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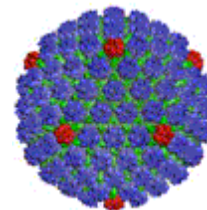
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# Single Particle Reconstruction part 2

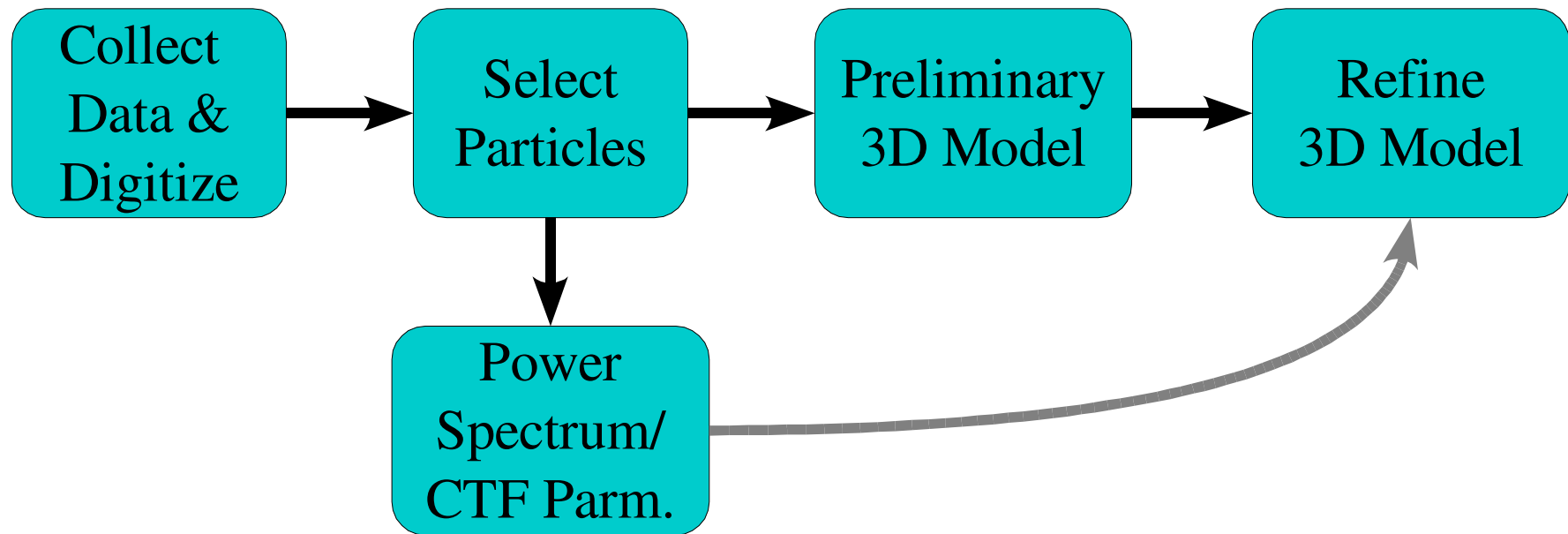
Steven Ludtke

National Center for Macromolecular Imaging  
Baylor College of Medicine

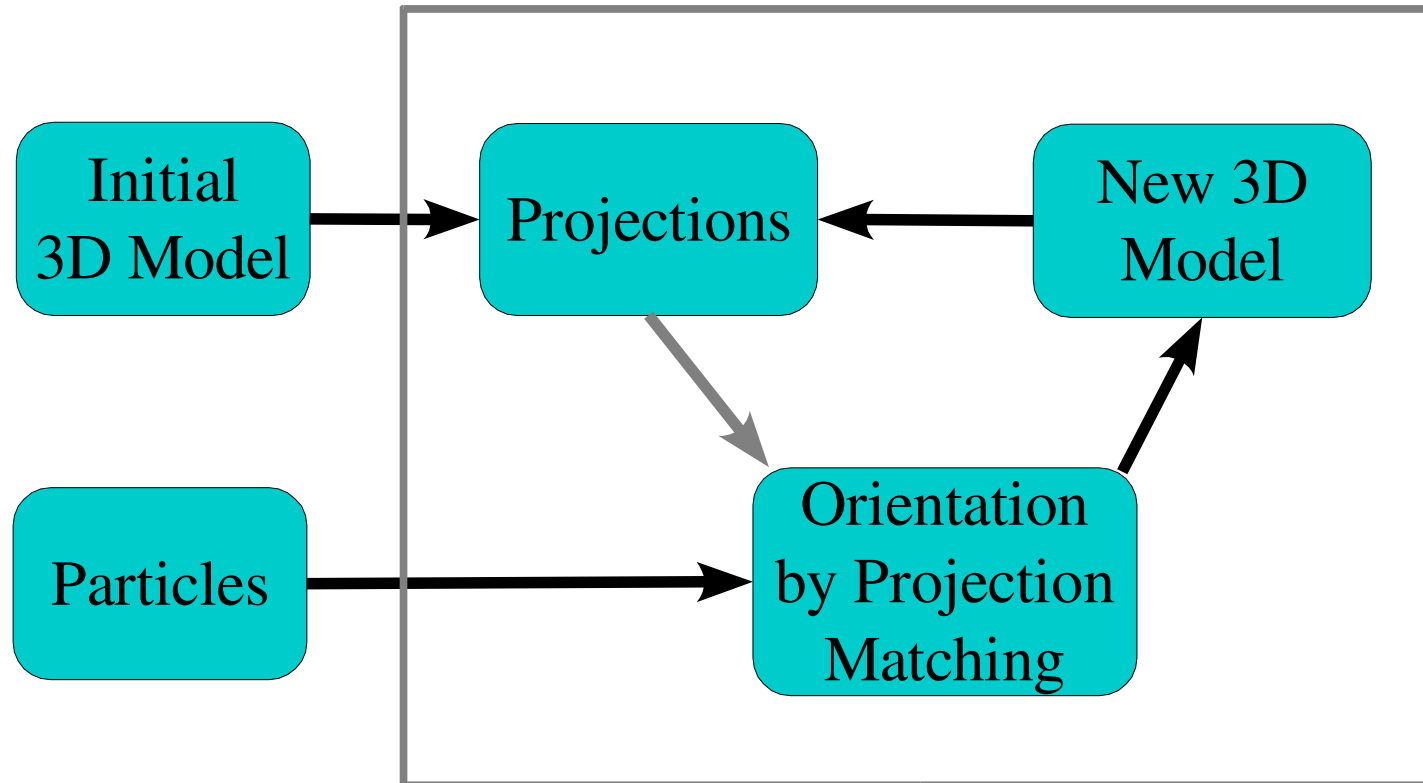
*Extensive video lectures and lecture notes on single particle analysis:  
[http://ncmi.bcm.tmc.edu/events/workshops/workshops\\_19](http://ncmi.bcm.tmc.edu/events/workshops/workshops_19)*



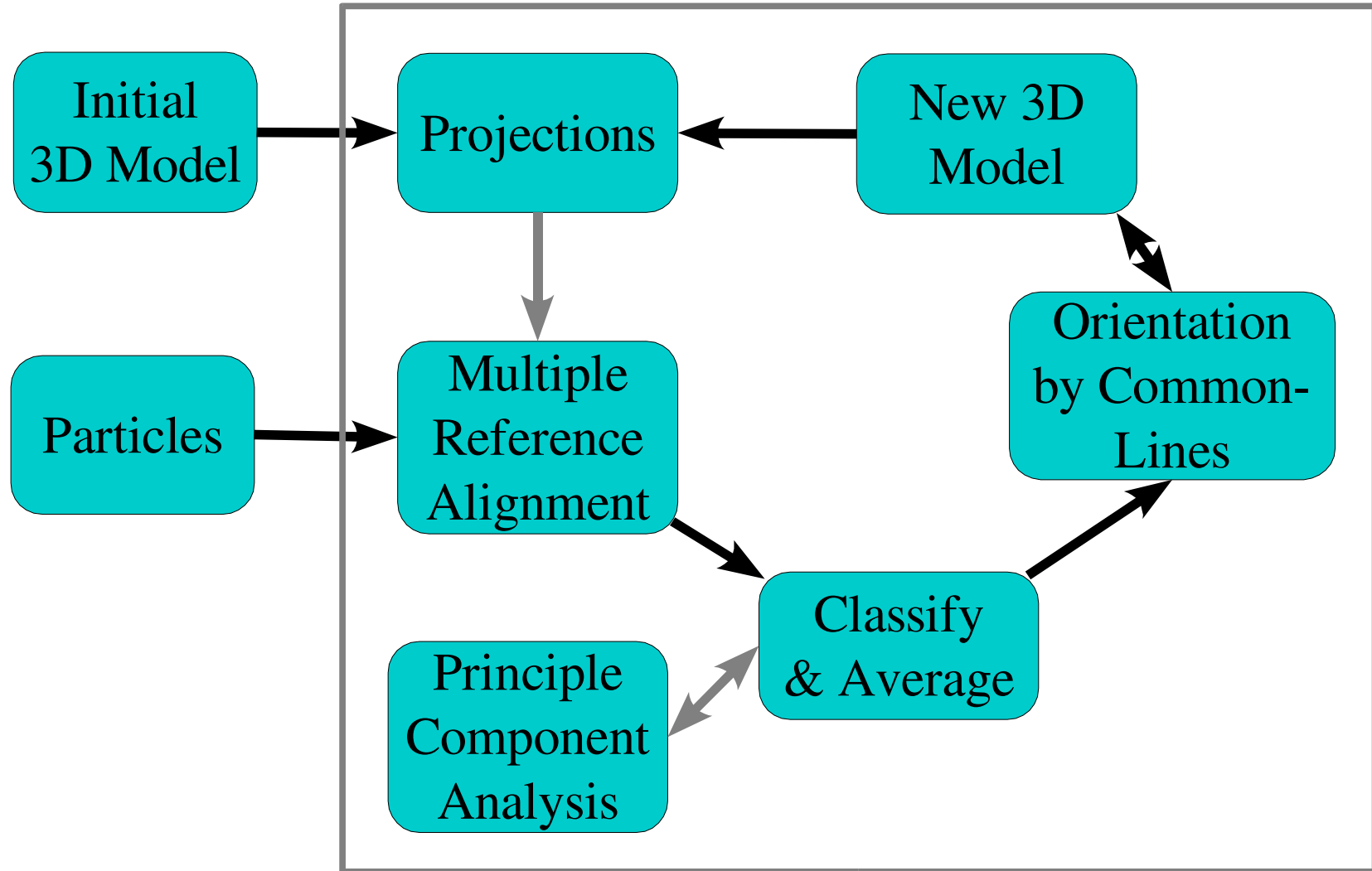
# The Reconstruction Process



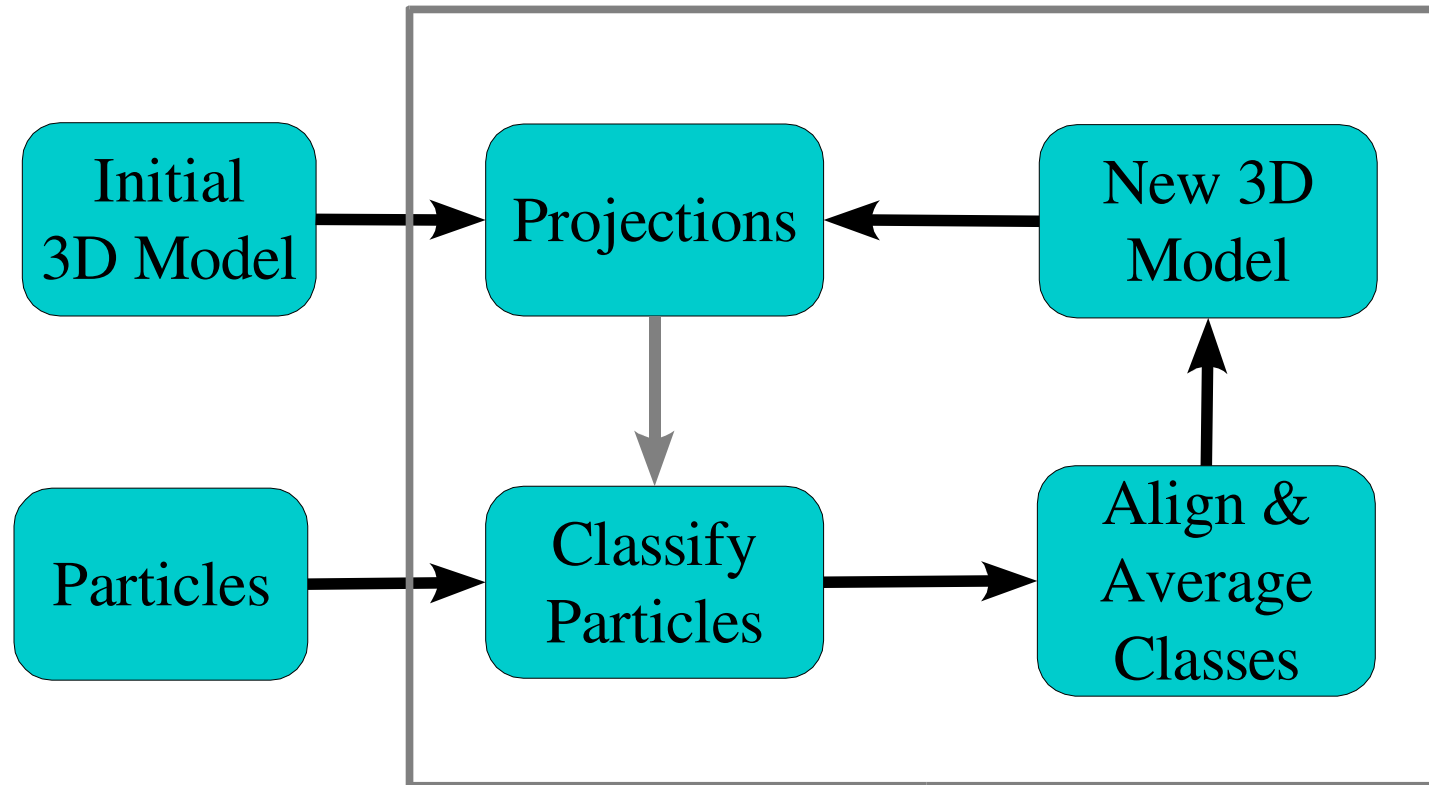
# Typical Refinement - Spider



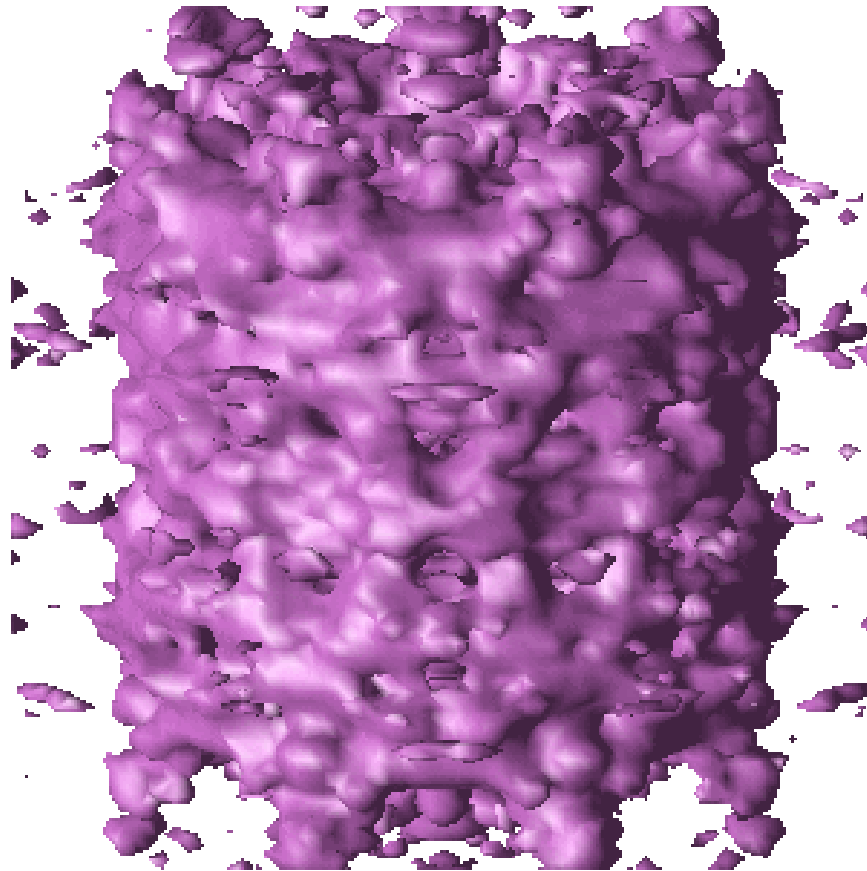
# Typical Refinement - Imagic



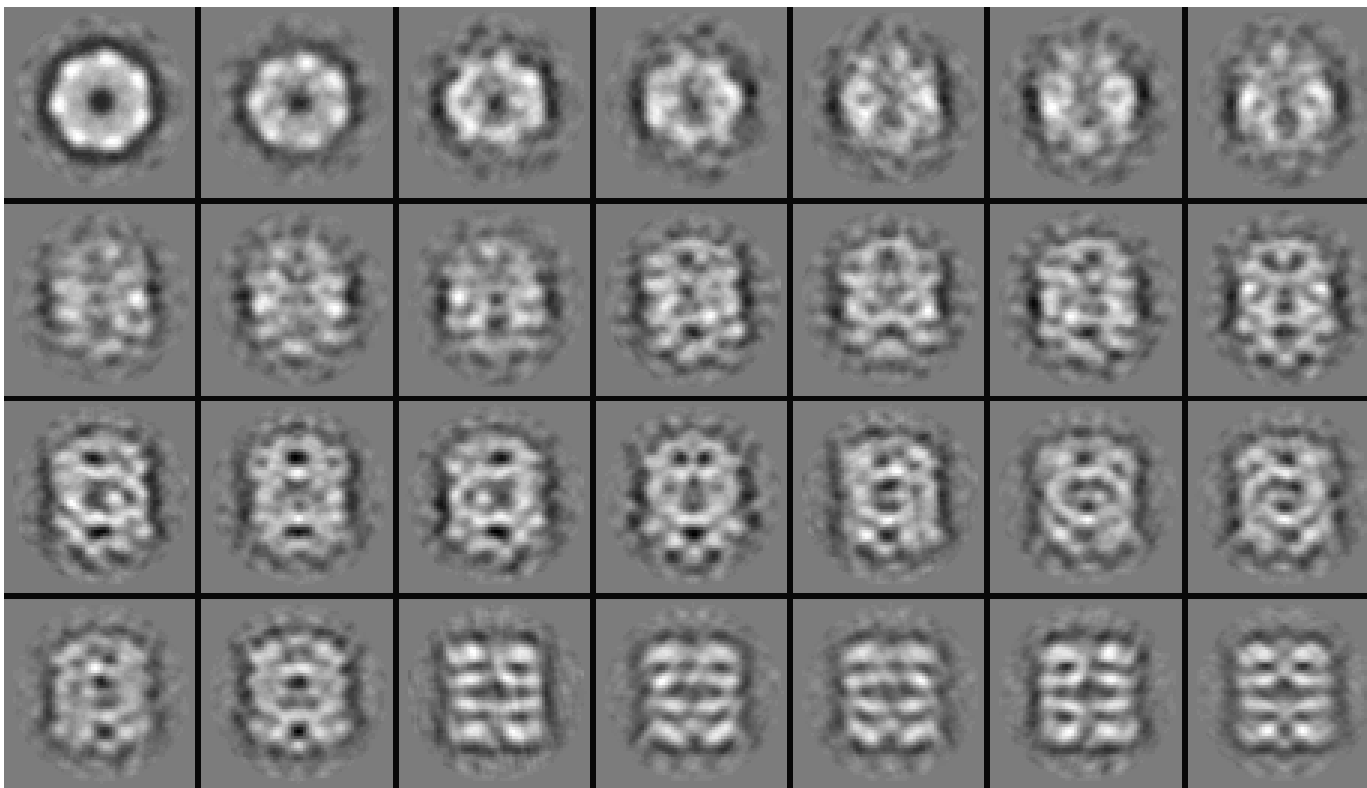
# Typical Refinement - EMAN



# Preliminary Model

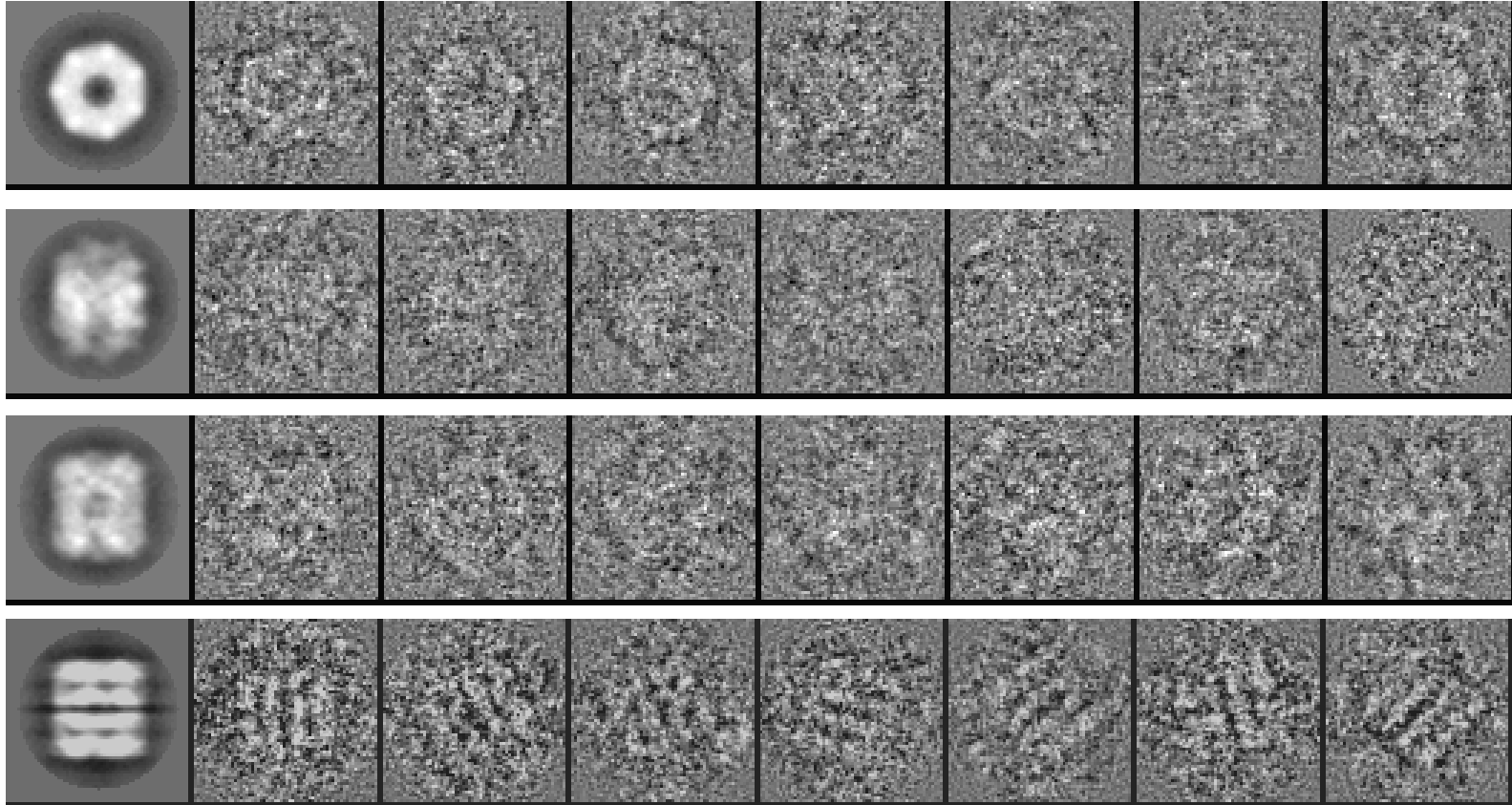


# Projections

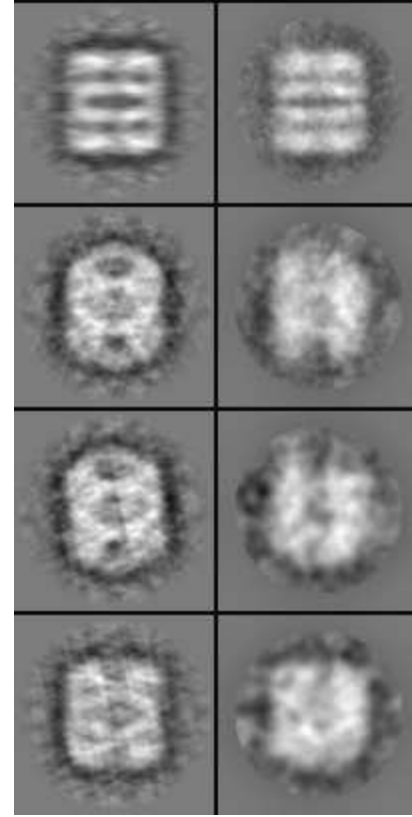
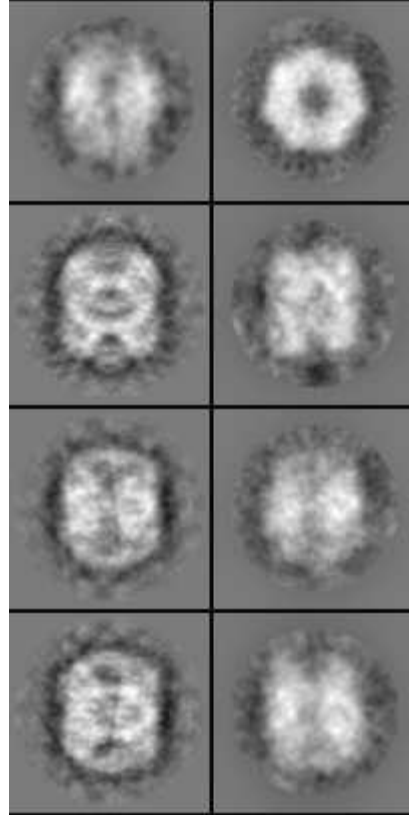
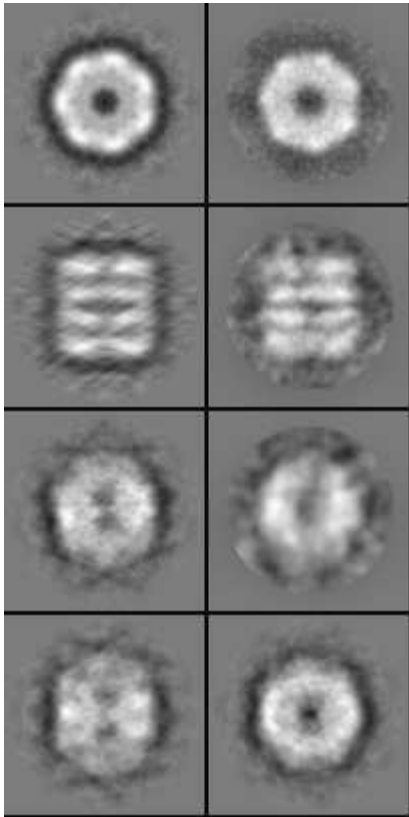




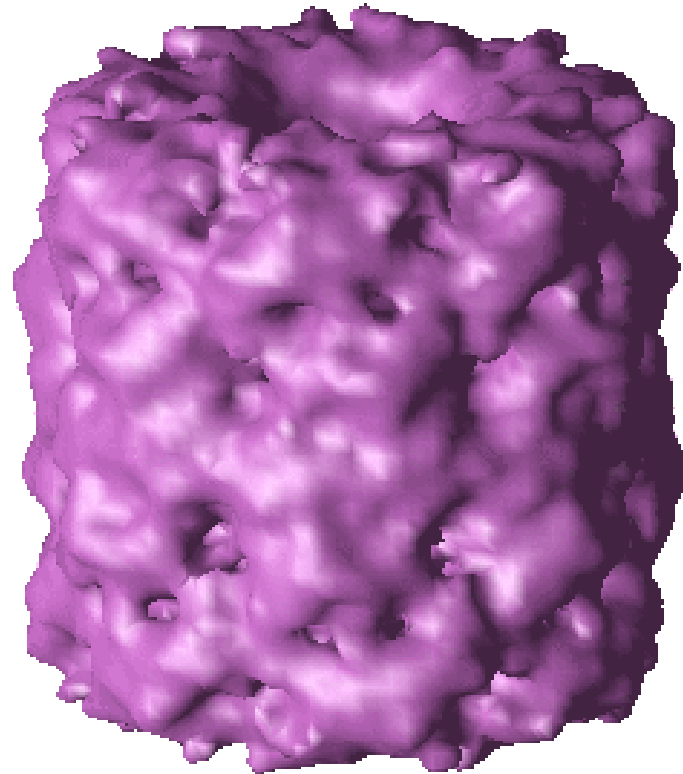
# Classification



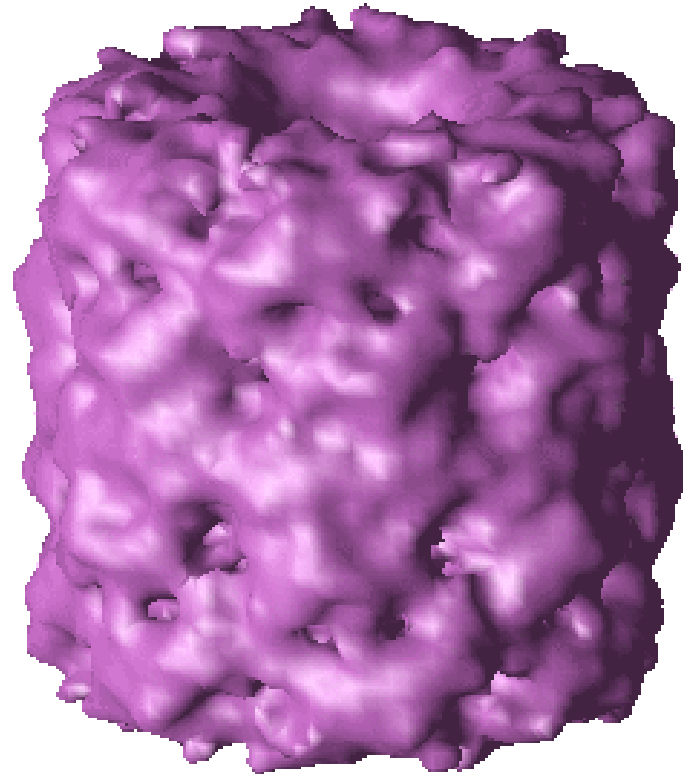
# Class Averages



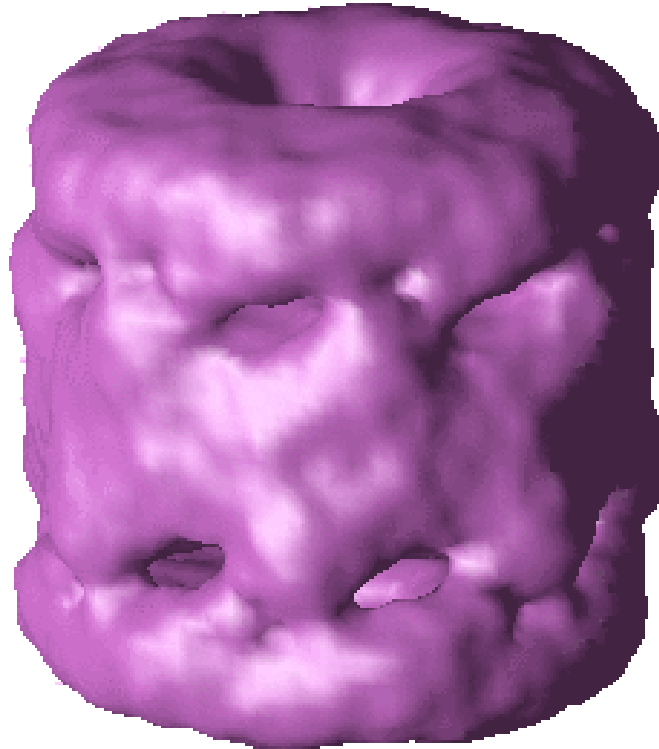
# Iteration 1



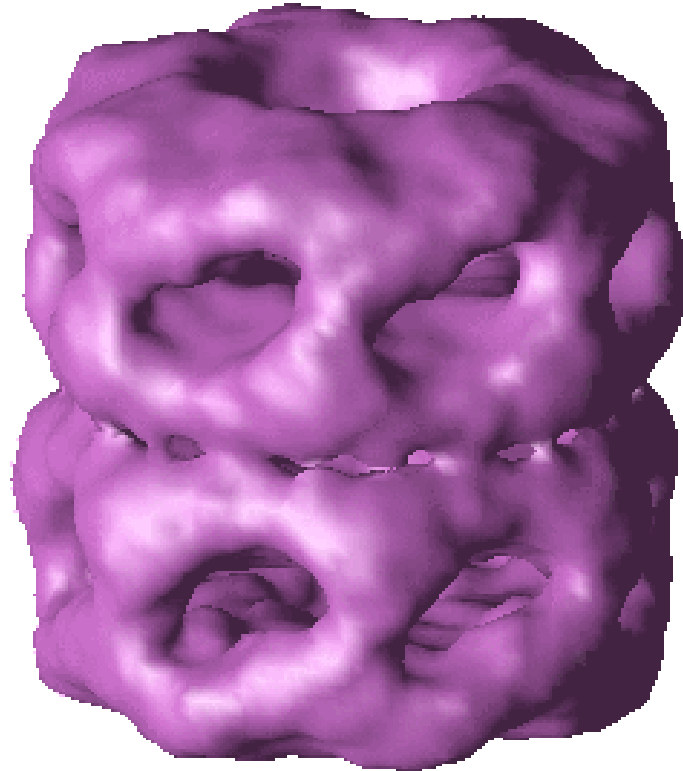
# Iteration 1



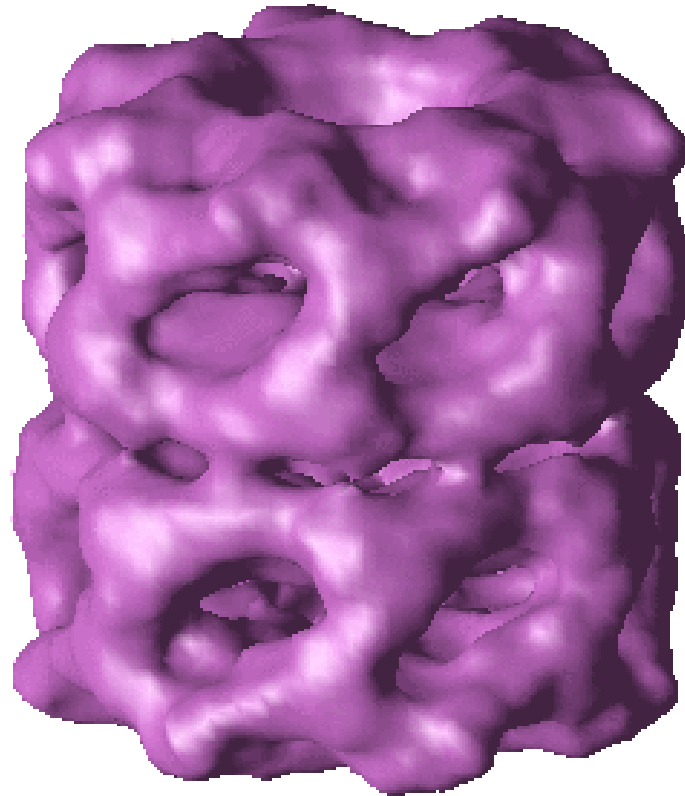
# Iteration 2



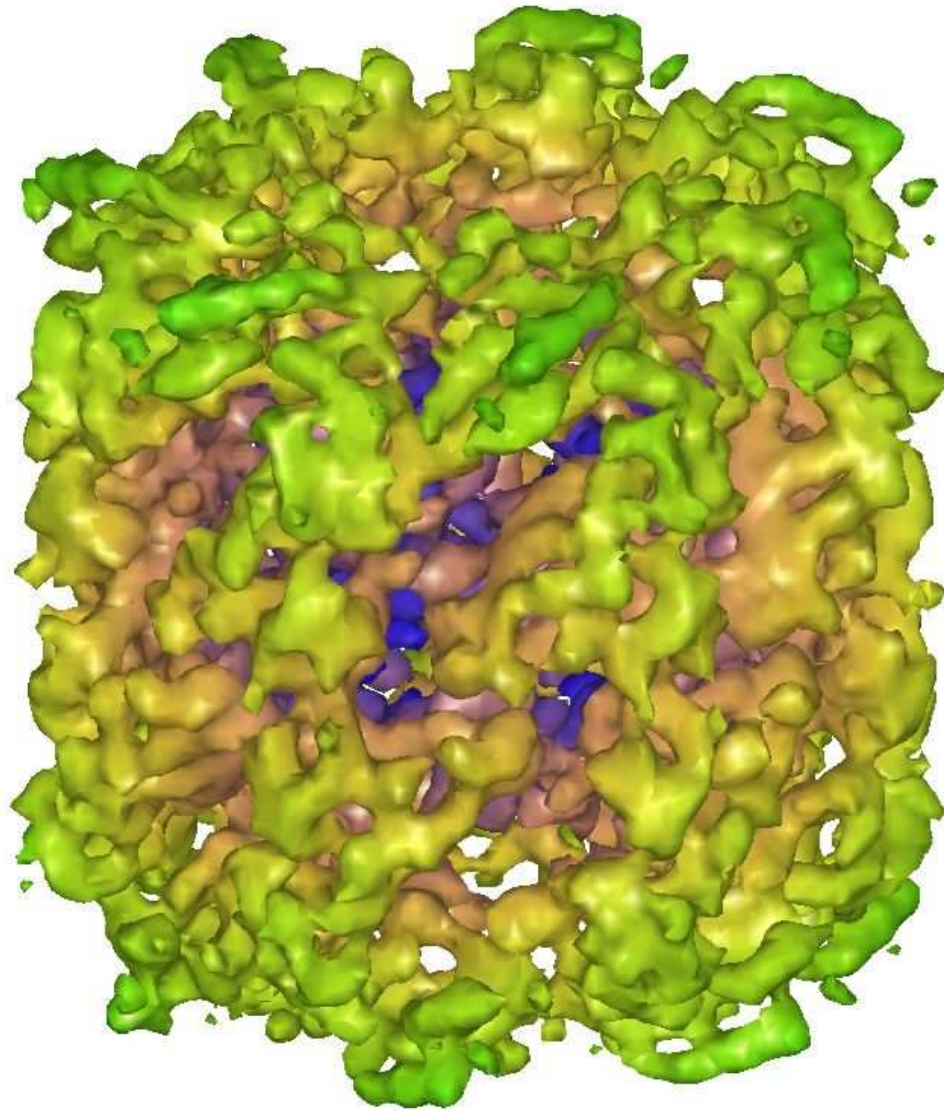
# Iteration 3



# Iteration 4

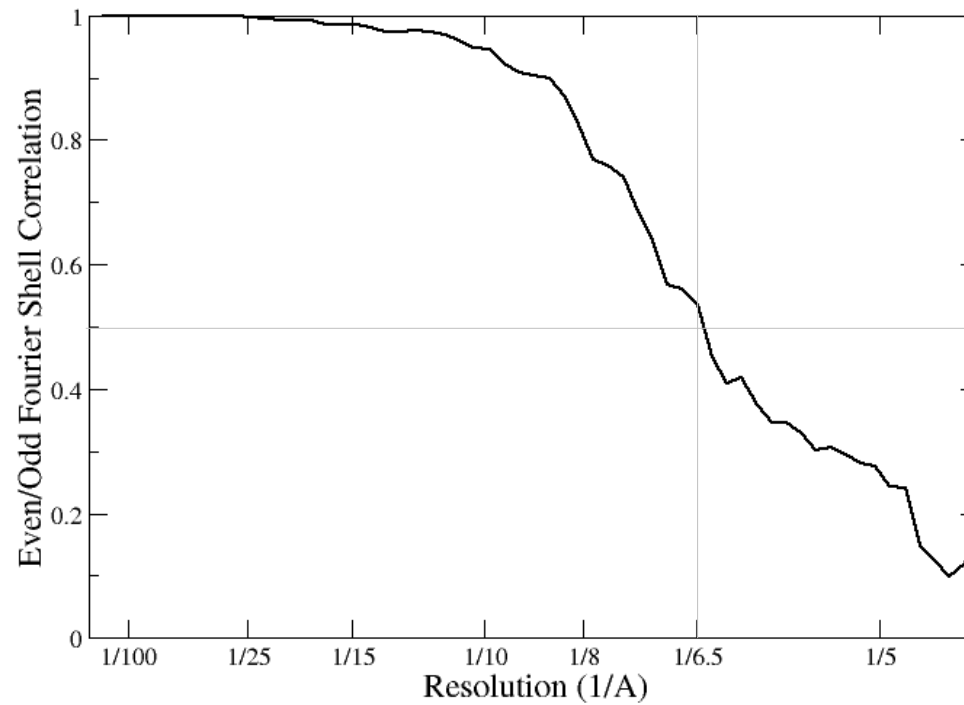


# GroEL Reconstruction at 6.5 Å



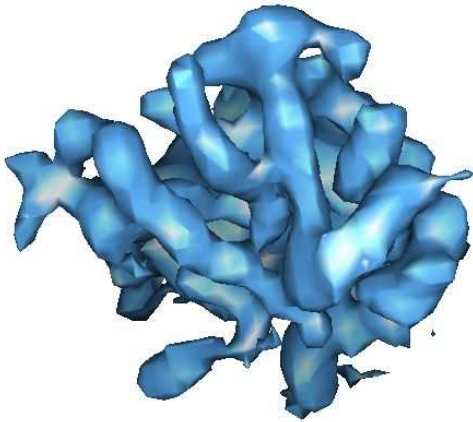


# GroEL Reconstruction at 6.5 Å

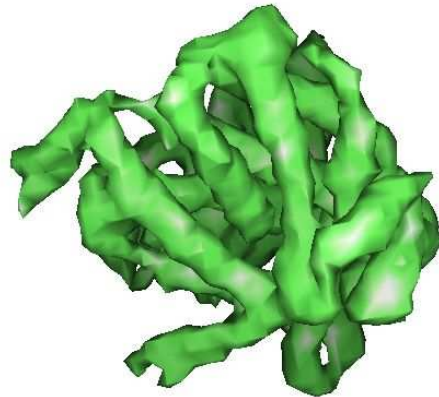


# X-ray Structure Comparison

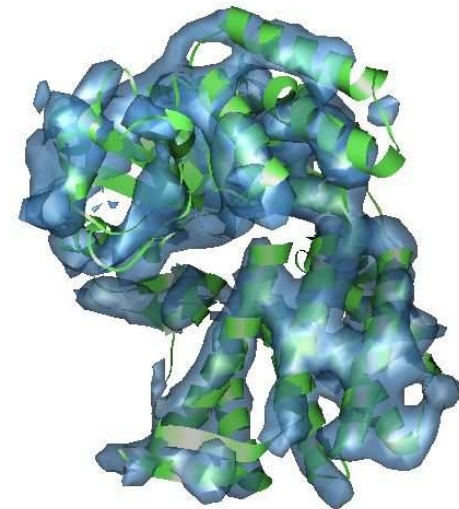
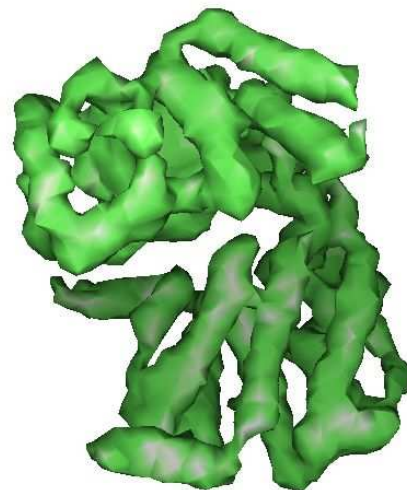
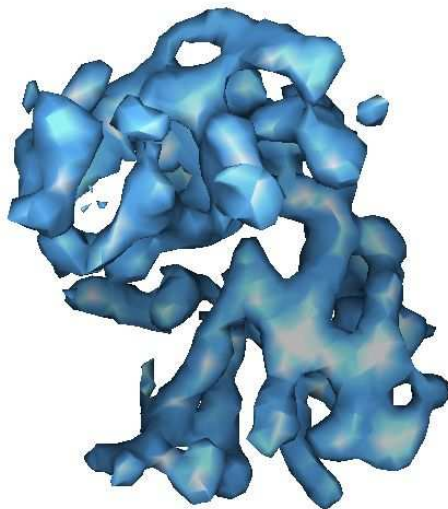
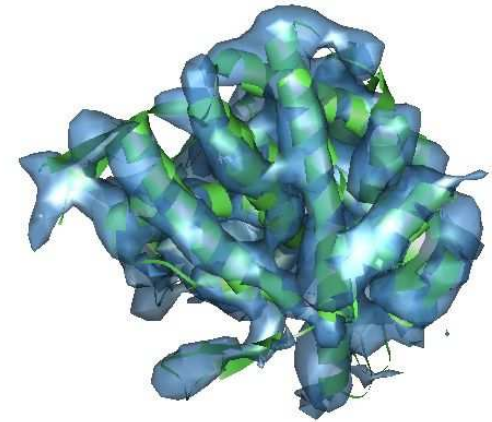
Cryo-EM



X-ray



Cryo-EM  
with ribbon



# Model Bias

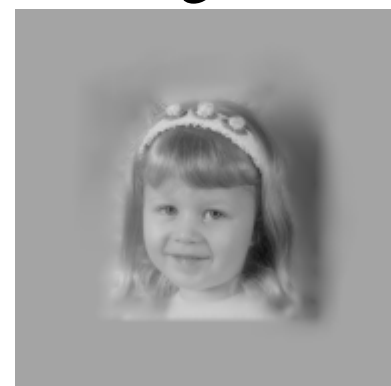
Base



Noisy



Align to

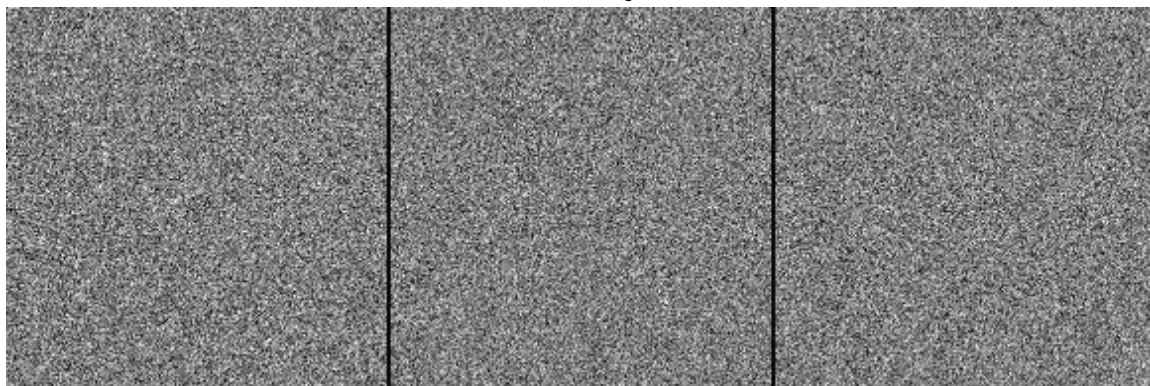


# Model Bias

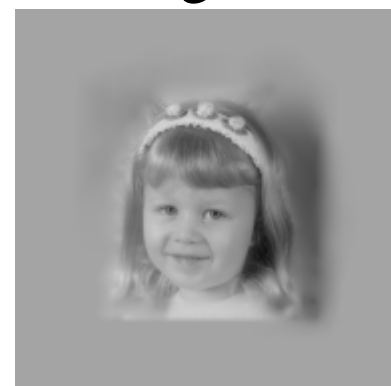
Base



Noisy



Align to



25

100

250

1000

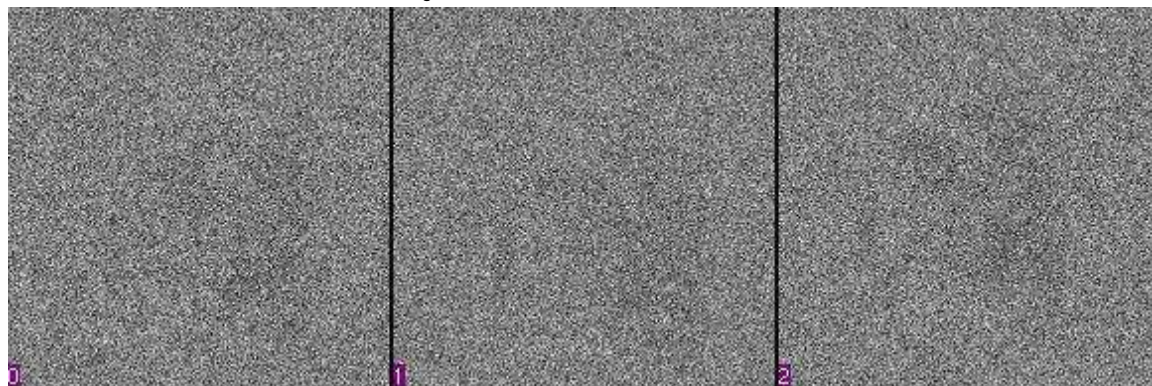
2000

# Model Bias

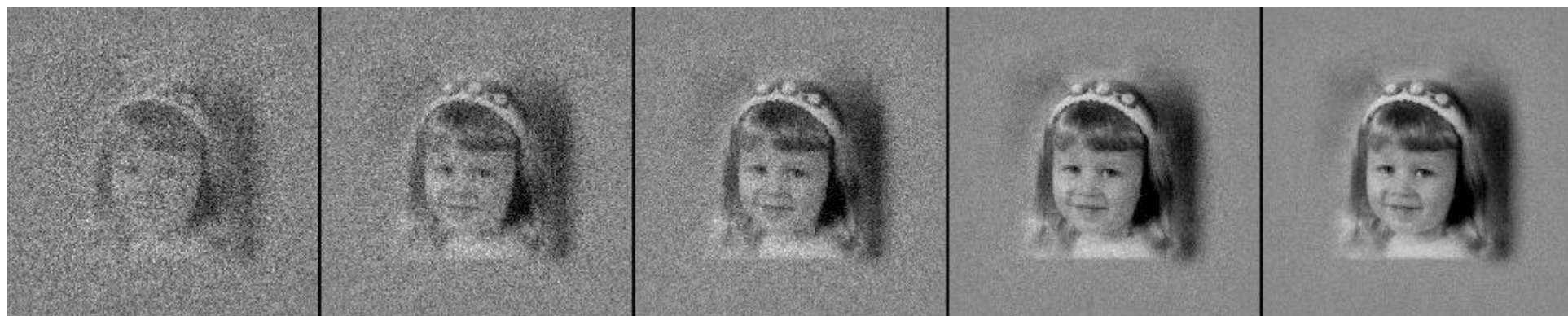
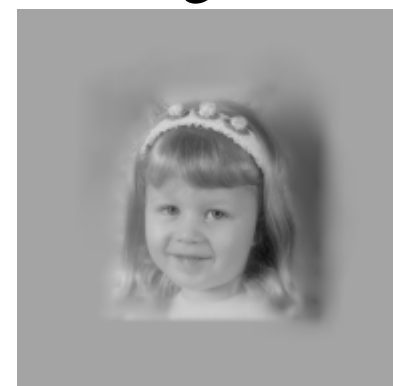
Base



Noisy (~10% contrast)



Align to



25

100

250

1000

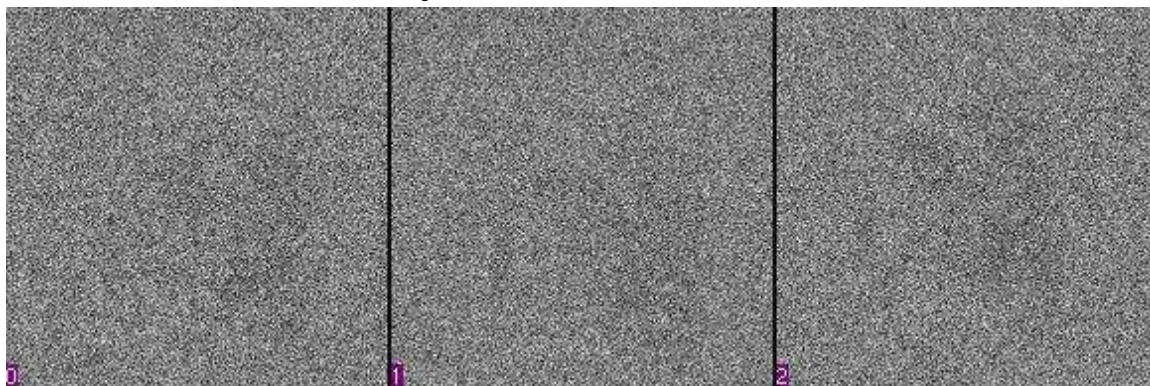
2000

# Model Bias

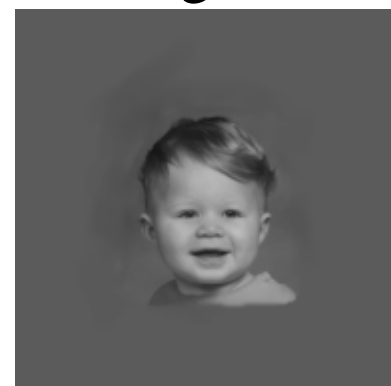
Base



Noisy (~10% contrast)



Align to



# Model Bias

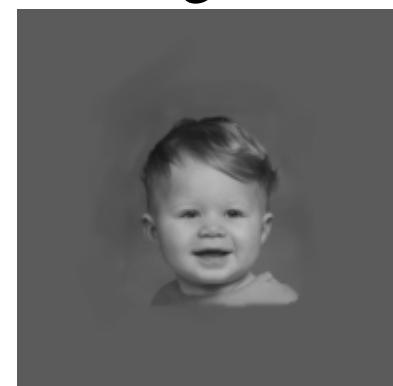
Base



Noisy (~10% contrast)



Align to



25

100

250

1000

2000

# Model Bias

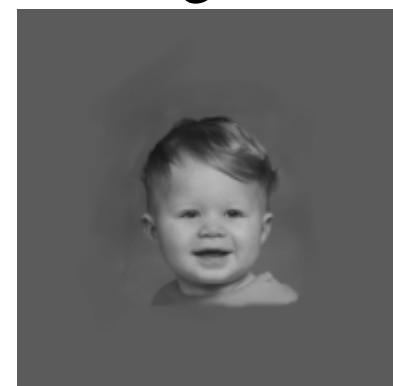
Base



Noisy



Align to



25

100

250

1000

2000



# Model Bias

Base

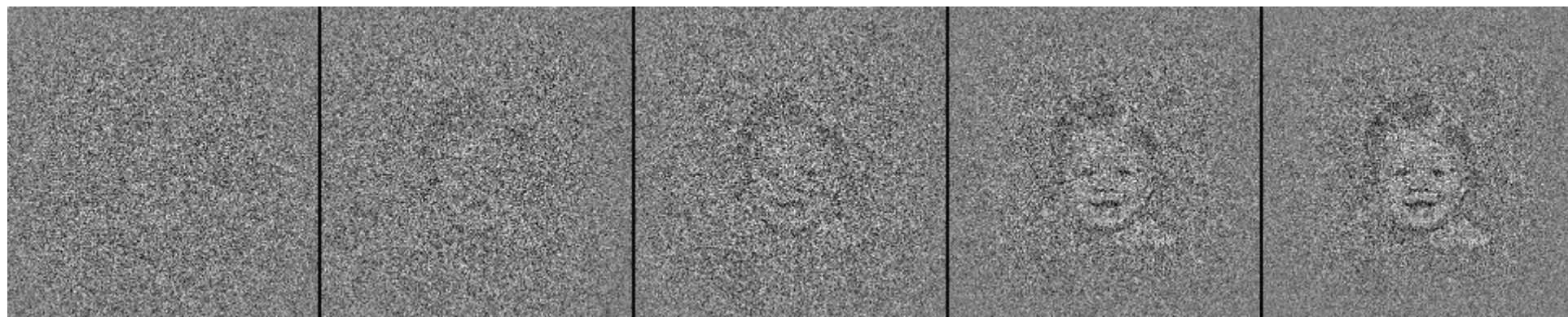


Noisy



Align to

Iter x4



25

100

250

1000

2000

# Model Bias

Base

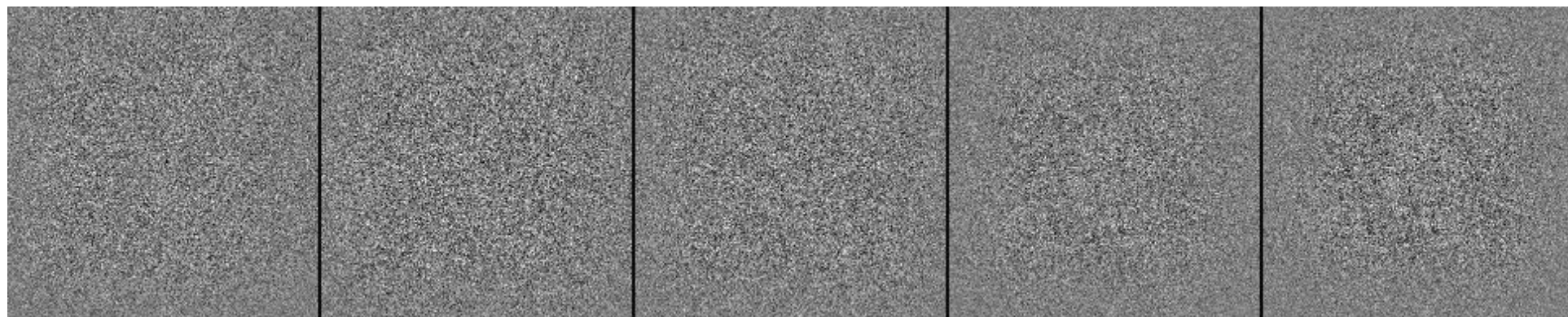


Noisy



Align to

Iter x8



25

100

250

1000

2000

# Model Bias

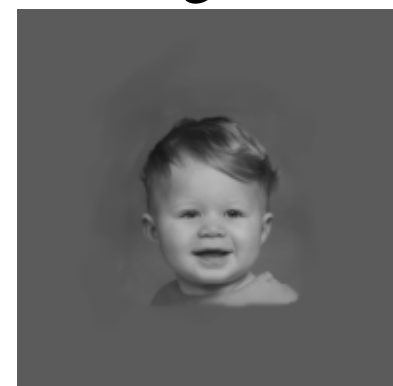
Base



Noisy (~10% contrast)



Align to



25

100

250

1000

2000

# Model Bias

Base



Noisy



Align to



Iter x4



25

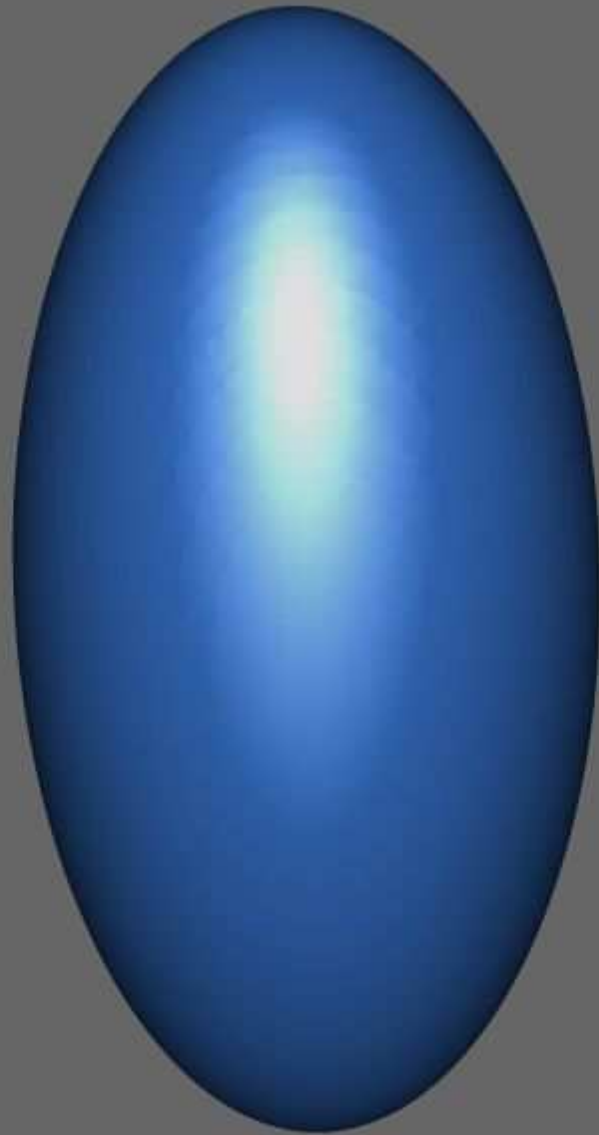
100

250

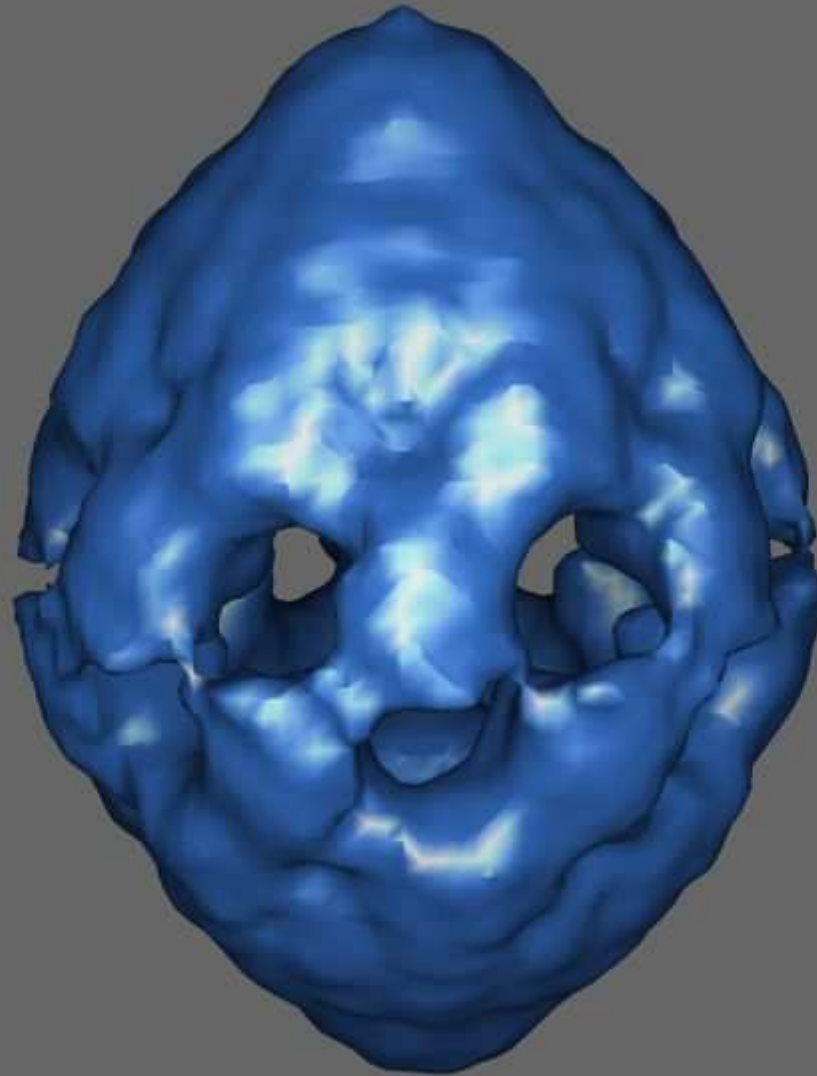
1000

2000

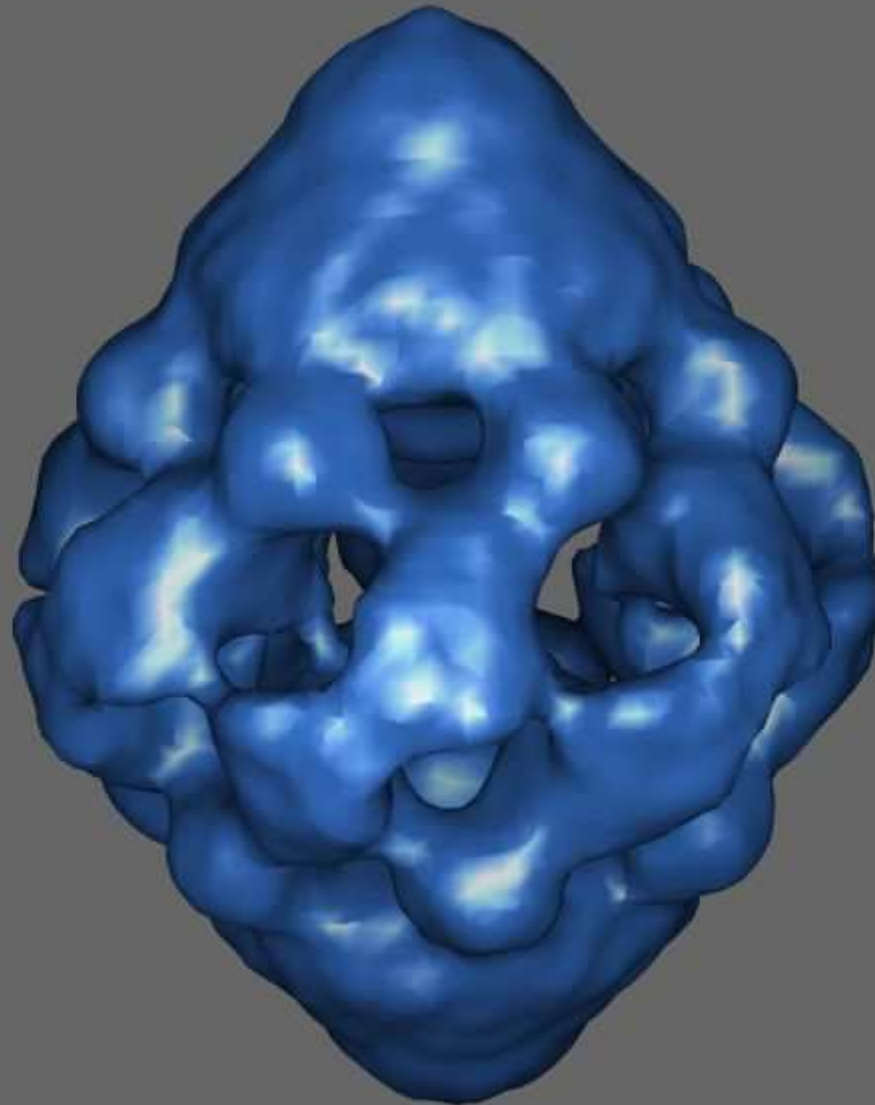
# Refine from Gaussian Ellipsoid



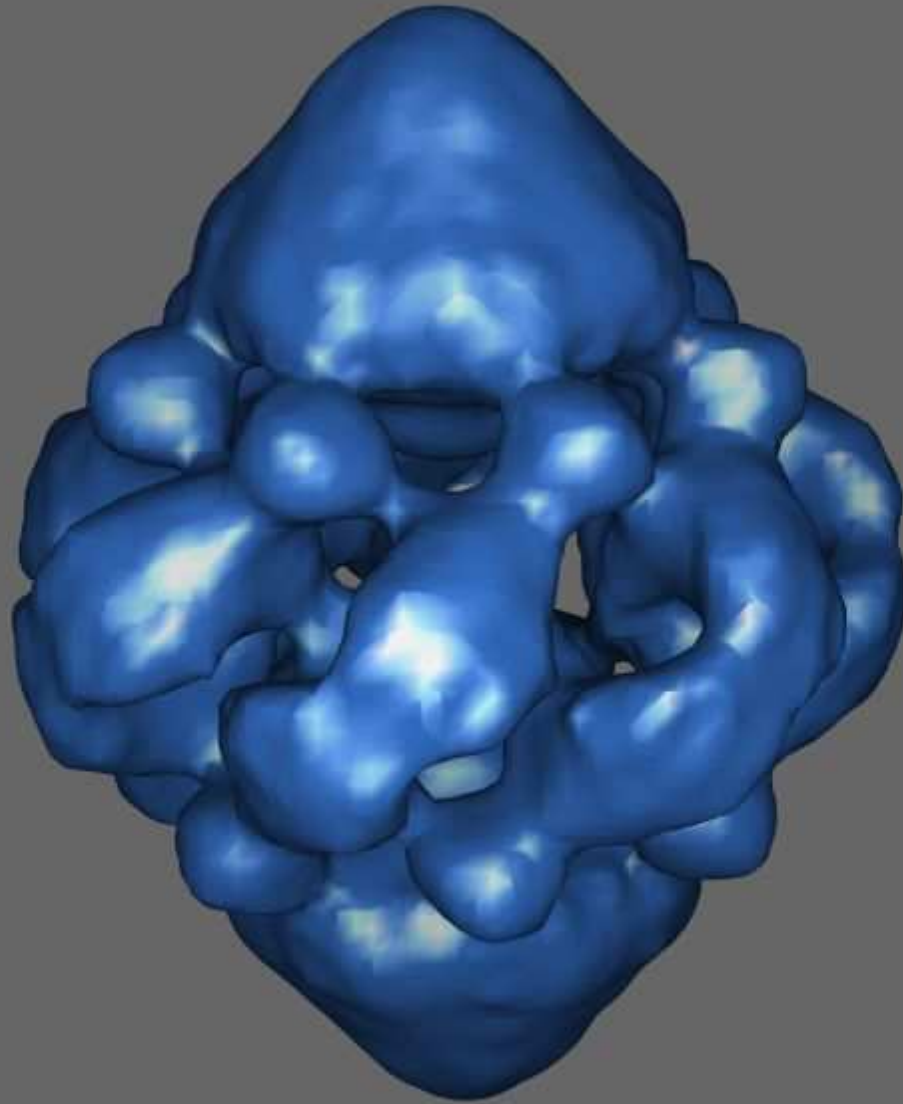
# Refine from Gaussian Ellipsoid



# Refine from Gaussian Ellipsoid



# Refine from Gaussian Ellipsoid

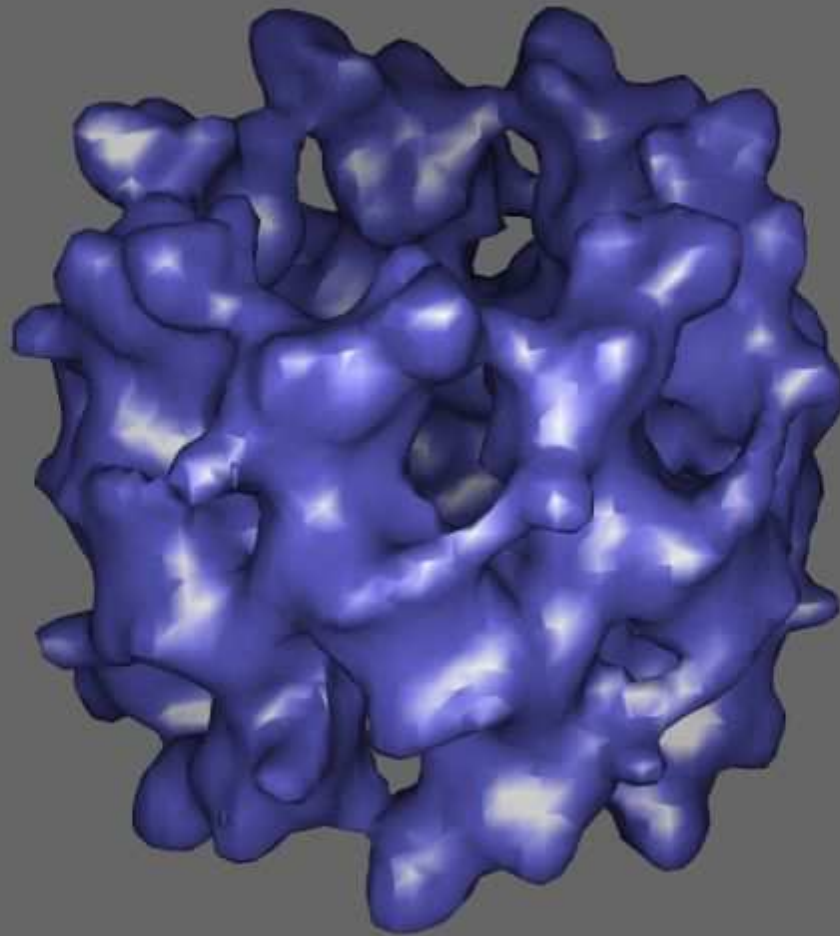




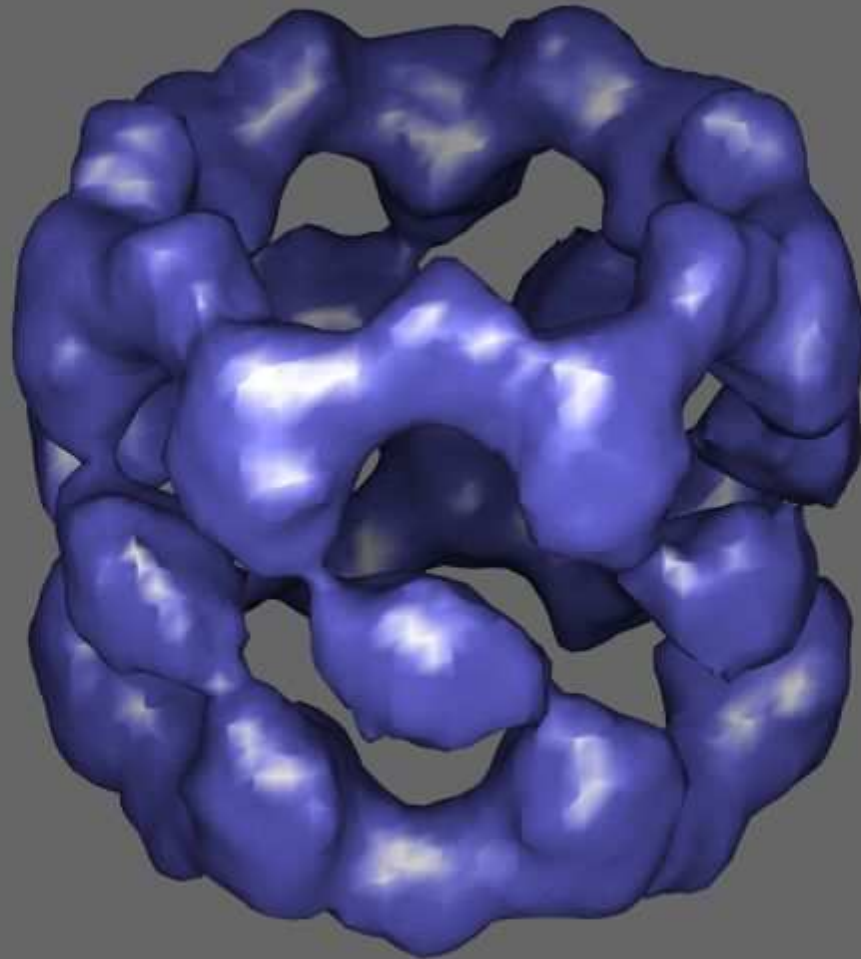
# Refine from Gaussian Ellipsoid



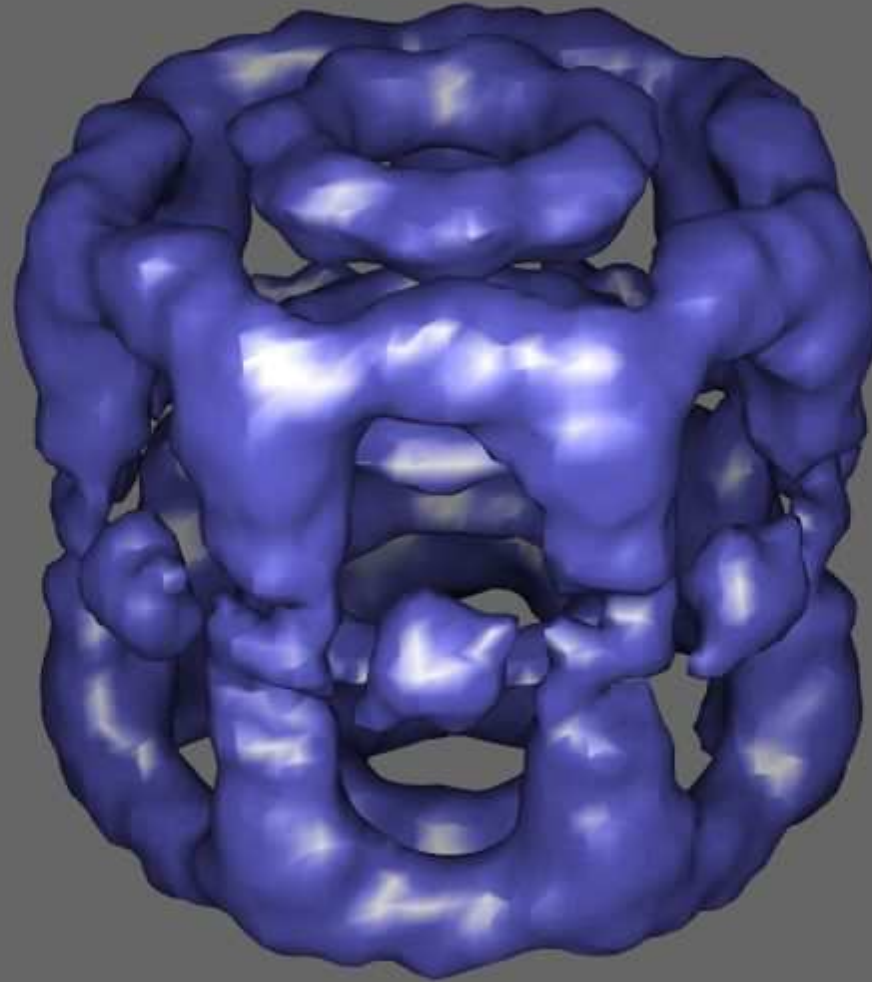
# Refine from Gaussian Ellipsoid



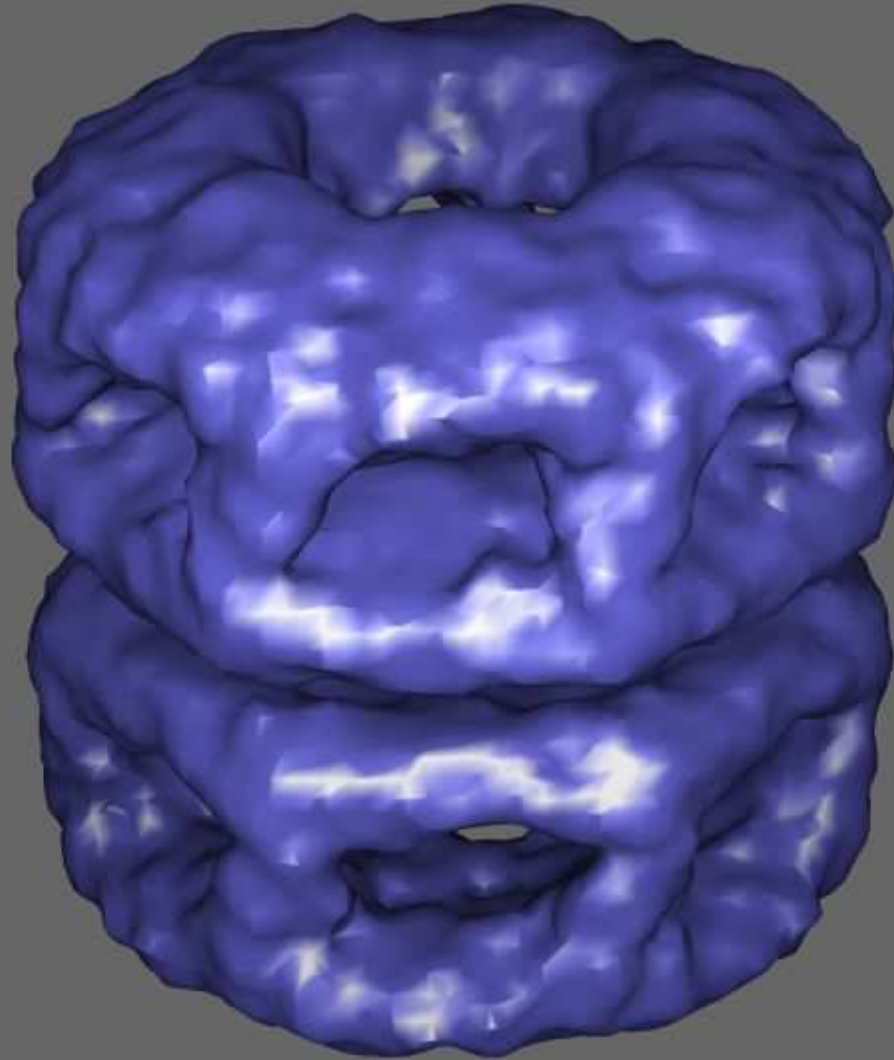
# Refine from Gaussian Ellipsoid



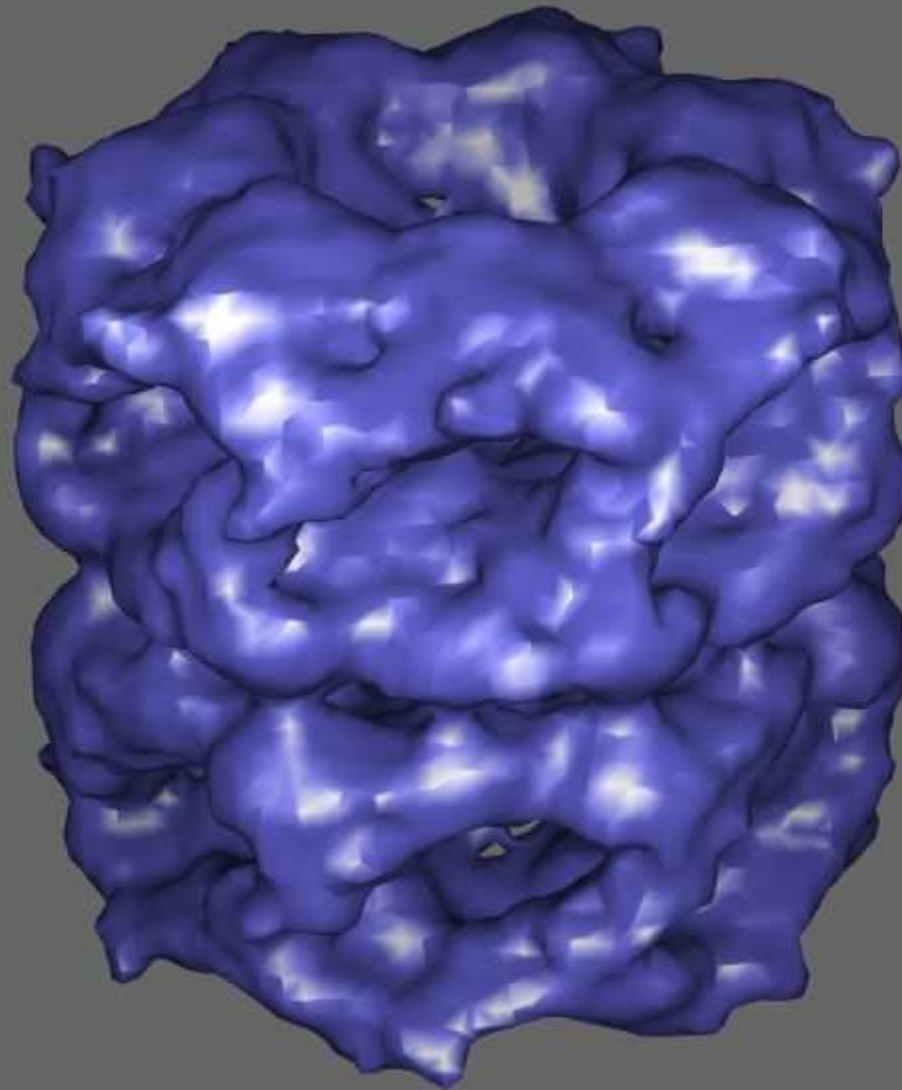
# Refine from Gaussian Ellipsoid



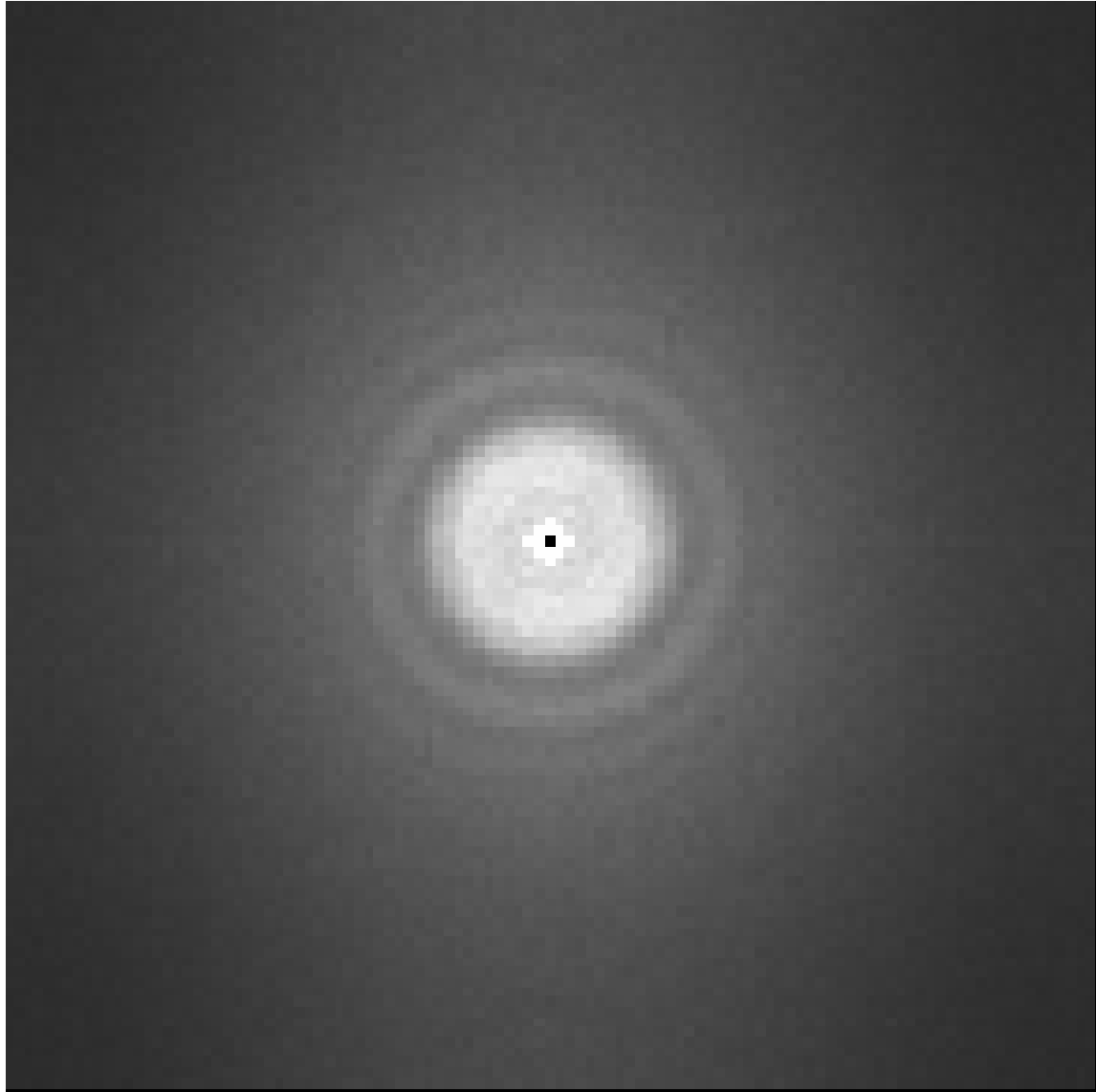
# Refine from Gaussian Ellipsoid



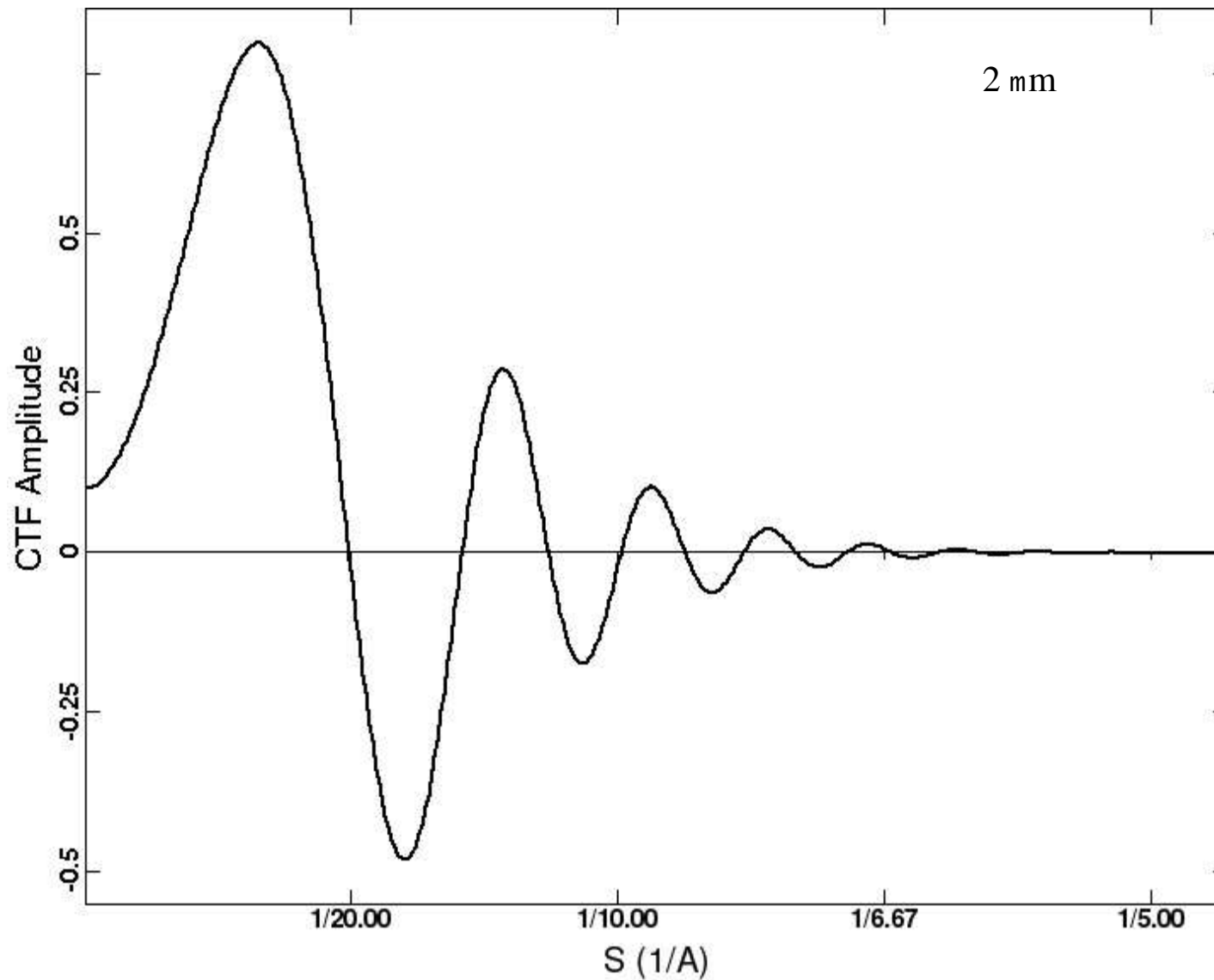
# Refine from Gaussian Ellipsoid



# CTF Correction

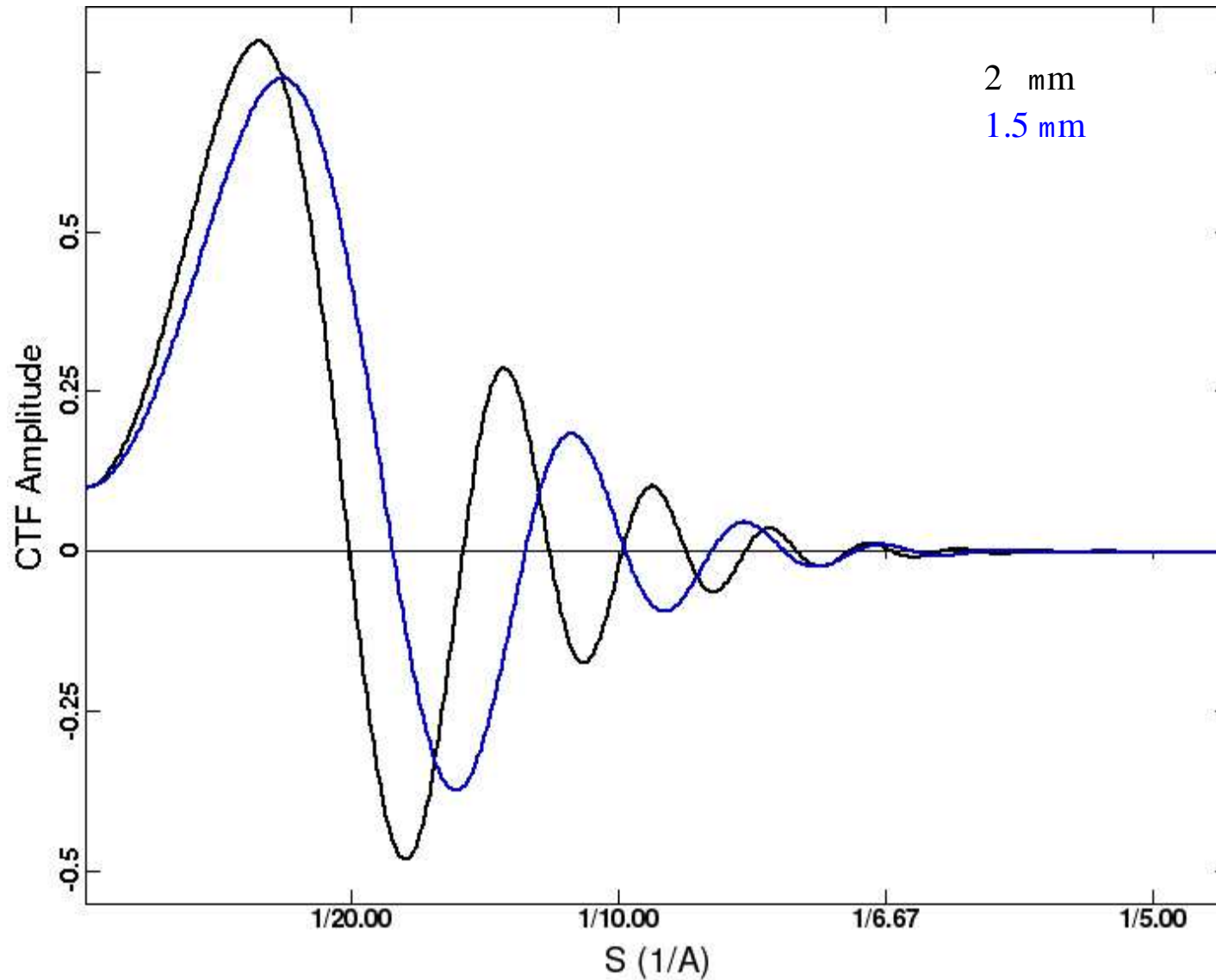


# Contrast Transfer Function

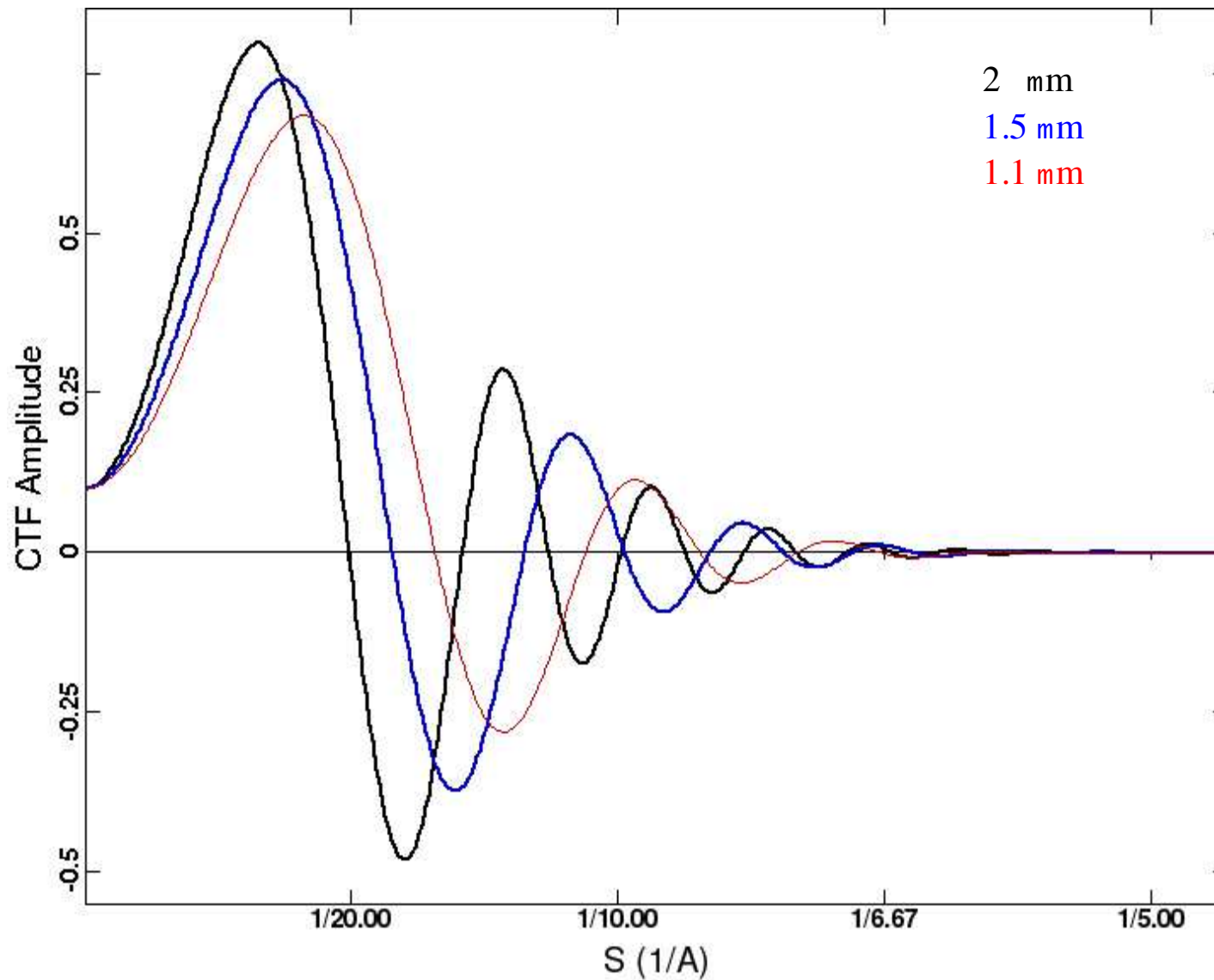




# Contrast Transfer Function



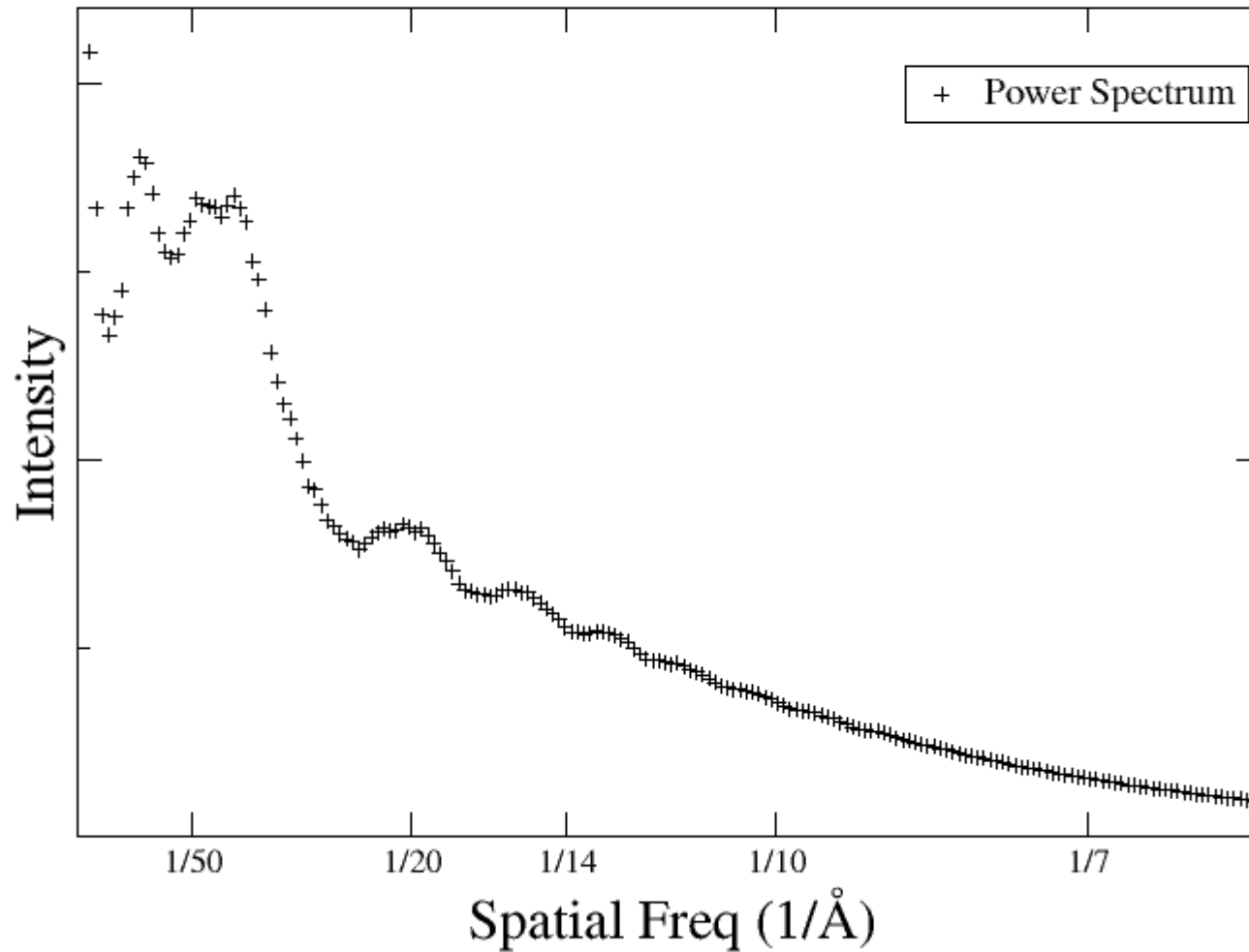
# Contrast Transfer Function



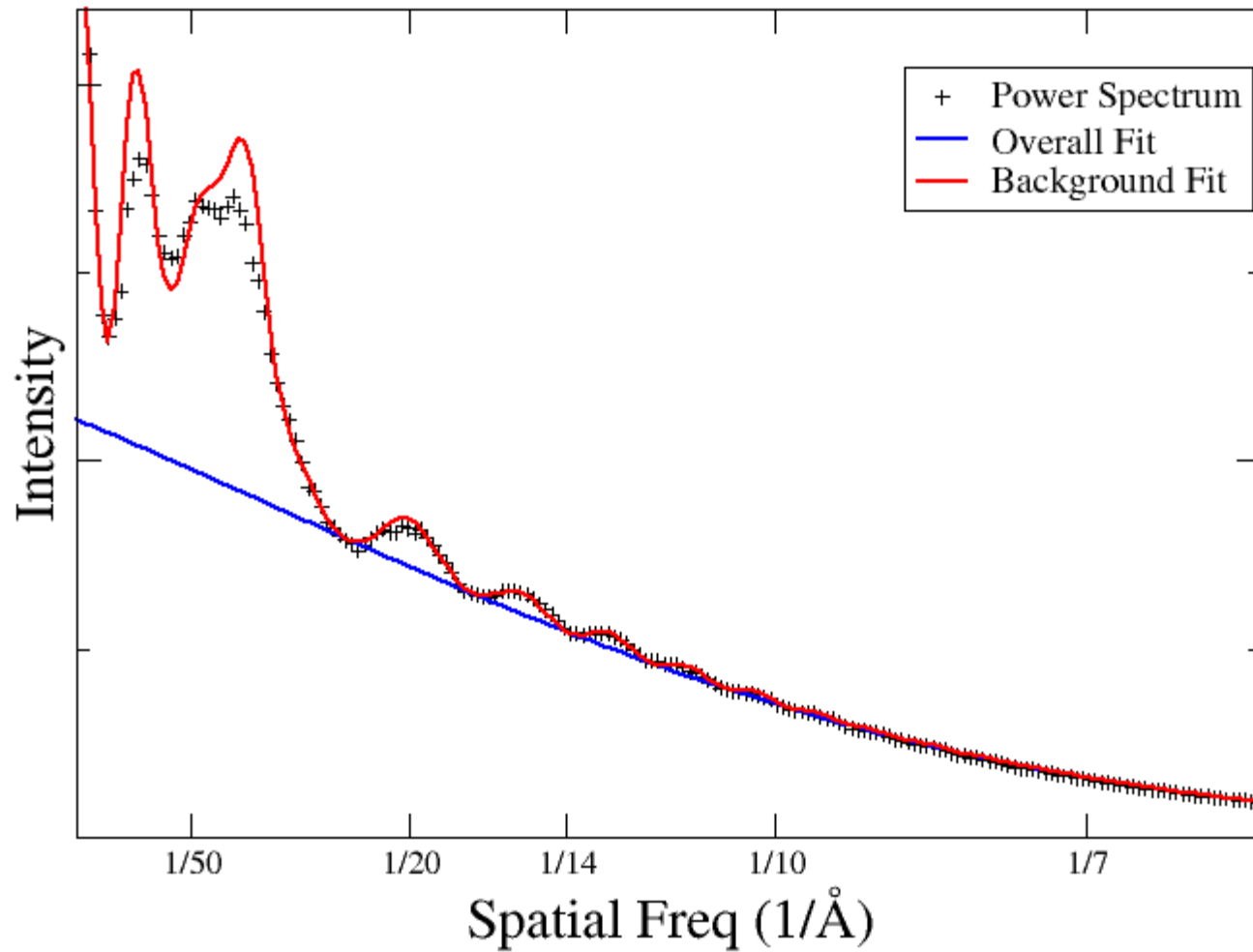
# CTF Correction

CTF Demo

# CTF Parameter Determination



# CTF Parameter Determination



# Contrast Transfer Function

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

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

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

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

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

M=Measured Data    F=1D Structure Factor  
C=CTF    E=Envelope    N=Background Noise

# CTF Correction

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

$$k_i = ?$$

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

# CTF Correction

(stigmatic, no drift)

$$\bar{T}(s, \theta) = \frac{\text{Wiener Filter}}{1 + F^2(s) R(s)} \sum_i \frac{\text{CTF Correction}}{C_i(s) E_i(s)} \frac{\text{SNR Weight}}{R_i(s)} \bar{M}_i(s, \theta)$$

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

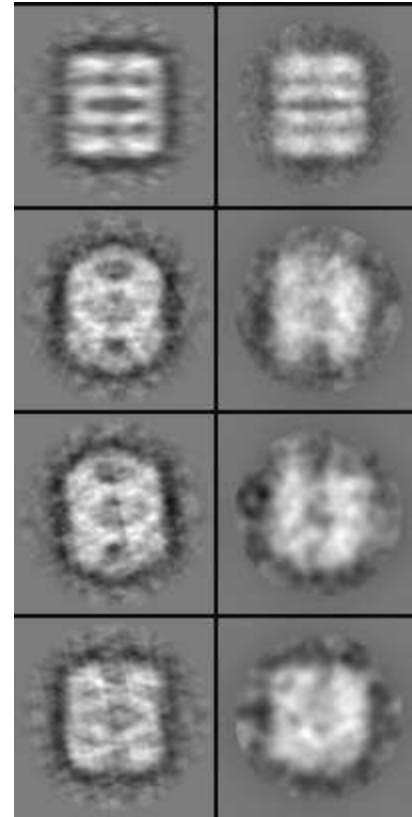
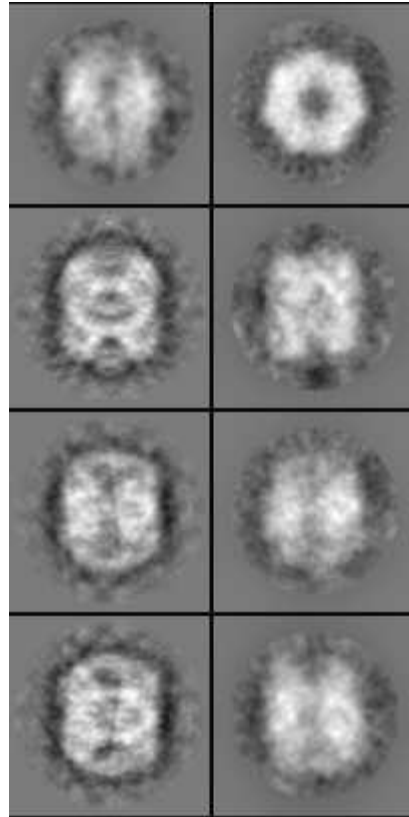
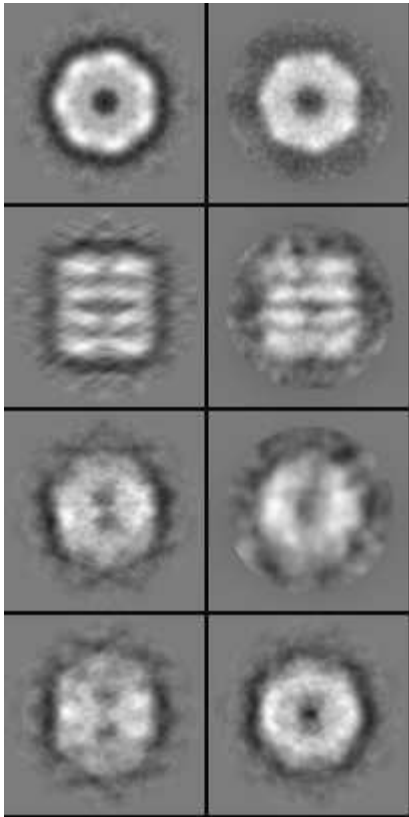
$$R(s) = \sum_i \frac{C_i^2(s) E_i^2(s)}{N_i^2(s)}$$

T=Corrected Image    M=Measured Data

F=1D Structure Factor    C=CTF    E=Envelope    N=Background

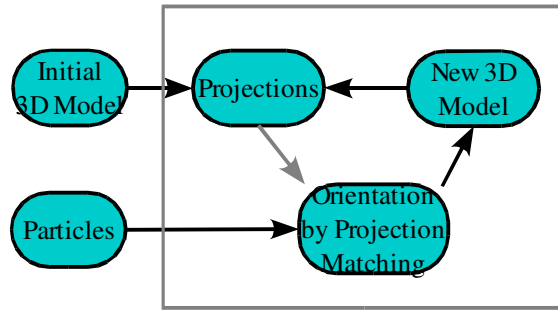


# Class Averages

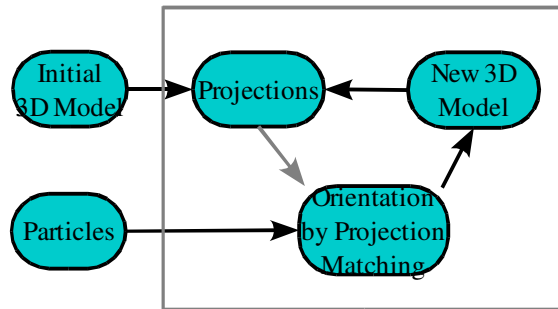


# CTF Correction - Spider

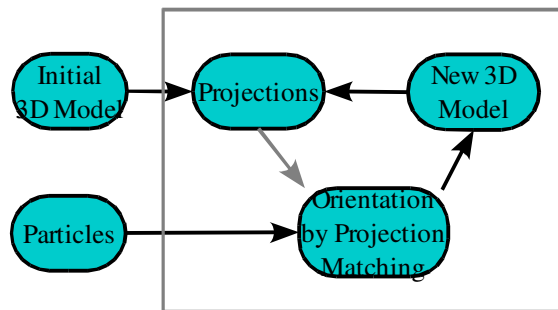
Defocus 1



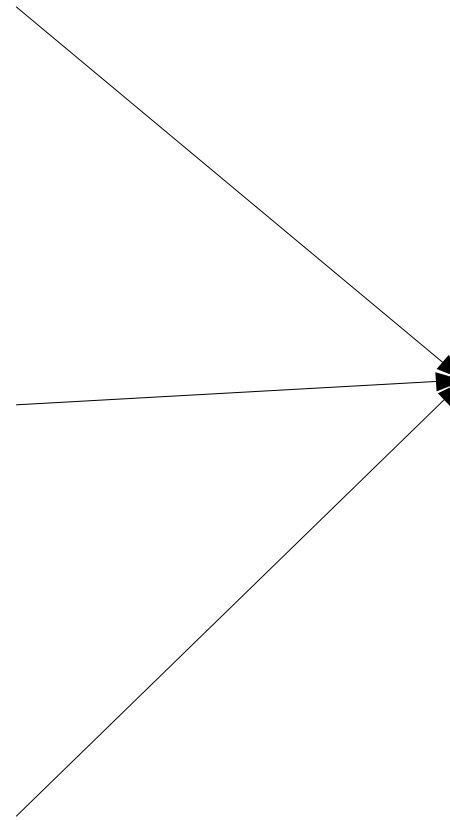
Defocus 2



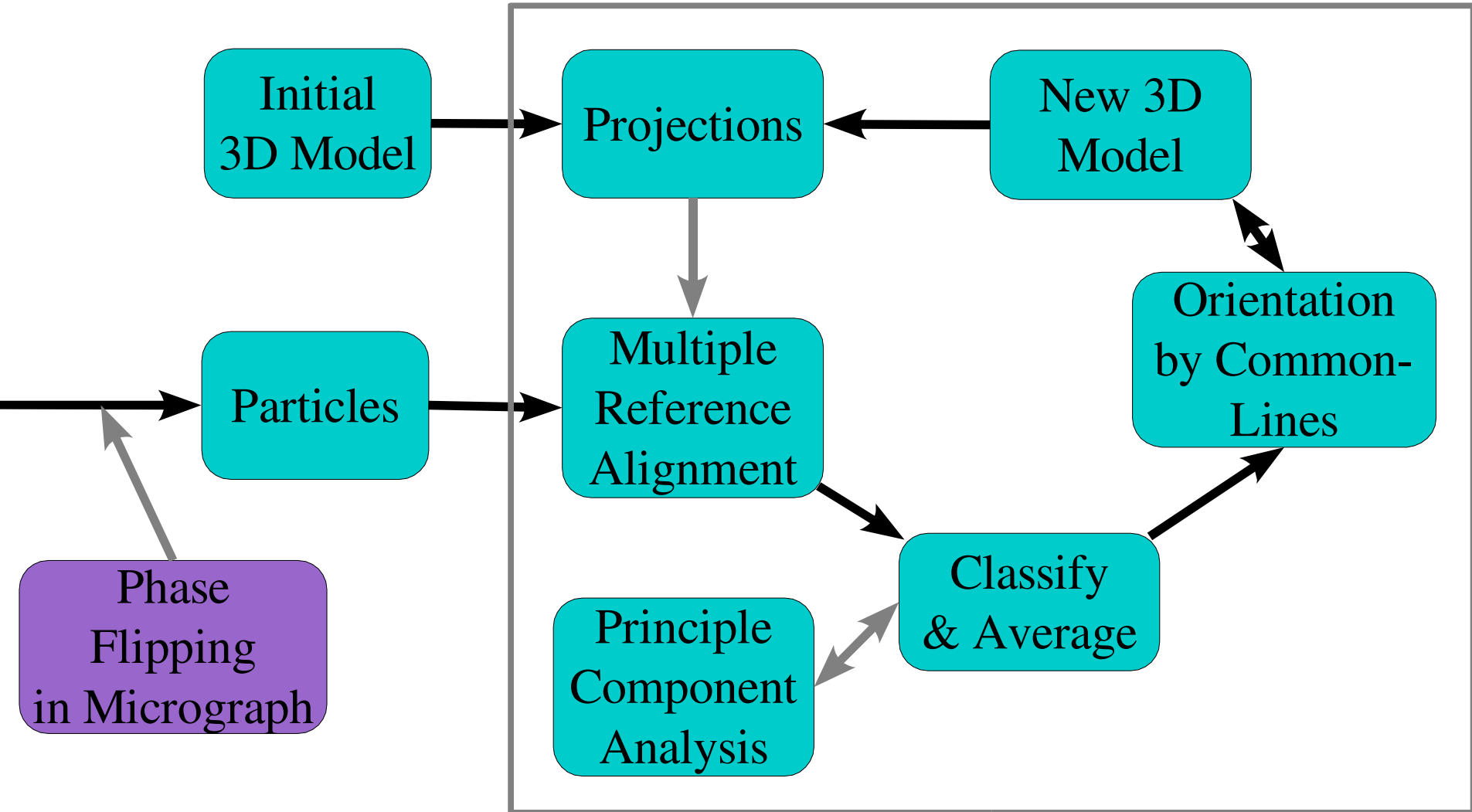
Defocus 3



CTF Correct  
and Merge in 3D



# CTF Correction - Imagic

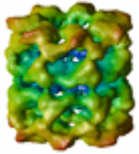




# Acknowledgements

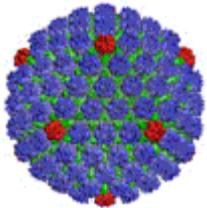
Baylor College of Medicine

- Wah Chiu
- Joanita Jakana



UT SW Medical Center

- David Chuang
- Jiu-Li Song



Thanks to:



National Center for Research Resources

National Partnership for Advanced

Computational Infrastructure (NPACI)

Agouron Institute