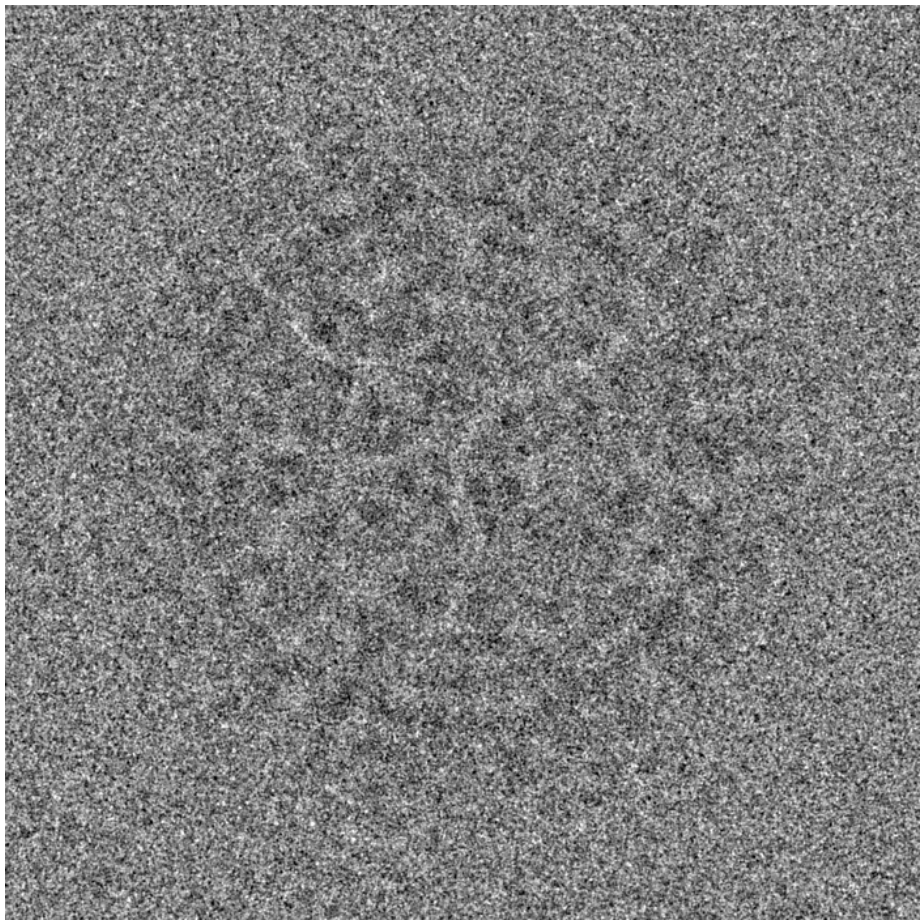
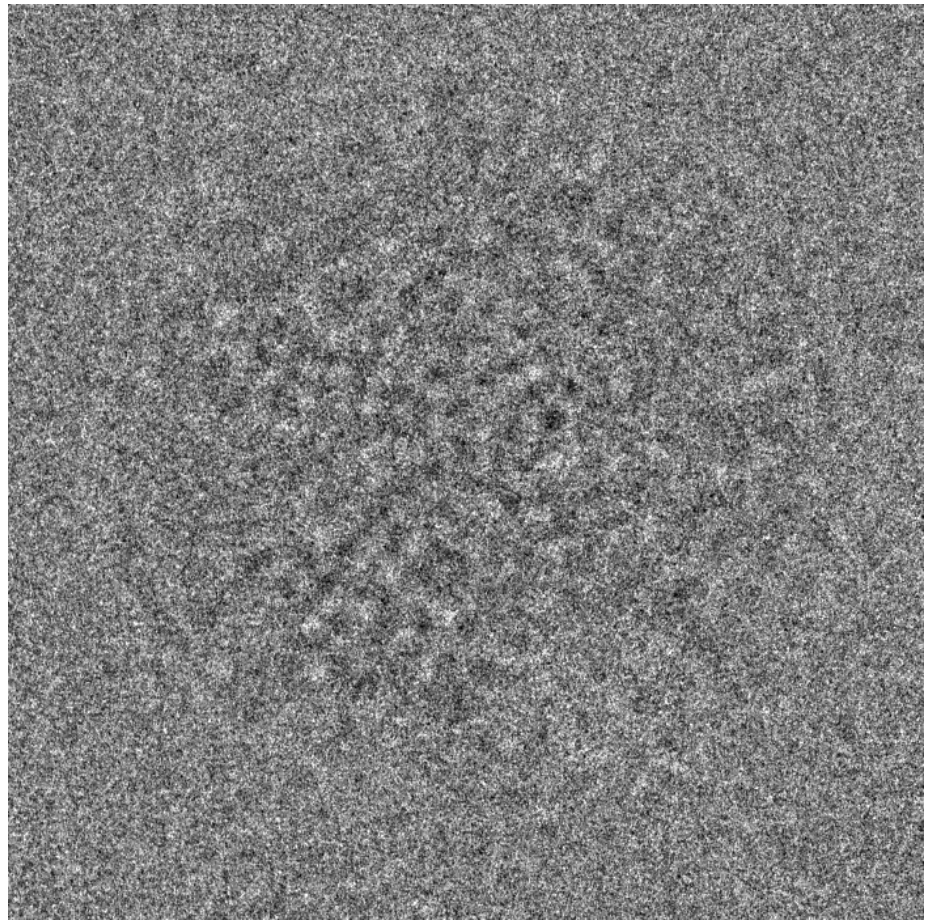
Correcting the Image

N. Grigorieff

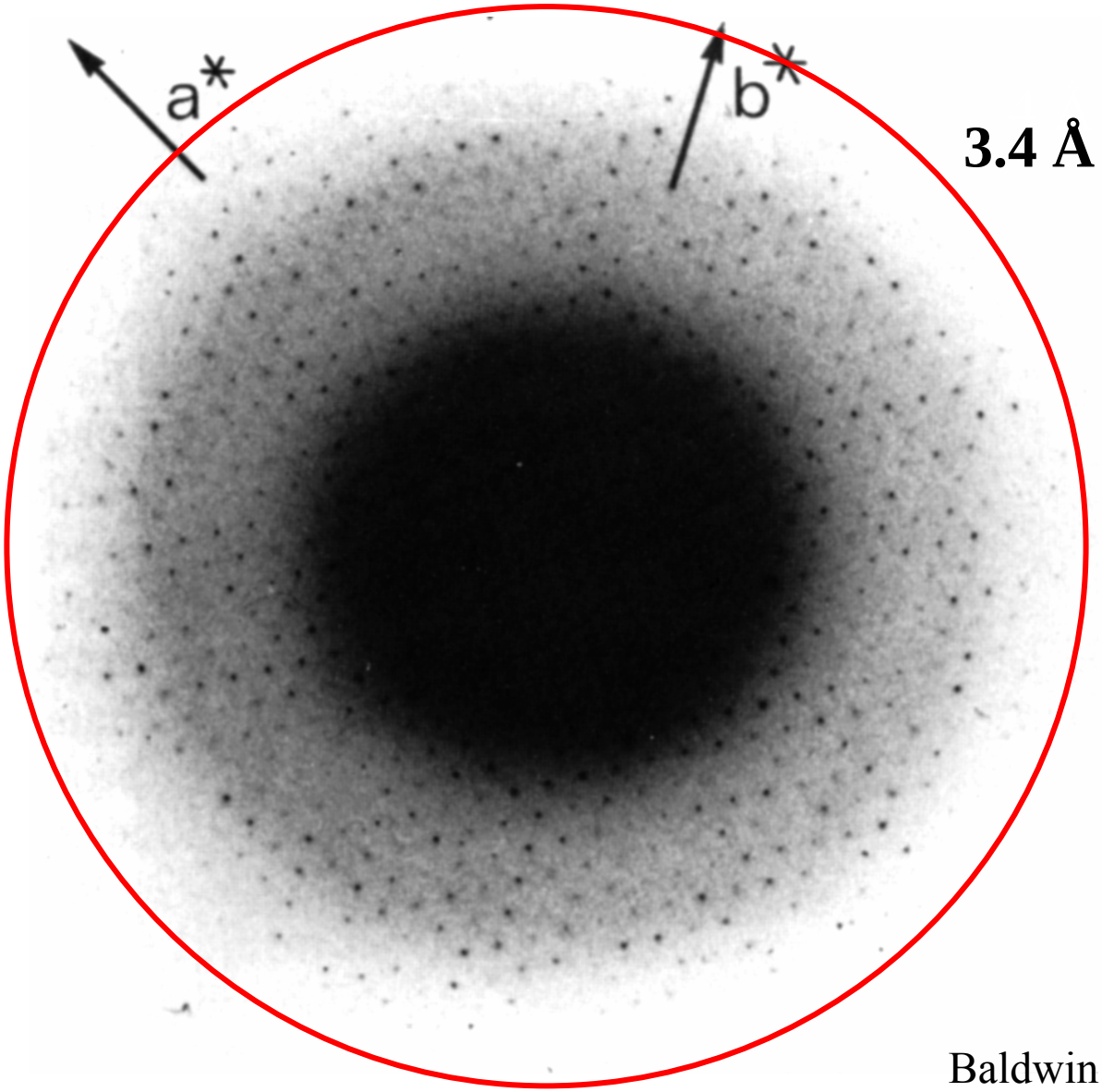
What is “Wrong” with the Image?





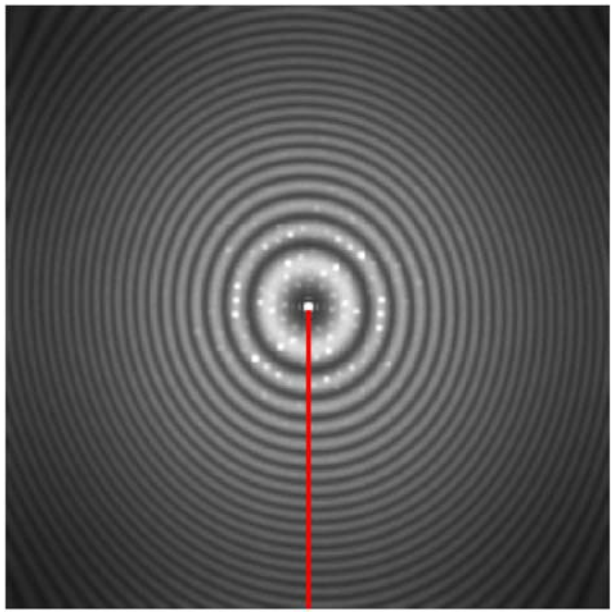
Xing Zhang 2007

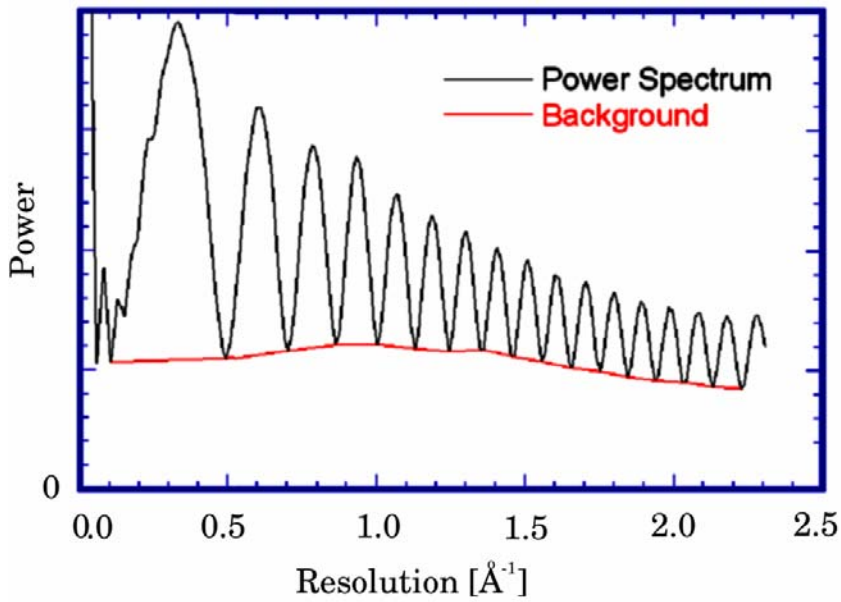
Electron Diffraction



Baldwin & Henderson 1984

Image Contrast





$$P(\mathbf{k}) = \left[\left(S(\mathbf{k}) + B_i(\mathbf{k})\right) ENV_i(\mathbf{k}) CTF(\mathbf{k})\right]^2 MTF^2(\mathbf{k})$$

$$+ B_s^2(\mathbf{k}) MTF^2(\mathbf{k}) + B_e^2$$

S = signal

B_i = imaged background

ENV_i = CTF envelope

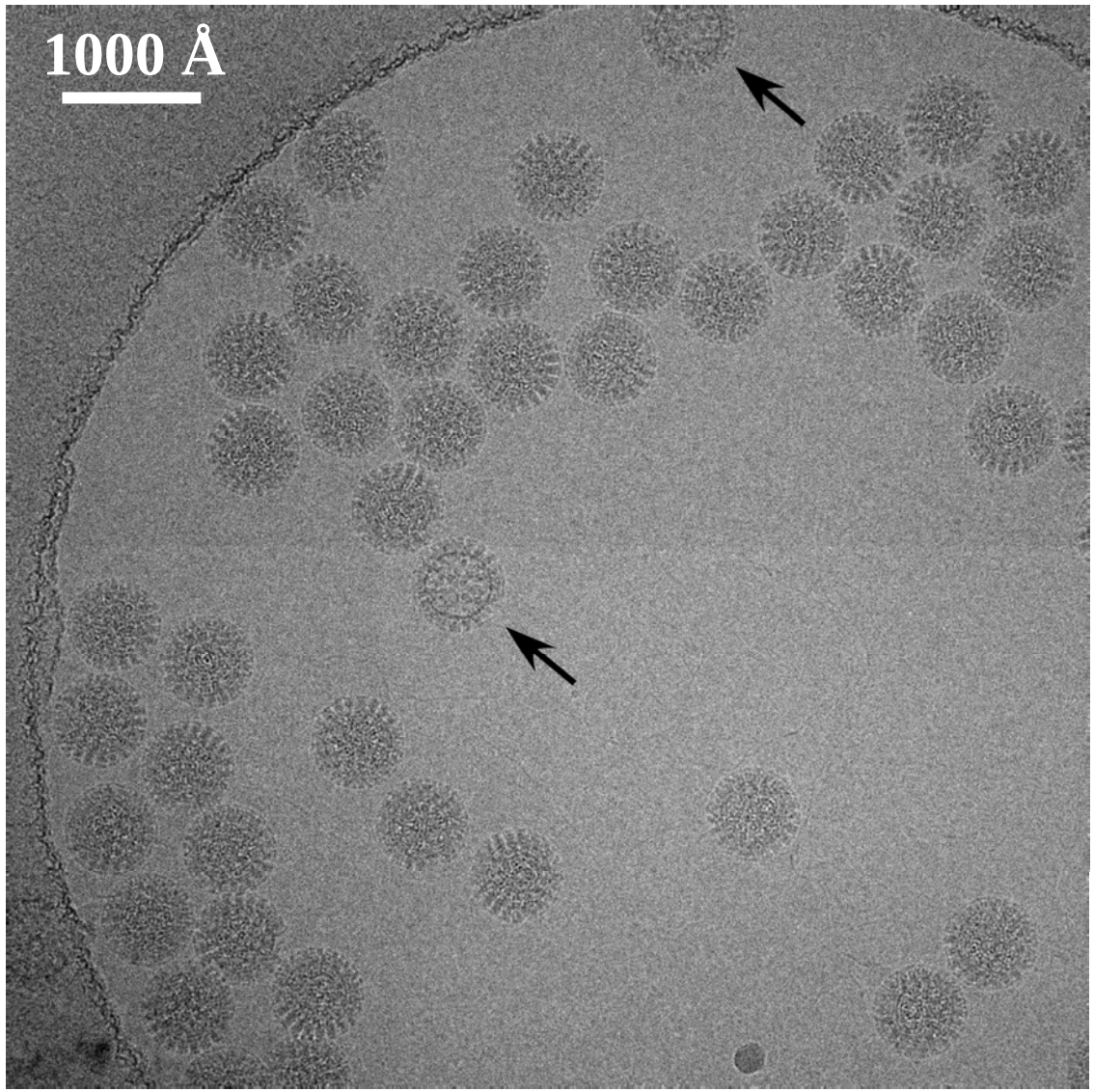
MTF = Detector MTF B_s = shot noise B_e = detector noise

Zeng et al. 2007

Things to Worry About

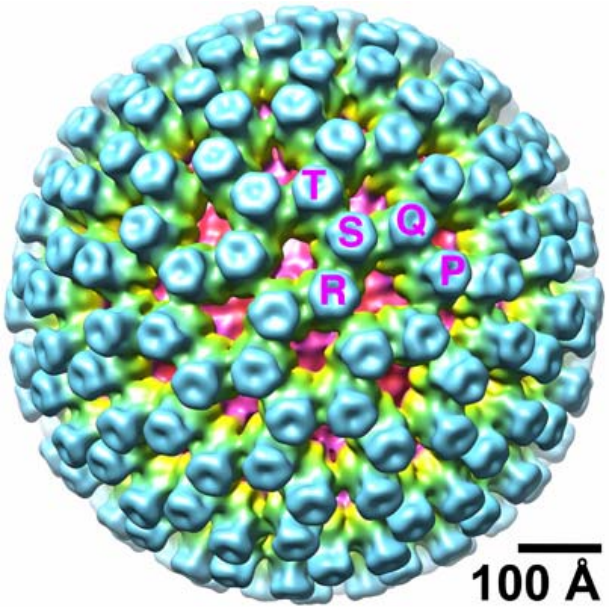
- Quality
  - Resolution, contrast
- Contrast transfer function
  - Defocus, astigmatism, beam tilt, envelope
- Magnification
  - Variations from image to image/within images
- Ewald sphere (for large objects)
- Contrast normalization
- Inelastic and multiple scattering
  - Energy filter, diffraction theory

Rotavirus Double-Layer Particles

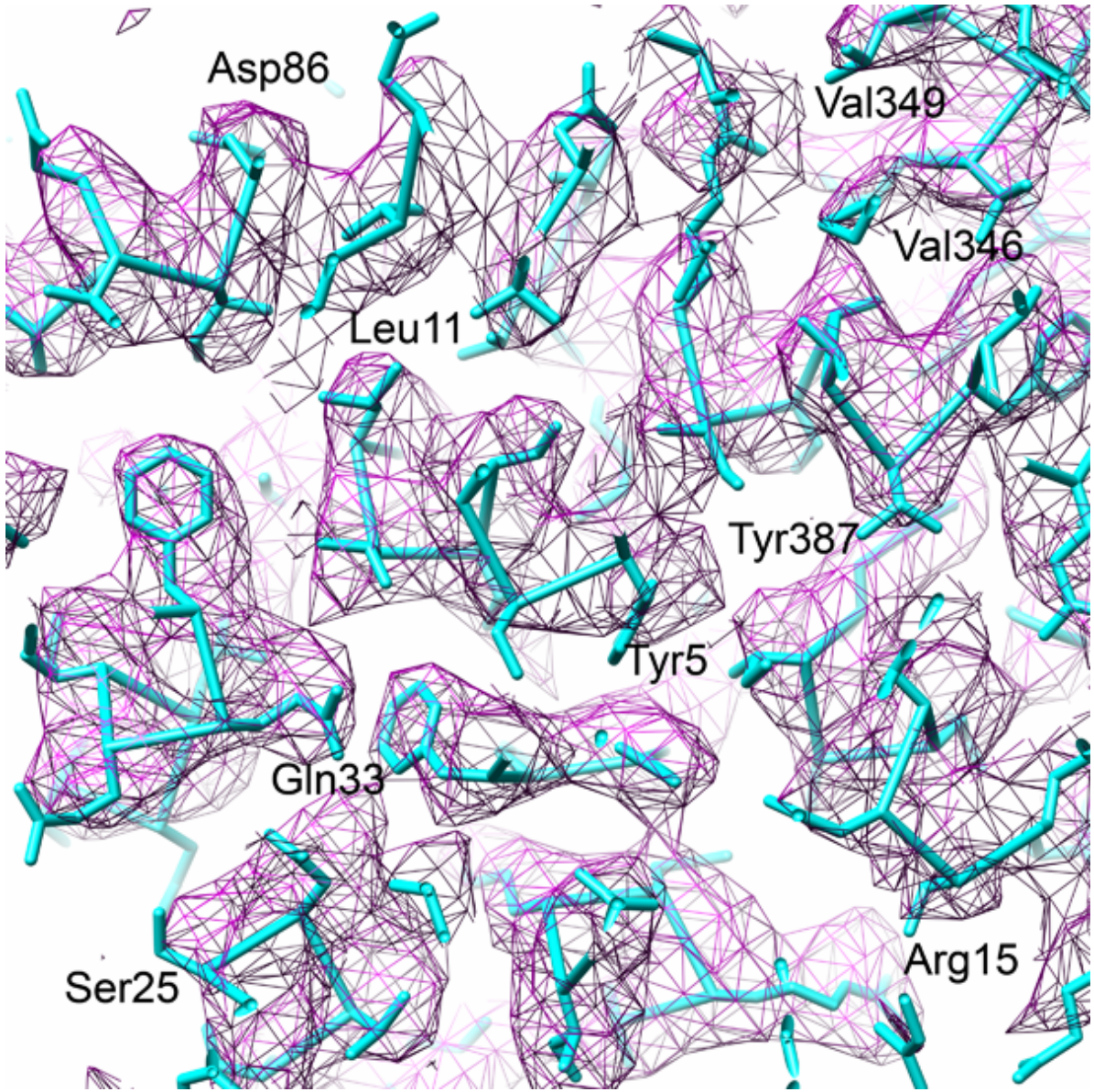


Side Chains

Rotavirus
double-layer
particles



8400 particles



Xing Zhang
Ethan Settembre 2007

Image Quality - Grids

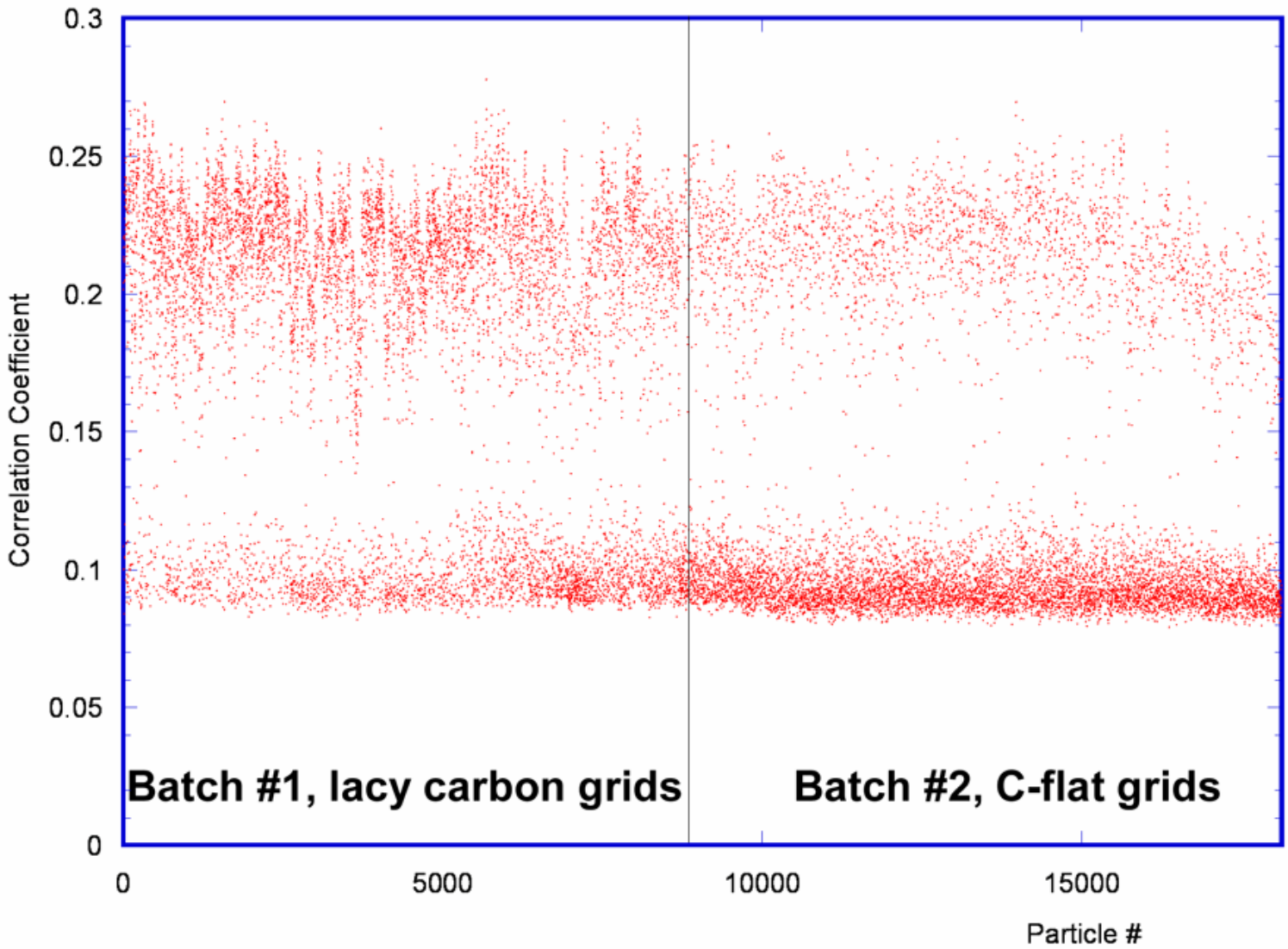


Image Quality – Location on Grid

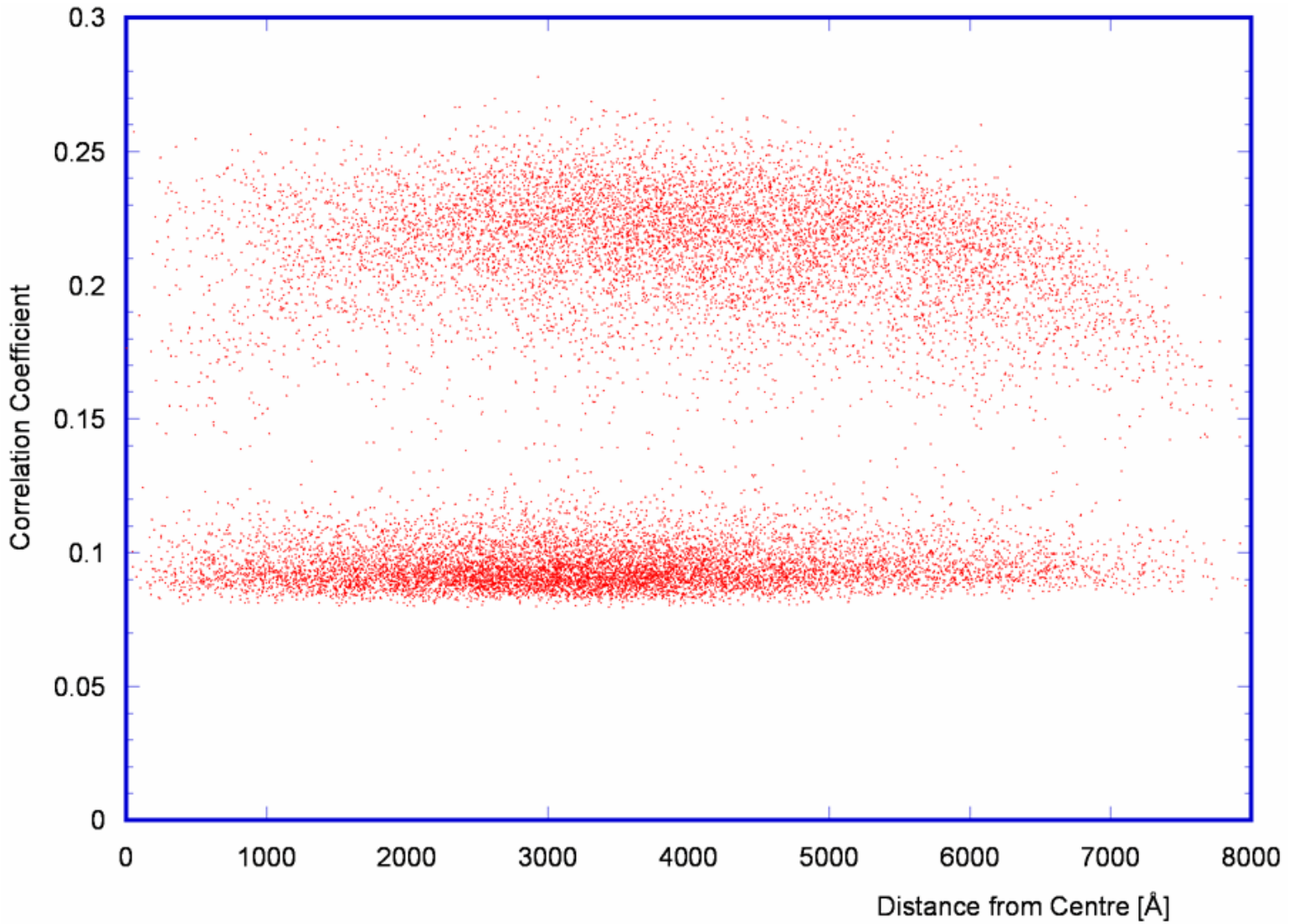
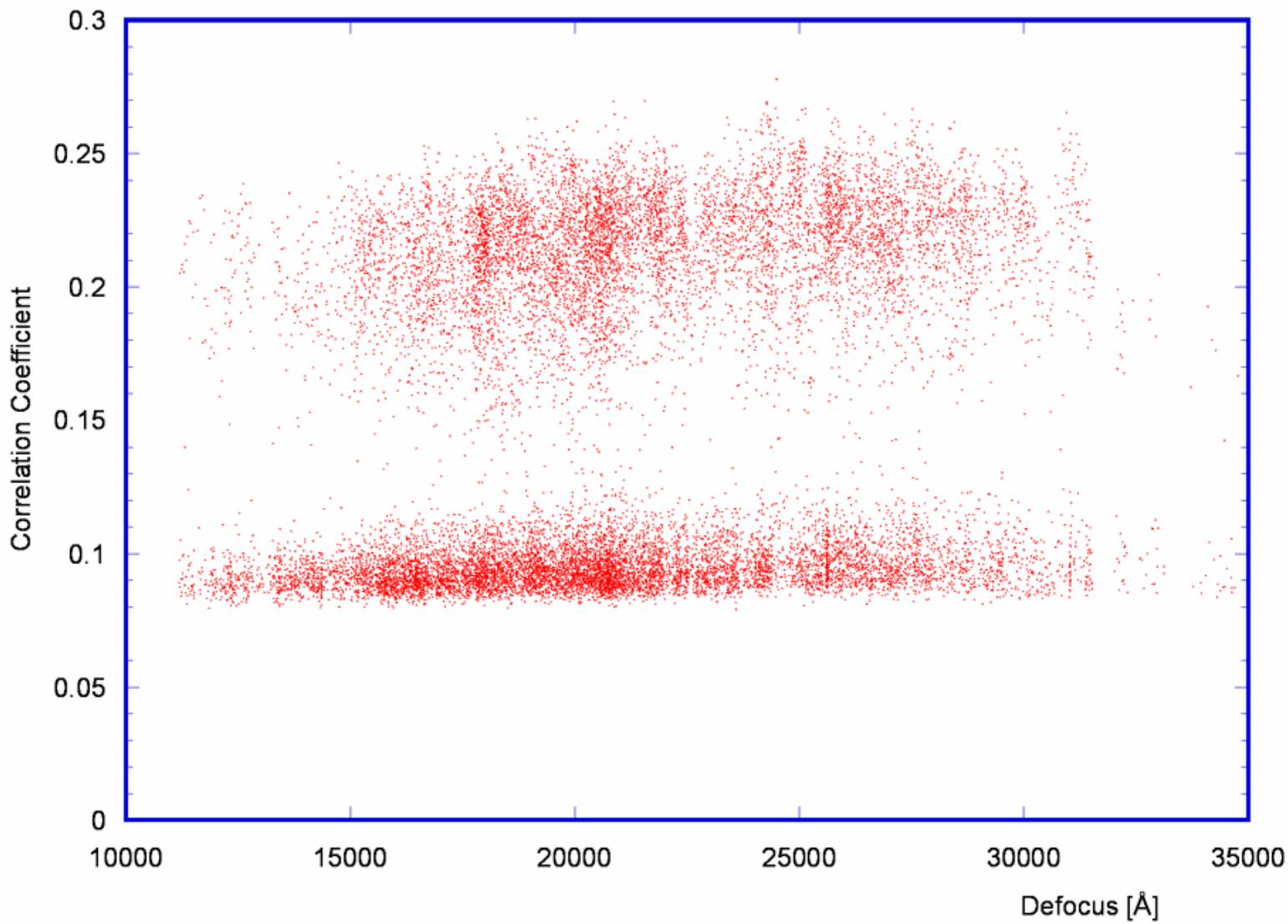
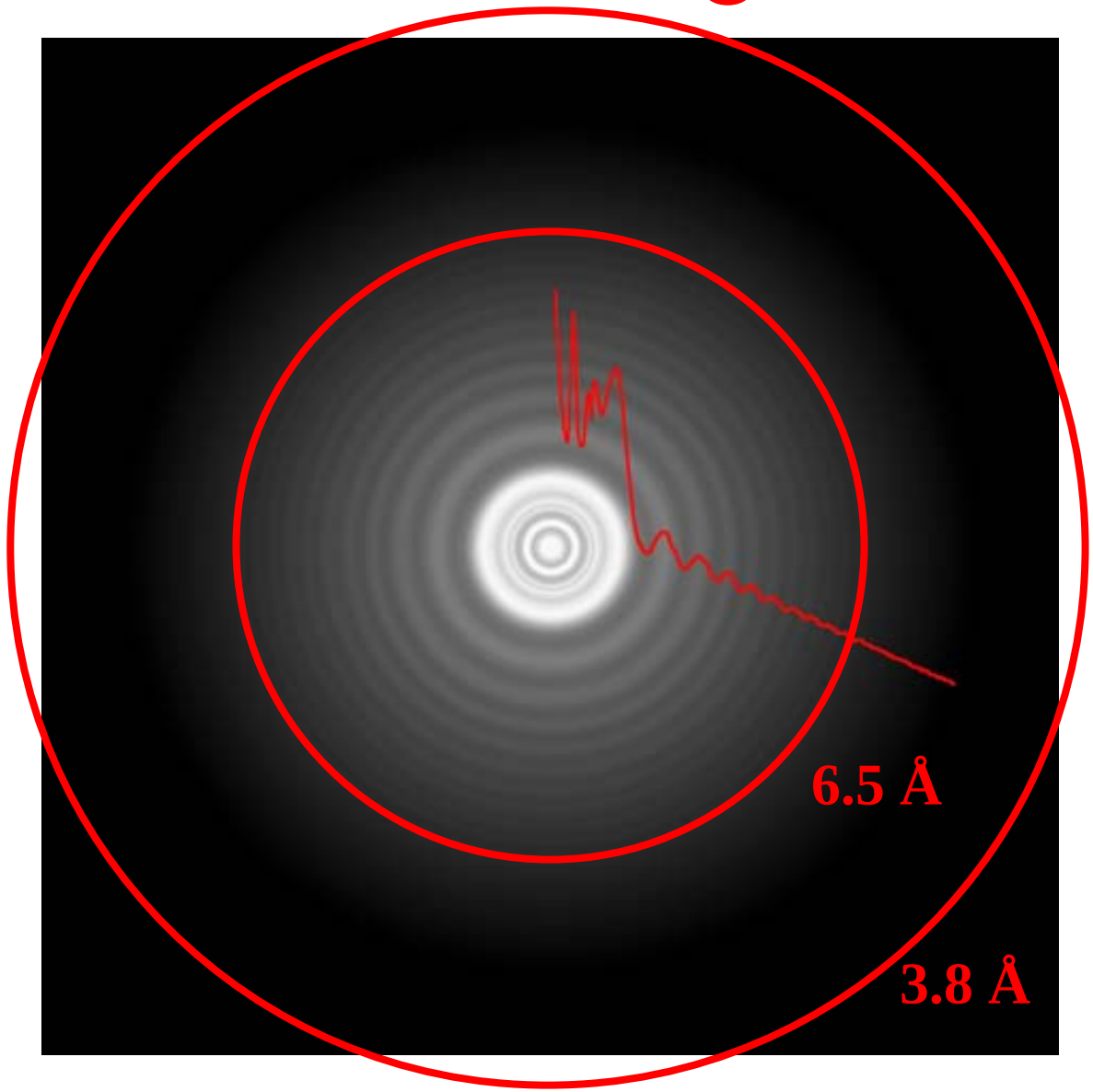


Image Quality - Defocus



Thin Rings



Determining the CTF

CTFFIND3

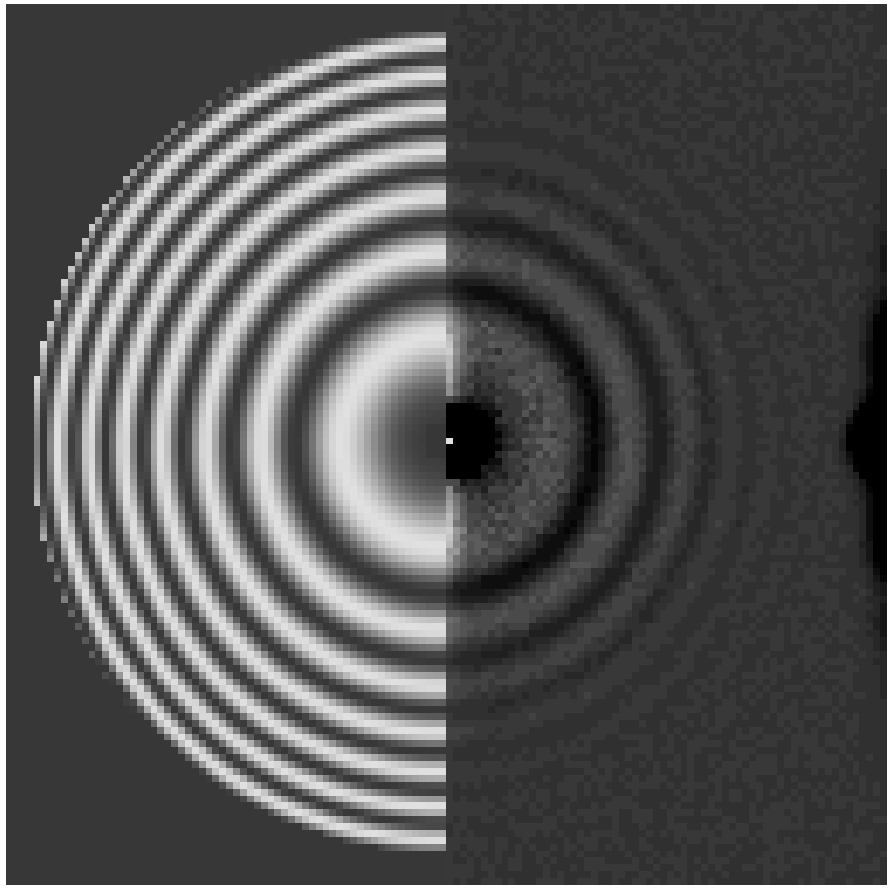


Image power spectrum

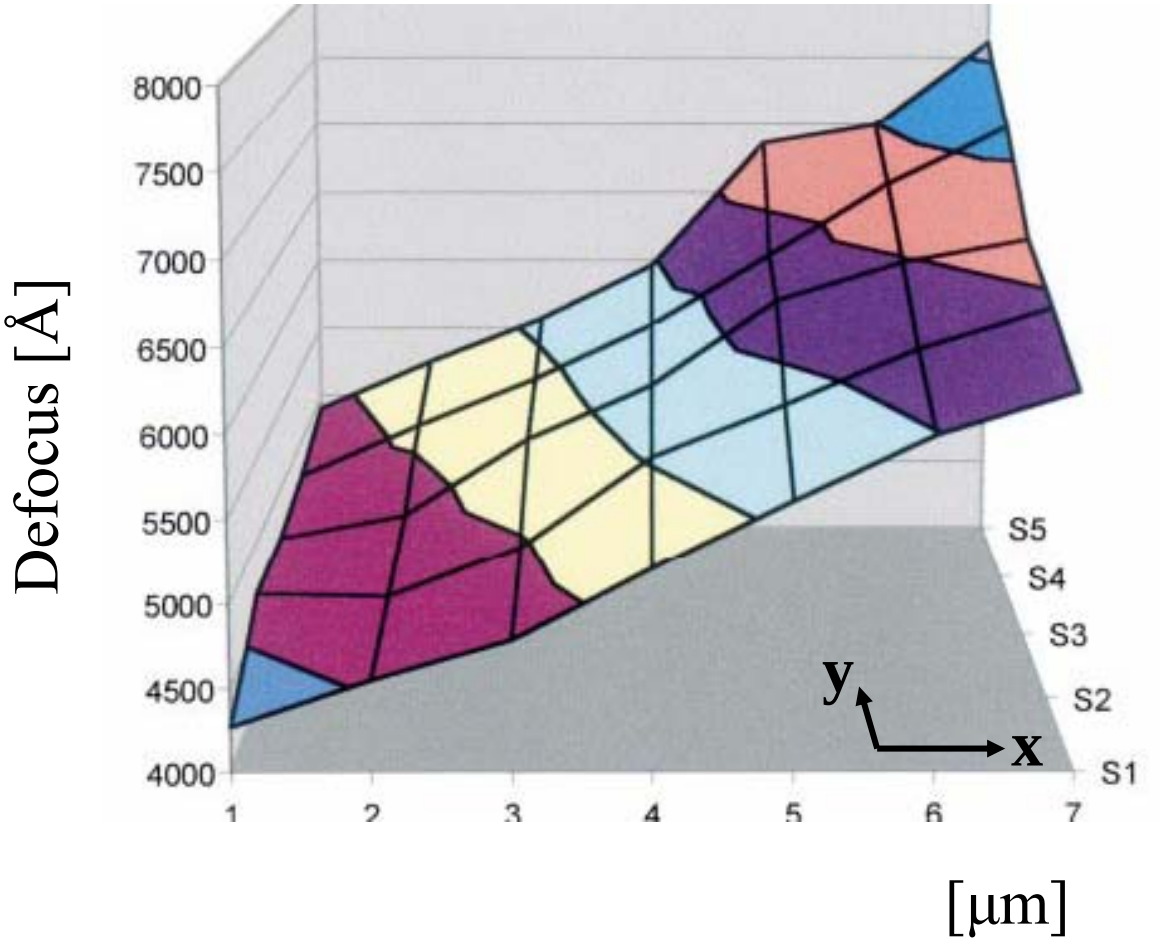
Model

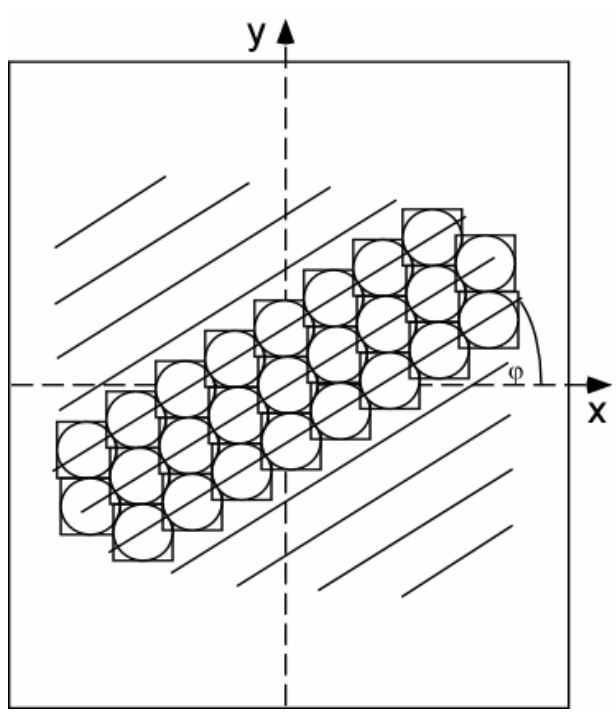
Experiment

$E = 120$ kV, $\Delta f = 21000$ Å, $C_s = 2$ mm, $A = 0.15$

Mindell & Grigorieff 2003

Defocus Gradient



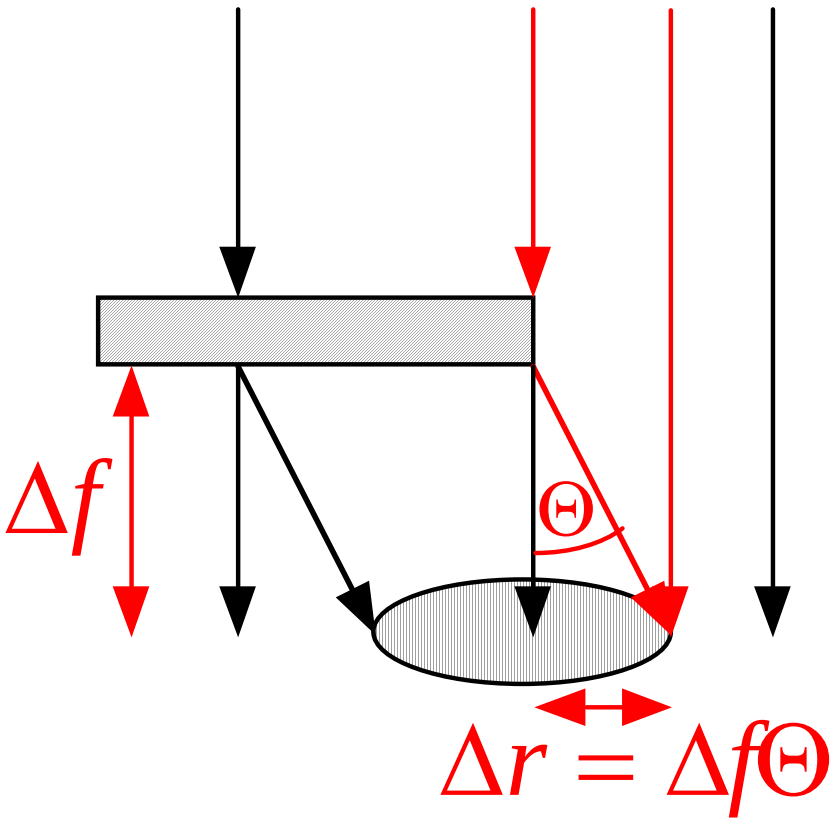


CTFTILT

van Heel et al. 2000

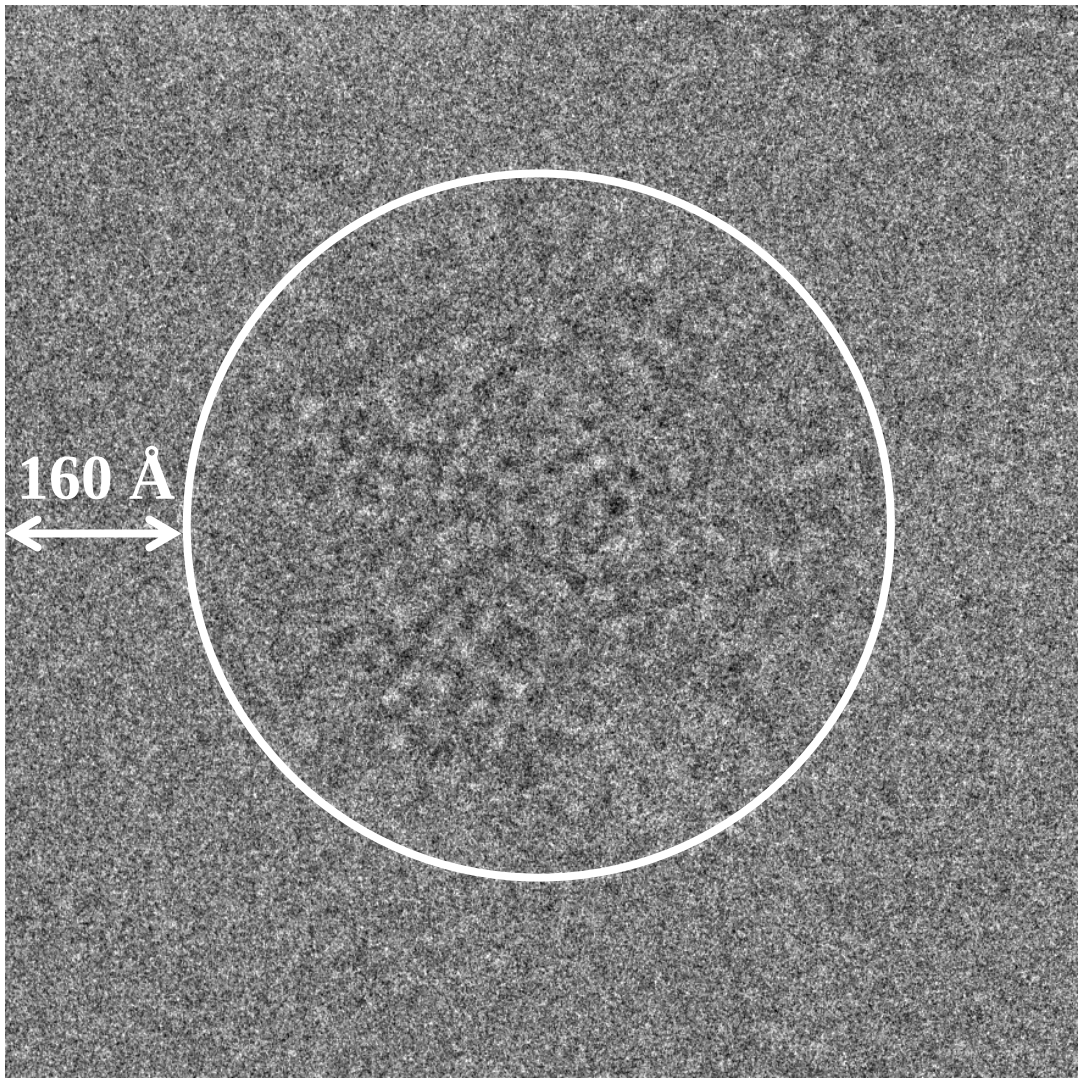
Mindell & Grigorieff 2003

Displaced Information

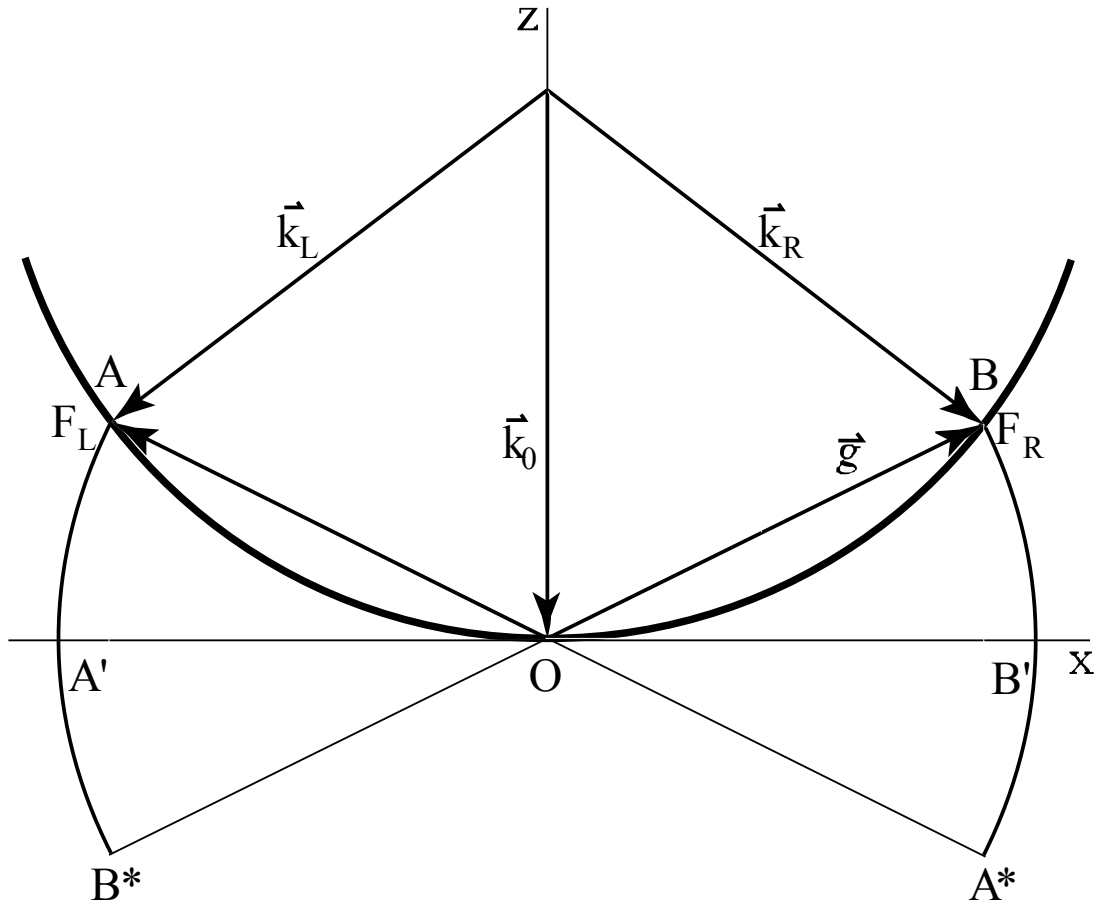


Margins

$$\Delta r = \Delta f \Theta$$
$$= 25000\text{Å} \times 5.3\text{mrad}$$
$$= 130\text{Å}$$



Ewald Sphere



Phase error = 60° for 700 Å particle at 300 kV

Normalization

$$\hat{I}(x,y) = \frac{I(x,y) - \text{avg}(I)}{\sqrt{\text{var}(I)}}$$

$$\hat{I}(x,y) = \frac{I(x,y) - \text{avg}[\text{bg}(I)]}{\sqrt{\text{var}[\text{bg}(I)]}}$$

Sorzano et al. 2004

Maximum Likelihood

Structure for *n*+1 iteration

$$A^{(n+1)} = \frac{1}{N} \sum_{i=1}^{N} \frac{\int X_i(\phi) p_i\left(\phi, \Theta^{(n)}\right) d\phi}{\int p_i\left(\phi, \Theta^{(n)}\right) d\phi}$$

Probability function

$$p_i(\phi, \Theta) = \left(\frac{1}{\sqrt{2\pi}\sigma}\right)^M \exp\left[-\frac{|X_i(\phi) - A|^2}{2\sigma^2}\right] f(\phi \mid \Theta)$$

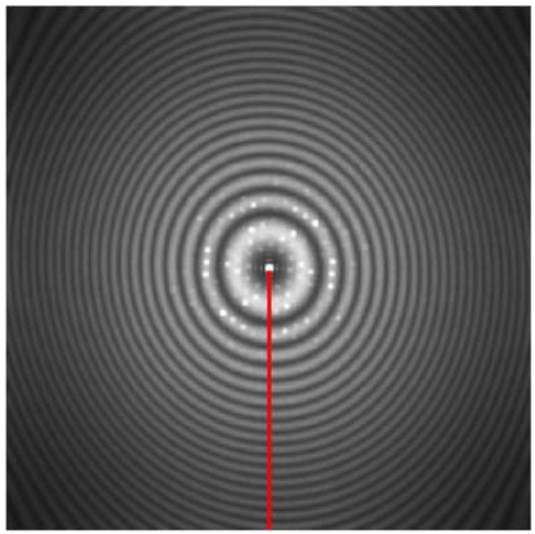
X_i: *i*th image
Θ: model parameters

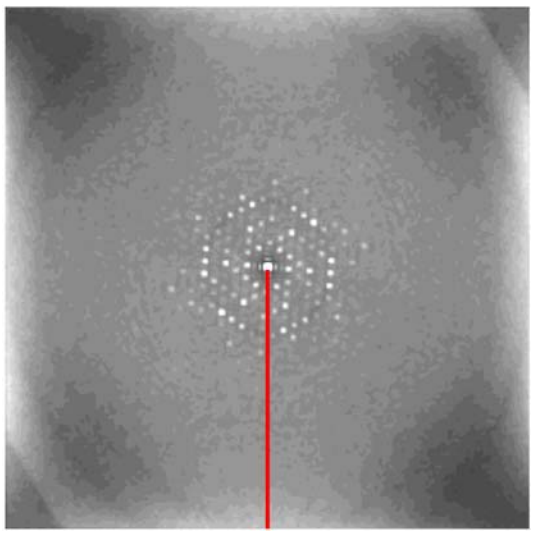
N: # of images
σ: noise in images

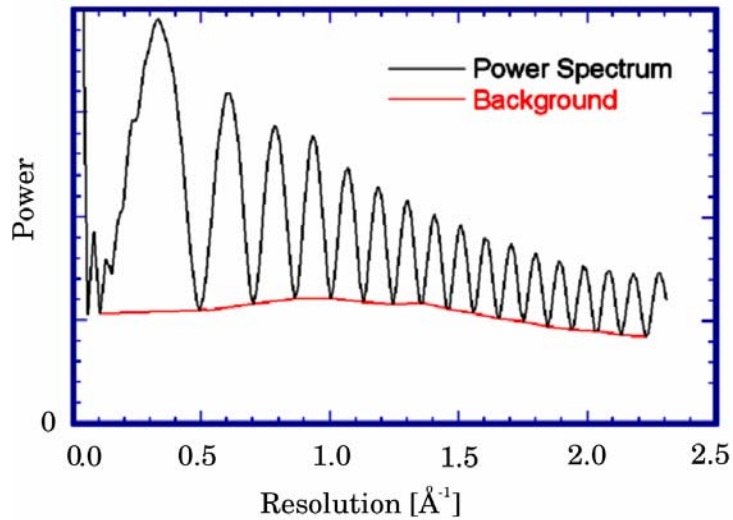
ϕ: alignment parameters
f: positional probab.

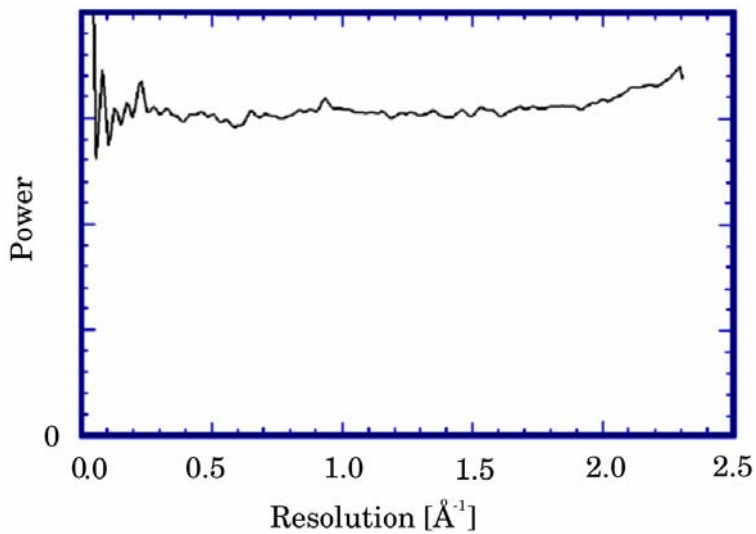
Sigworth 1998

Noise Model



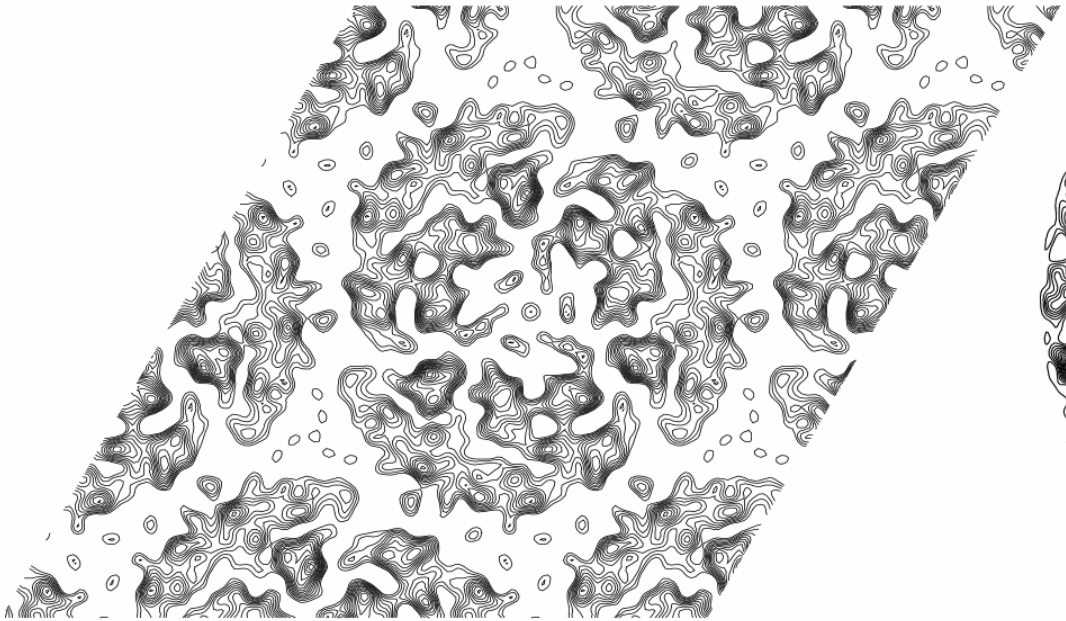




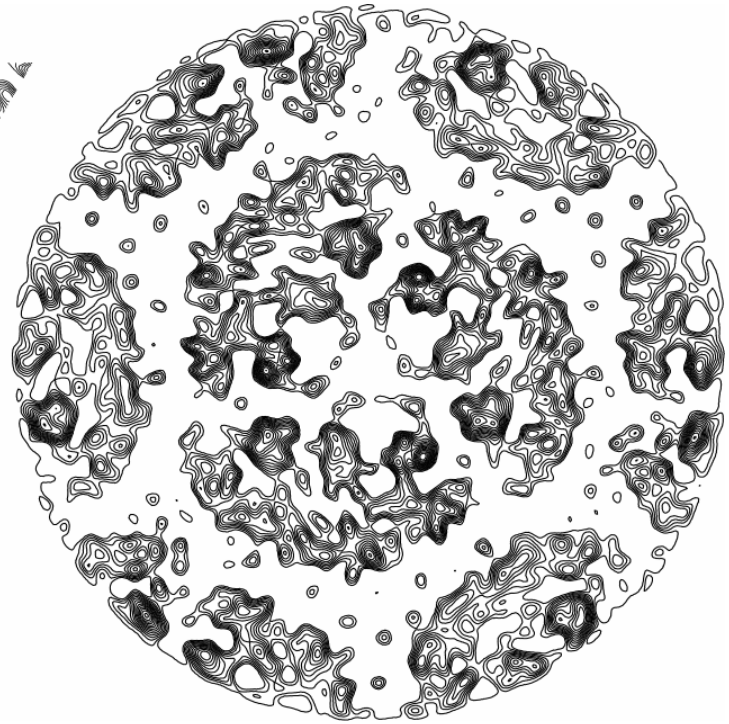


Zeng et al. 2007

ML processing of 2D crystals

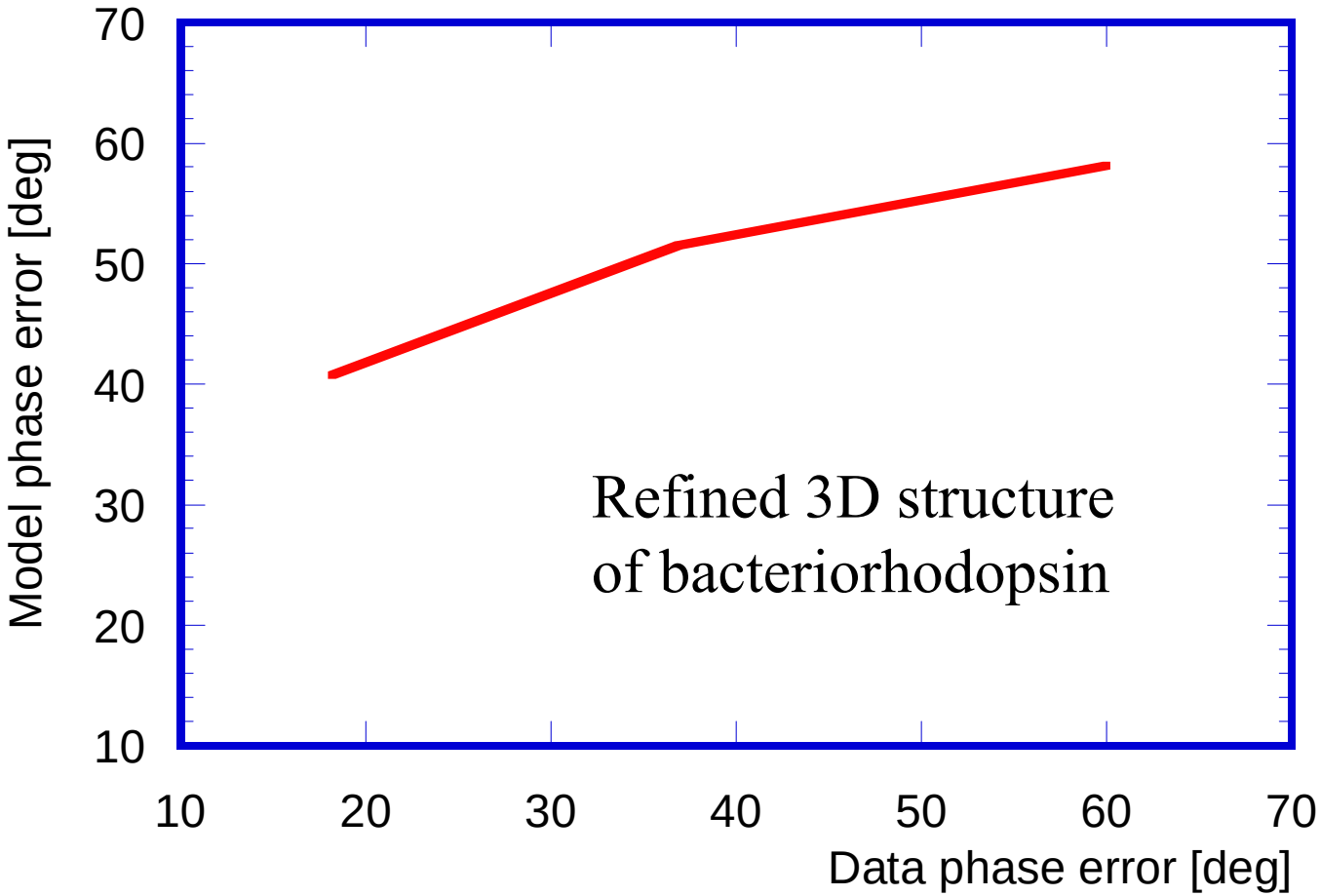


Crystallography



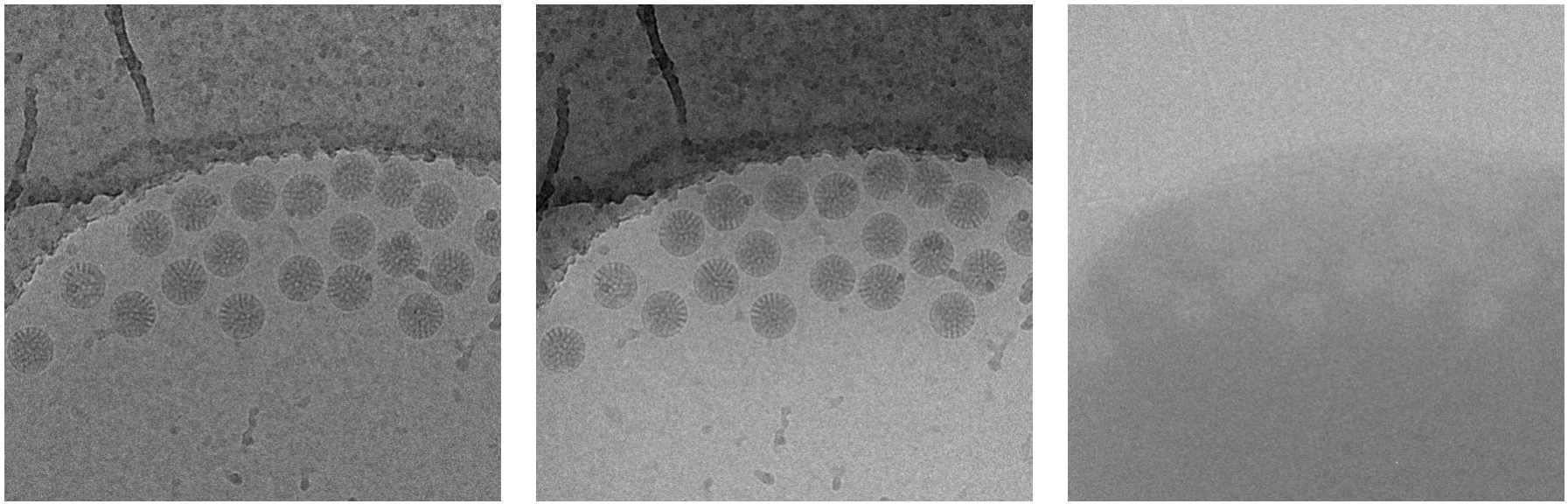
Alignment of individual unit cells using ML approach

Systematic Error



Grigorieff et al. 1996

Inelastic Image



Unfiltered 0 eV 25 eV

300 kV, 6 μm underfocus, 15 eV energy window

Chen Xu 2007

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- Contrast normalization
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  - Energy filter, diffraction theory

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- Rotavirus DLP — Xing Zhang, Ethan Settembre (Harrison lab)
- Purple membrane — Fritz Zemlin, Erich Beckmann
- ML of 2D crystals — Xiangyan Zeng (Stahlberg lab)
- CTFFIND3/TILT — Joe Mindell
- Ewald sphere — Matthias Wolf, David DeRosier
- Inelastic scattering — Chen Xu

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