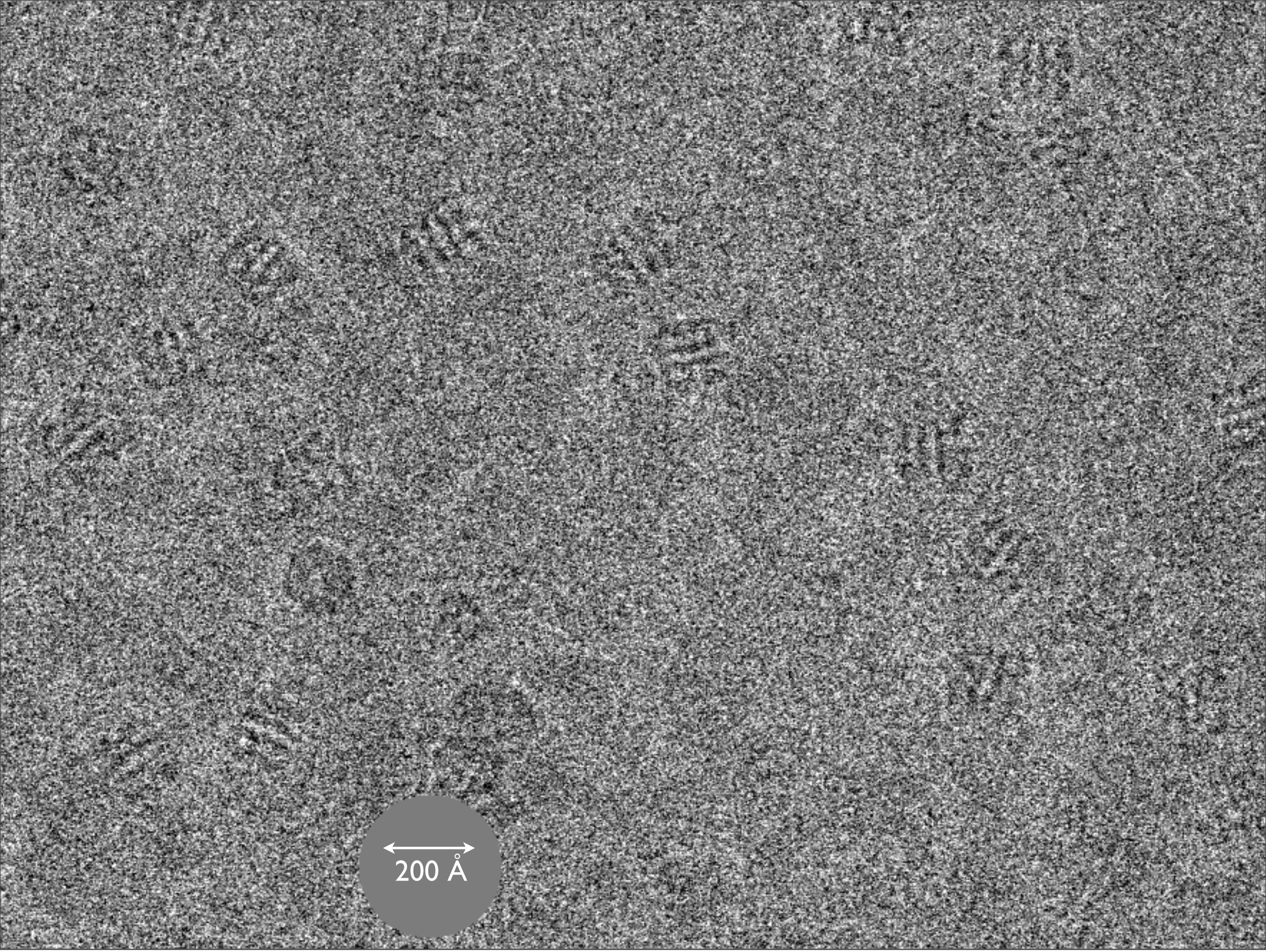
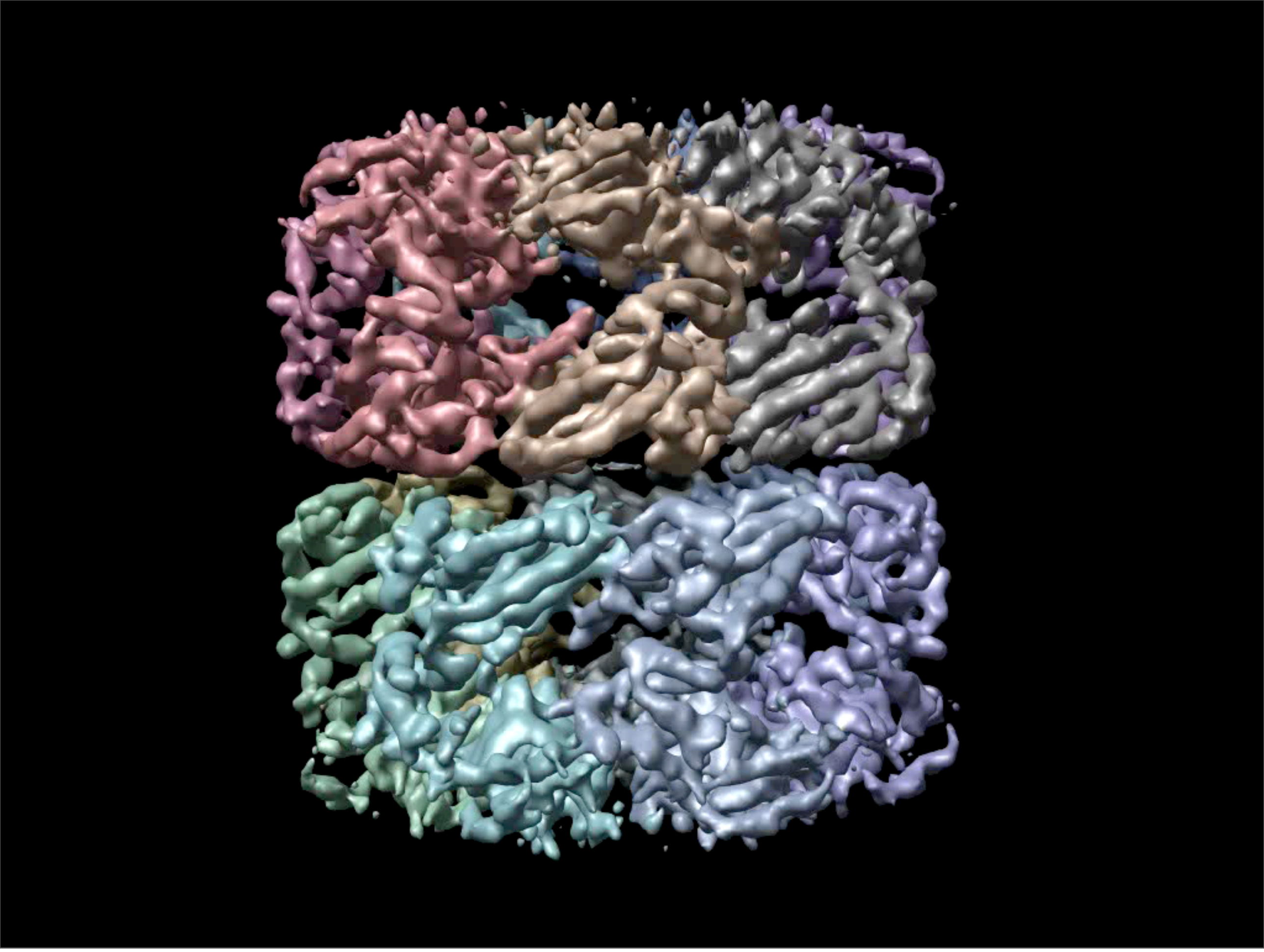

200 Å



Canonical SPA in EMAN

- Image Acquisition
- Particle Picking
- 2-D Analysis
- Symmetry/Low Resolution Model
- Determine CTF Parameters
- High Resolution Refinement
- Post-processing
- Dynamics Analysis

Image Aquisition

- Pick your defocus range
  - Envelope function related to defocus even on modern FEG scopes
  - As close to focus as possible while still able to locate the particles → +1 μm, random values (for EMAN)
    - Focal Pairs ?
- CCD
  - Adequate sampling. For low symmetry, 2/5 Nyquist is a good rule of thumb (res=5*A/pix)
- Film
  - Scanner is important. Bit depth less important than scanner envelope function (e2scannereval.py)

Image Aquisition

- Avoid continuous carbon substrate
- Decreases contrast
- Difficult to separate c-film CTF from specimen
- Makes CTF correction difficult

Particle Picking

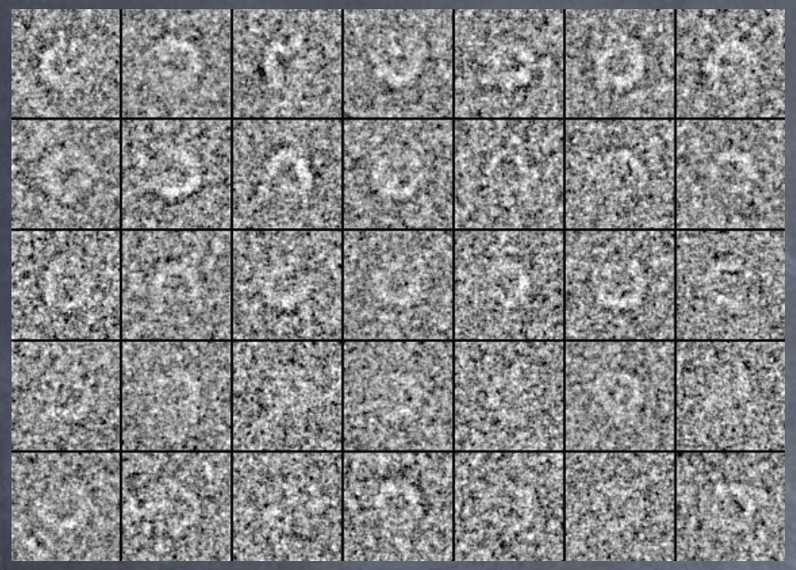
- boxer, batchboxer, helixboxer, e2boxer.py
- Outside software ?
- manual or semi-automated process
- False positives are very dangerous, but also beware of excluding views you weren't expecting
- Mixing microscopes possible, but rarely worthwhile (tomorrow)

2-D Analysis

- Even if you know the quaternary structure, still worthwhile. May be surprises.
- At least 1000-2000 particles with uniform orientation distribution, perhaps fewer if symmetry or preferred orientation
- Look for dynamics or degradation
- 'shrink' particles for speed
- # classes ≤ # particles/20
- refine2d.py **not** startnrclasses

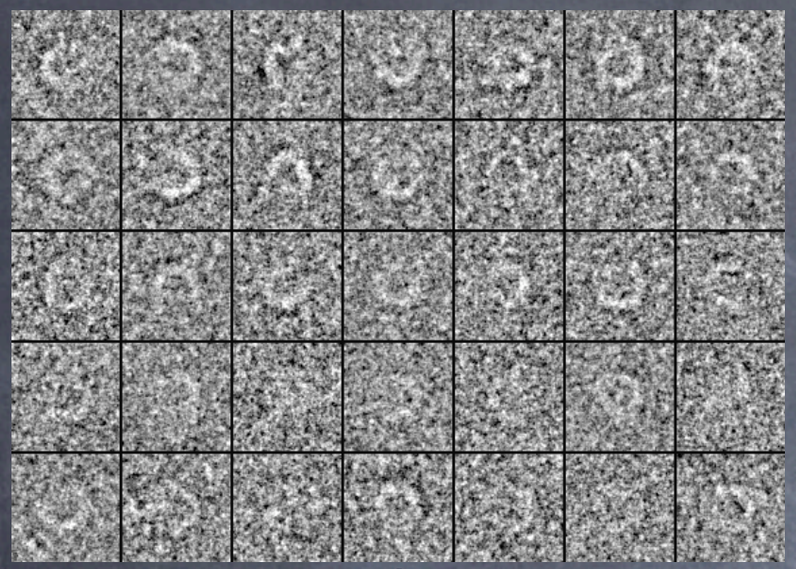
2-D Refinement

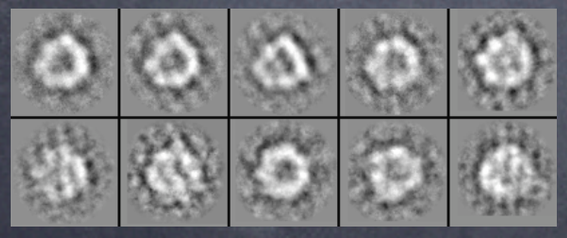
Particles



2-D Refinement

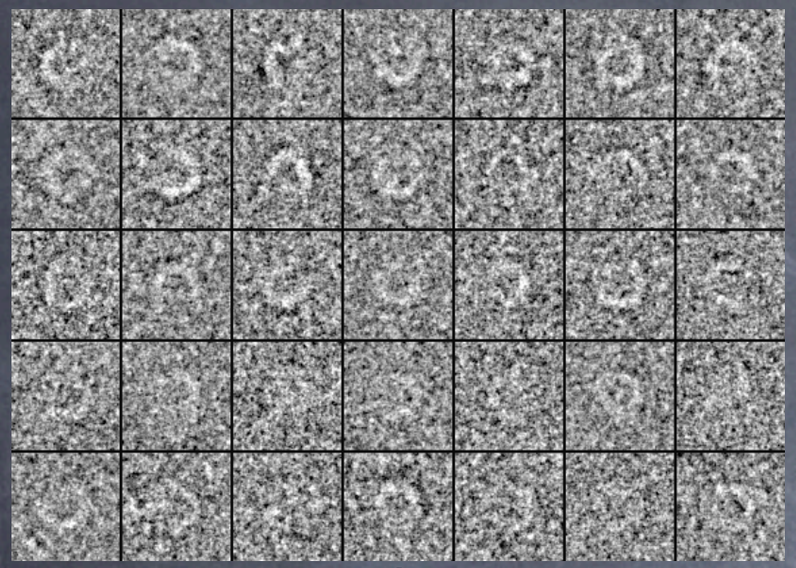
Particles



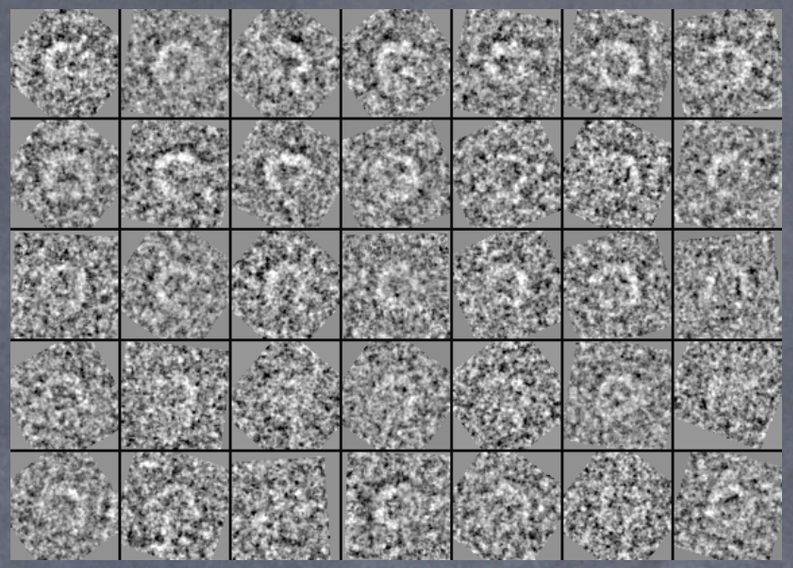


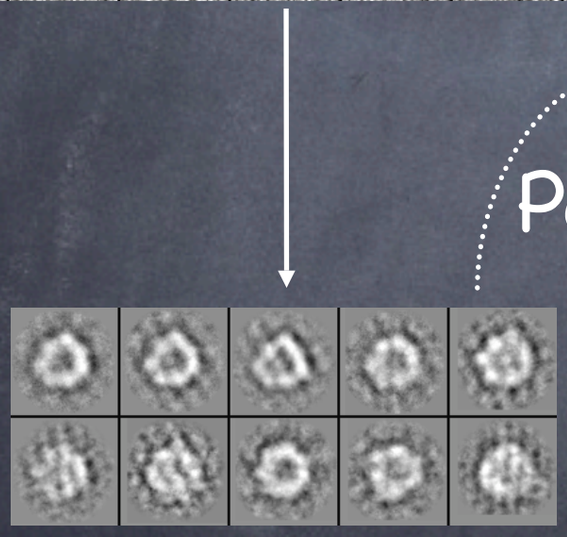
2-D Refinement

Particles



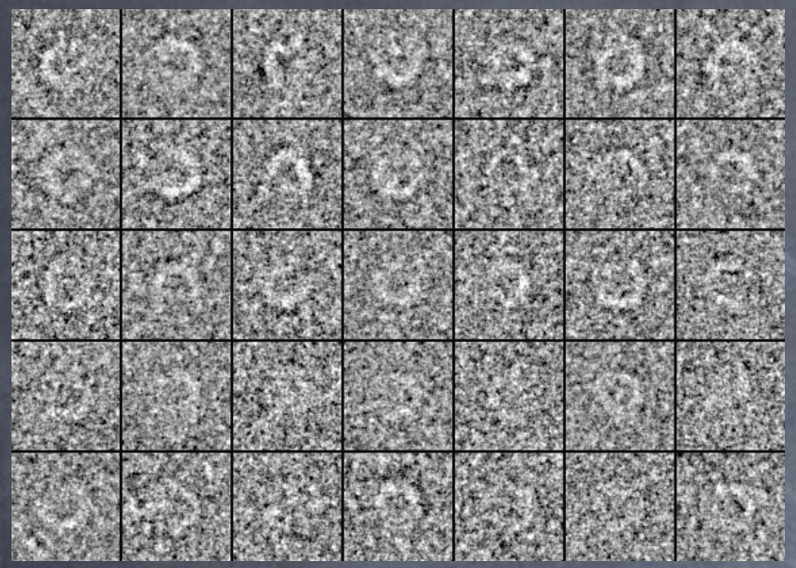
Align Particles



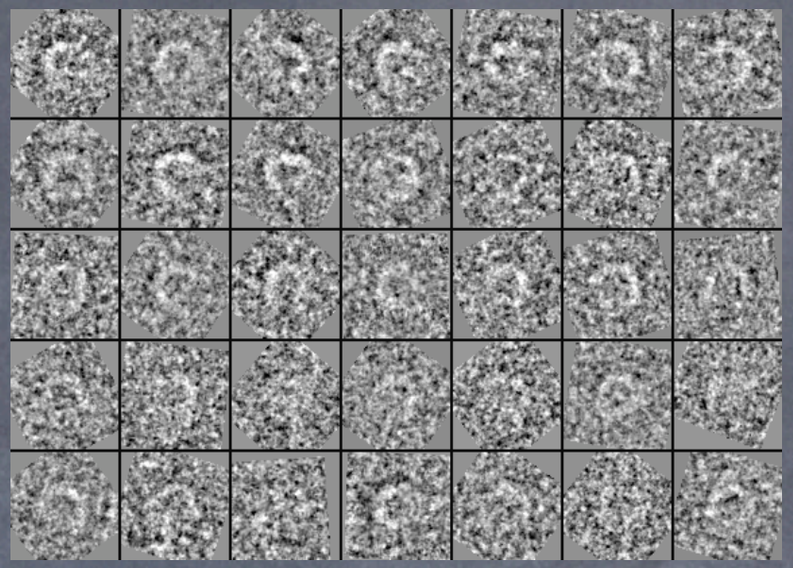


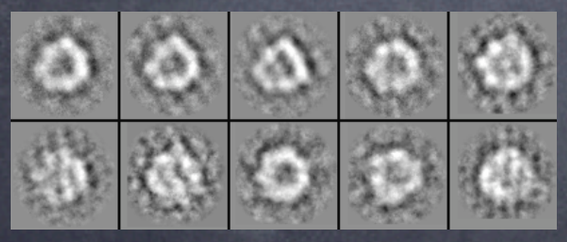
2-D Refinement

Particles

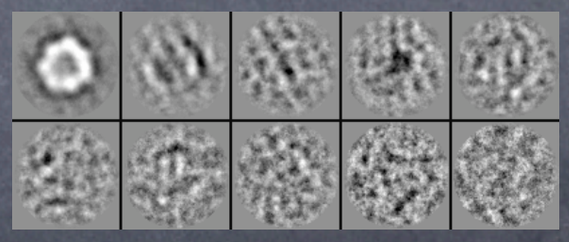


Align Particles



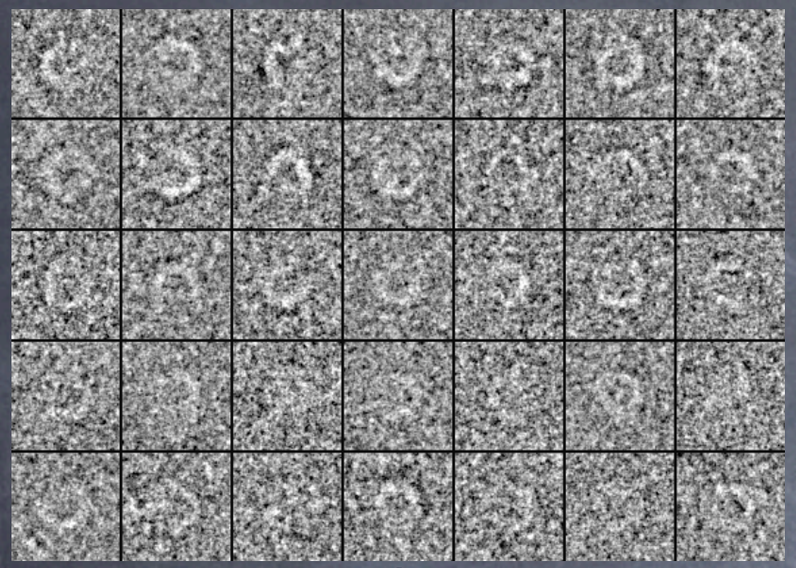


SVD/PCA

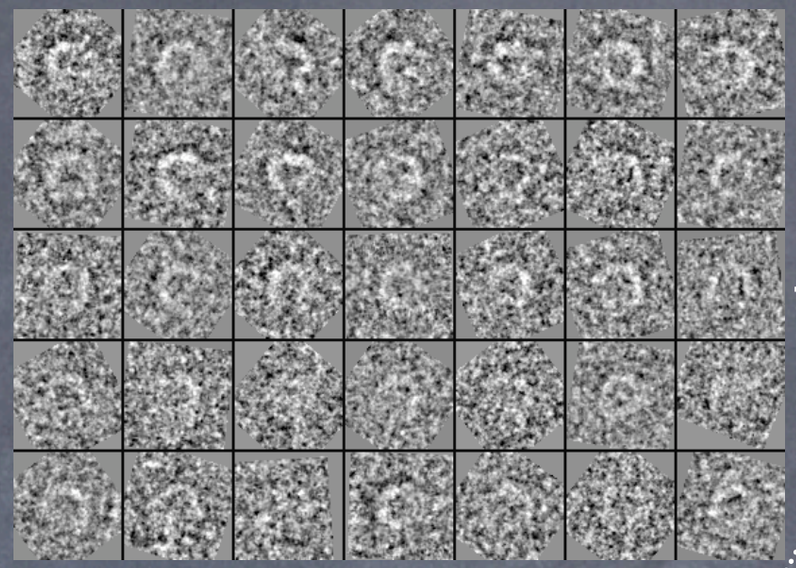


2-D Refinement

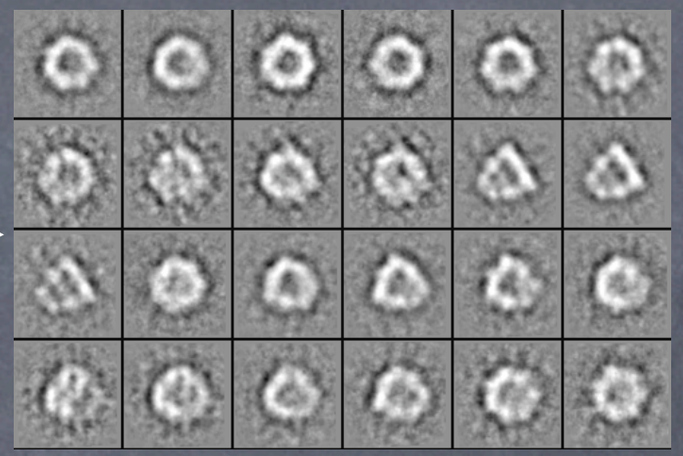
Particles

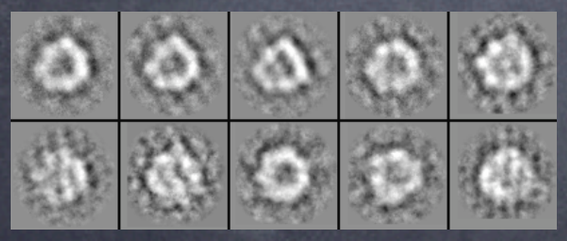


Align Particles

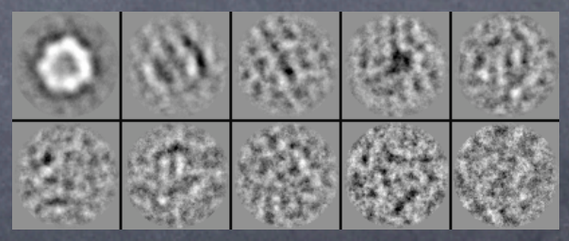


k-means classification in SVD subspace



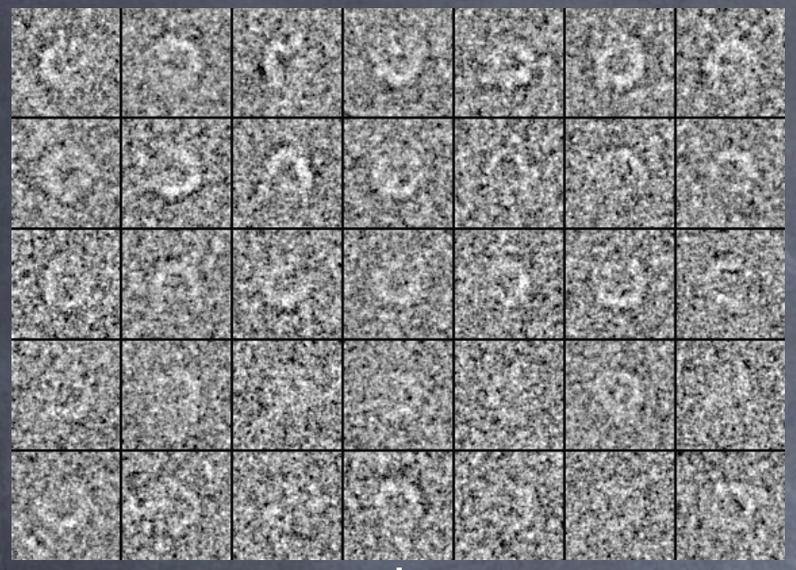


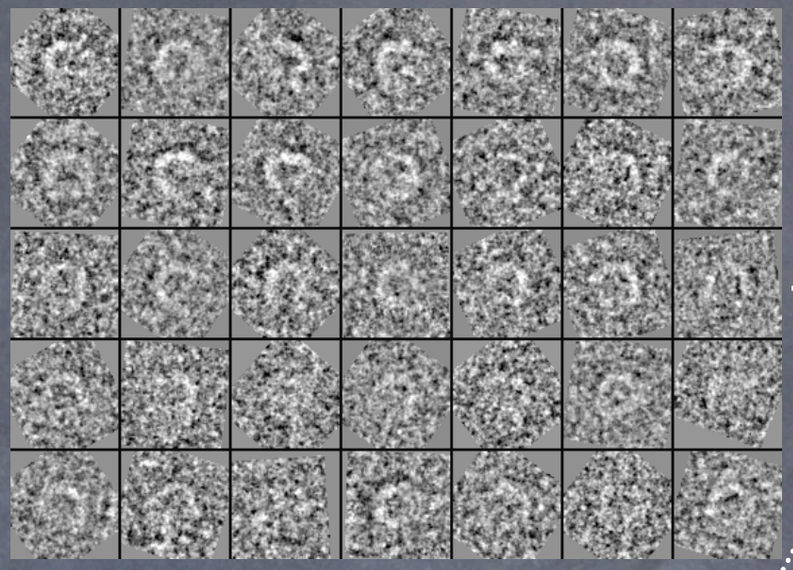
SVD/PCA

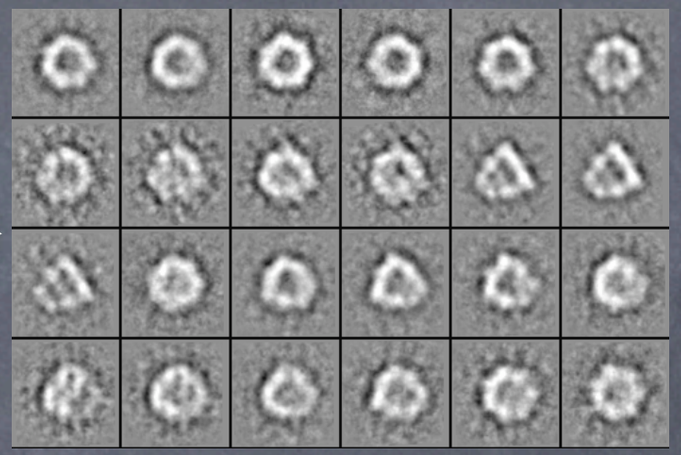


2-D Refinement

Particles

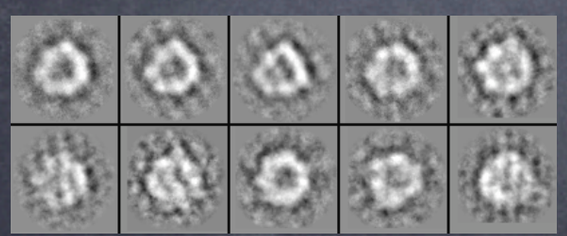




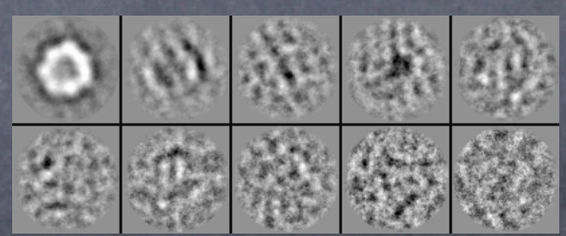


k-means classification in SVD subspace

Align Particles

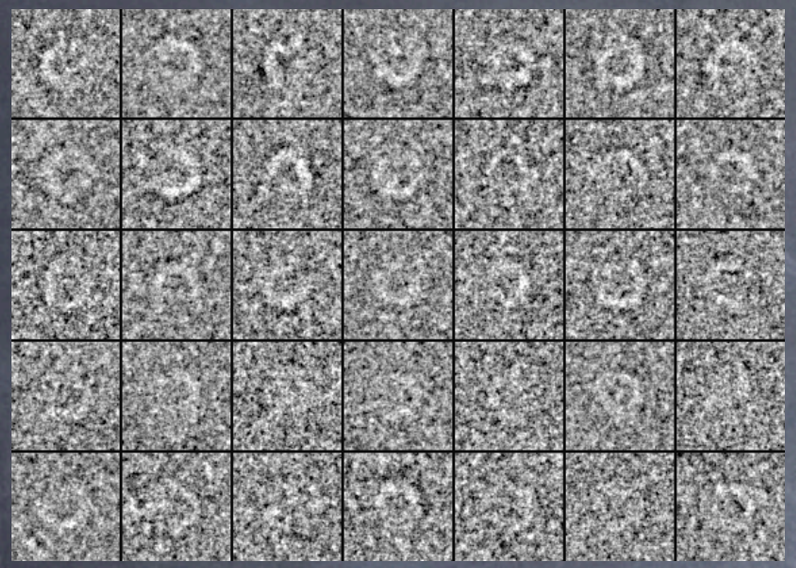


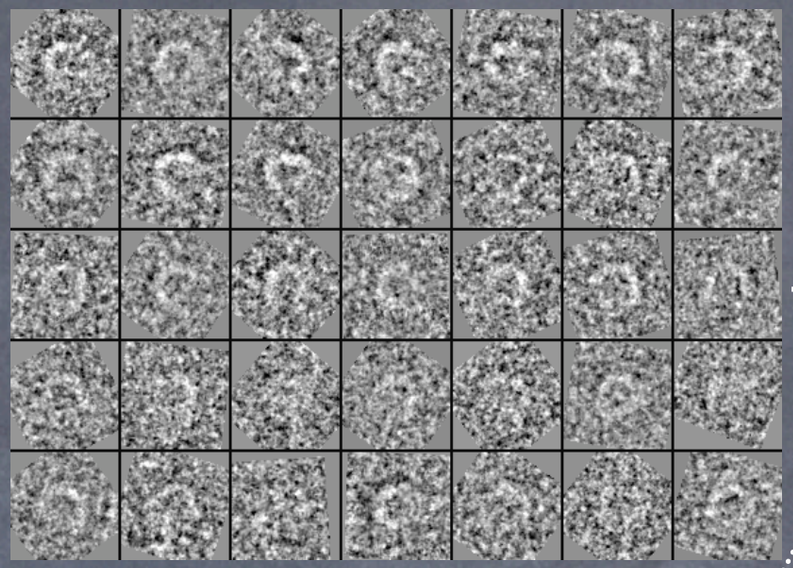
SVD/PCA

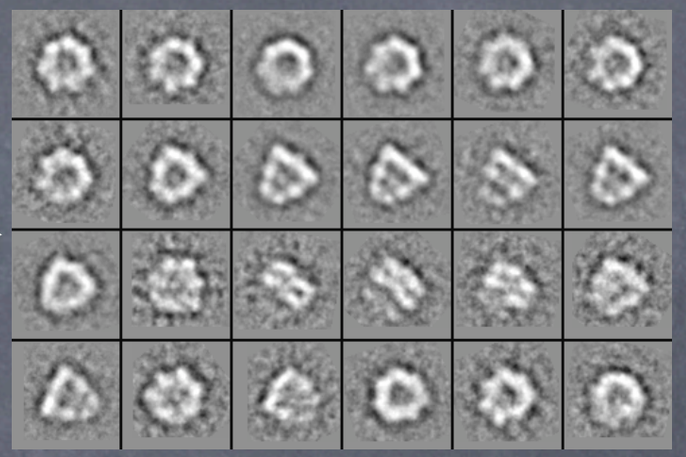


2-D Refinement

Particles

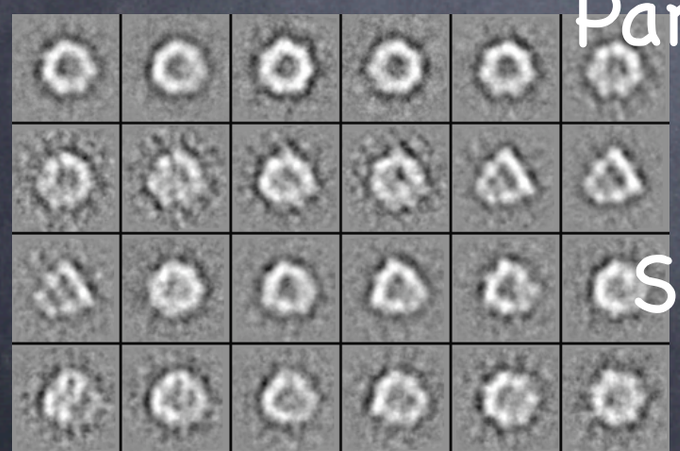




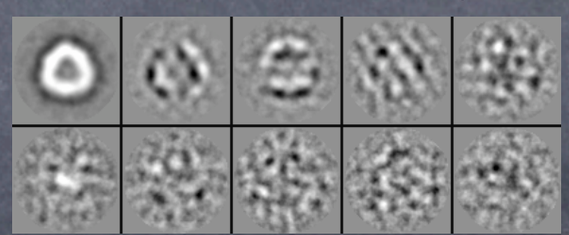


k-means classification in SVD subspace

Align Particles

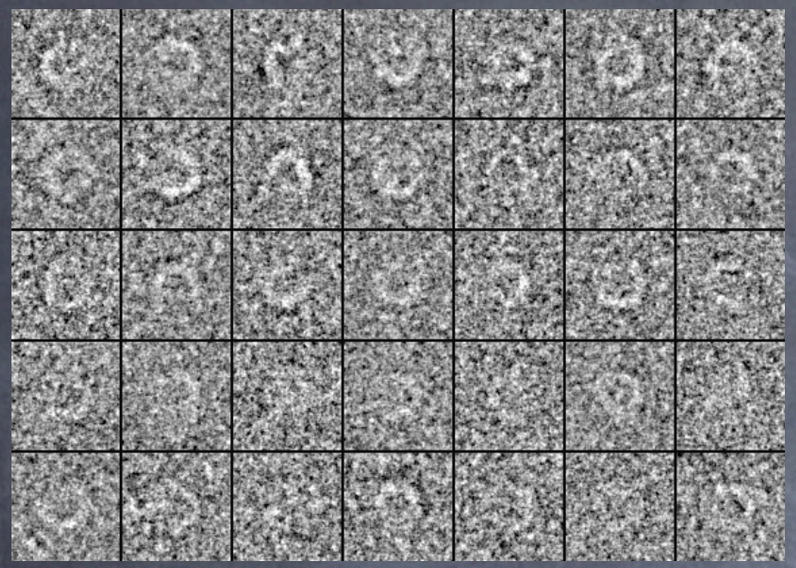


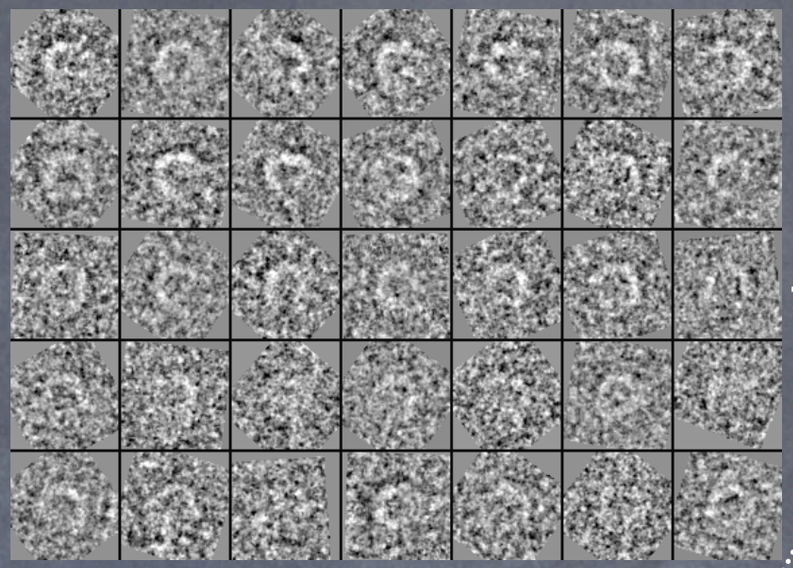
SVD/PCA

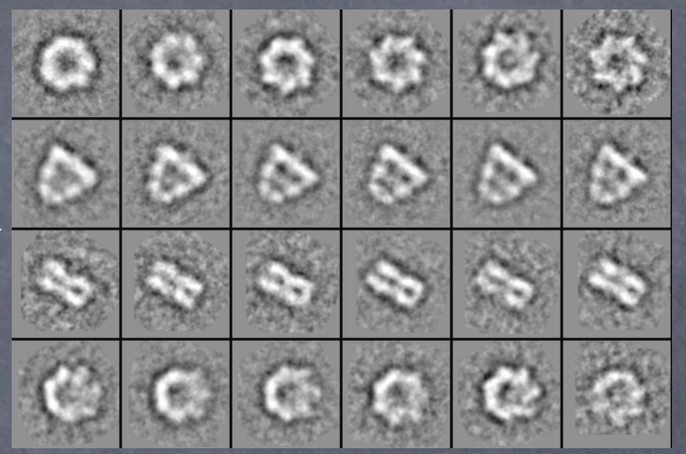


2-D Refinement

Particles

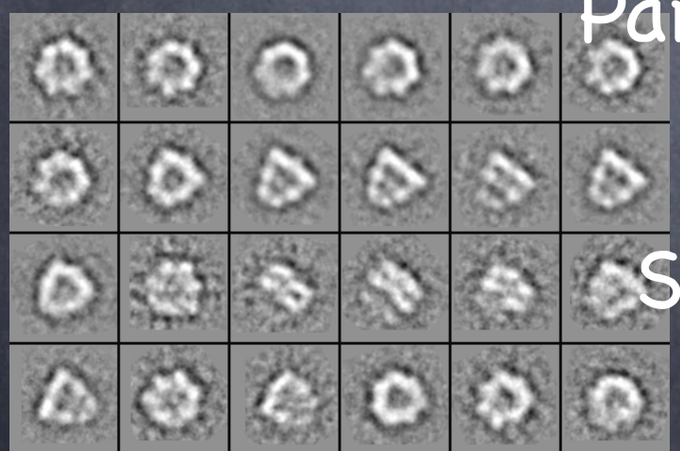




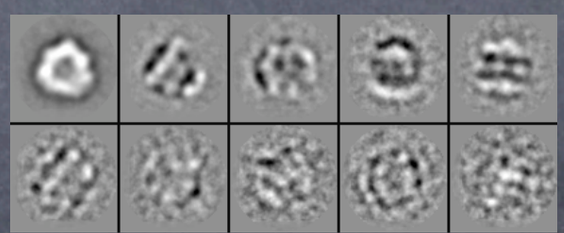


k-means classification in SVD subspace

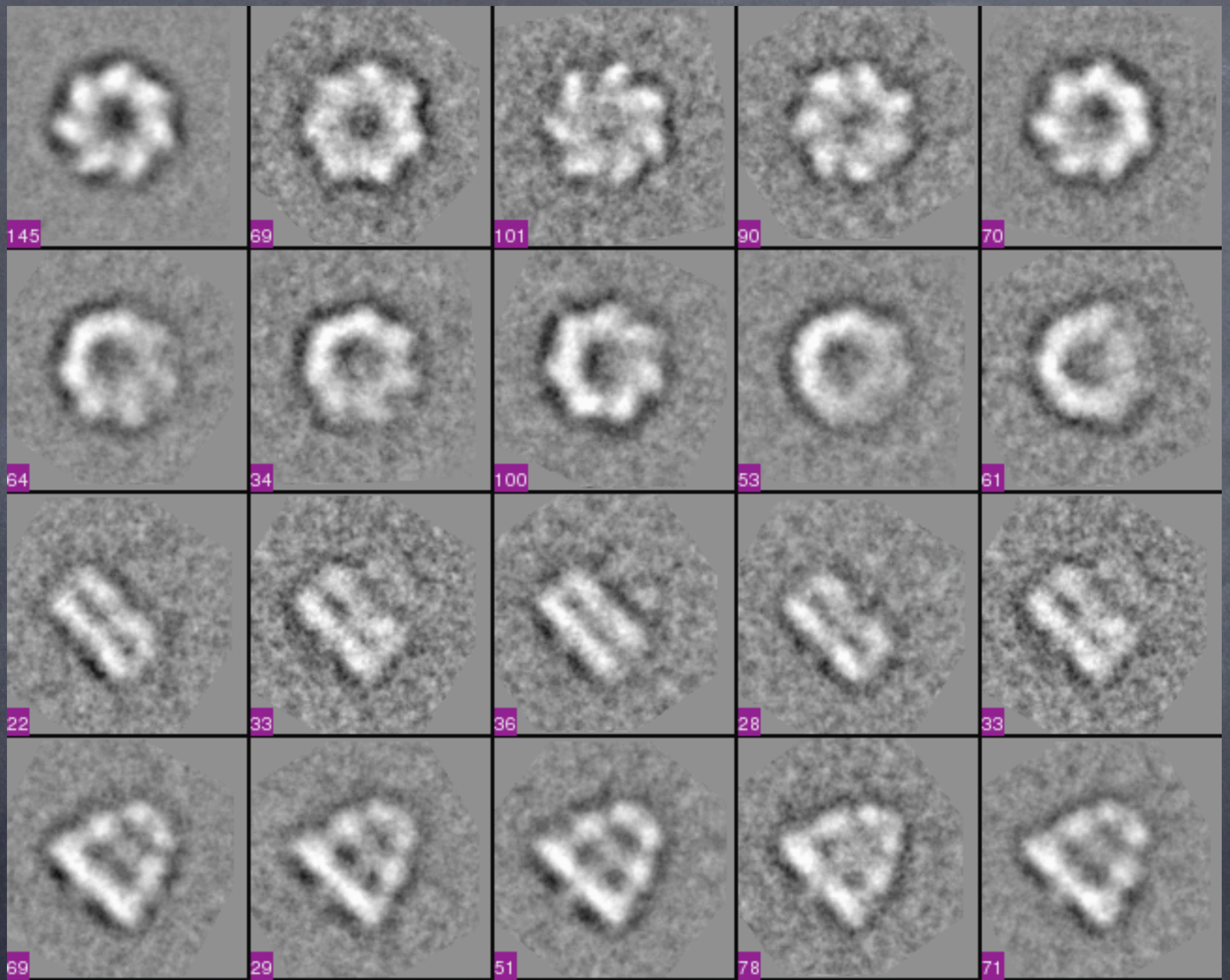
Align Particles



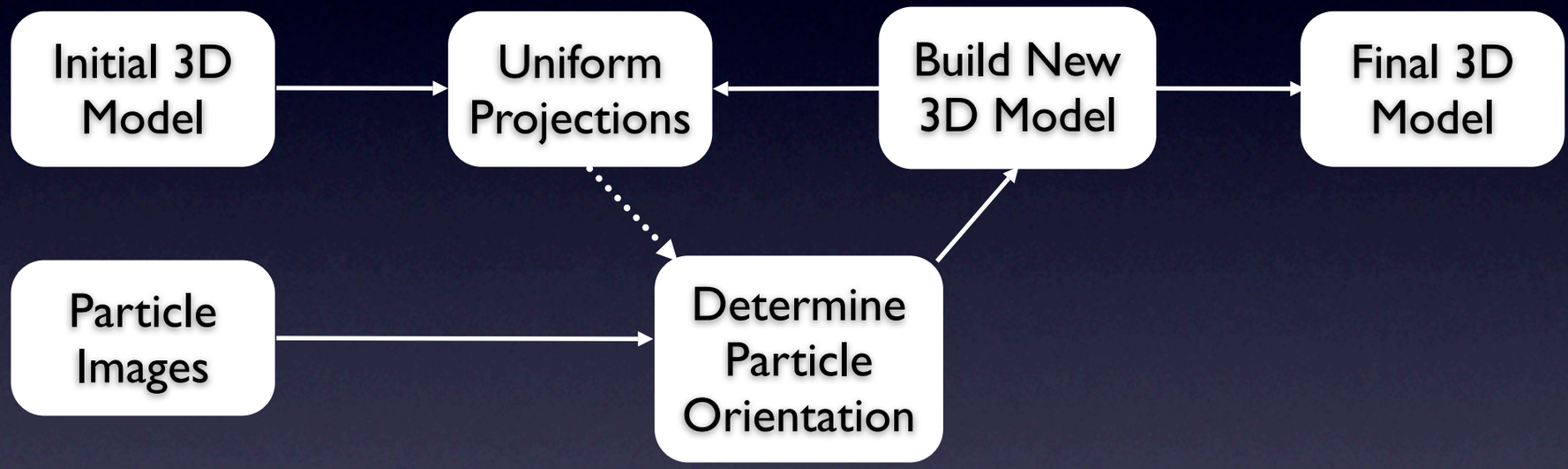
SVD/PCA

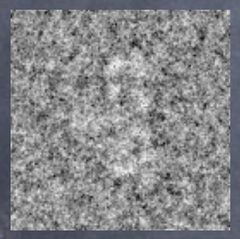


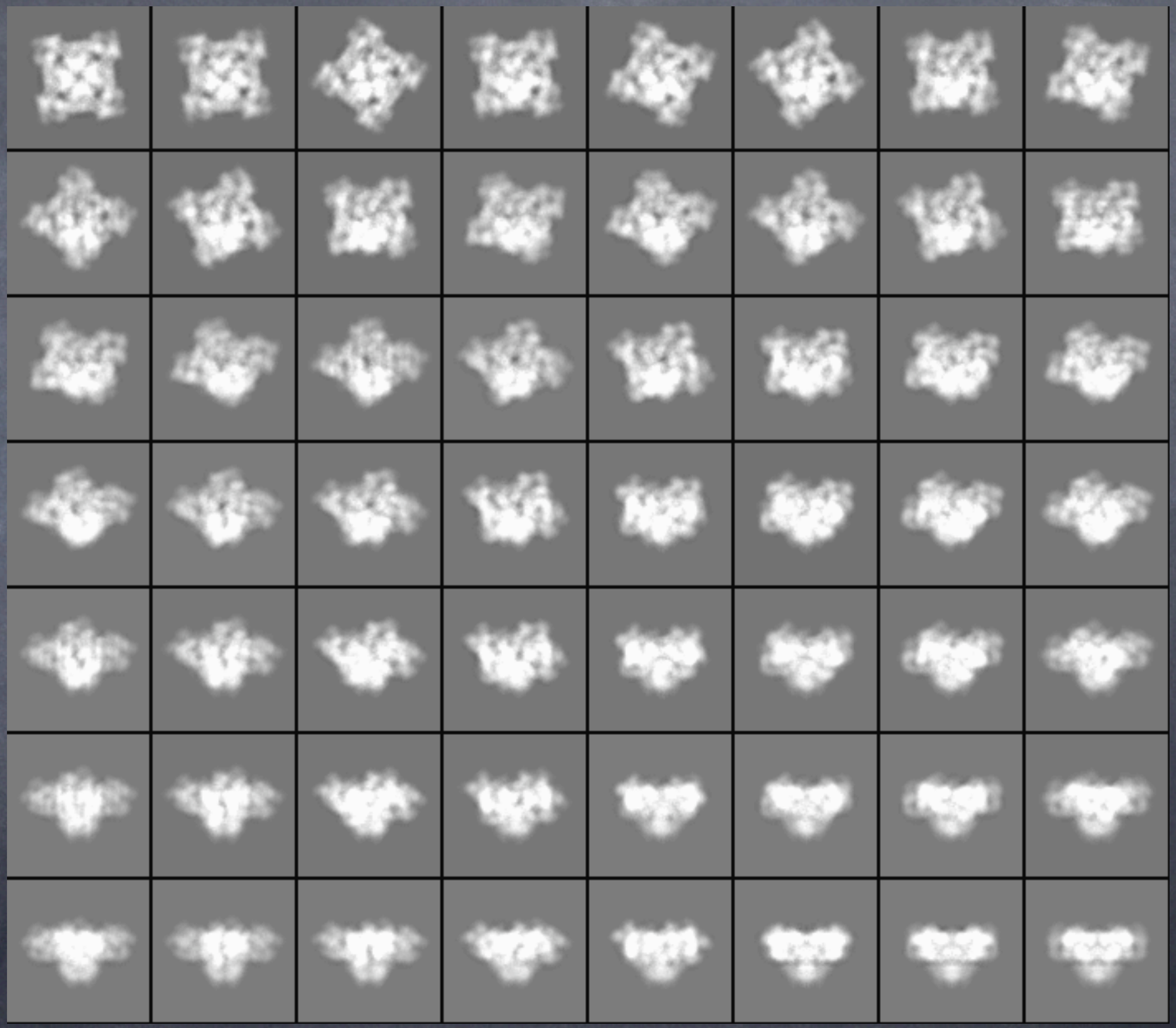
After 9 iterations


145
69
101
90
70
64
34
100
53
61
22
33
36
28
33
69
29
51
78
71
100 Å

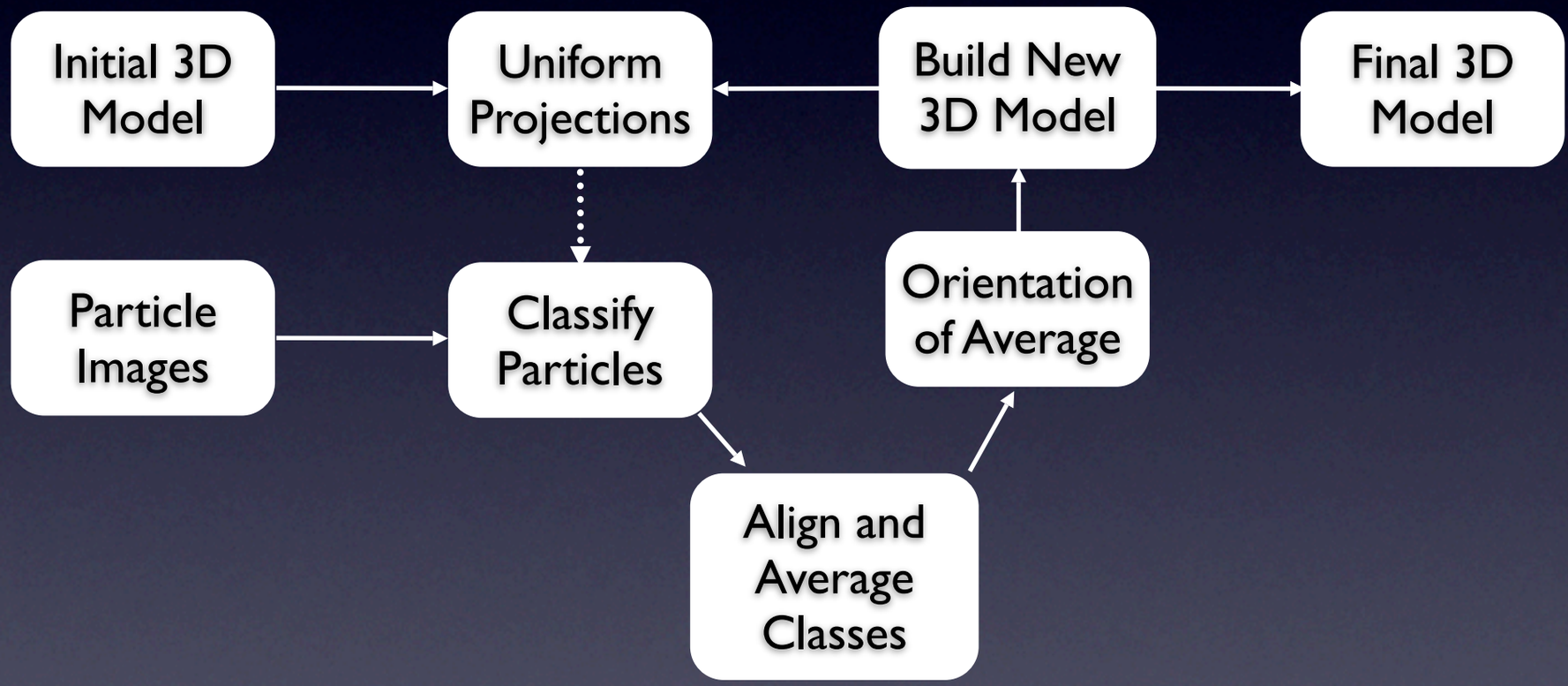
3-D Refinement
(Typical) Spider Approach



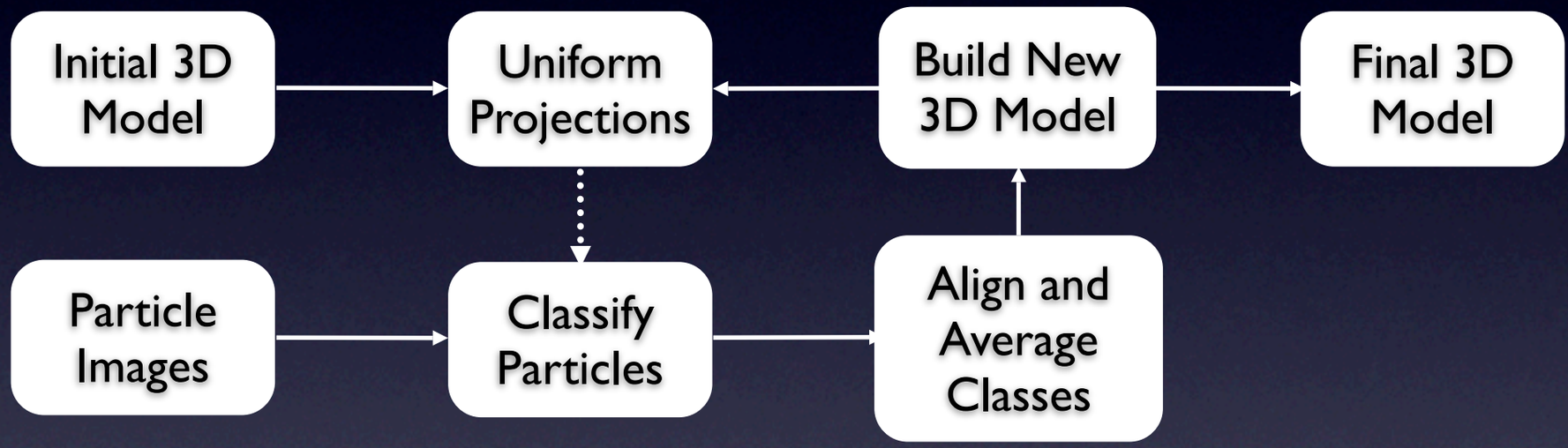


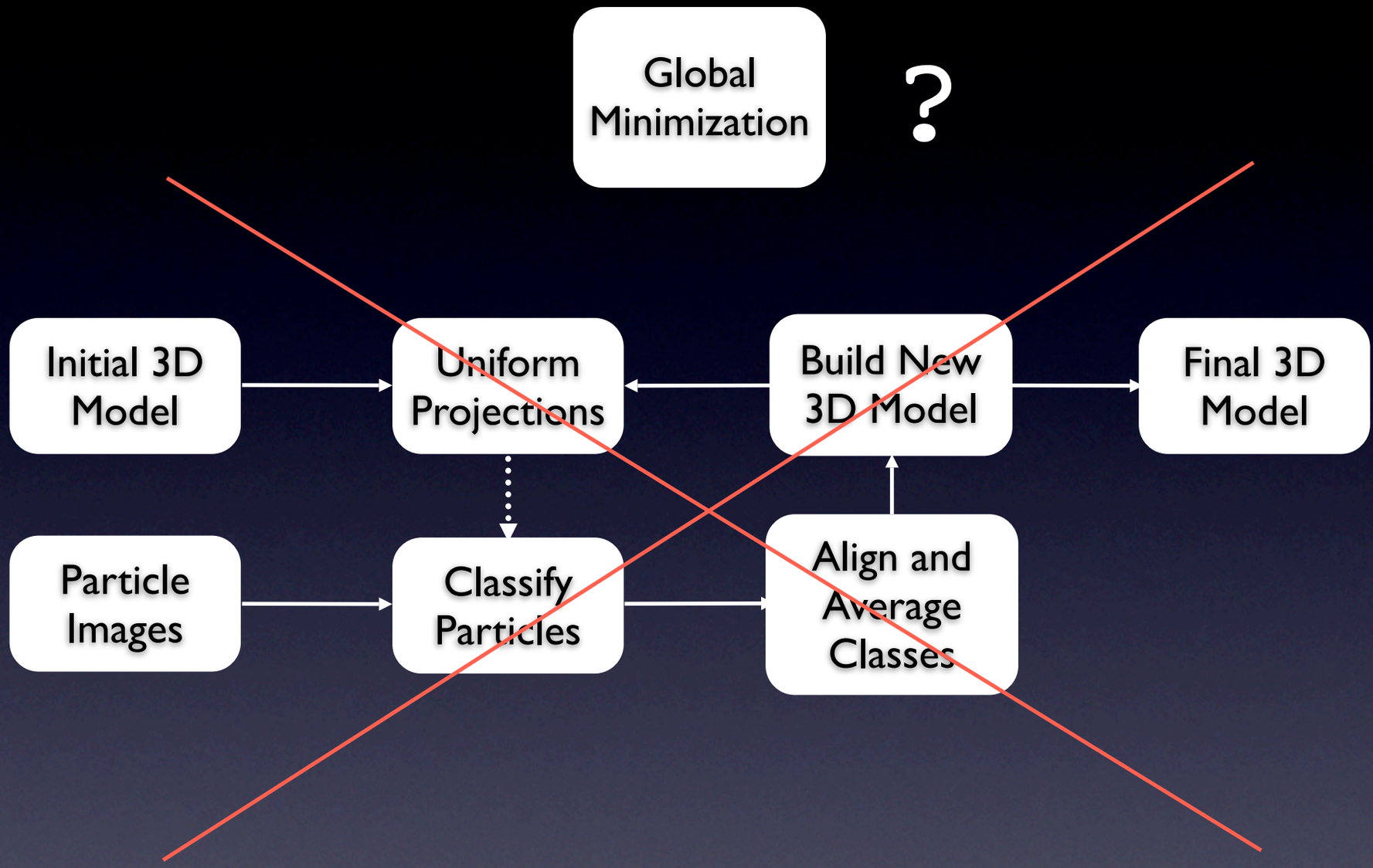


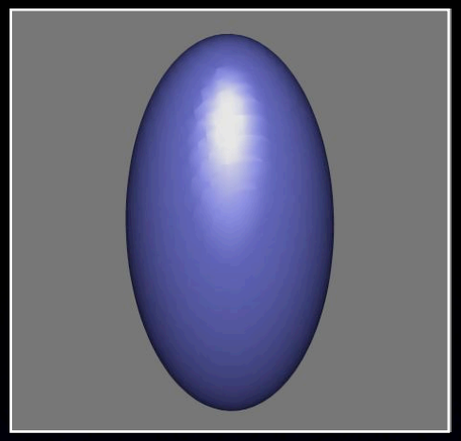
3-D Refinement
(Typical) Imagic Approach

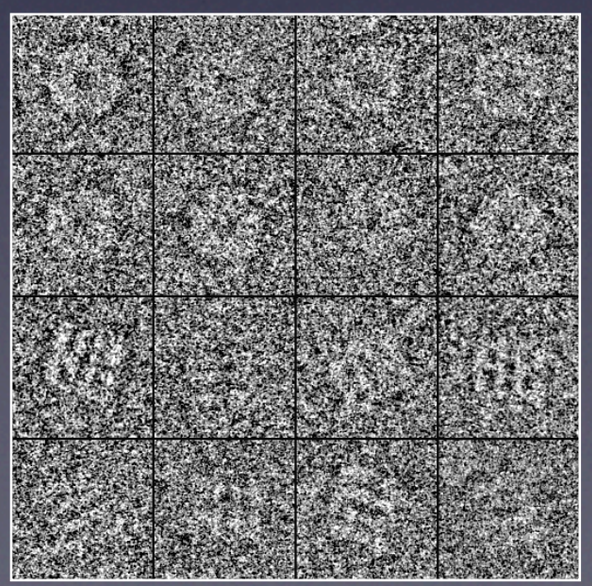


3-D Refinement
EMAN Approach




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





Initial 3D Model

Uniform Projections

Build New 3D Model

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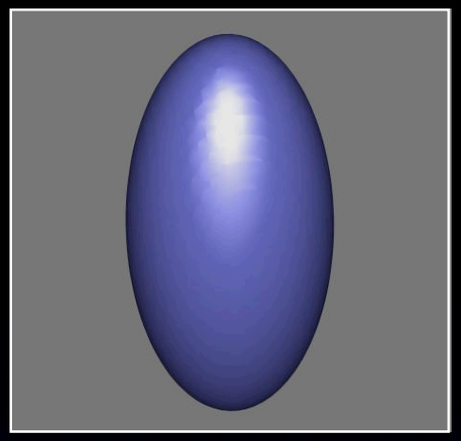

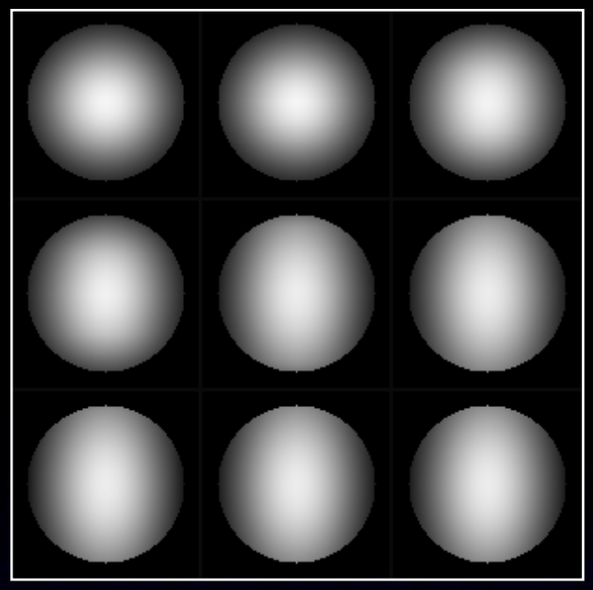

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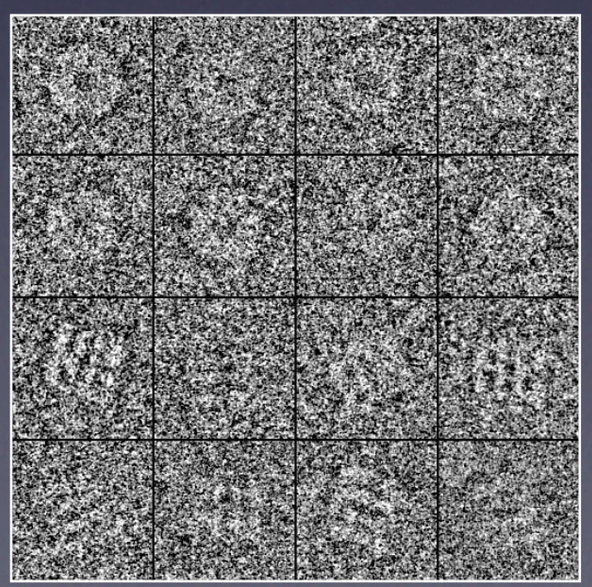

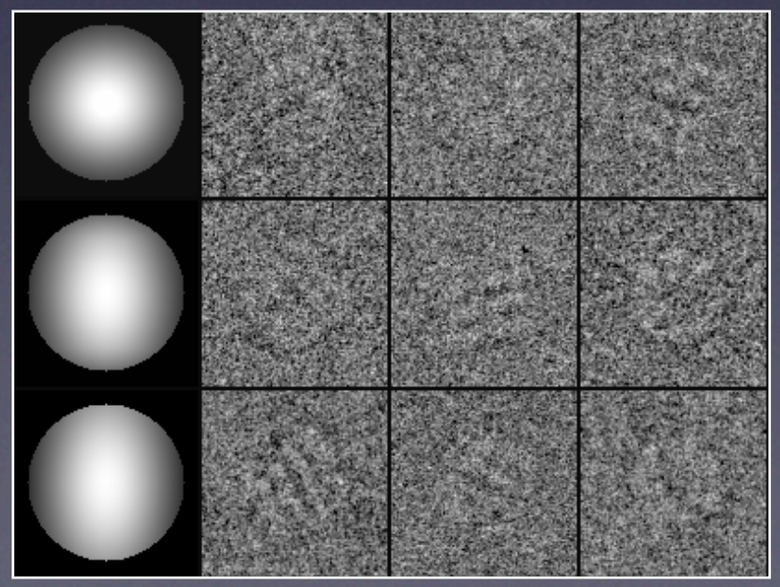

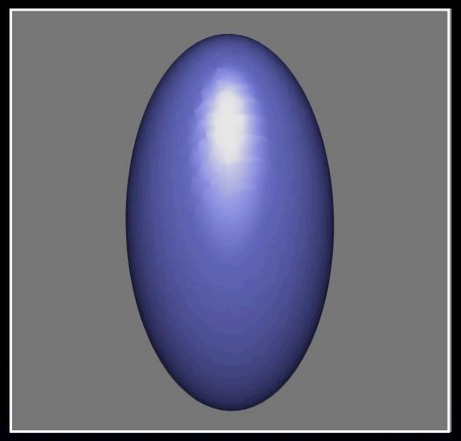

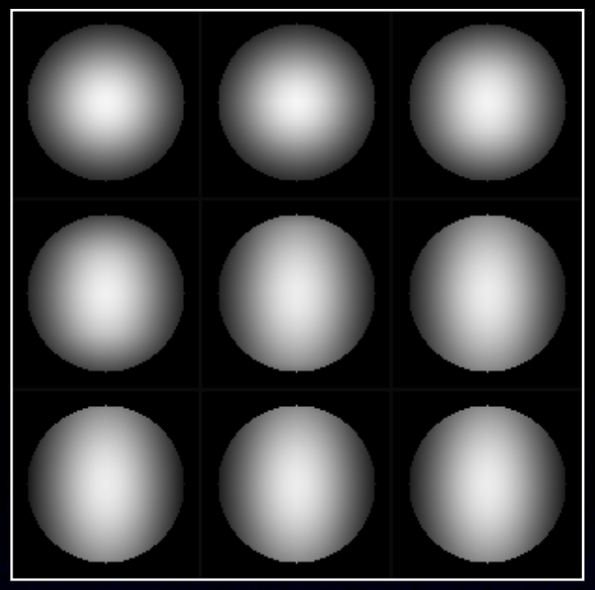

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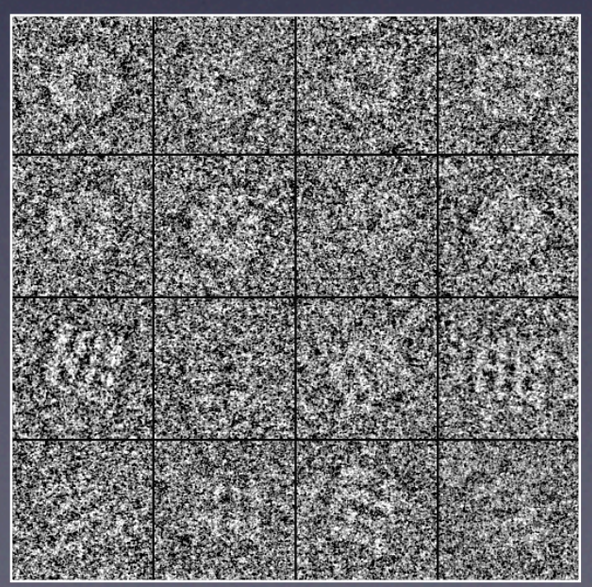

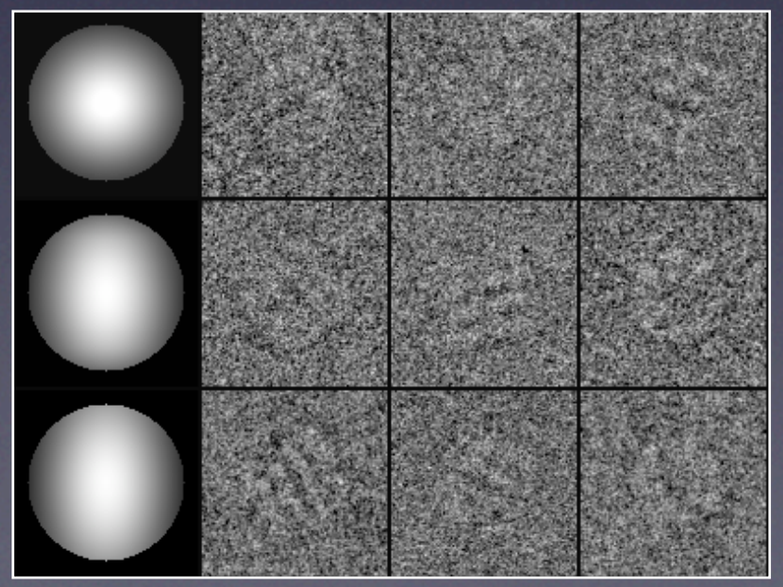

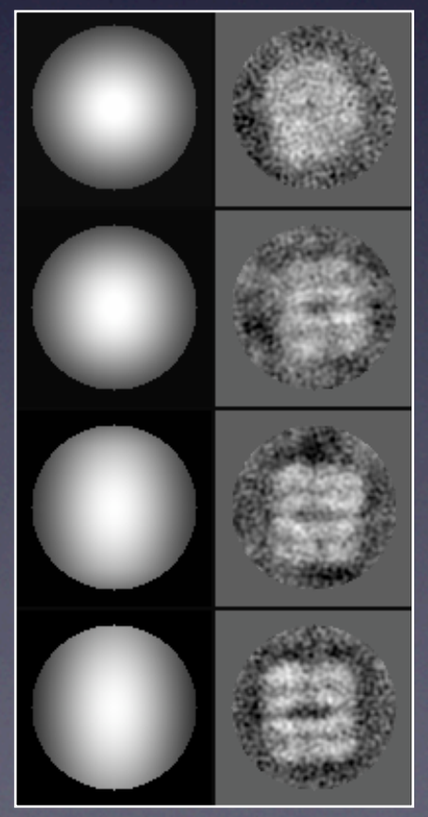

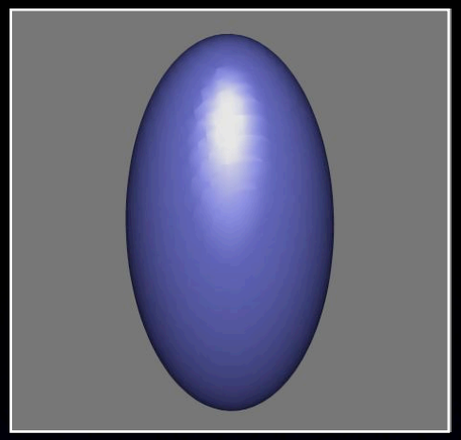

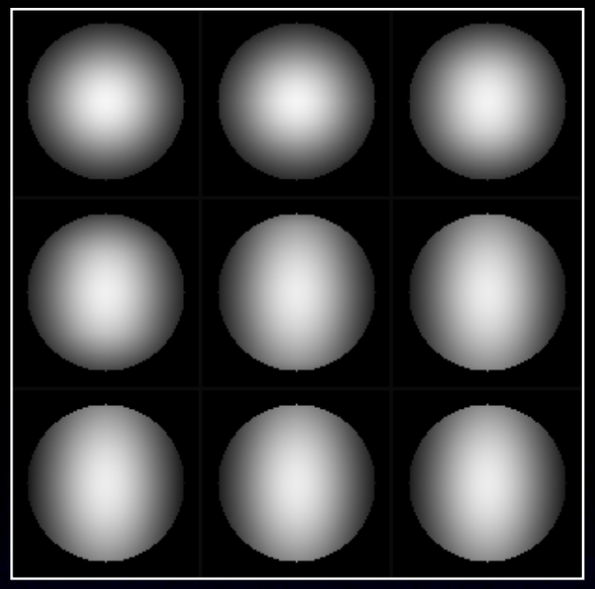

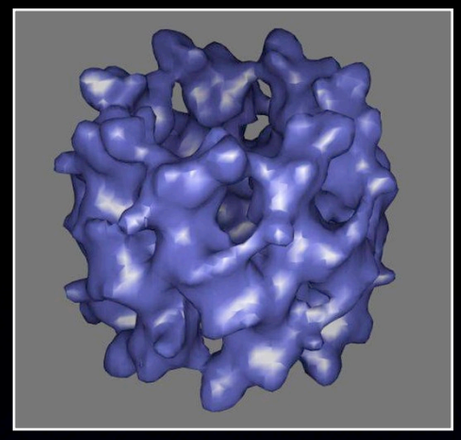

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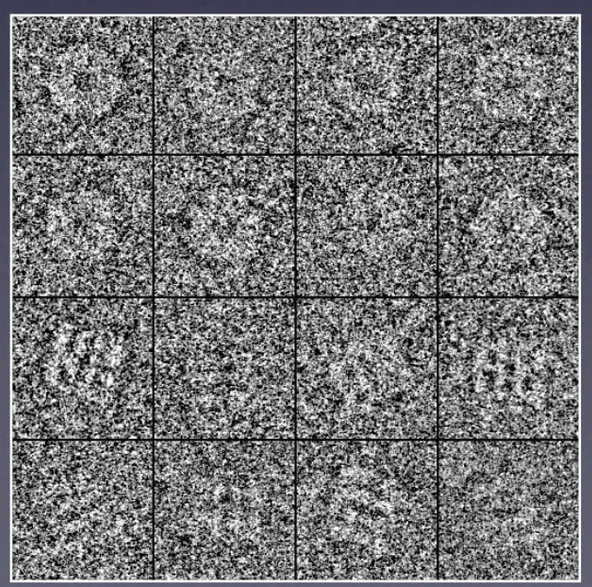
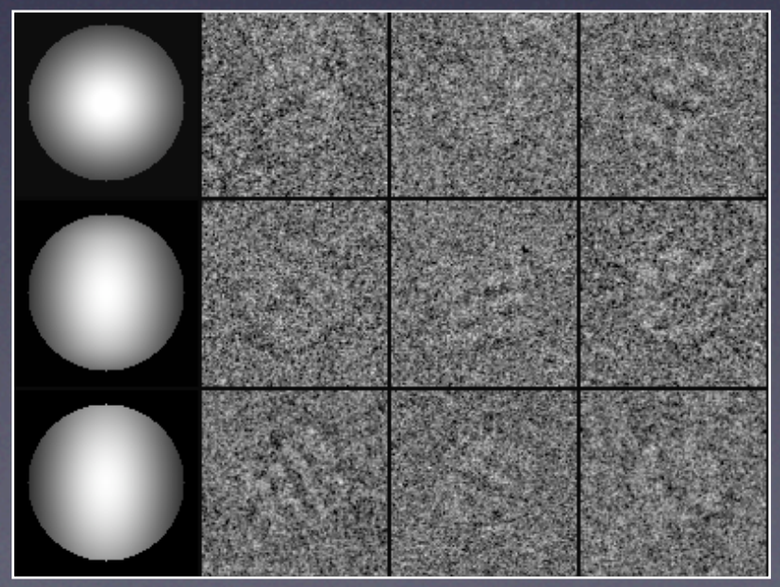
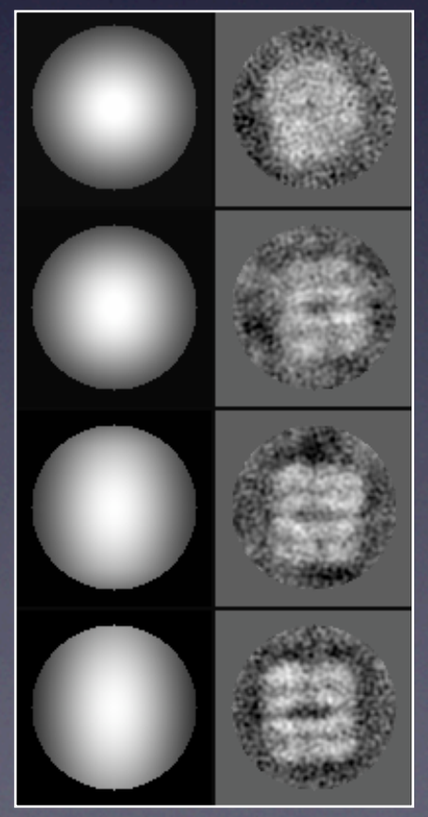
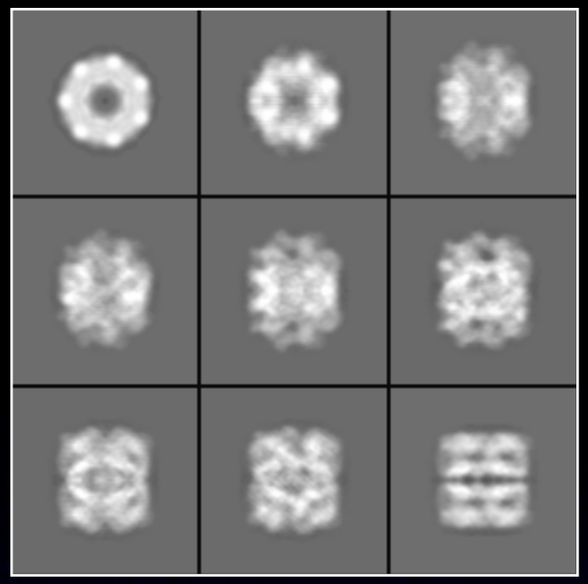
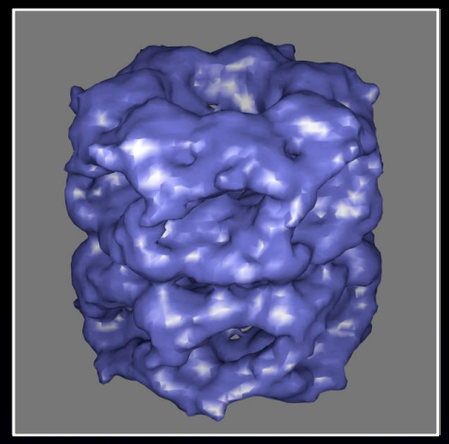
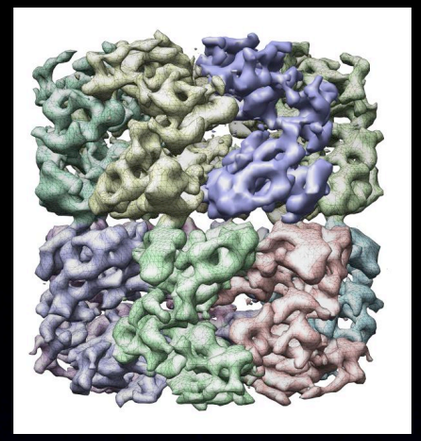












Initial 3D Model

Uniform Projections

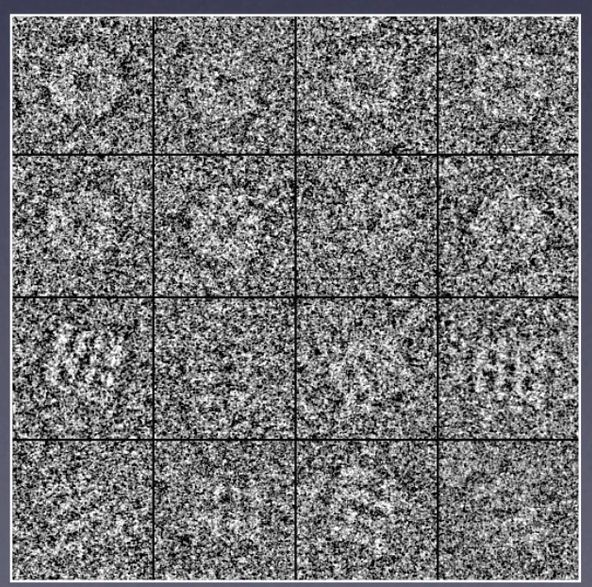
Build New 3D Model

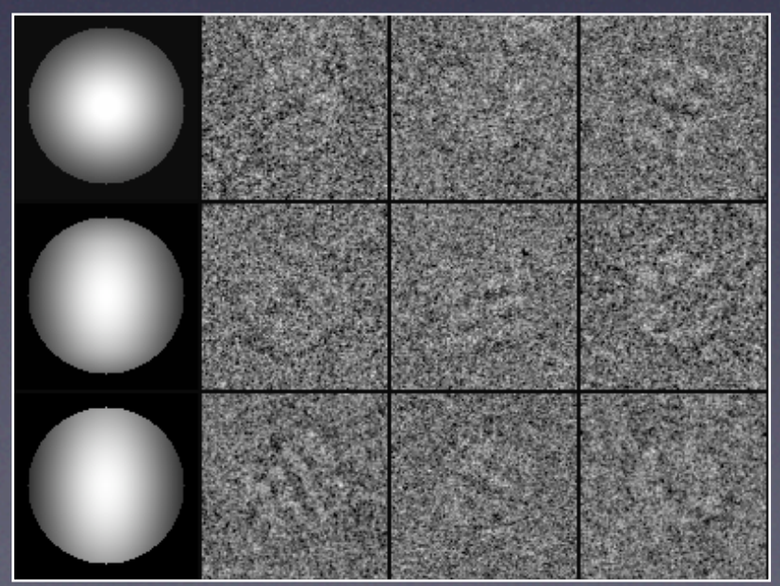
Final 3D Model

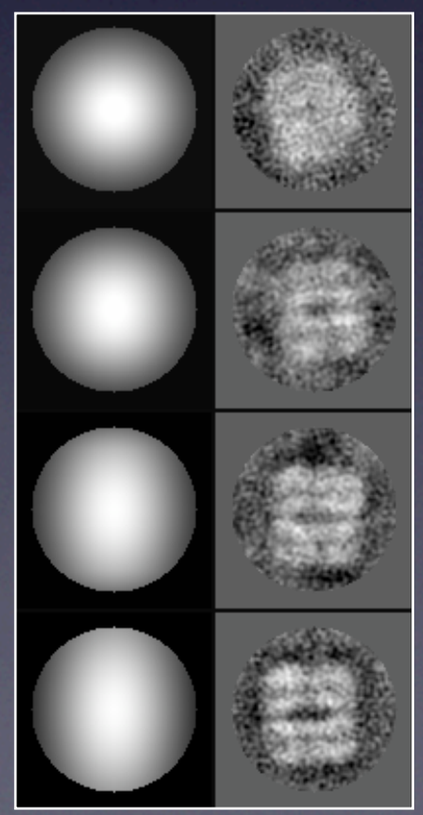
Particle Images

Classify Particles

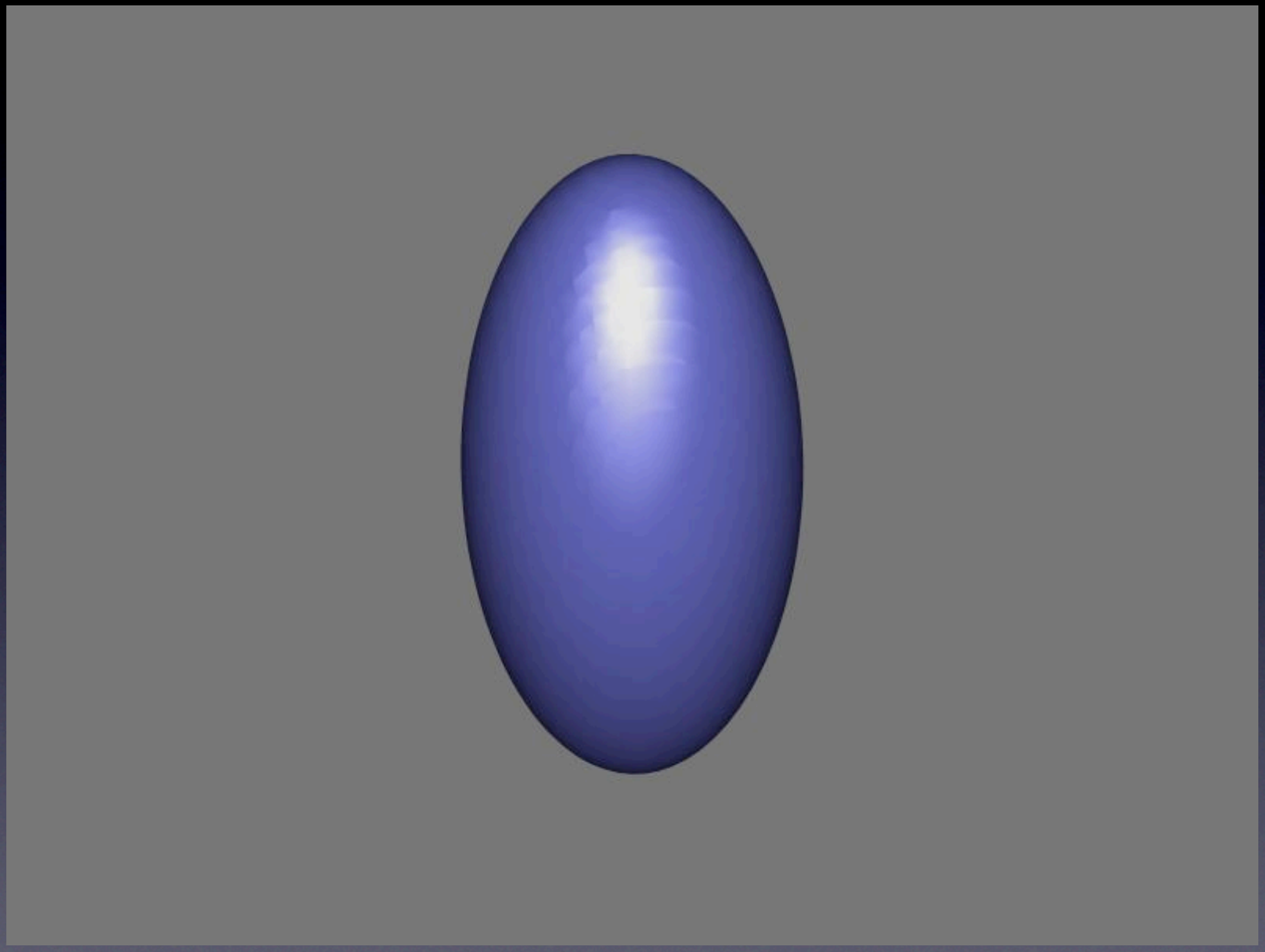
Align and Average Classes



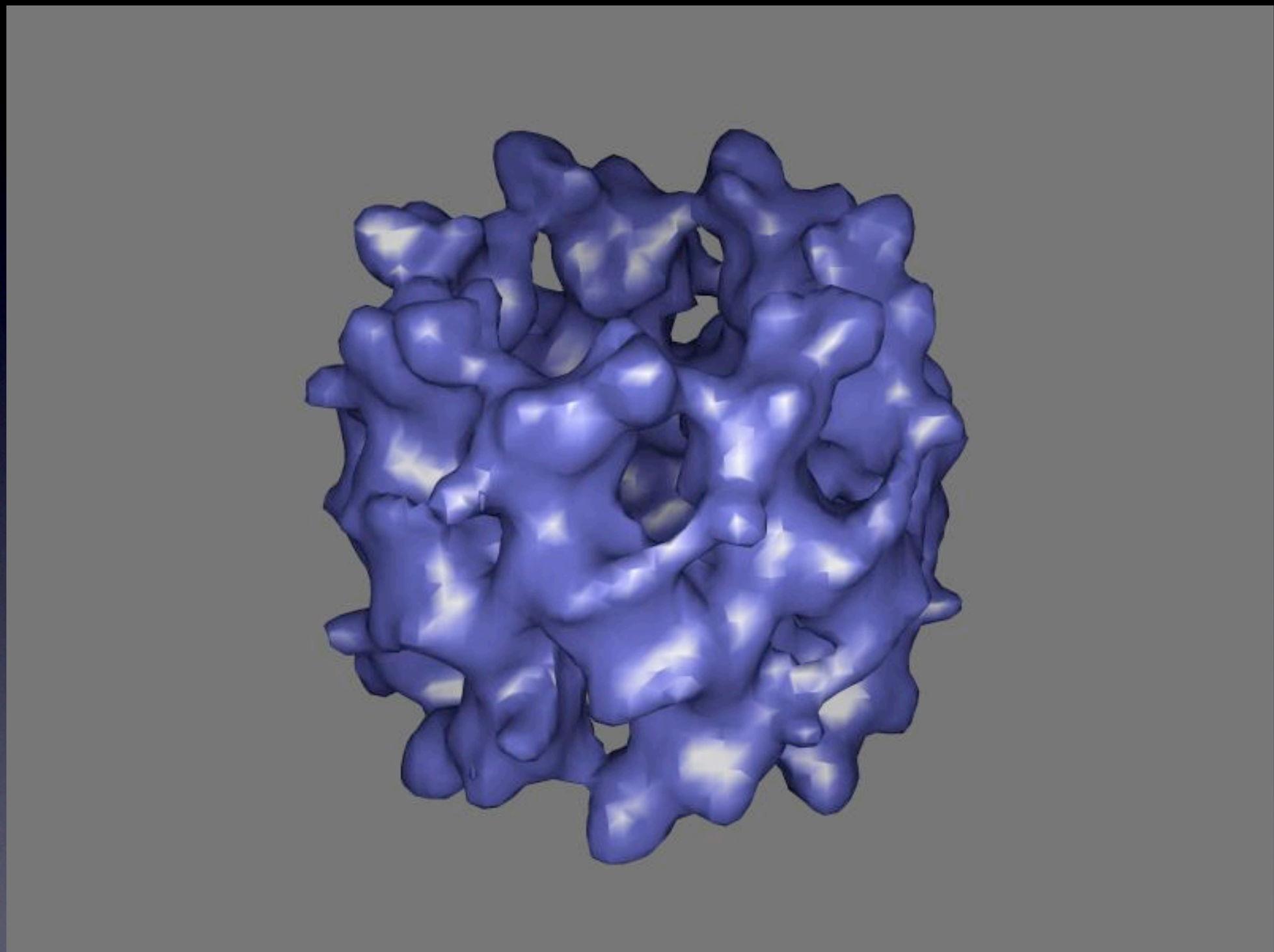




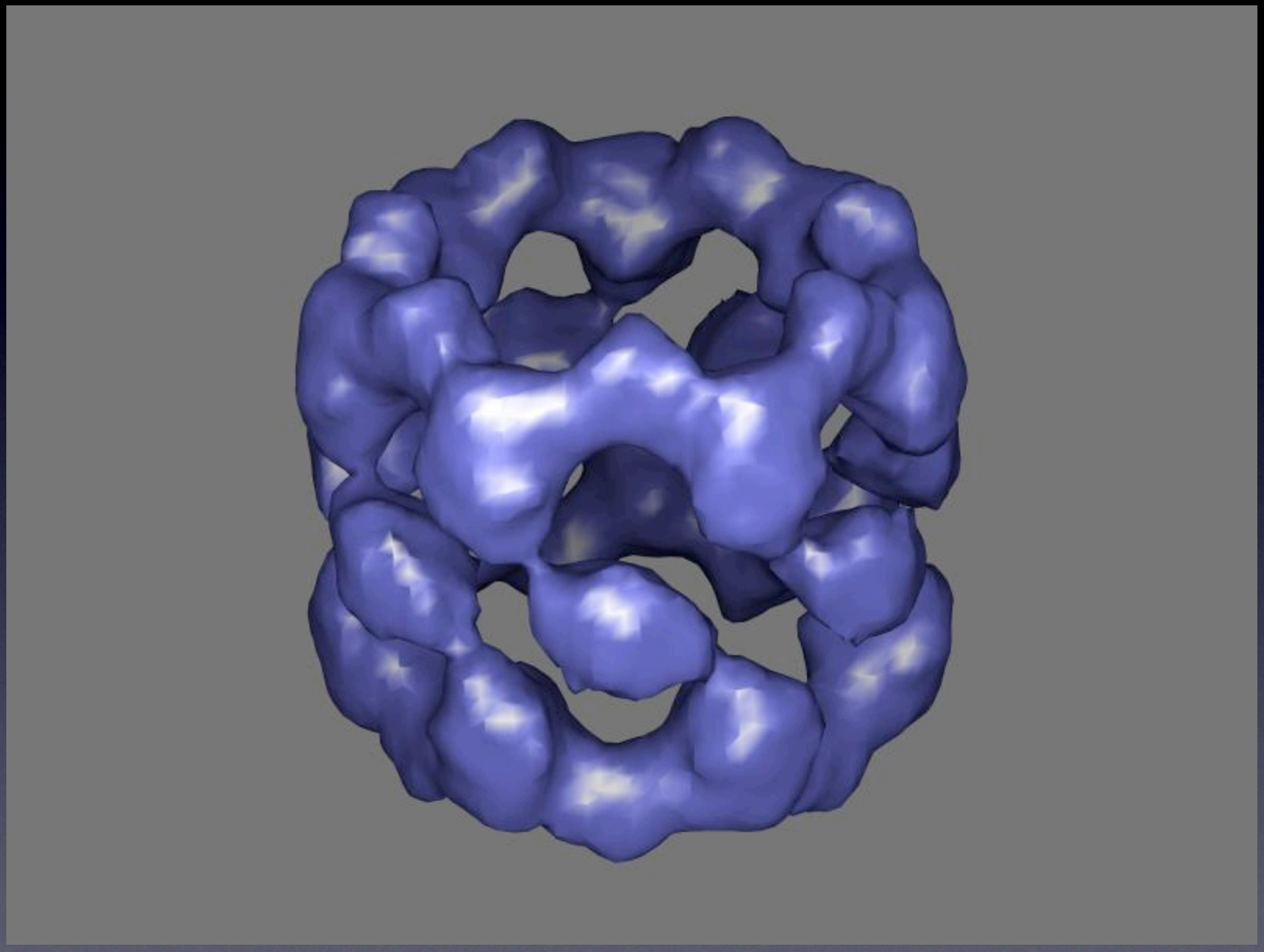
Refine from Gaussian Ellipsoid



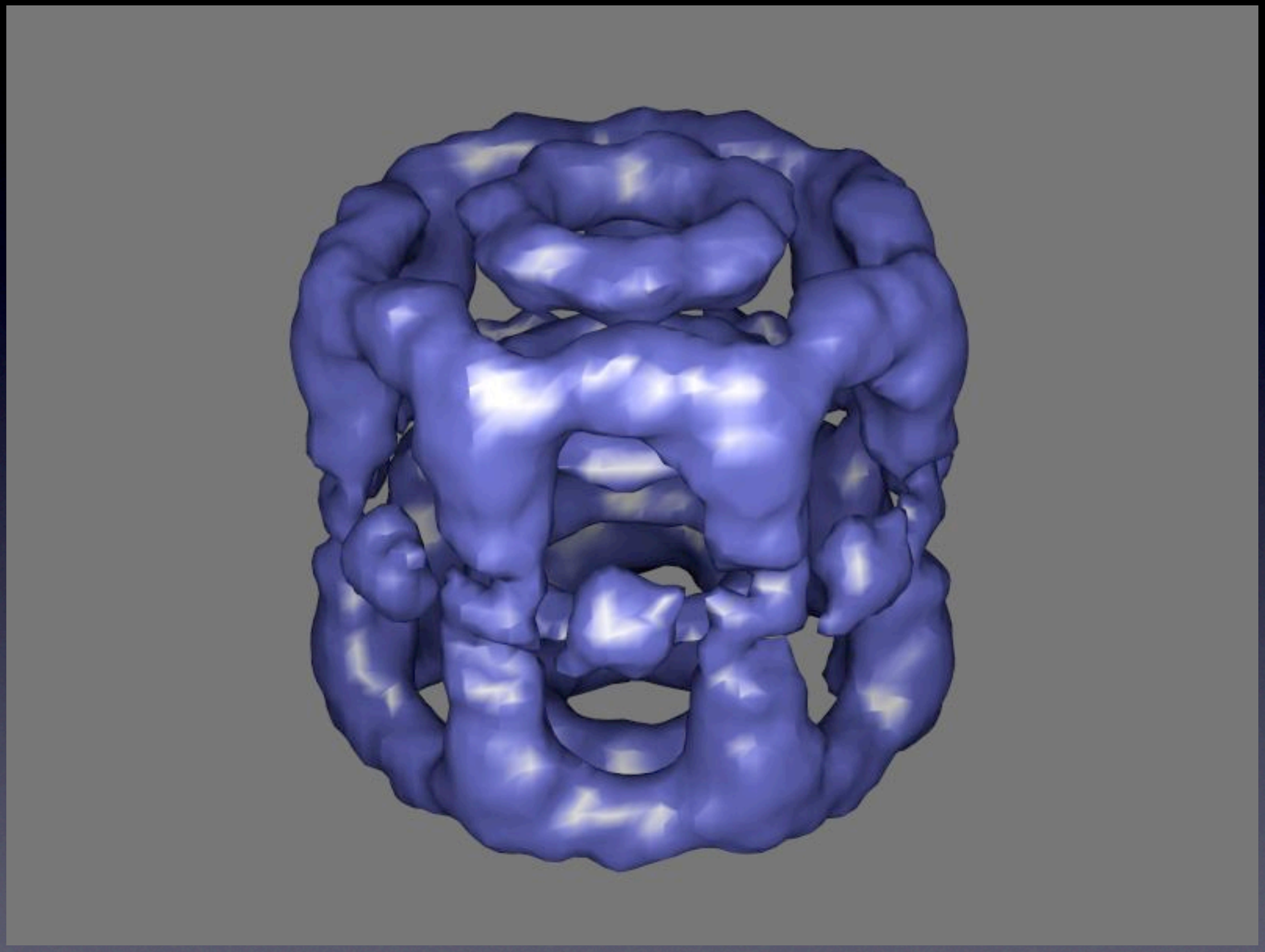
Iteration 1



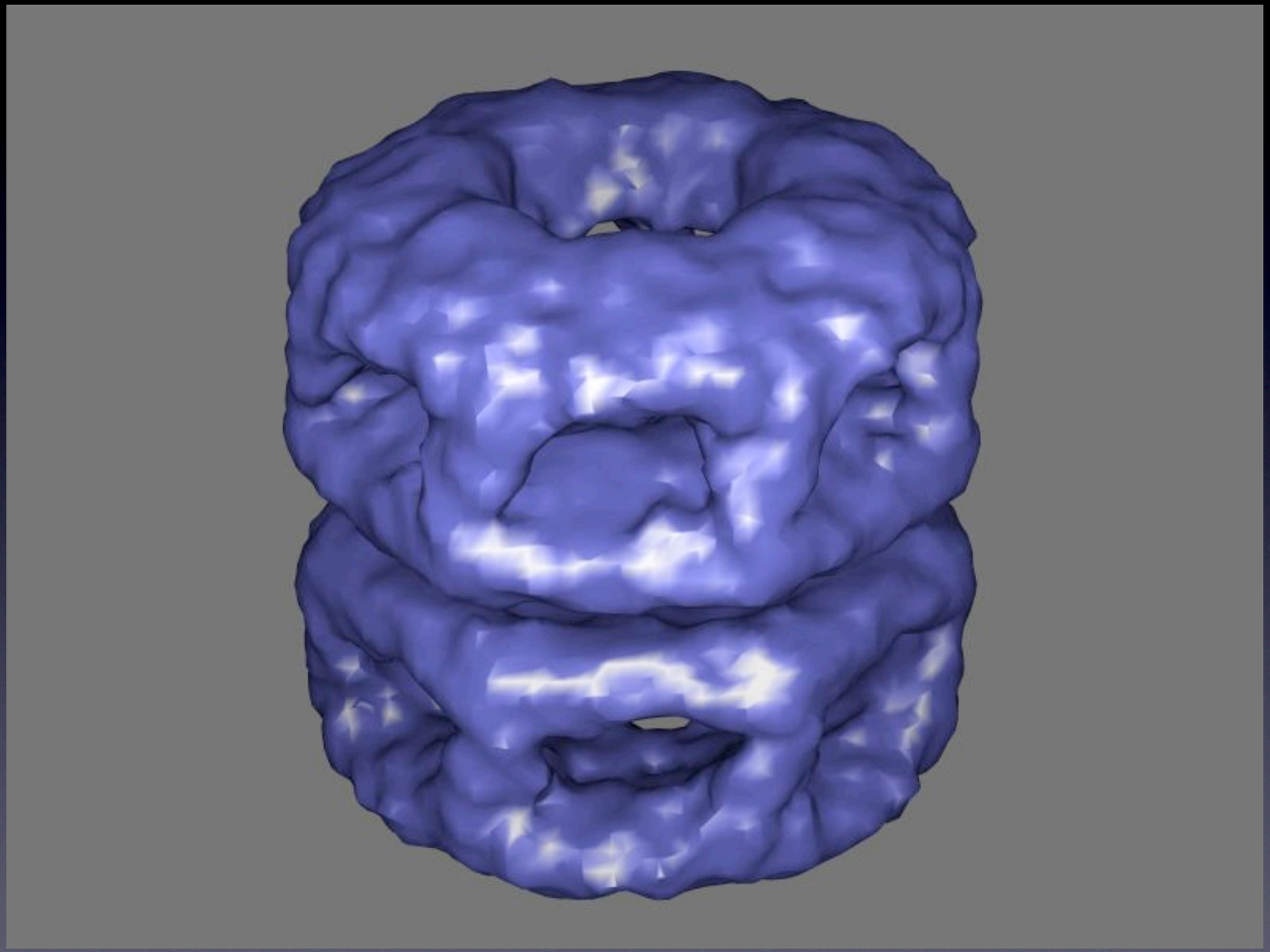
Iteration 2



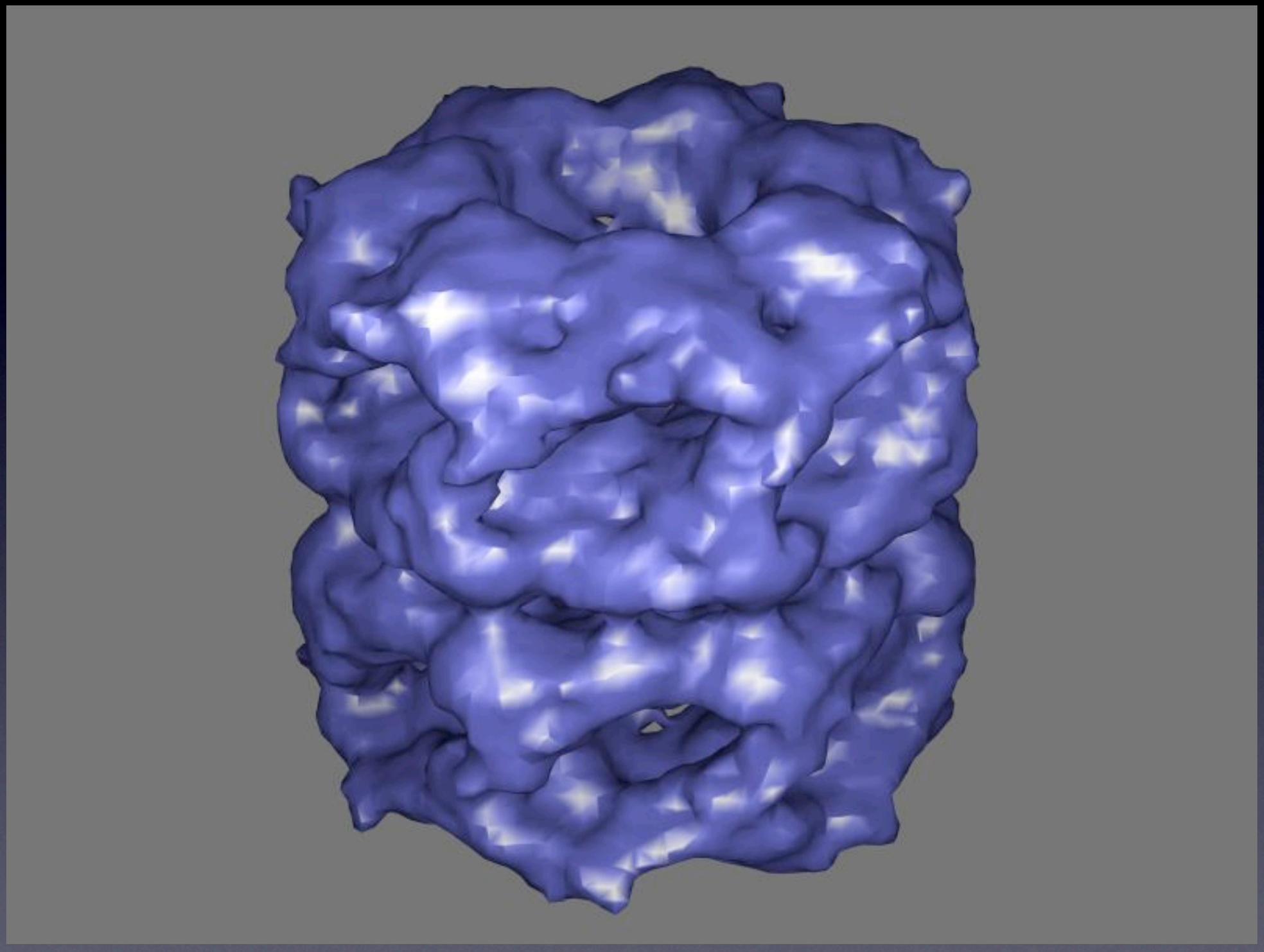
Iteration 3



Iteration 4



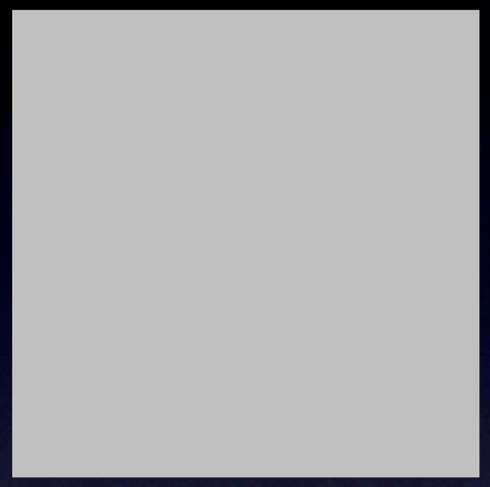
Iteration 5



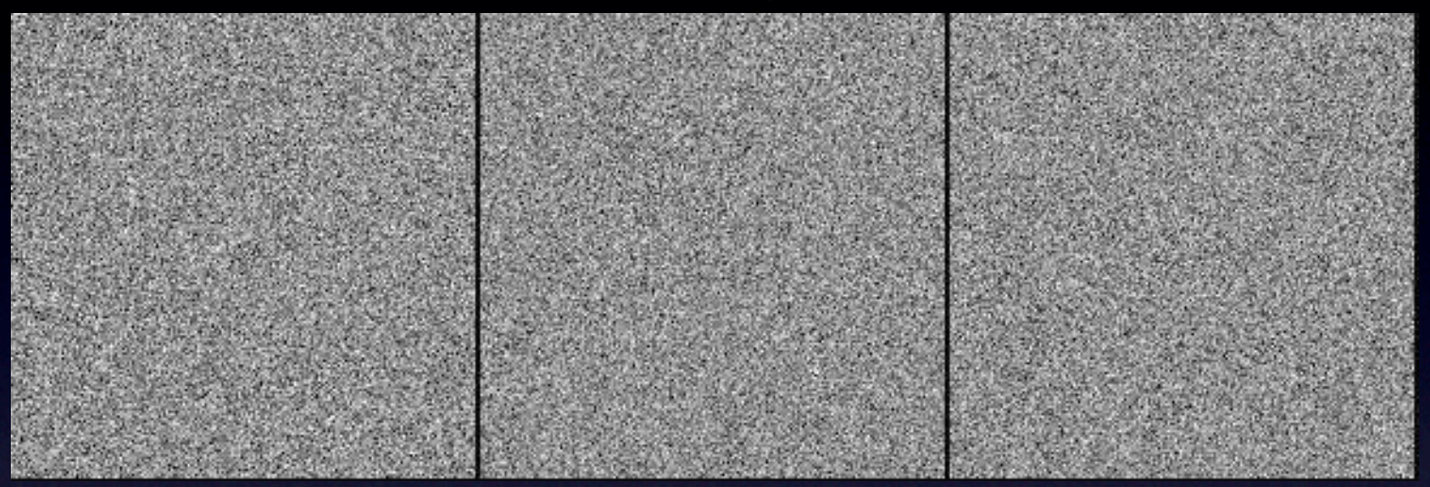
Model Bias ?

Model Bias

Base



Noisy



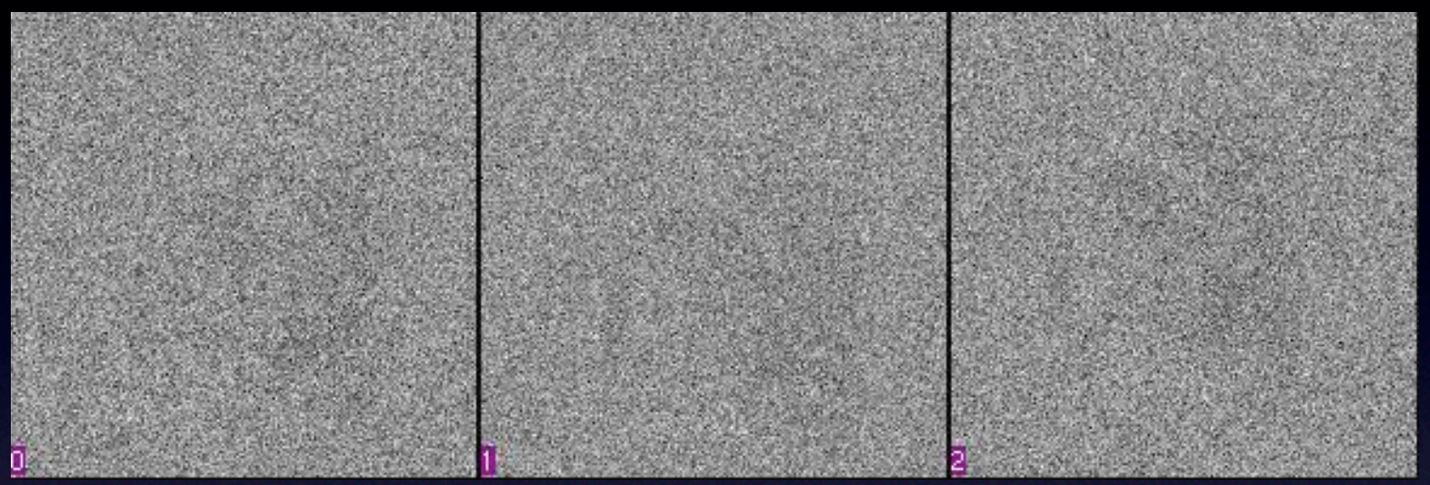
Align to

25 100 250 1000 2000

Model Bias

Base

Noisy (~10% contrast)



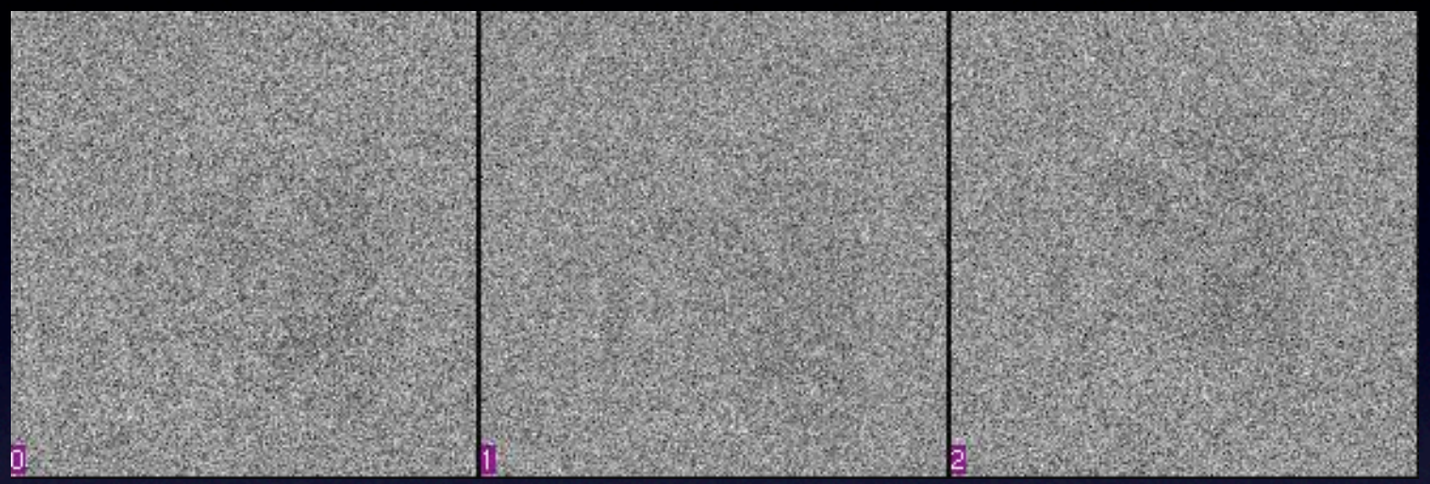
Align to

25 100 250 1000 2000

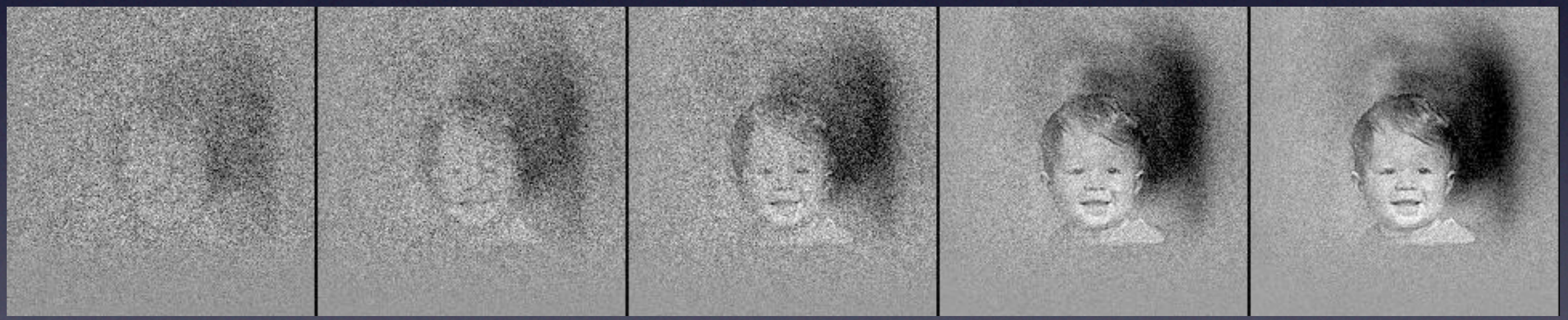
Model Bias

Base

Noisy (~10% contrast)



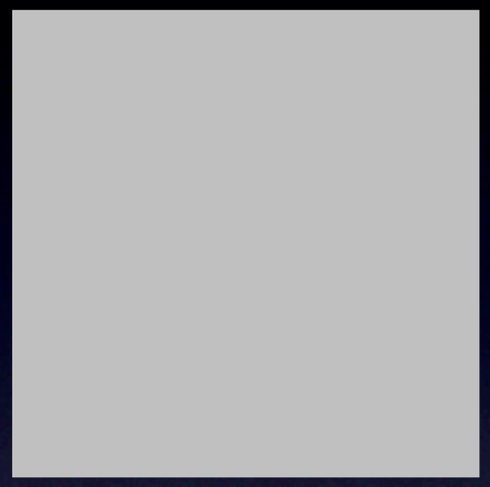
Align to



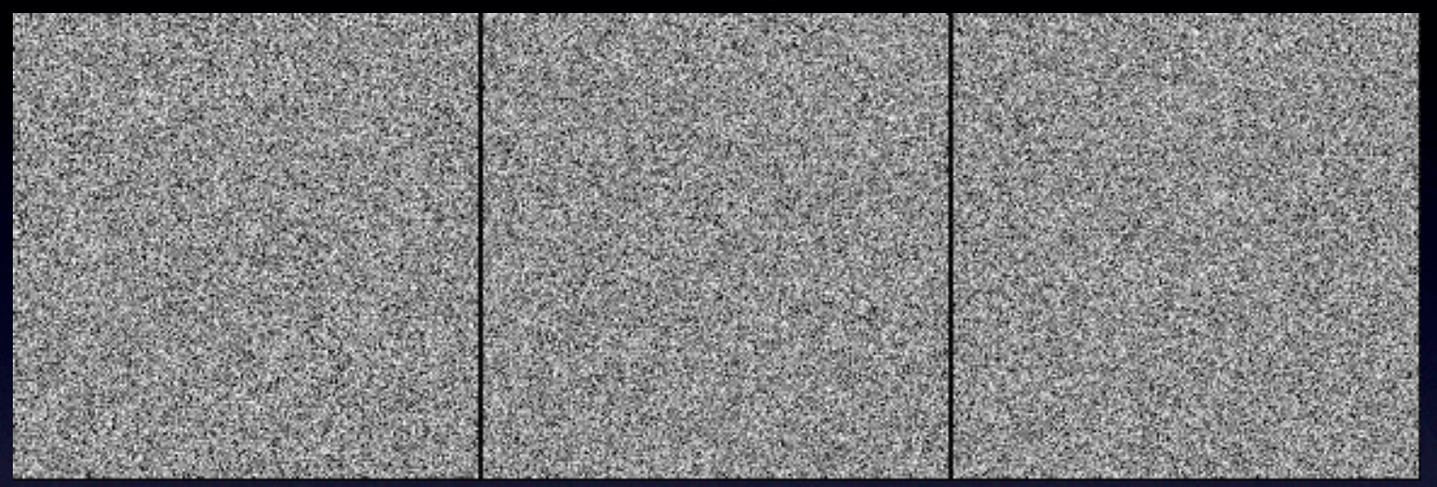
25 100 250 1000 2000

Model Bias

Base



Noisy

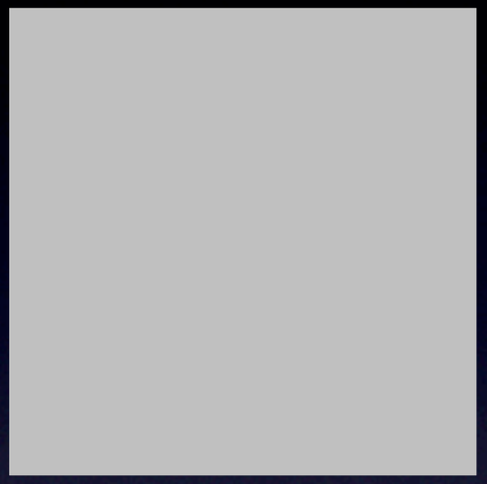


Align to

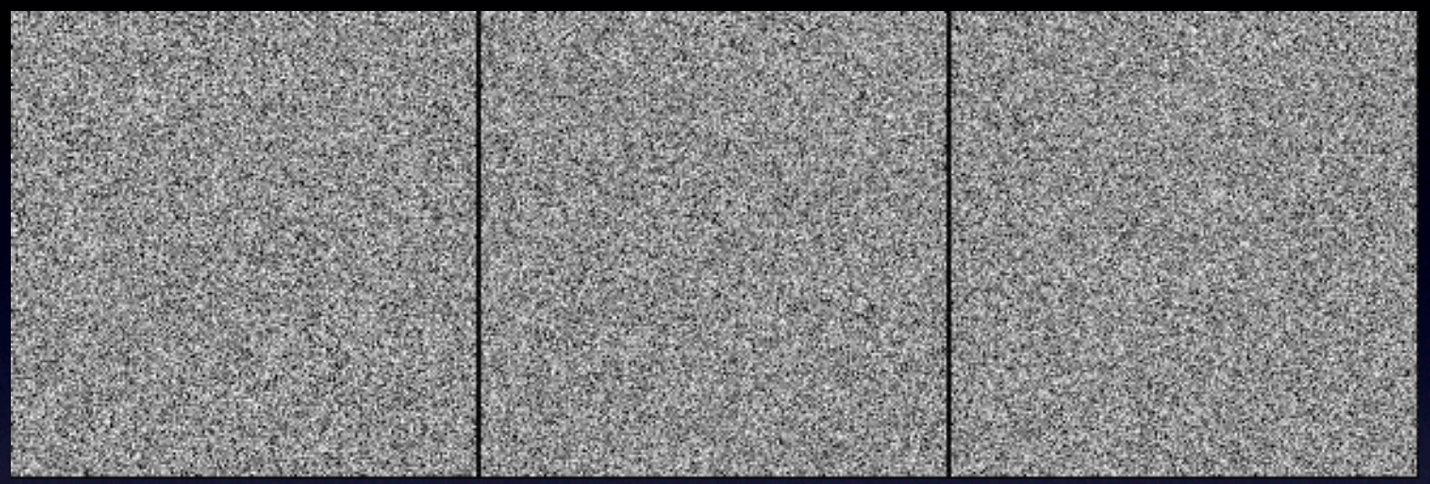
25 100 250 1000 2000

Model Bias

Base

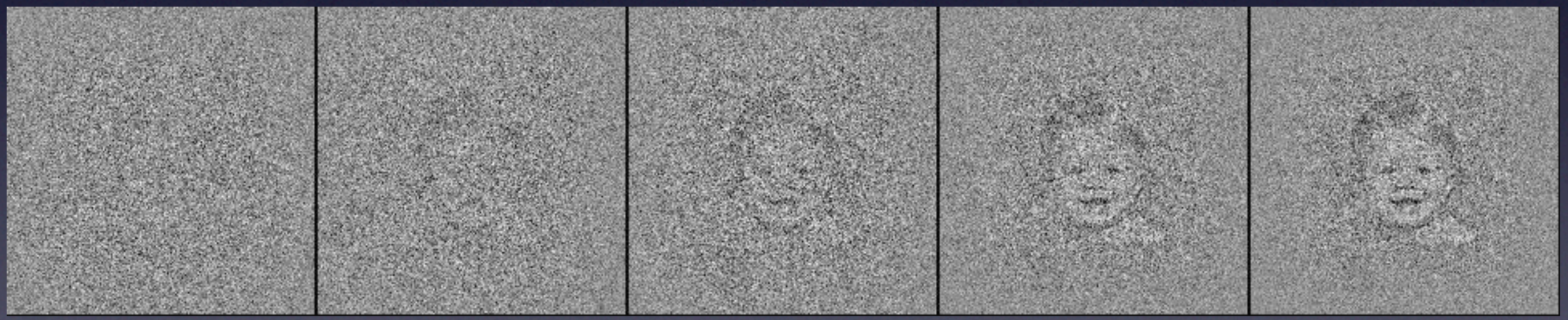


Noisy



Align to

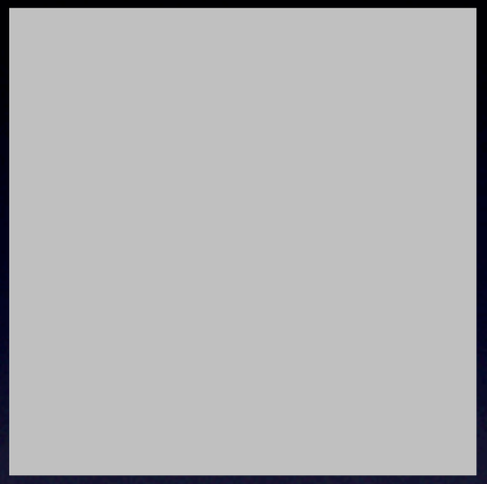
Iter x4



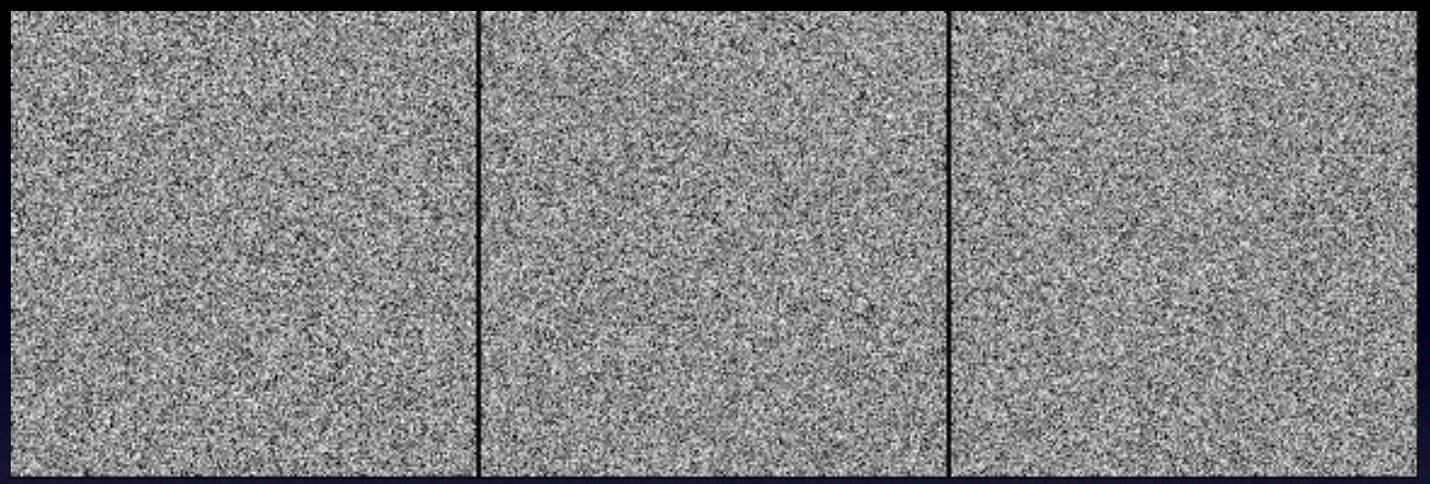
25 100 250 1000 2000

Model Bias

Base

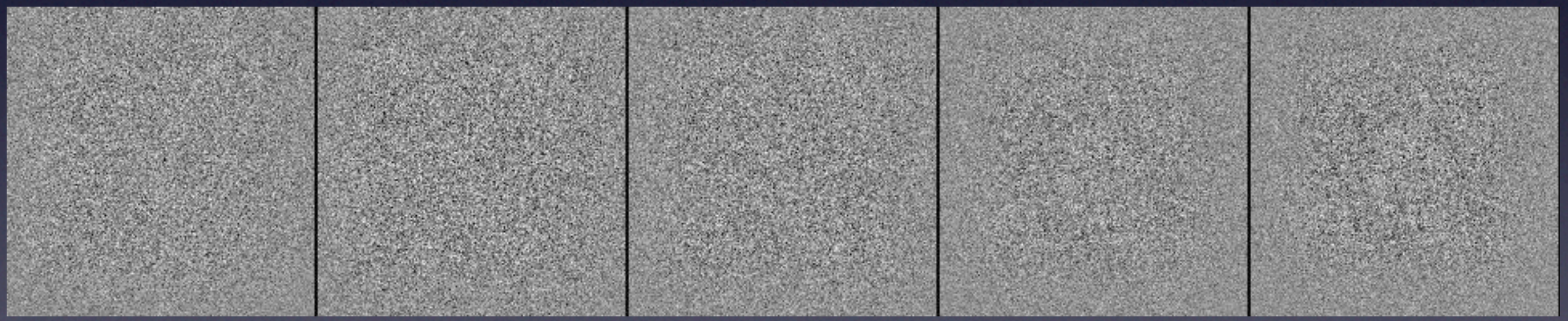


Noisy



Align to

Iter x8

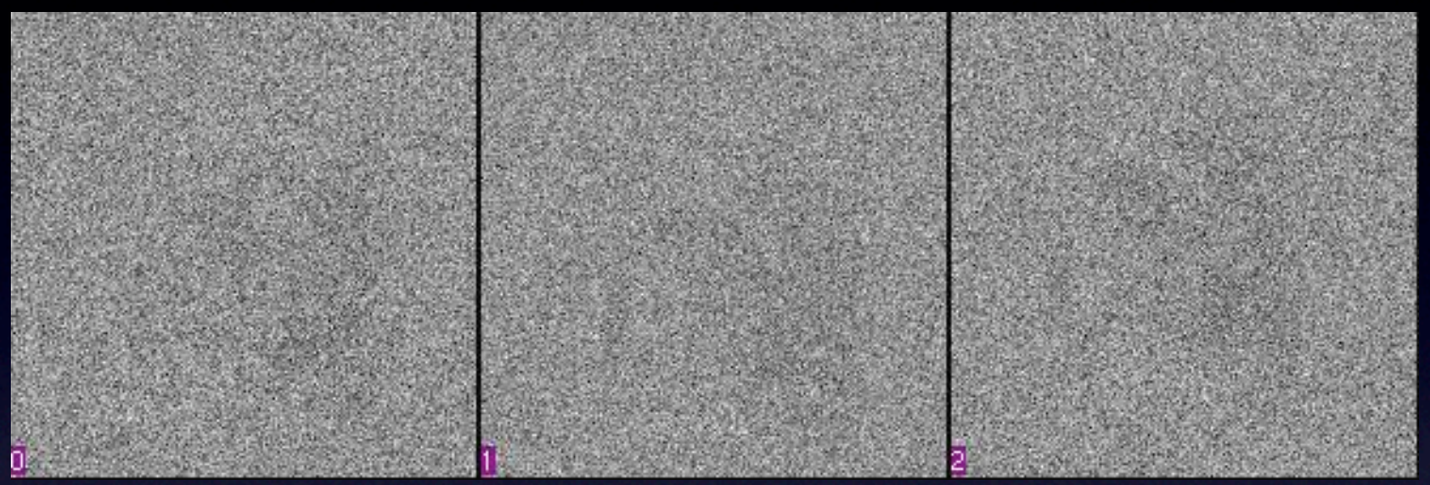


25 100 250 1000 2000

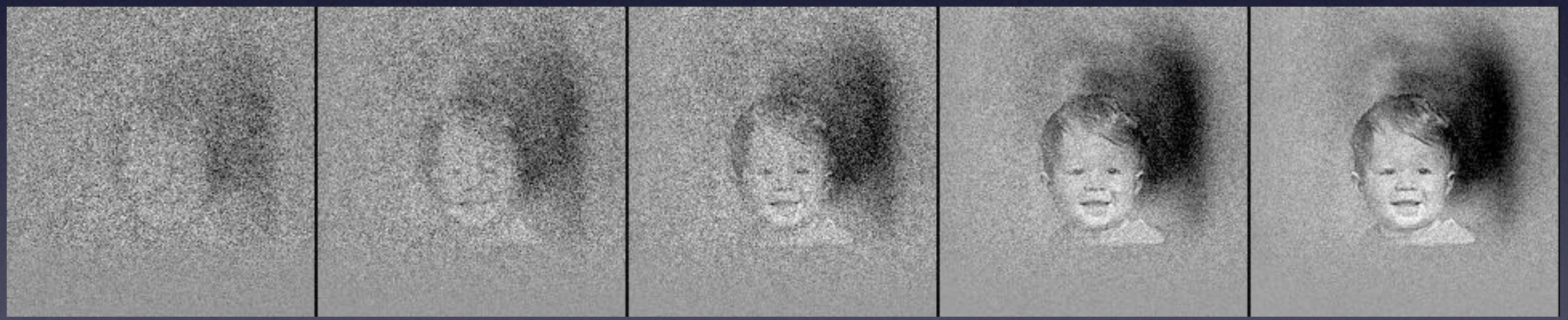
Model Bias

Base

Noisy (~10% contrast)



Align to

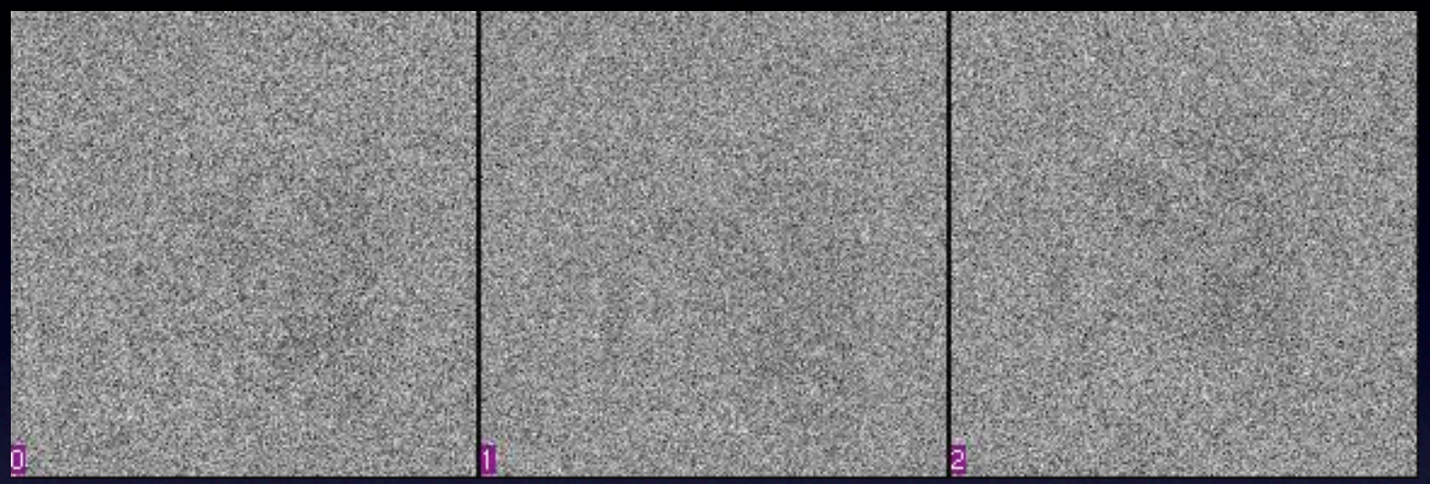


25 100 250 1000 2000

Model Bias

Base

Noisy

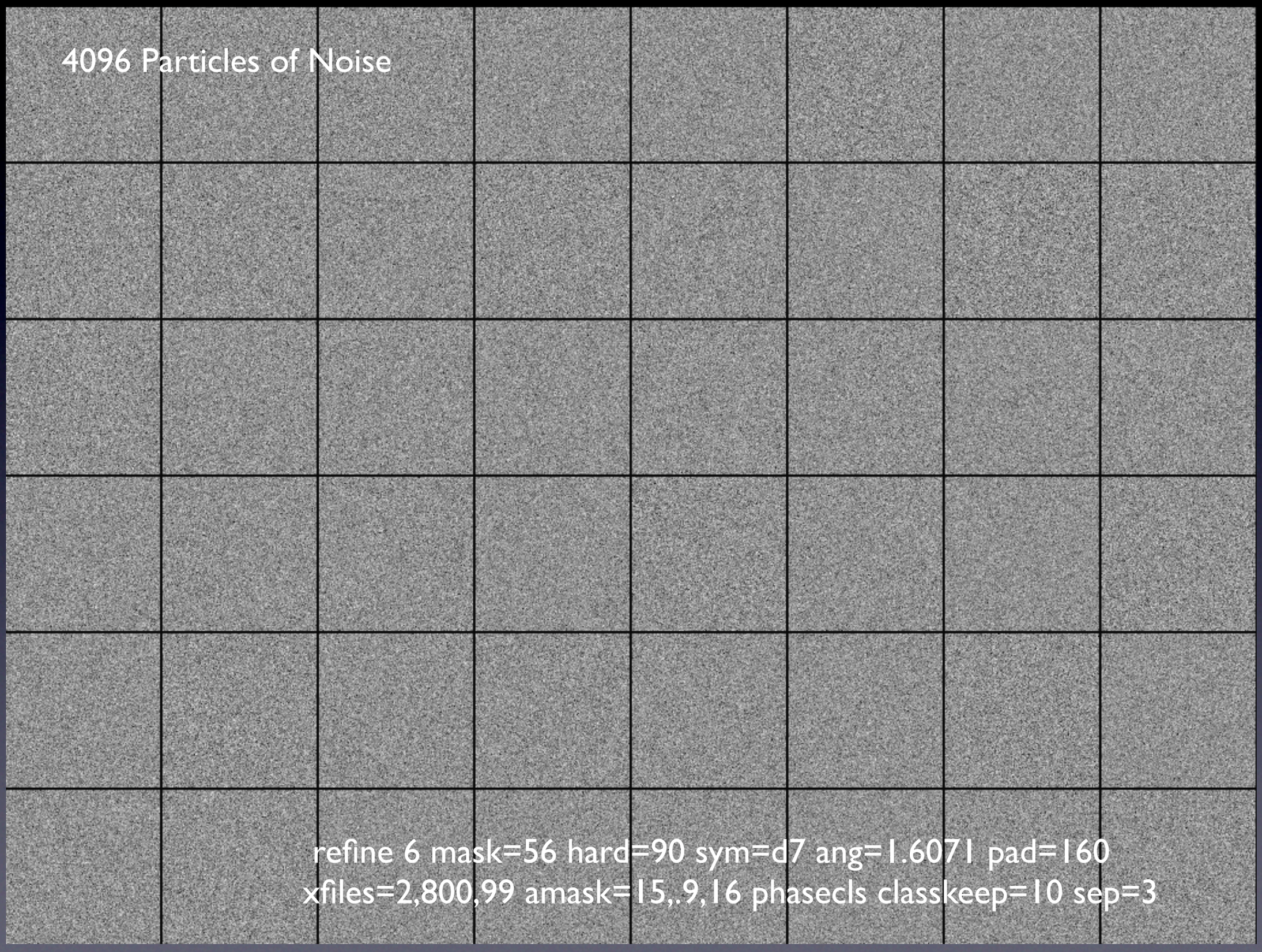


Align to

Iter x4

25 100 250 1000 2000

How About 3-D ?

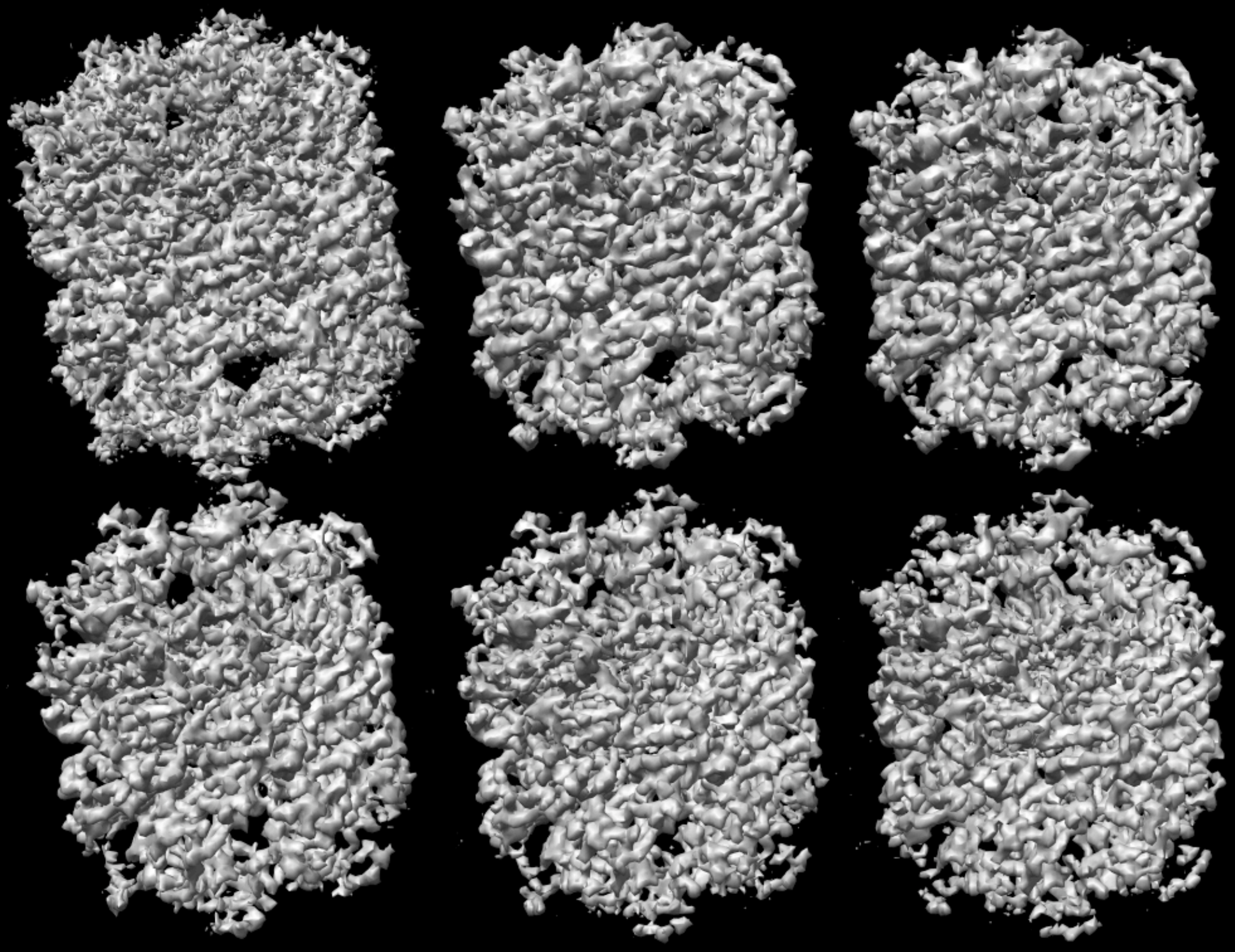

4096 Particles of Noise
refine 6 mask=56 hard=90 sym=d7 ang=1.6071 pad=160
xfiles=2,800,99 amask=15,.9,16 phasecls classkeep=10 sep=3

no iteration

Initial Model

1 Iter.

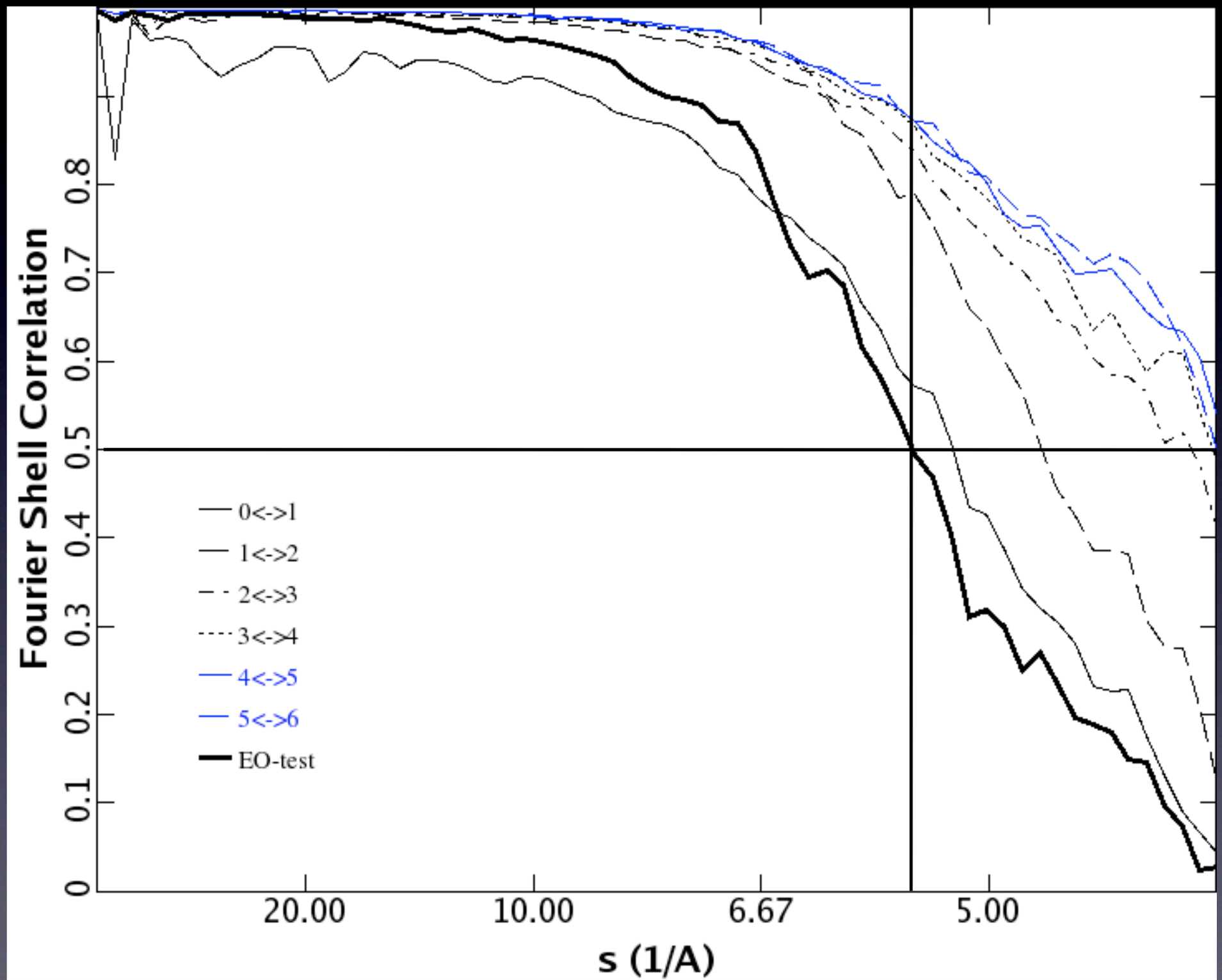
2 Iter.



3 Iter.

4 Iter.

5 Iter.

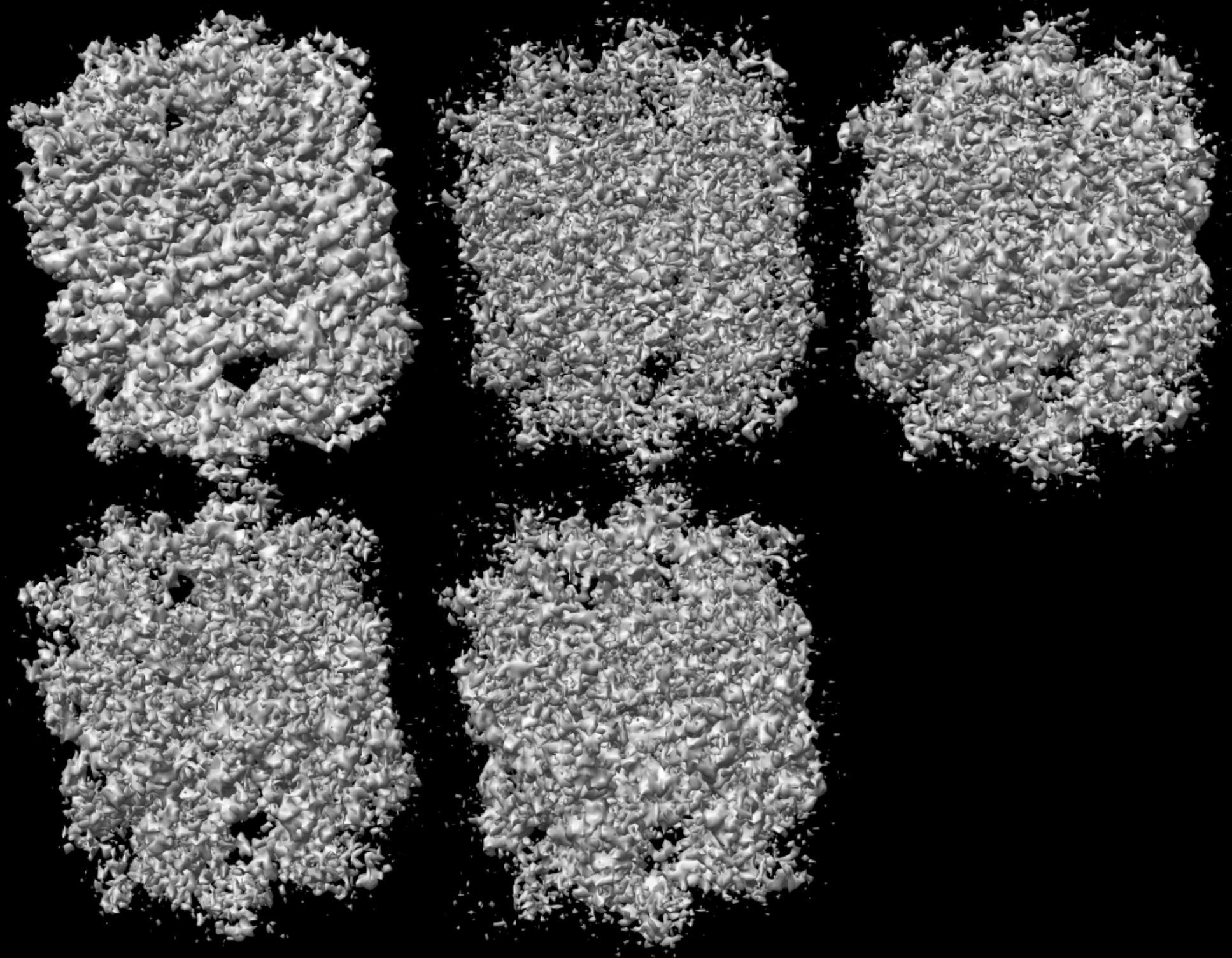

Fourier Shell Correlation
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0<->1
1<->2
2<->3
3<->4
4<->5
5<->6
EO-test
20.00
10.00
6.67
5.00
s (1/A)

1 iteration

Initial Model

1 Iter.

2 Iter.



3 Iter.

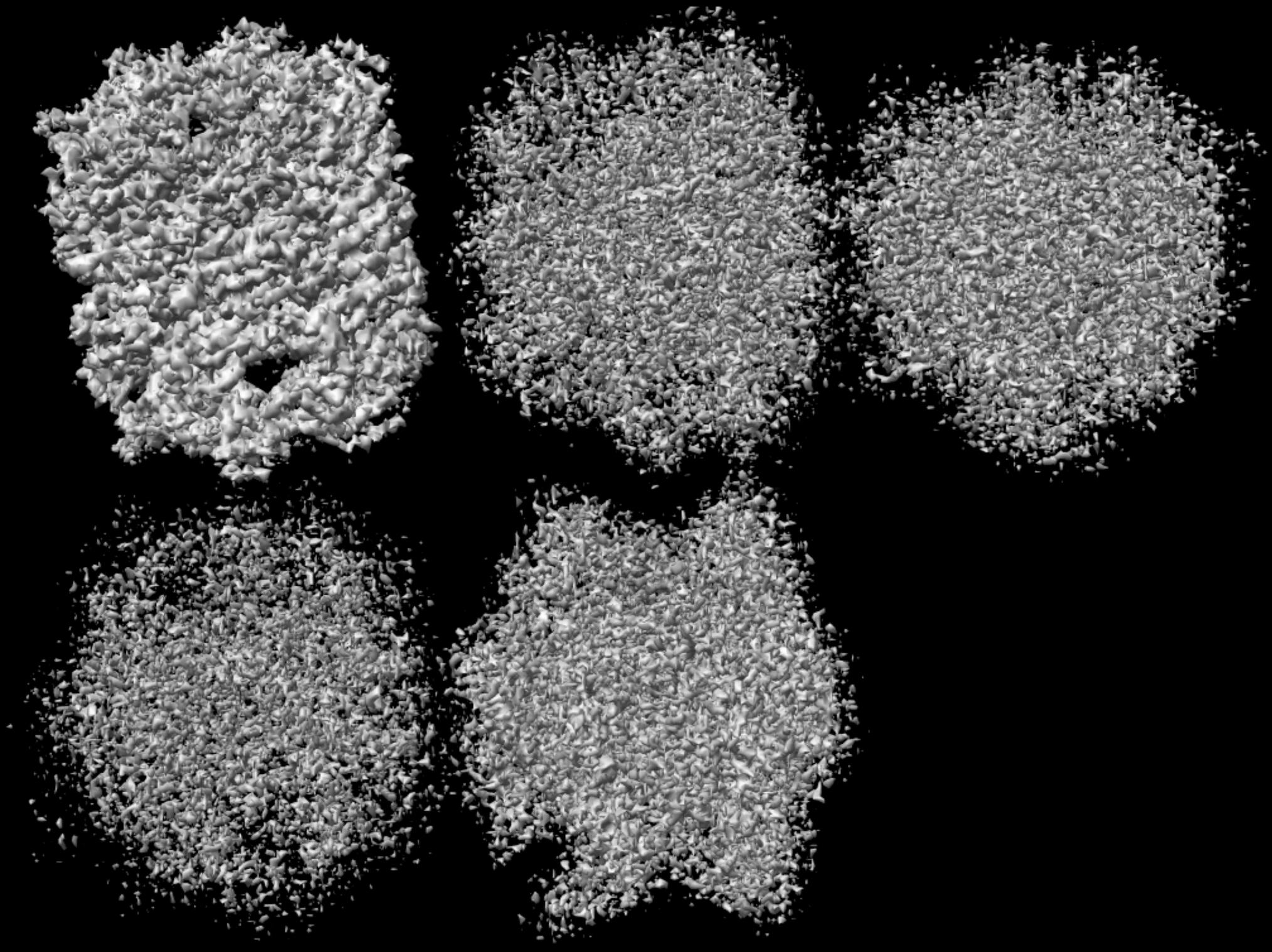
4 Iter.

6 iterations

Initial Model

1 Iter.

2 Iter.



3 Iter.

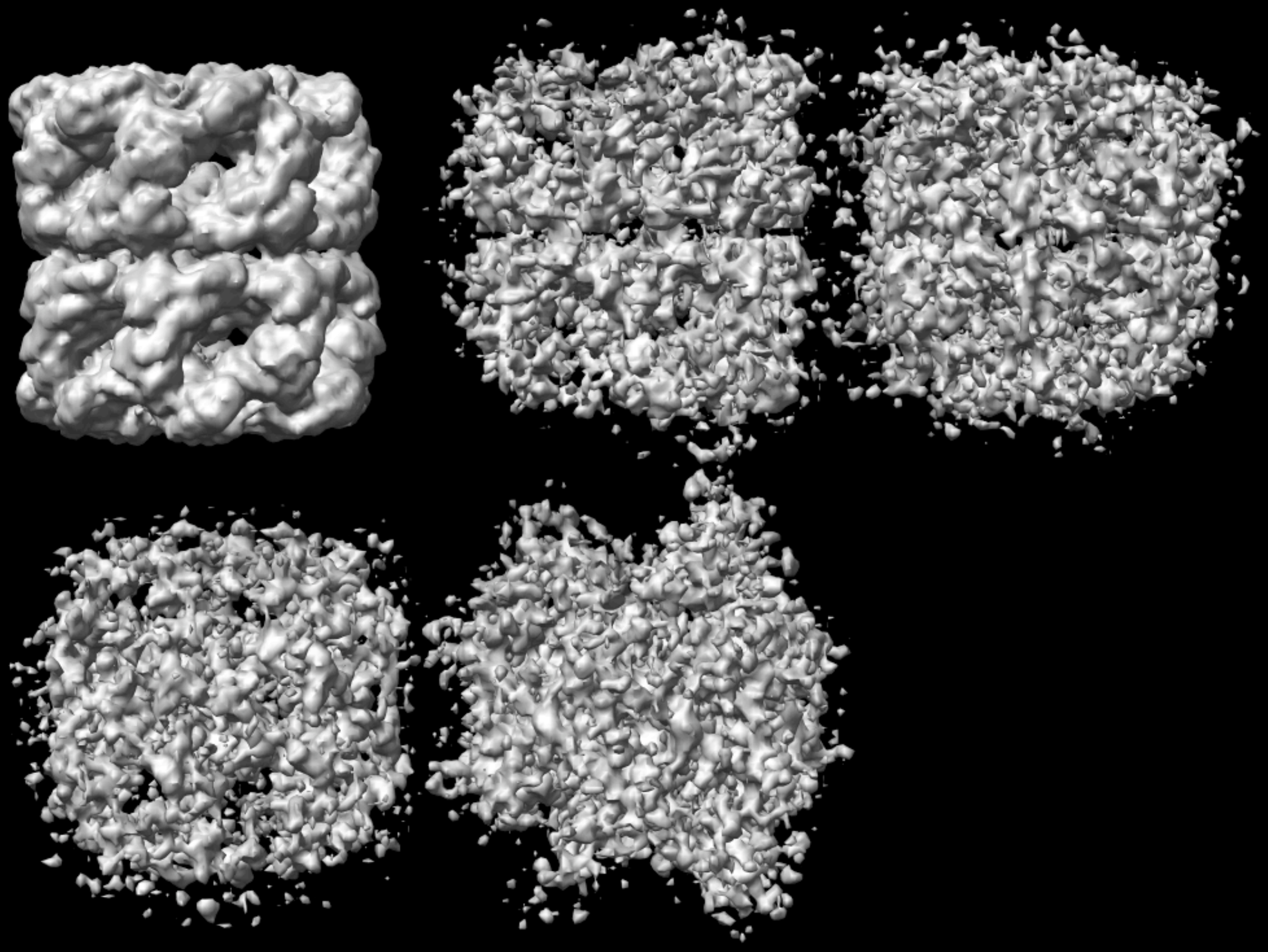
4 Iter.

6 iterations
(8 A lowpass)

Initial Model

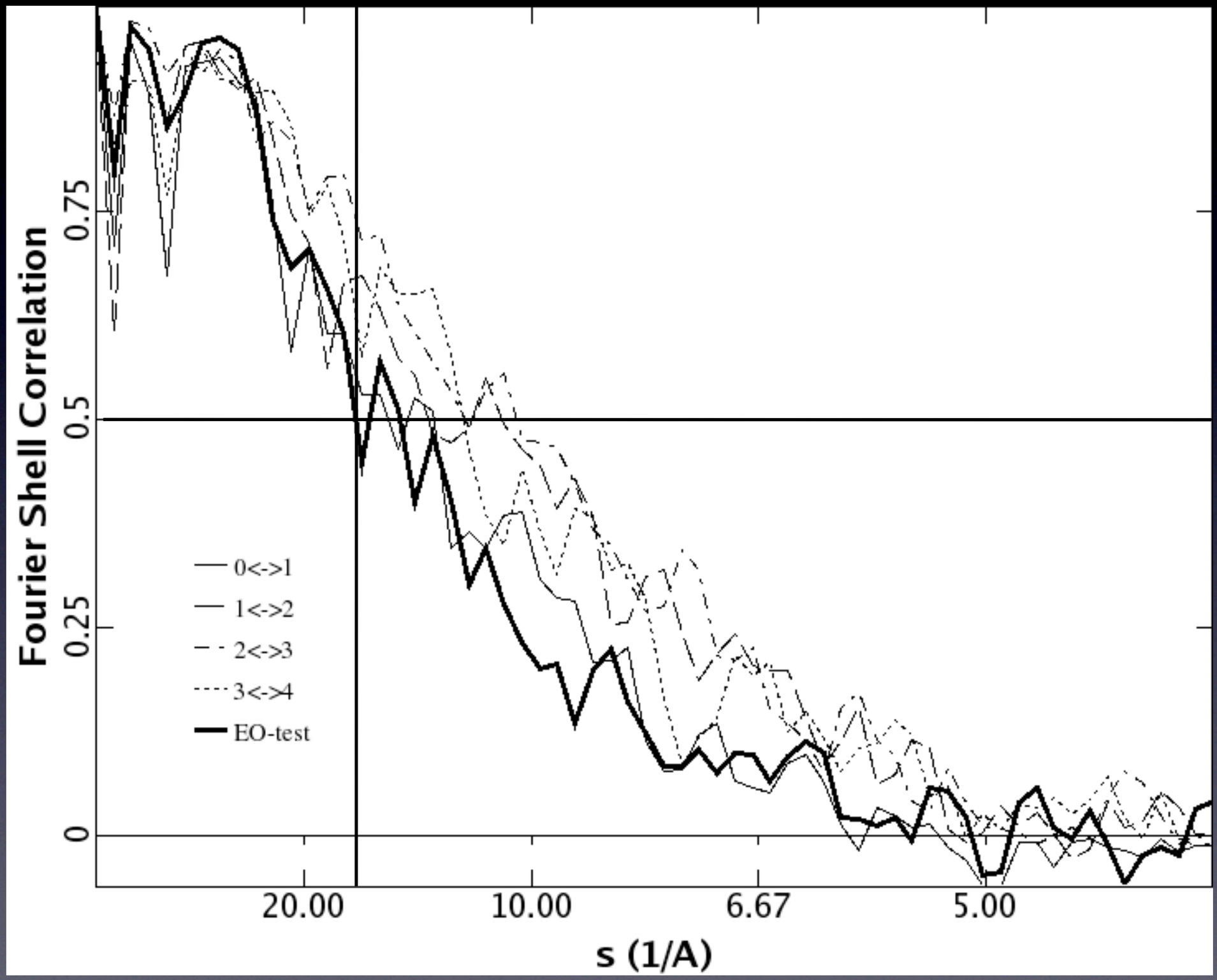
1 Iter.

2 Iter.



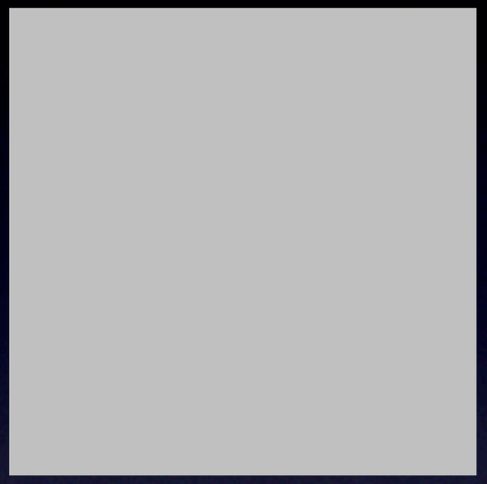
3 Iter.

4 Iter.

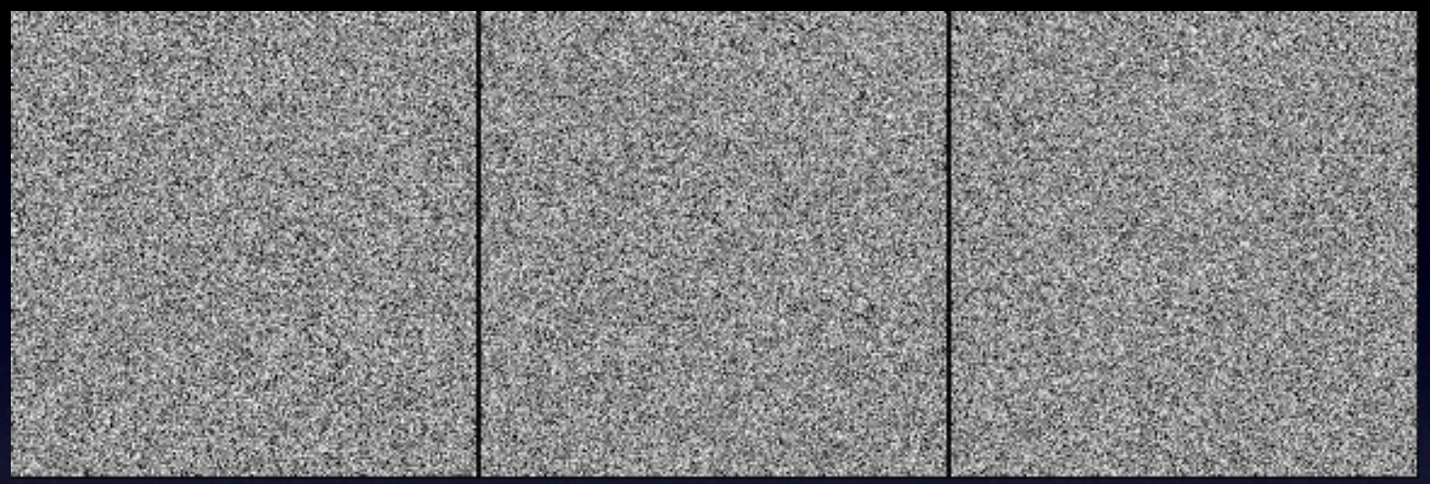

Fourier Shell Correlation
0.75
0.5
0.25
0
0<->1
1<->2
2<->3
3<->4
EO-test
20.00
10.00
6.67
5.00
s (1/A)

Model Bias

Base

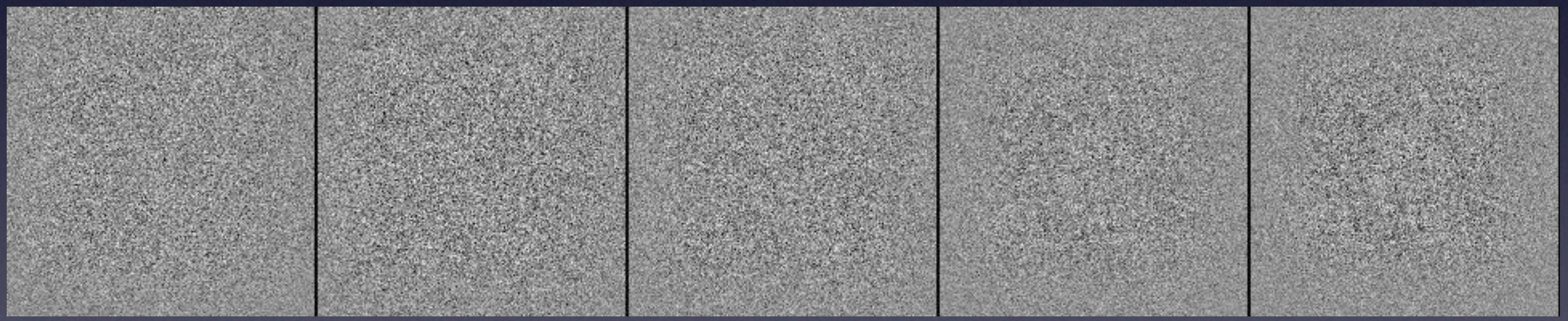


Noisy



Align to

Iter x8

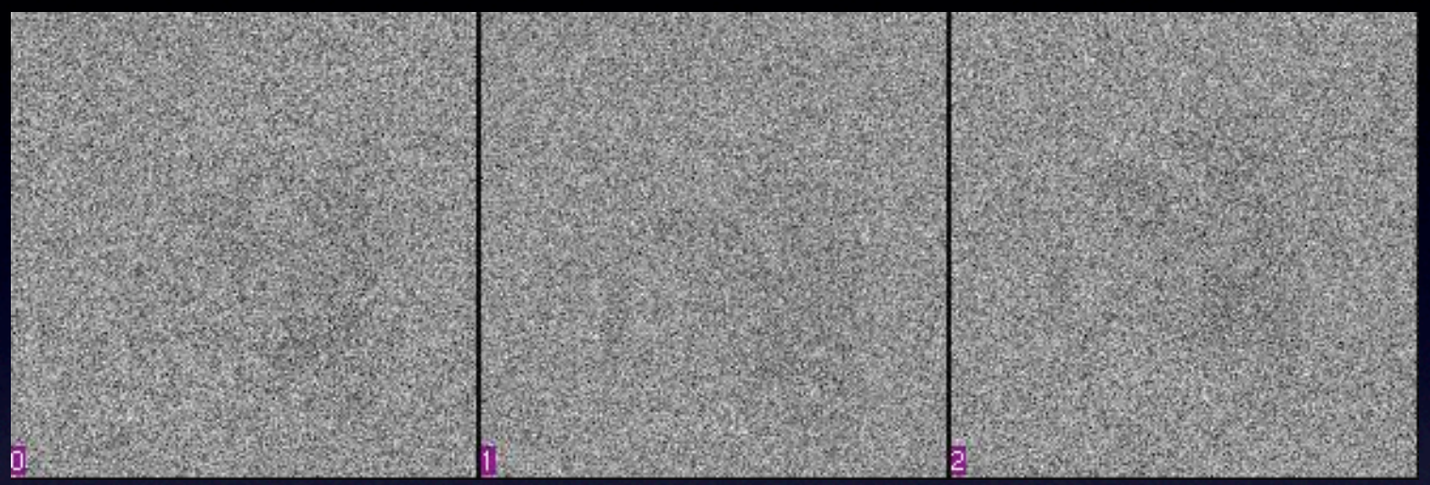


25 100 250 1000 2000

Model Bias

Base

Noisy



Align to

Iter x4

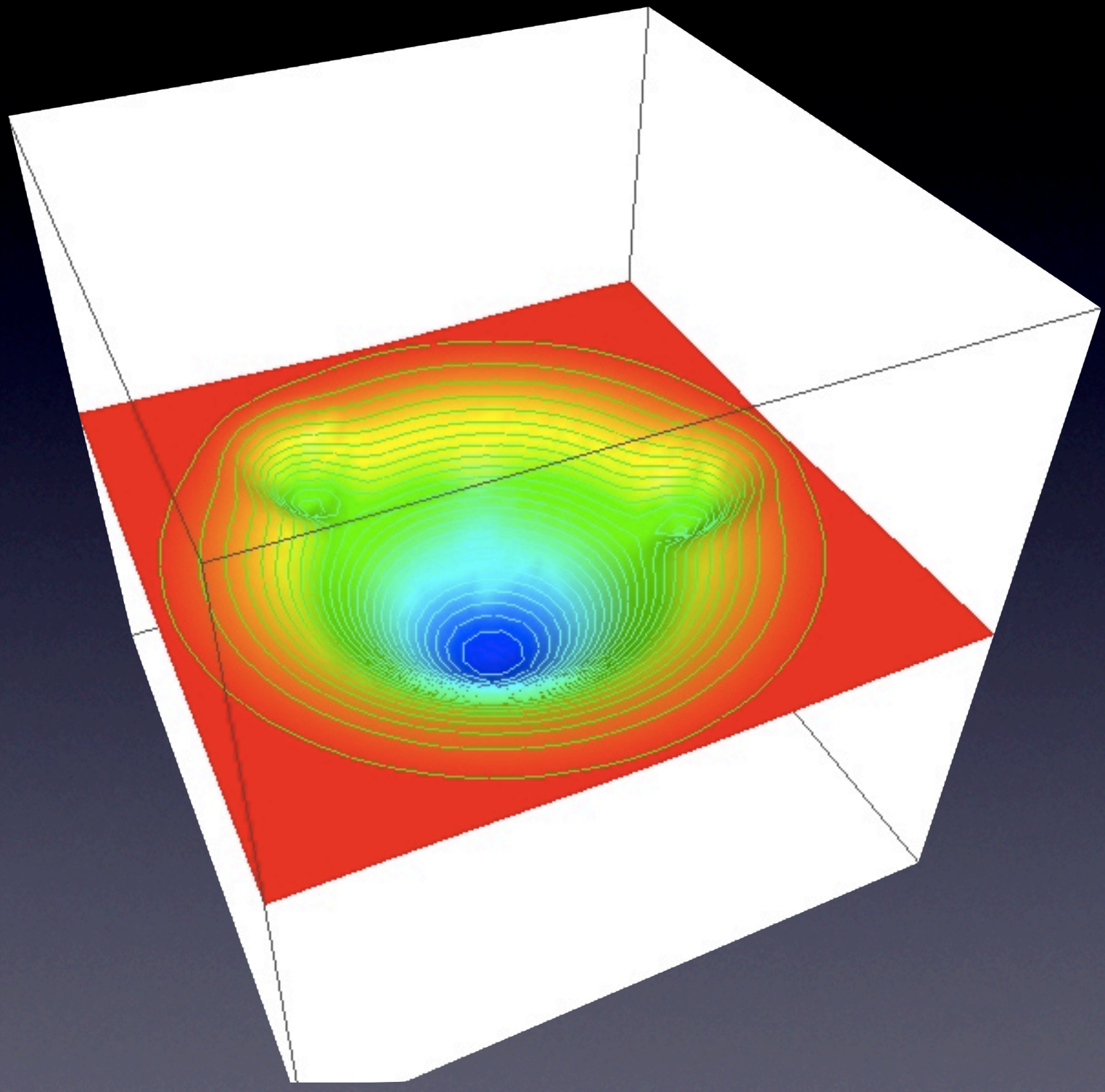
25 100 250 1000 2000

How Do we Stop This ?

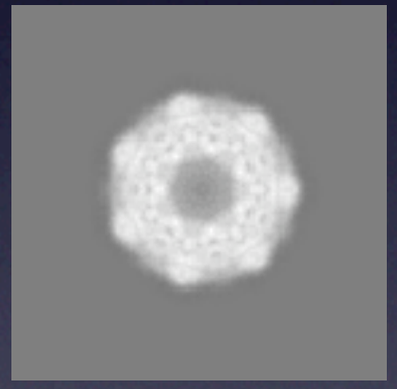
- (In EMAN) use classiter>3 for a few rounds
- Always refine from multiple starting models

(note - you can shrink the data first)

- If the results are not effectively the same, try to establish which one is correct by looking at self consistency of projections/class-averages
- Compare with results of 2D analysis



Measures of Similarity



← ? →

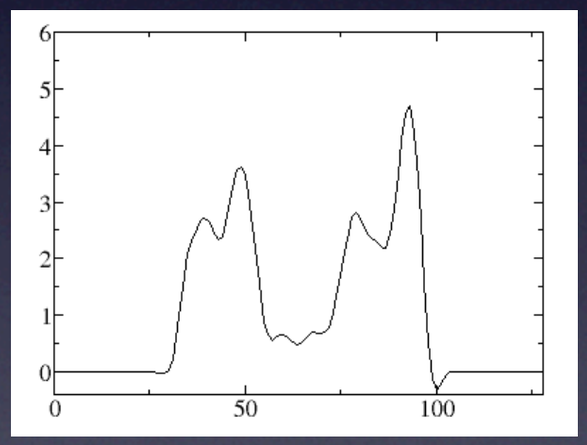
← ? →

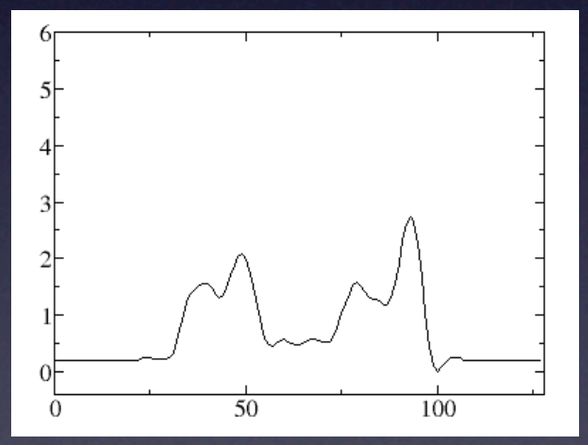
← ? →

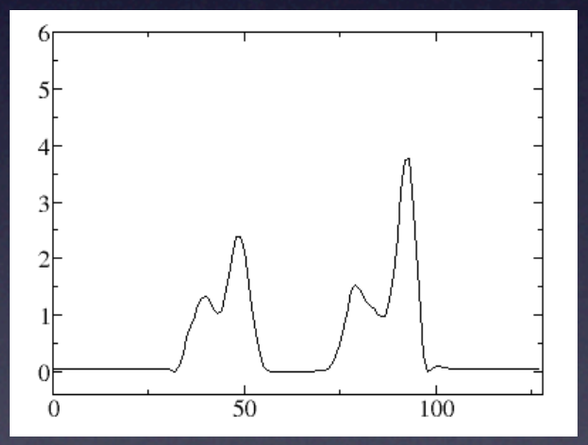
Measures of Similarity

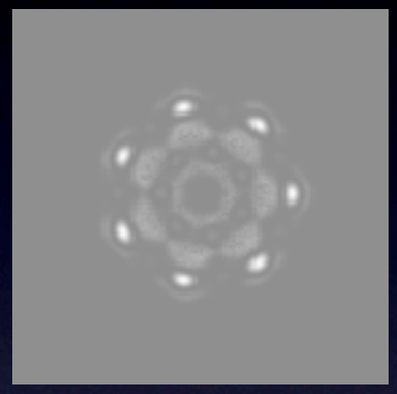
- Correlation coefficient
- Variance (equivalent)
- Phase Residual
- FSC
- Mutual Information
- etc...

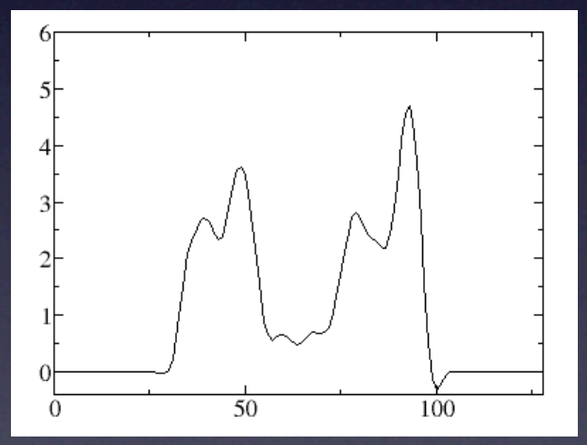
( - )² = 

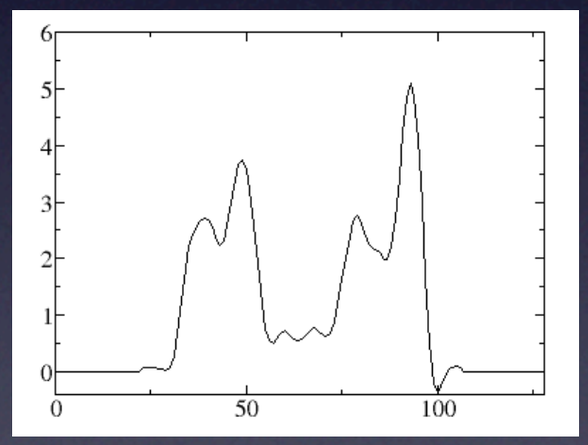


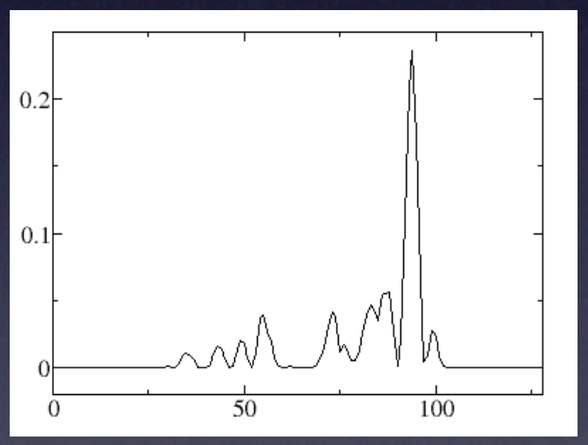


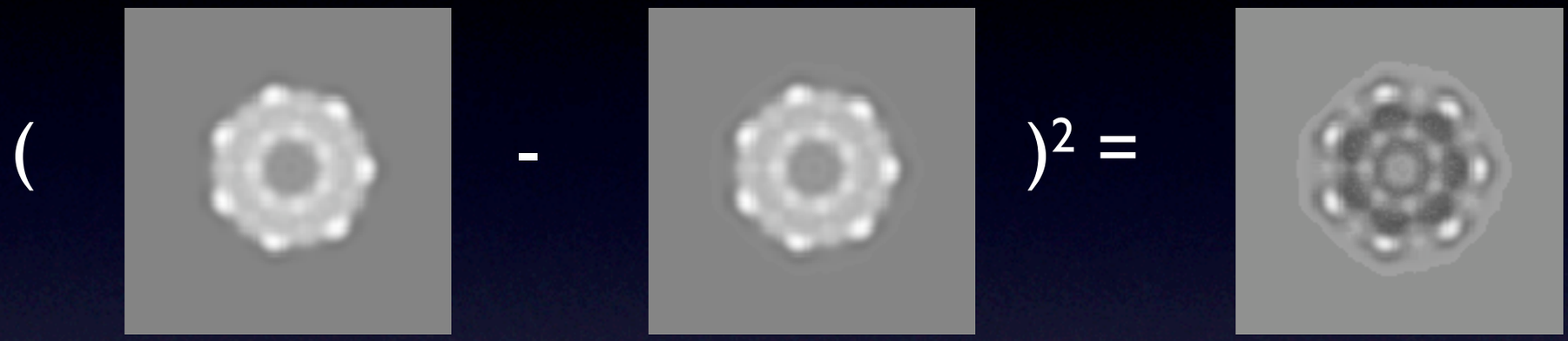


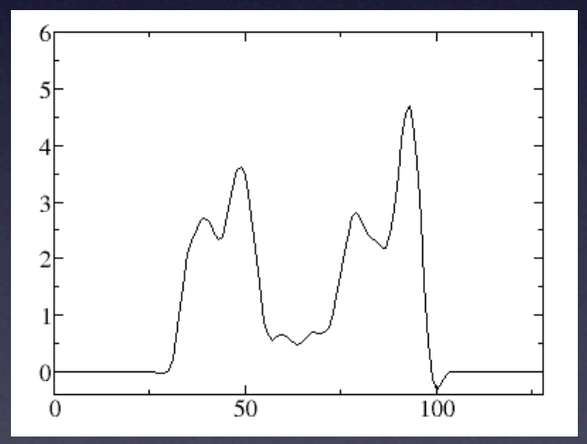
( - )² = 

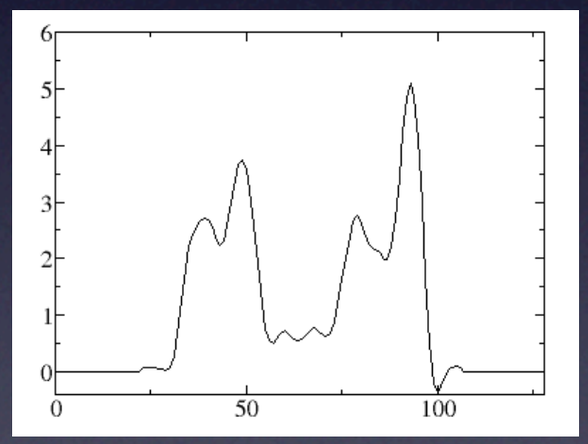


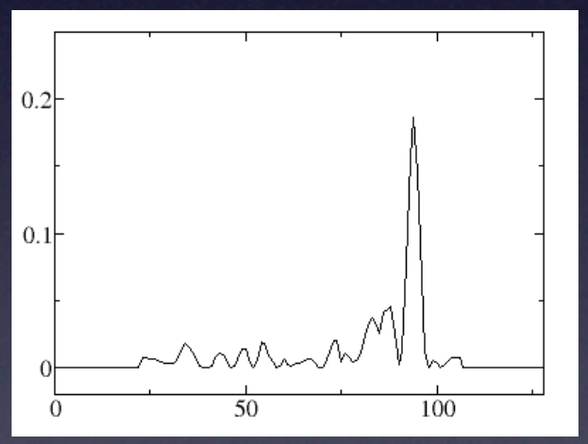


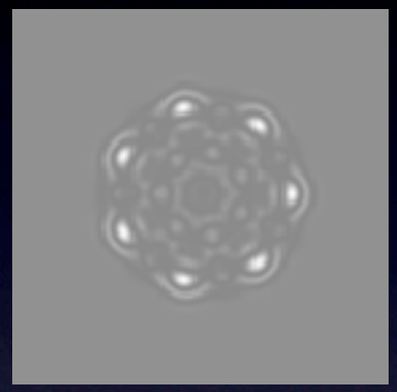


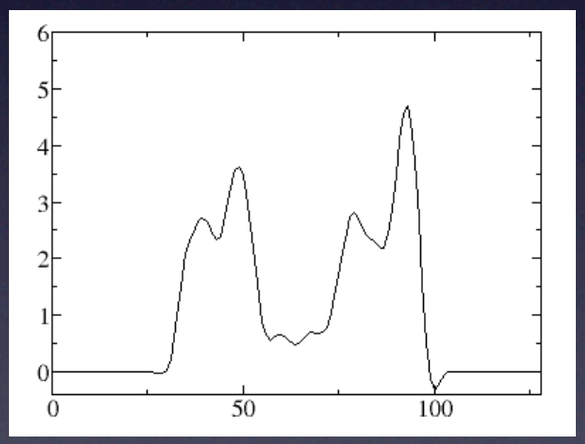

(-)² =

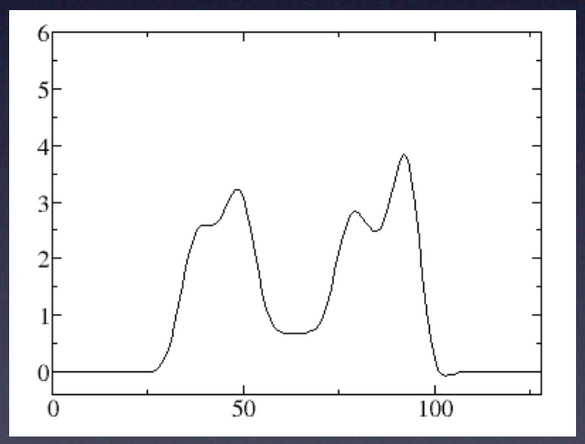

0
1
2
3
4
5
6
0
50
100

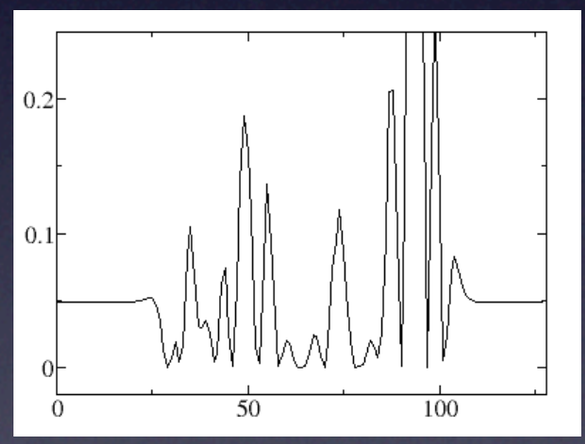

0
1
2
3
4
5
6
0
50
100


0
0.1
0.2
0
50
100

( - )² = 







One Answer ...

- Wiener filter particle
- Filter reference to match
- Normalize reference density to particle
- Calculate variance

Canonical SPA in EMAN

- Image Acquisition
- Particle Picking
- 2-D Analysis
- Symmetry/Low Resolution Model
- Determine CTF Parameters
- High Resolution Refinement
- Post-processing
- Dynamics Analysis

Canonical SPA in EMAN

- Image Acquisition
- Install EMAN (EMAN2?)
- Particle Picking
- 2-D Analysis
- Symmetry/Low Resolution Model
- Determine CTF Parameters
- High Resolution Refinement
- Post-processing
- Dynamics Analysis