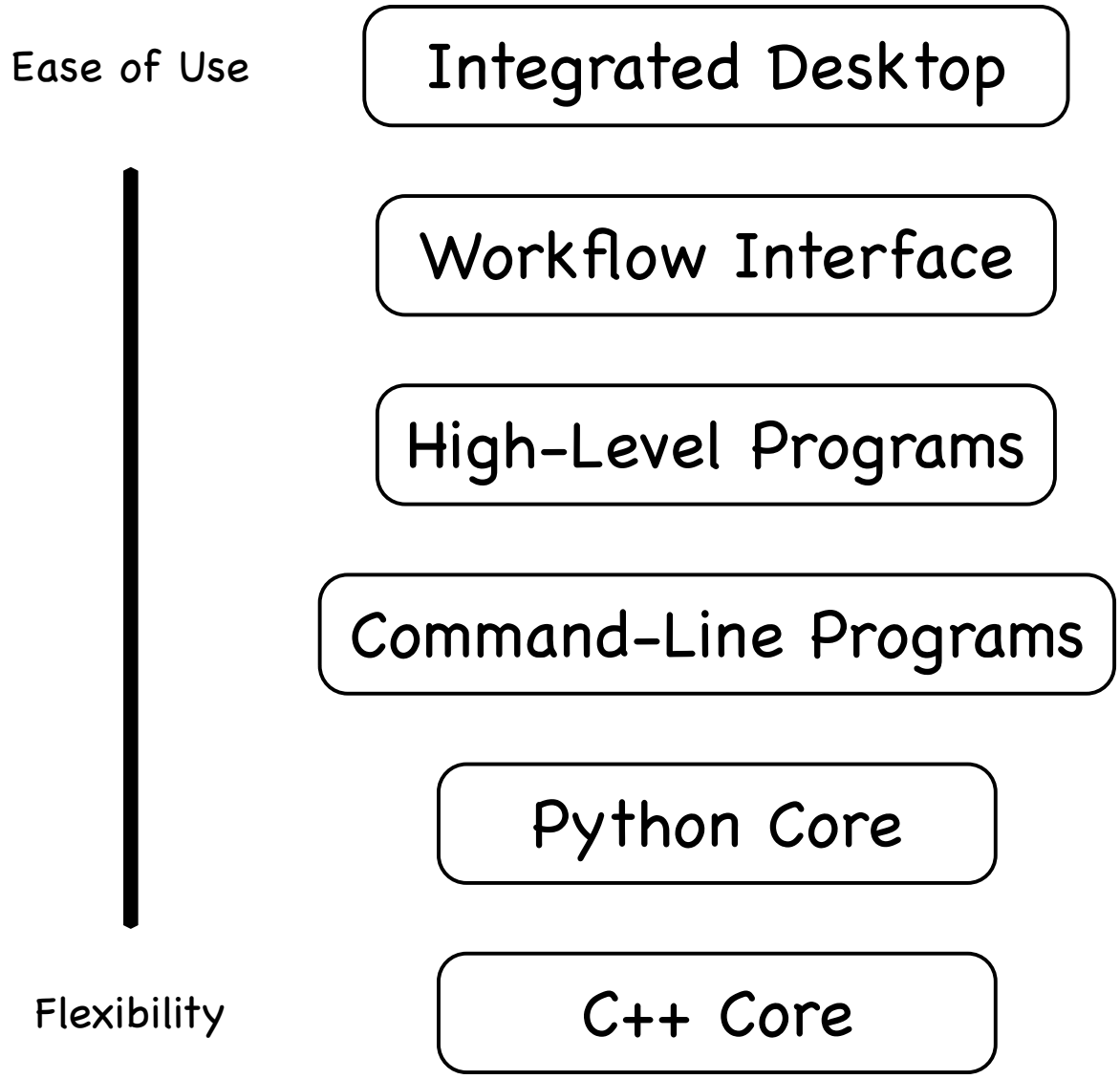
EMAN2

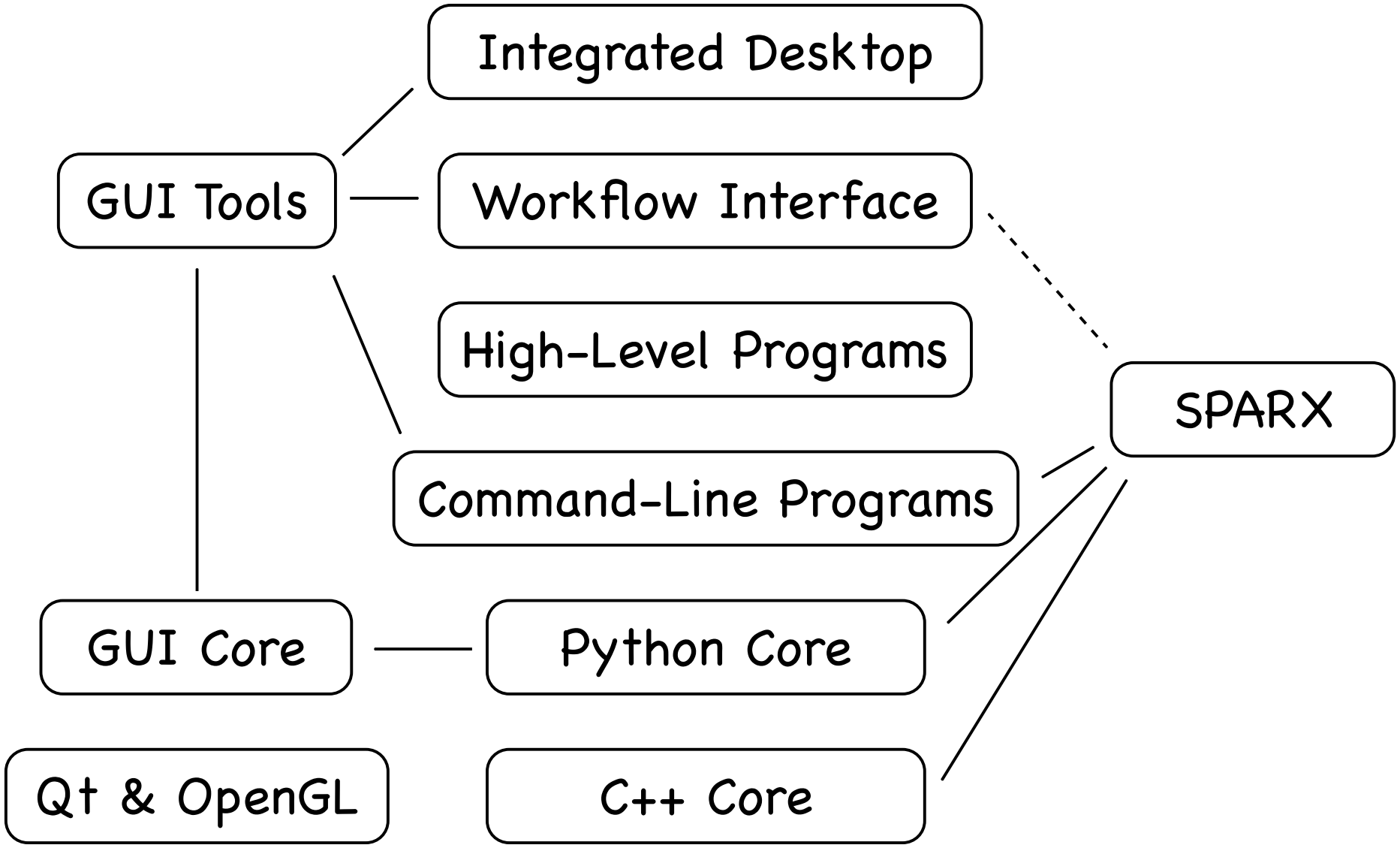
Changes to Tutorial

- Small set for ribosome -> ~2000 ptcl
- Use far from focus images for the small sets.
- Note that many tasks really need a large/ high resolution monitor.

EMAN2 Architecture



EMAN2 Architecture



File Formats

BDB +

MRC	R/W	IMAGIC	R/W
SPIDER	R/W	HDF5	R/W
PIF	R/W	ICOS	R/W
VTK	R/W	PGM	R/W
Amira	R/W	Xplor	W
Gatan DM2	R	Gatan DM3	R
TIFF	R/W	Scans-a-lot	R
LST	R/W	PNG	R/W
Video-4-Linux	R	JPEG	W

BDB

- Embedded database
  - No server, easy maintenance
  - Fast
  - Multiple processors on 1 machine - No file corruption !
  - Arbitrary metadata (header information)
- Widely used (now owned by Oracle, but free)
- EMAN2DB directories

BDB Warnings

- PLEASE READ:

  http://blake.bcm.edu/emanwiki/EMAN2/DatabaseWarning

- e2bdb.py -c
- Do NOT move files within an EMAN2DB directory around !
- If you need to remove files from an EMAN2DB directory: e2bdb.py -c
- Do NOT delete or rename directories containing EMAN2DB directories without running e2bdb.py -c first.
- If you DO get a message saying there is a database error: e2bdb.py -c
- Beware of network mounted filesystems.

Extensible Core

Type	Description	#
Processor	Generic image processing algorithms, filters, masks, thresholds, etc.	175
Aligner	Algorithms used to align 2 images or volumes to each other	22
Projector	Routines to generate 2-D projections of 3-D objects	6
Reconstructor	Routines to reconstruct 3-D objects from 2-D projections	11
Cmp	Similarity metrics used to compare two images or volumes	10
Averager	Average together stacks of images in various ways	7
Analyzer	Perform various operations on sets of images, such as classification or PCA	6
Orientgen	Routines describing how projections cover the asymmetric triangle	6

Processors

(categories & examples)

- filter
  - filter.lowpass.gauss
  - filter.homomorphic.tophat
- mask
  - mask.sharp
  - mask.gaussian
- math
  - math.sqrt
  - math.laplacian
- misc
  - misc.localnorm
- normalize
  - normalize
  - normalize.edgemean
- testimage
  - testimage.scurve
- threshold
  - threshold.binary
  - threshold.clampminmax
- xform
  - xform.centerofmass
  - xform.fourierorigin.tocenter

Similarity Metrics
(cmp)

With Default options, SMALLER -> more similar

- dot - dot product (negative by default)
- frc - Fourier ring correlation (weighted)
- optvariance - 'optimized variance' (EMAN1)
- phase - mean phase error
- quadmindot - Worst of quadrant dot products
- sqeuclidean - sum $(a-b)^2/n$

Programs

- Command-Line Programs (EMAN2)

syntax:

e2<name>.py --help

e2<name>.py <file> [--option=value] [--option] [-O]

<> - required parameter

[] - optional parameter

GUI

- e2workflow.py - Primary workflow dialog
- e2display.py - General image/volume display
- e2boxer.py - Interactive particle picker
- e2helixboxer.py - Filament picker
- e2tomoboxer.py - Interactive tomogram picker
- e2ctf.py - Various CTF operations
- e2eulerxplor.py - Look at particle orientations
- e2simmxxplor.py - Evaluate how well orientations can be determined
- e2cmpxplor.py - Evaluate how different similarity metrics work

High Level Programs

- e2refine2d.py - reference free class-averages
- e2initialmodel.py - Make initial models from a few class-averages
- e2refine.py - Standard single particle analysis 3-D refinement
- e2eotest.py - even/odd test for resolution assessement
- e2refinevariance.py - Compute a variance map
- e2refinemulti.py - multiple map simultaneous refinement
- e2classifyligand.py - Split data into 2 groups based on 2 models
- e2refinetofrealign.py - Set up for a Frealign run based on an EMAN2 refinement
- e2runfrealign.py - Execute Frealign
- e2refinefromfrealign.py - Process the results of a Frealign run

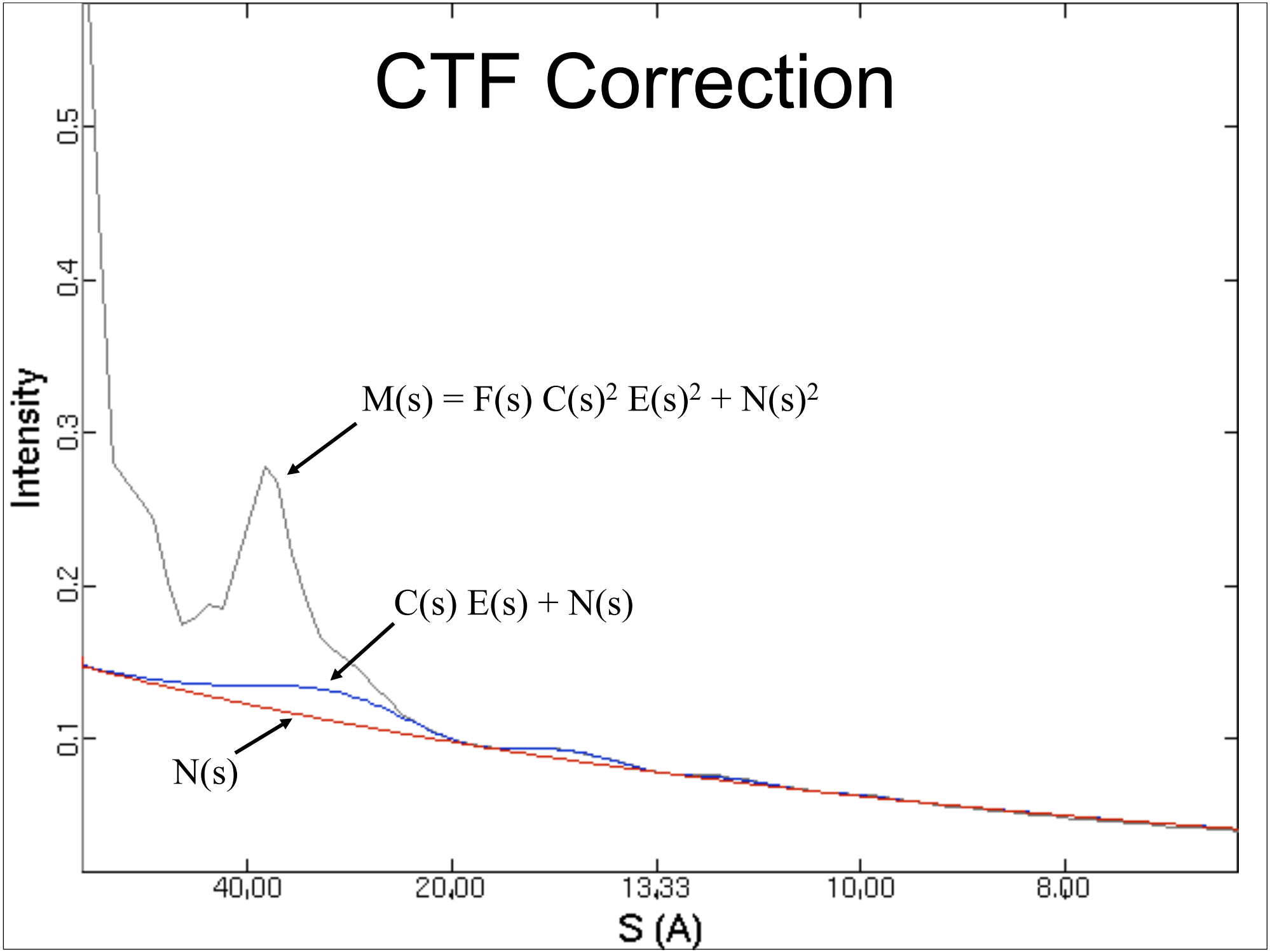
Utility Programs

- e2version.py - Display version info
- e2speedtest.py - Test machine performance
- e2help.py - Documentation for modular functions
- e2bdb.py - database manipulation and querying
- e2iminfo.py - general image information tool
- e2proc2d.py - 2d image processing of stacks and single images
- e2proc3d.py - 3d image processing of 3-D stacks and single volumes
- e2parallel.py - Used for some parallelism operations
- e2.py - Python command-line for EMAN2

Which to Choose ?

- Get started on the tutorial
  - GroEL
  - mm-cpn
  - e-coli ribosome + SecY

CTF Correction



Measured Image

Ideal Particle

Random Noise

$$\overline{M}(s,\theta) = \overline{F}(s,\theta)C(s)E(s) + \overline{N}(s,\theta)$$

$$C(s) = \sqrt{1-Q^2}\sin\gamma + Q\cos\gamma$$

$$\gamma = -\pi\left(\frac{1}{2}C_S\lambda^3 s^4 - \Delta Z\lambda s^2\right)$$

$$E(s) = e^{-Bs^2}$$

$$\overline{M}(s,\theta) = \overline{F}(s,\theta)C(s)E(s) + \overline{N}(s,\theta)$$

$$C(s) = \sqrt{1-Q^2}\sin\gamma + Q\cos\gamma$$

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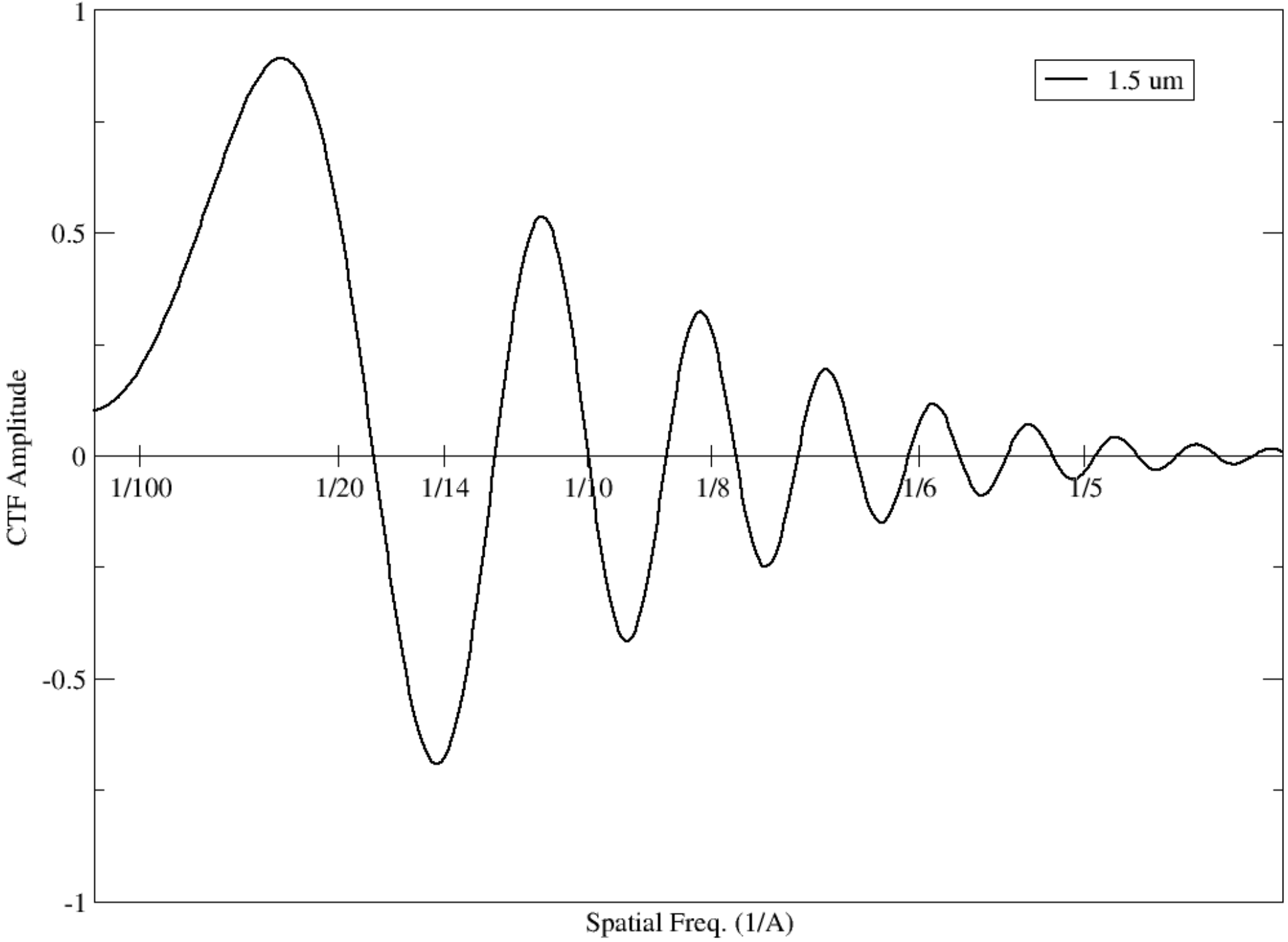
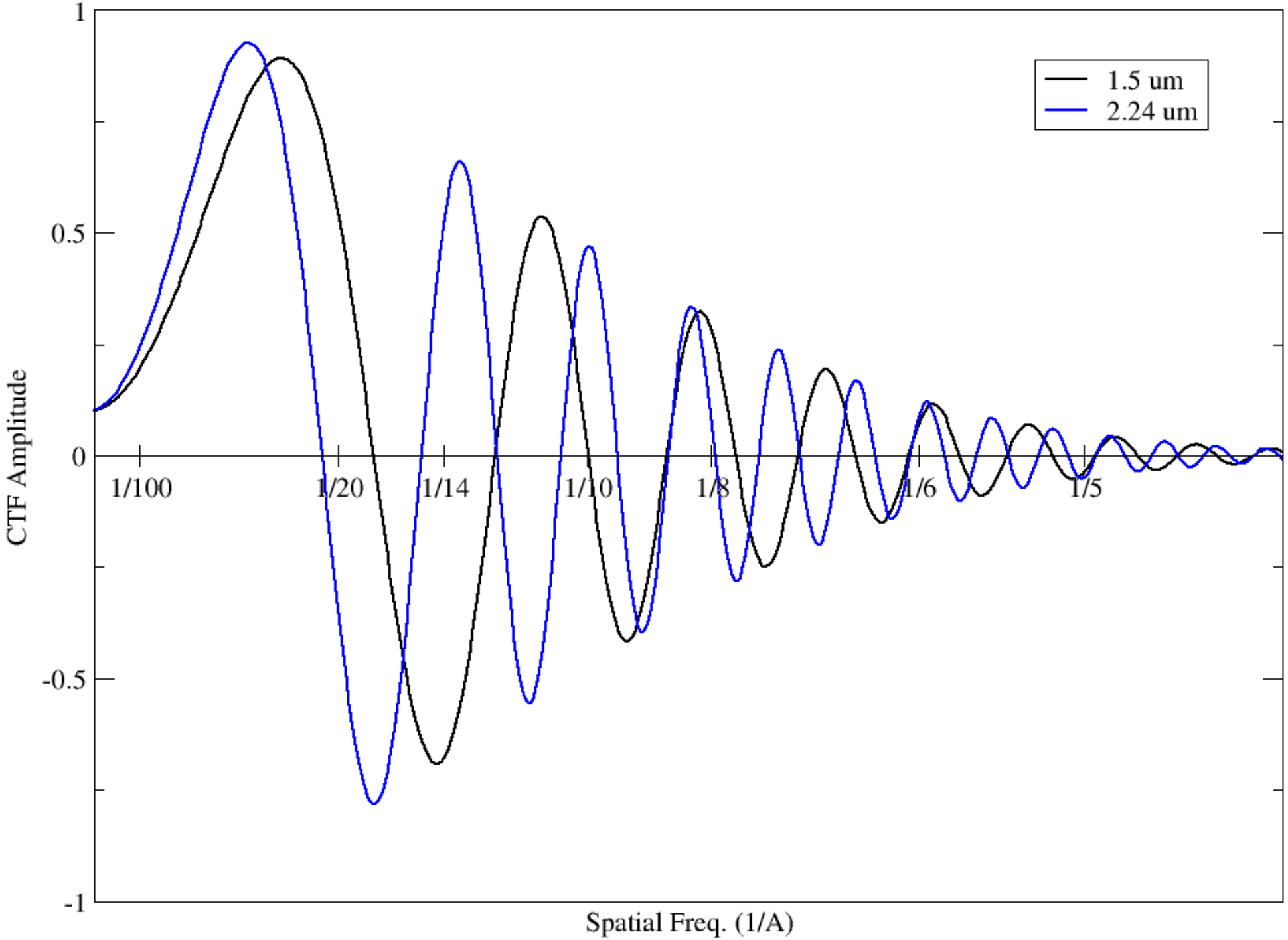

1
0.5
0
-0.5
-1
CTF Amplitude
1.5 um
1/100
1/20
1/14
1/10
1/8
1/6
1/5
Spatial Freq. (1/A)

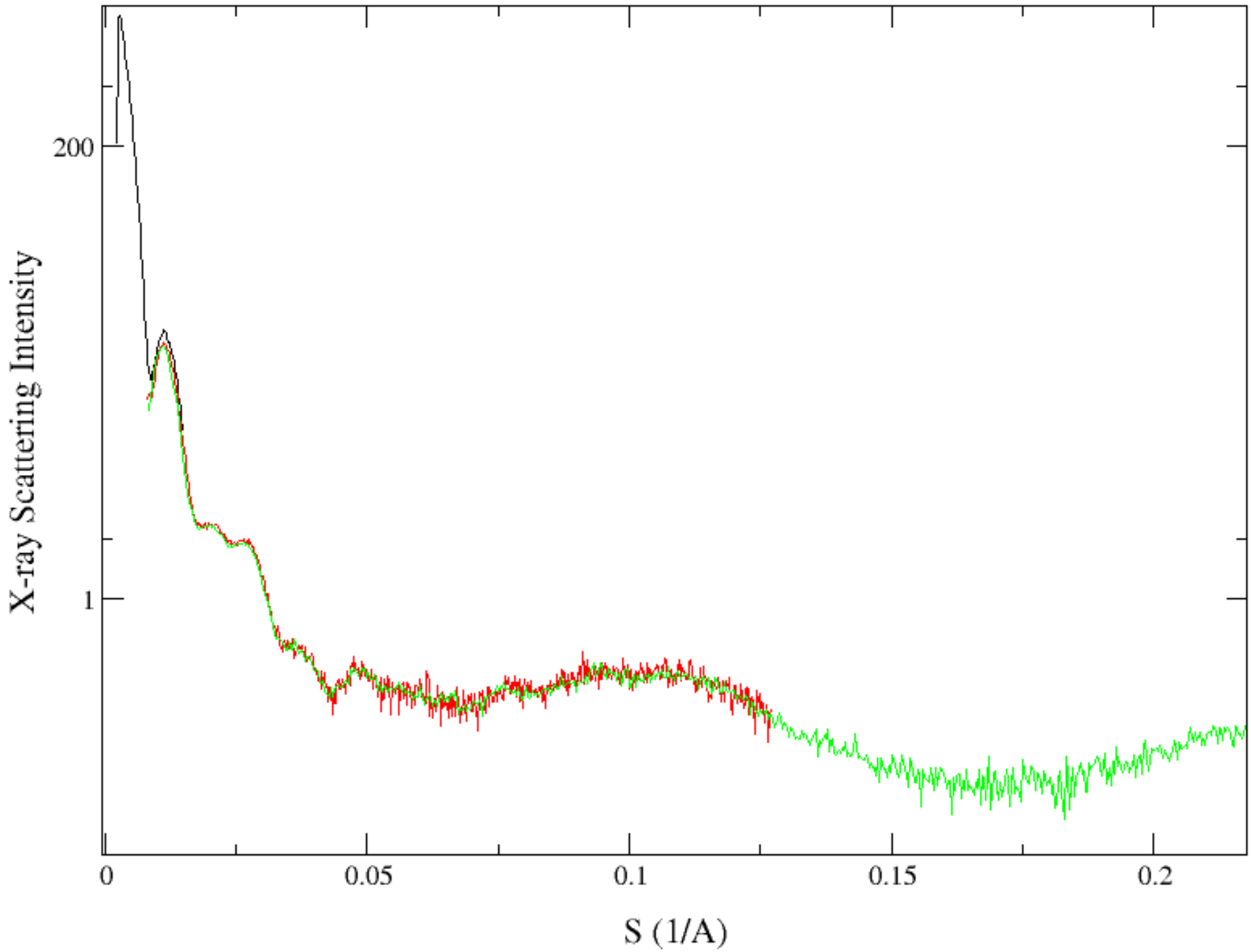

1
0.5
0
-0.5
-1
CTF Amplitude
1/100
1/20
1/14
1/10
1/8
1/6
1/5
Spatial Freq. (1/A)
1.5 um
2.24 um

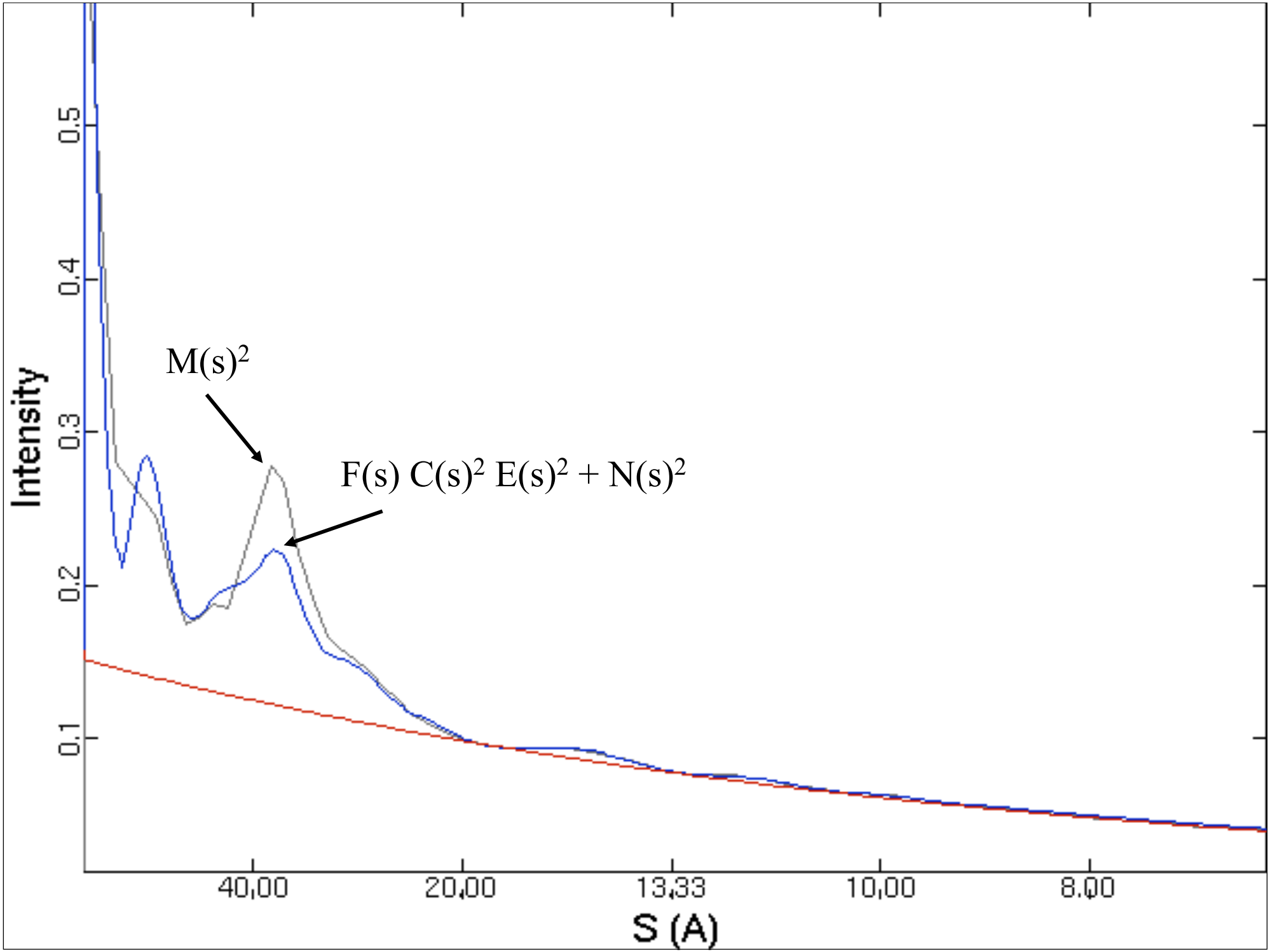
CTF Correction

Wiener Filter | CTF Correction | SNR Weight

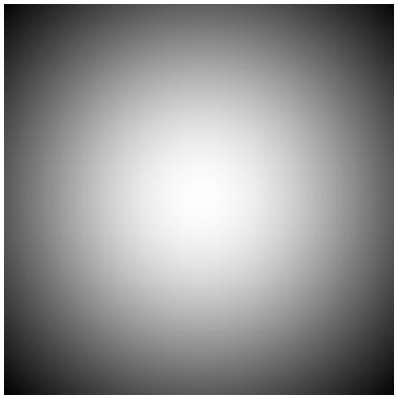
$$\overline{T}(s,\theta)=\frac{F^2(s)R(s)}{1+F^2(s)R(s)}\sum_i \frac{1}{C_i(s)E_i(s)}\frac{R_i(s)}{R(s)}\overline{M}_i(s,\theta)$$

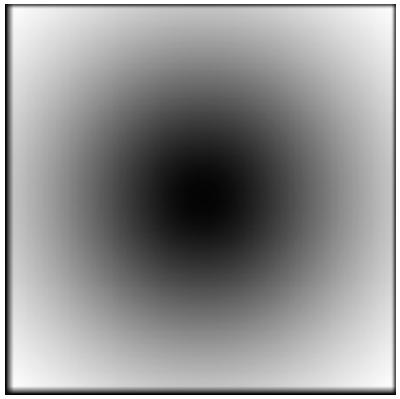
$$R_i(s)=\frac{C_i^2(s)E_i^2(s)}{N_i^2(s)} \qquad R(s)=\sum_i \frac{C_i^2(s)E_i^2(s)}{N_i^2(s)}$$

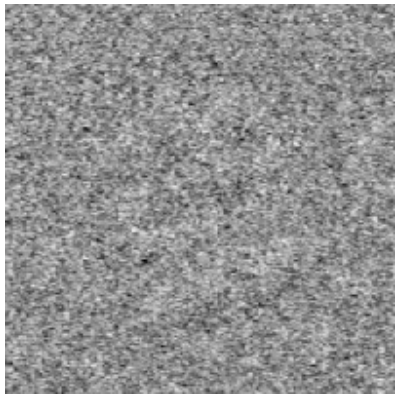

X-ray Scattering Intensity
200
1
0
0.05
0.1
0.15
0.2
S (1/A)

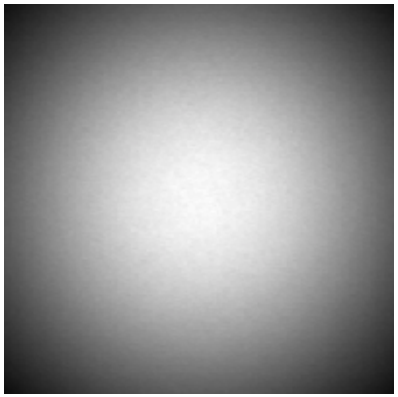

Intensity
0.5
0.4
0.3
0.2
0.1
M(s)2
F(s) C(s)2 E(s)2 + N(s)2
40,00
20,00
13,33
10,00
8.00
S (A)

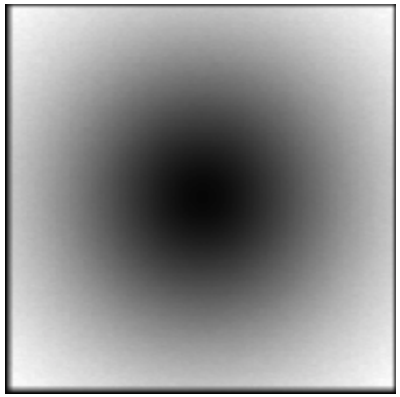
CTF Correction in EMAN2



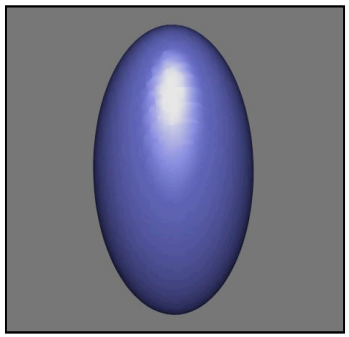


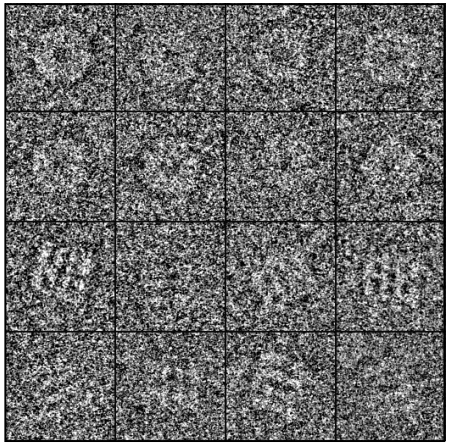




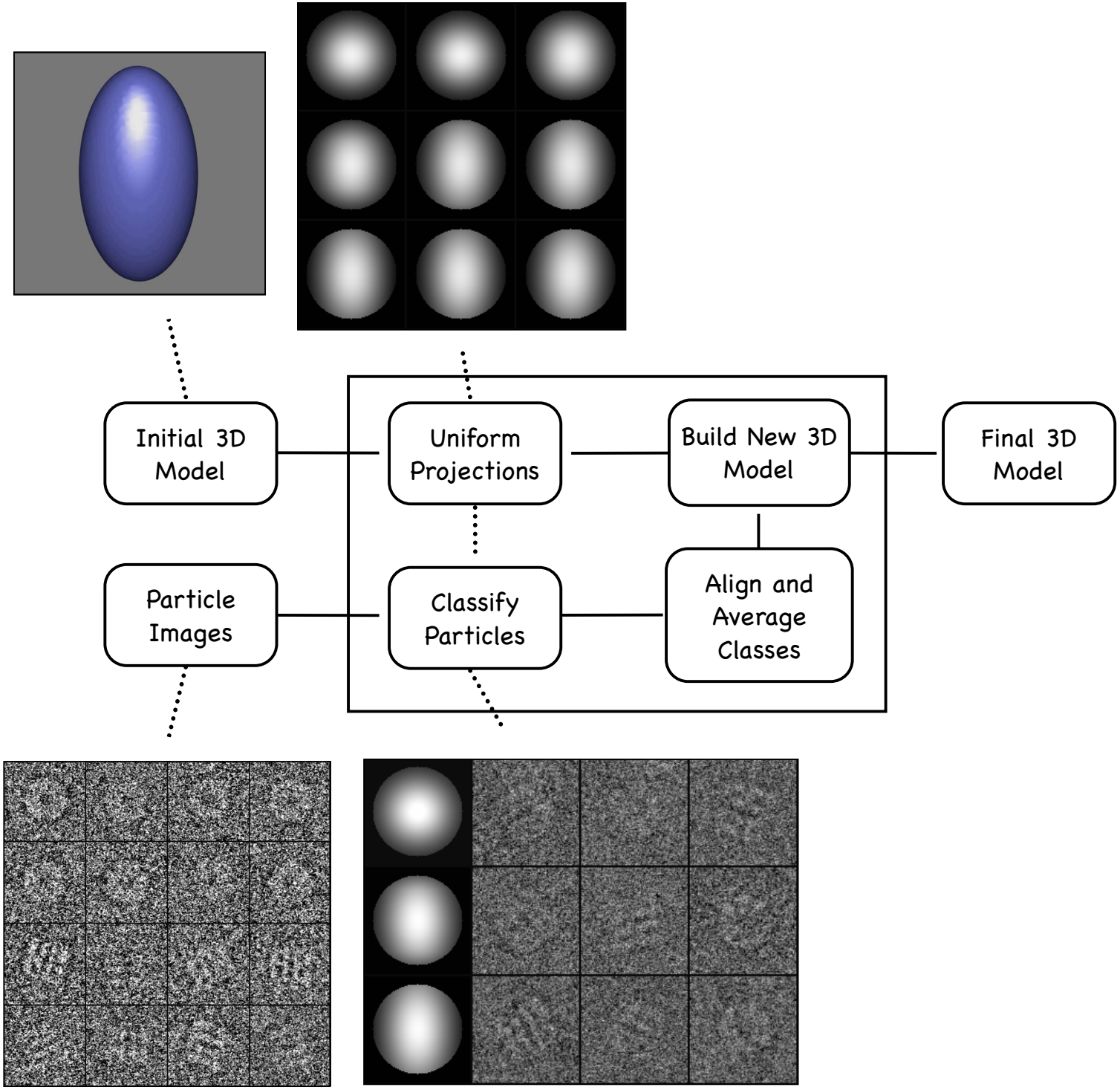


e2ctf demo





Initial 3D Model
Particle Images
Uniform Projections
Classify Particles
Build New 3D Model
Align and Average Classes
Final 3D Model


Initial 3D Model
Uniform Projections
Build New 3D Model
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Particle Images
Classify Particles
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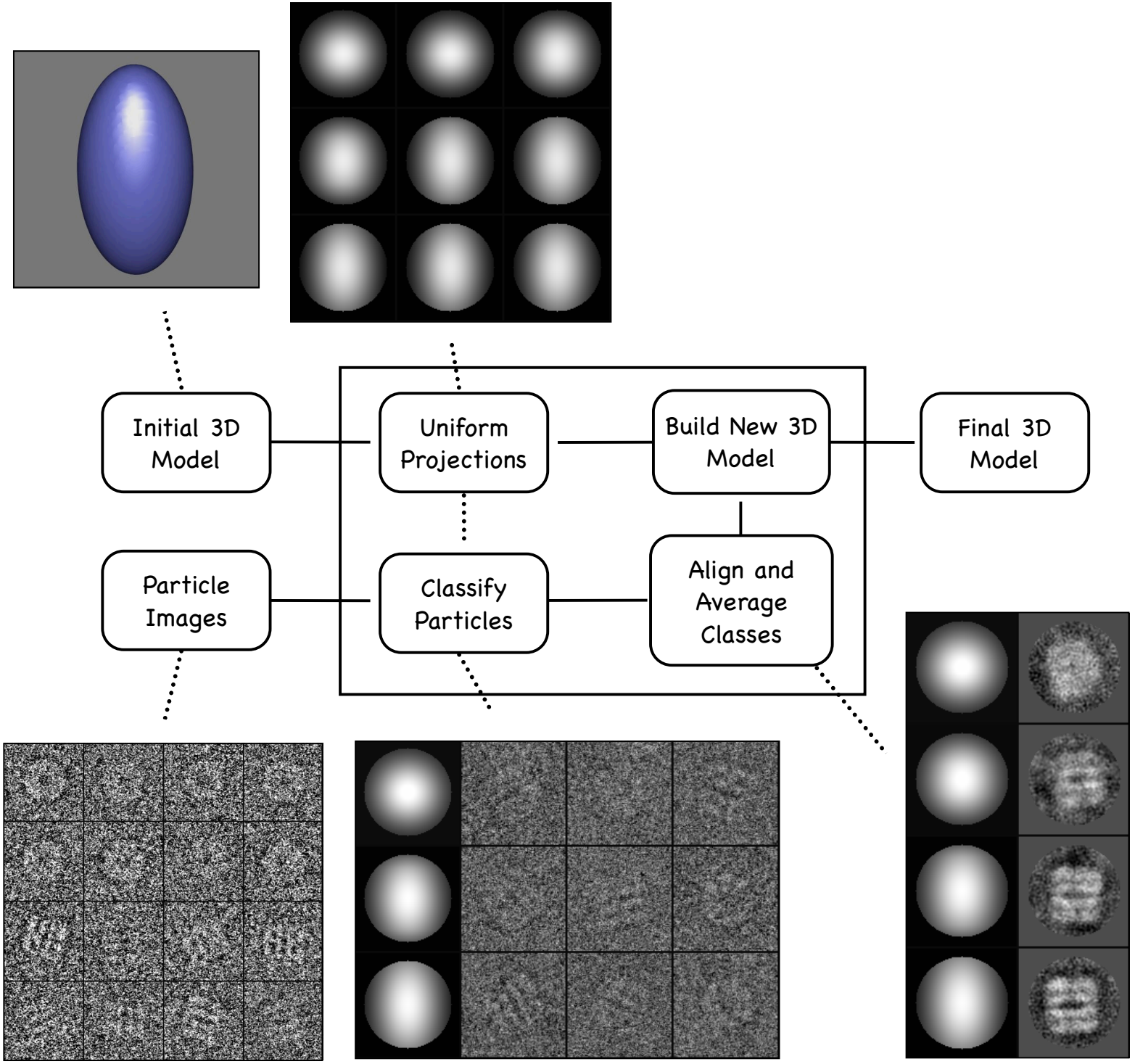
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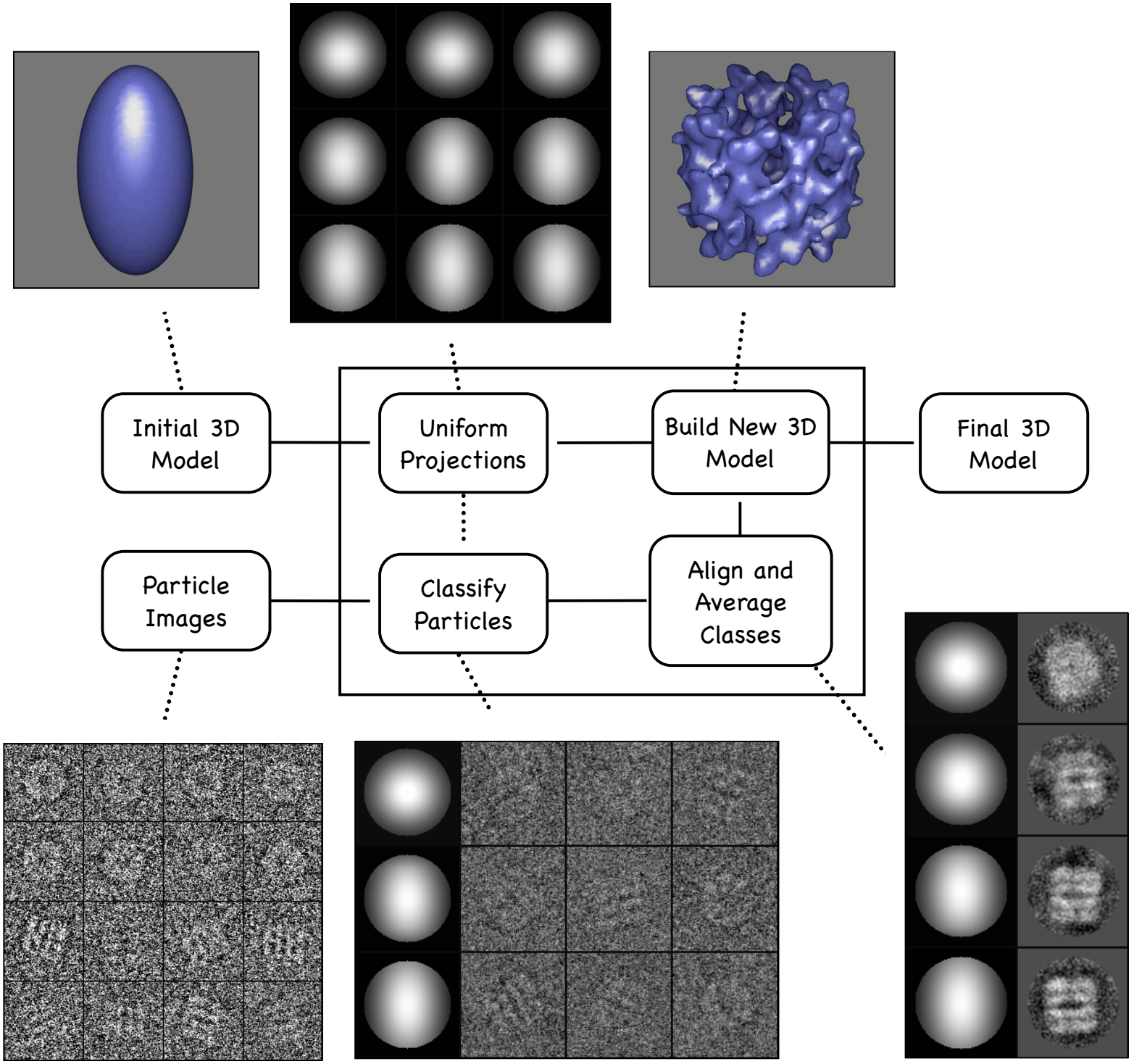
Initial 3D Model
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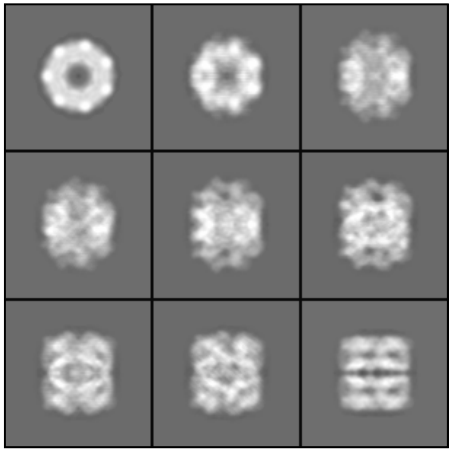
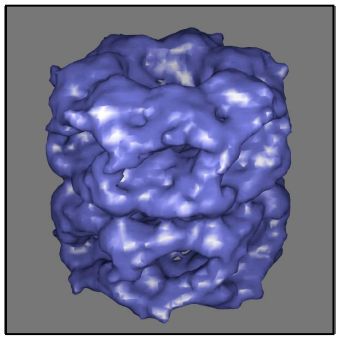
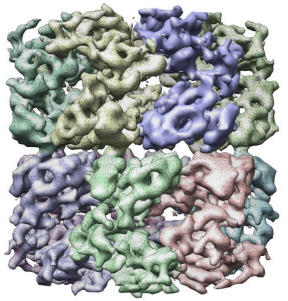






Initial 3D Model

Uniform Projections

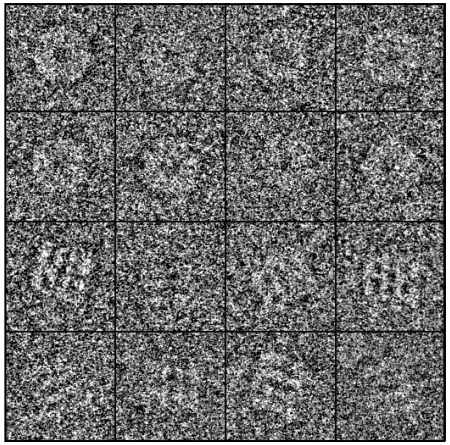
Build New 3D Model

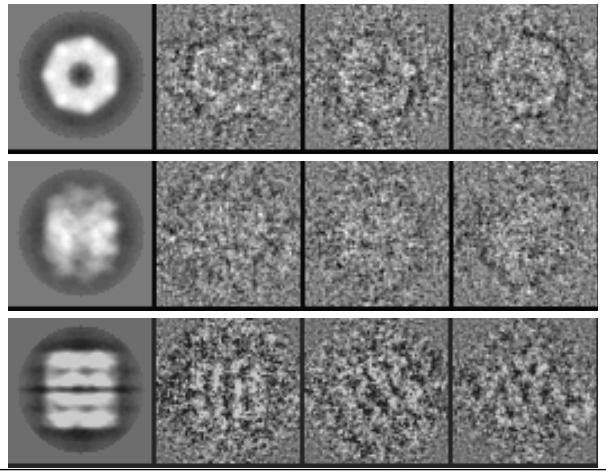
Final 3D Model

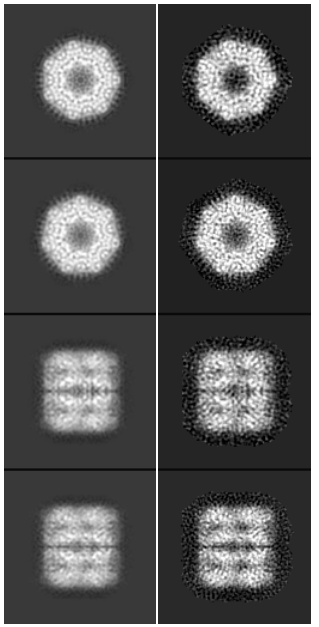
Particle Images

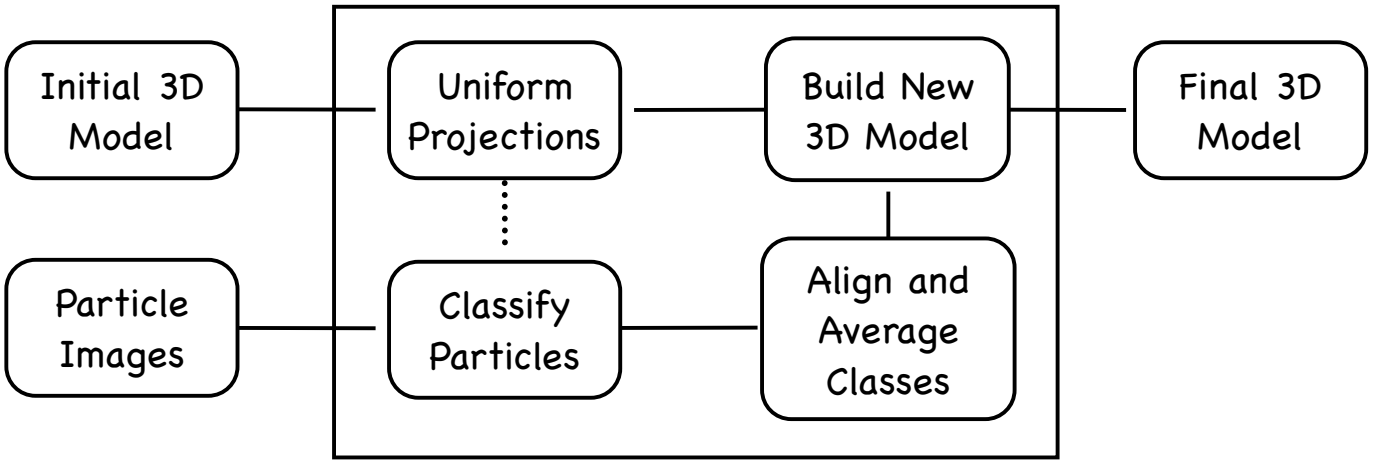
Classify Particles

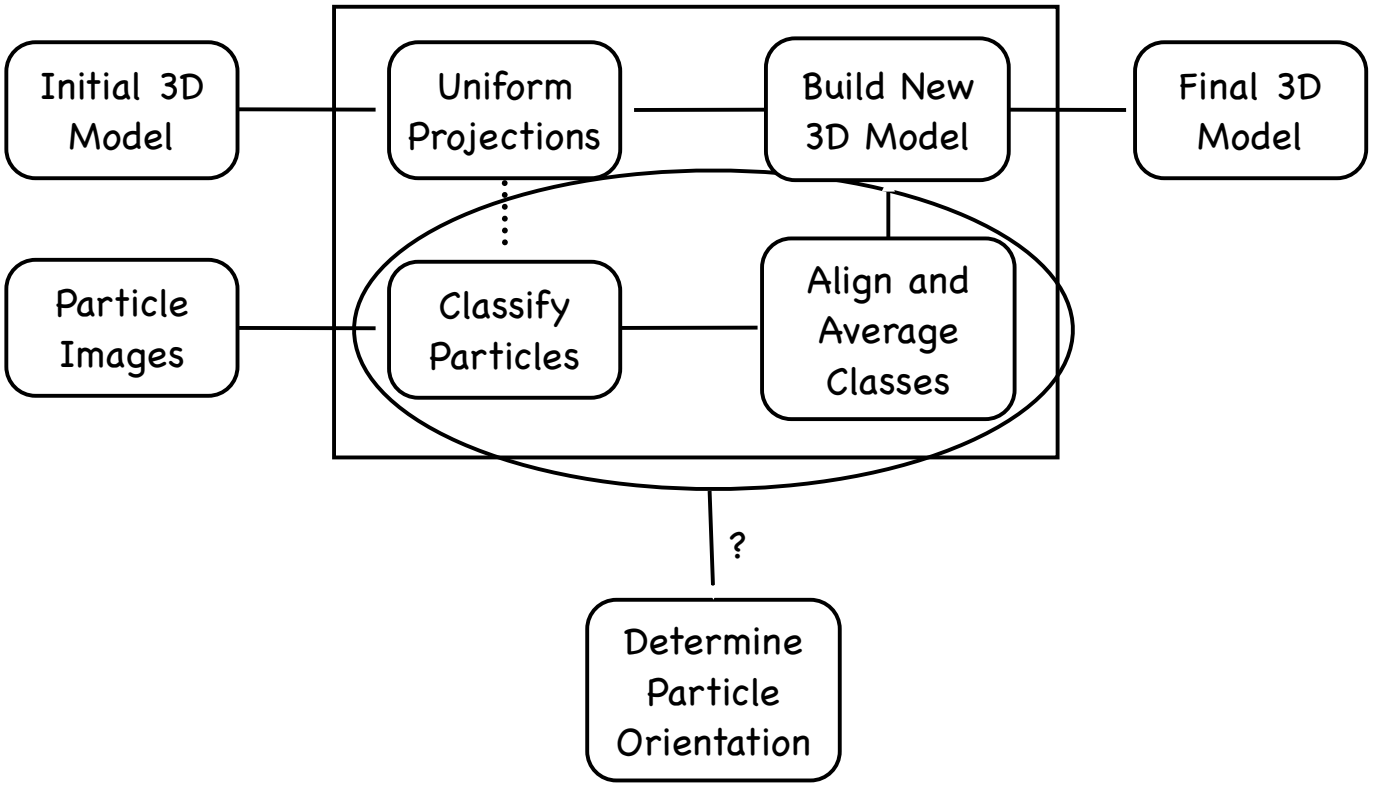
Align and Average Classes

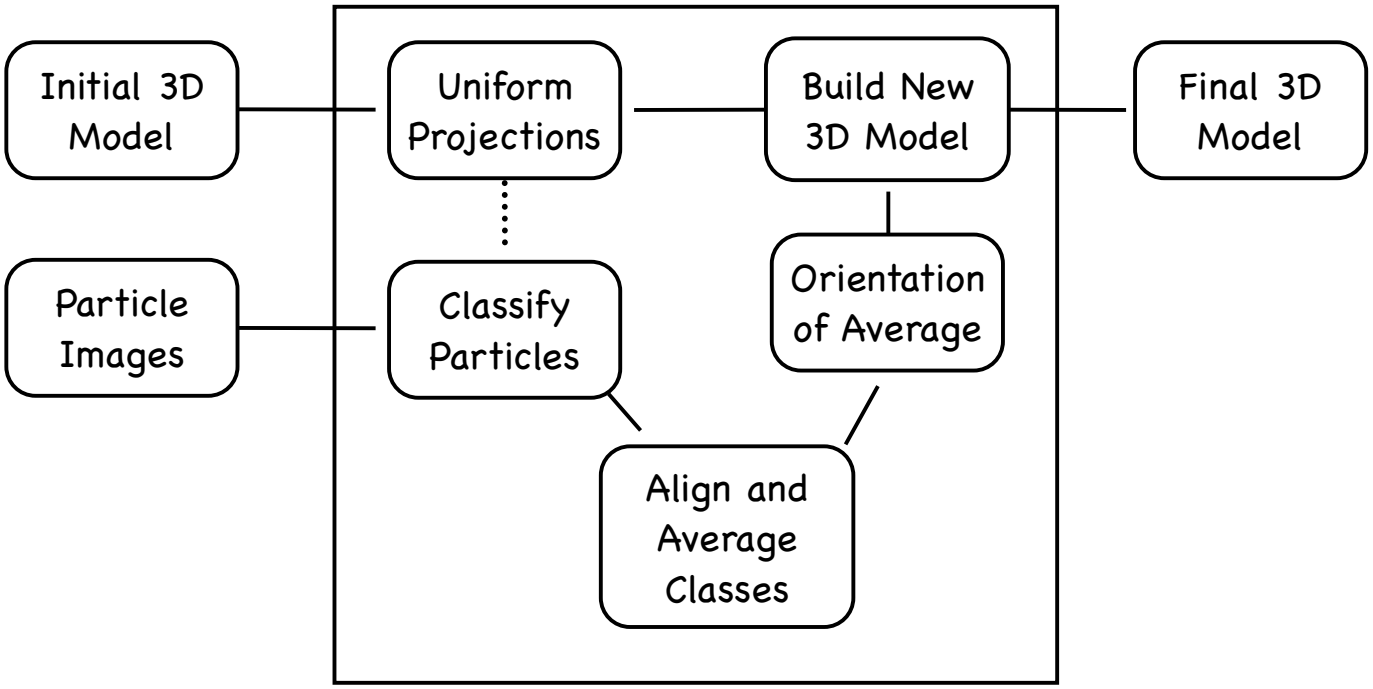


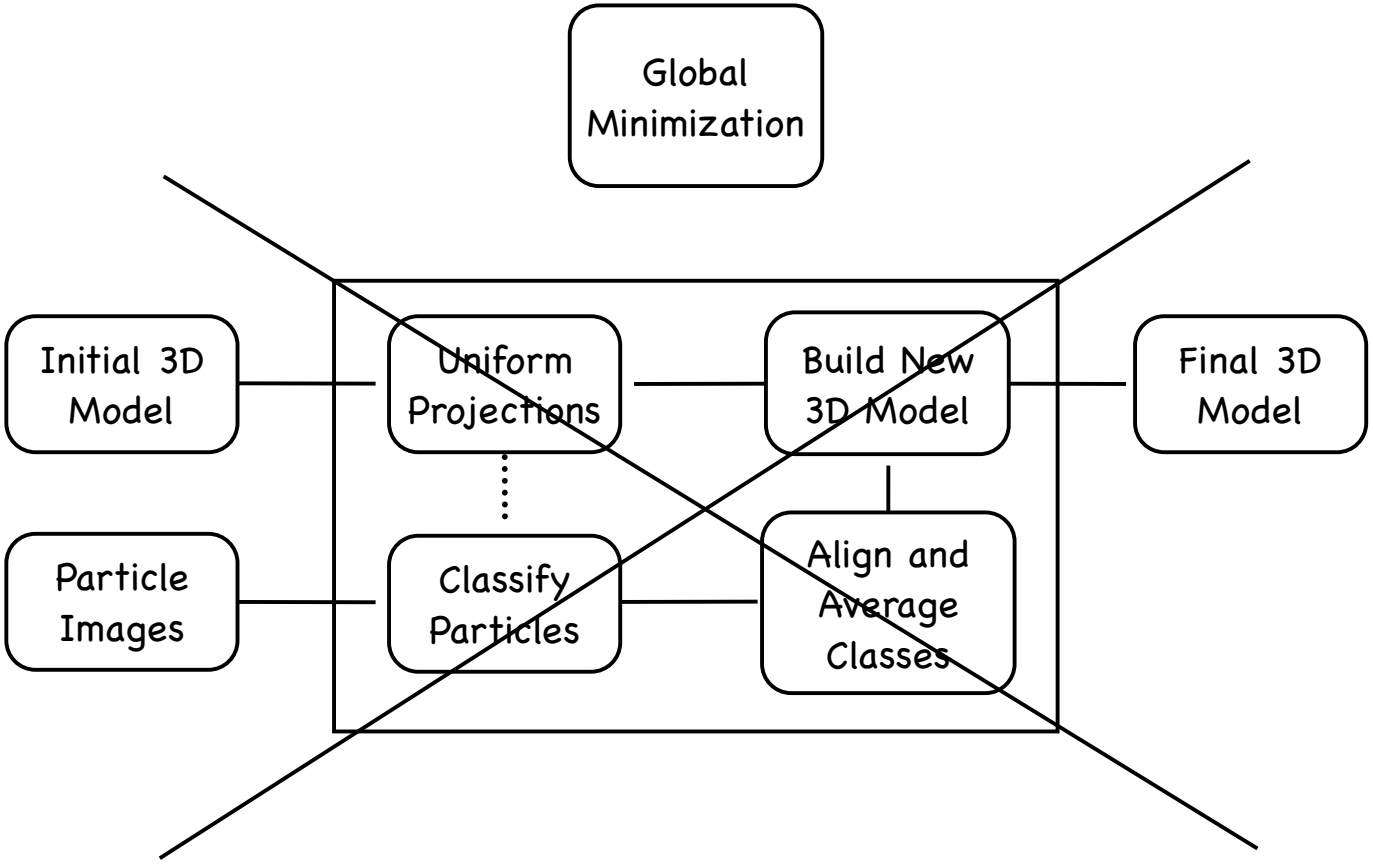





Initial 3D Model
Particle Images
Uniform Projections
Classify Particles
Build New 3D Model
Align and Average Classes
Final 3D Model


Initial 3D Model
Uniform Projections
Build New 3D Model
Final 3D Model
Particle Images
Classify Particles
Align and Average Classes
?
Determine Particle Orientation


Initial 3D Model
Uniform Projections
Build New 3D Model
Final 3D Model
Particle Images
Classify Particles
Orientation of Average
Align and Average Classes


Global Minimization
Initial 3D Model
Particle Images
Uniform Projections
Classify Particles
Build New 3D Model
Align and Average Classes
Final 3D Model

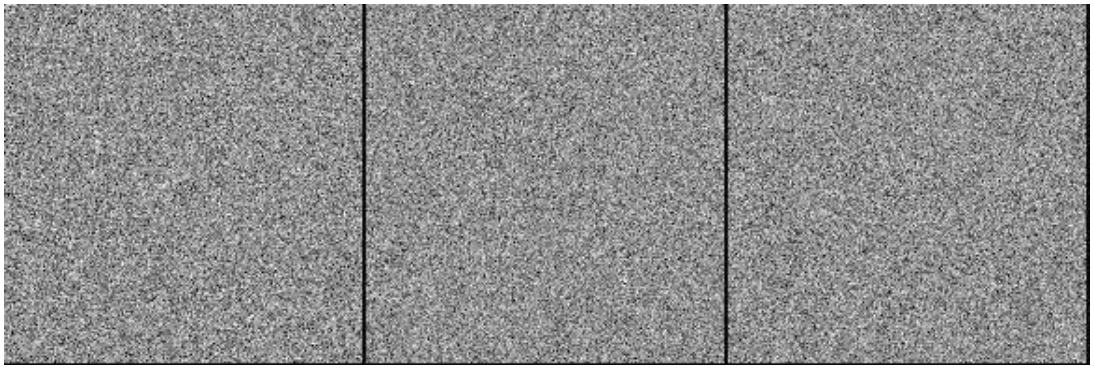
Model Bias ?

Model Bias

Base

Noisy

Align to

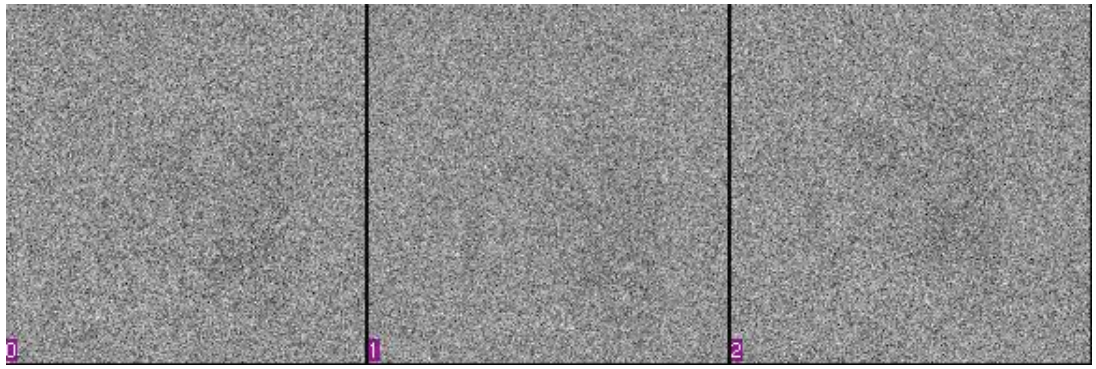


25 100 250 1000 2000

Model Bias

Base

Noisy (~10% contrast)



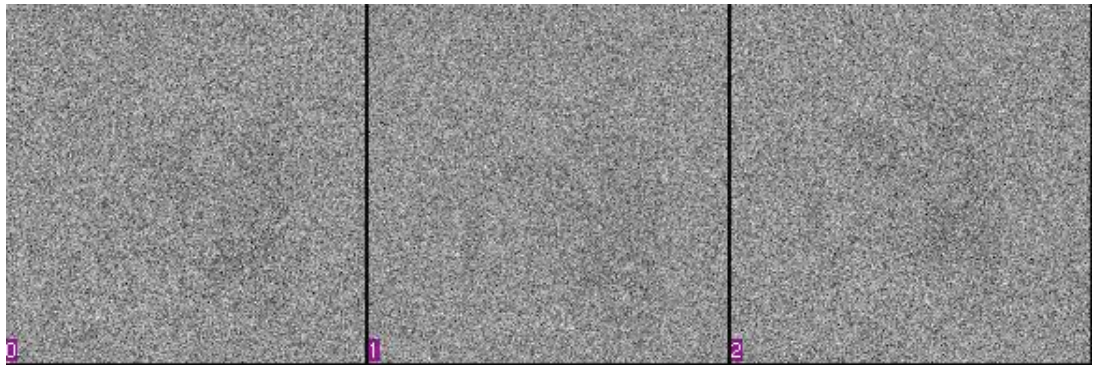
Align to

25 100 250 1000 2000

Model Bias

Base

Noisy (~10% contrast)



Align to

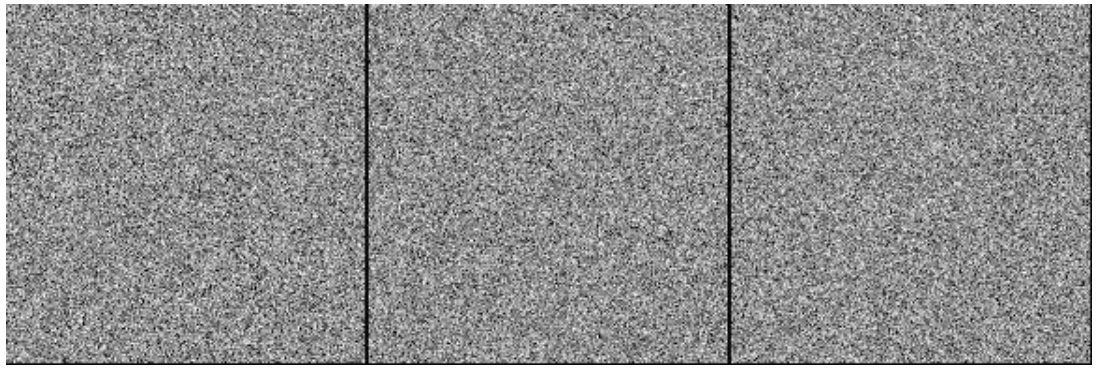
25 100 250 1000 2000

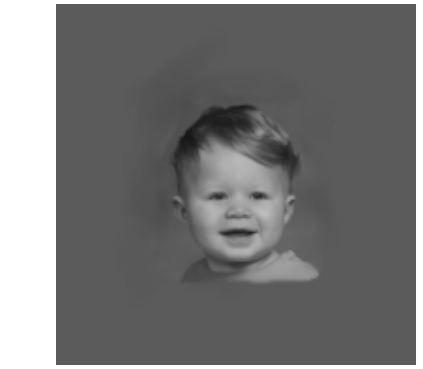
Model Bias

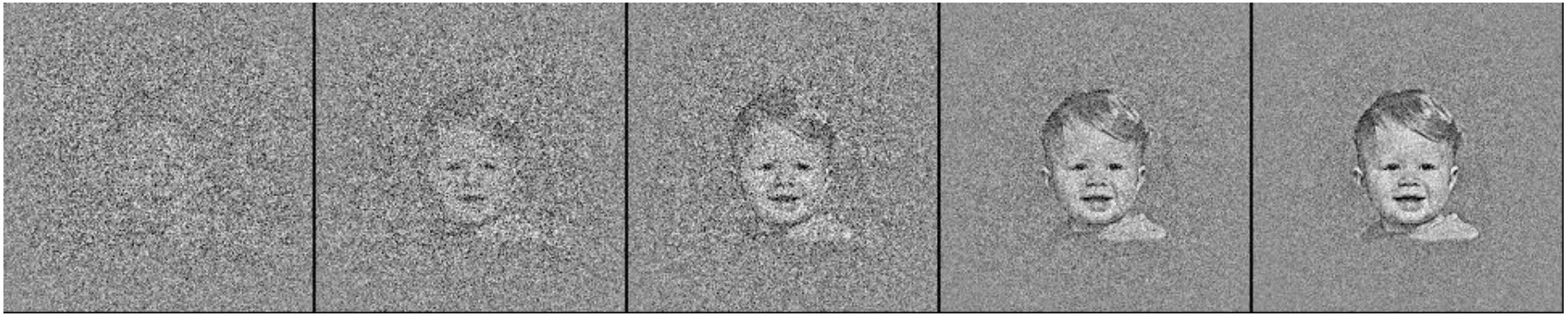
Base

Noisy

Align to







25 100 250 1000 2000

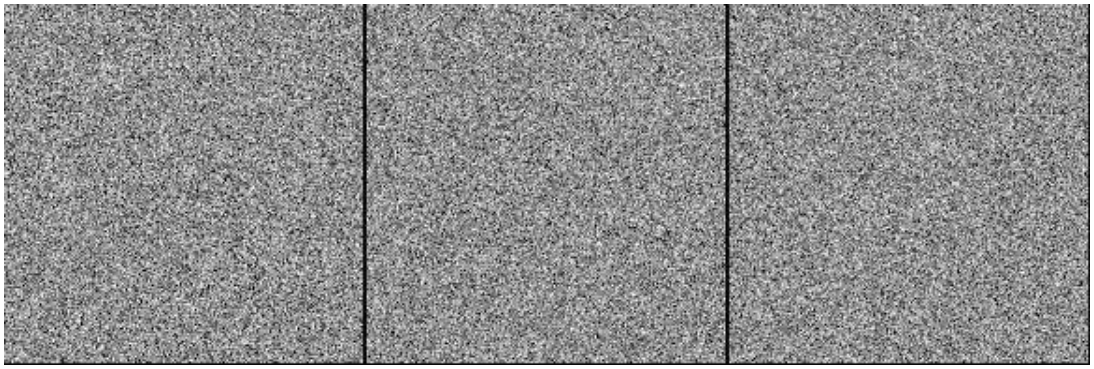
Model Bias

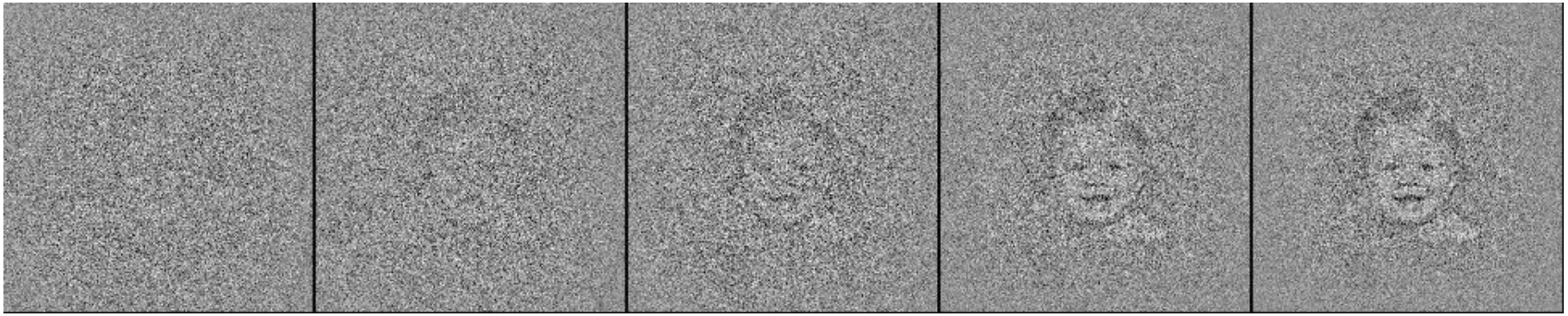
Base

Noisy

Align to

Iter x4





25 100 250 1000 2000

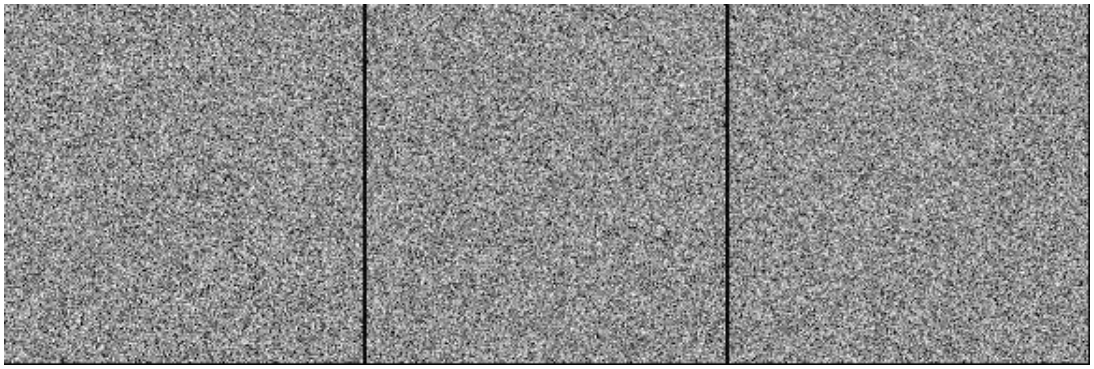
Model Bias

Base

Noisy

Align to

Iter x8

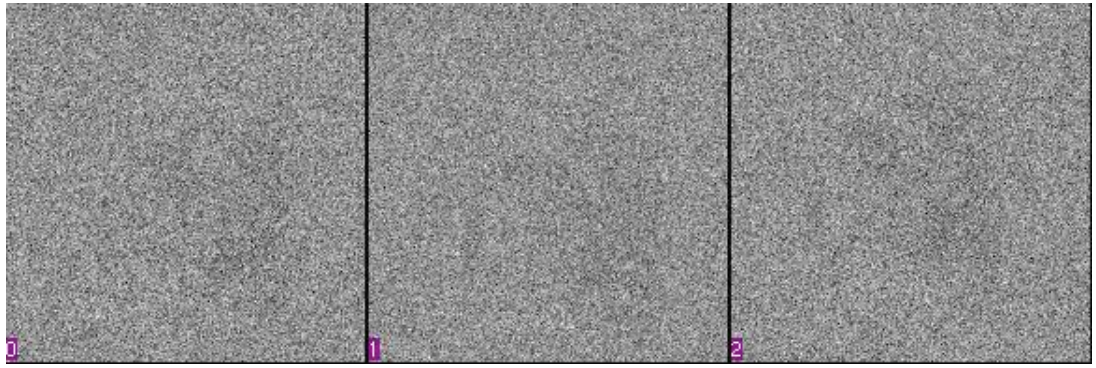


25 100 250 1000 2000

Model Bias

Base

Noisy (~10% contrast)



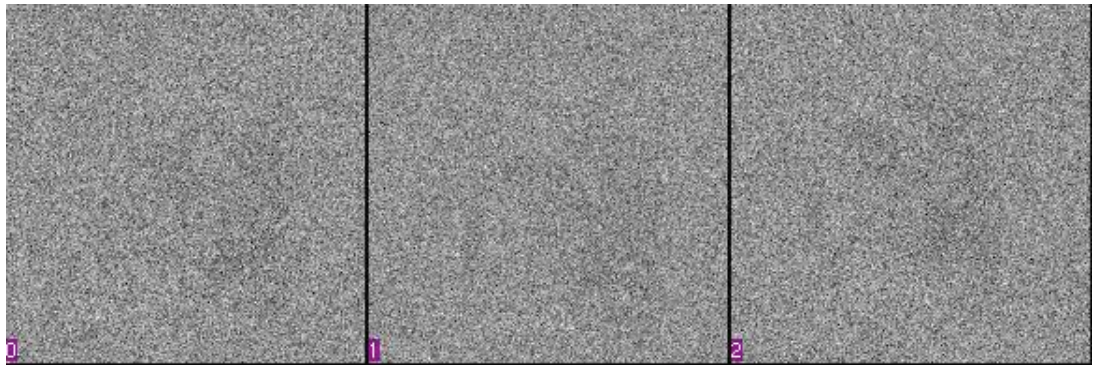
Align to

25 100 250 1000 2000

Model Bias

Base

Noisy

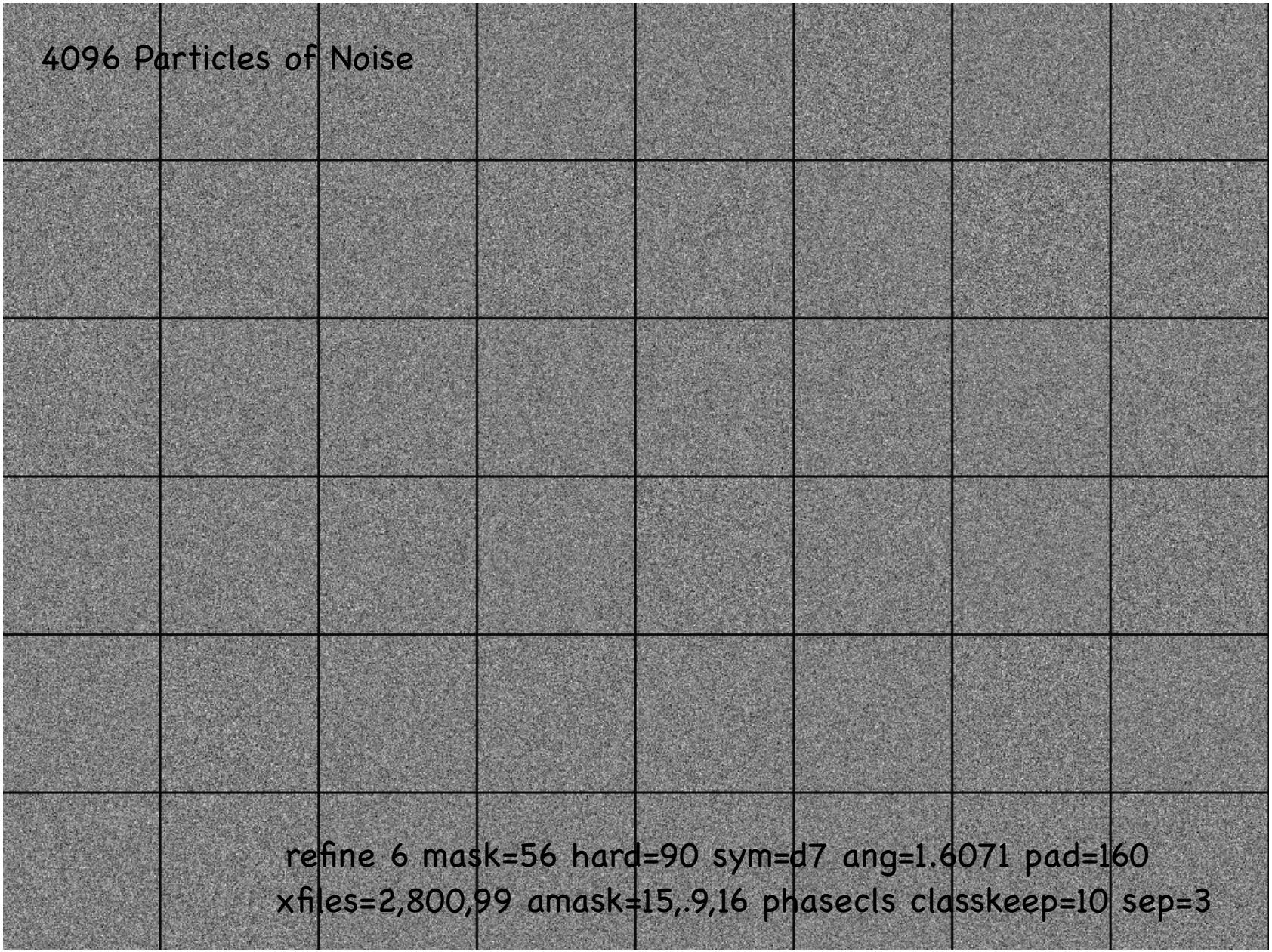


Align to

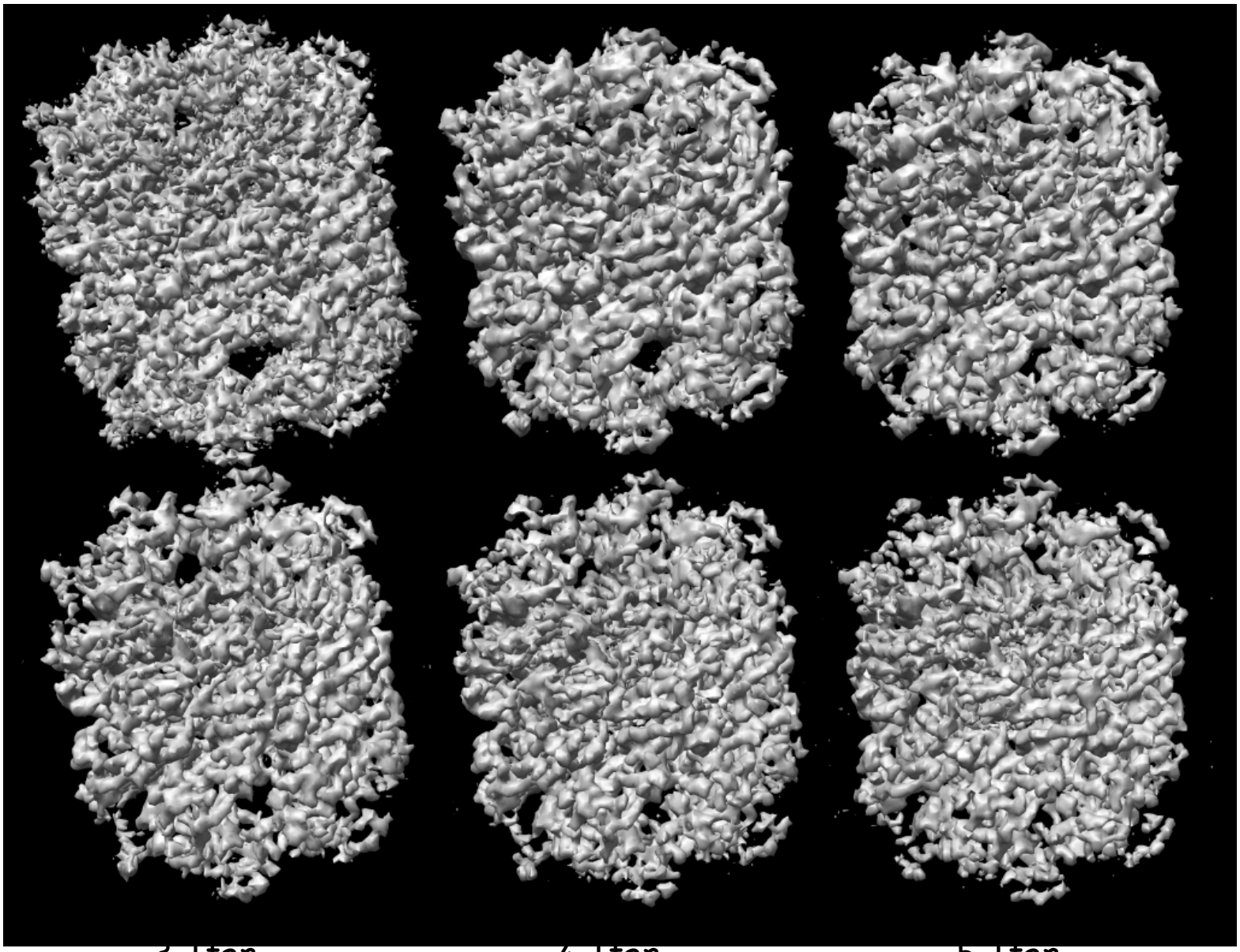
Iter x4

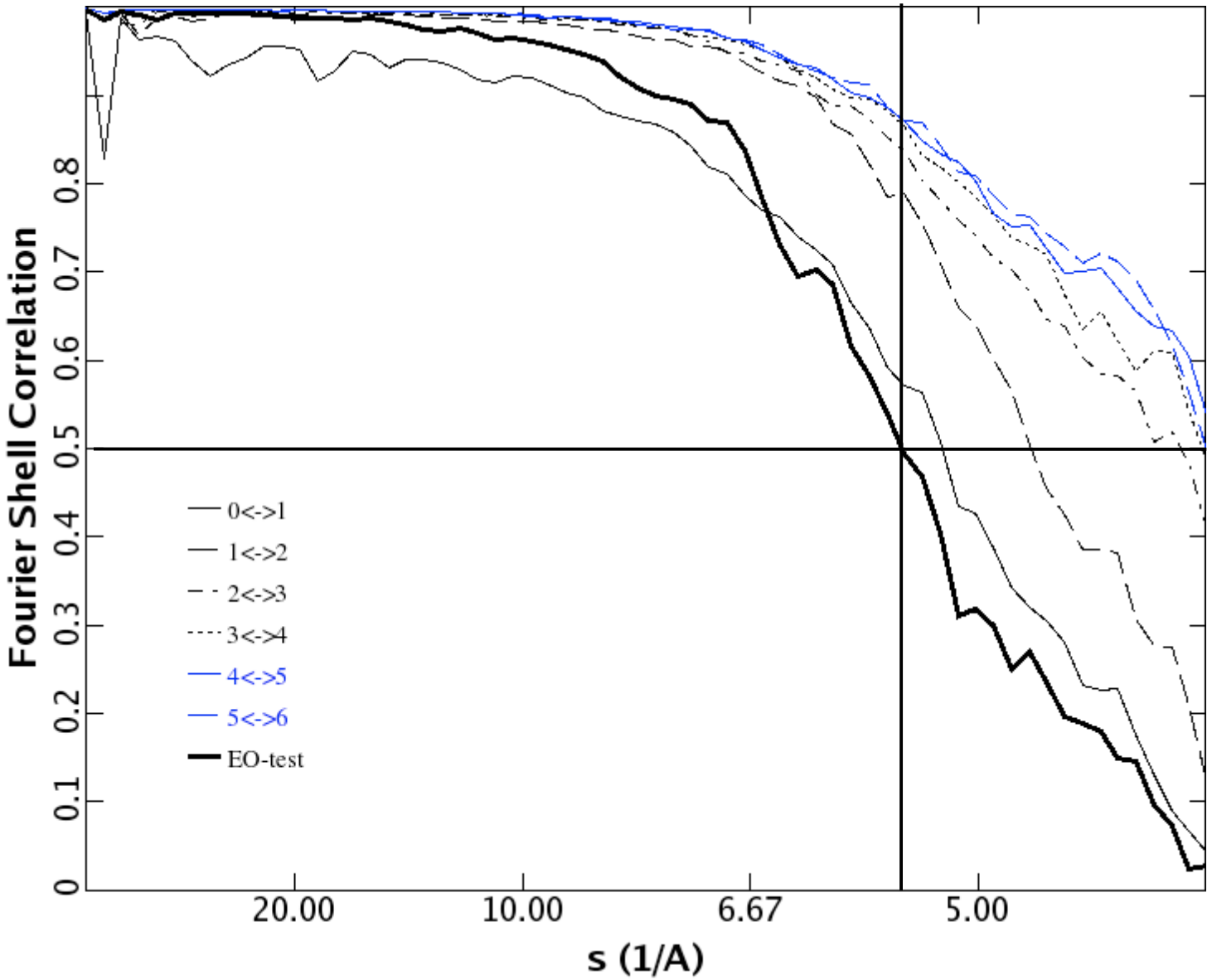
25 100 250 1000 2000

How About 3-D ?


4096 Particles of Noise
refine 6 mask=56 hard=90 sym=d7 ang=1.6071 pad=160
xfiles=2,800,99 amask=15,.9,16 phasecls classkeep=10 sep=3

no iteration



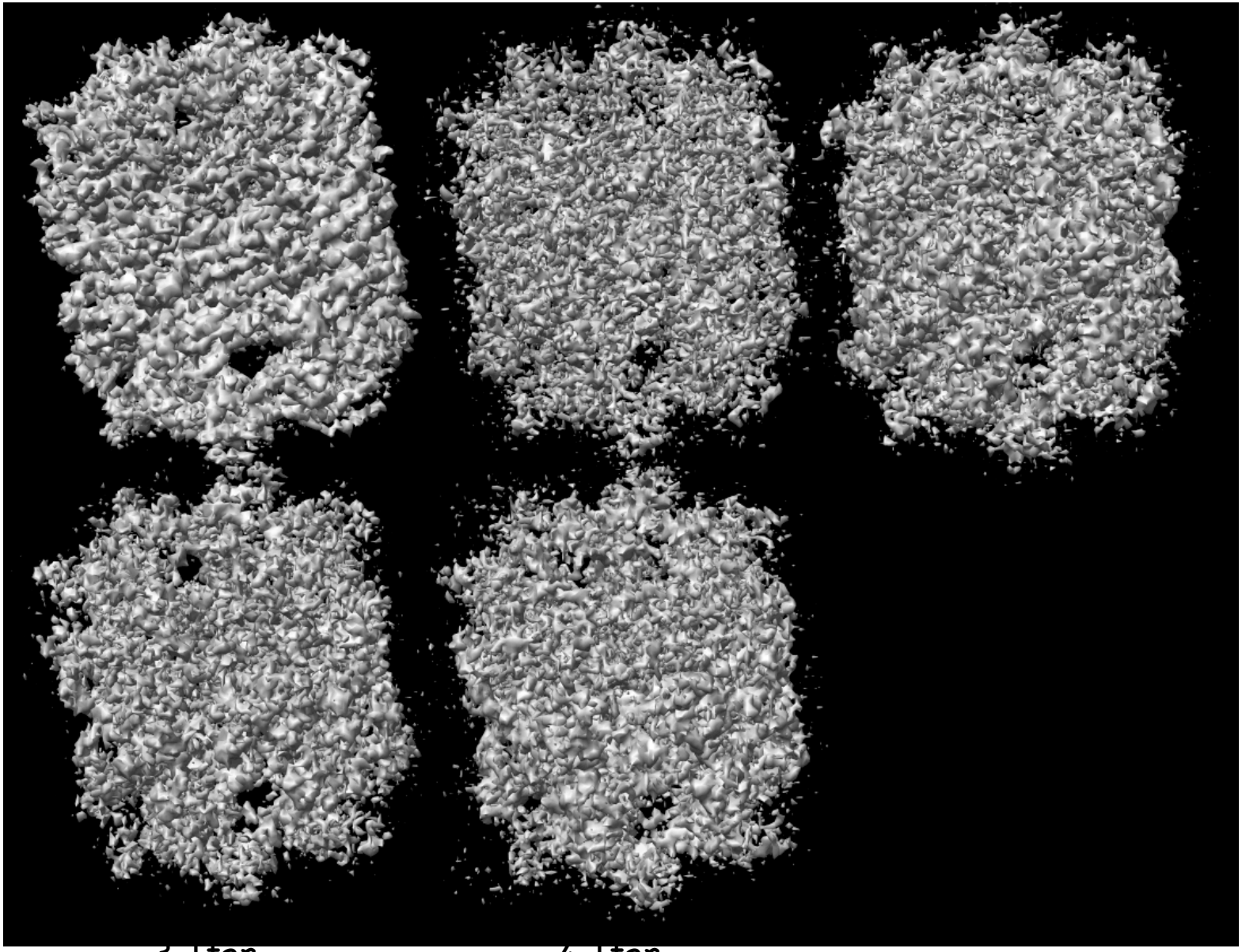

Fourier Shell Correlation
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
20.00
10.00
6.67
5.00
s (1/A)
0<->1
1<->2
2<->3
3<->4
4<->5
5<->6
EO-test

1 iteration

Initial Model

1 Iter.

2 Iter.



3 Iter.

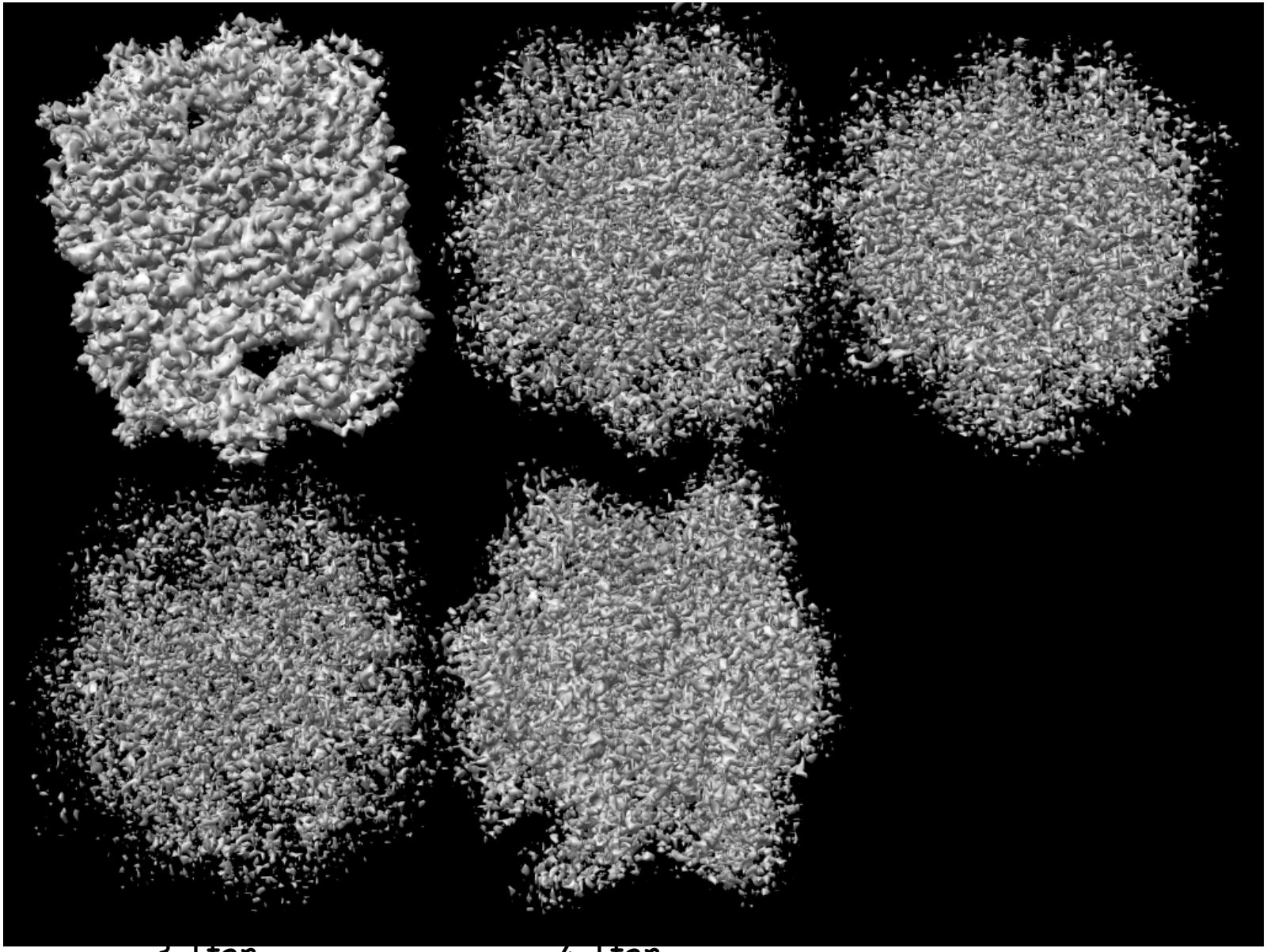
4 Iter.

6 iterations

Initial Model

1 Iter.

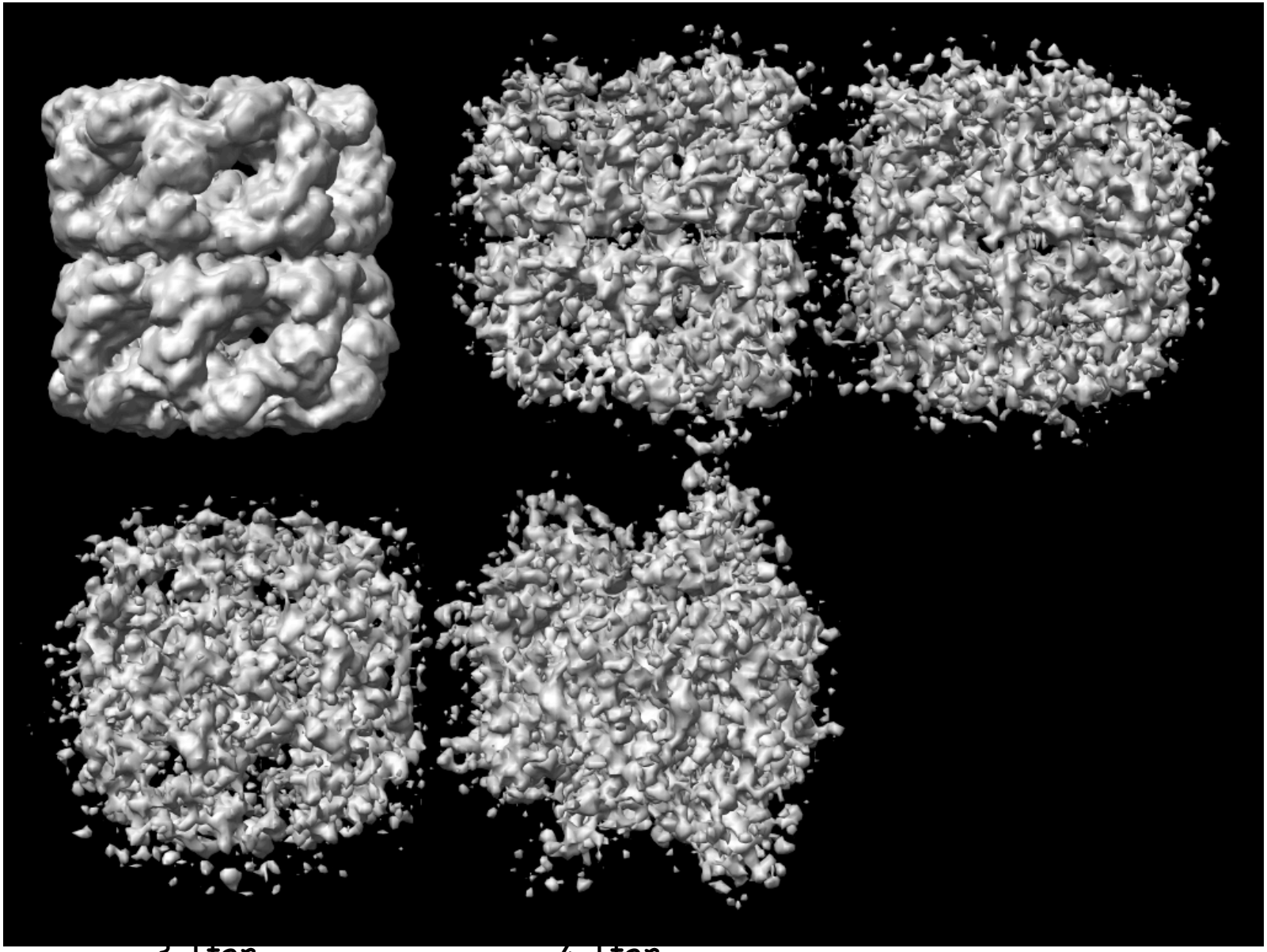
2 Iter.

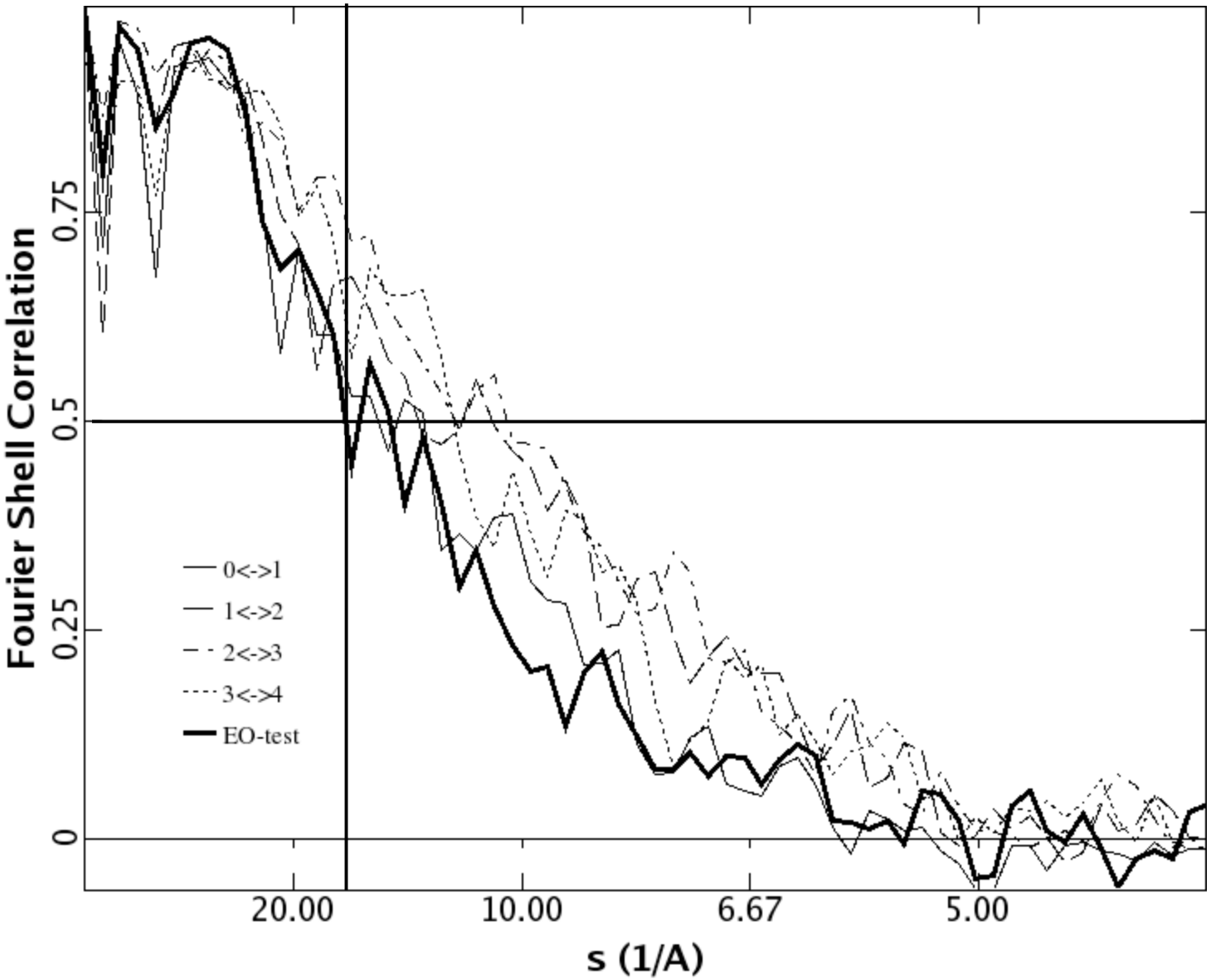


3 Iter.

4 Iter.

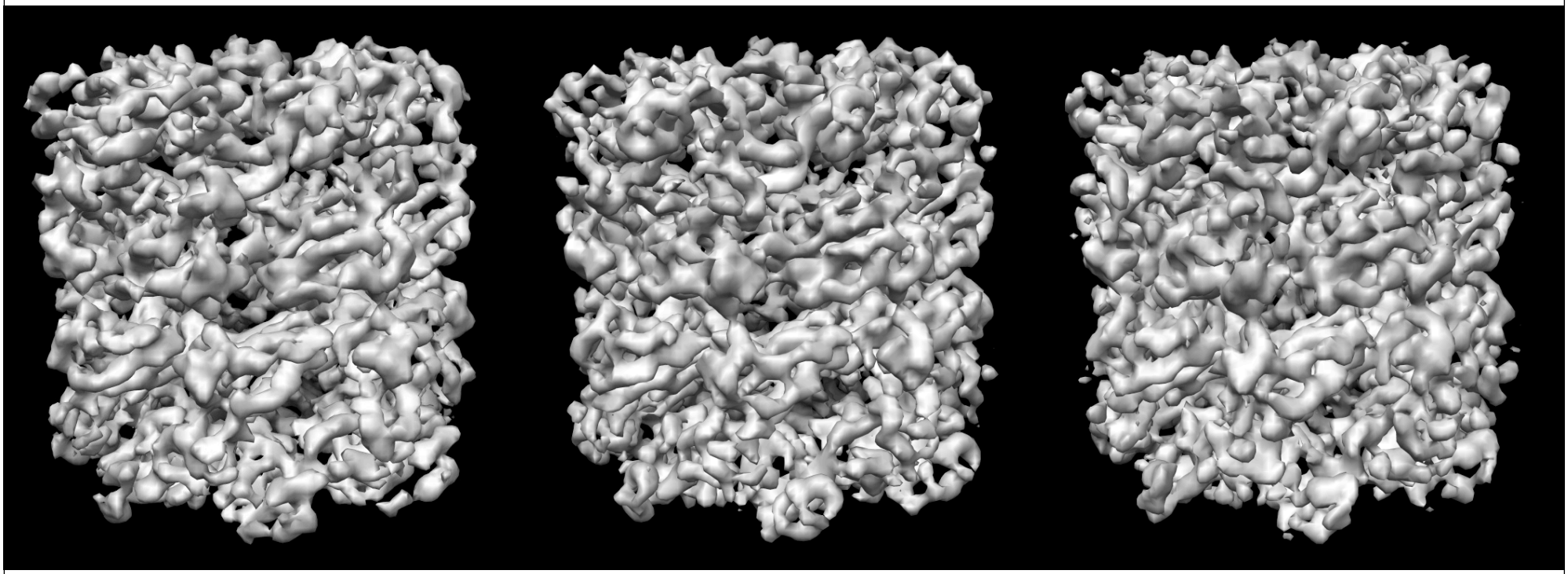
6 iterations
(8 A lowpass)



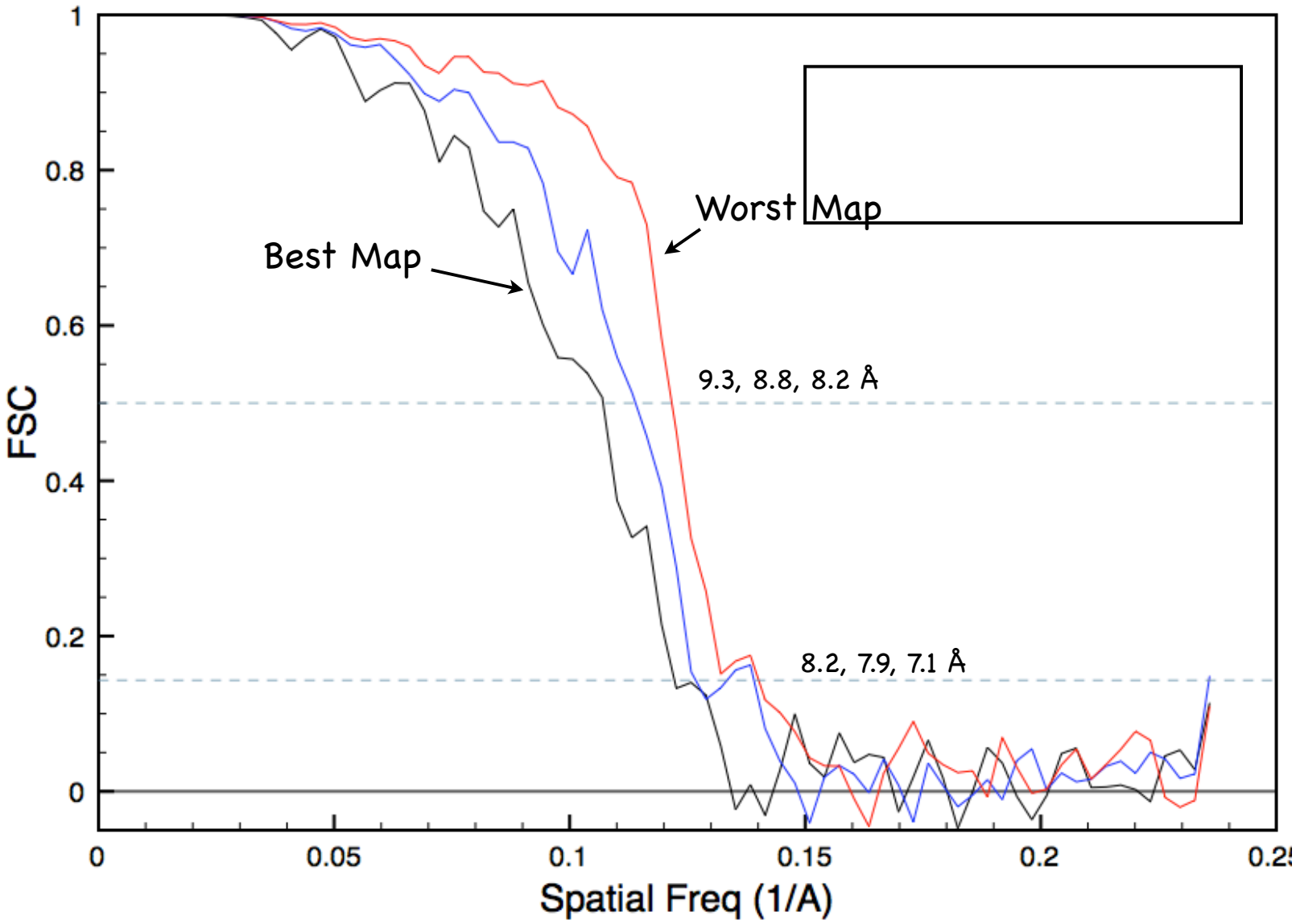

Fourier Shell Correlation
0
0.25
0.5
0.75
20.00
10.00
6.67
5.00
s (1/A)
0<->1
1<->2
2<->3
3<->4
EO-test

How Do we Stop This ?

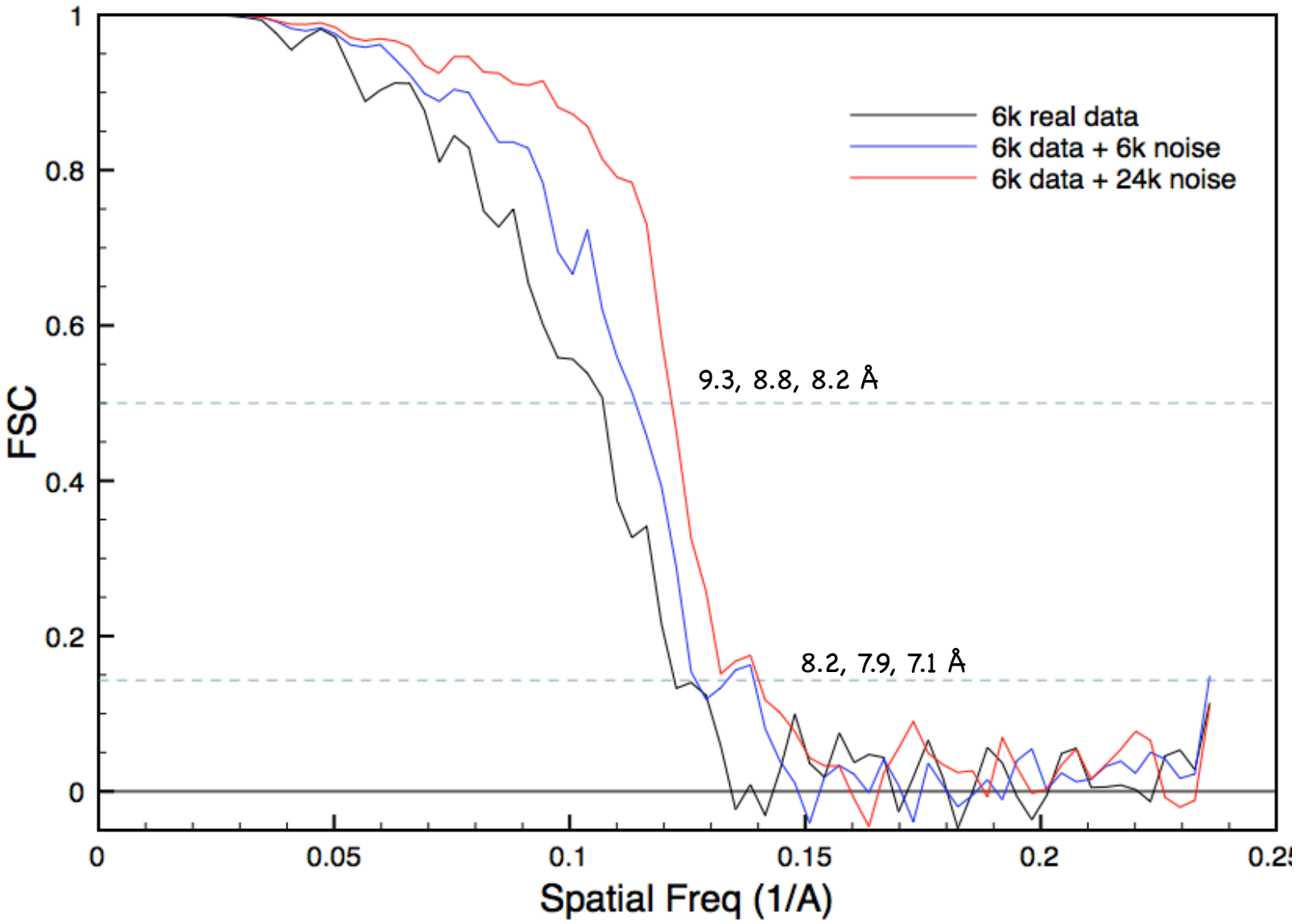
- (In EMAN) use classiter>3 for a few rounds
- Use several different (random) starting models and insure that you get a good answer
- Compare 3D models with results of 2D analysis



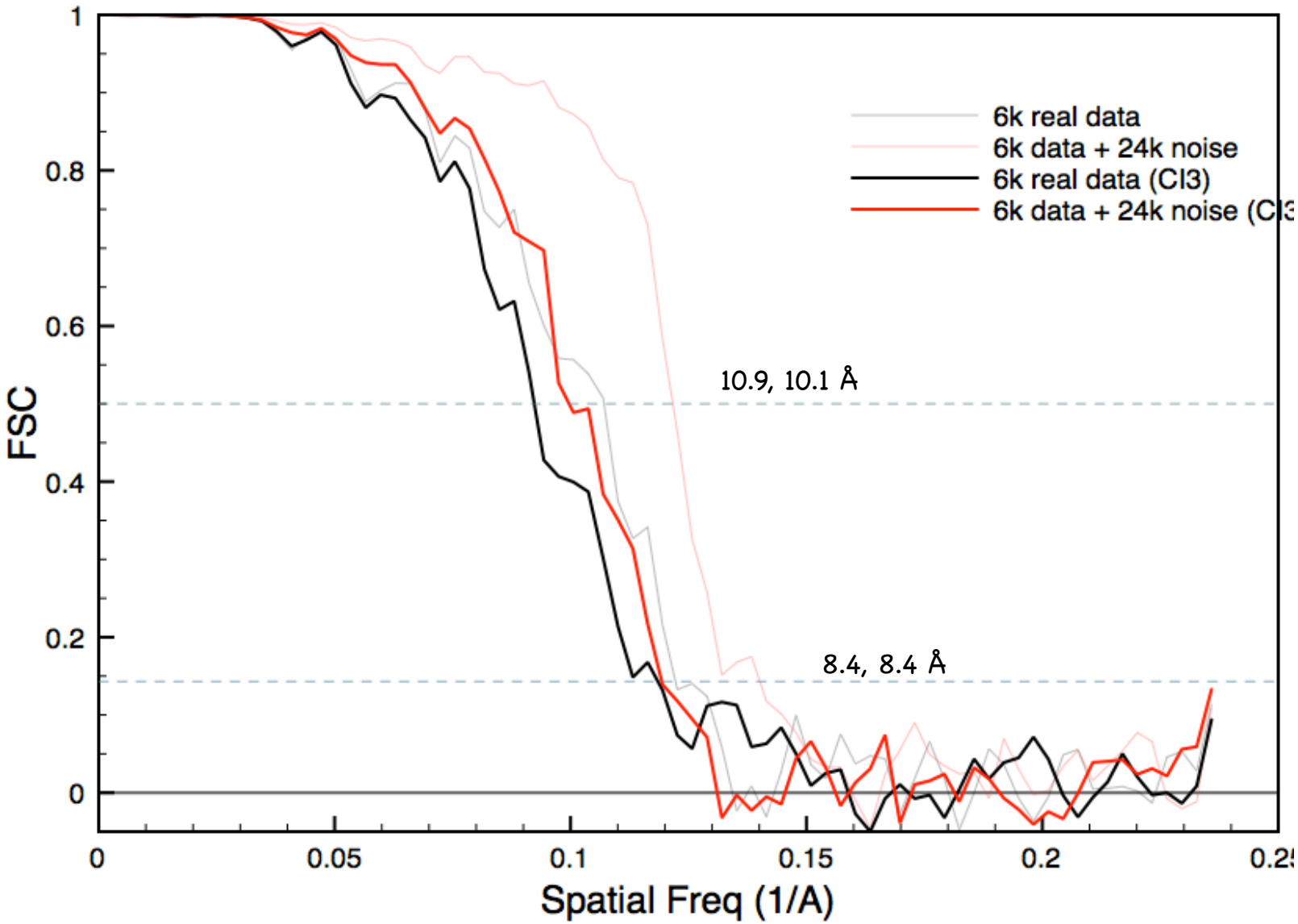
???


1
0.8
0.6
0.4
0.2
0
FSC
0
0.05
0.1
0.15
0.2
0.25
Spatial Freq (1/A)
Worst Map
Best Map
9.3, 8.8, 8.2 Å
8.2, 7.9, 7.1 Å

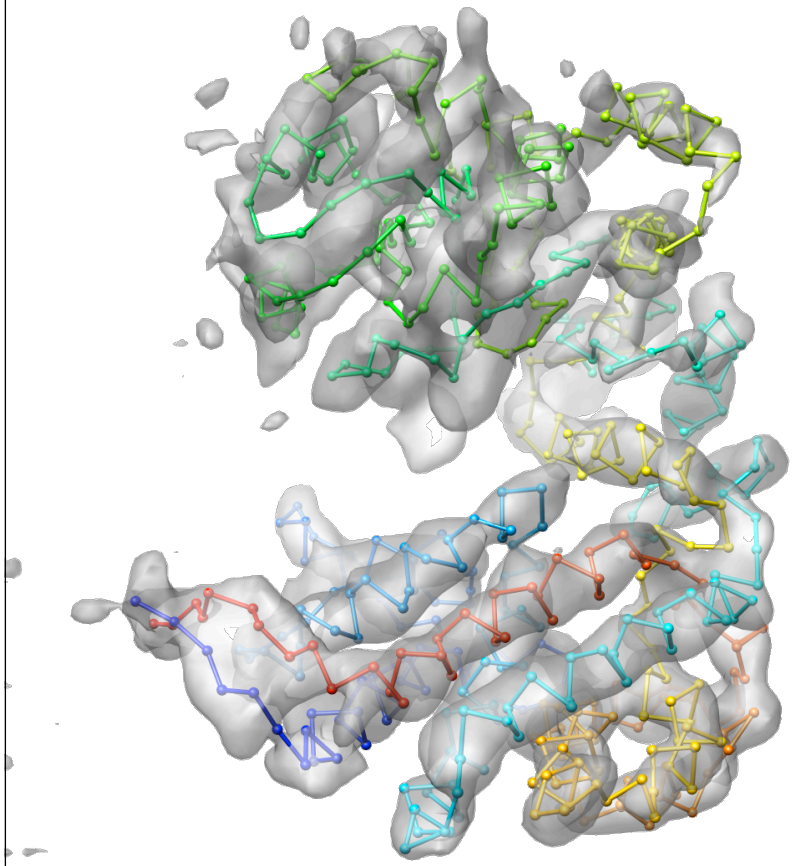
Adding Noise



Adding Noise with Classiter



Acknowledgements



EMAN2

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