NCMI
National Center for Macromolecular Imaging

Methods for High Resolution Refinement
in Single Particle Processing

Steve Ludtke

Asst. Professor, Biochemistry, BCM
Co-director,NCMI

NCRR

BCM
Baylor College of Medicine

Single Particle Reconstruction with EMAN

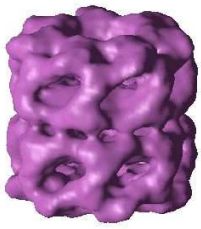
GroEL

Donghua Chen
Joanita Jakana
Wah Chiu

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

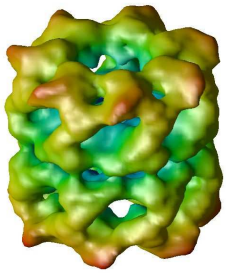
EMAN: http://ncmi.bcm.tmc.edu/eman

GroEL 2000 (15 Å)



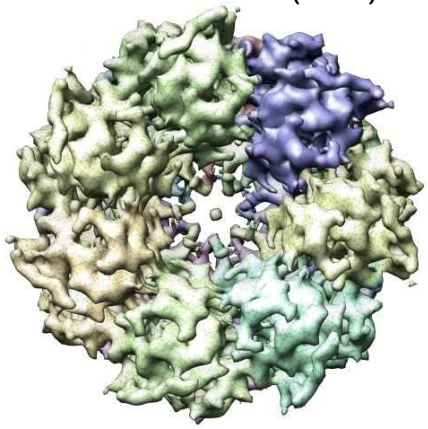
5000 particles, JEOL 4000

GroEL 2001 (11.5 Å)



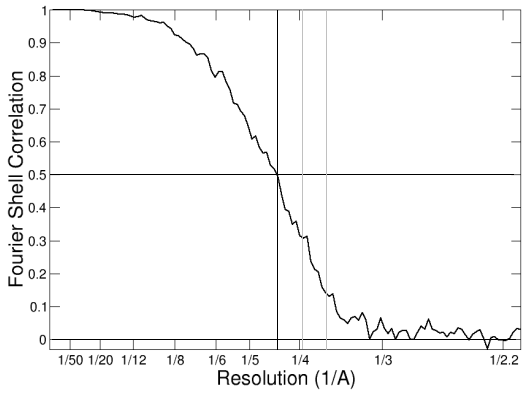
5000 particles, JEOL 4000

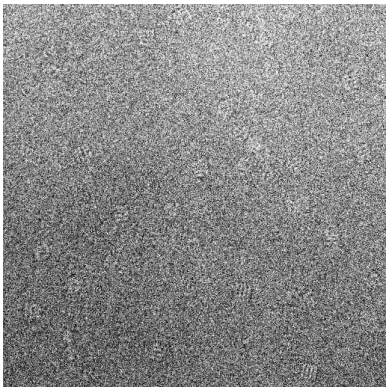
GroEL 2003 (6 Å)

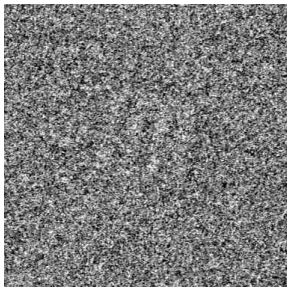


30,000 particles, JEOL 2010F

2005

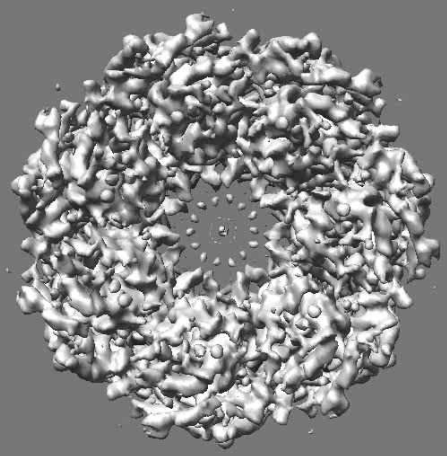






Jeol 3000
7 Days of imaging, 910 micrographs
1.06 Å/pix, Nikon 9000 scanner
135 used, 34,868 particles

Animation Unavailable in PDF version



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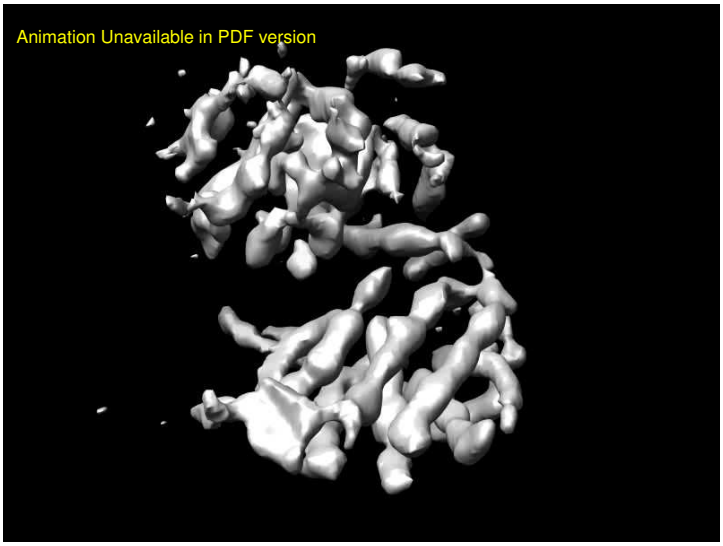

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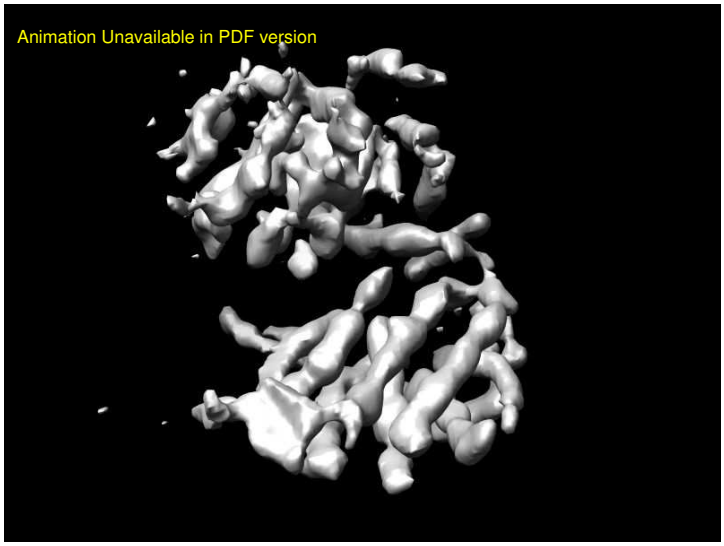

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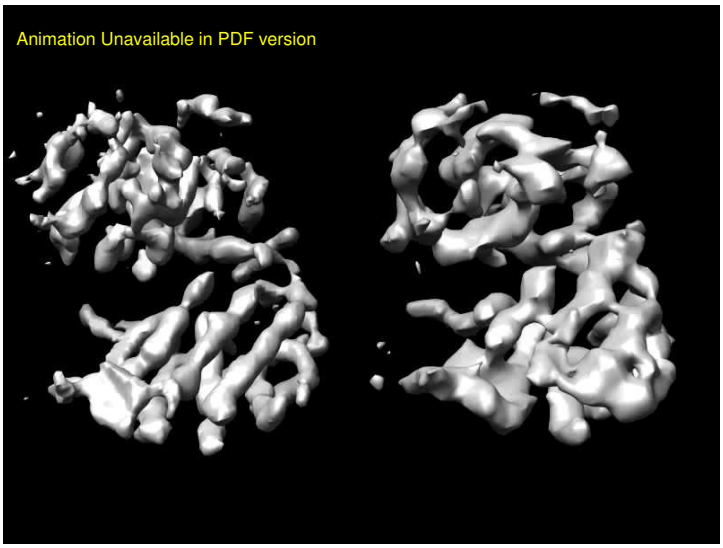

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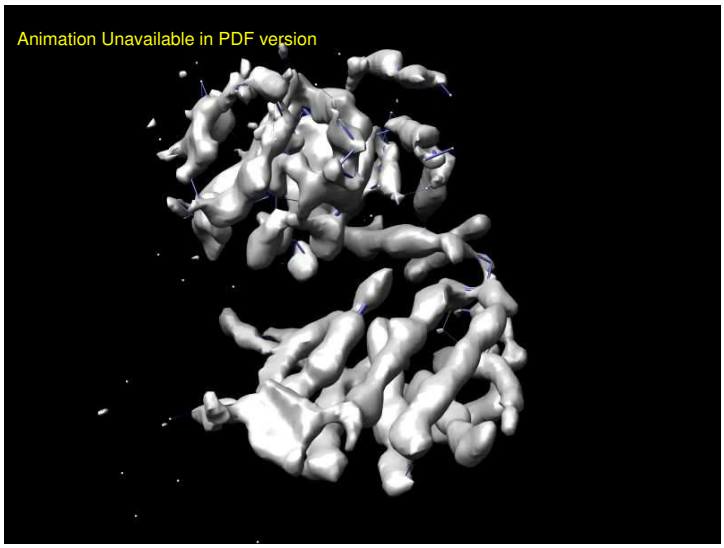

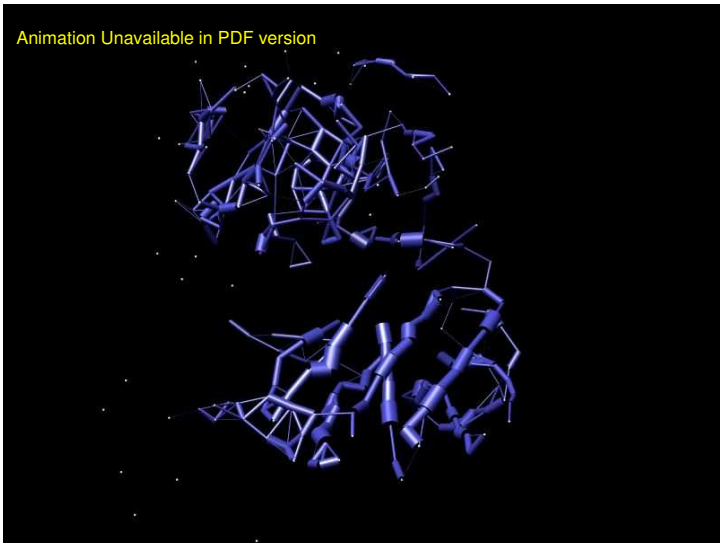
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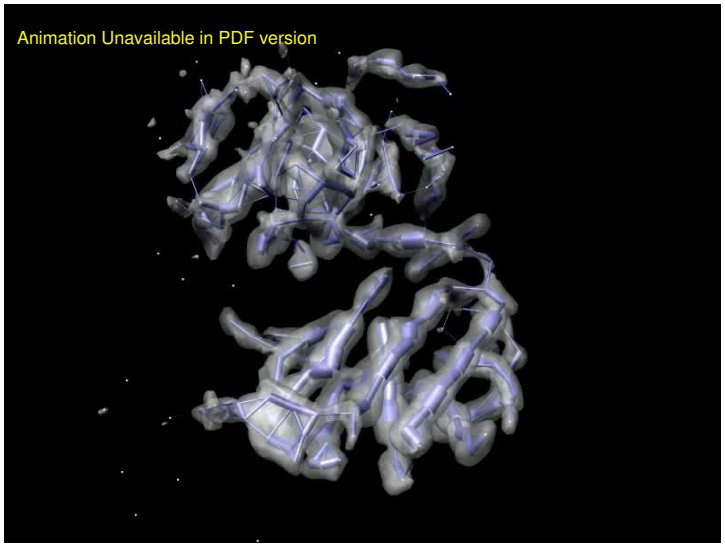
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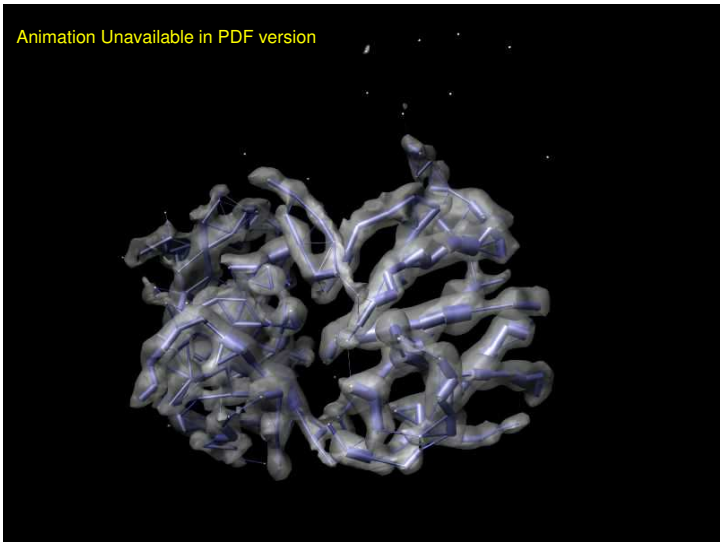
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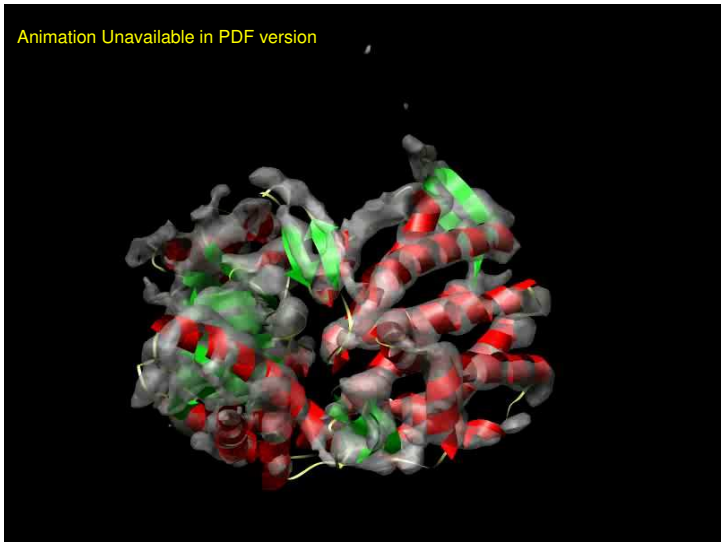
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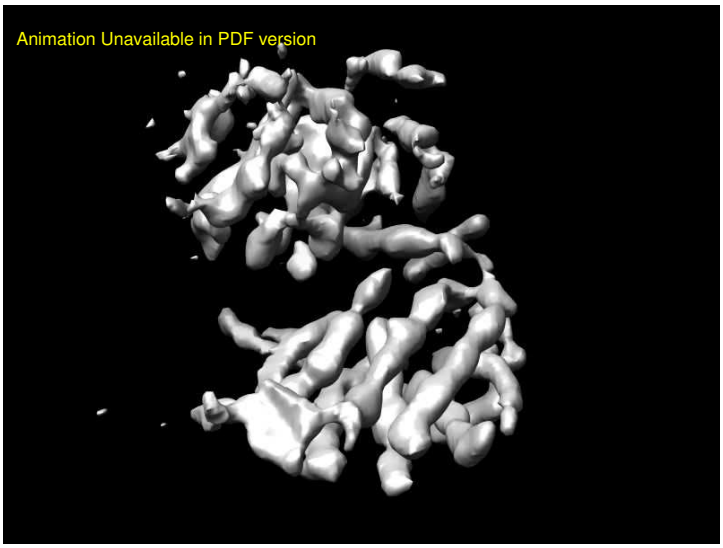
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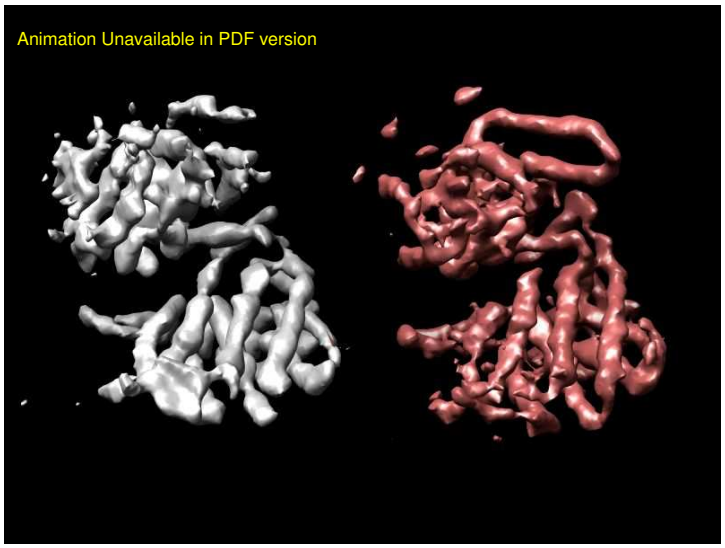
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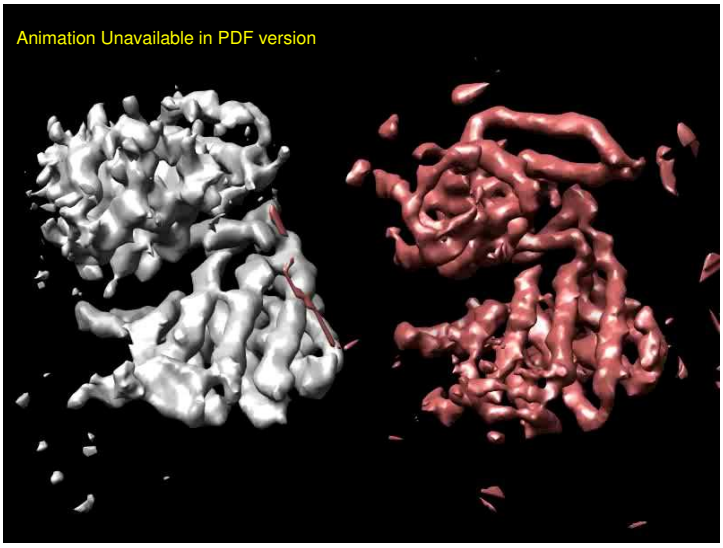

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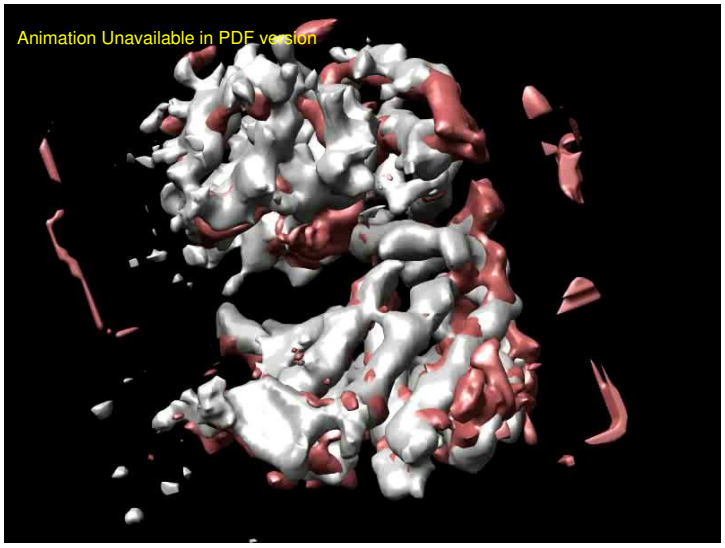

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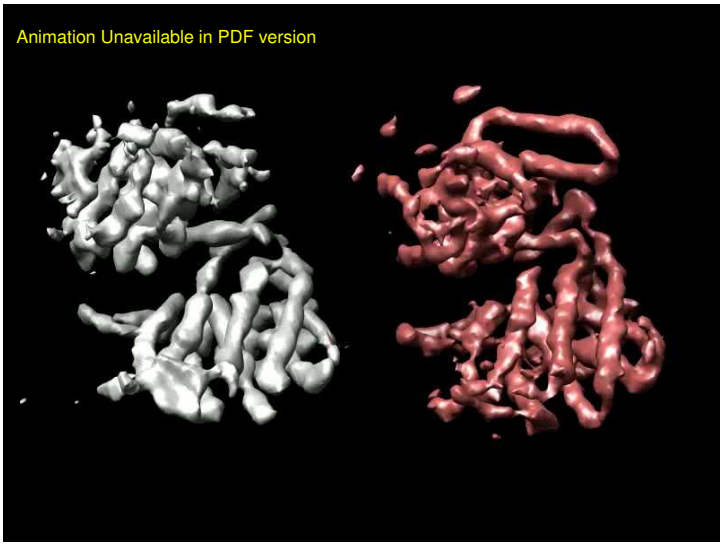
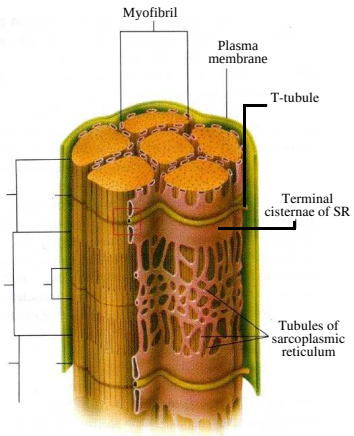


Ca^{2+} Release Channel

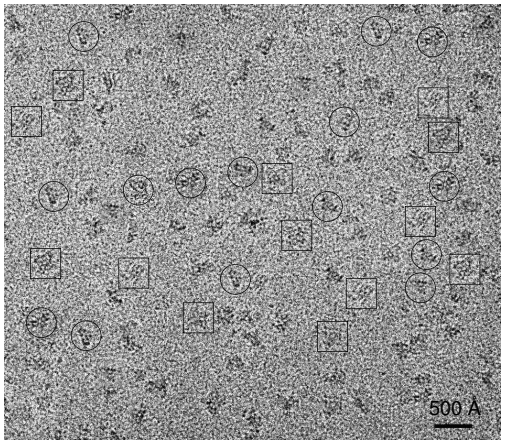
Irina Serysheva
Wah Chiu
Susan Hamilton



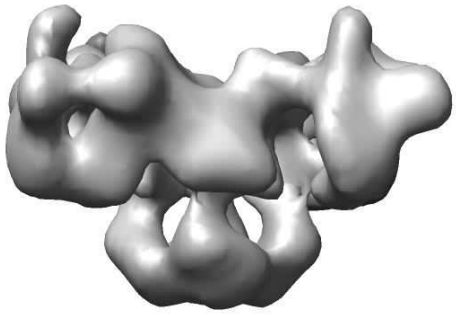
Ca^{2+} Release Channel

- **SR membrane, triggered by DHPR in T-tubule**
- **Homotetramer**
- **~2200 kDa**
- **Releases Ca^{++} which initiates cross-bridge cycle**

200 kV image of ice-embedded RyR1 (no continuous CF)



~30 Å Resolution



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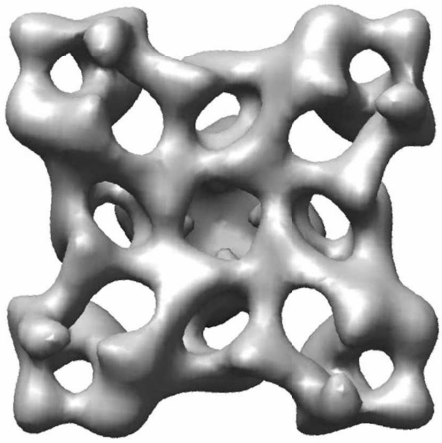
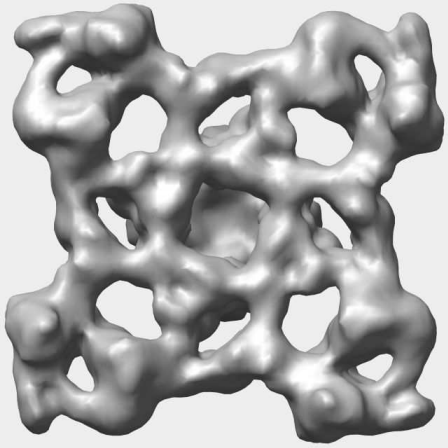
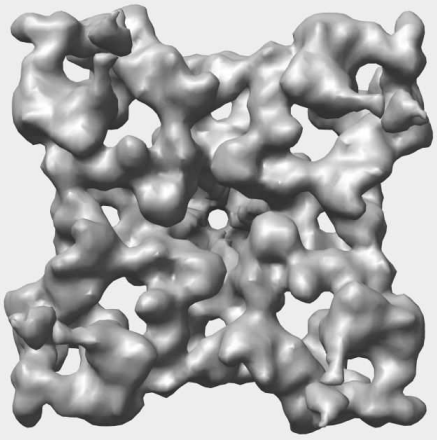


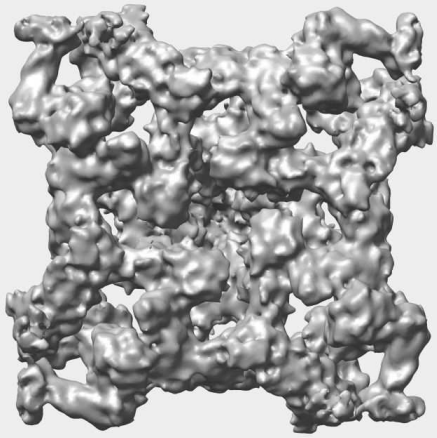
20 Å Resolution



14 Å Resolution



9.6 Å Resolution



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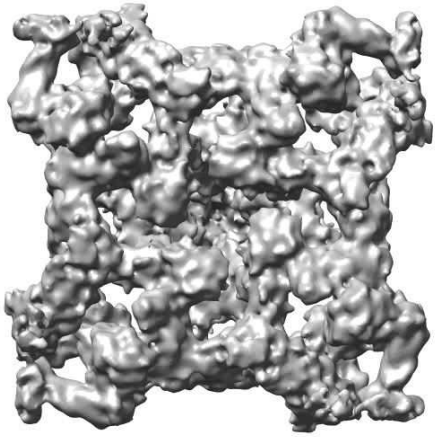

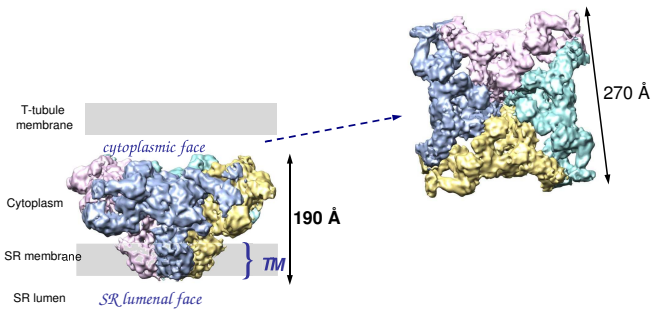

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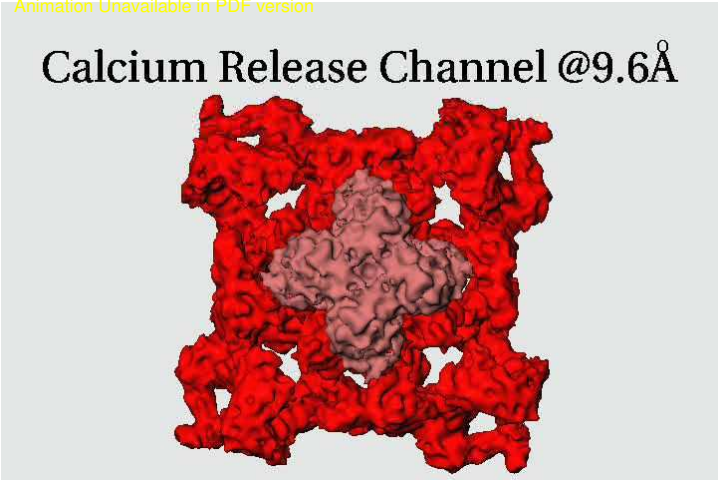
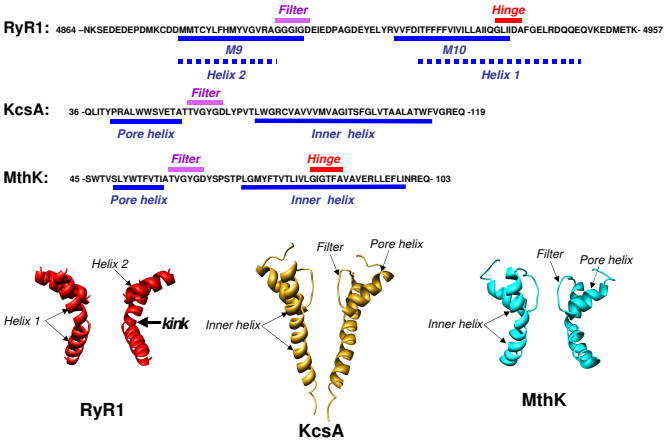
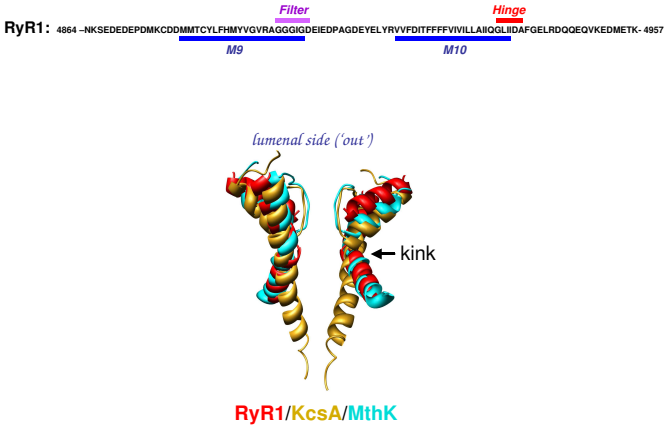


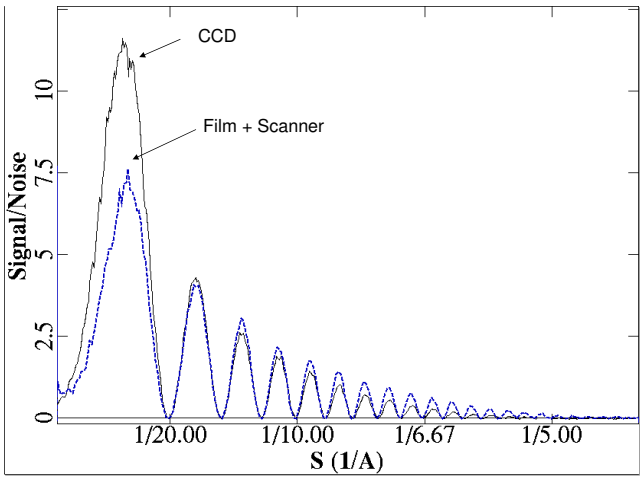
Sequence assignment of observed helices

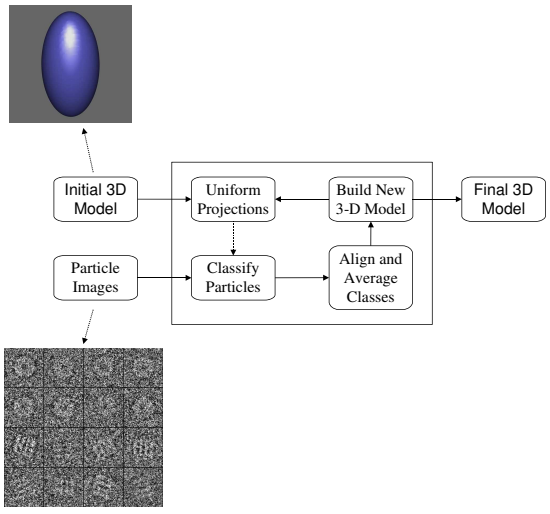


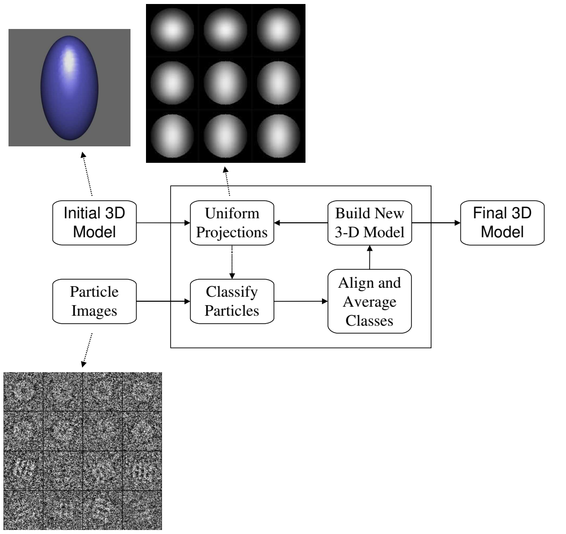
Sequence assignment of observed helices

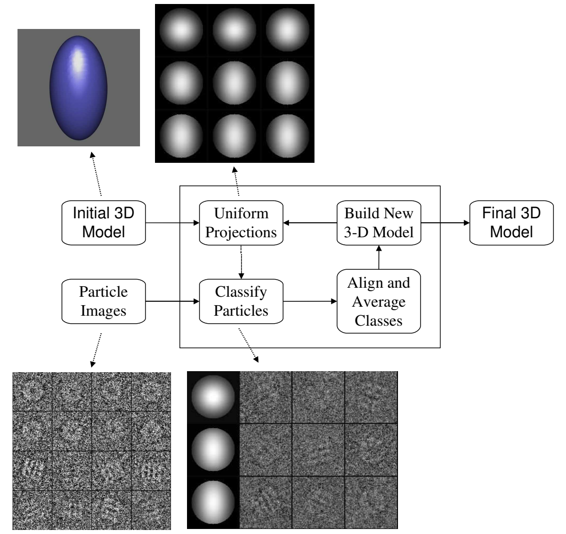


CCD vs. Film






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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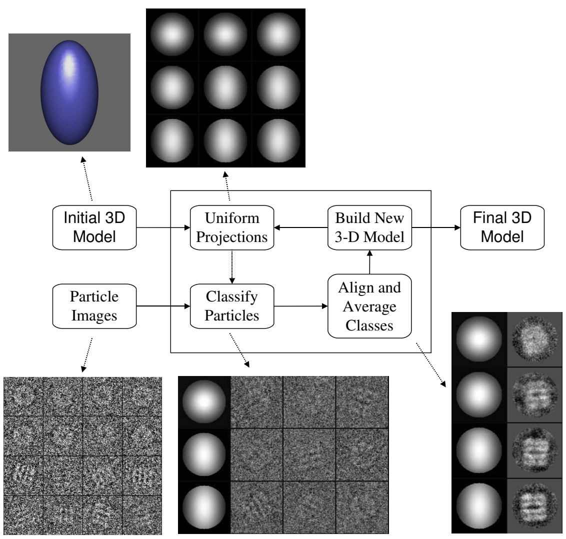
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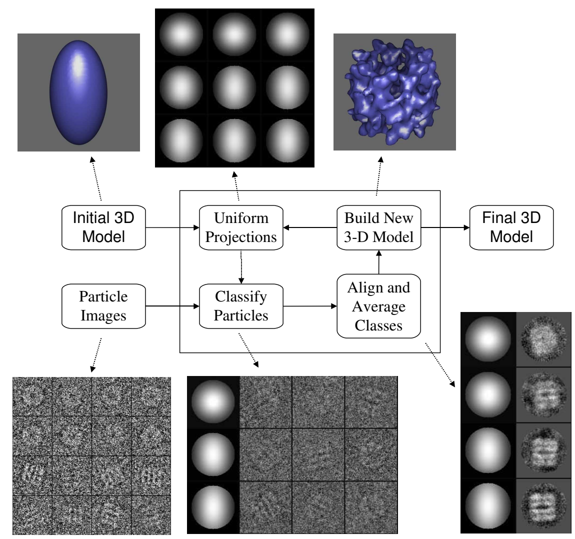
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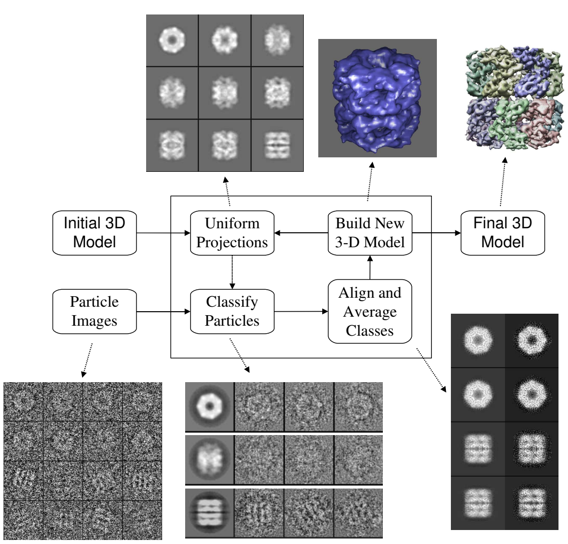
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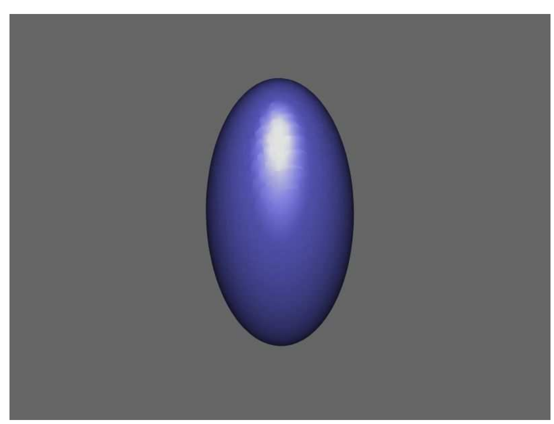
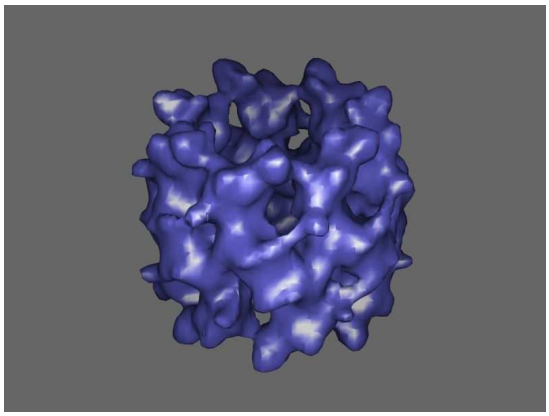
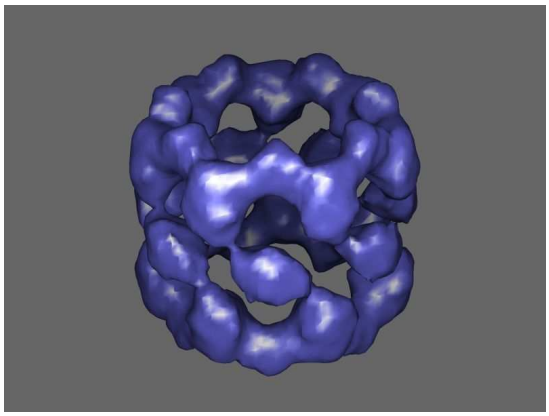
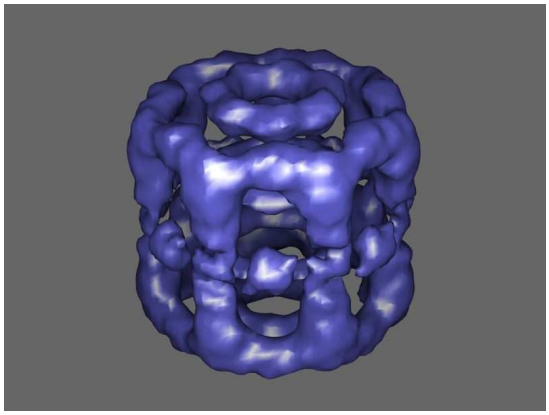
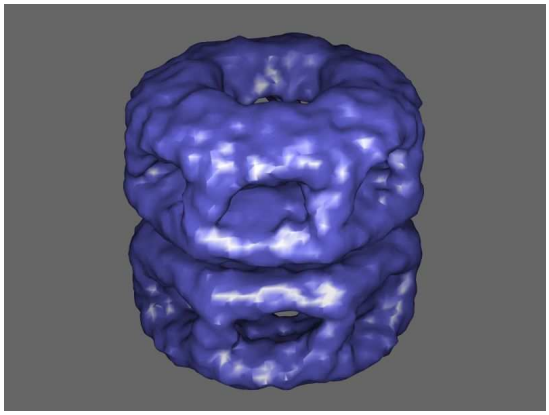
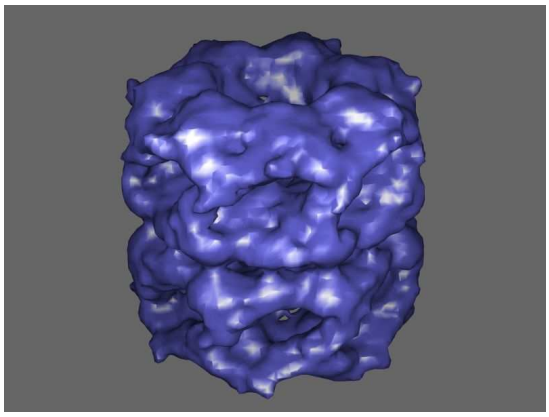












How do we get to Higher Resolutions?

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

Contrast Transfer Function

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

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

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

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

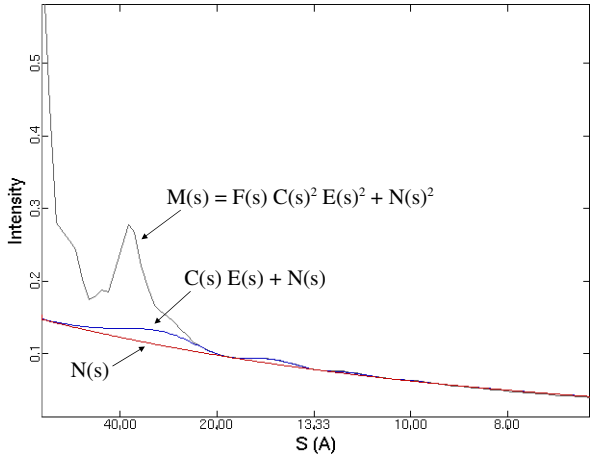
$$|N^2| = n_1 e^{n_2 s + n_3 s^2 + n_4\sqrt{s}}$$

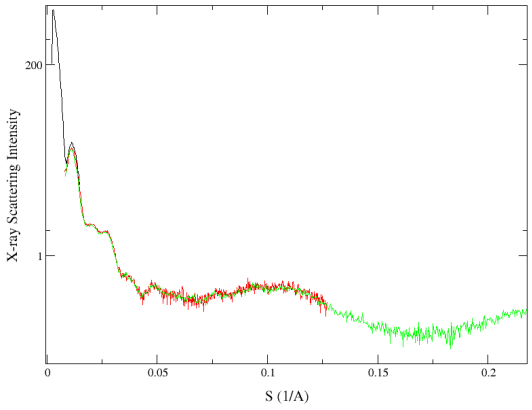
$$M(s)^2 = F(s)^2 C(s)^2 E(s)^2 + N(s)^2$$

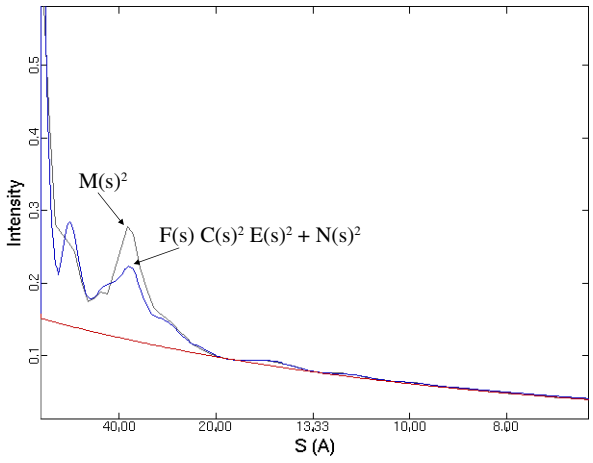
8 Parameters

ΔZ - Defocus
Q - Amplitude Contrast
B - Gaussian Envelope Width
k - Signal Amplitude

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







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)*

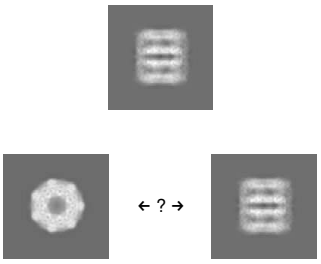
CTF Correction

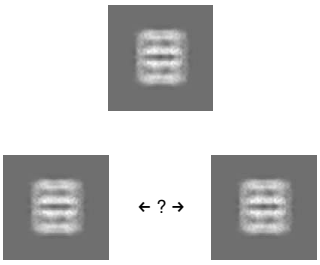
Wiener Filter | CTF Correction | SNR Weight

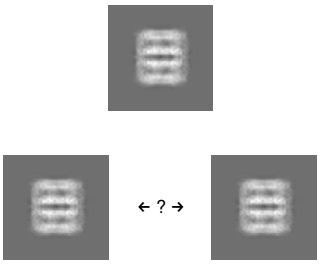
$$\overline{T}(s,\theta)=\frac{F^2(s)R(s)}{1+F^2(s)R(s)}\sum_i \frac{1}{C_i(s)E_i(s)}\frac{R_i(s)}{R(s)}\overline{M}_i(s,\theta)$$

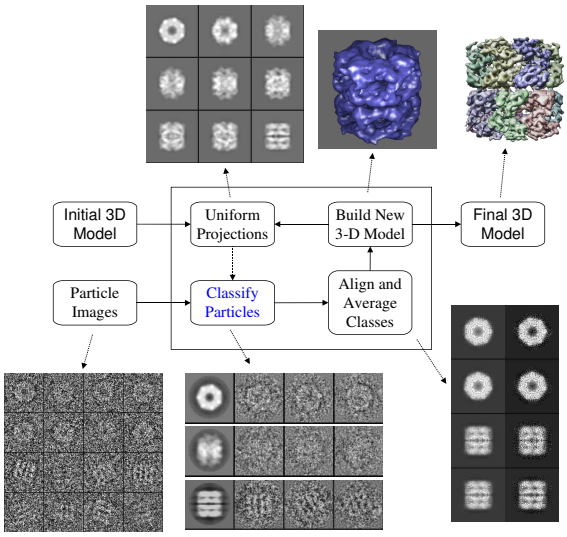
$$R_i(s)=\frac{C_i^2(s)E_i^2(s)}{N_i^2(s)} \qquad R(s)=\sum_i \frac{C_i^2(s)E_i^2(s)}{N_i^2(s)}$$

Image Classification

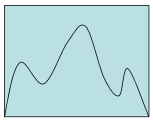








Alignment/Registration



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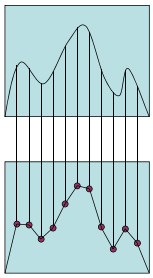
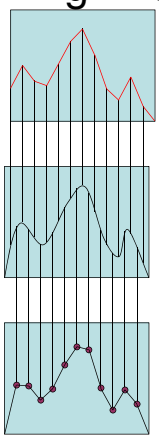
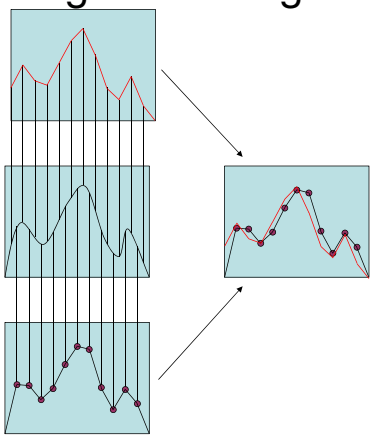
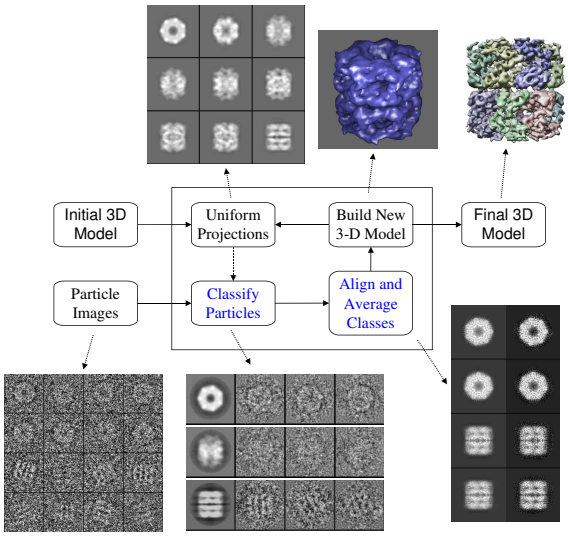


Alignment/Registration



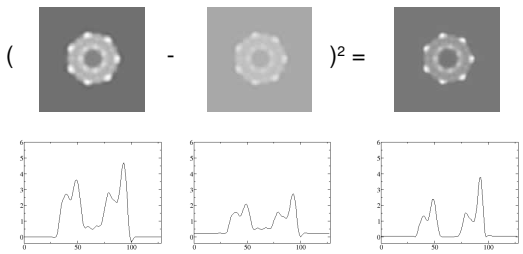
Alignment/Registration

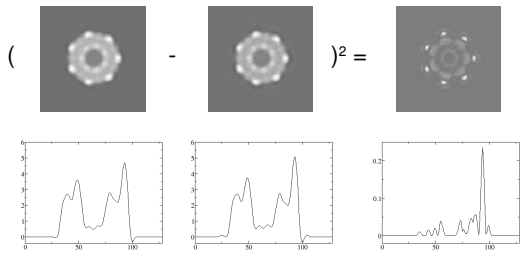


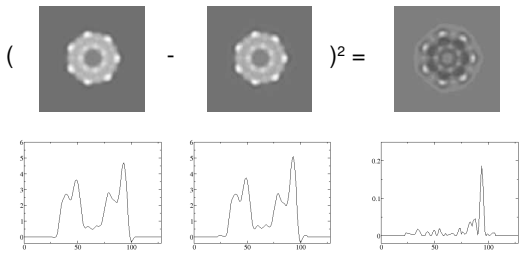


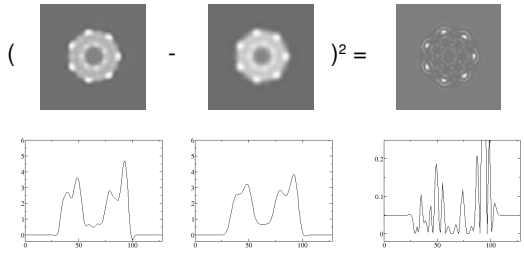
Measures of Similarity

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



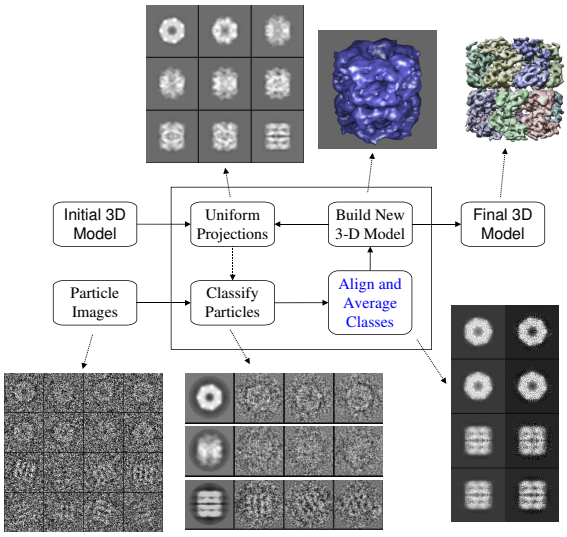




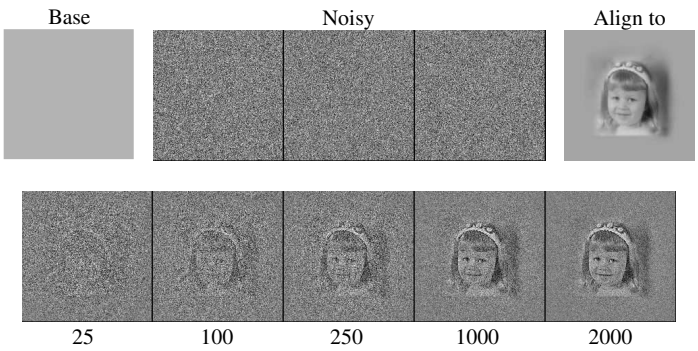


And the Answer is…

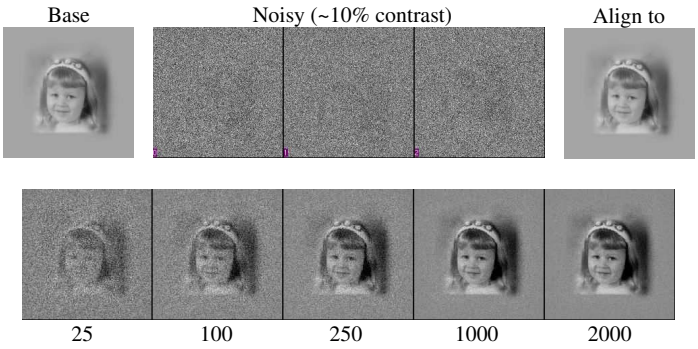
- Wiener filter particle
- Filter reference to match
- Normalize reference density to particle
- Calculate variance



Model Bias



Model Bias



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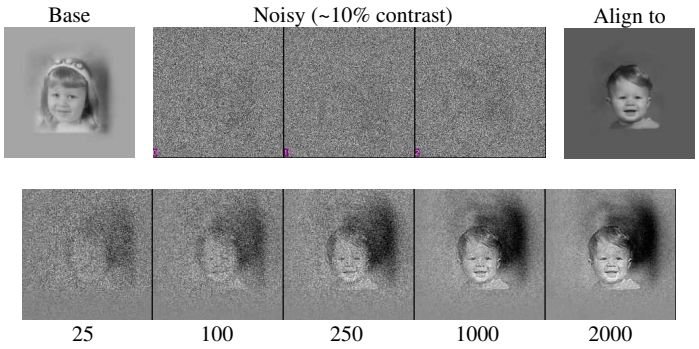

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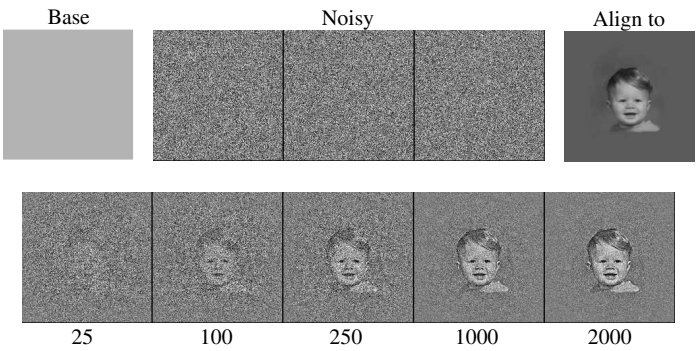

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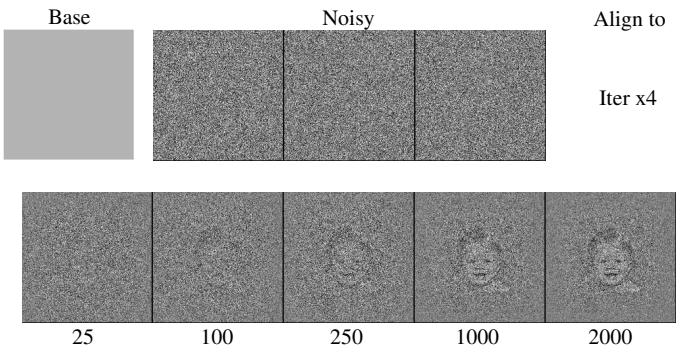

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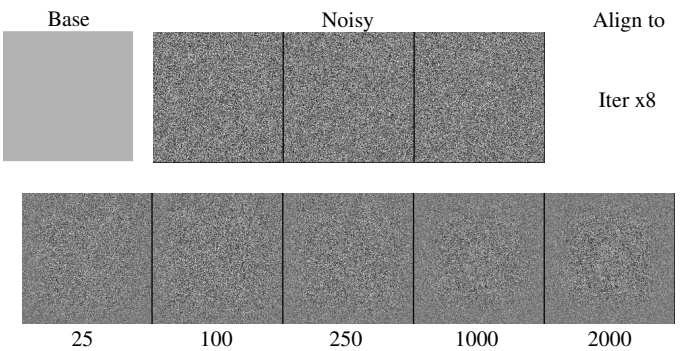

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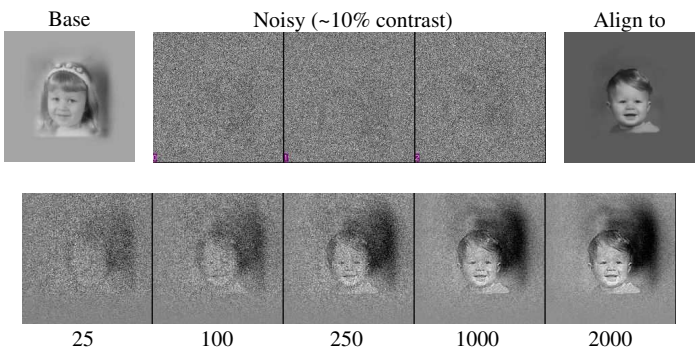
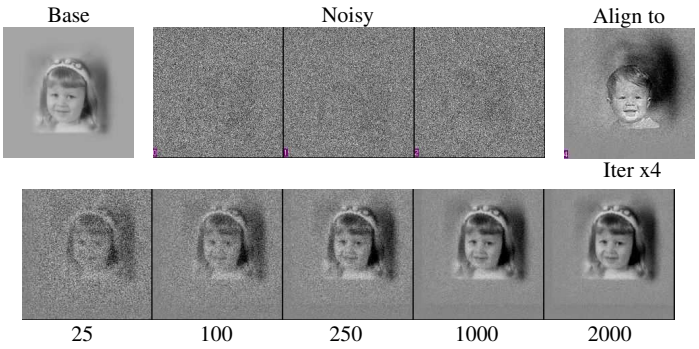
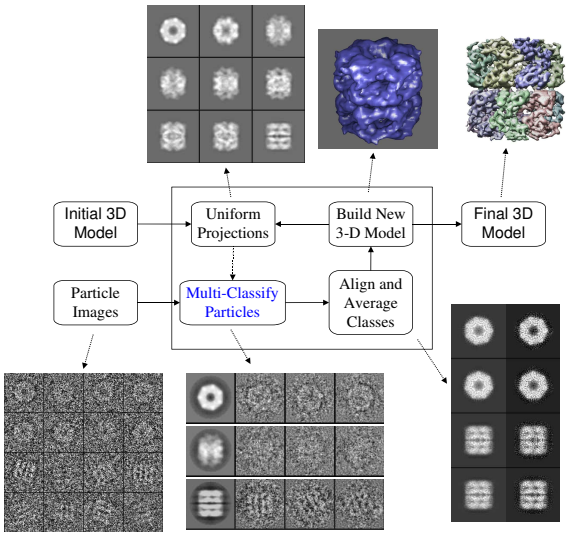


Model Bias





- Each particle -> best n classes
- More restrictive exclusion from class-avg

The Future

- Better similarity criteria
- Improved CTF model
- Per-particle CTF (at least defocus)
- Beam tilt
- Better 3-D reconstruction
- New refinement methodologies