Methods for High Resolution Refinement in Single Particle Processing

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Co-director,NCMI

NCRR

BCM
Baylor College of Medicine

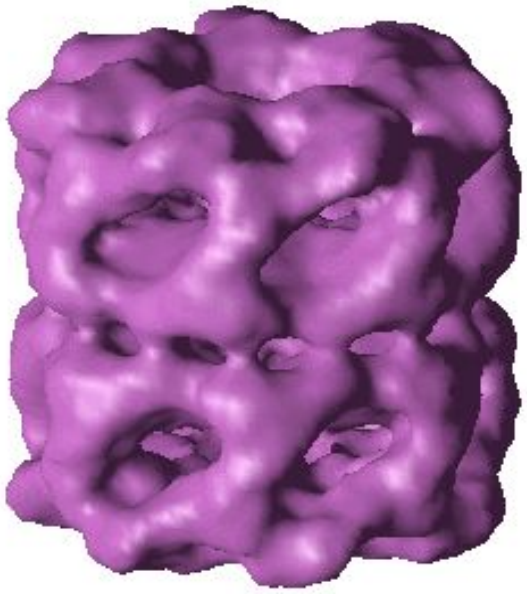
GroEL at 4.5 Å Resolution Using EMAN

Donghua Chen
Joanita Jakana
Wah Chiu

Jiu-Li Song (UT-SW Med)
David Chuang (UT-SW Med)

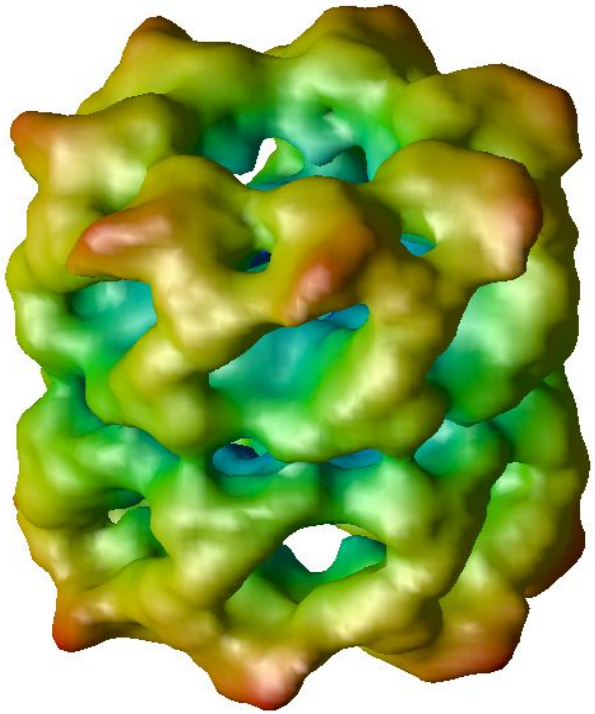
EMAN: http://ncmi.bcm.tmc.edu/eman

GroEL 2000 (15 Å)



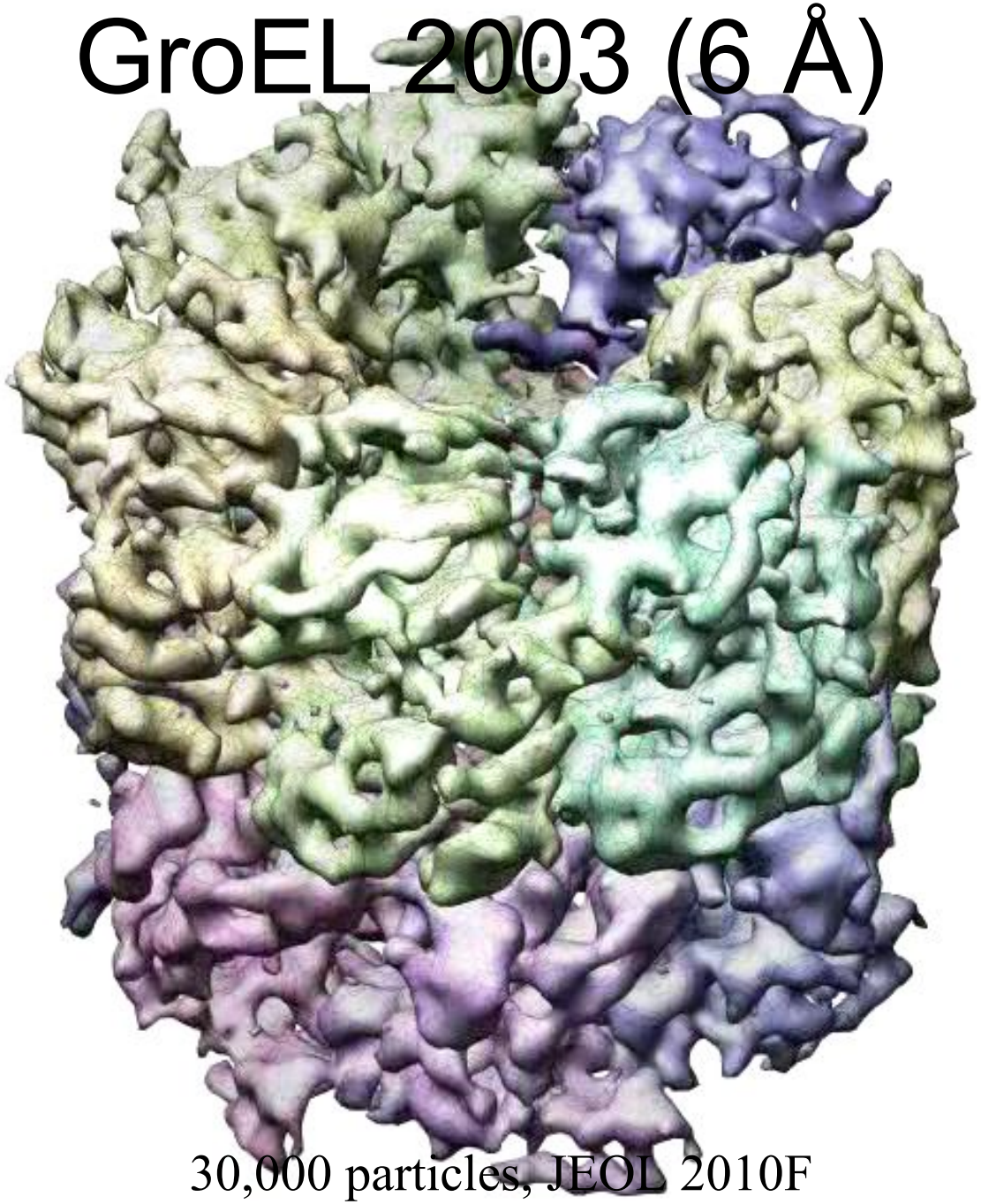
5000 particles, JEOL 4000

GroEL 2001 (11.5 Å)



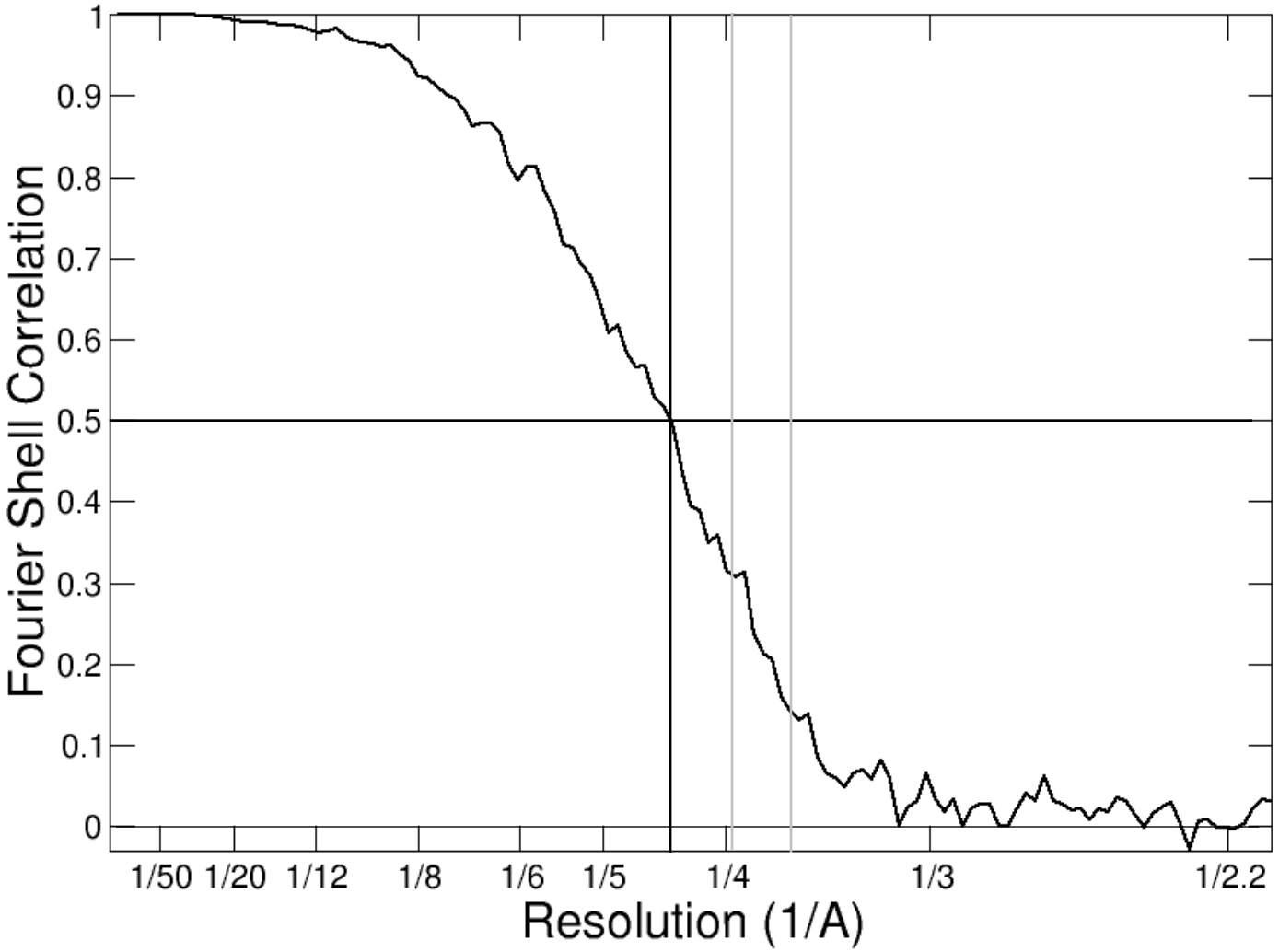
5000 particles, JEOL 4000

GroEL 2003 (6 Å)

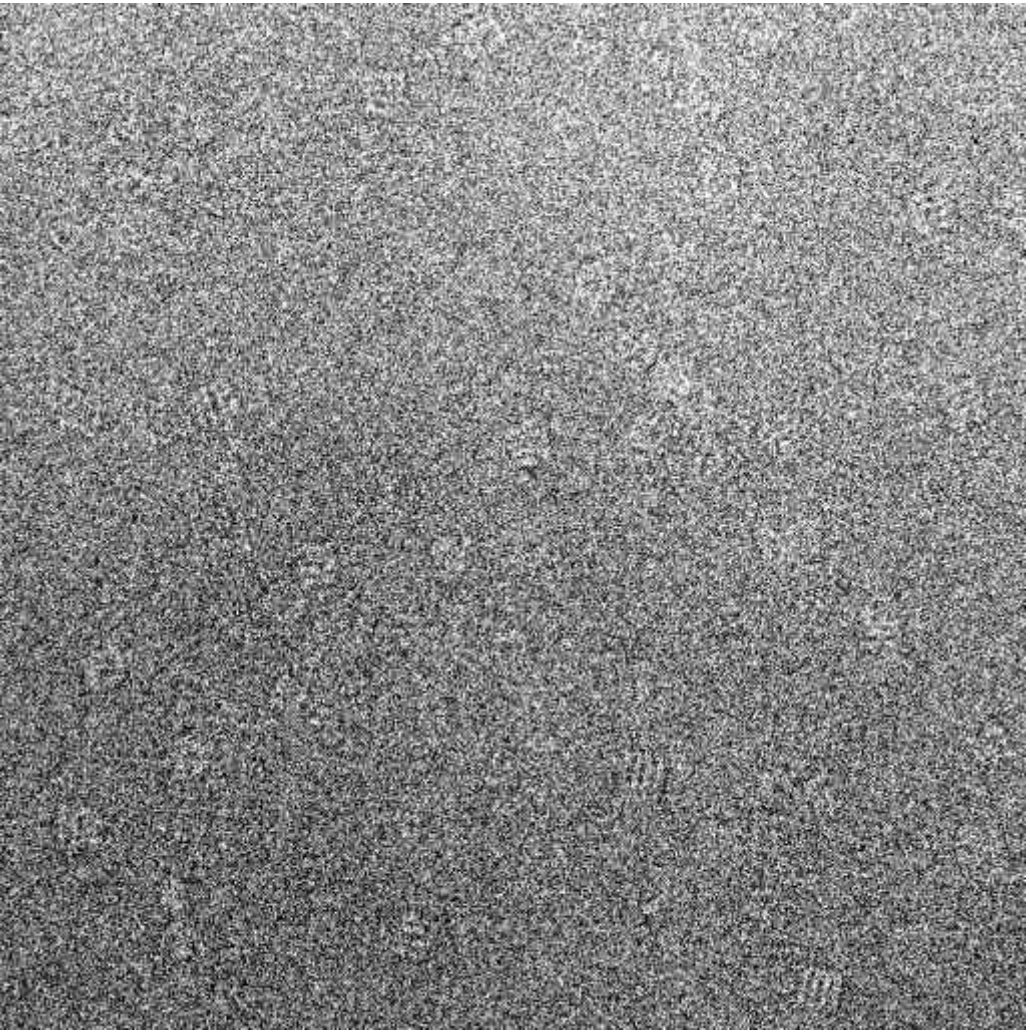


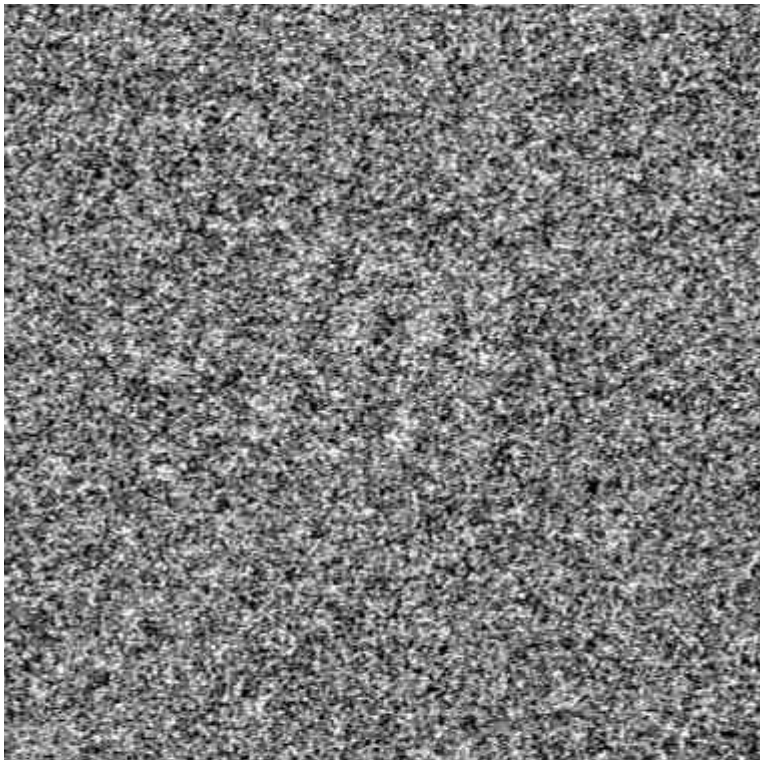
30,000 particles, JEOL 2010F

2005

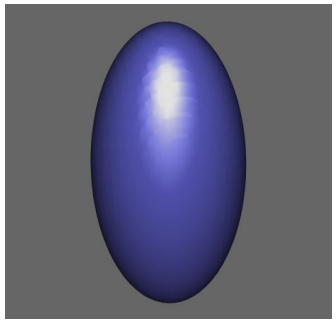

Fourier Shell Correlation
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1/50 1/20 1/12 1/8 1/6 1/5 1/4 1/3 1/2.2
Resolution (1/A)

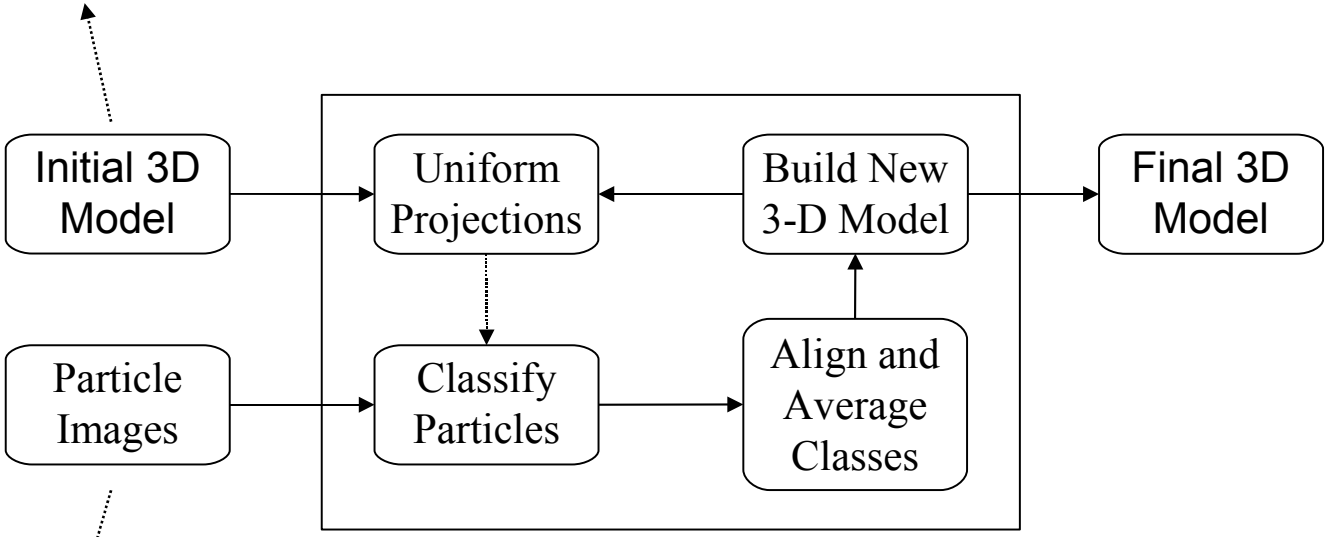
GroEL 4.5 A Animation Sequence

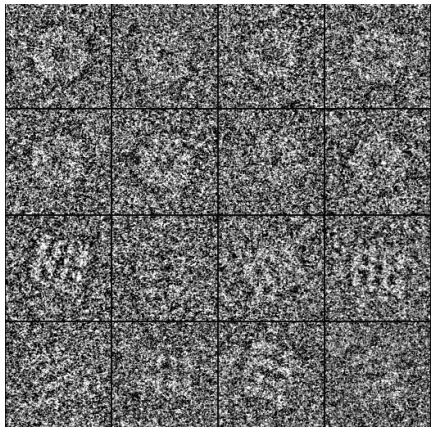


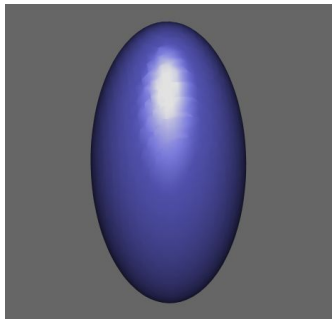


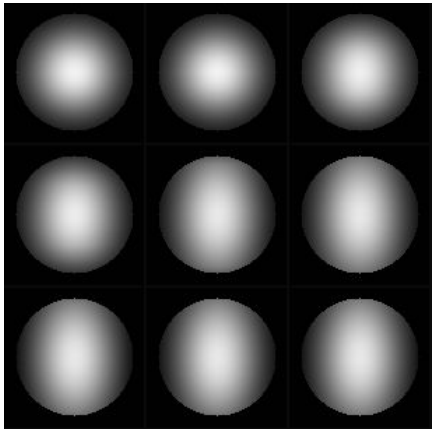
Jeol 3000
7 Days of imaging, 910 micrographs
1.06 Å/pix, Nikon 9000 scanner
135 used, 34,868 particles




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







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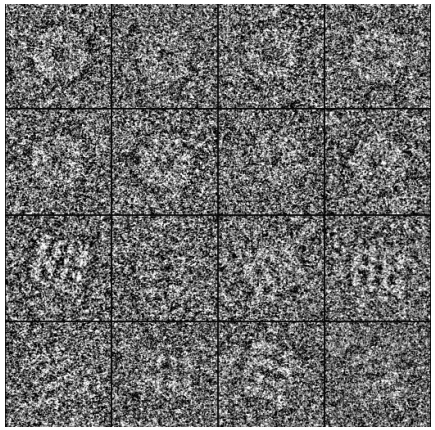

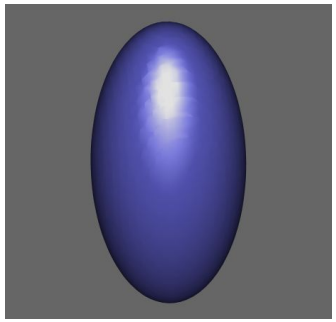

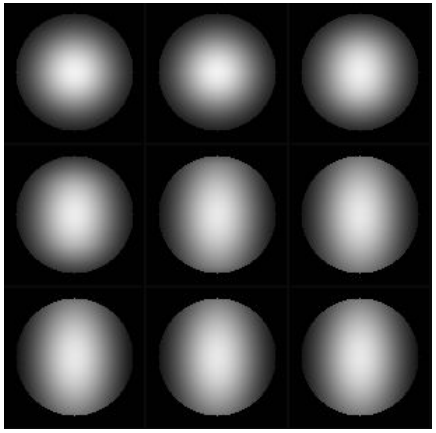

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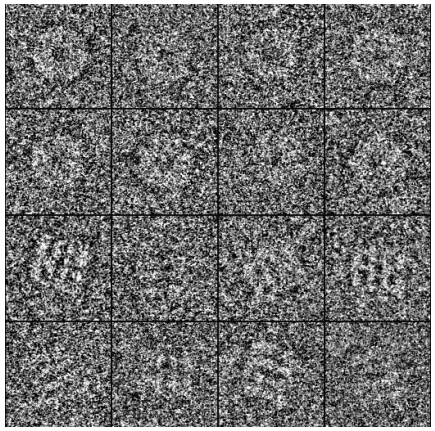

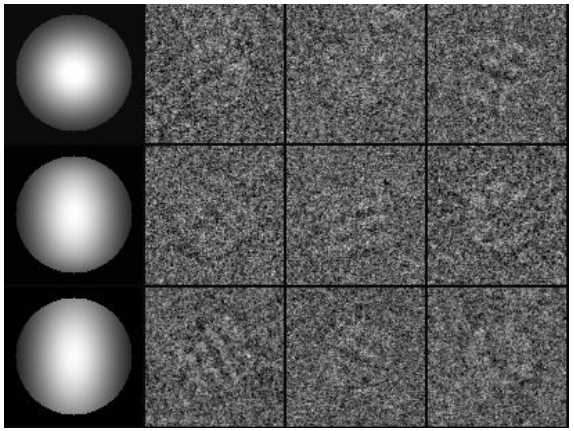

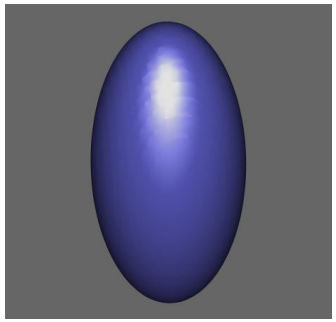

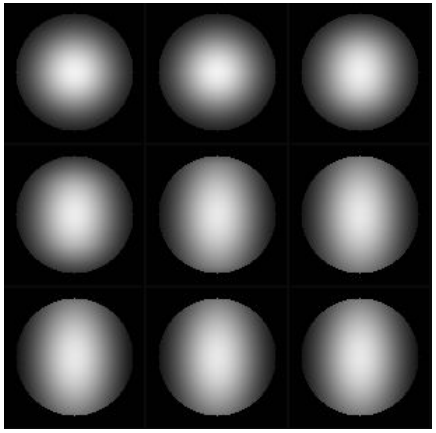

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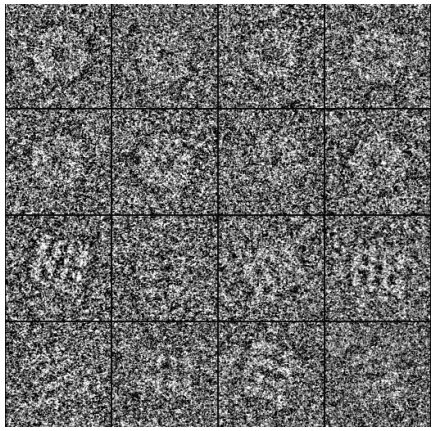

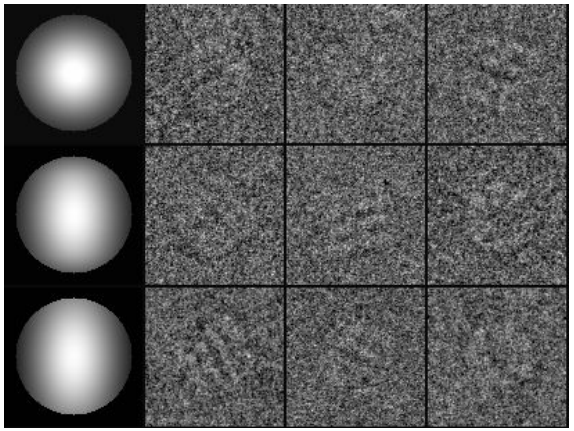

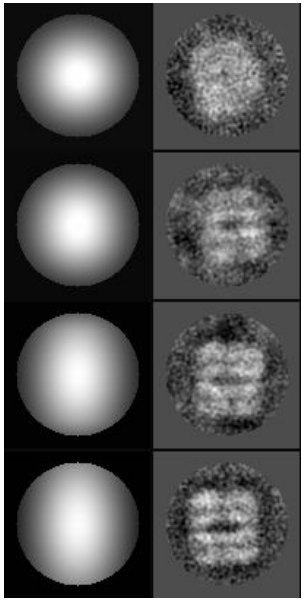

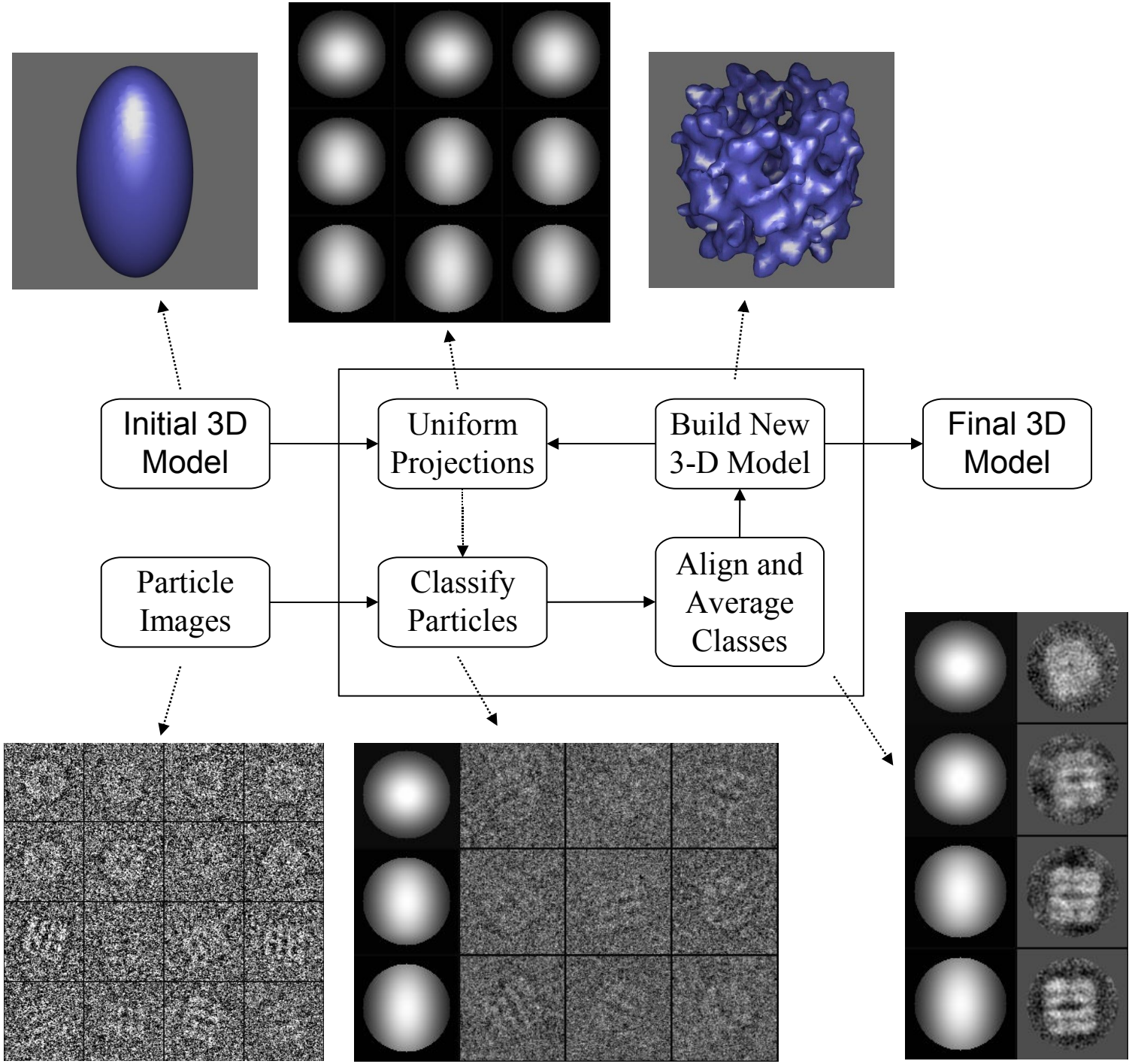
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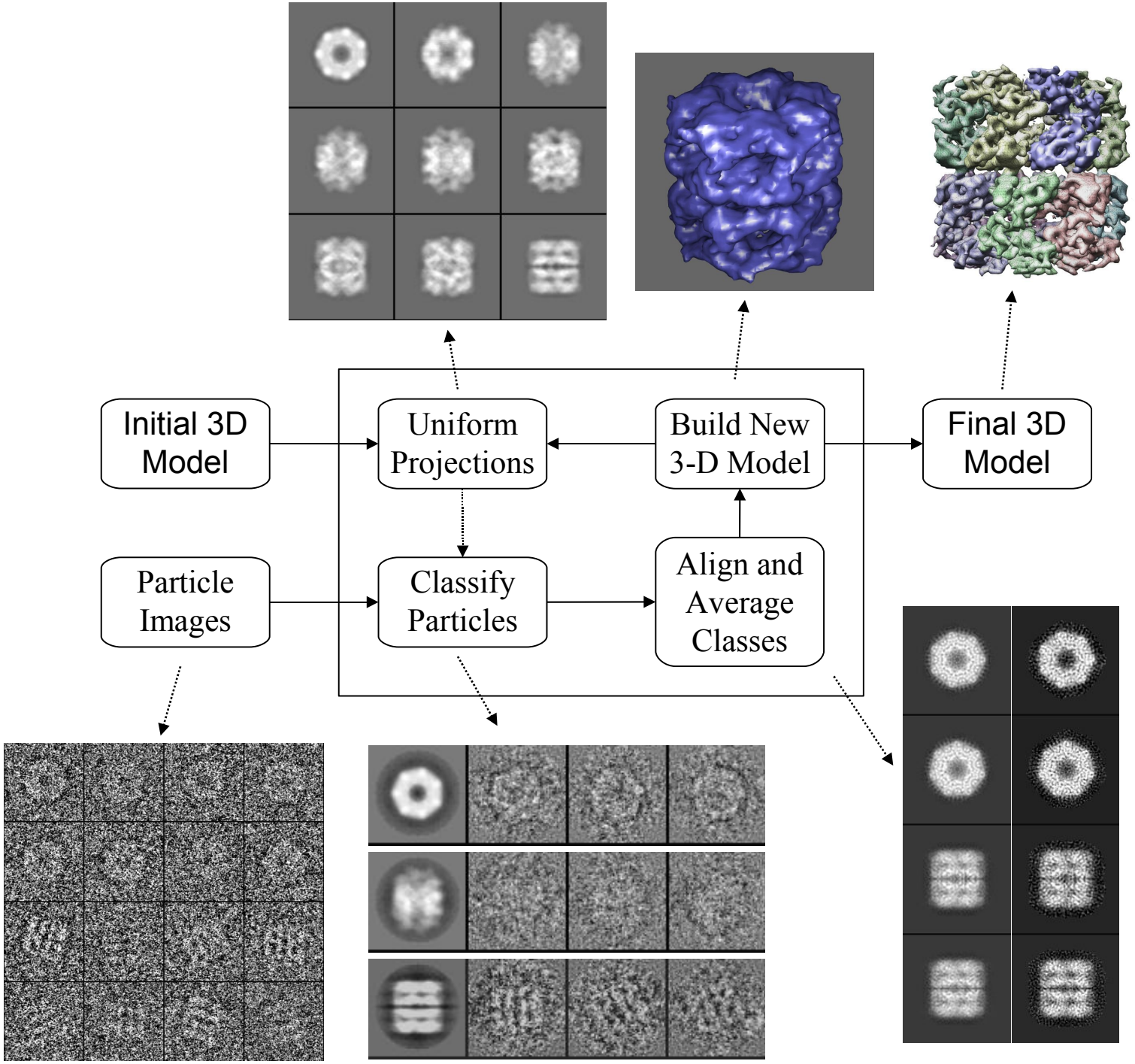
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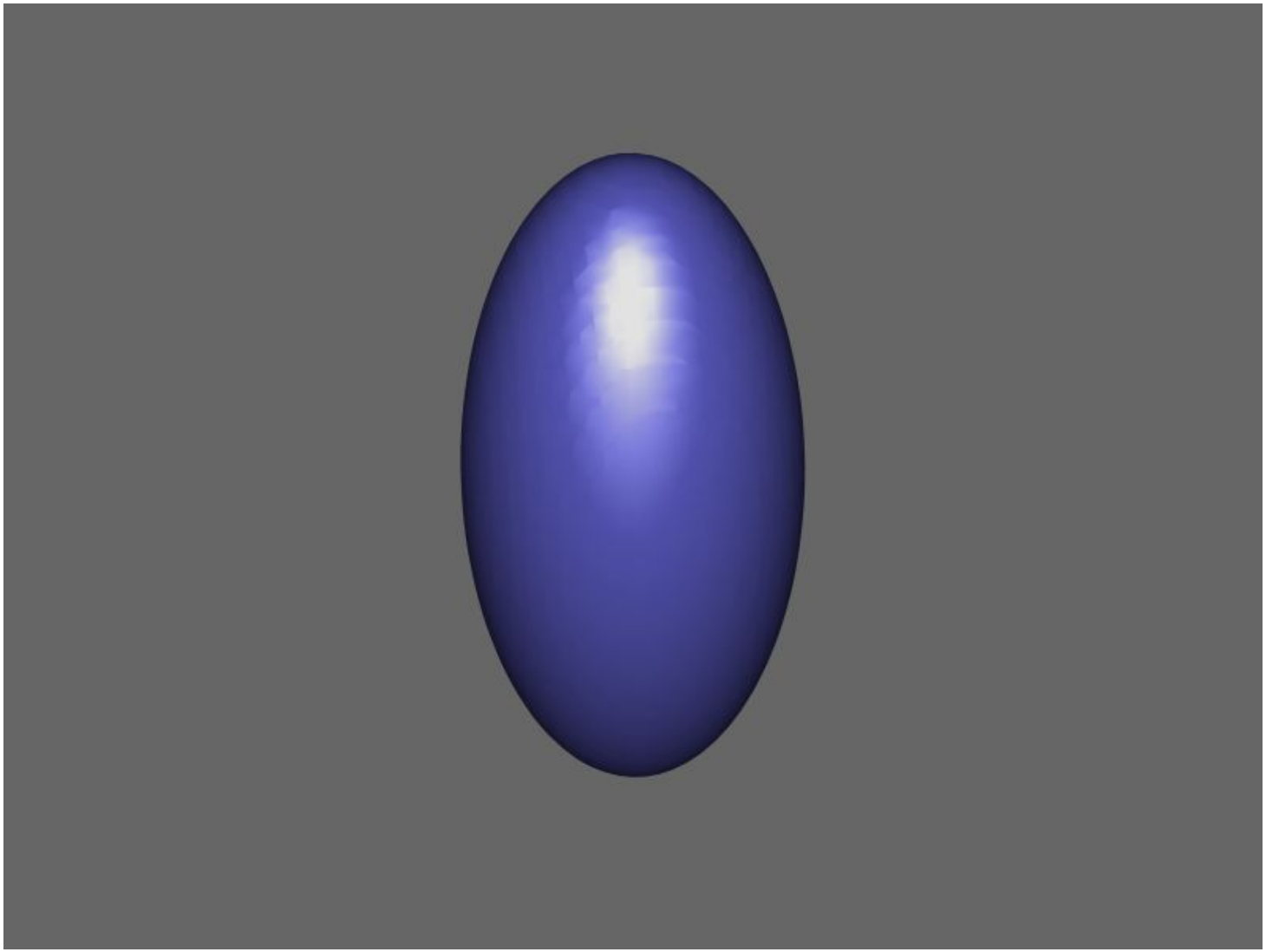
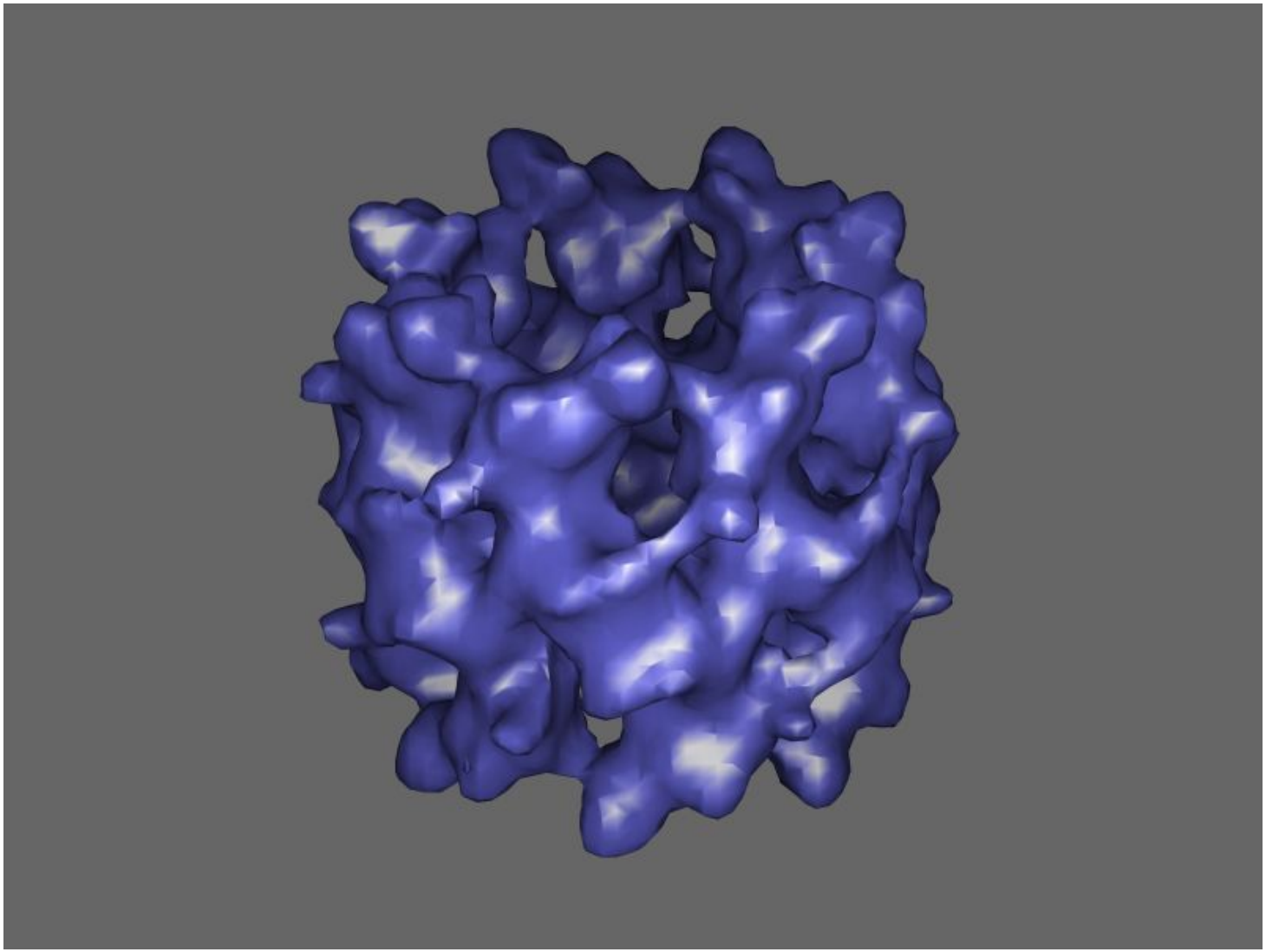
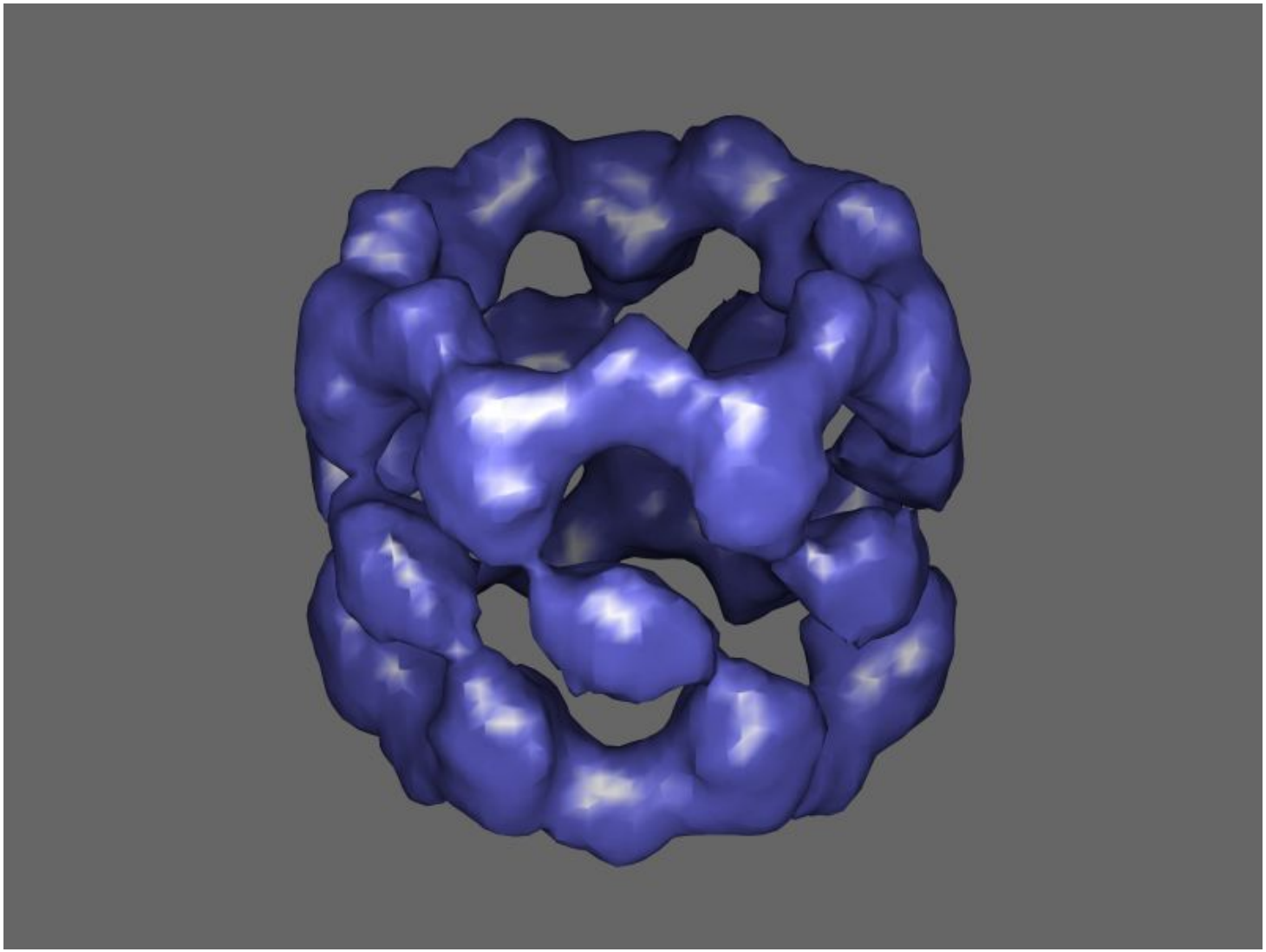
Refine from Gaussian Ellipsoid



Iteration 1



Iteration 2



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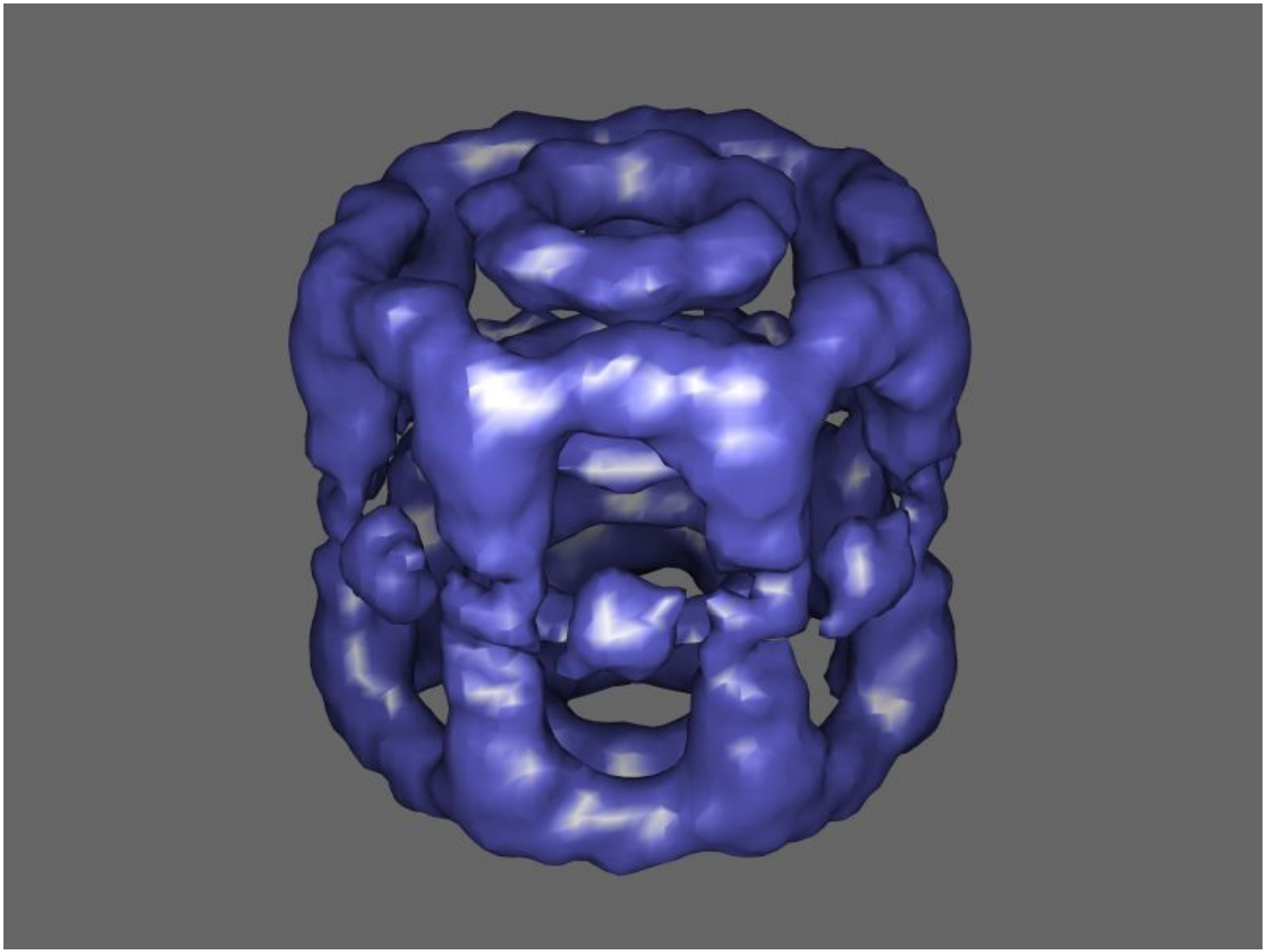

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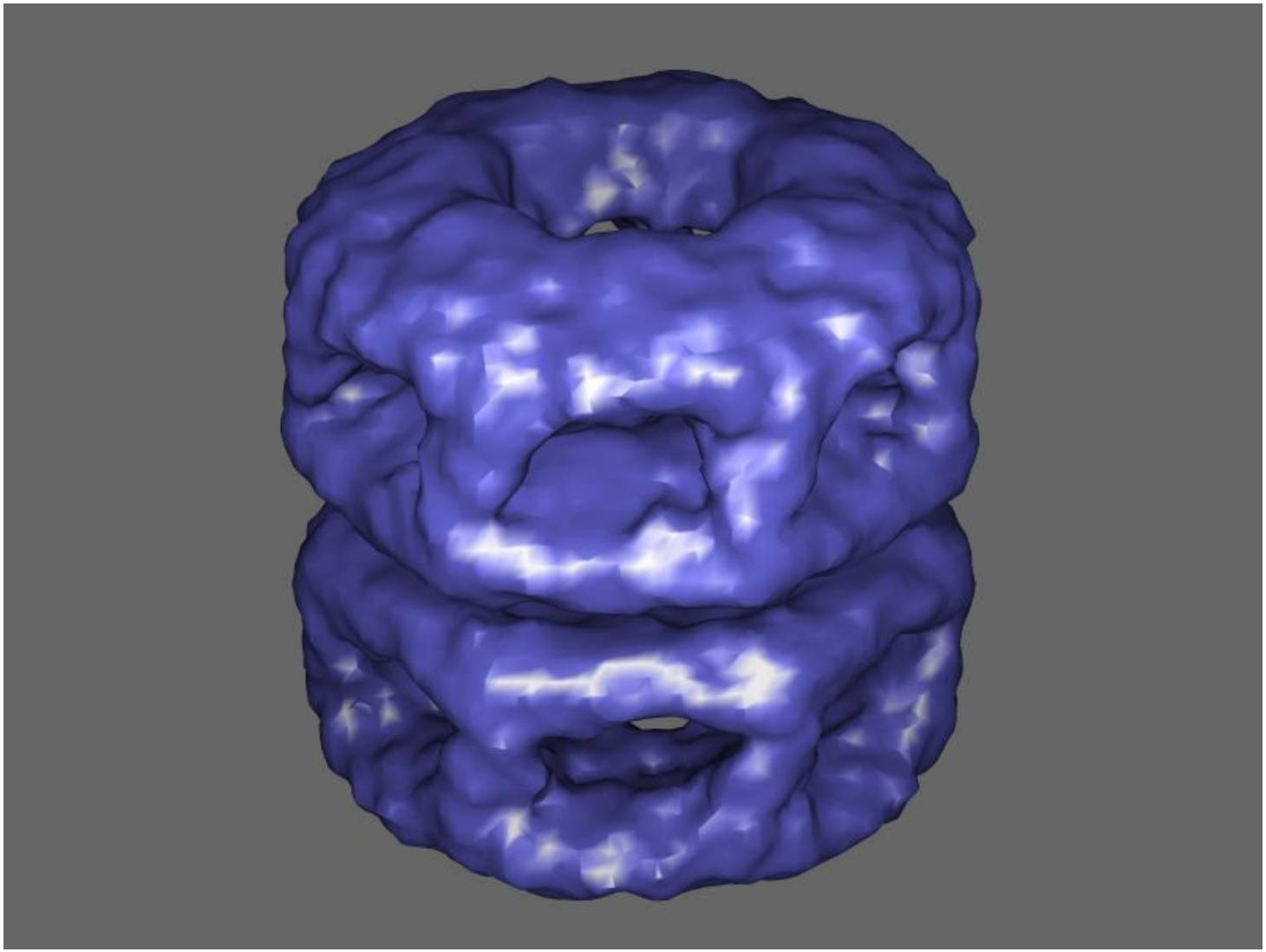
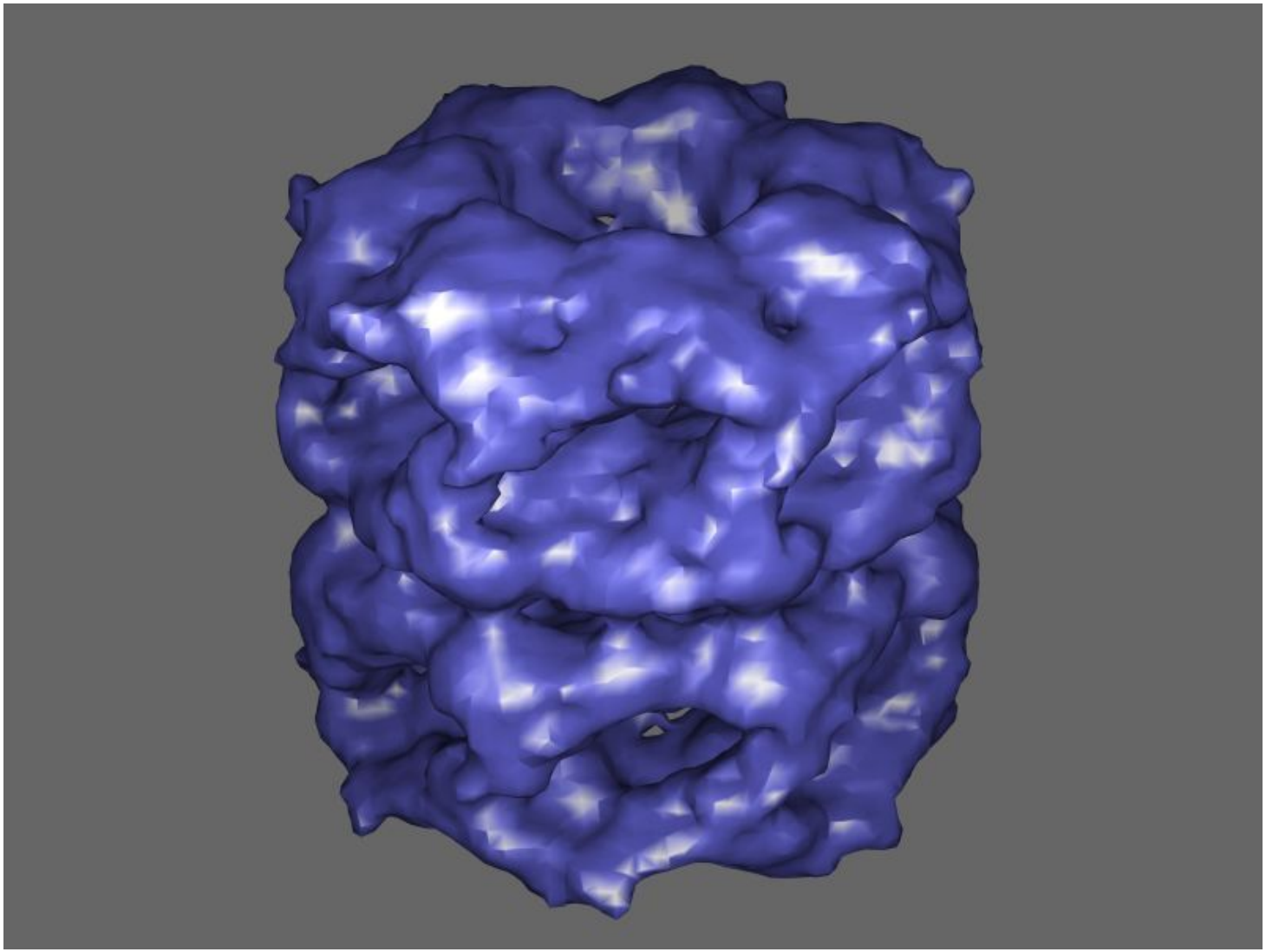


Iteration 5



How do we get to Higher Resolutions?

- Get a better microscope

How do we get to Higher Resolutions?

- Get a better microscope
- Find a better microscopist

How do we get to Higher Resolutions?

- Get a better microscope
- Find a better microscopist
- Algorithm Improvements

Contrast Transfer Function

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

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

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

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

$$\left|N^2\right| = n_1 e^{n_2 s + n_3 s^2 + n_4\sqrt{s}}$$

$$M(s)^2 = F(s)^2 C(s)^2 E(s)^2 + N(s)^2$$

8 Parameters

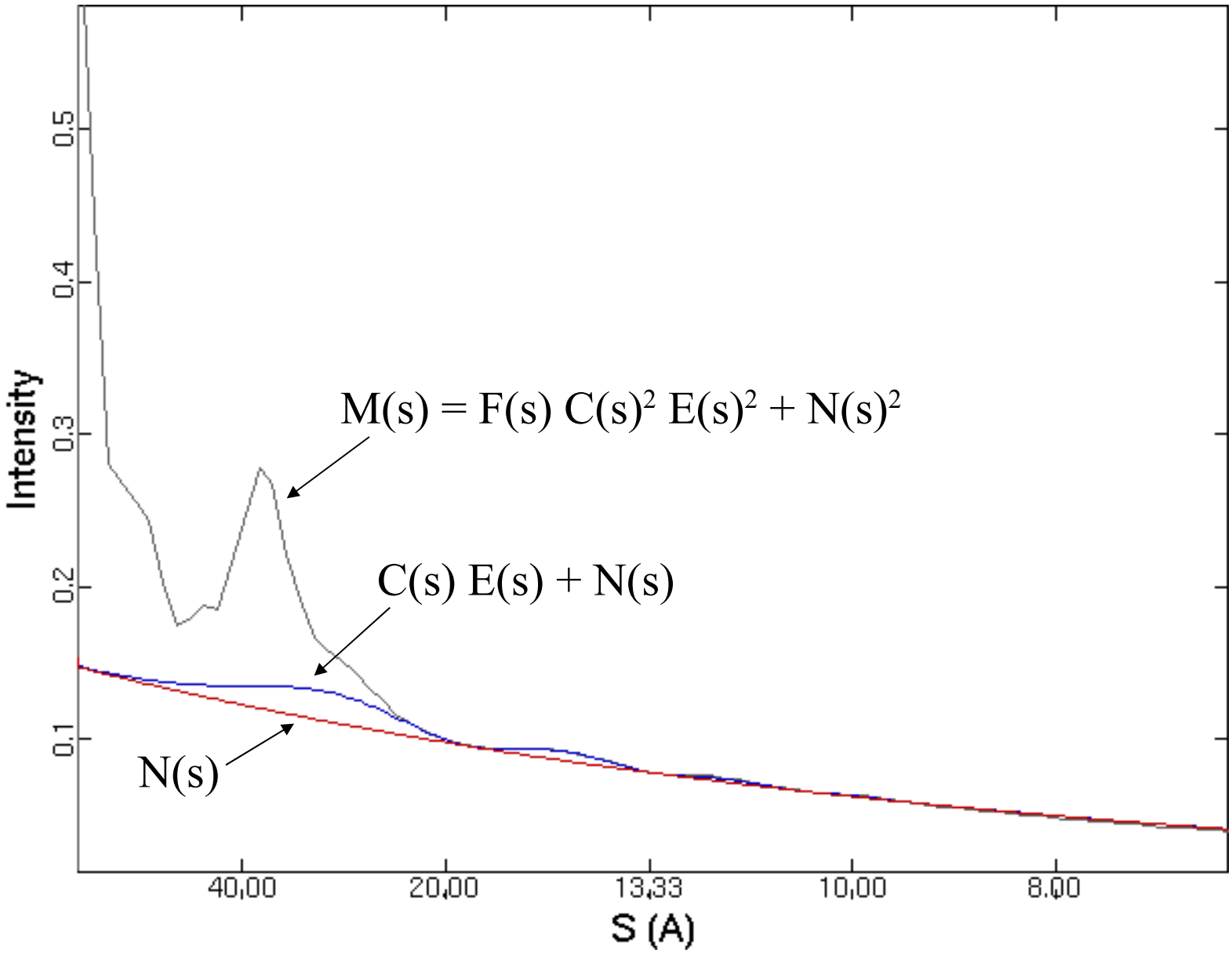
ΔZ - Defocus

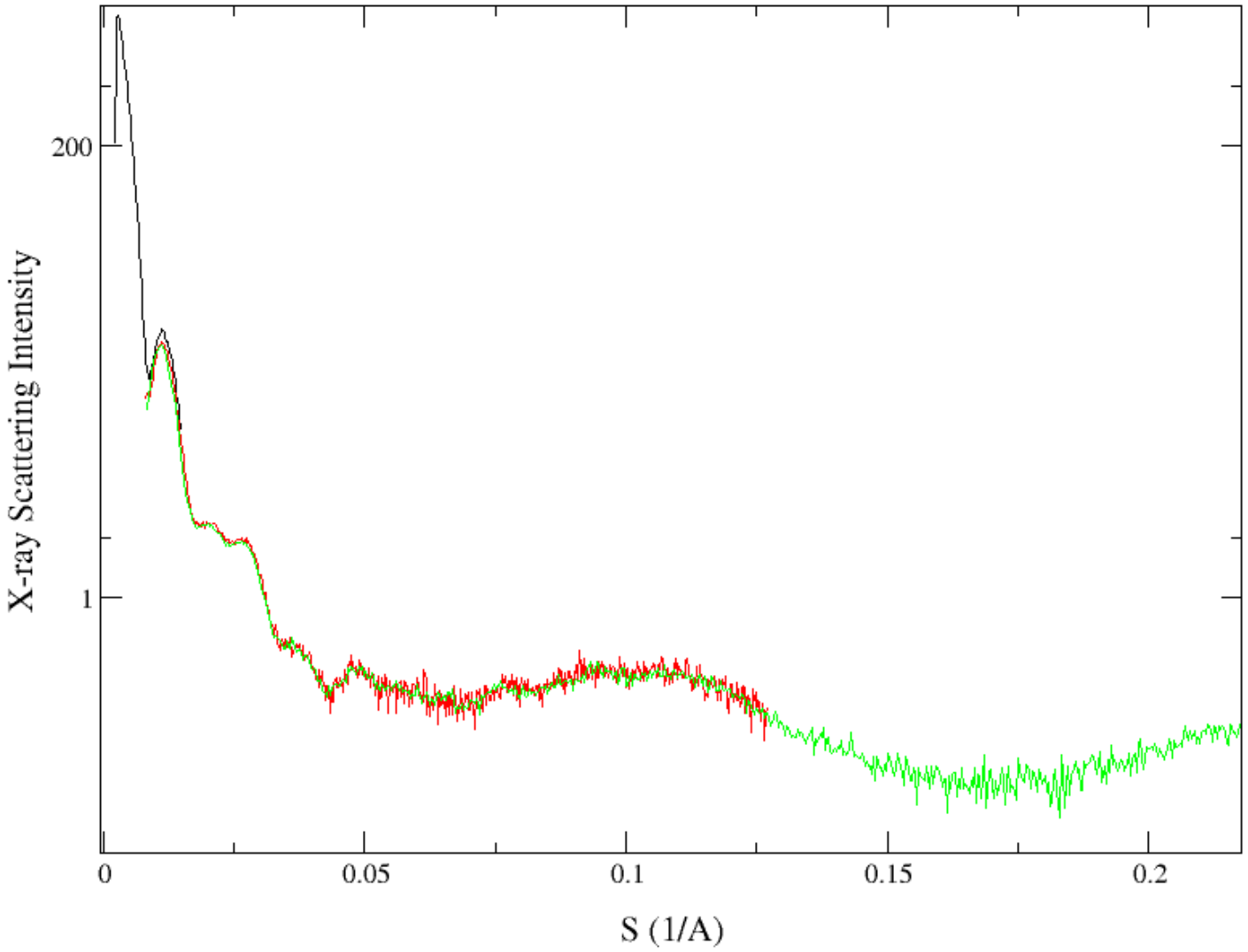
Q - Amplitude Contrast

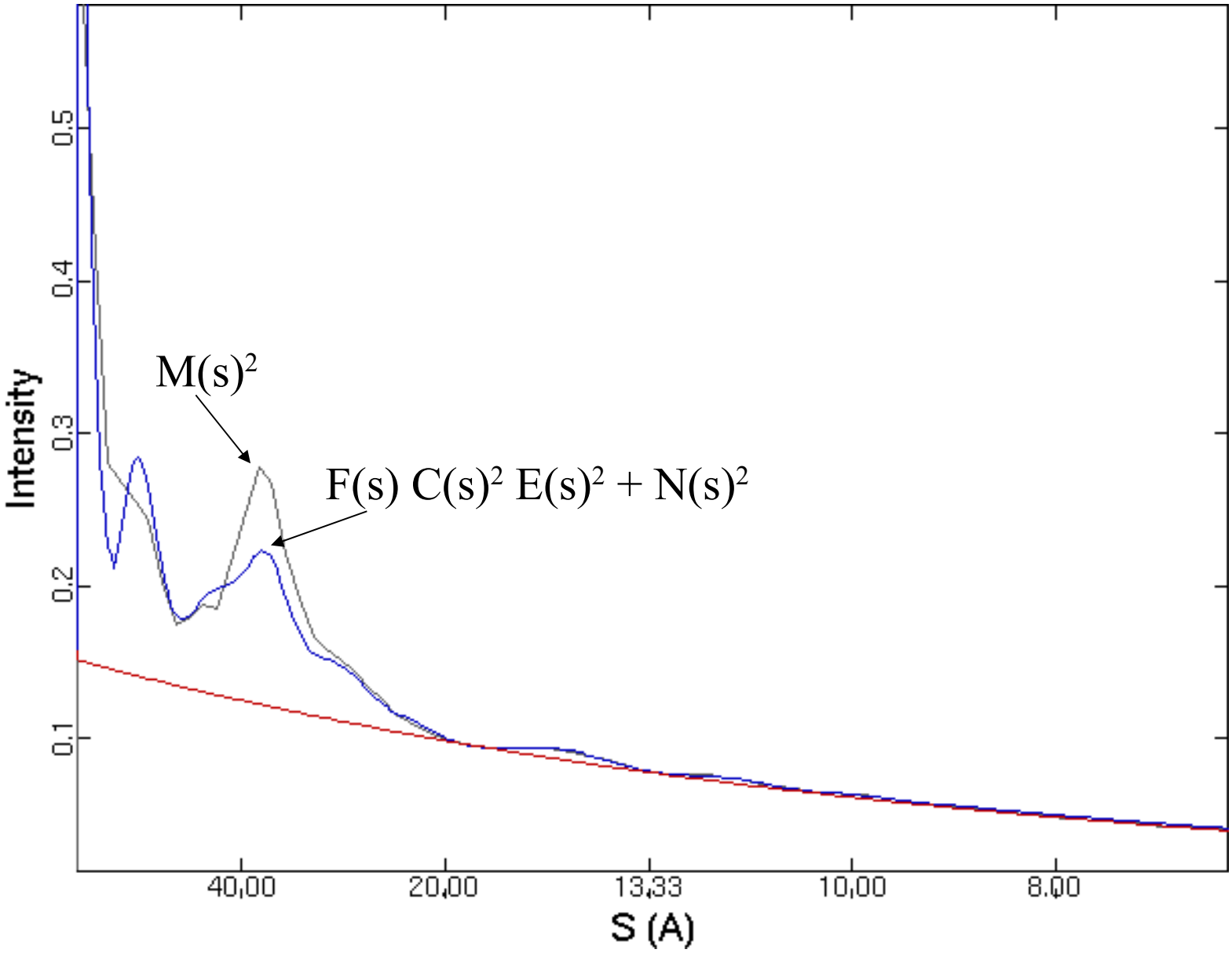
B - Gaussian Envelope Width

k - Signal Amplitude

$n_{1\text{-}4}$ - Noise Parameters


Intensity
0.5
0.4
0.3
0.2
0.1
M(s) = F(s) C(s)² E(s)² + N(s)²
C(s) E(s) + N(s)
N(s)
40,00
20,00
13,33
10,00
8,00
S (A)


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

$$\overline{T}(s,\theta)=\sum_i k_i \overline{M}_i(s,\theta)$$

$$k_i=?$$

- Maximize SNR of *T(s,q)*
- Minimize variance between *T(s,q)* and *F(s,q)*

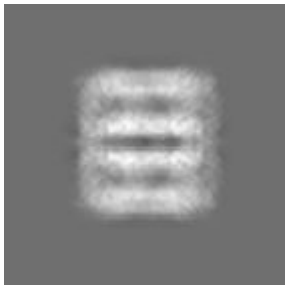
CTF Correction

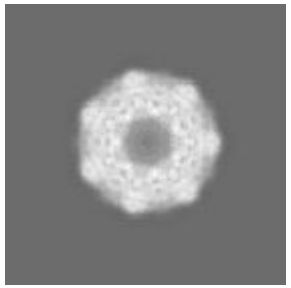
Wiener Filter | CTF Correction | SNR Weight

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

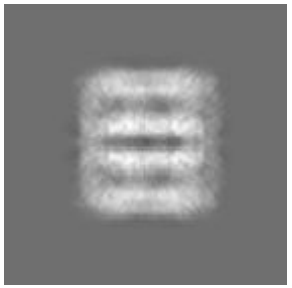
$$R_i(s)=\frac{C_i^2(s)E_i^2(s)}{N_i^2(s)} \qquad R(s)=\sum_i \frac{C_i^2(s)E_i^2(s)}{N_i^2(s)}$$

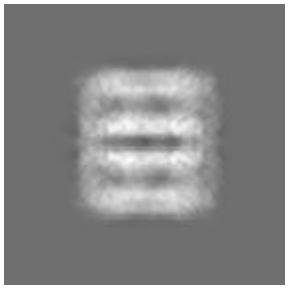
Image Classification

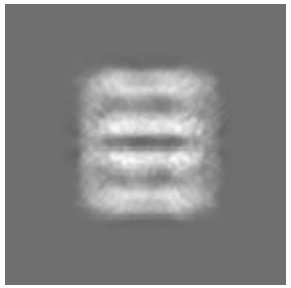




← ? →







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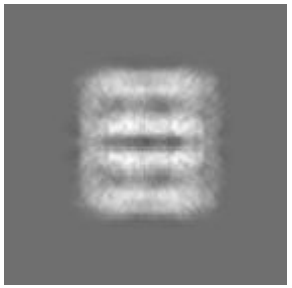

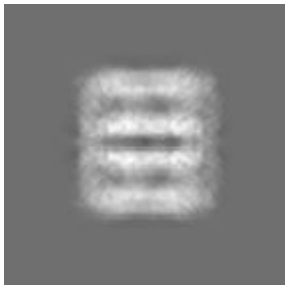

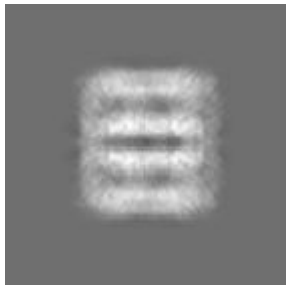

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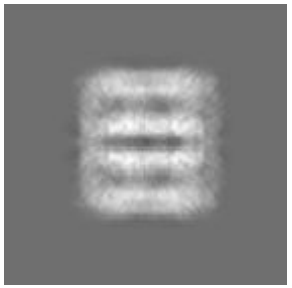

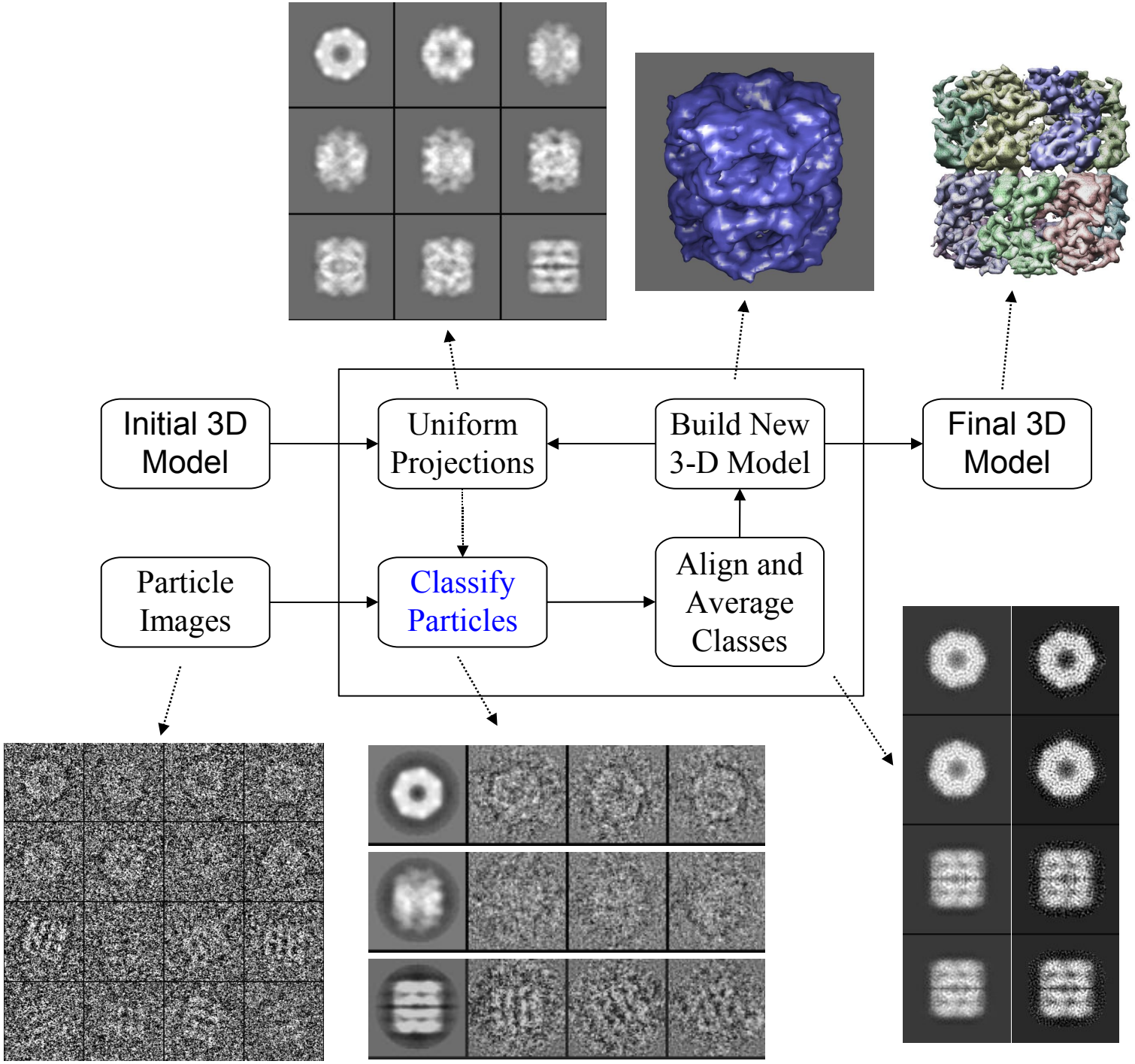
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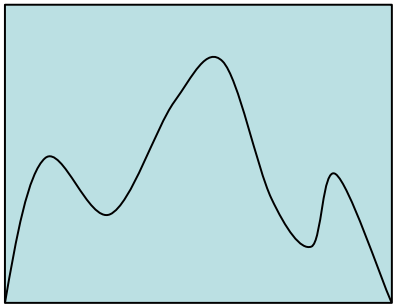
Alignment/Registration



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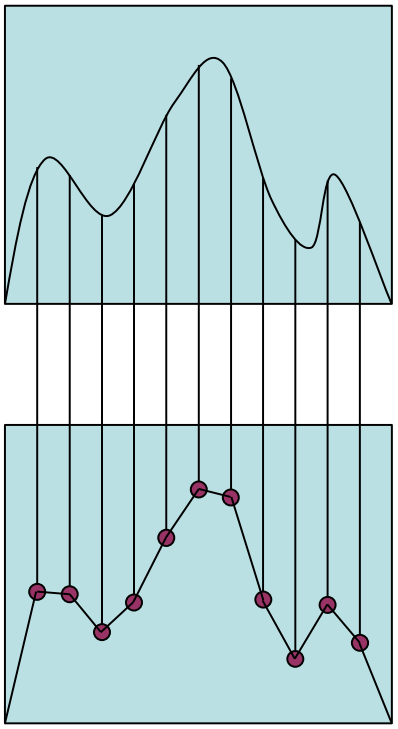

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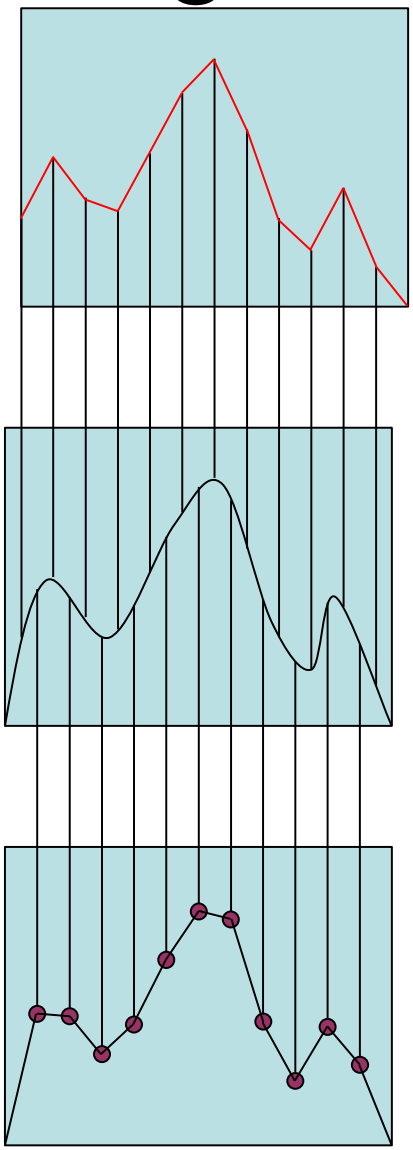

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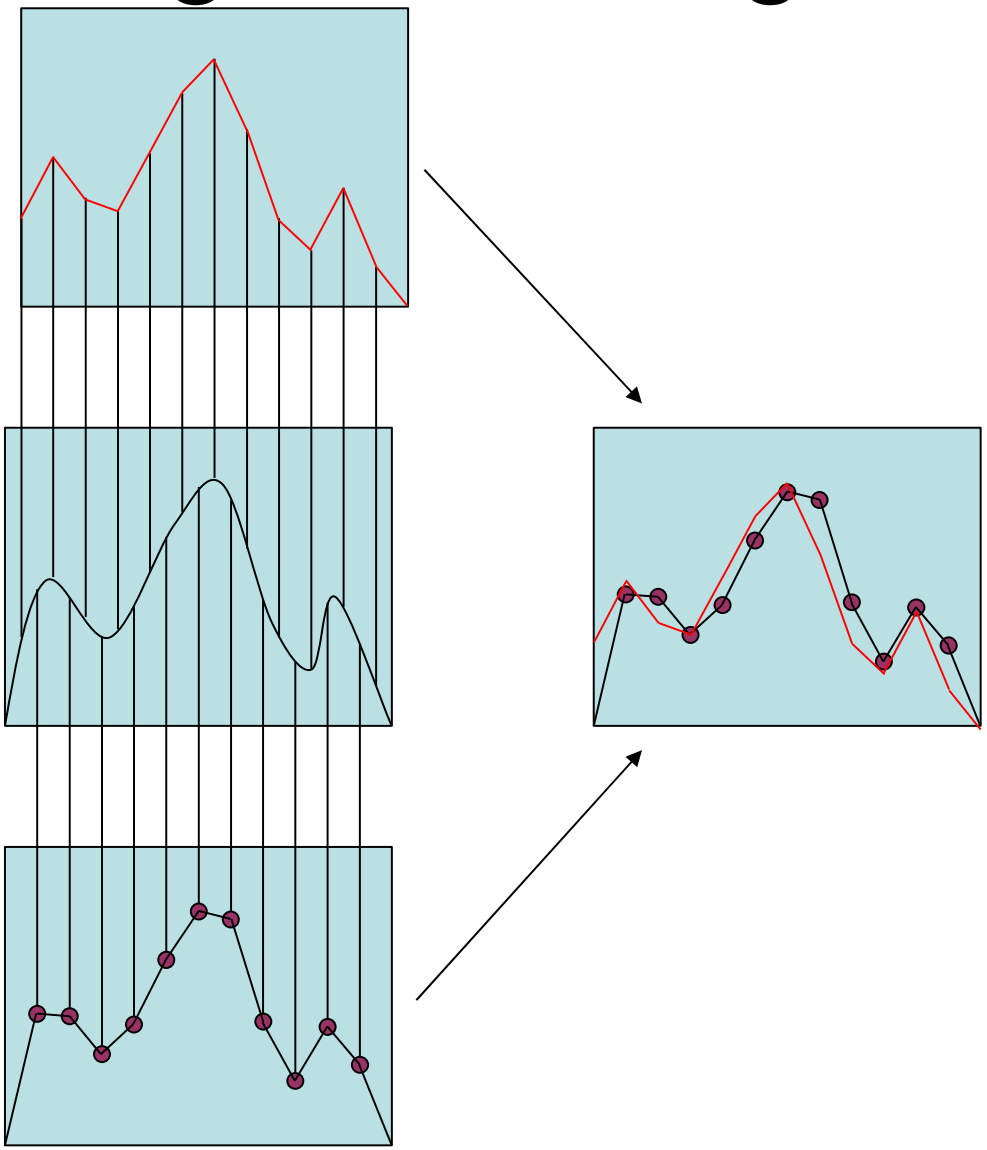

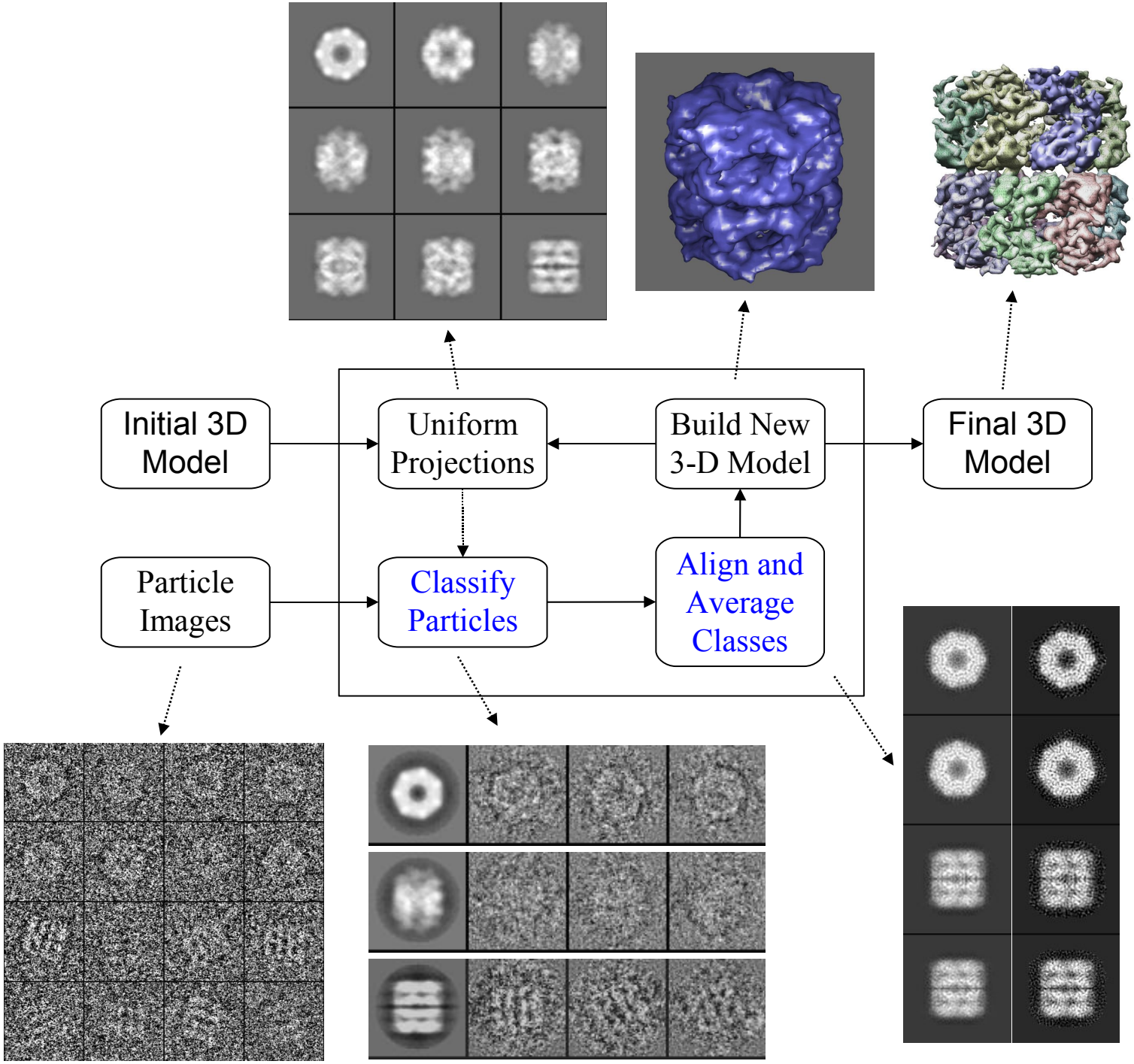
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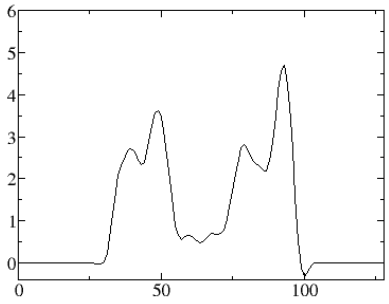
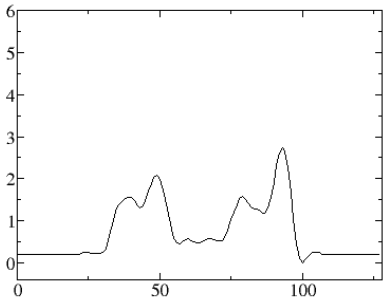
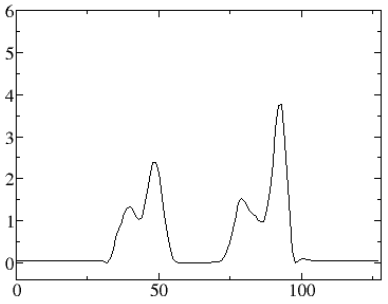
Measures of Similarity

- Correlation Coefficient
- Variance (transformed density)
- Variance (matched filter)
- Phase Residual
- Mutual Information
- etc.


(
-
)2 =


6
5
4
3
2
1
0
0
50
100


6
5
4
3
2
1
0
0
50
100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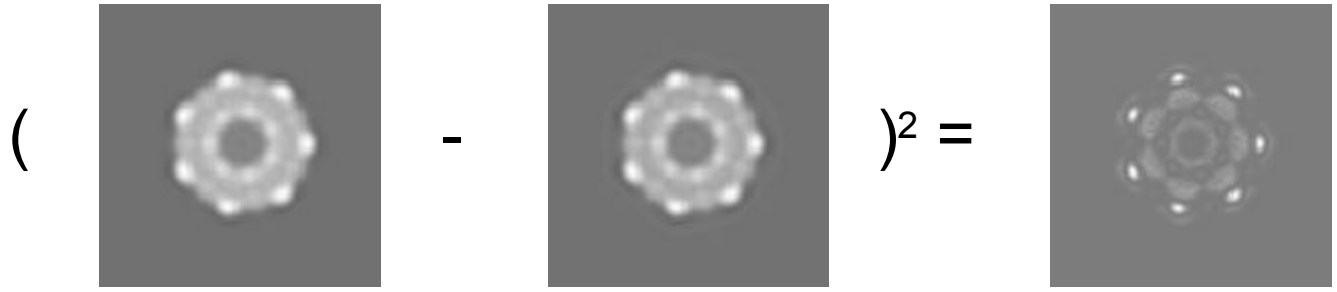
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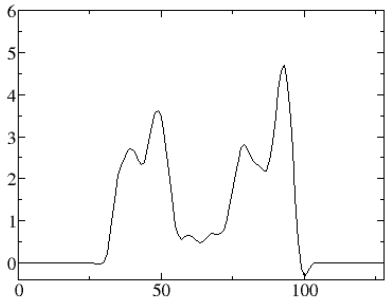
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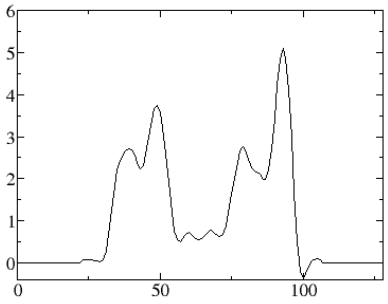
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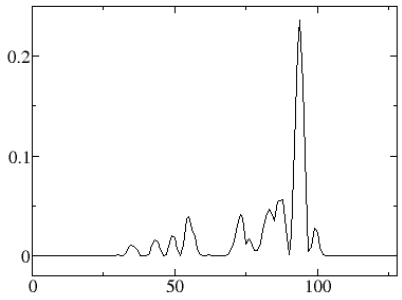
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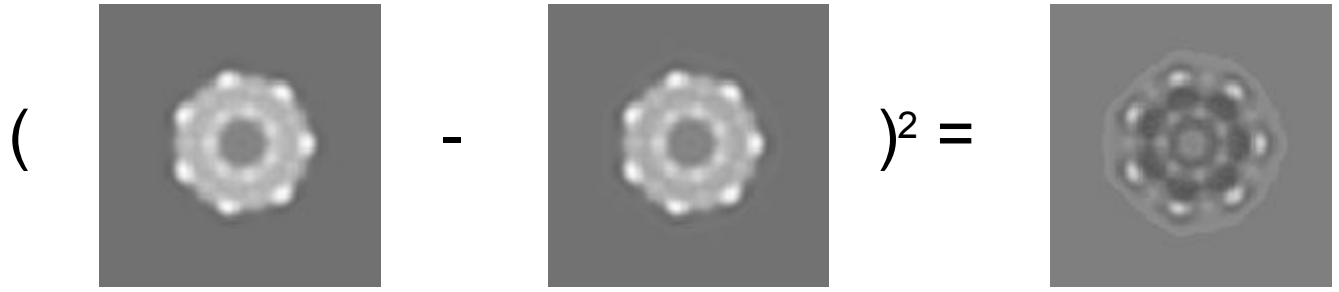
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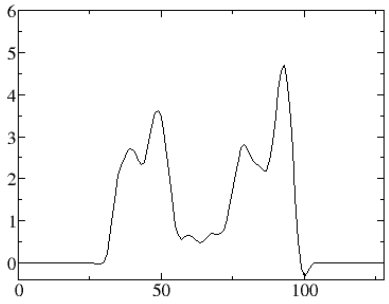
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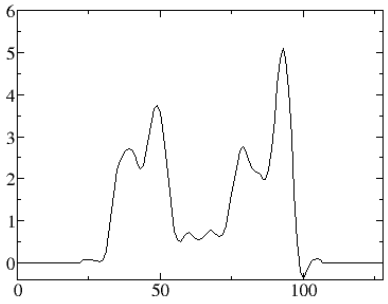
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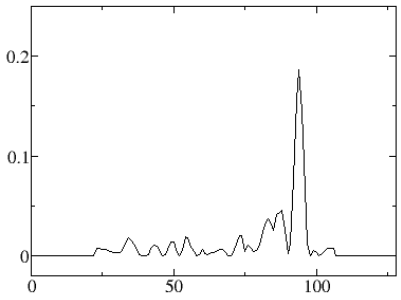
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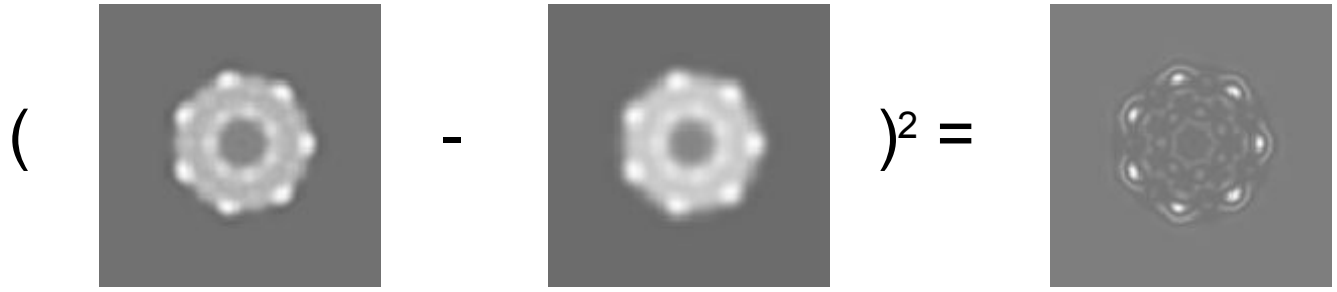
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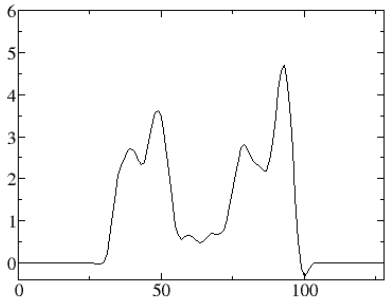
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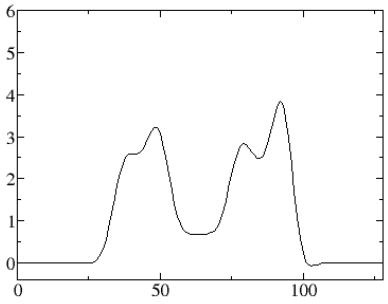
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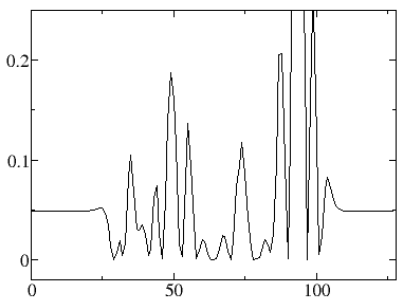
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0.1
0
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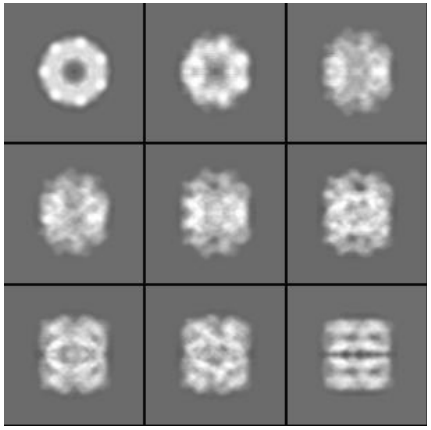
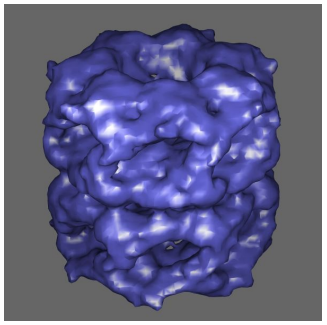
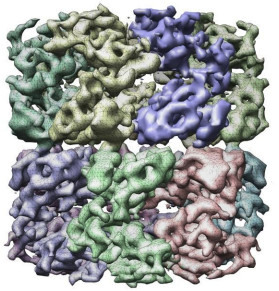
And the Answer is…

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







Initial 3D Model

Uniform Projections

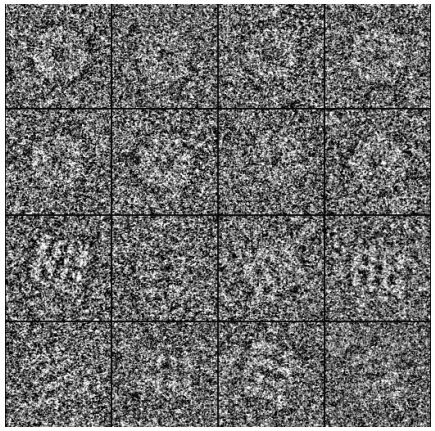
Build New 3-D Model

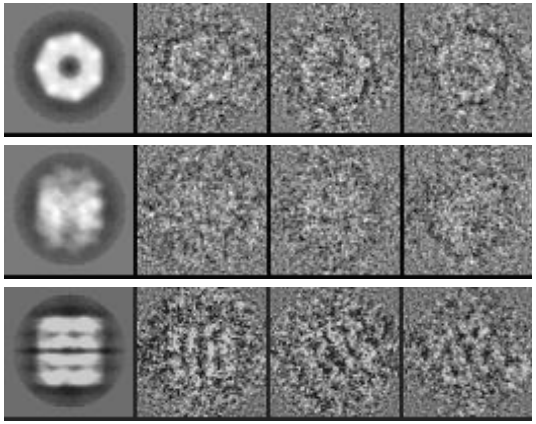
Final 3D Model

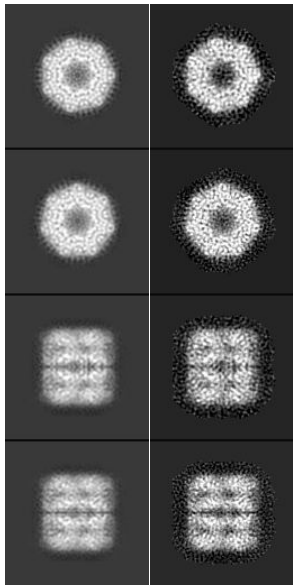
Particle Images

Classify Particles

Align and Average Classes



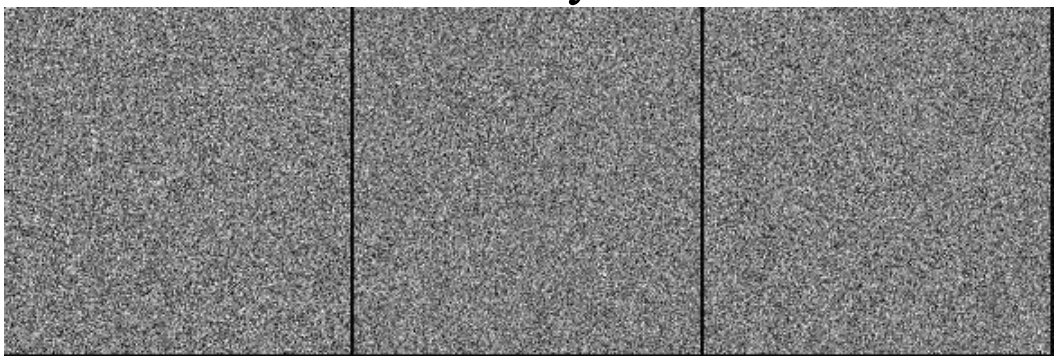




Model Bias

Base

Noisy



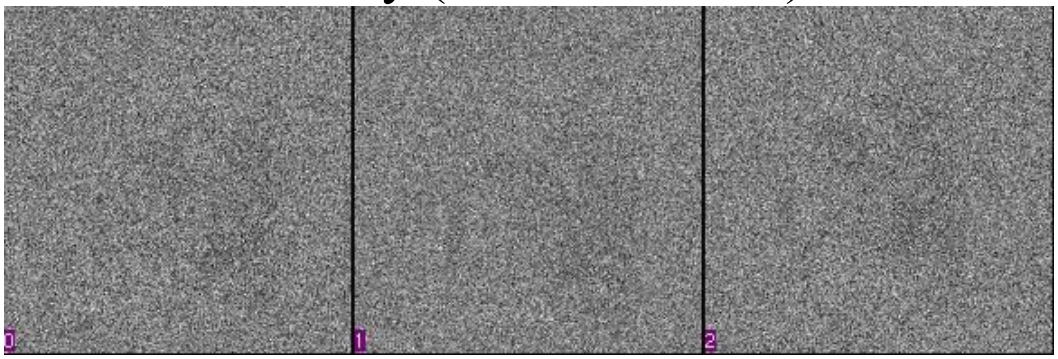
Align to

25 100 250 1000 2000

Model Bias

Base

Noisy (~10% contrast)



Align to

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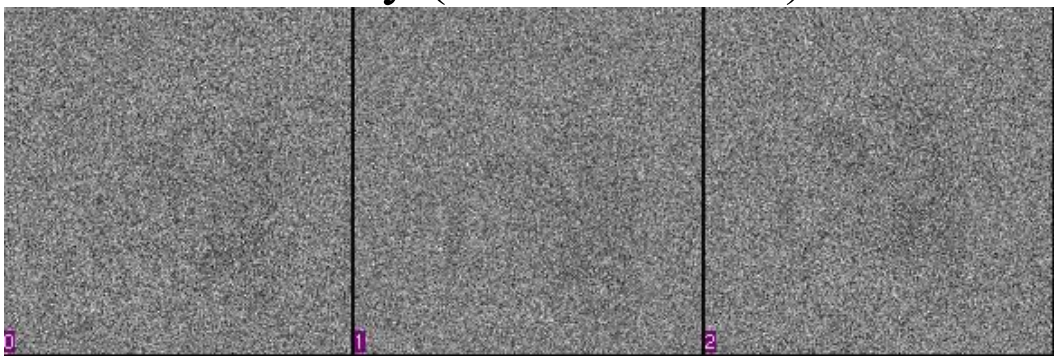
Model Bias

Base

Noisy (~10% contrast)



Align to

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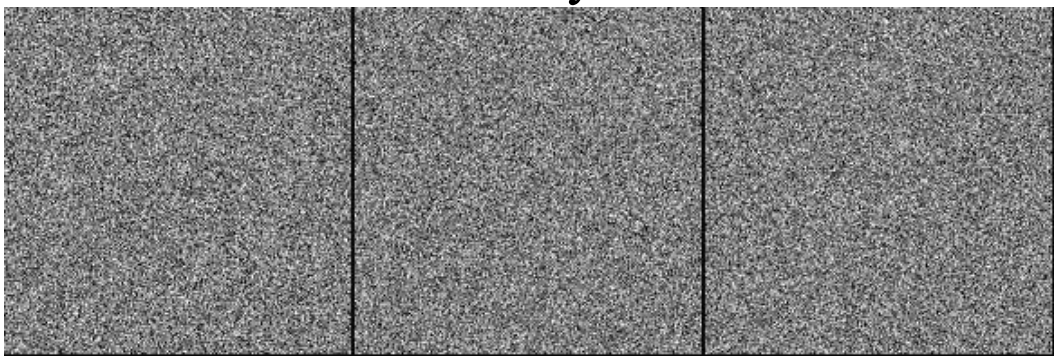
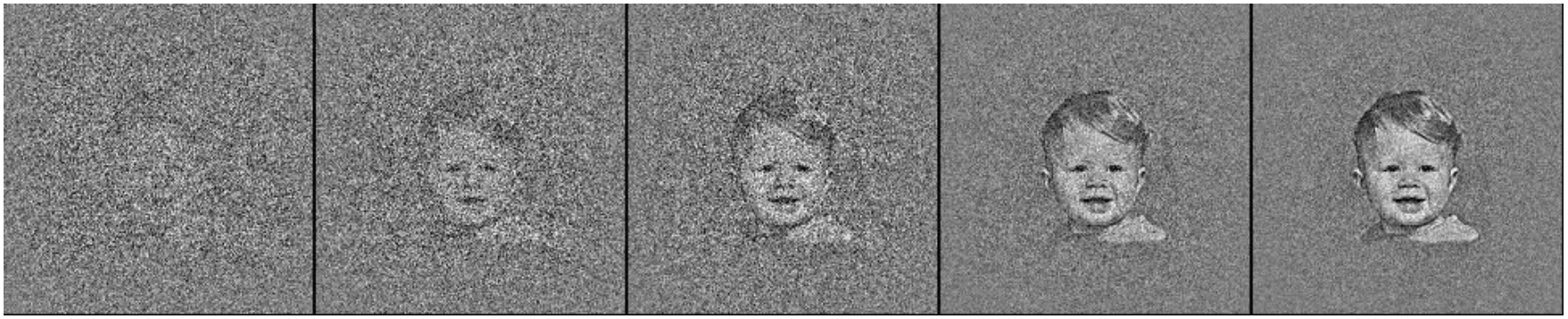
Model Bias

Base

Noisy



Align to



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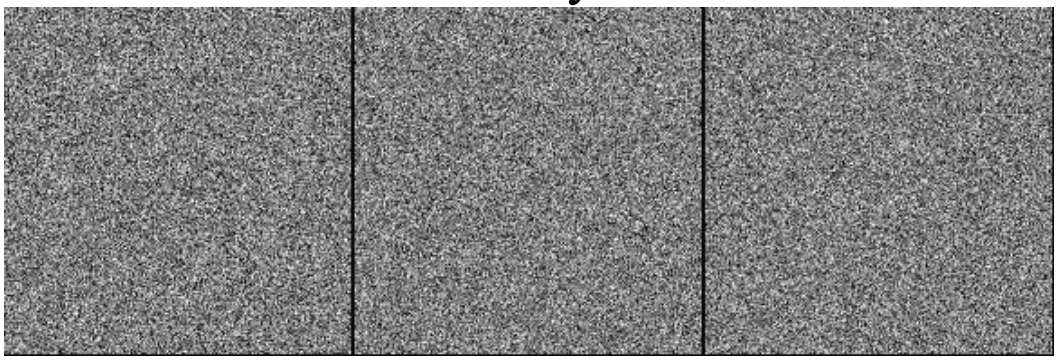
Model Bias

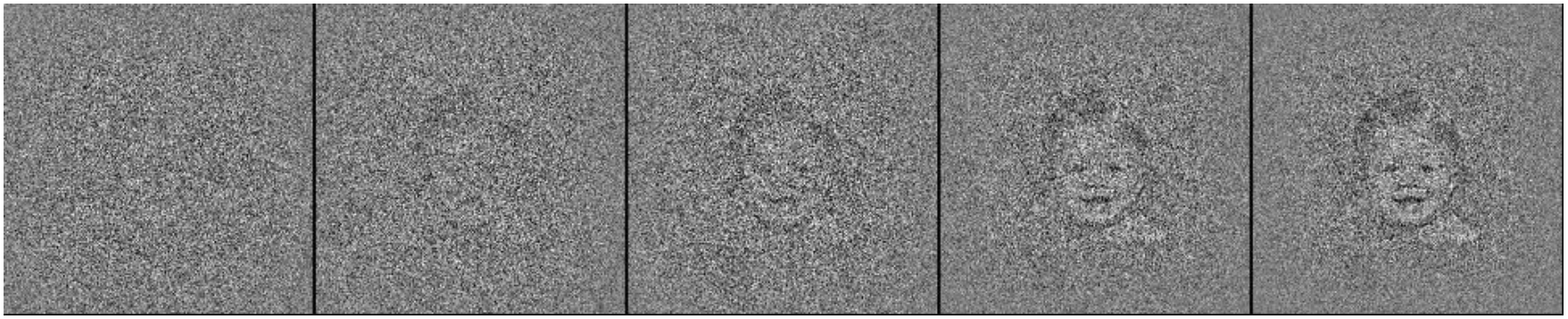
Base

Noisy



Align to

Iter x4



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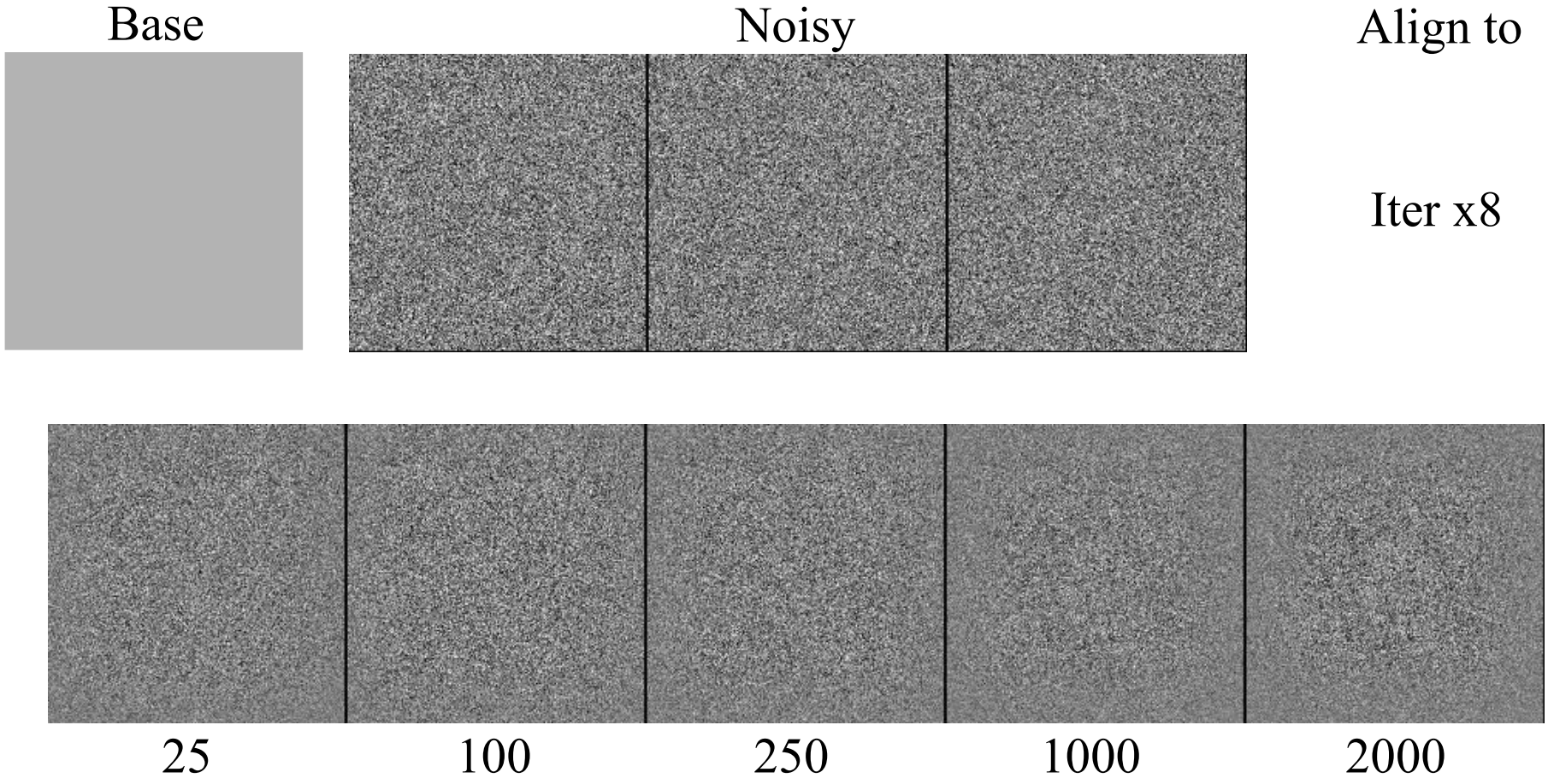
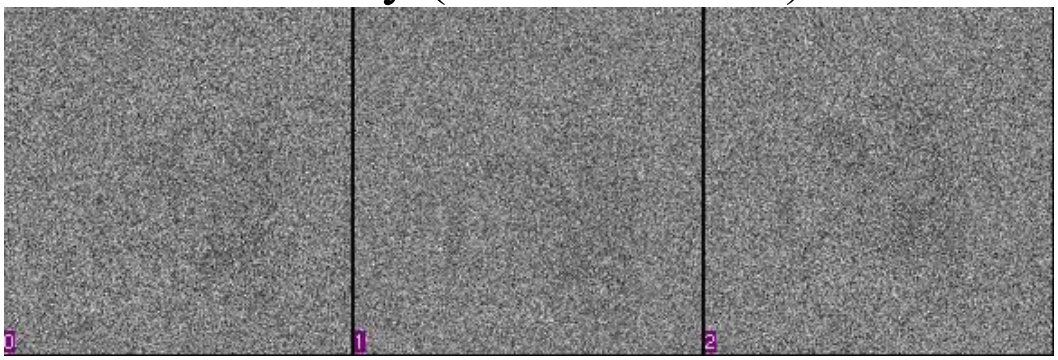
Model Bias



Model Bias

Base

Noisy (~10% contrast)



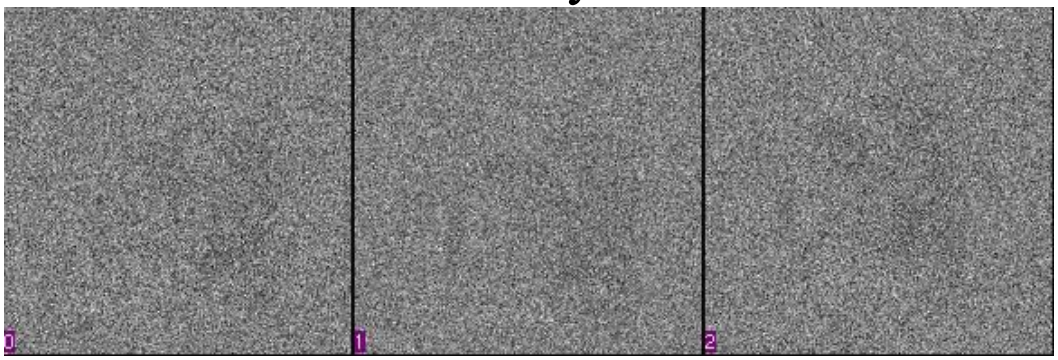
Align to

25 100 250 1000 2000

Model Bias

Base

Noisy



Align to

Iter x4

25 100 250 1000 2000

The Future

- EMAN2

- Better similarity criteria
- Improved CTF model
- Better 3-D reconstruction
- New refinement methodologies