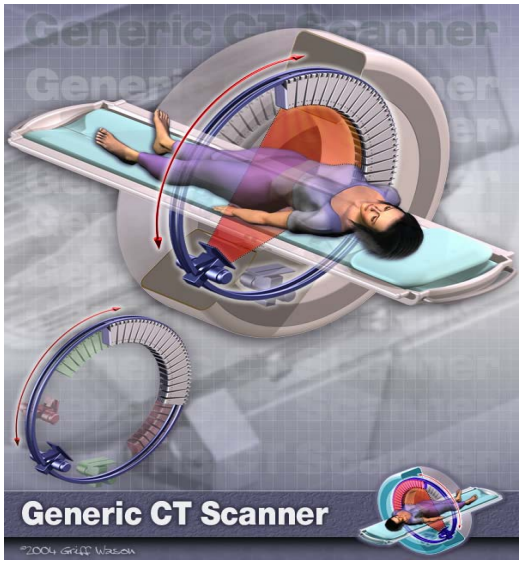
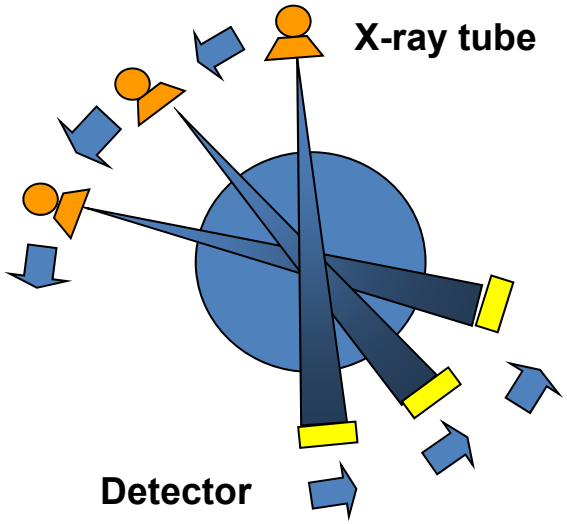
Lecture 8: Tomography (part 1)

1. Principles of Electron Tomography
2. Sample Preparation
3. Data Acquisition
4. Tomogram Reconstruction
5. Tomogram Denoising

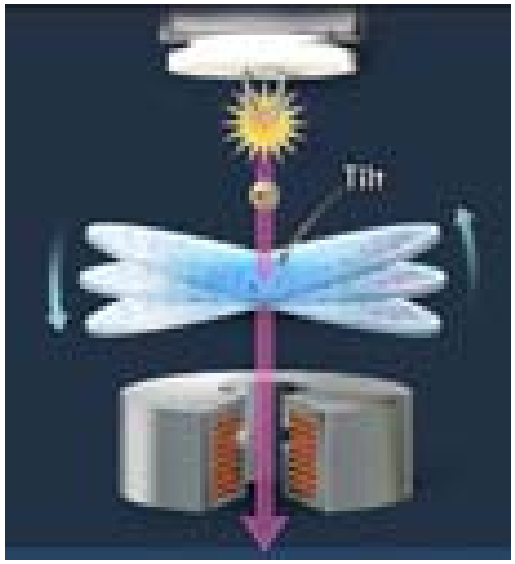
Principles of EM Tomography

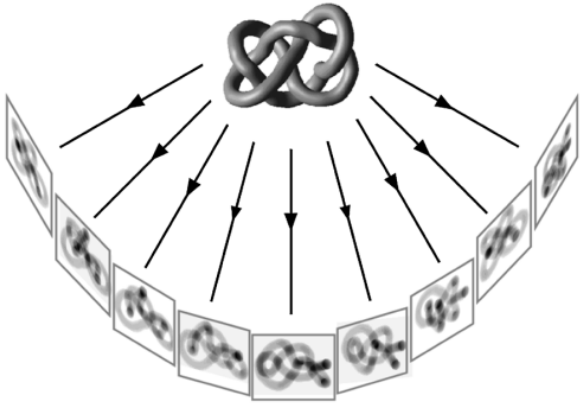
Computer Tomography



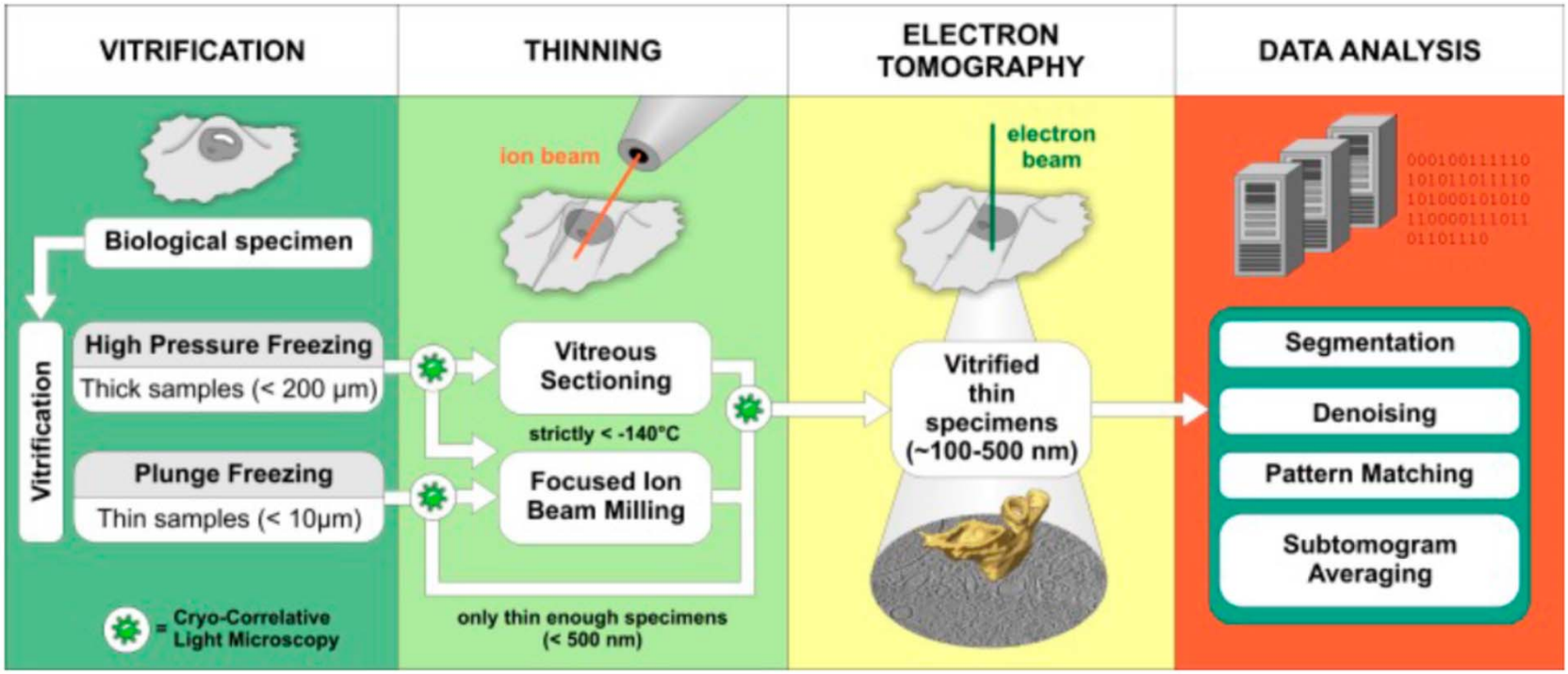


Electron Tomography



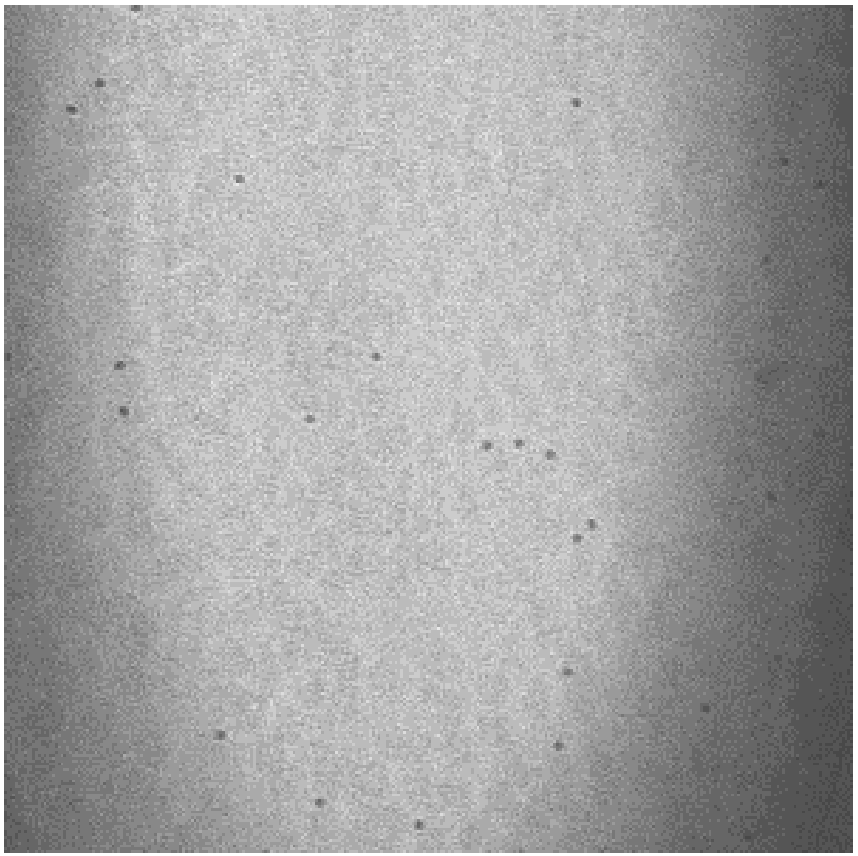


Workflow in Electron Tomography

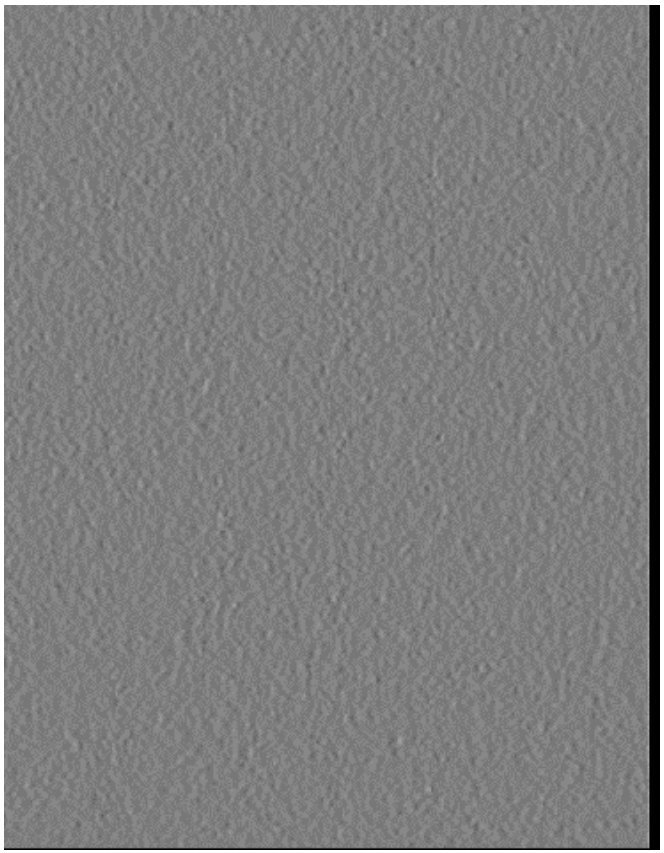


Principles of Electron Tomography

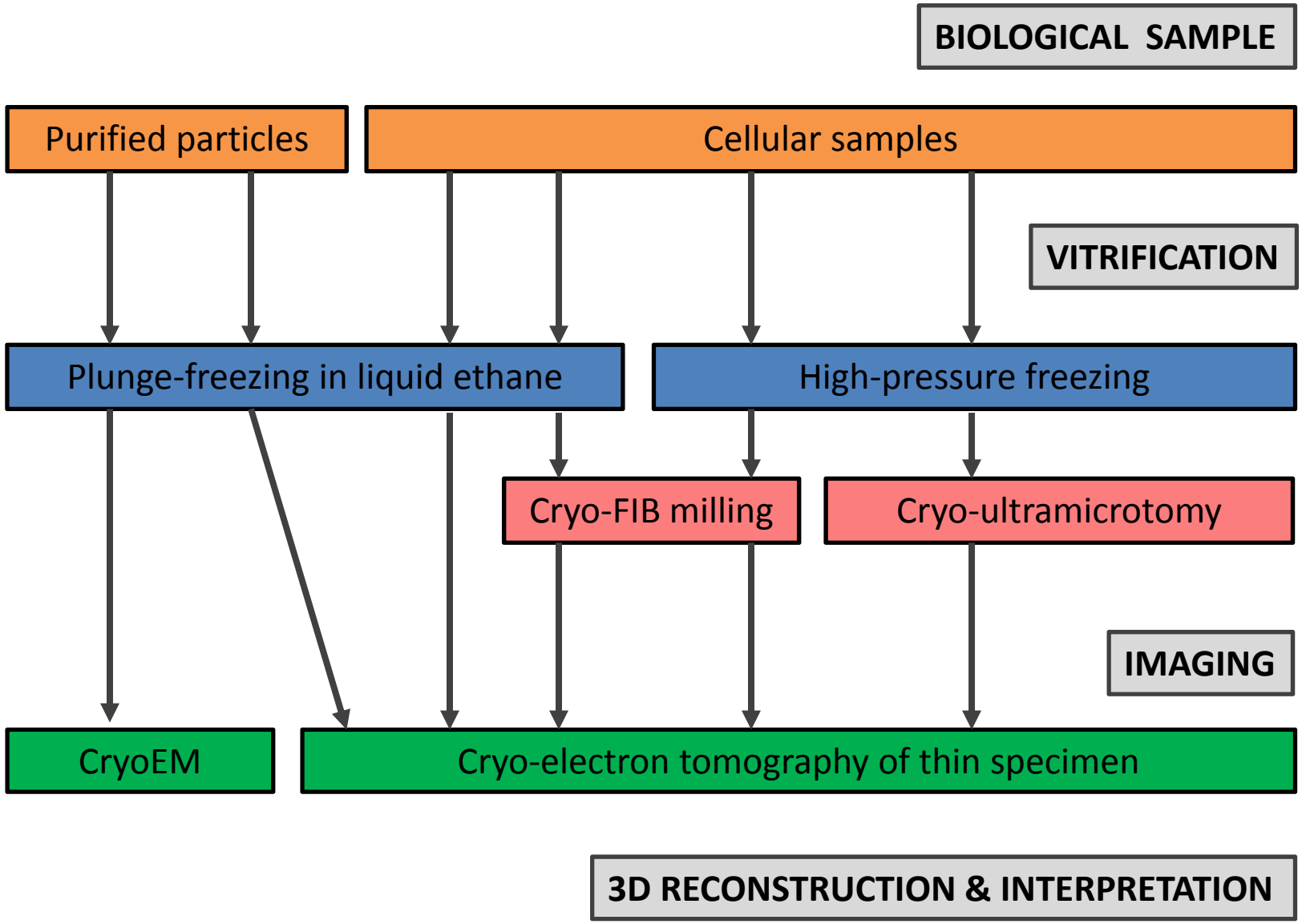
Aligned Tilt Series



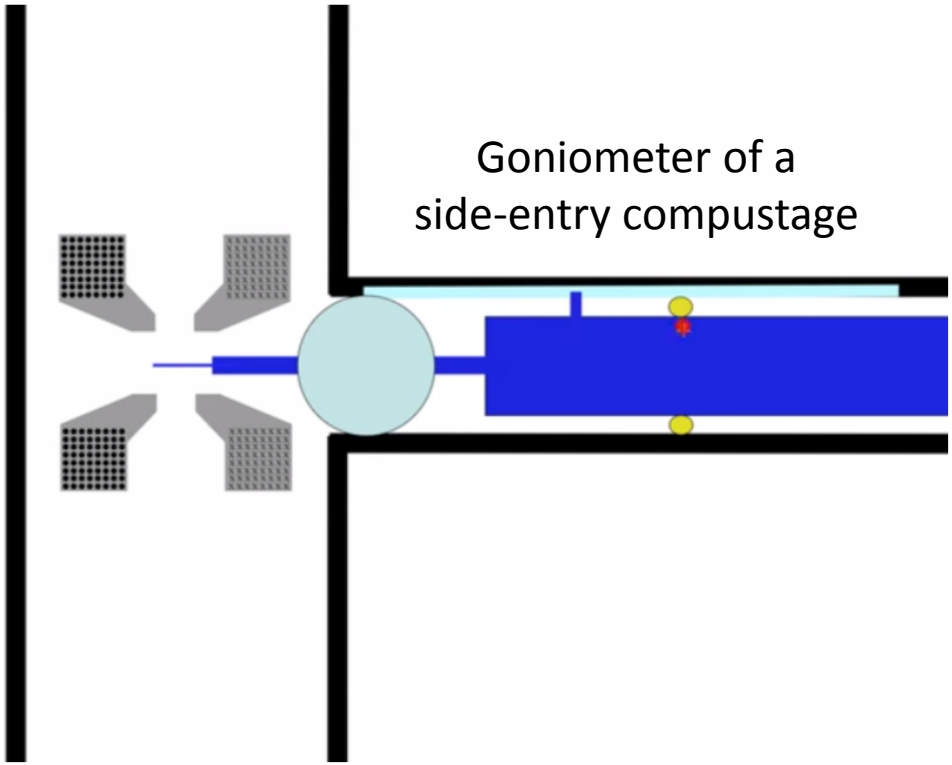
Reconstructed Tomogram

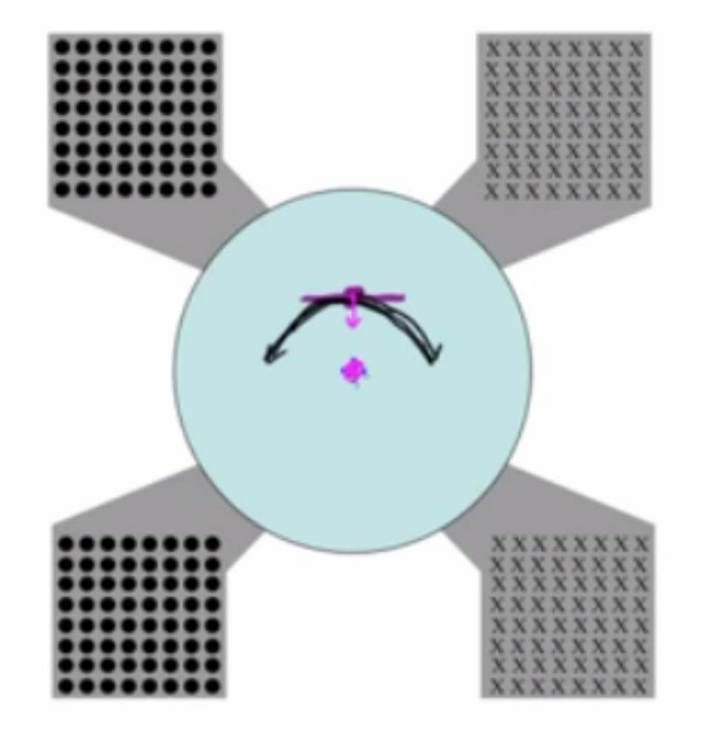


Sample Preparation for CryoET



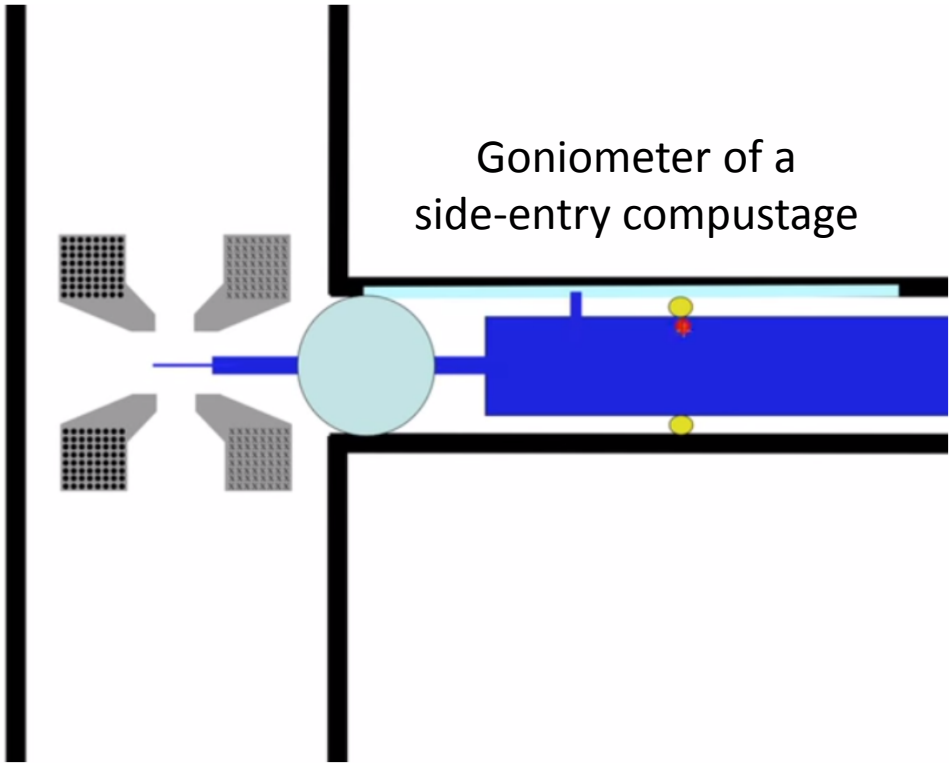
Acquisition of Tilt Series



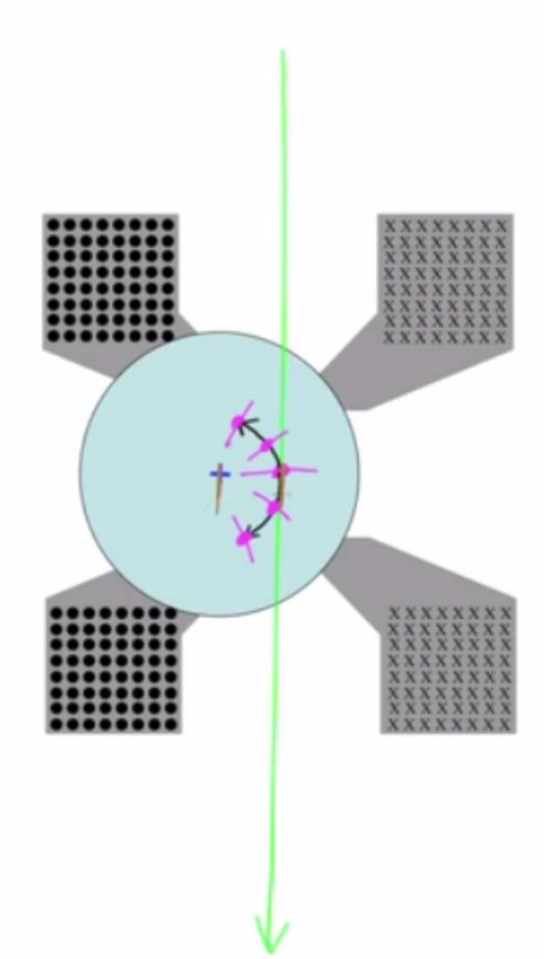


Eucentric height

Acquisition of Tilt Series

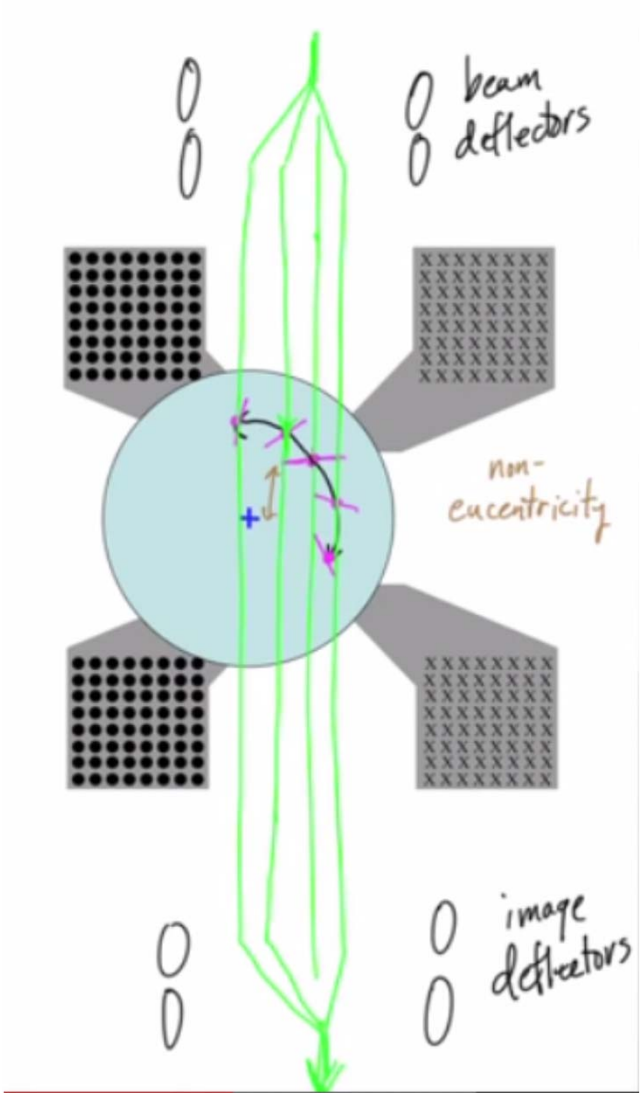


Goniometer of a
side-entry compustage



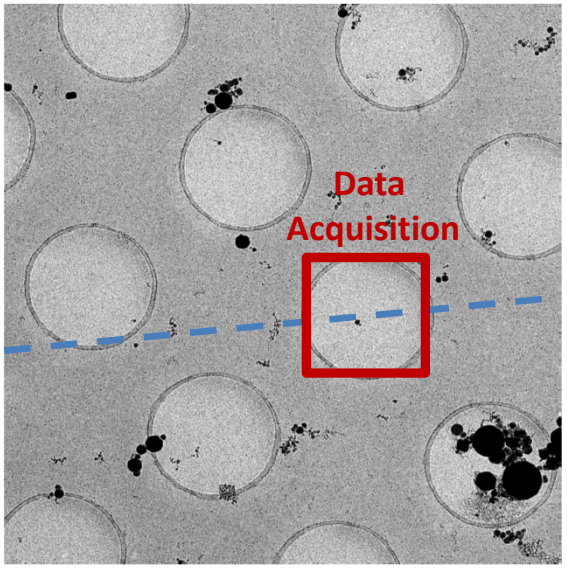
Tilt axis offset

Acquisition of Tilt Series

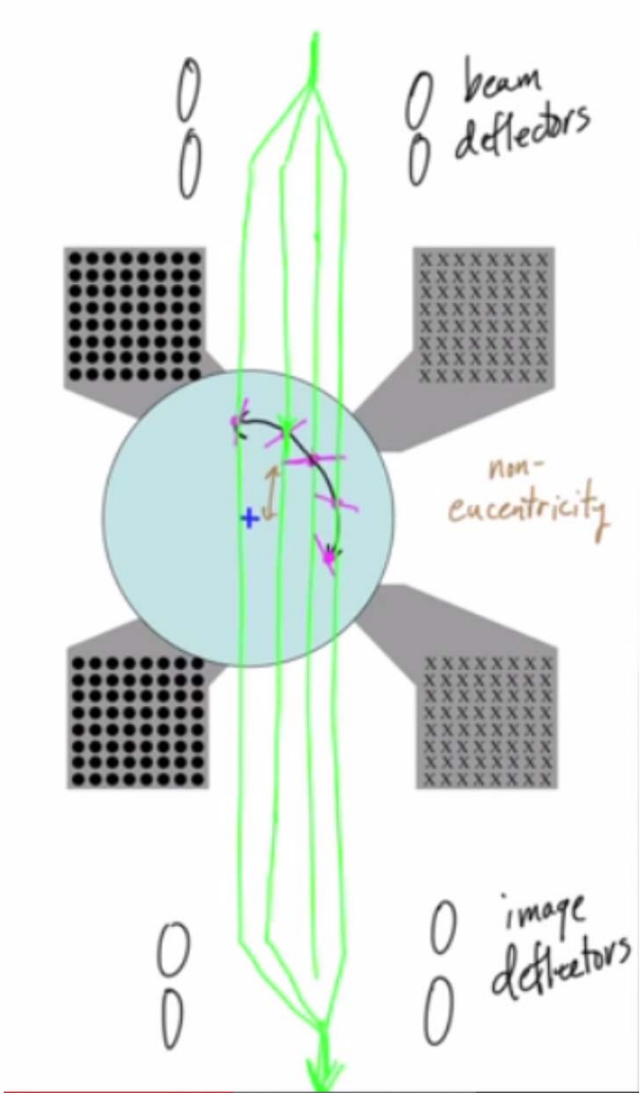


Predictive Method

- Collect few initial tilt images
- Determine image shifts
- Fit shift to a model of tilt geometry
- Predict and apply beam/image shifts
- Collect further images, refine model

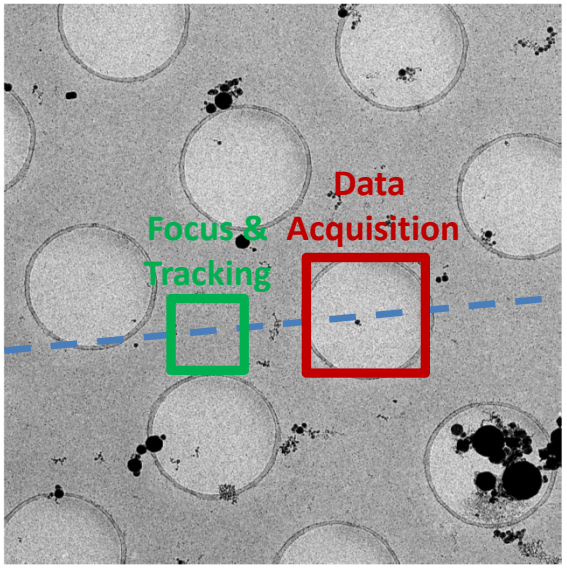


Acquisition of Tilt Series



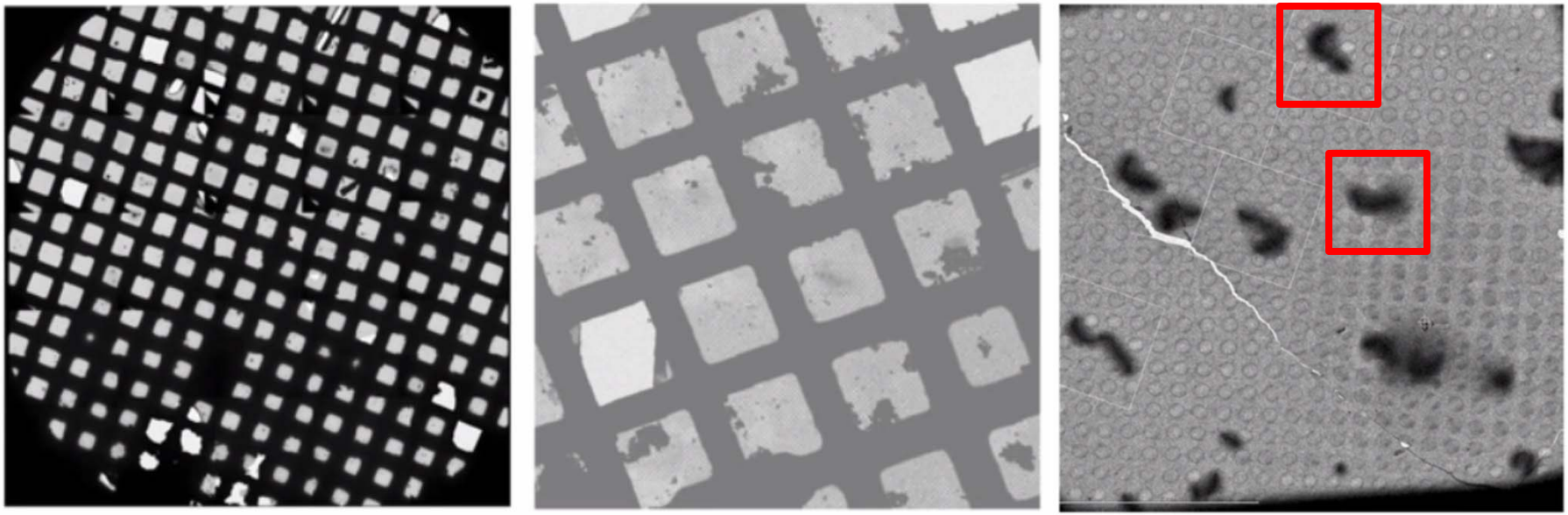
Focus Position Method

- Move to Focus, focus and center
- Move to Record, collect image
- Tilt, move to Focus, focus, center
- Move to Record, collect image
- Refine model of beam/image shifts



Automated Data Collection

Identify the target area of interest

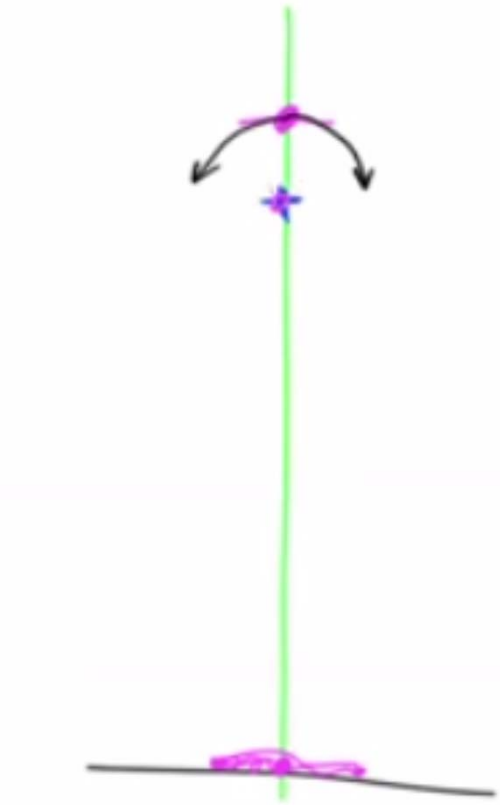


Set parameters for data collection:

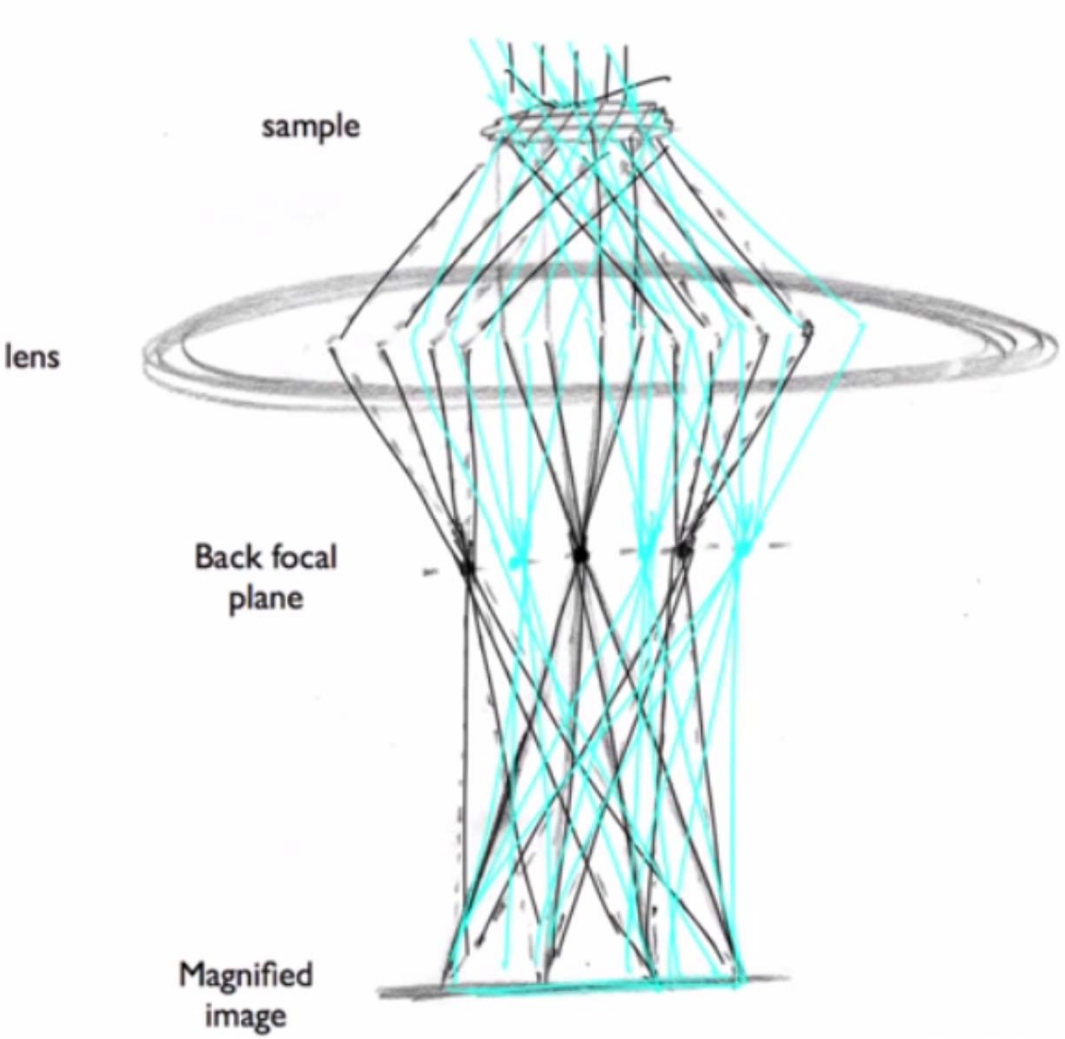
Range of tilt angles:	-60° to +60°
Angular step:	1° or 2°
Dose per image:	0.5-2.0 e^-/A^2
Dose distribution:	uniform vs. tilt-dependent

Automated Data Collection

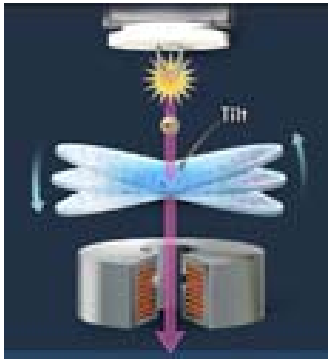
Automated determination of the Eucentric height

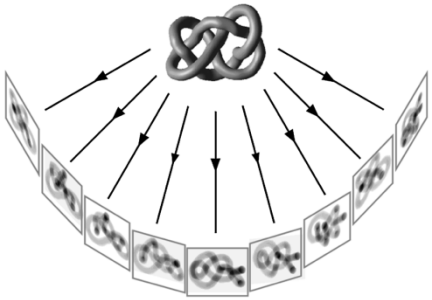


Automated focusing

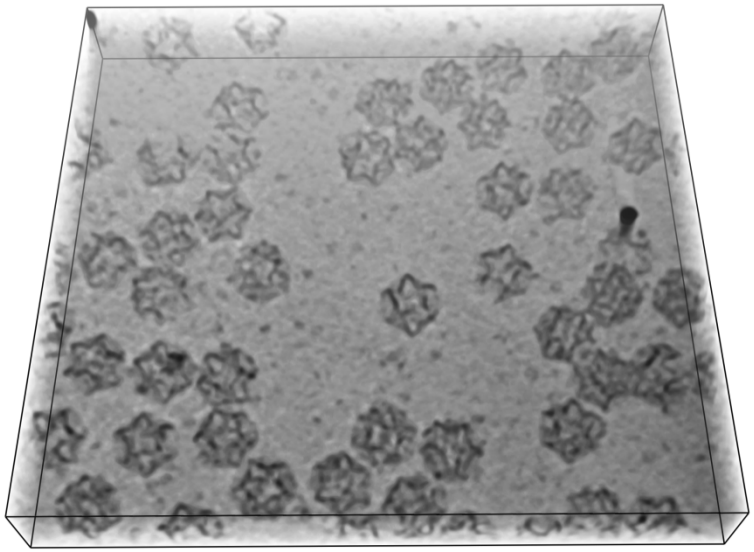


Electron Tomography

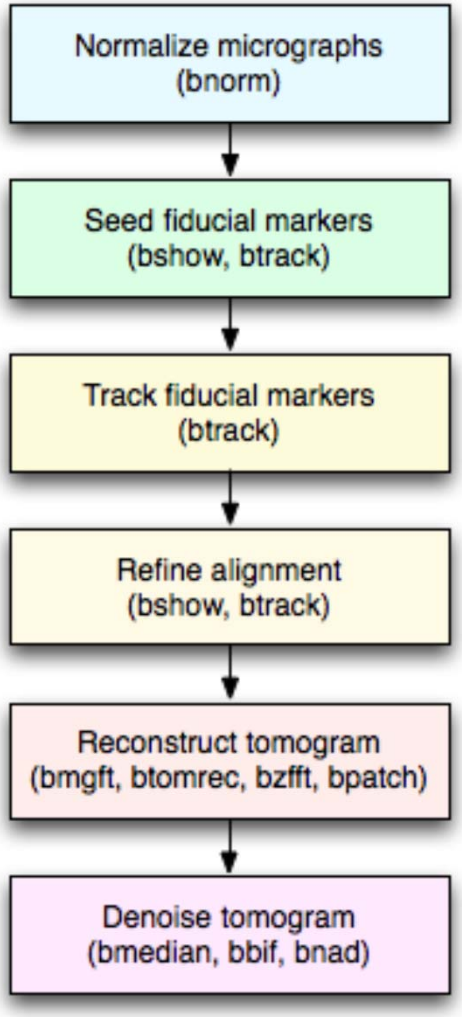




Data acquisition (tilt series)

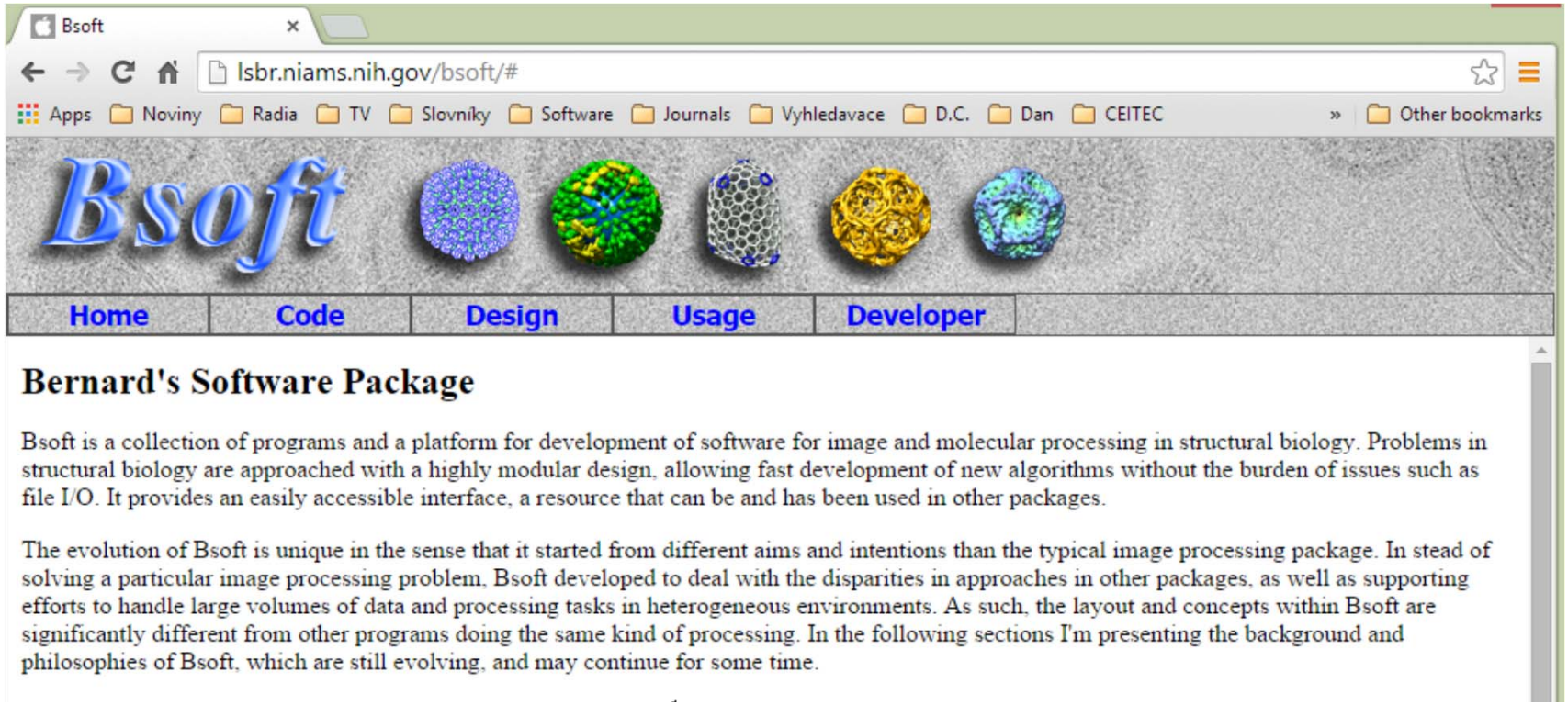


Reconstructed tomogram



Tomogram reconstruction workflow
(IMOD, Bsoft, EMAN2, Xmipp)

Image processing in Bsoft

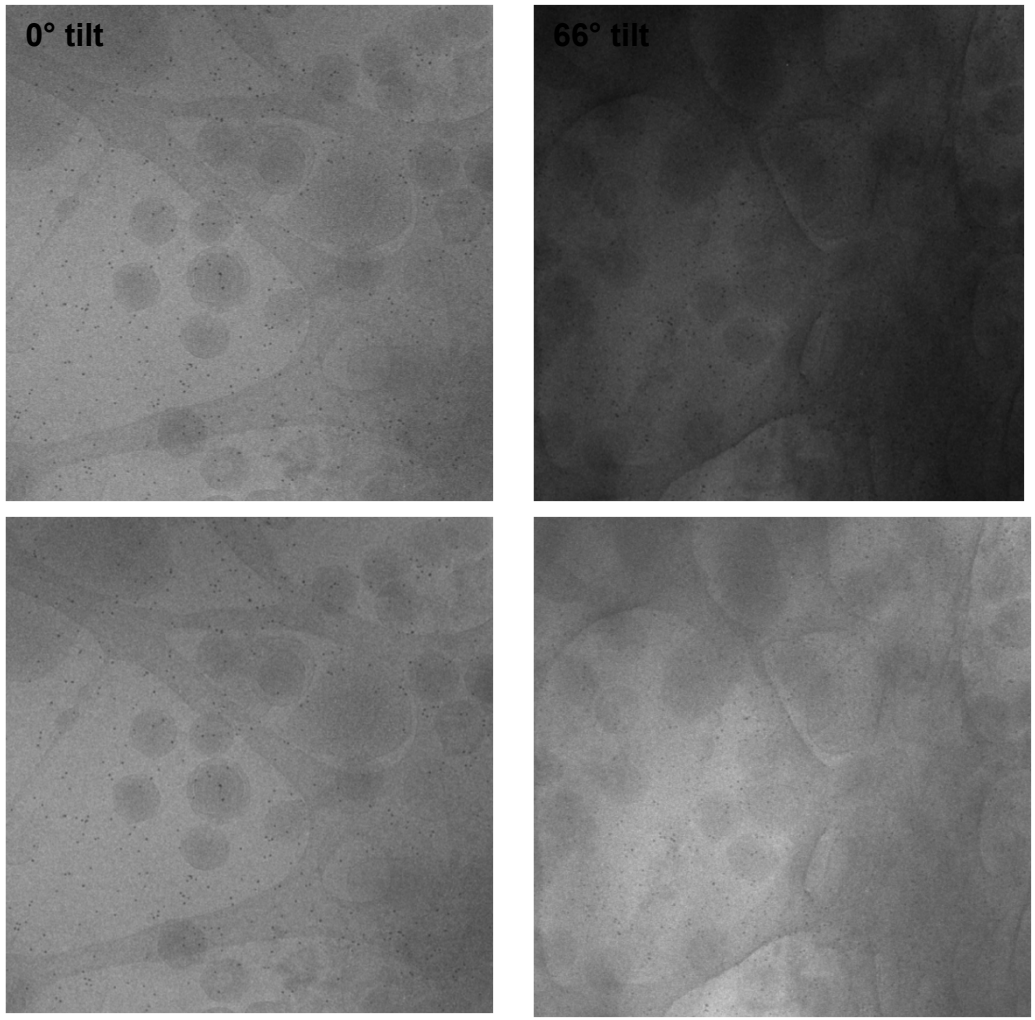


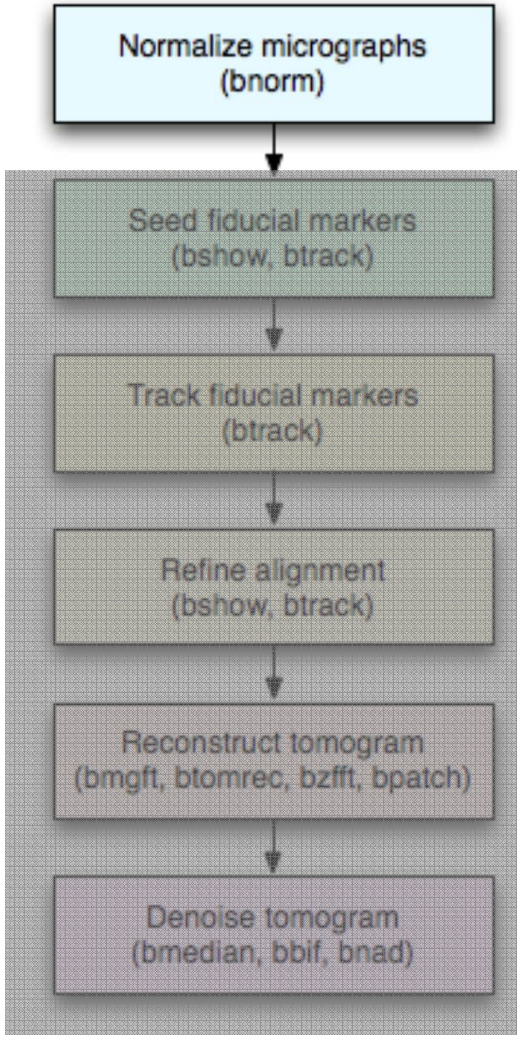
Images are stored in pif/mrc/tif files as a **set of 2D images** or as **slices of a 3D image**.

Parameters are stored in ASCII **star files** with predetermined organization for **micrographs**, **reconstructions** and **models**.

Heymann et al., *J. Struct. Biol.*(2008) 161, 232

Preprocessing of collected data

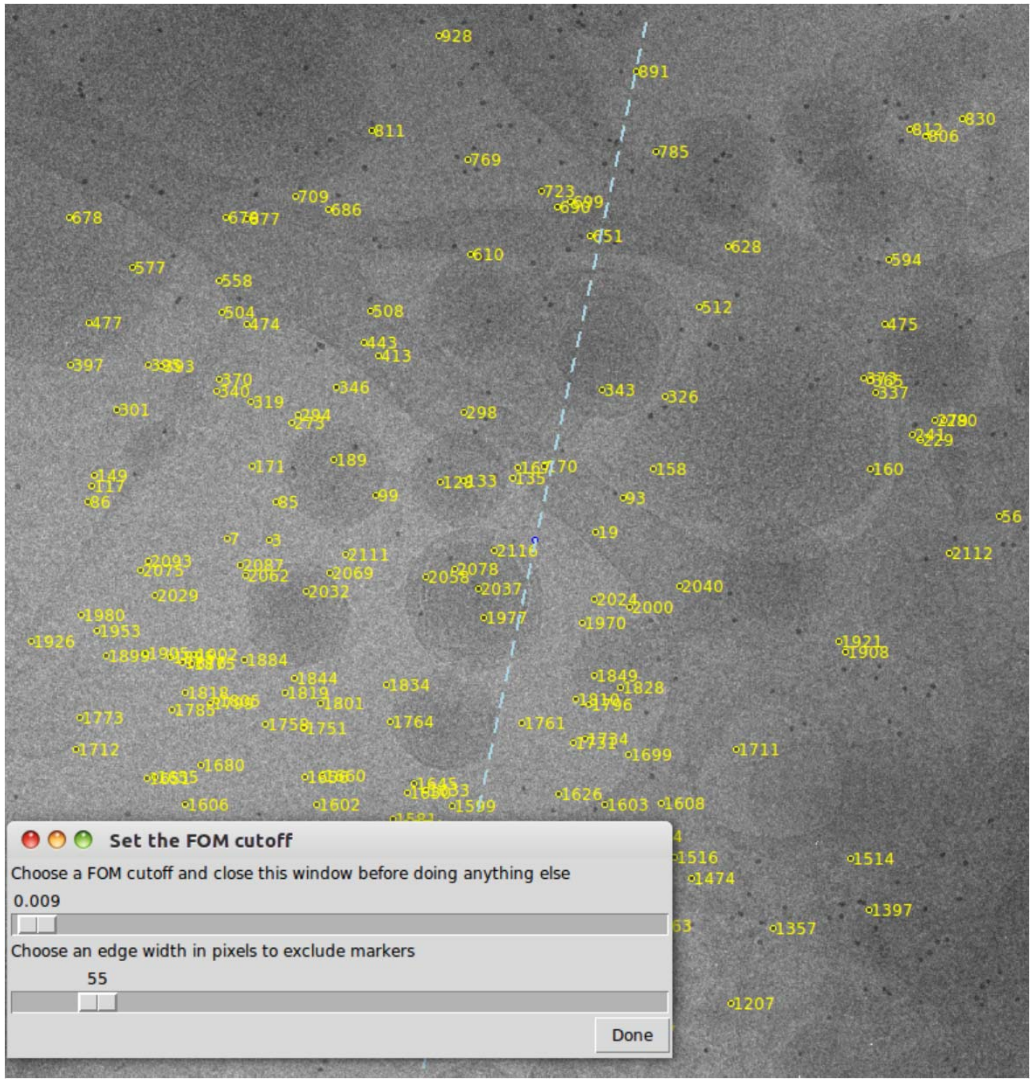


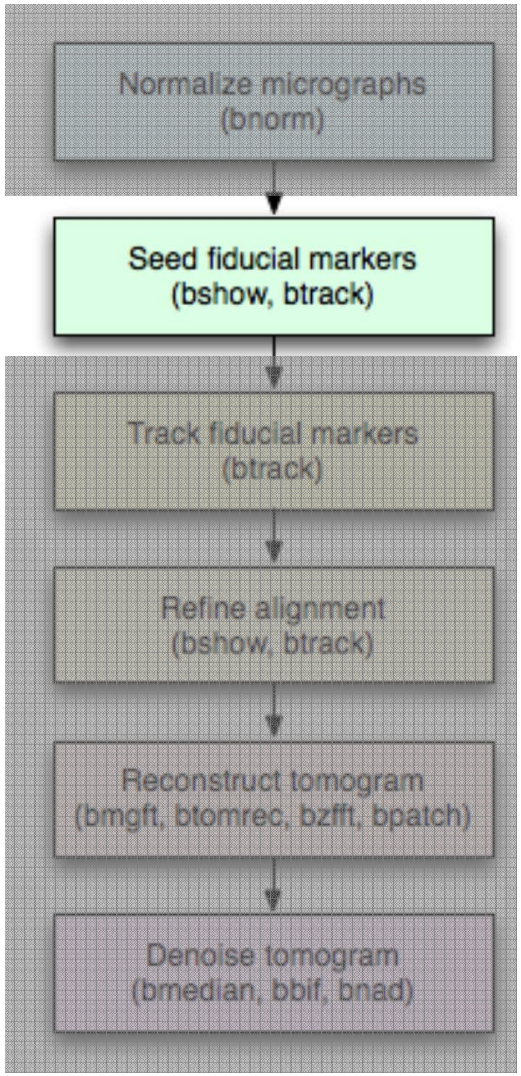


bnorm -ver 7 -images -rescale 127,10 -data byte -out output.star input.mrc output.pif

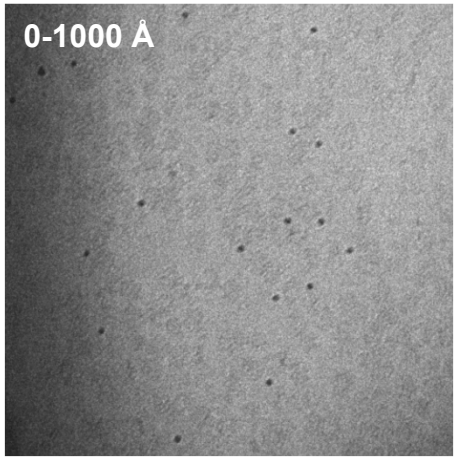
btomo -v 7 -sampling 8.05 -axis 78 -tilt -60,2 -gold 5 -out output.star input.star

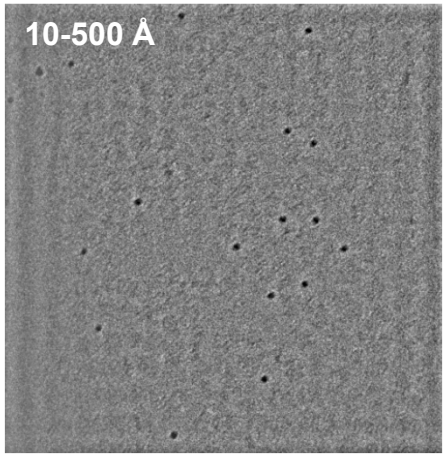
Finding fiducial markers for alignment of tilt series

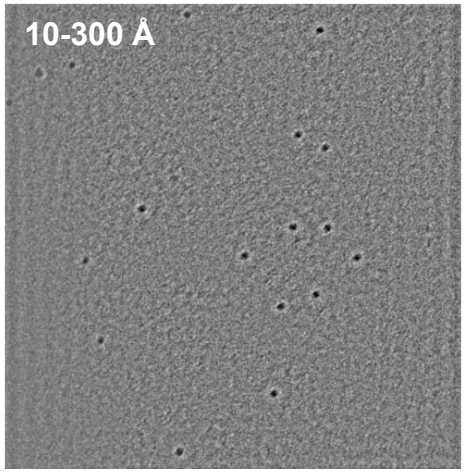


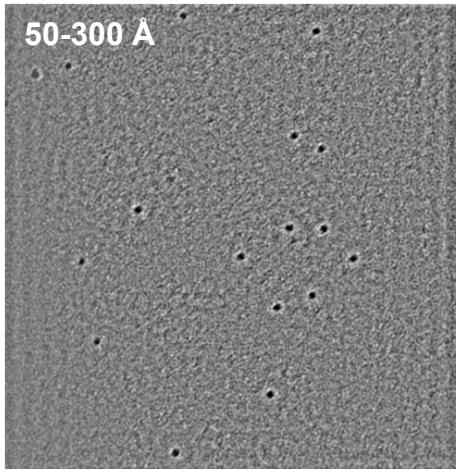


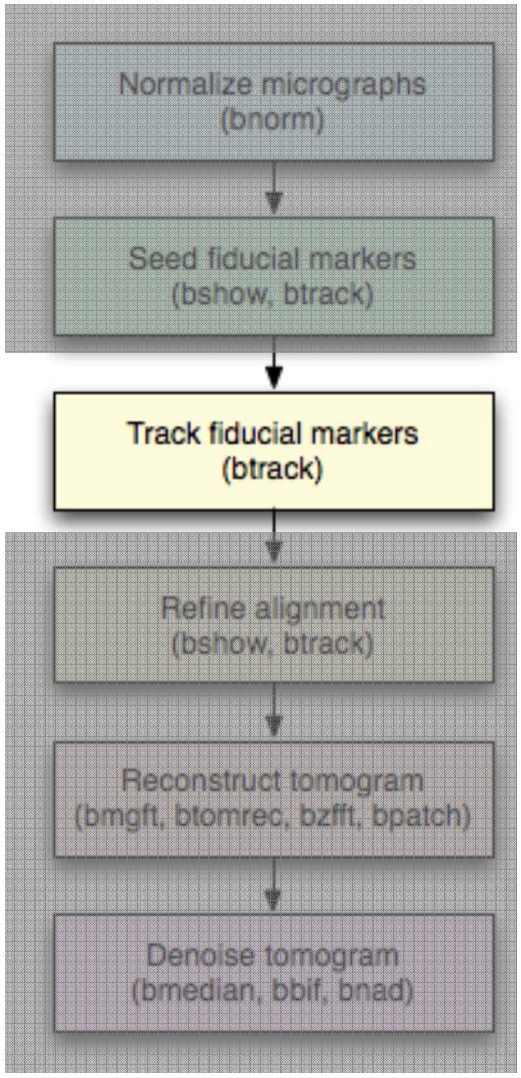
Tracking fiducial markers in all tilt images





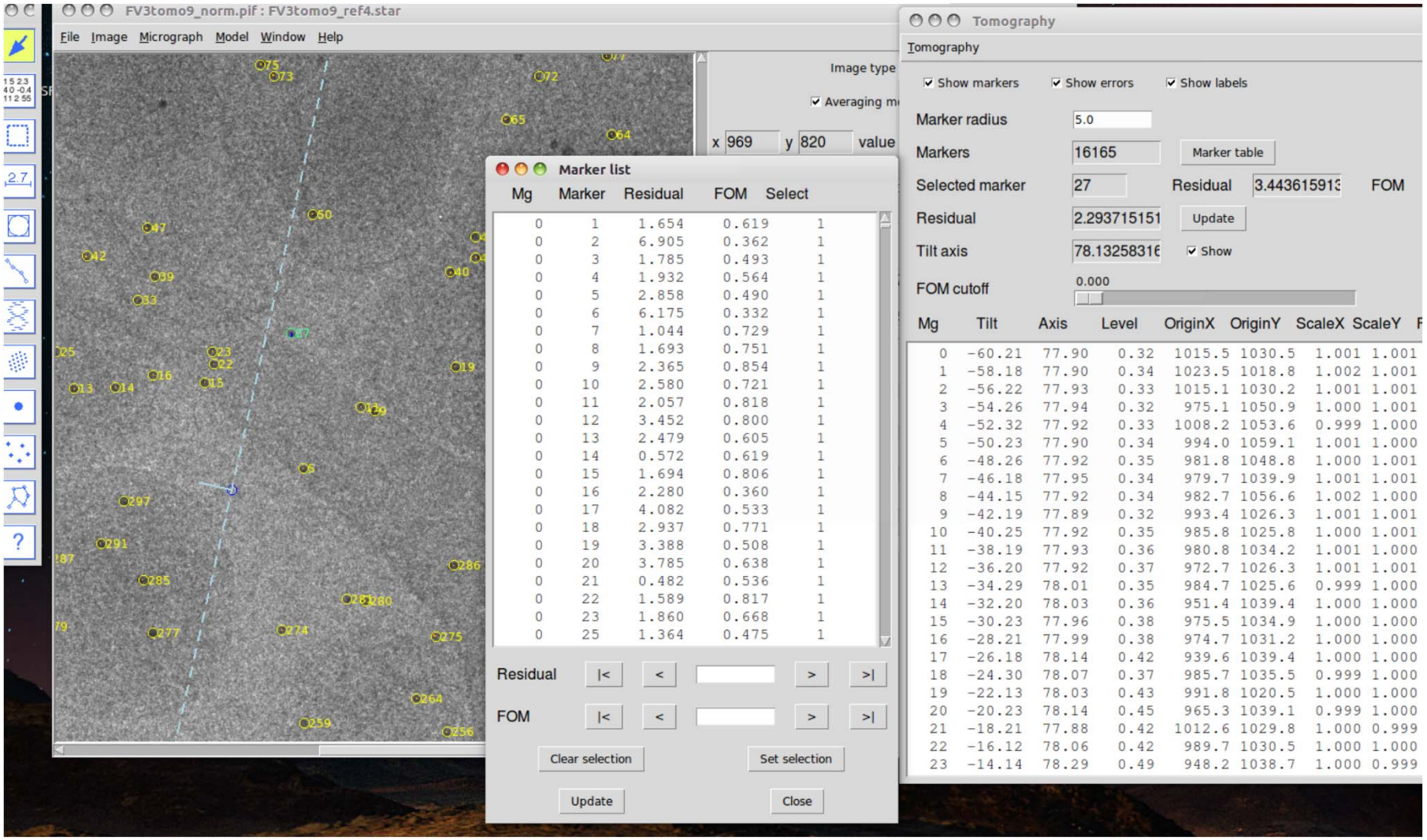






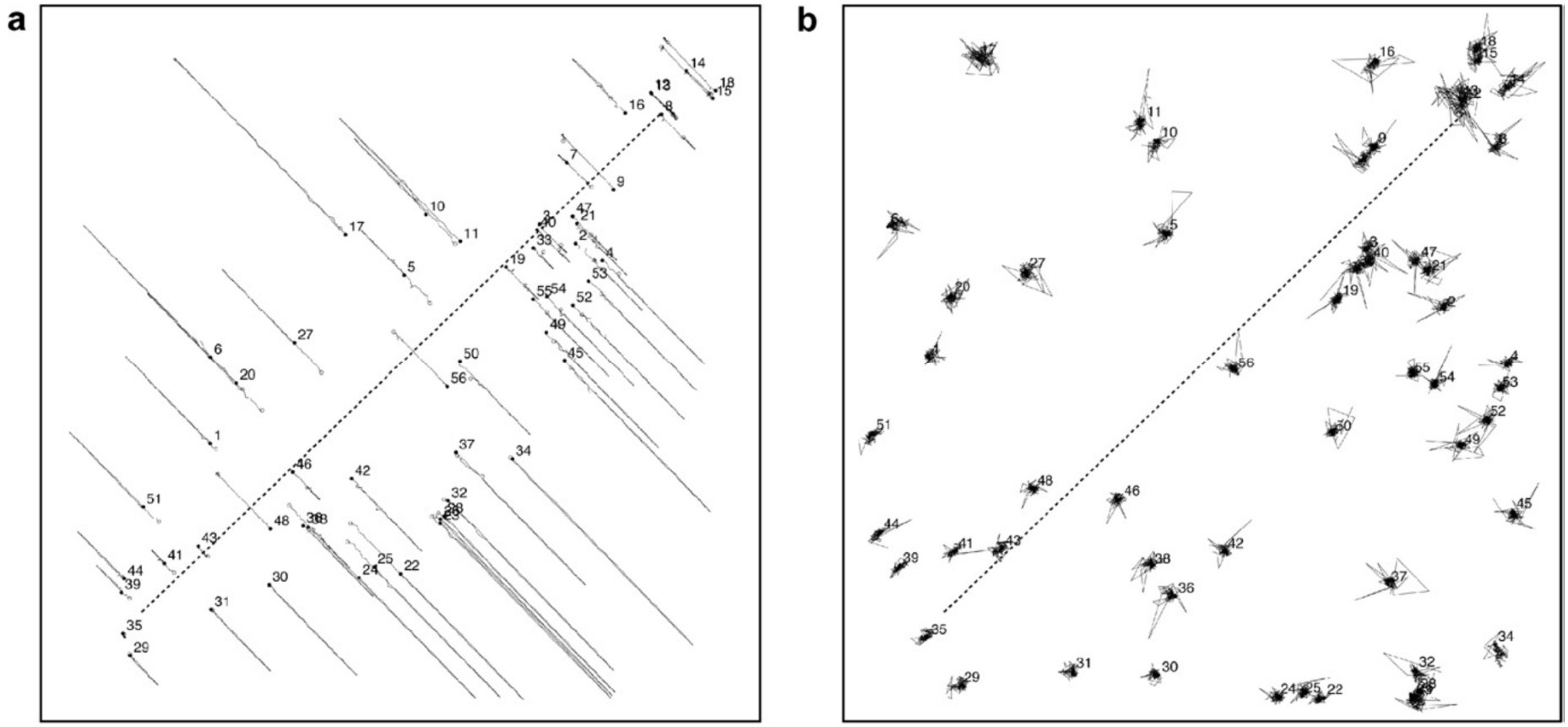
btrack -ver 1 -reset -axis 78 -exclude none -resol 15,300 -shift 1000 -update -track 5 -refine markers -out FV3tomo9_trk.star FV3tomo9_seed.star >& FV3tomo9_trk.log

Refinement of fiducial marker positions



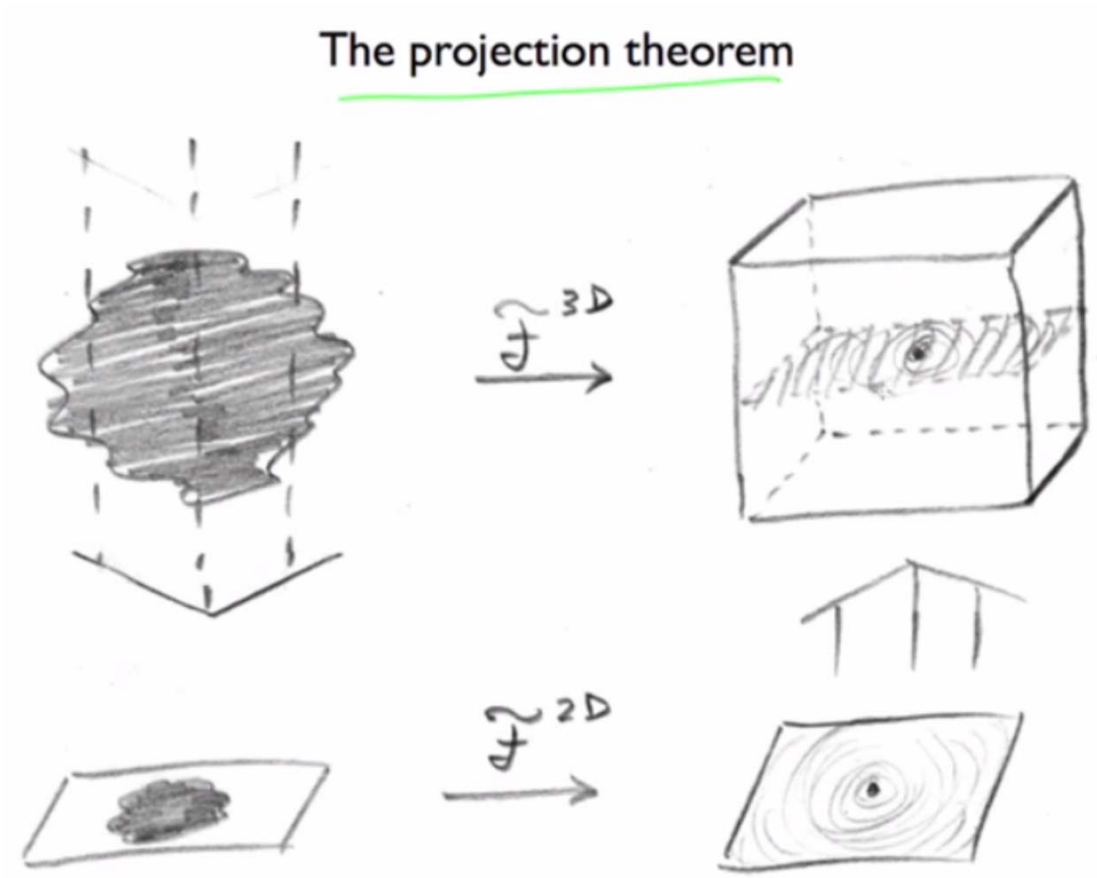
btrack -ver 1 -reset -refine 10,z,o,v -image FV3tomo9_align.pif -Post FV3tomo9_err.ps -out FV3tomo9_ref1.star FV3tomo9_ref0.star >& FV3tomo9_ref1.log

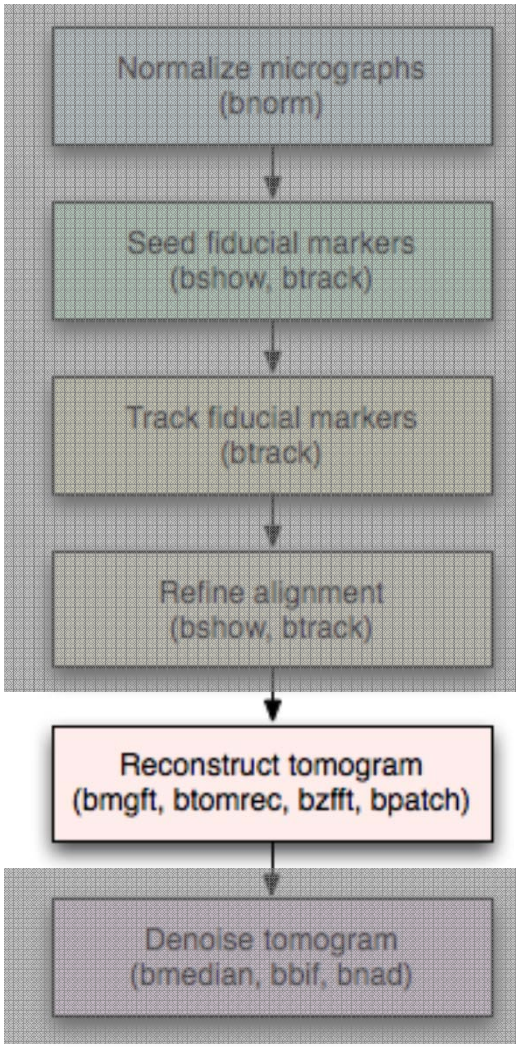
Refinement of fiducial marker positions



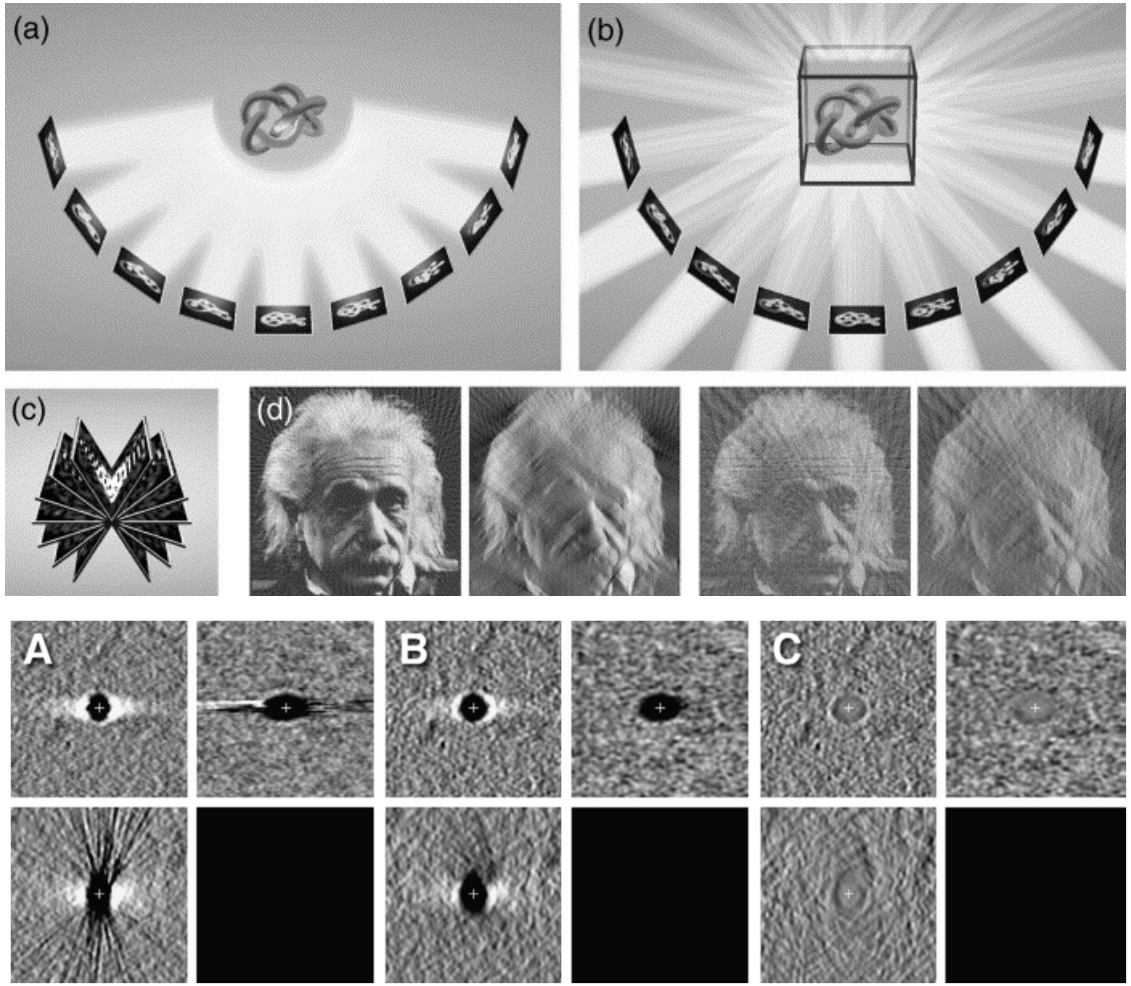
btrack -ver 1 -reset -refine 10,z,o,v -image FV3tomo9_align.pif -Post FV3tomo9_err.ps -out FV3tomo9_ref1.star FV3tomo9_ref0.star >& FV3tomo9_ref1.log

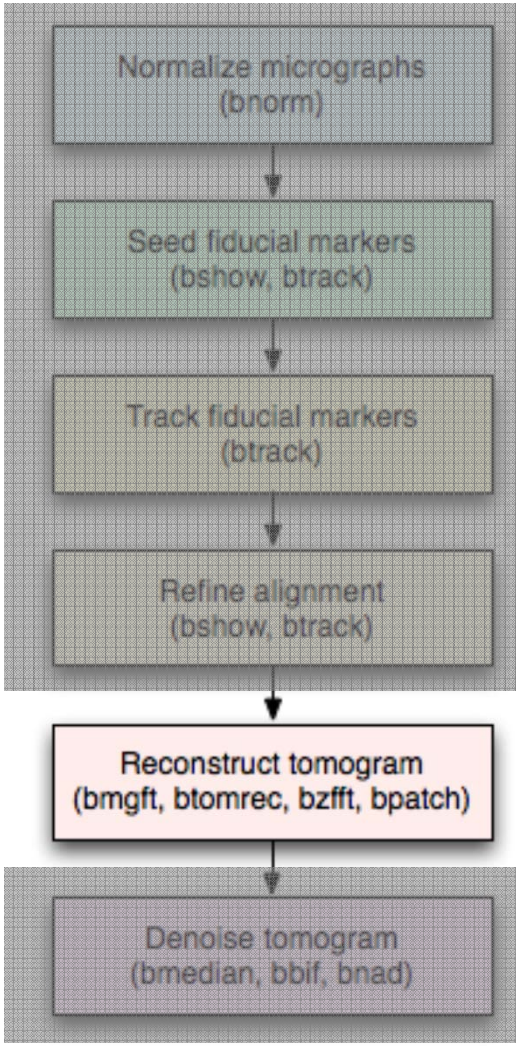
Tomogram reconstruction





Tomogram reconstruction

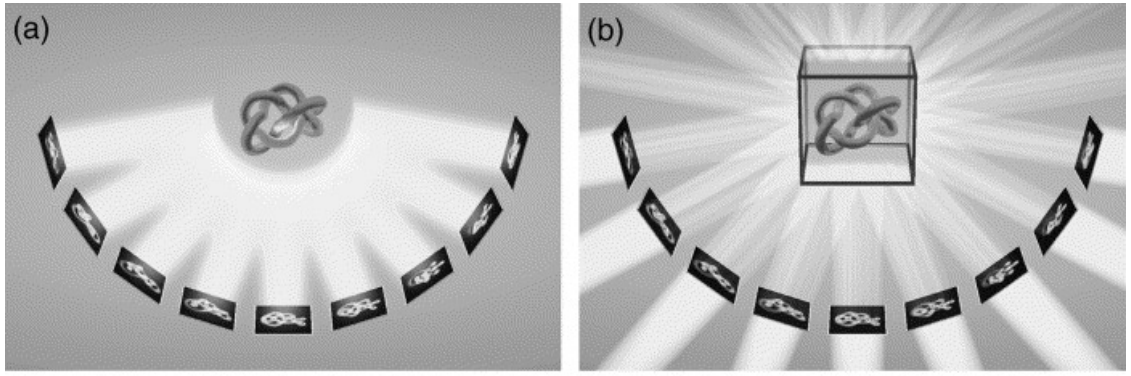


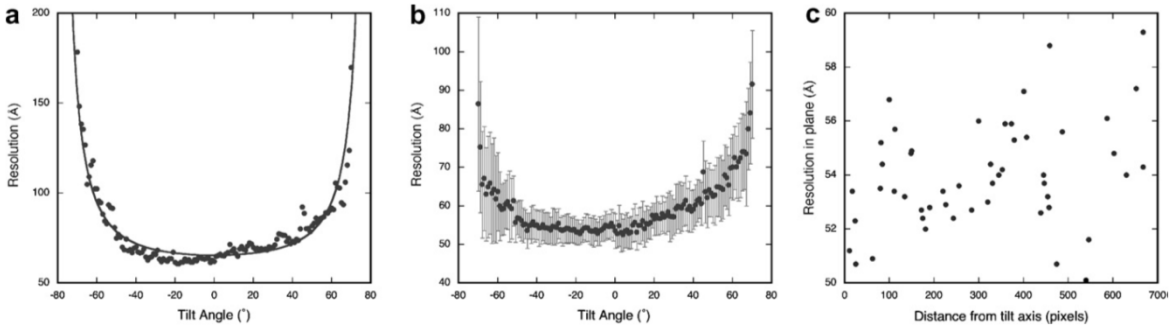


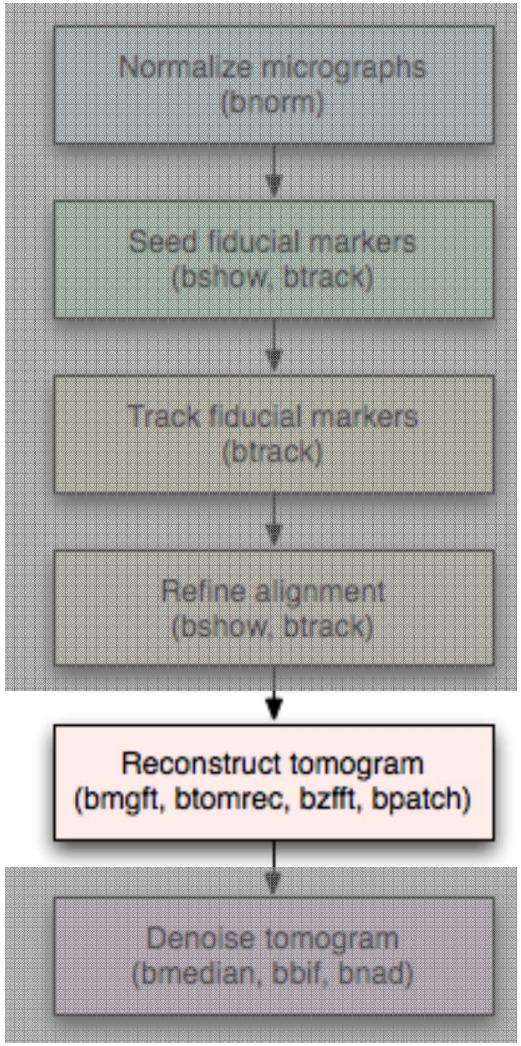
bmark -v 7 FV3tomo7_ref4.star

tomrec_PBS.tcsh -rec output.pif -resol 20 -size 2048,2048,550 -remove 10 -thick 20 -scale 1 -out output.star input.star

Tomogram reconstruction



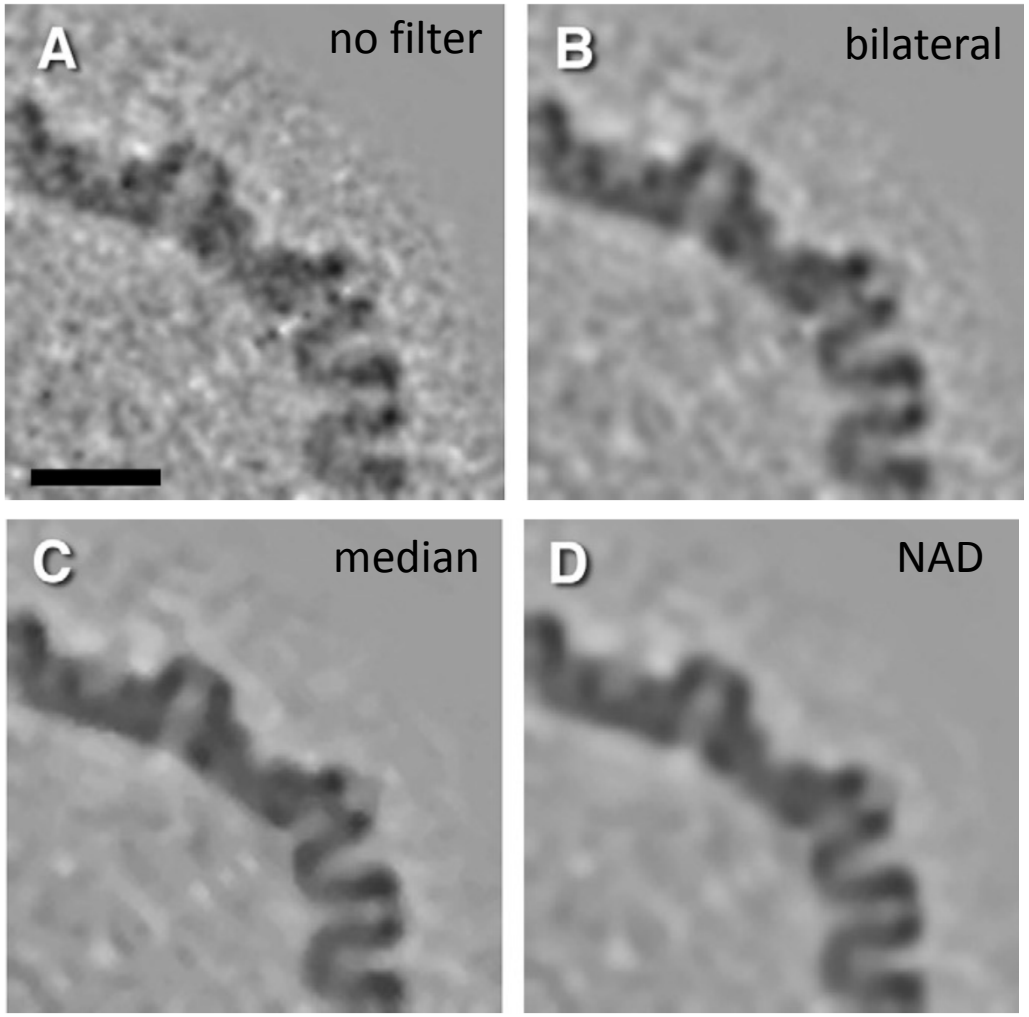


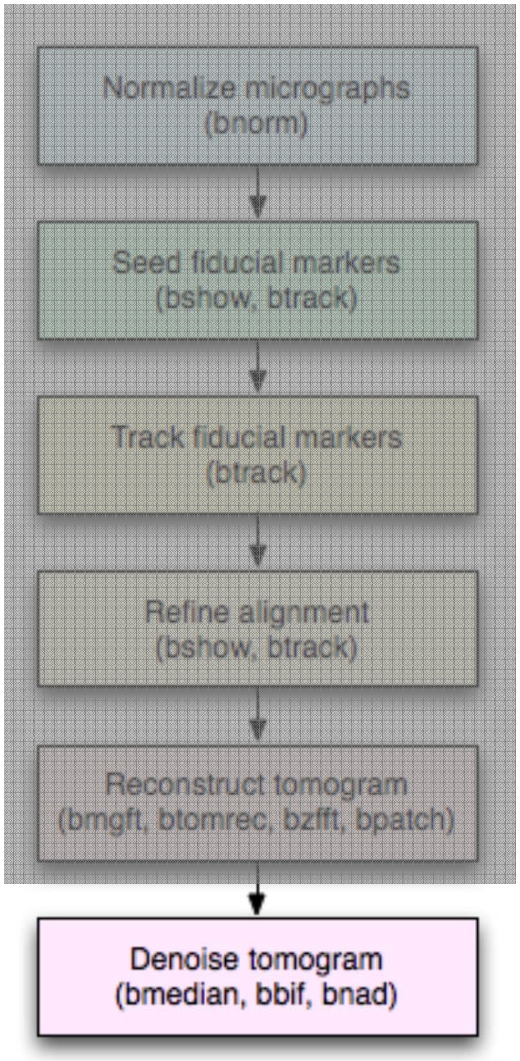


bmark -v 7 FV3tomo7_ref4.star

tomrec_PBS.tcsh -rec output.pif -resol 20 -size 2048,2048,550 -remove 10 -thick 20 -scale 1 -out output.star input.star

Denoising of reconstructed tomograms





tomnad_PBS.tcsh -size 300,300,550 -overlap 100,100,0 -iterations 30 input.pif output.pif