



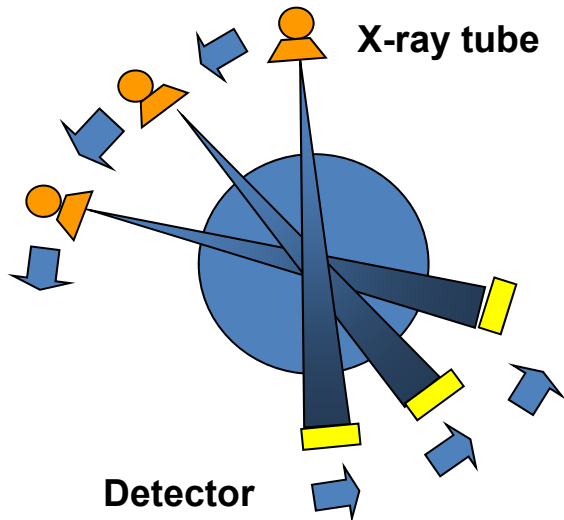
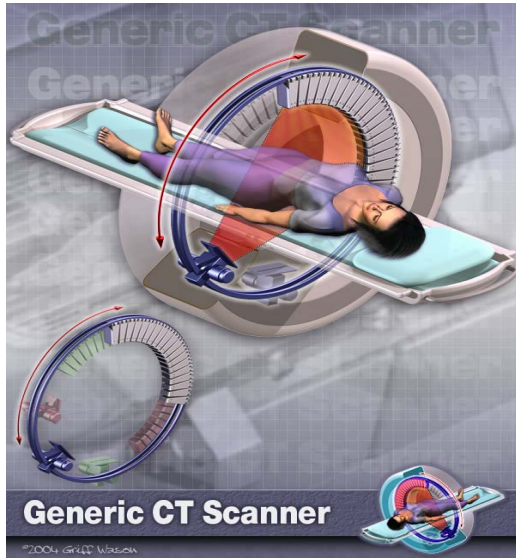
Lecture 8: Tomography (part 1)

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

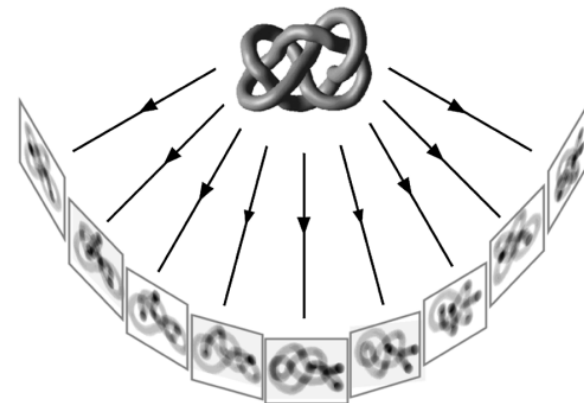
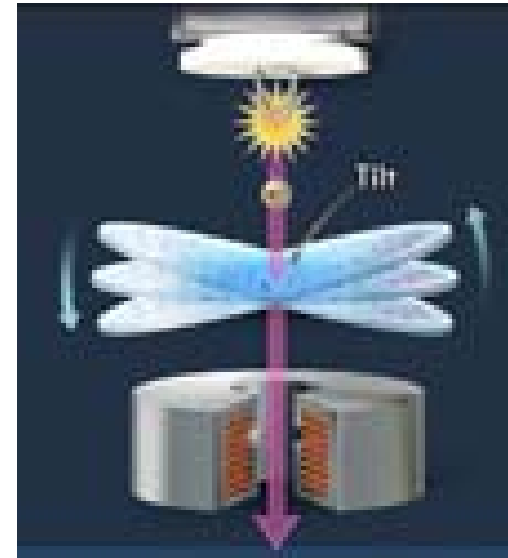


Principles of EM Tomography

Computer Tomography

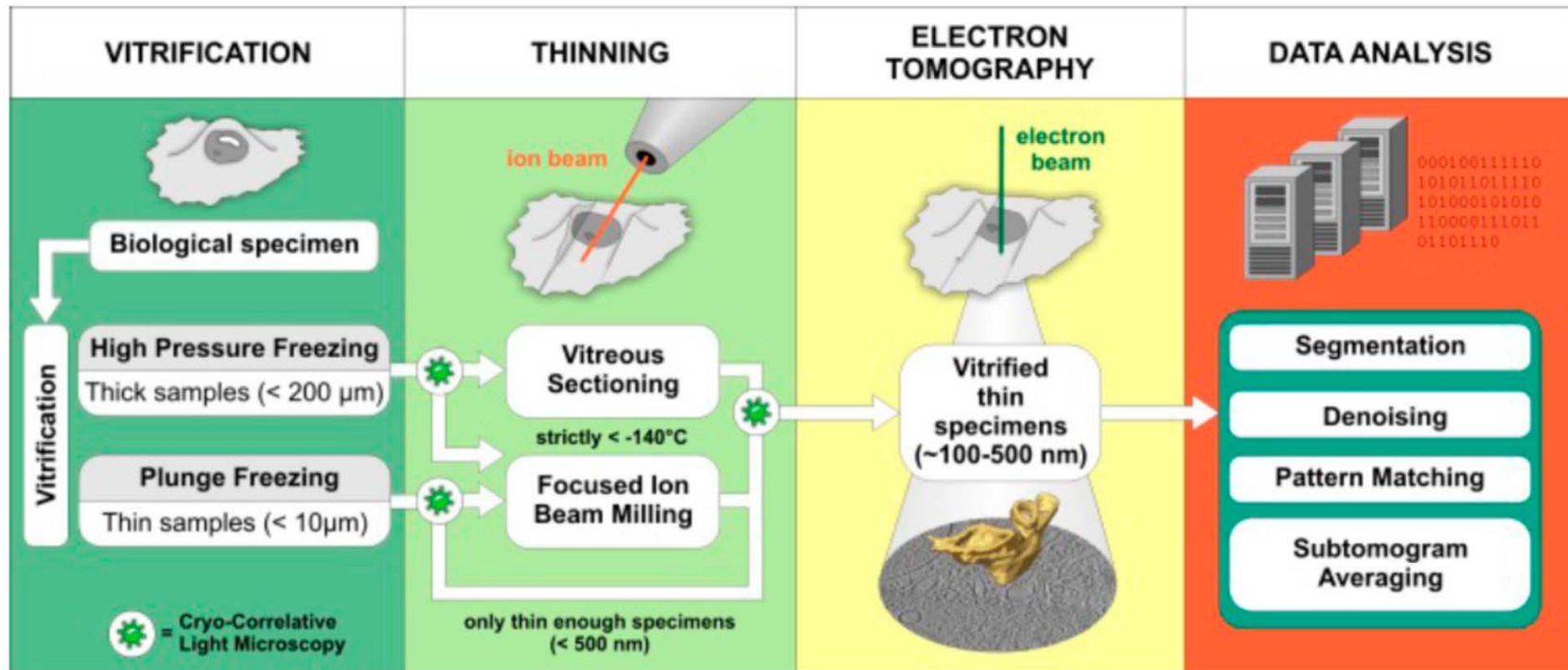


Electron Tomography





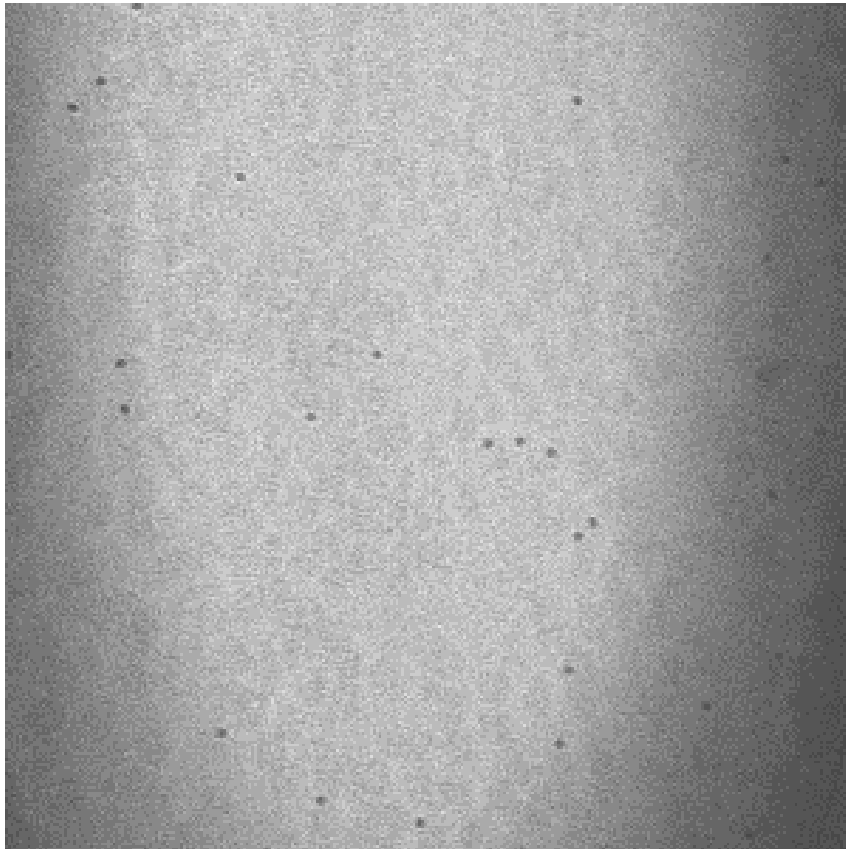
Workflow in Electron Tomography



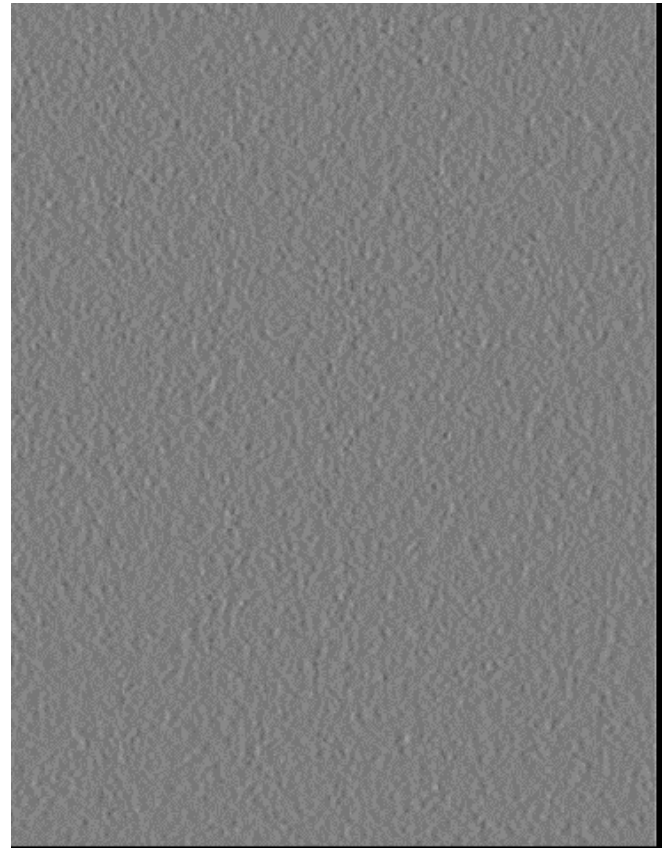


Principles of Electron Tomography

Aligned Tilt Series

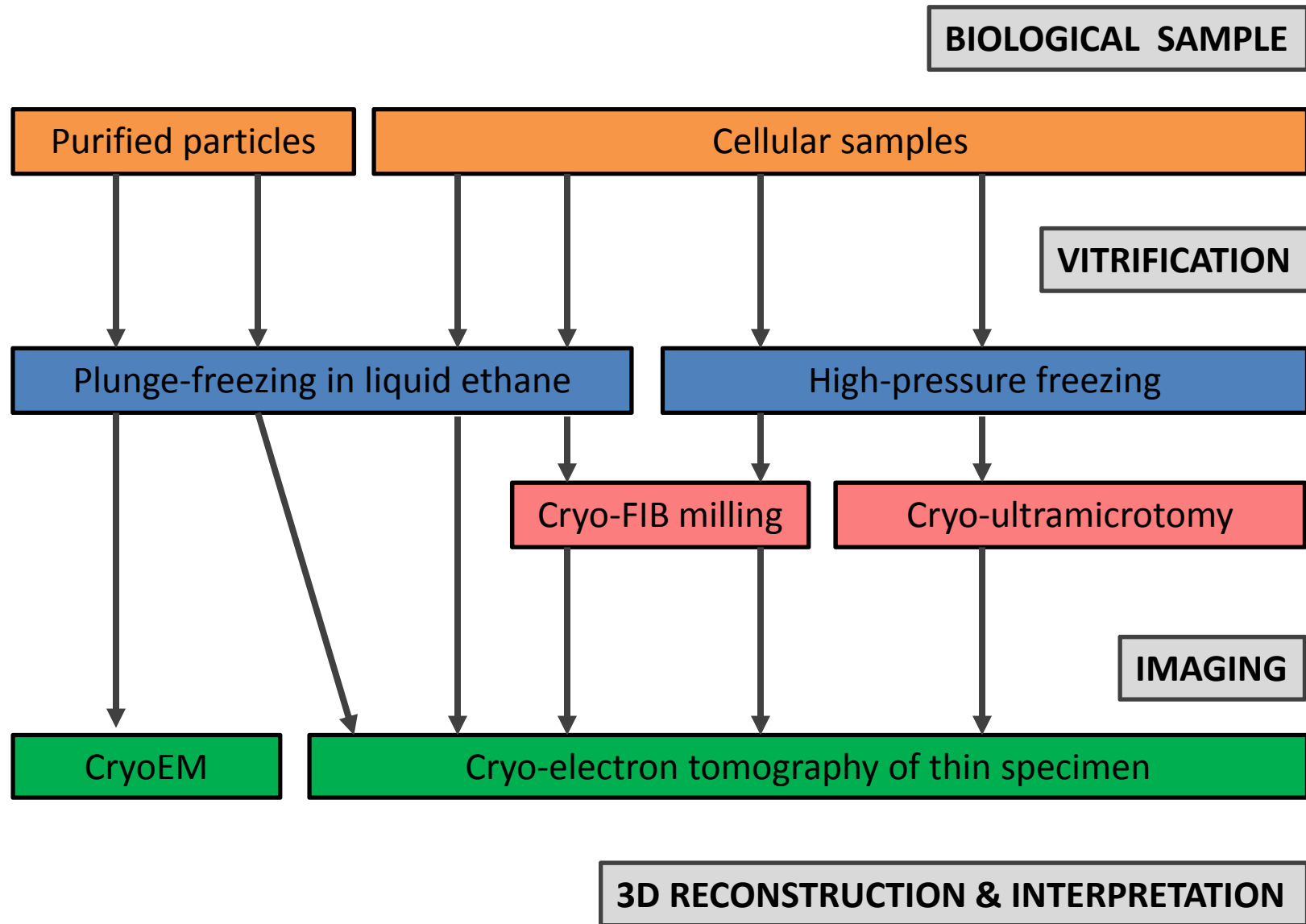


Reconstructed Tomogram



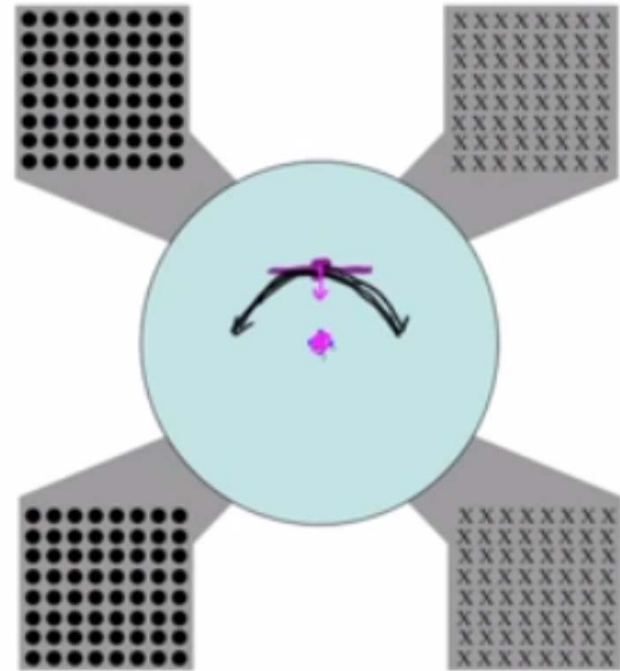
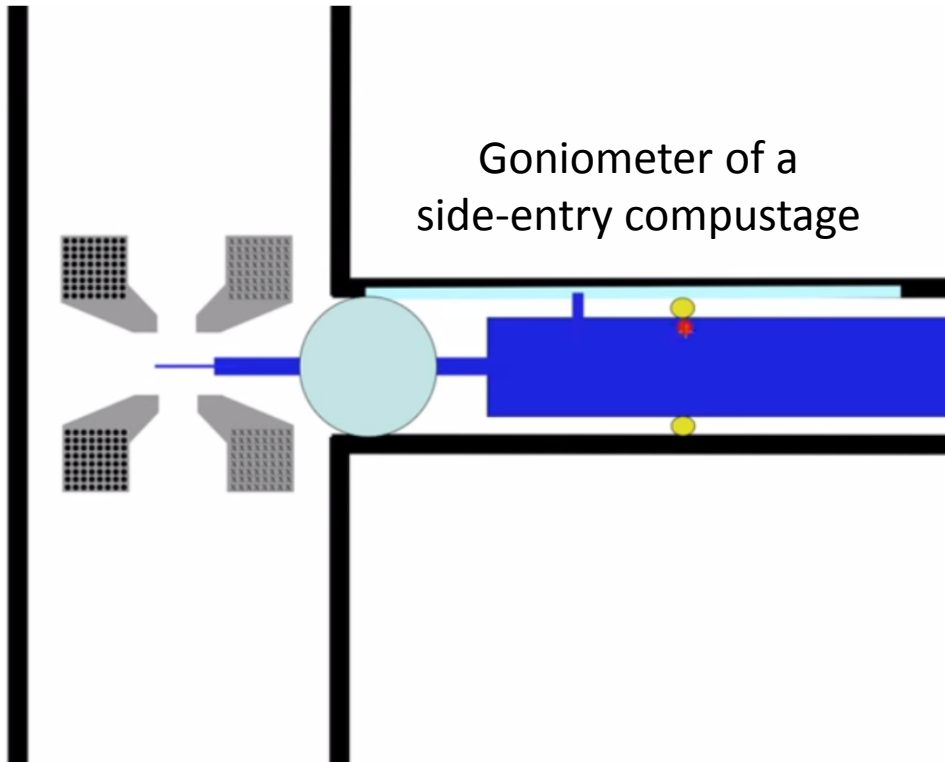


Sample Preparation for CryoET





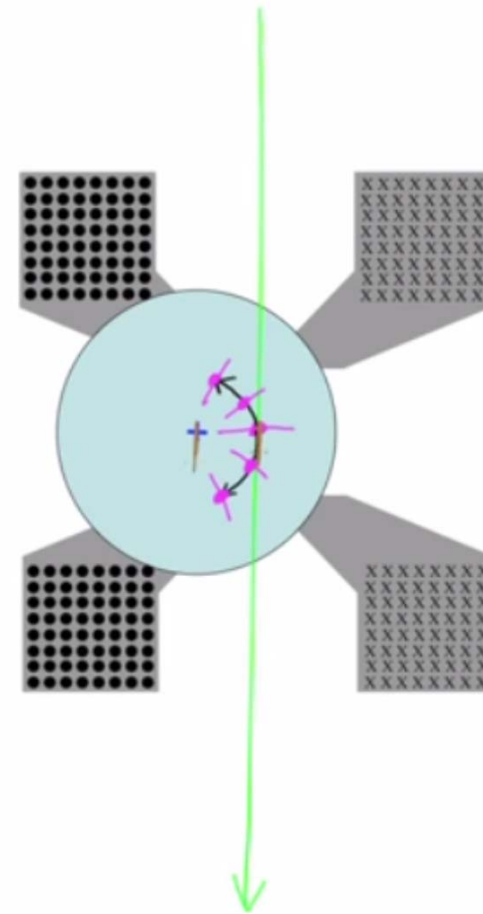
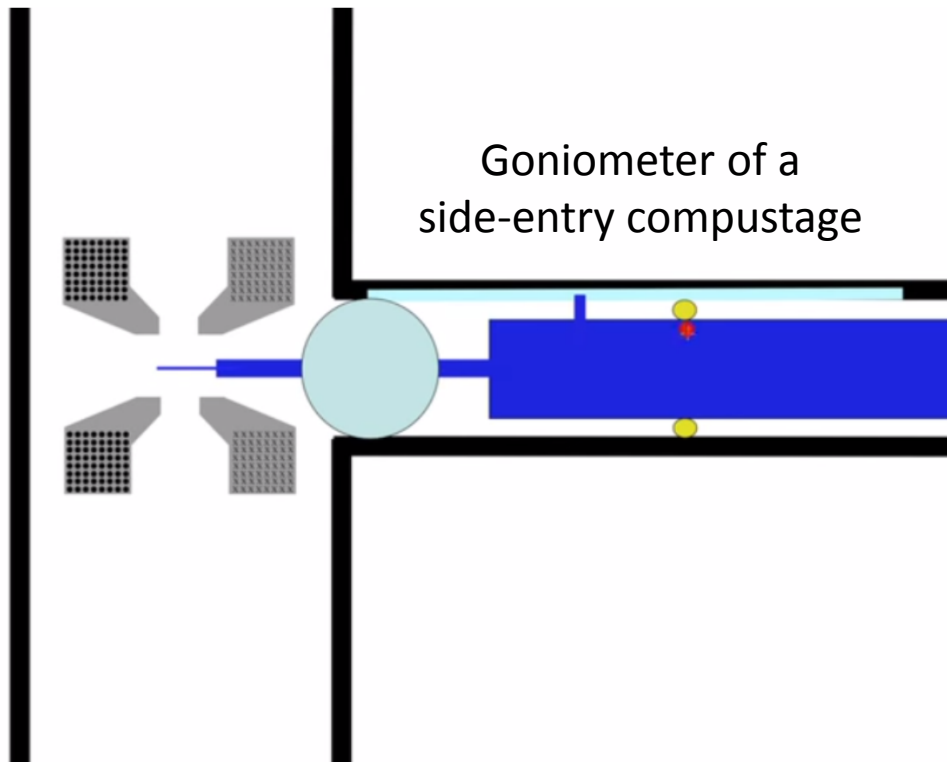
Acquisition of Tilt Series



Eucentric height

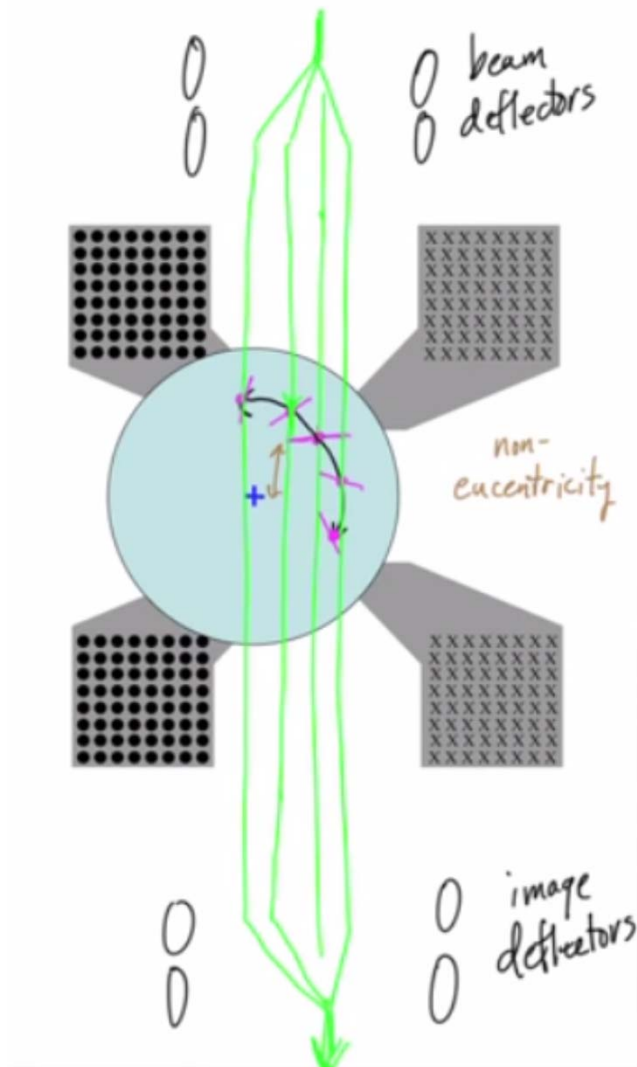


Acquisition of Tilt Series



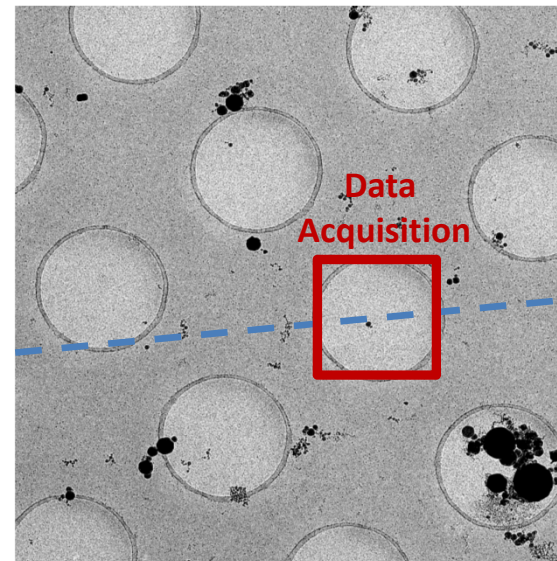


Acquisition of Tilt Series



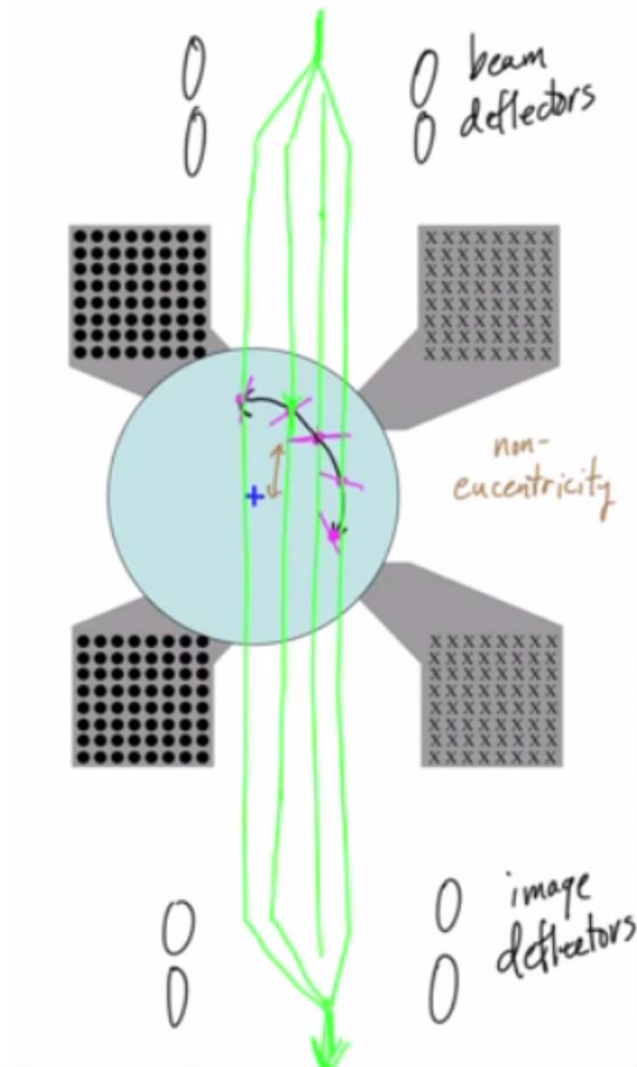
Predictive Method

- 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



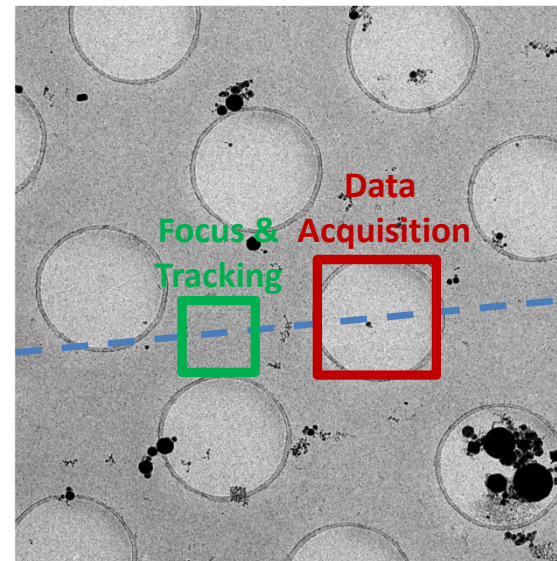


Acquisition of Tilt Series



Focus Position Method

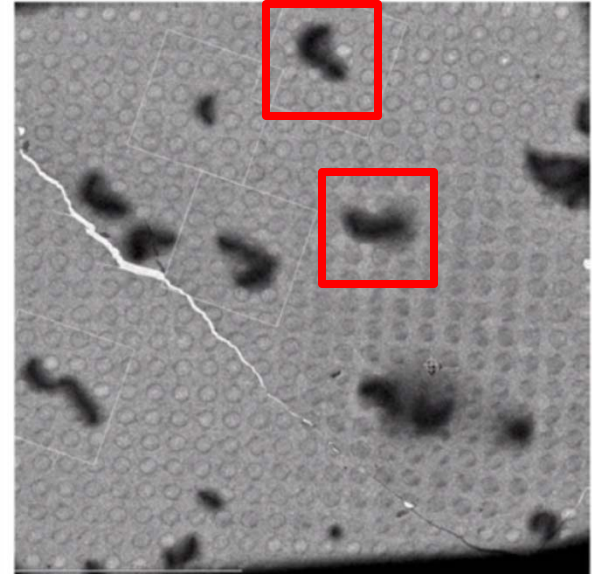
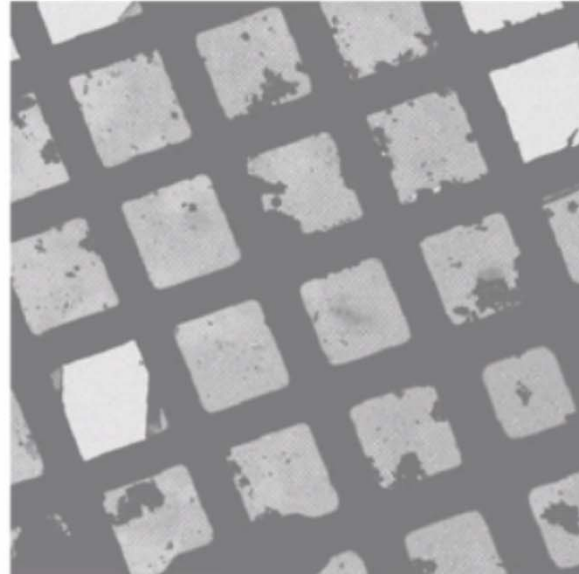
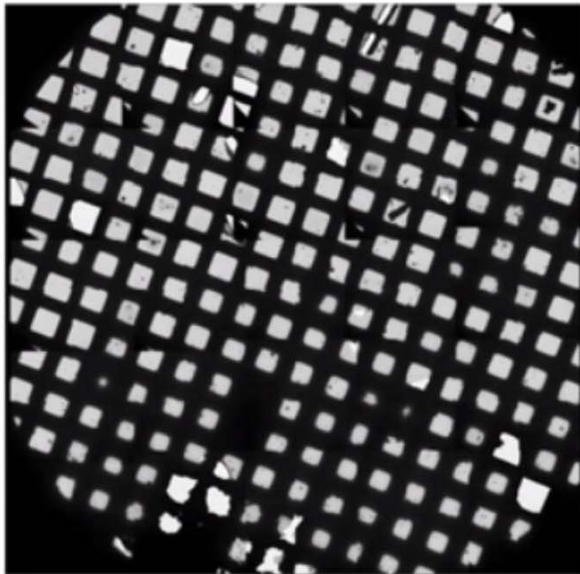
- Move to Focus, focus and center
- Move to Record, collect image
- Tilt, move to Focus, focus, center
- Move to Record, collect image
- Refine model of beam/image shifts





Automated Data Collection

Identify the target area of interest



Set parameters for data collection:

Range of tilt angles: -60° to $+60^\circ$

Angular step: 1° or 2°

Dose per image: $0.5-2.0 \text{ e}^-/\text{A}^2$

Dose distribution: uniform vs. tilt-dependent

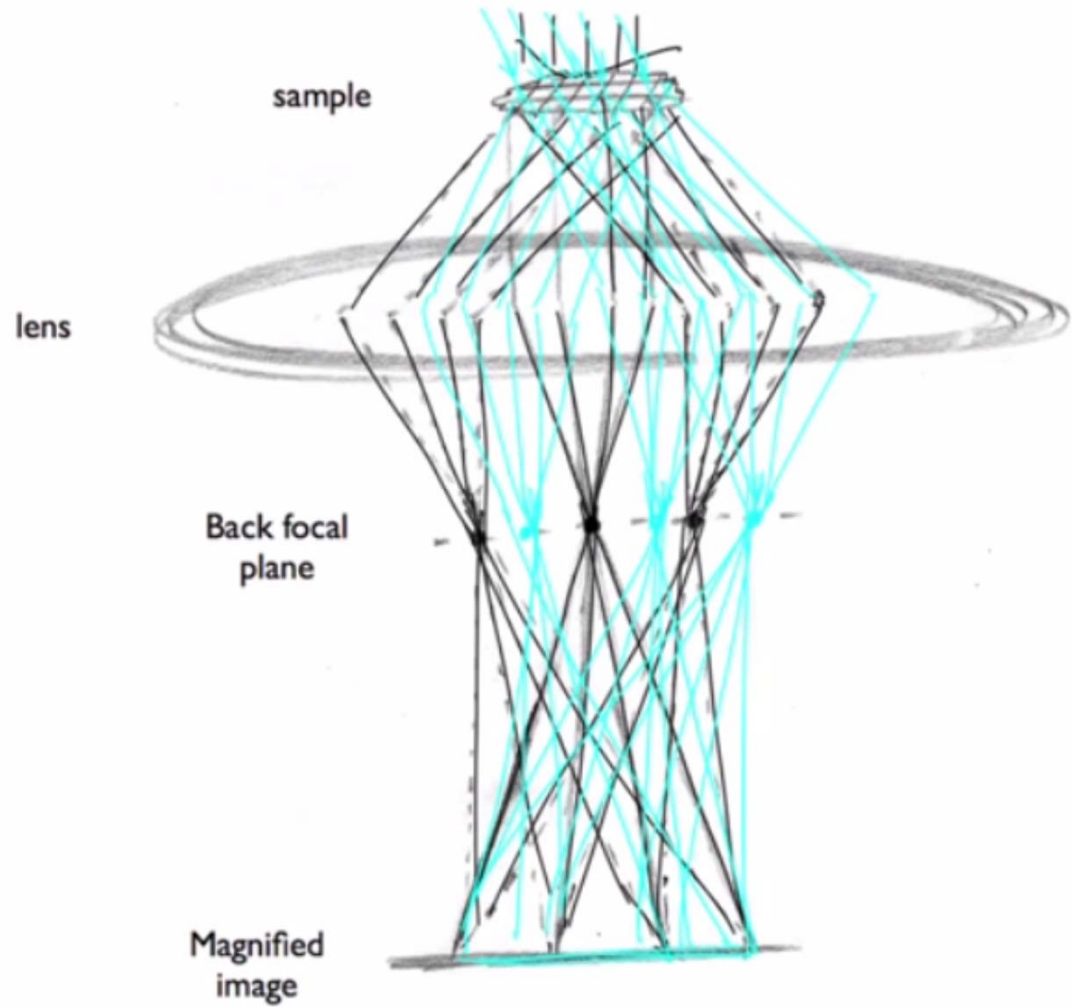


Automated Data Collection

Automated determination
of the Eucentric height



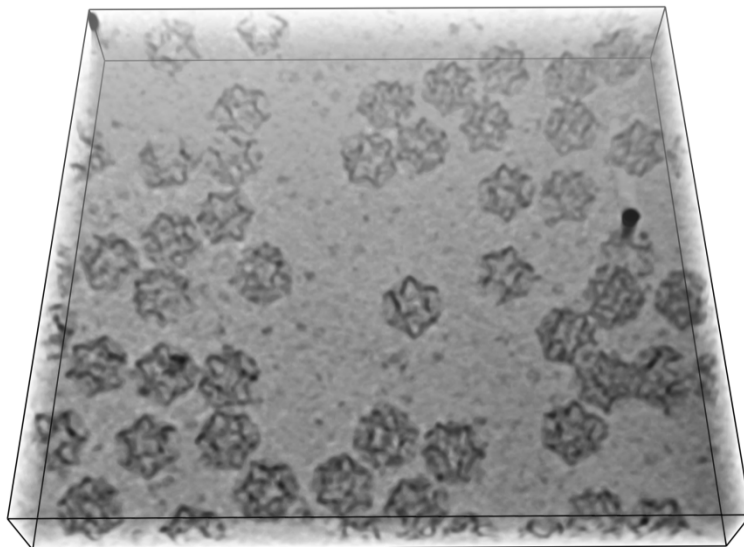
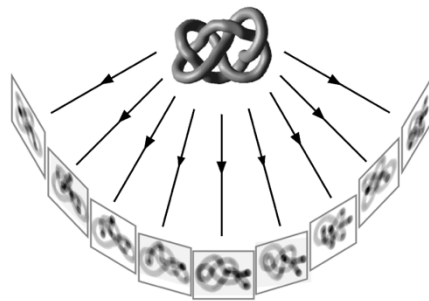
Automated focusing



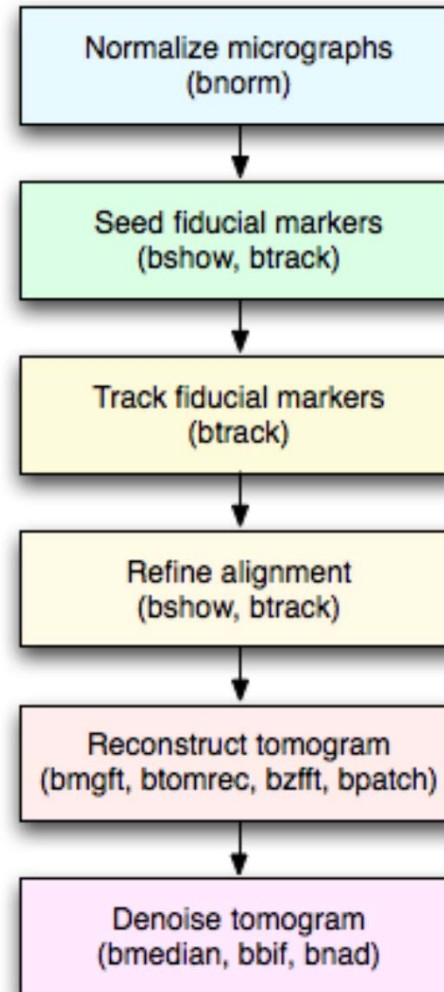
Electron Tomography



Data acquisition (tilt series)

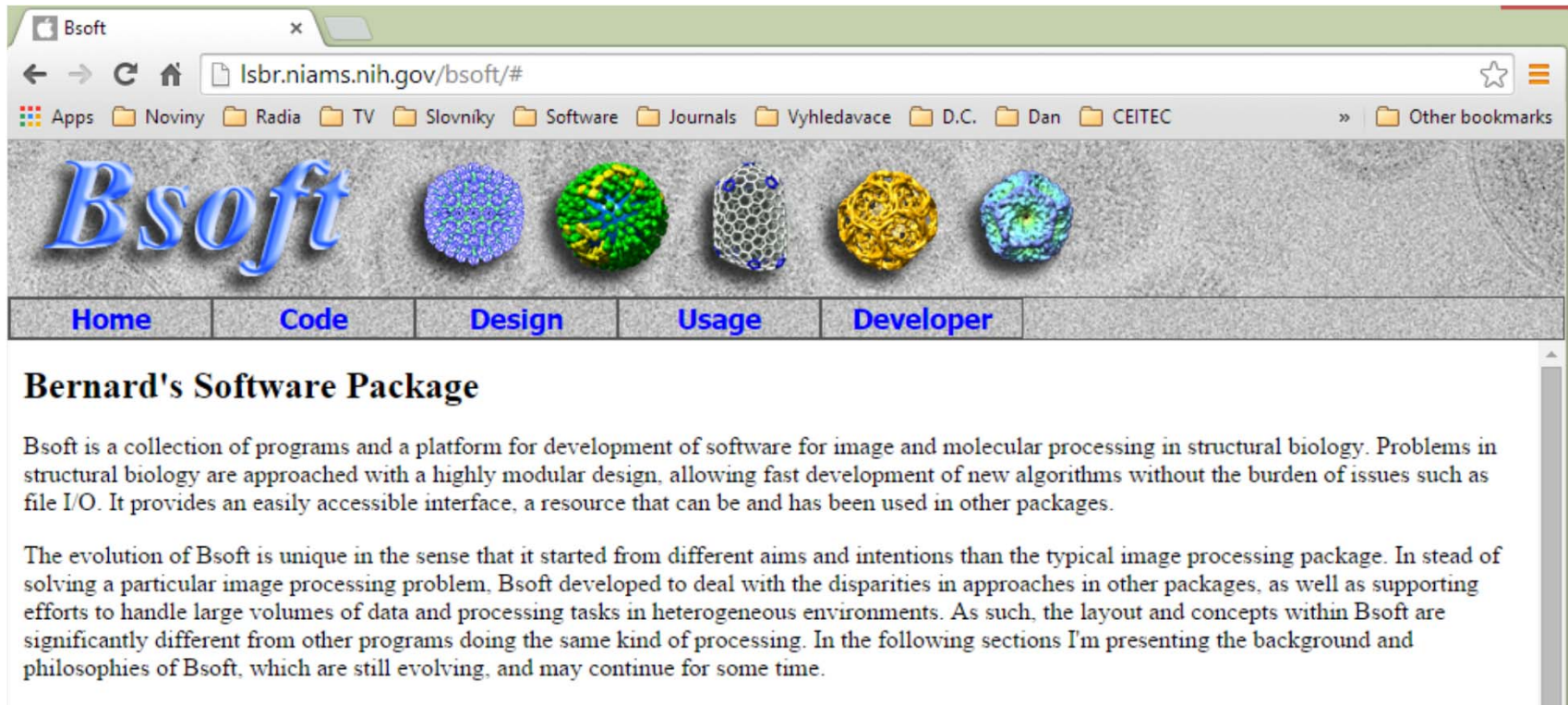


Reconstructed tomogram



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

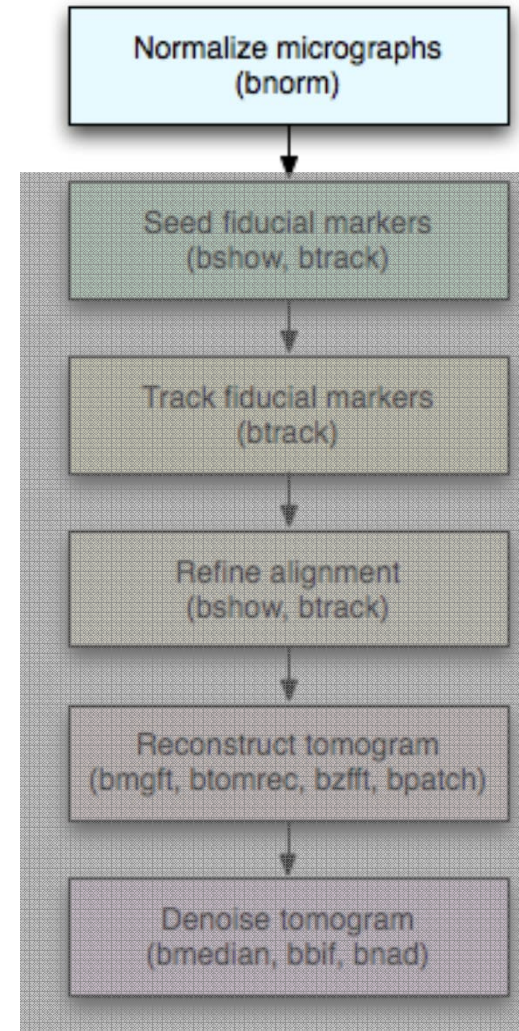
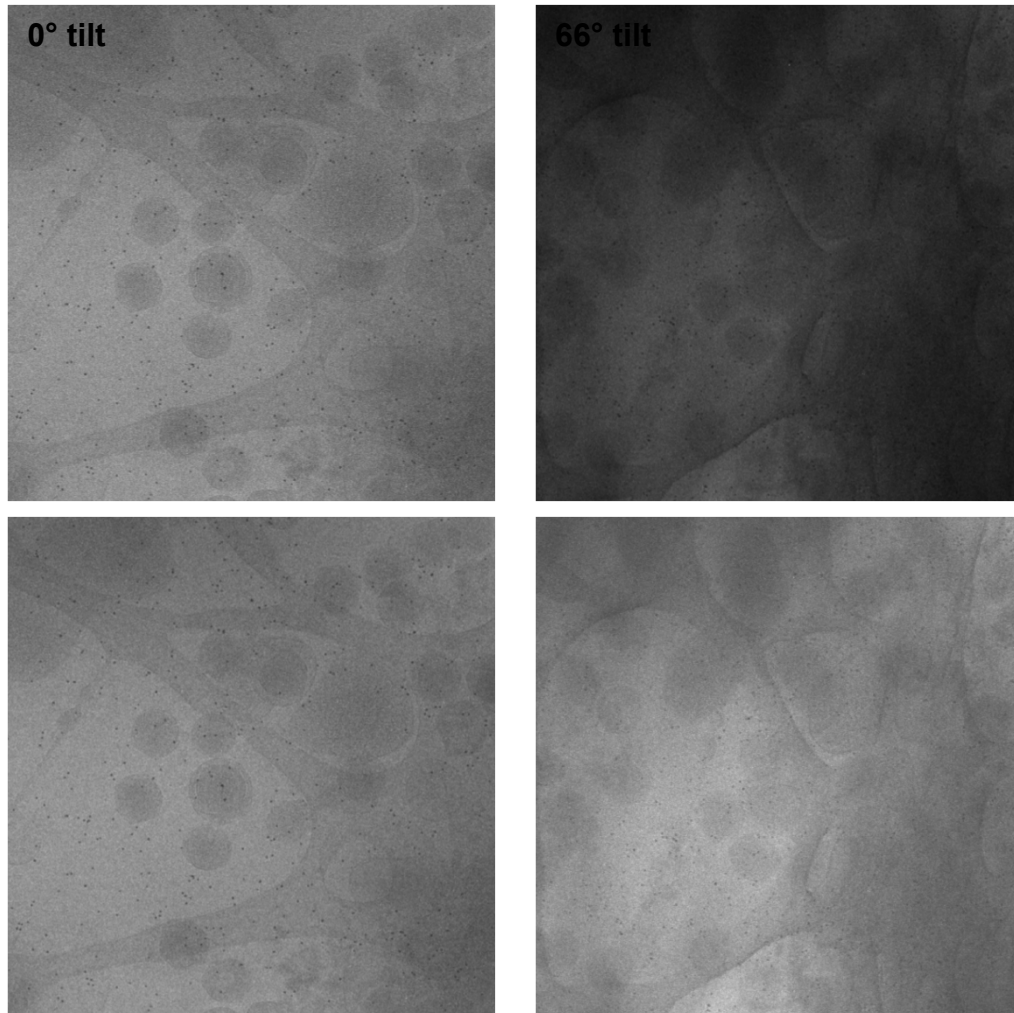
Image processing in Bsoft



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

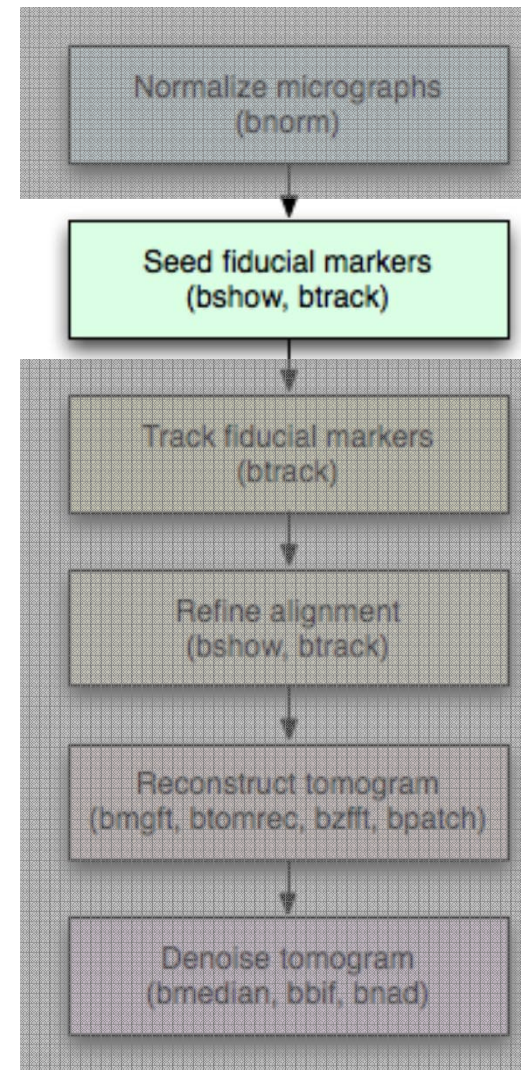
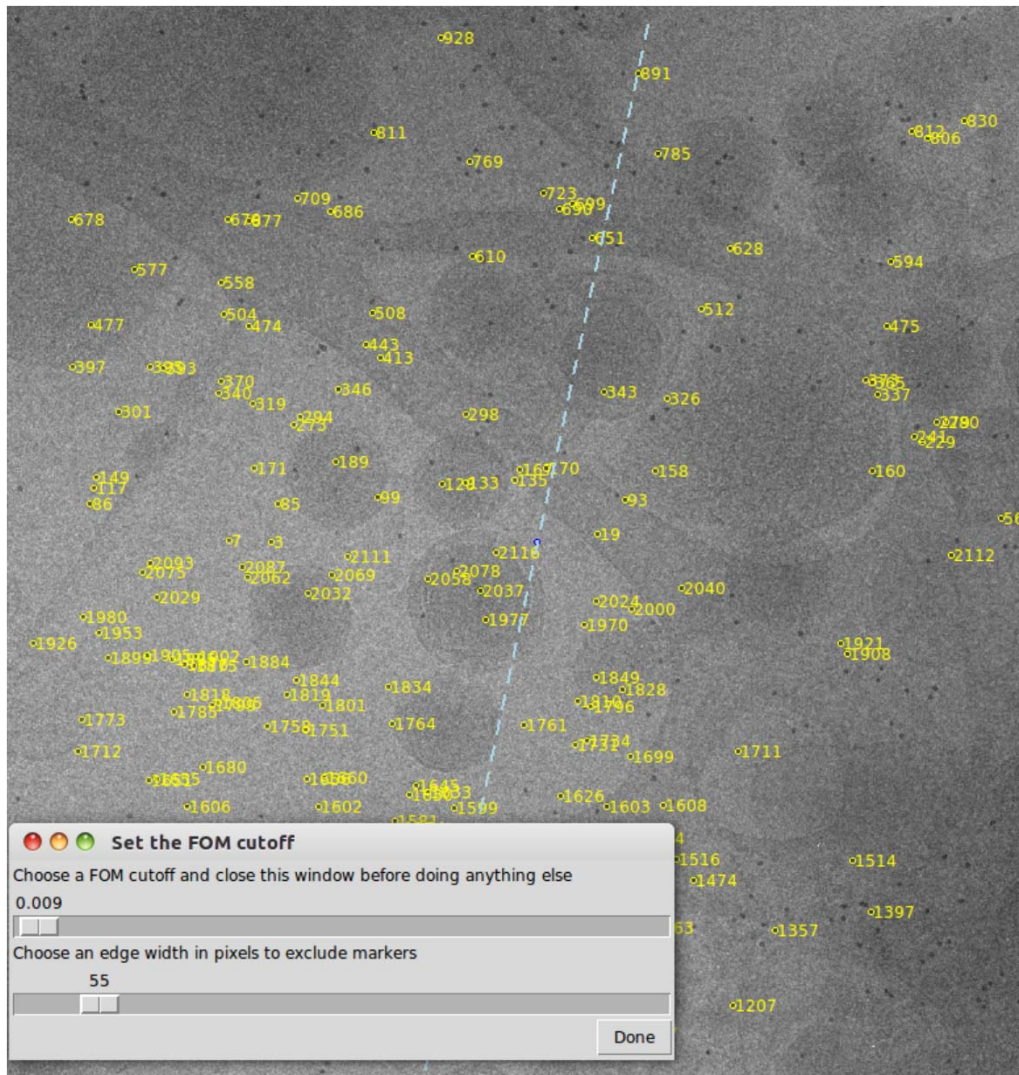
Parameters are stored in ASCII **star files** with predetermined organization for **micrographs, reconstructions and models**.

Preprocessing of collected data

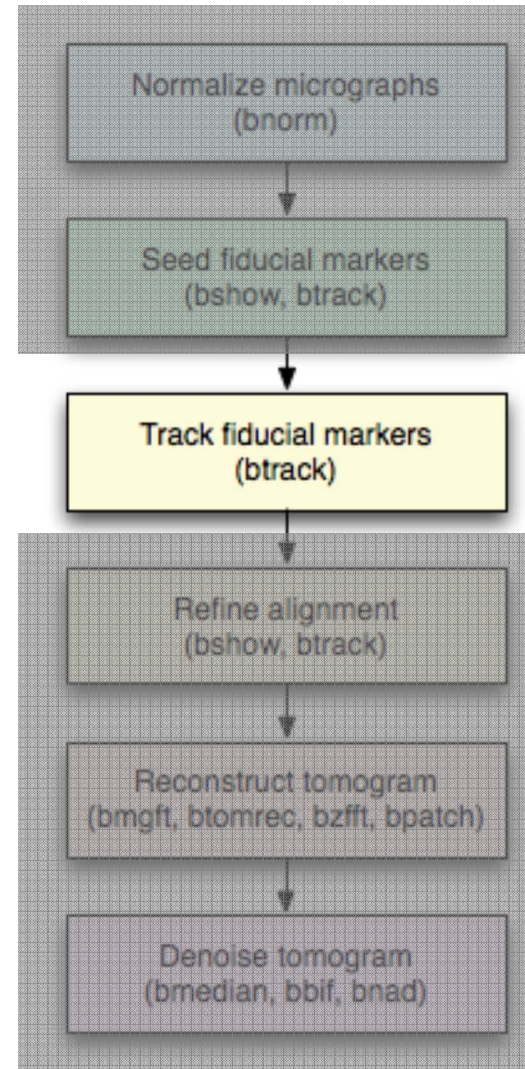
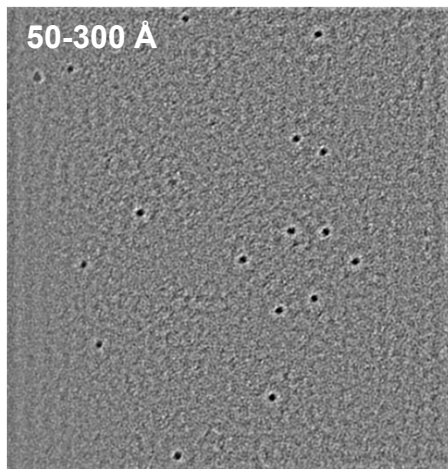
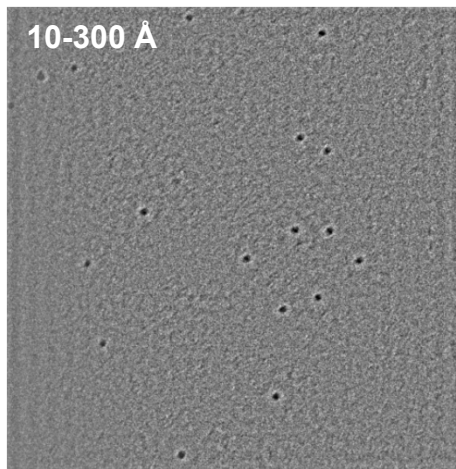
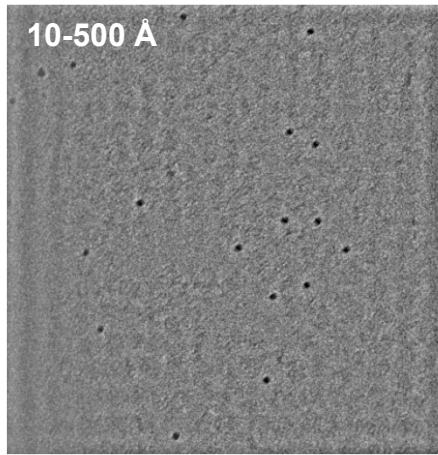
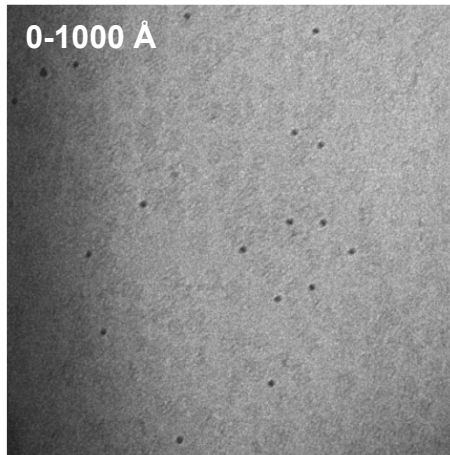


`bnorm -ver 7 -images -rescale 127,10 -data byte -out output.star input.mrc output.pif`
`btomo -v 7 -sampling 8.05 -axis 78 -tilt -60,2 -gold 5 -out output.star input.star`

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

Marker list

Mg	Marker	Residual	FOM	Select
0	1	1.654	0.619	1
0	2	6.905	0.362	1
0	3	1.785	0.493	1
0	4	1.932	0.564	1
0	5	2.858	0.490	1
0	6	6.175	0.332	1
0	7	1.044	0.729	1
0	8	1.693	0.751	1
0	9	2.365	0.854	1
0	10	2.580	0.721	1
0	11	2.057	0.818	1
0	12	3.452	0.800	1
0	13	2.479	0.605	1
0	14	0.572	0.619	1
0	15	1.694	0.806	1
0	16	2.280	0.360	1
0	17	4.082	0.533	1
0	18	2.937	0.771	1
0	19	3.388	0.508	1
0	20	3.785	0.638	1
0	21	0.482	0.536	1
0	22	1.589	0.817	1
0	23	1.860	0.668	1
0	25	1.364	0.475	1

Tomography

Tomography

Show markers Show errors Show labels

Marker radius: 5.0

Markers: 16165

Selected marker: 27 Residual: 3.443615913 FOM

Residual: 2.293715151

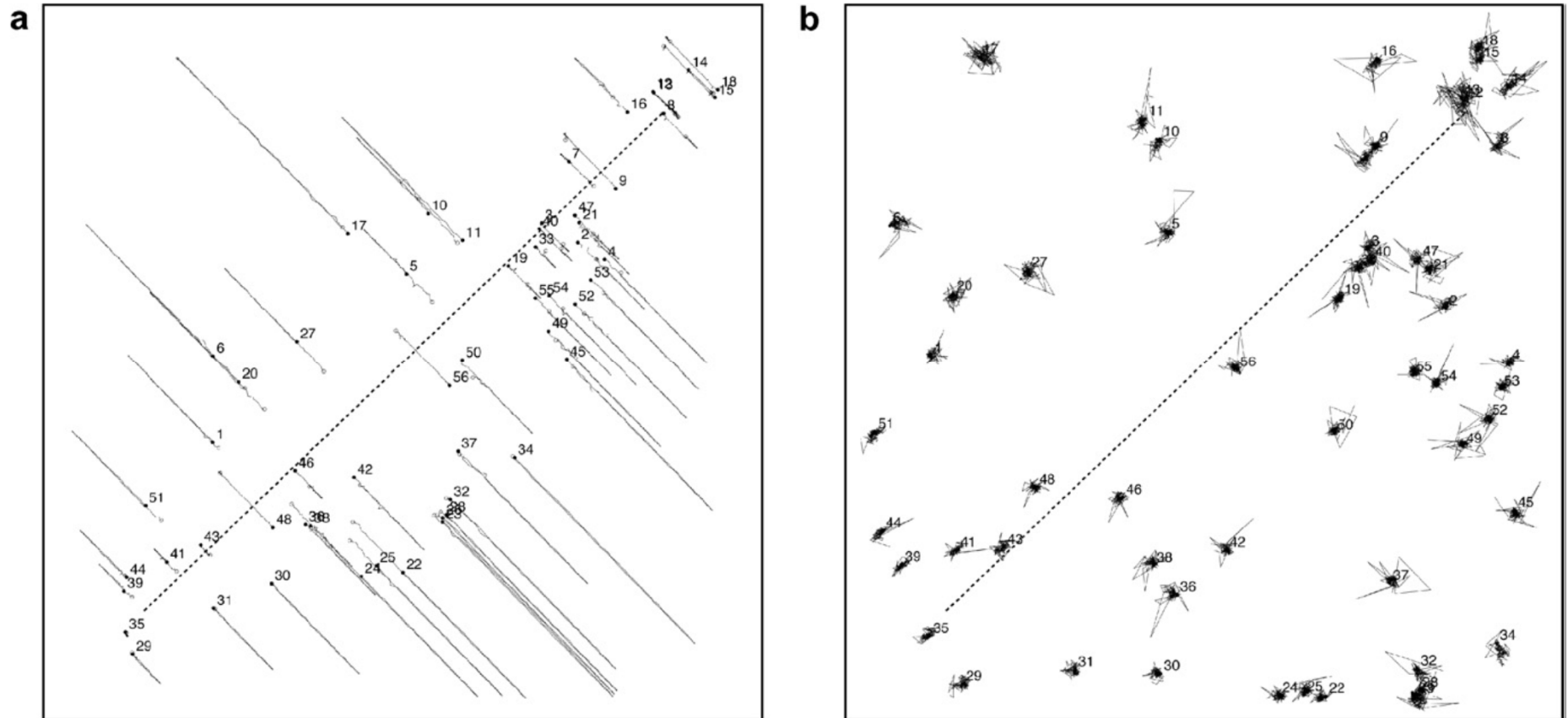
Tilt axis: 78.13258316 Show

FOM cutoff: 0.000

Mg	Tilt	Axis	Level	OriginX	OriginY	ScaleX	ScaleY
0	-60.21	77.90	0.32	1015.5	1030.5	1.001	1.001
1	-58.18	77.90	0.34	1023.5	1018.8	1.002	1.001
2	-56.22	77.93	0.33	1015.1	1030.2	1.001	1.001
3	-54.26	77.94	0.32	975.1	1050.9	1.000	1.001
4	-52.32	77.92	0.33	1008.2	1053.6	0.999	1.000
5	-50.23	77.90	0.34	994.0	1059.1	1.001	1.000
6	-48.26	77.92	0.35	981.8	1048.8	1.000	1.001
7	-46.18	77.95	0.34	979.7	1039.9	1.001	1.001
8	-44.15	77.92	0.34	982.7	1056.6	1.002	1.000
9	-42.19	77.89	0.32	993.4	1026.3	1.001	1.001
10	-40.25	77.92	0.35	985.8	1025.8	1.000	1.001
11	-38.19	77.93	0.36	980.8	1034.2	1.001	1.000
12	-36.20	77.92	0.37	972.7	1026.3	1.001	1.001
13	-34.29	78.01	0.35	984.7	1025.6	0.999	1.000
14	-32.20	78.03	0.36	951.4	1039.4	1.000	1.000
15	-30.23	77.96	0.38	975.5	1034.9	1.000	1.000
16	-28.21	77.99	0.38	974.7	1031.2	1.000	1.000
17	-26.18	78.14	0.42	939.6	1039.4	1.000	1.000
18	-24.30	78.07	0.37	985.7	1035.5	0.999	1.000
19	-22.13	78.03	0.43	991.8	1020.5	1.000	1.000
20	-20.23	78.14	0.45	965.3	1039.1	0.999	1.000
21	-18.21	77.88	0.42	1012.6	1029.8	1.000	0.999
22	-16.12	78.06	0.42	989.7	1030.5	1.000	1.000
23	-14.14	78.29	0.49	948.2	1038.7	1.000	0.999

```
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
```

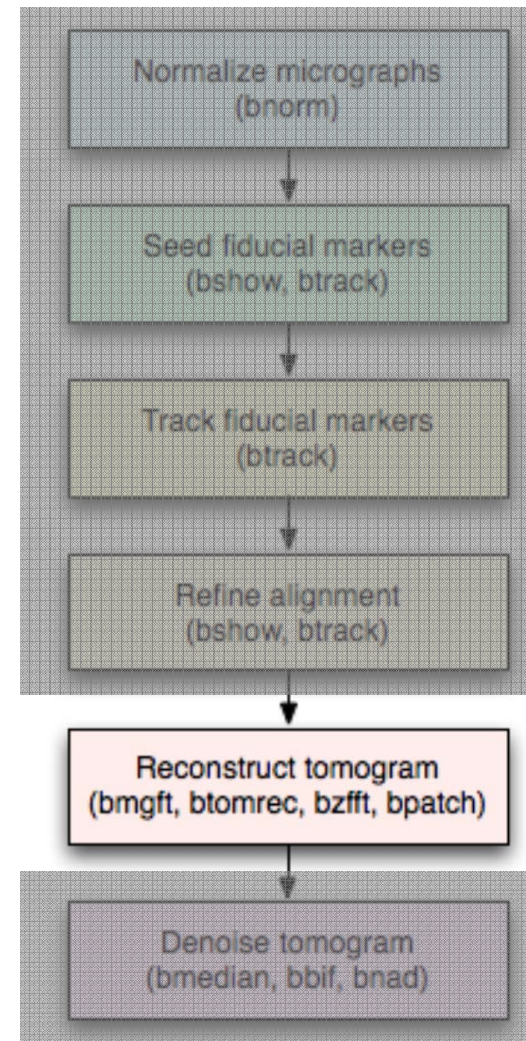
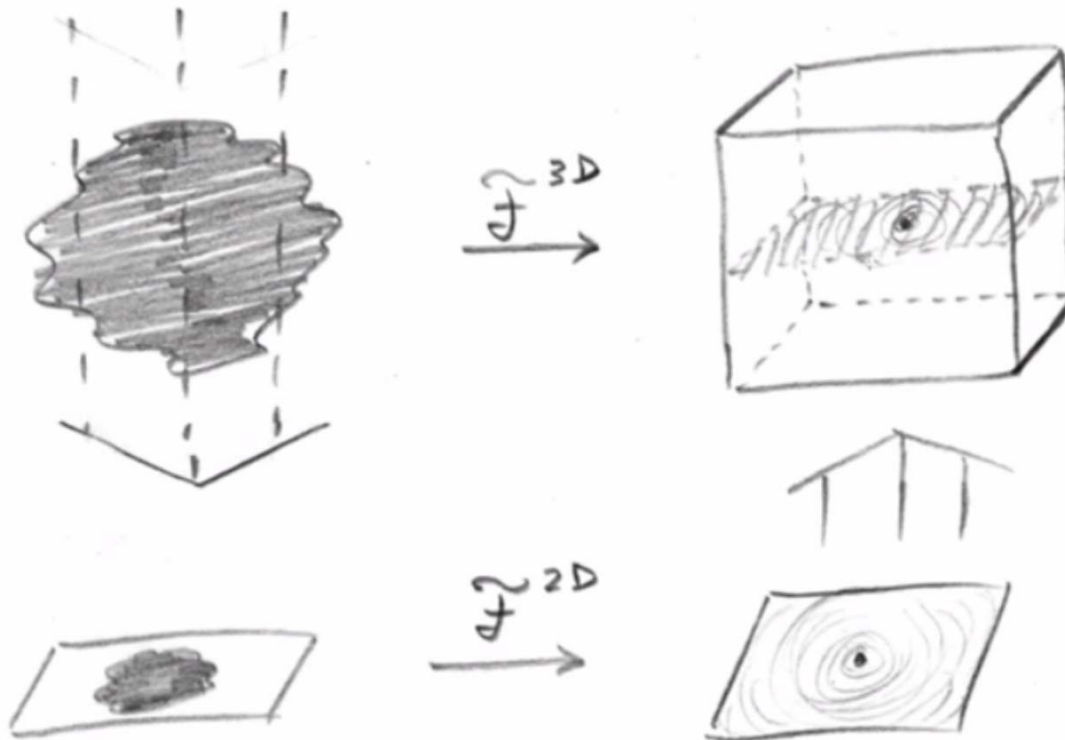
Refinement of fiducial marker positions



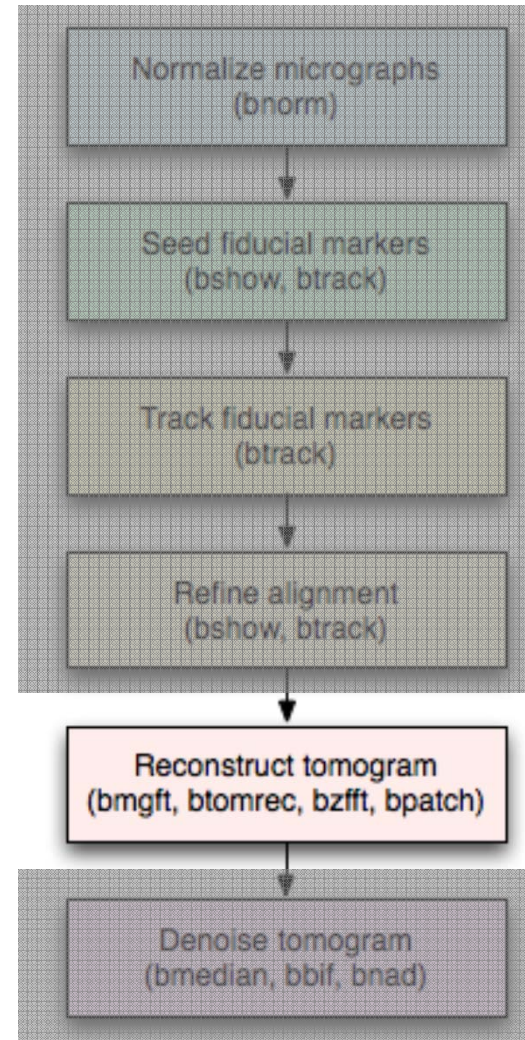
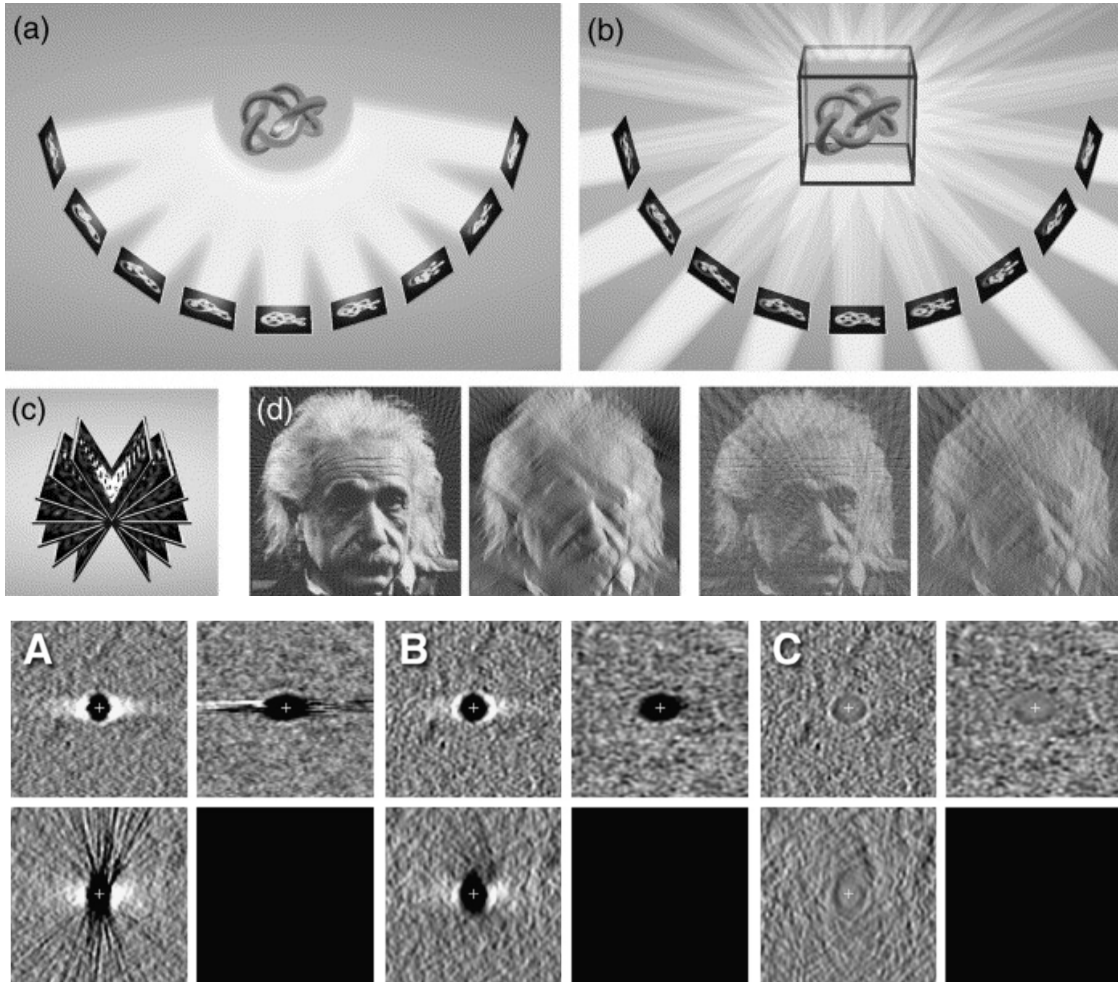
```
btrack -ver 1 -reset -refine 10,z,o,v -image FV3tomo9_align.pif -Post FV3tomo9_err.ps  
-out FV3tomo9_ref1.star FV3tomo9_ref0.star >& FV3tomo9_ref1.log
```

Tomogram reconstruction

The projection theorem



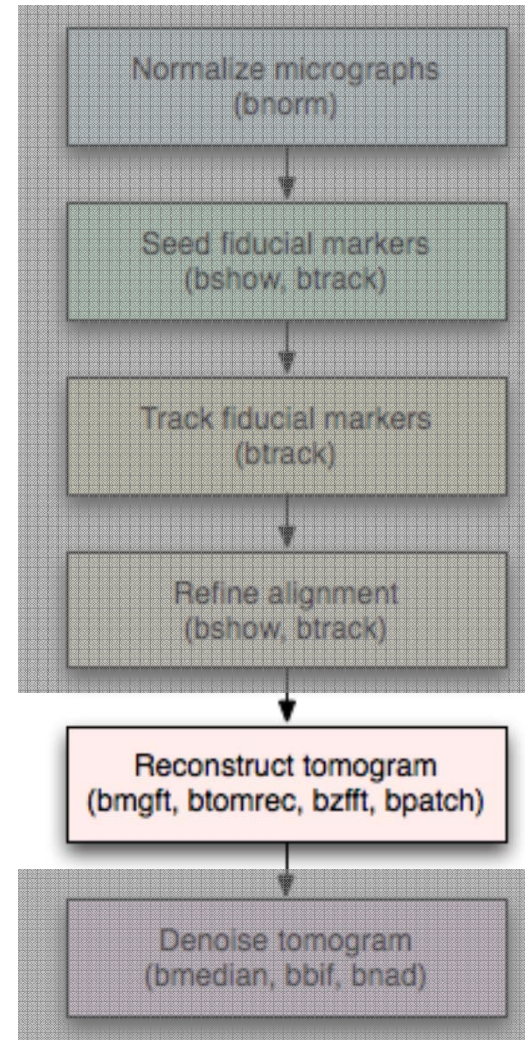
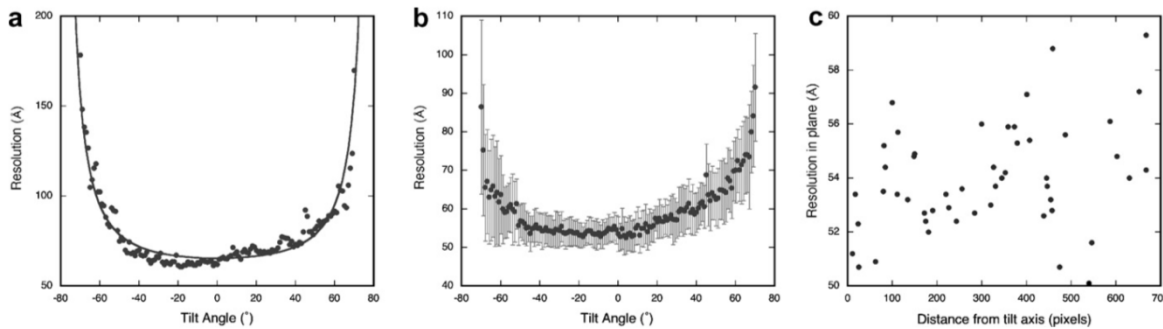
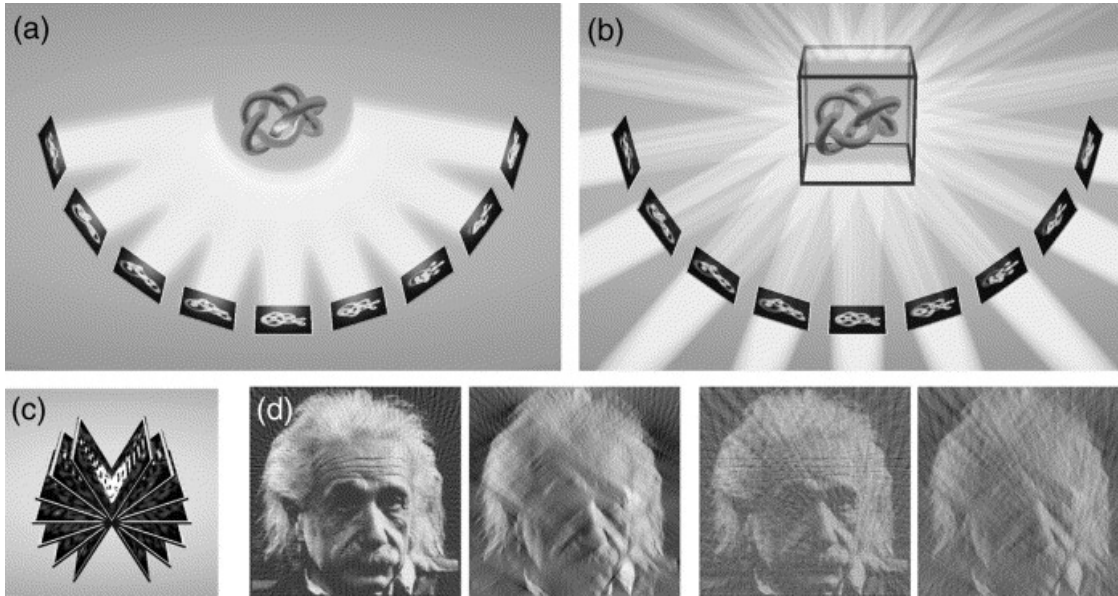
Tomogram reconstruction



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

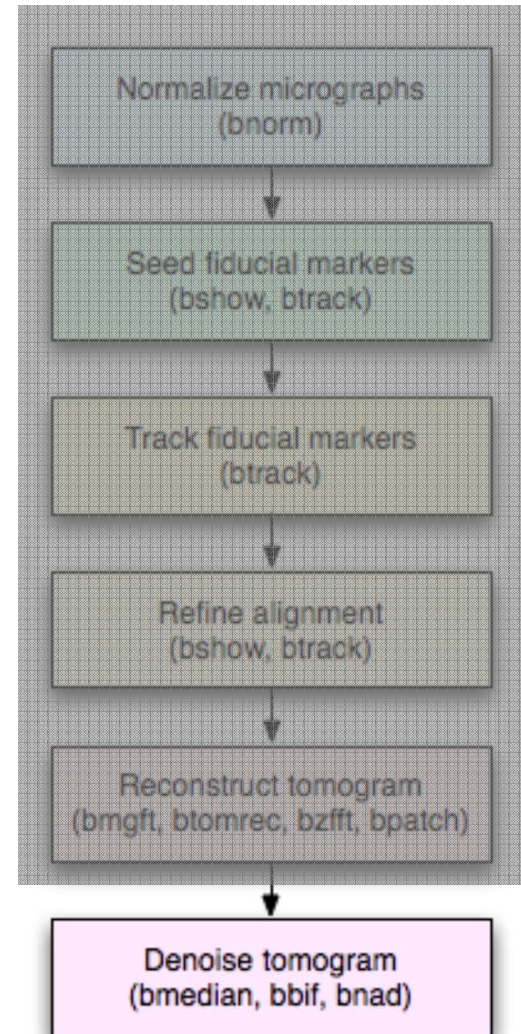
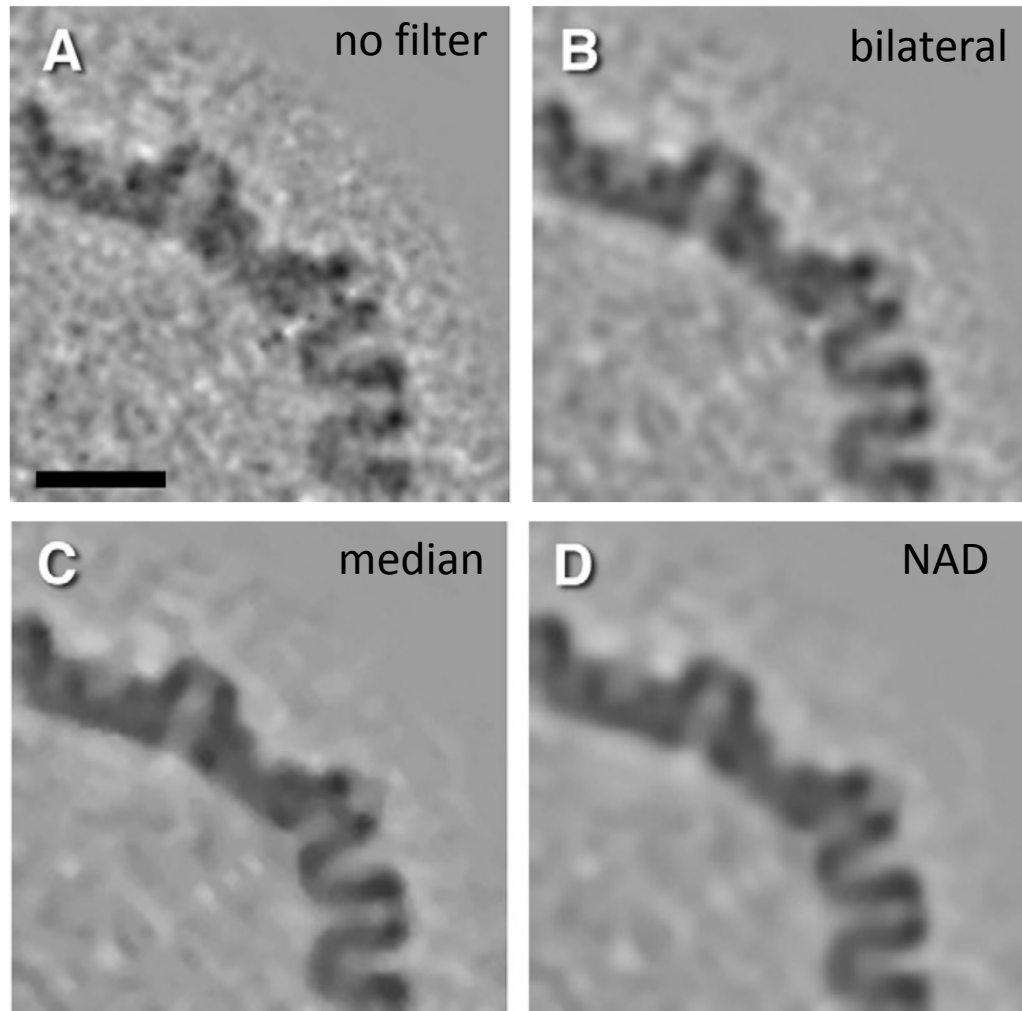
Tomogram reconstruction



`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`

Denosing of reconstructed tomograms



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