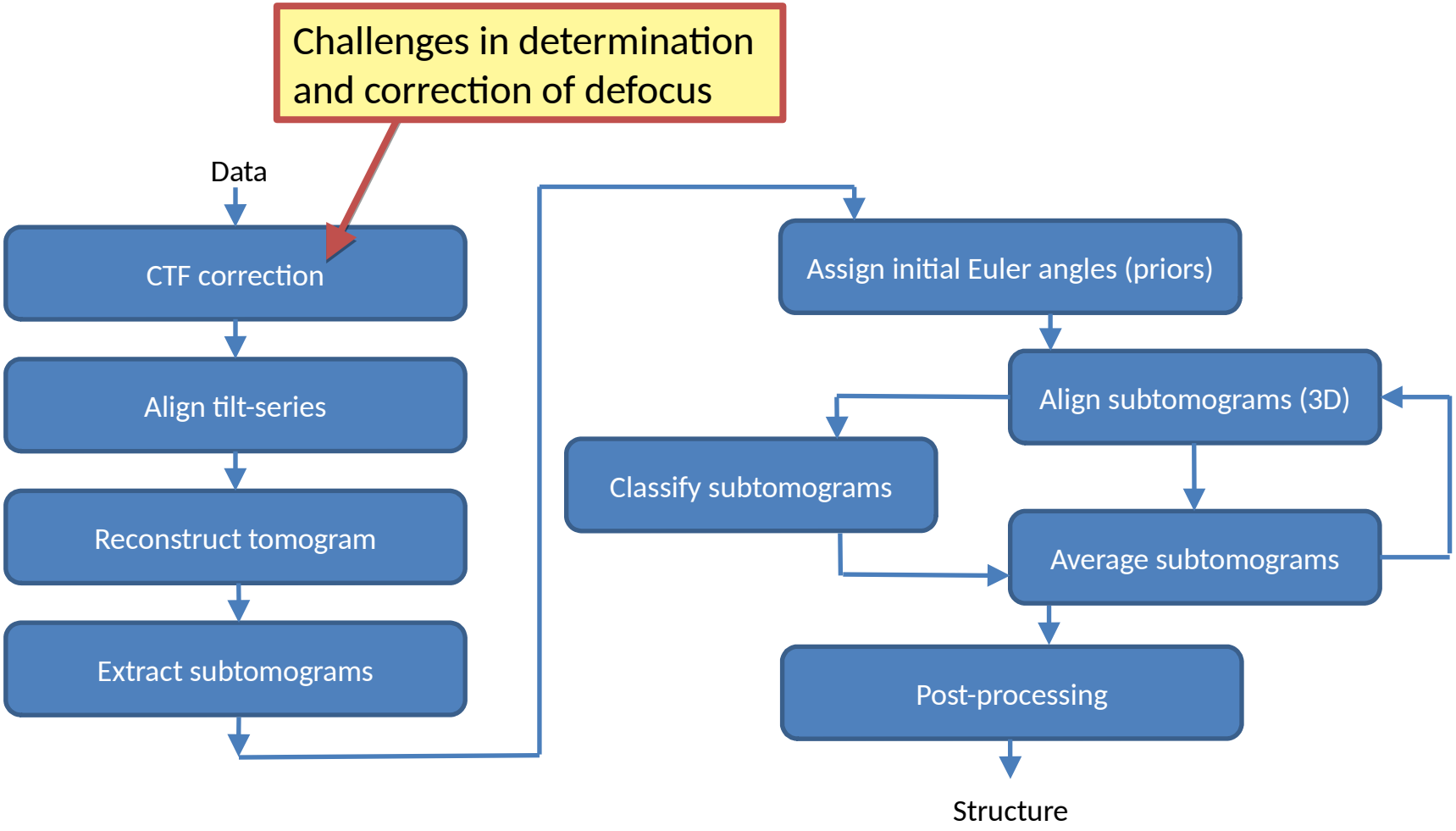
Himes and Zhang emClarity (biorxiv)

Subtomogram averaging

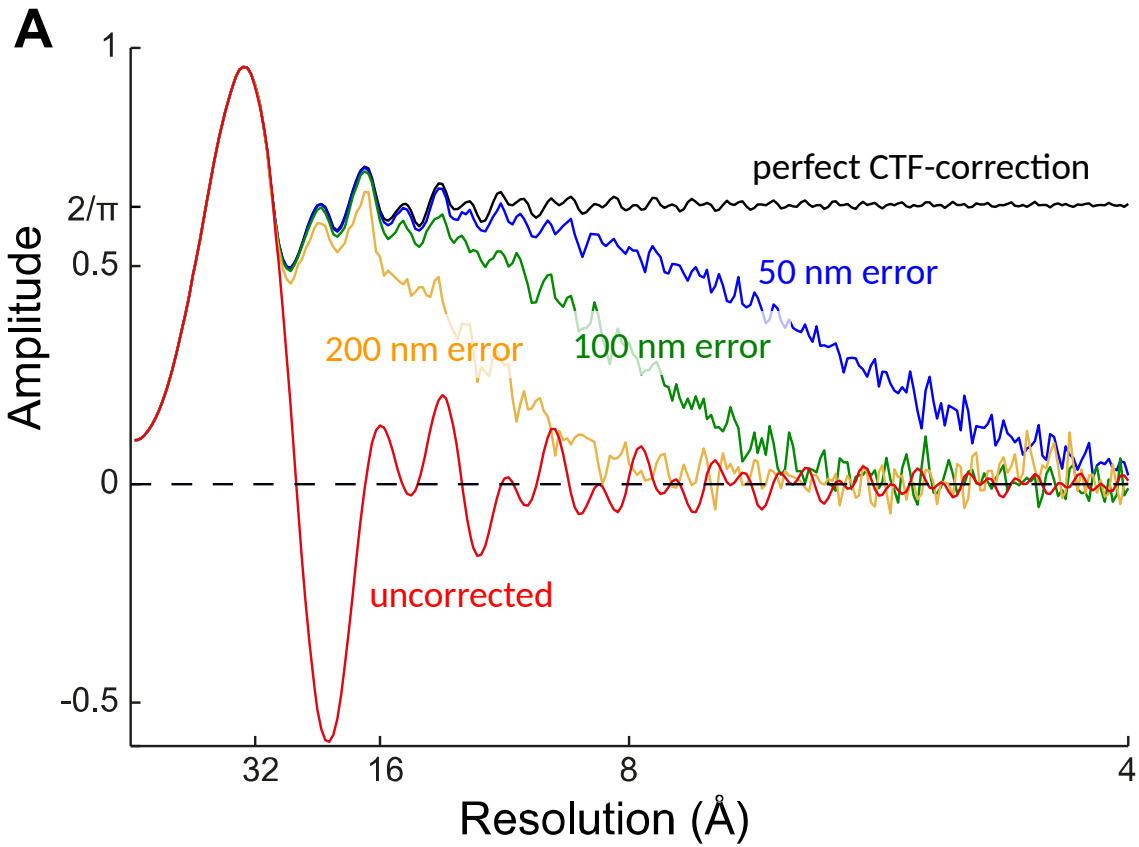


Another idea is to iteratively refine the tilt images of individual particles against the final structure. This requires constraining the relative orientations (otherwise the sample could be studied by single particle reconstruction).

Challenges in data processing



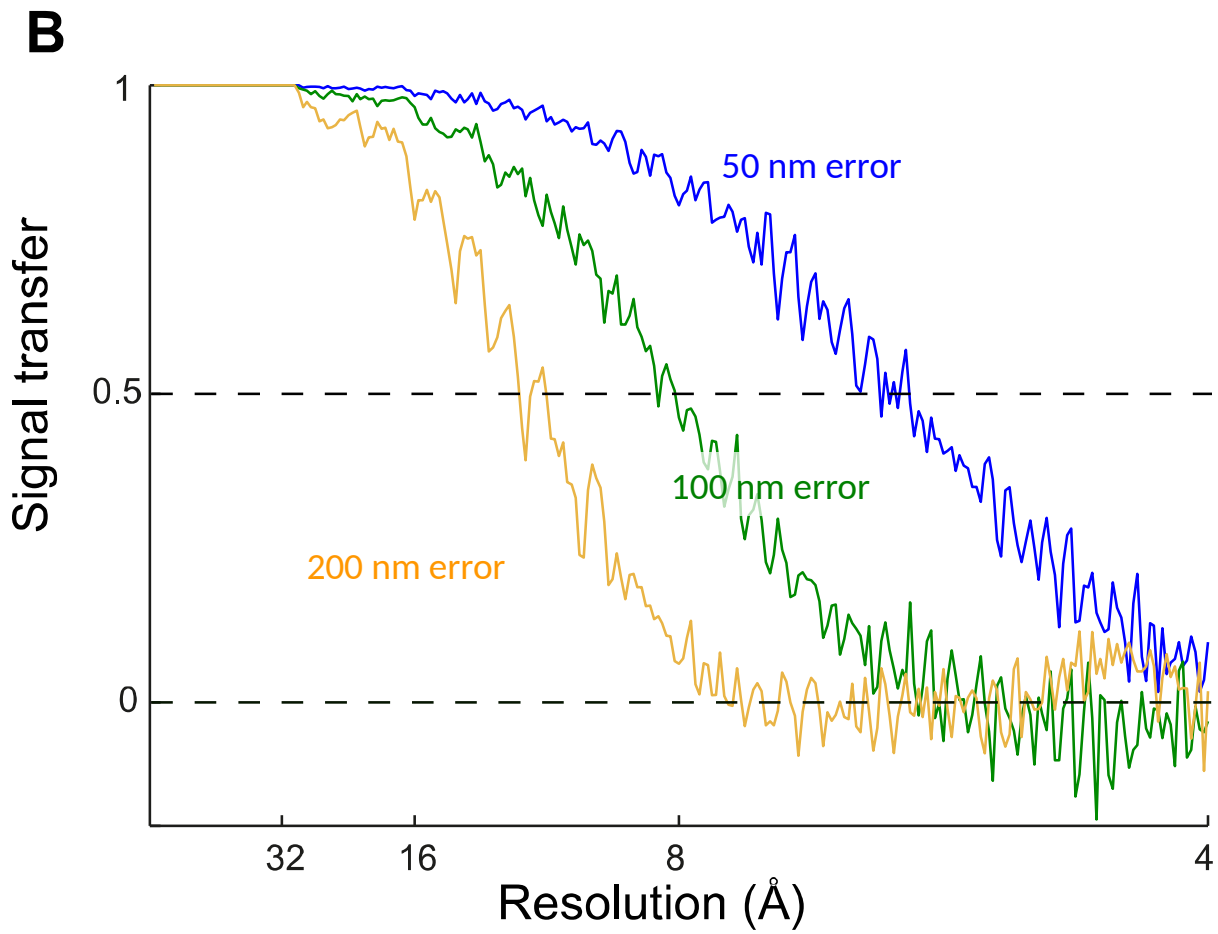
At which resolution will errors in defocus estimation/correction be limiting?



Simulation of CTF of final average from multiple tomograms with mixed defoci

Schur et al. JSB 2013

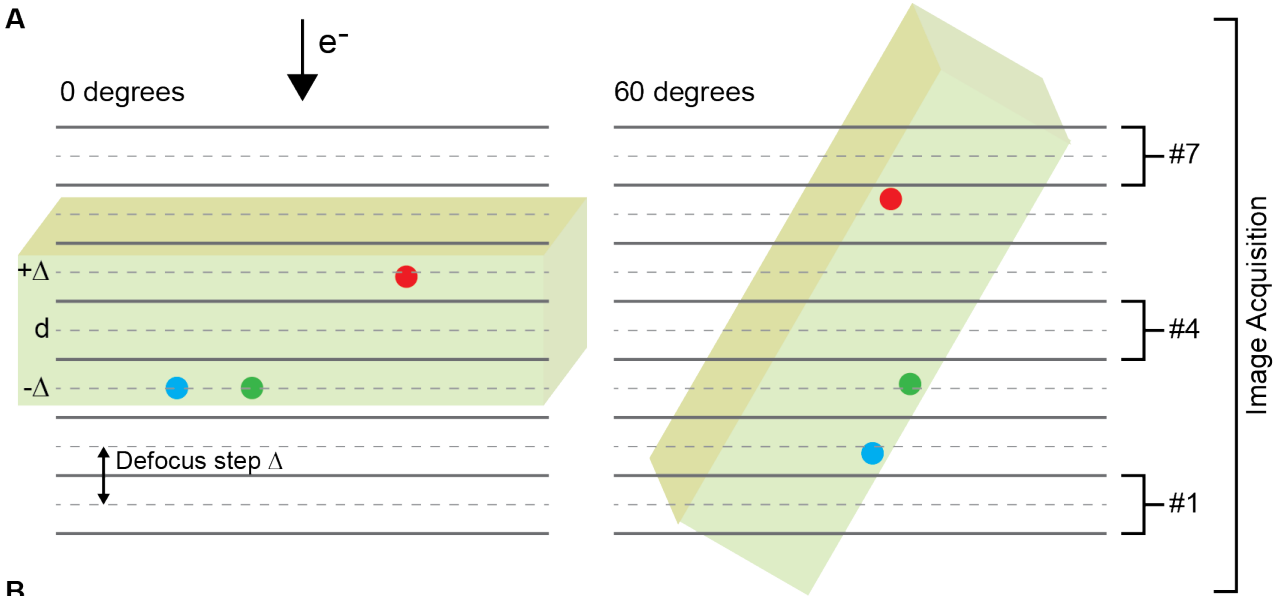
At which resolution will errors in defocus estimation/correction be limiting?



Simulation of effect on signal transfer of error in defocus estimation
(sigma of normally distributed error)

Schur et al. JSB 2013

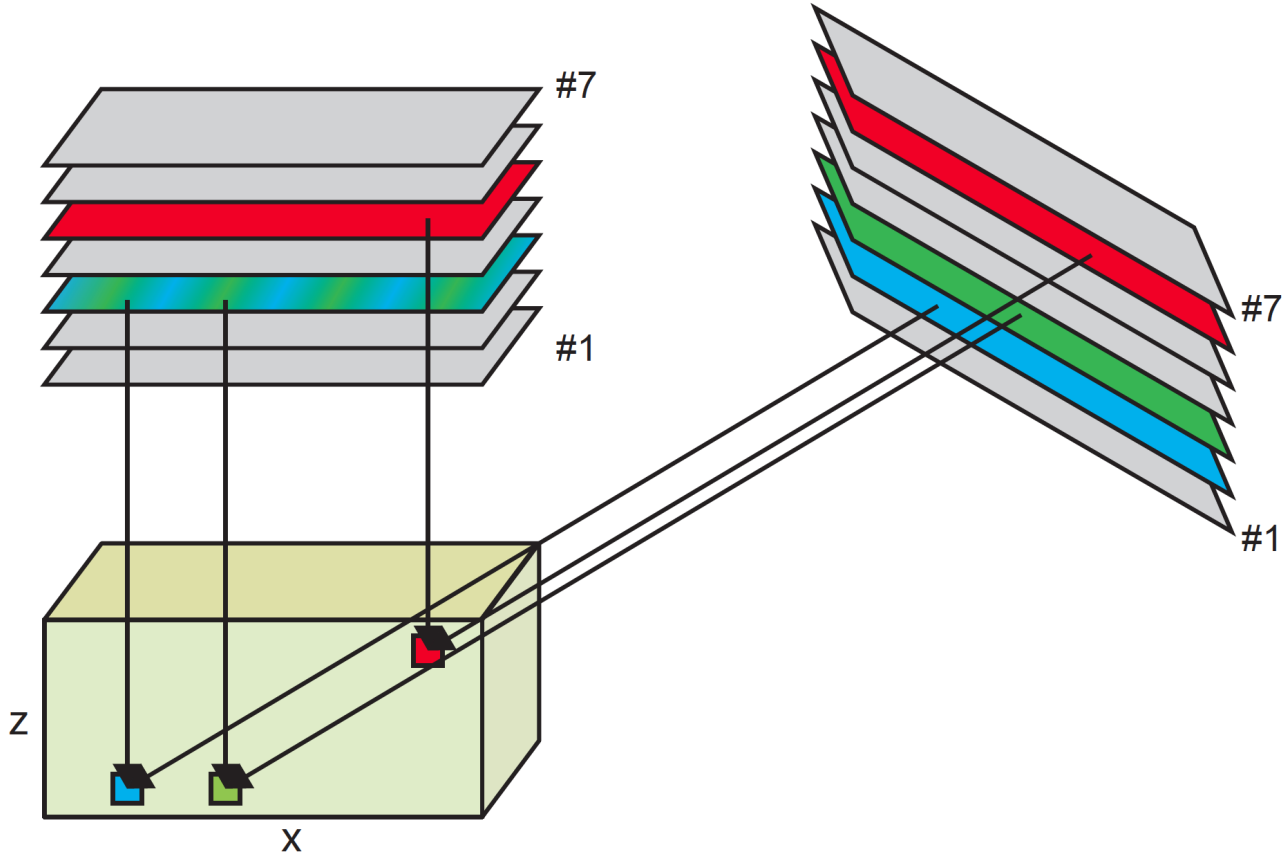
Defocus gradients in the sample



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

Turonova et al. JSB 2017

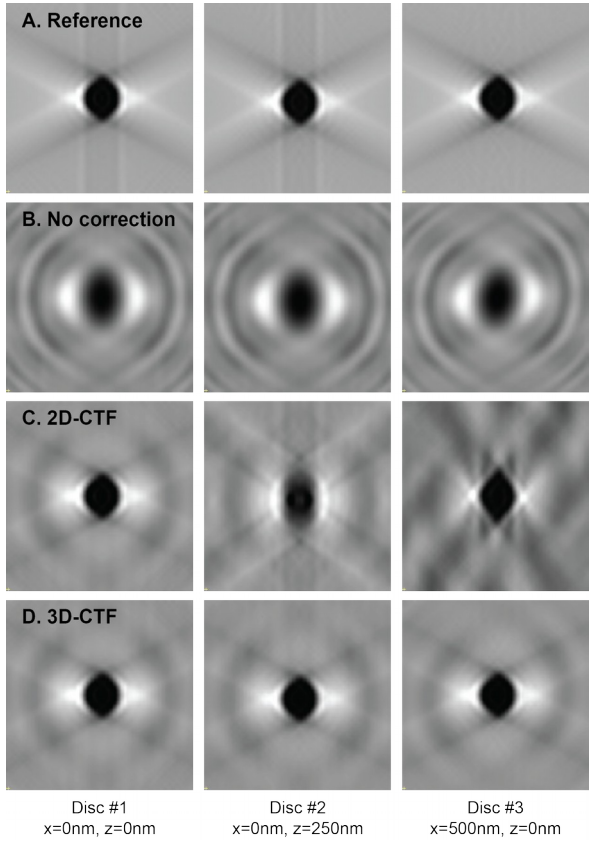
Defocus gradients in the sample



For 3D CTF correction, each voxel is reconstructed using appropriately CTF-multiplied tilt images

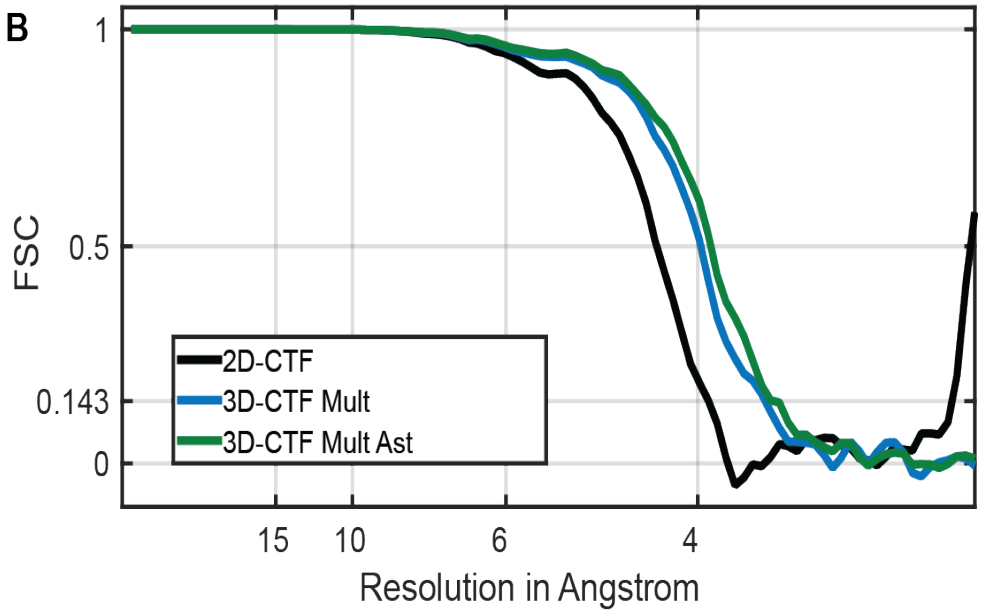
Turonova et al. JSB 2017

Defocus gradients in the sample



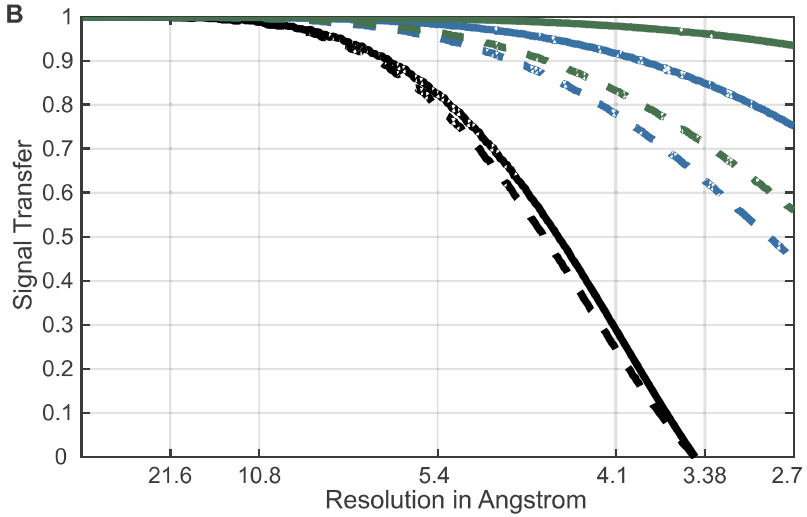
Turonova et al. JSB 2017

Defocus gradients in the sample

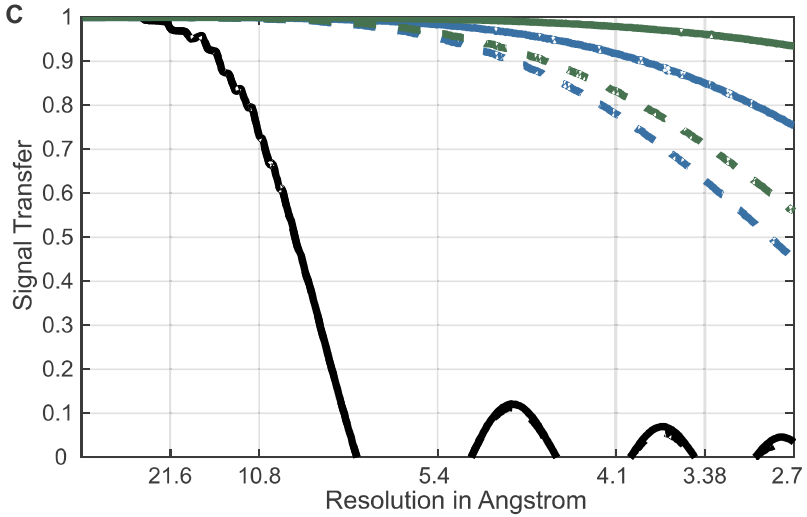


Turonova et al. JSB 2017

Defocus gradients in the sample



100 nm sample



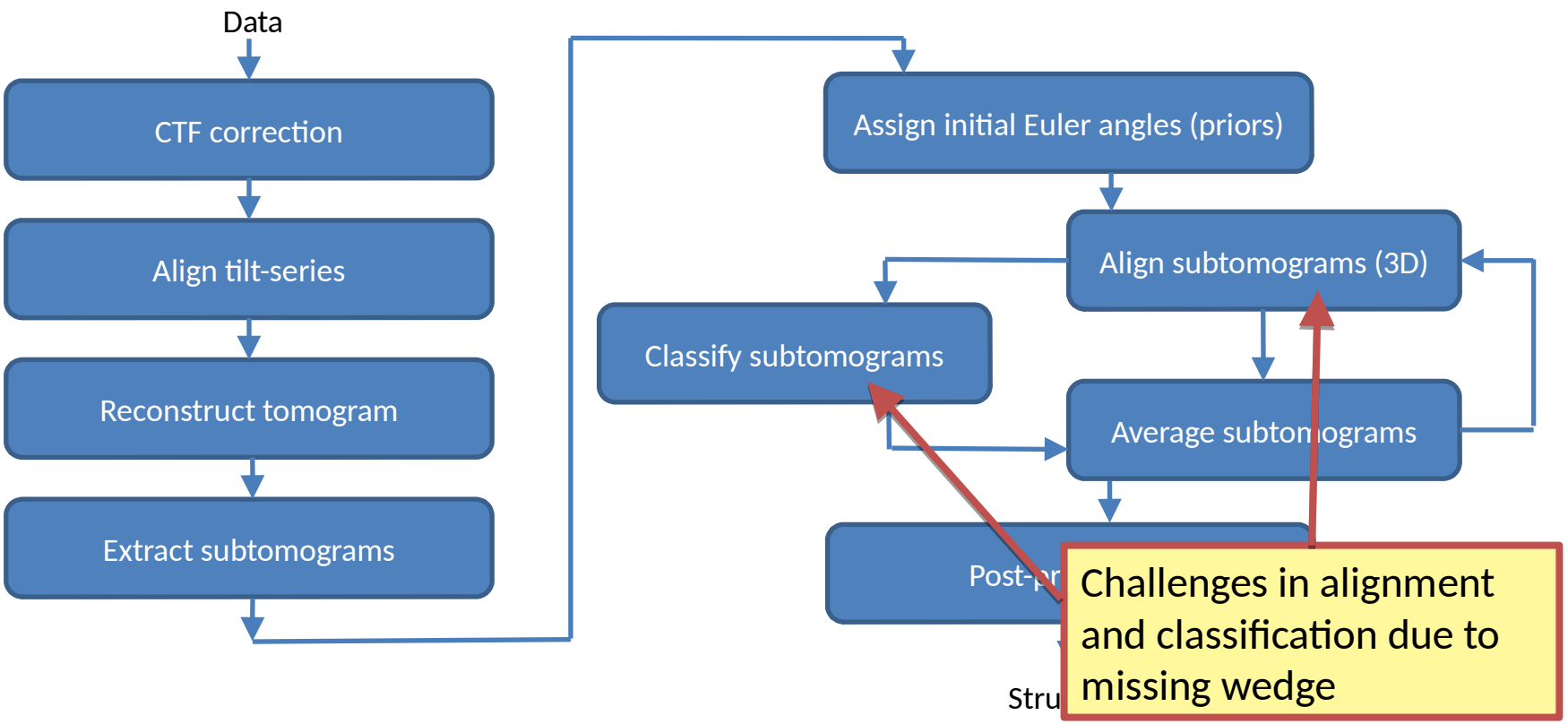
500 nm sample

Black – 2DCTF
Blue – 3DCTF 30nm
Green – 3DCTF 15nm

Solid – no error
Dashed – 12nm sd normal error in determination

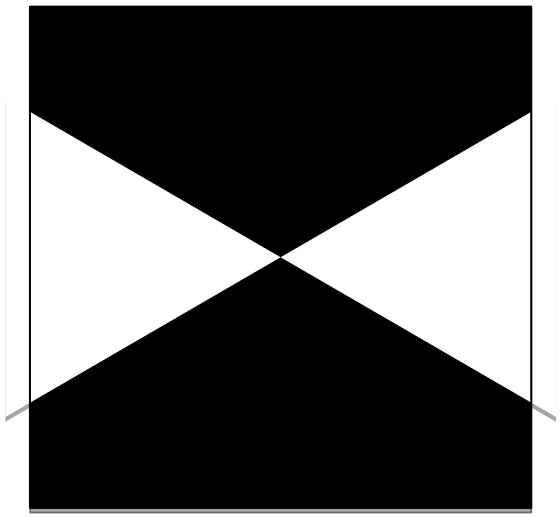
Turonova et al. JSB 2017

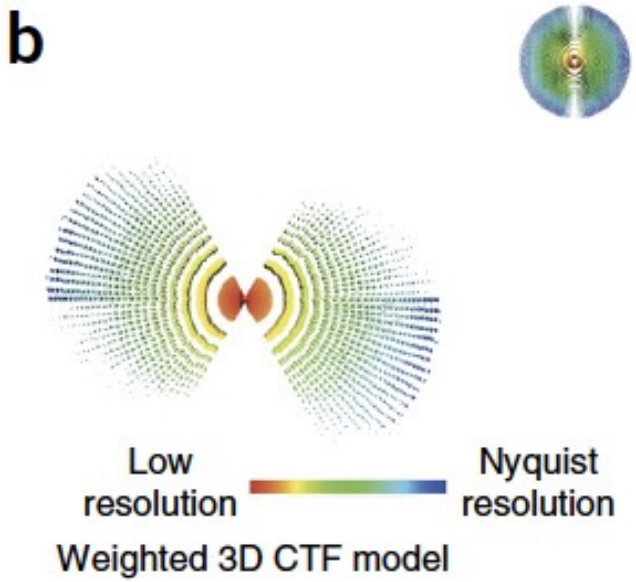
Challenges in data processing



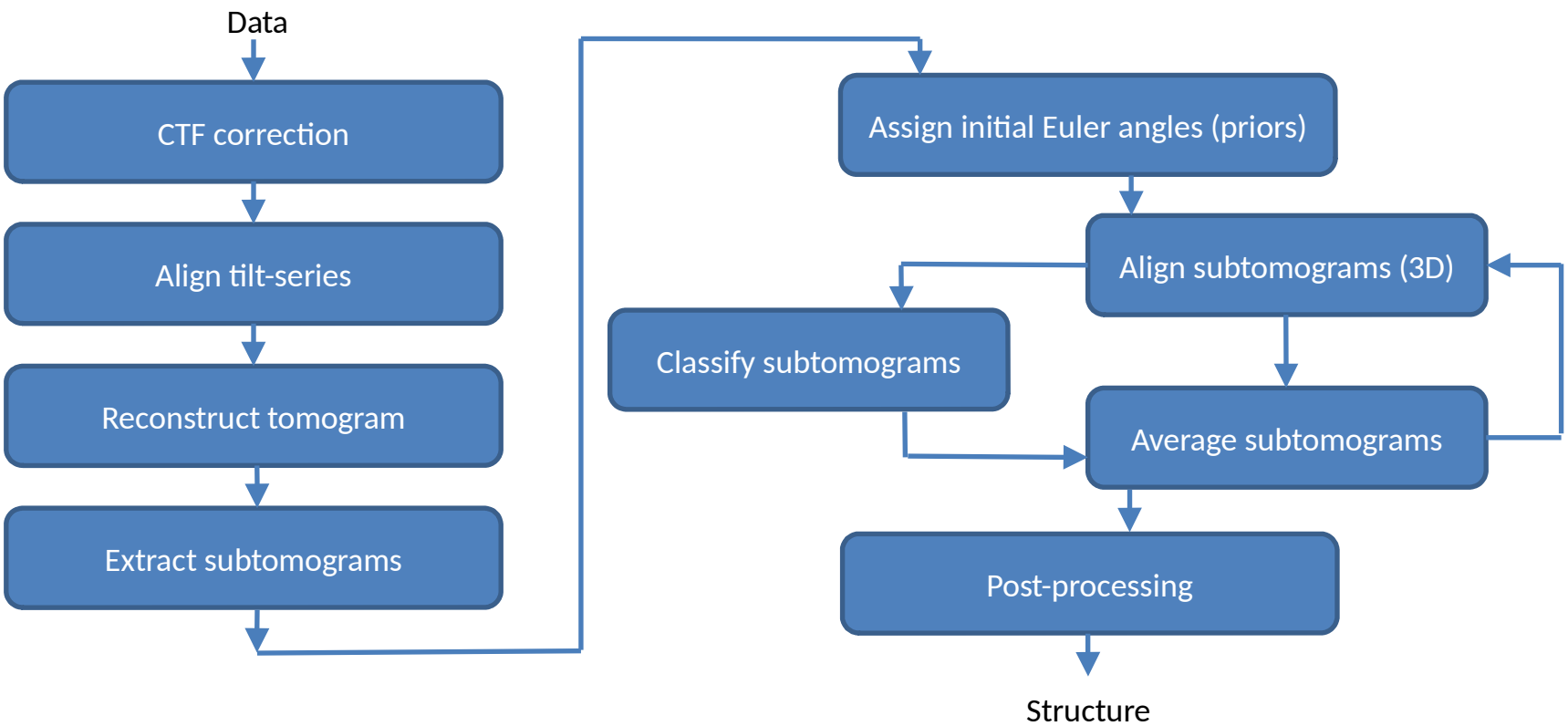
Challenges in data processing

- Most algorithms are “wedge compensated”. We can improve on the traditional binary model for the missing wedge – eg use a 3D-CTF model as in Bharat and Scheres 2016, Wan et al 2017, Himes and Zhang (biorxiv).





Challenges in data processing



Time consuming processing of large 3D datasets

GPU acceleration, new algorithms etc…

Challenges in data processing

We can learn from single-particle reconstruction.

Many ideas and algorithms can be adapted to subtomogram averaging.

What happens at the boundary between single particle and subtomogram averaging?

At the boundary between single particle and subtomogram averaging

If you can do single particle reconstruction then you probably should (because data collection is much faster)

Subtomogram averaging to generate starting models

Subtomogram averaging to aid helical reconstruction

Constrained alignment approaches

Software for subtomogram averaging

Dynamo (Castano-Diez, Basel)

PEET (Heumann and Mastronarde, Boulder)

PyTOM (Foerster, Utrecht)

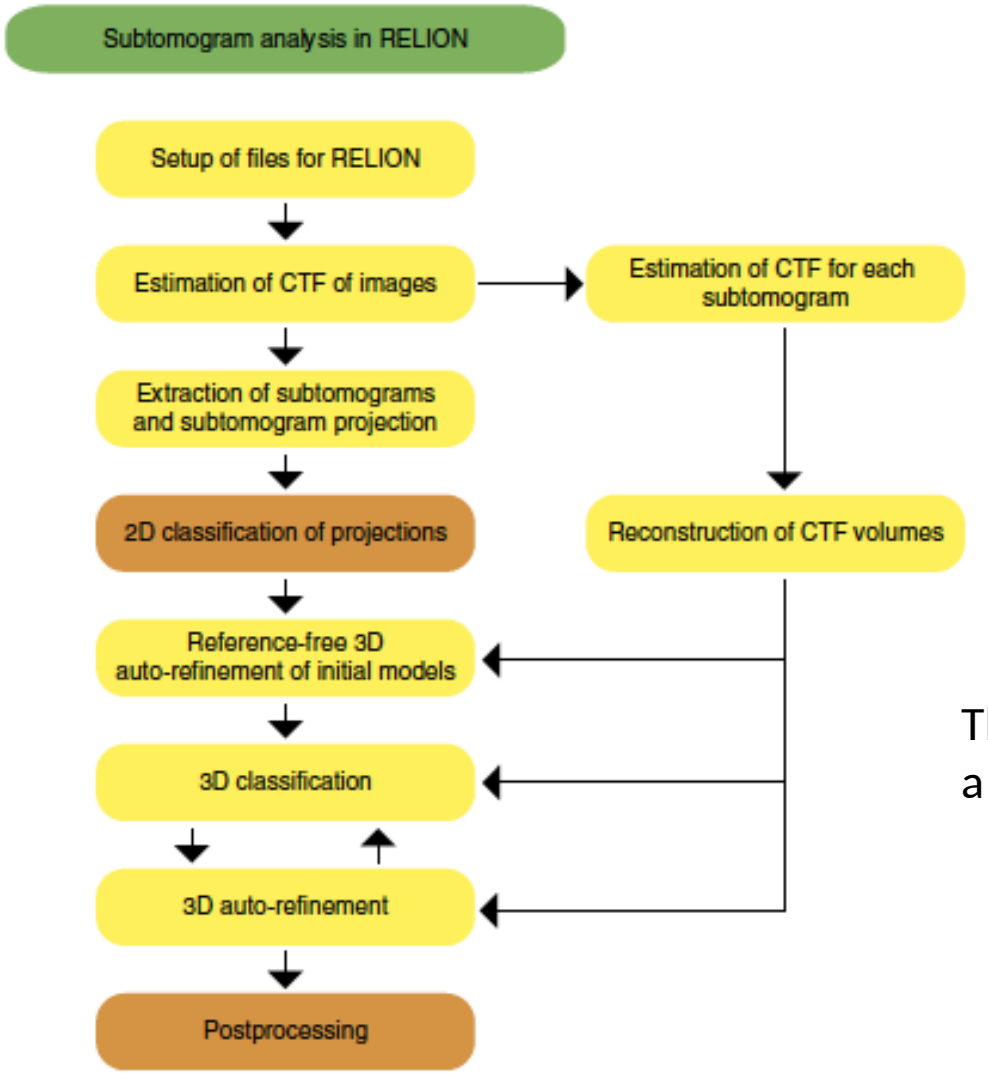
RELION (Bharat and Scheres, LMB)

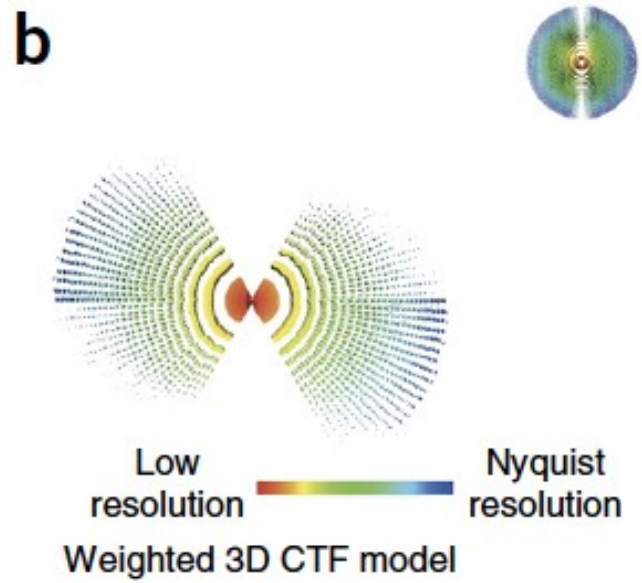
emClarity (Himes and Zhang, Pitsburg)

Articles by many labs cited in:

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

Subtomogram averaging





The current RELION approach uses a 3D CTF model.

Bharat and Scheres 2016