CTF & Filtration

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Baylor College of Medicine

Baylor College of Medicine

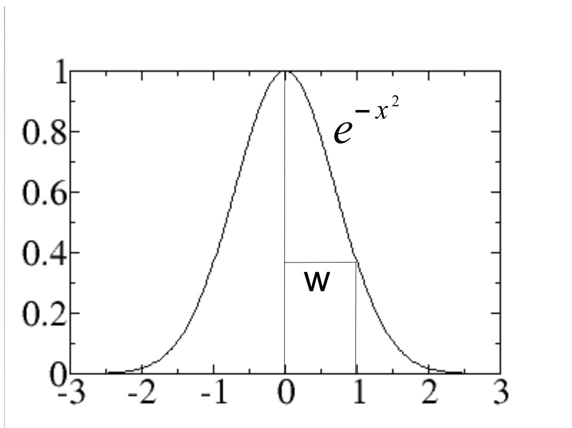
VERNA & MARRS McLEAN DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

NCMI National Center for Macromolecular Imaging

Gaussians

Gaussian or 'Normal' Distribution:

$$f(x) = e^{-\left(\frac{x}{w}\right)^2}$$

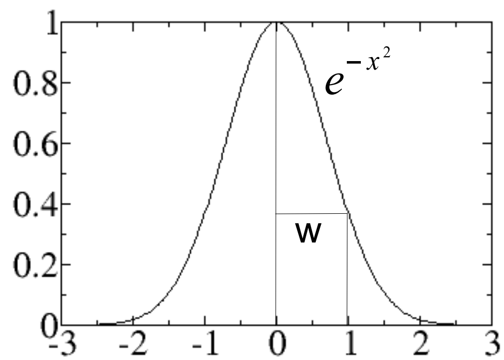


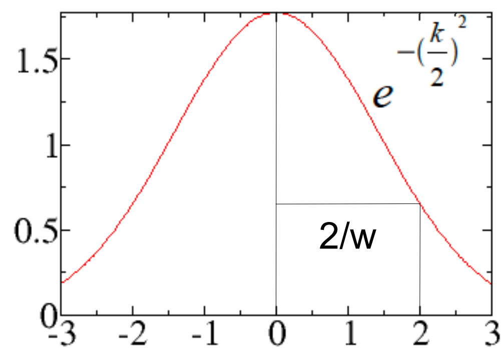
Gaussians are everywhere

Gaussians

Gaussian or 'Normal' Distribution:

$$f(x) = e^{-\left(\frac{x}{w}\right)^2} \qquad \int_{-\infty}^{\infty} e^{-ikx} e^{-\left(\frac{x}{w}\right)^2} dx = w\sqrt{\pi}\, e^{-\left(\frac{kw}{2}\right)^2}$$





Gaussians are everywhere

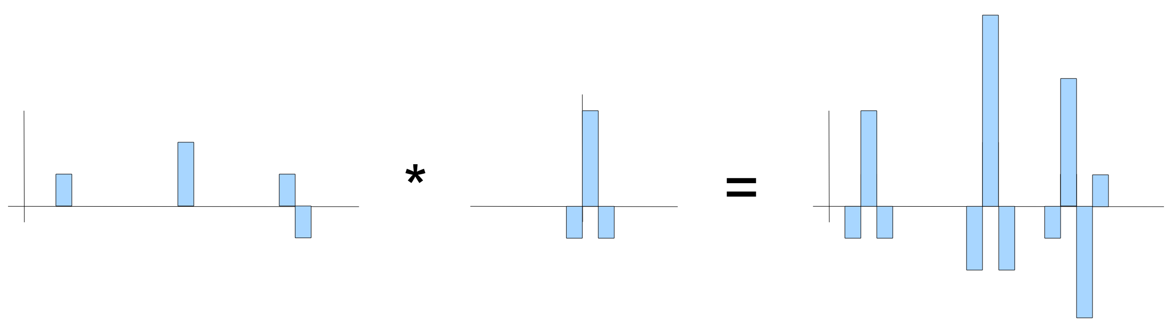
Filtration -> Convolution

Continuous Real Space Convolution:

$$f(x)*g(x)=\int_{-\infty}^{\infty} f(t)g(x-t)dt$$

Discrete Real Space Convolution:

$$f_i*g_i=\sum_{t=-\infty}^{\infty} f_t g_{i-t}$$



Convolution/Filtration

$$h(x) = f(x) * g(x) = \int_{-\infty}^{\infty} f(t)\, g(x-t)\, dt$$

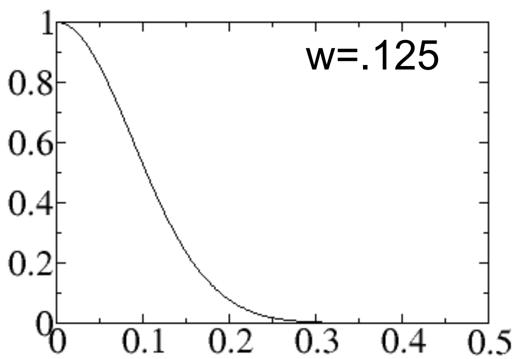
$$\bar{H}(k) = \bar{F}(k)\, \bar{G}(k)$$

Test Image

$f(x,y)$

$$f(x,y)\ \overrightarrow{FFT}\ \bar{F}(k_x,k_y)\cdot G(k_x,k_y)=\bar{F}'(k_x,k_y)\ \overrightarrow{IFT}\ f'(x,y)$$

Image Filtration
Gaussian Lowpass

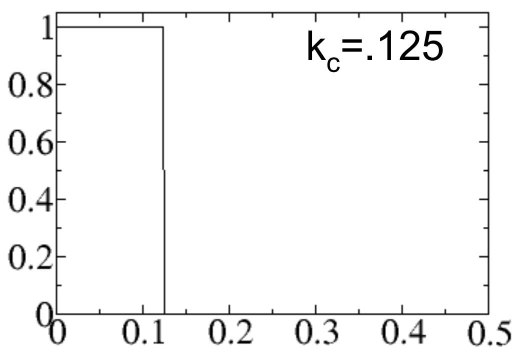


$$e^{-\left(\frac{k}{w}\right)^2}$$

$$f'(x,y)$$

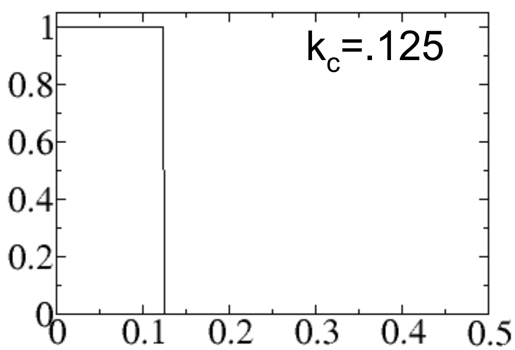
$$\bar{F}'(k_x,k_y)=e^{-\frac{k_x^2+k_y^2}{w^2}}\bar{F}(k_x,k_y)$$

Image Filtration
Sharp Lowpass



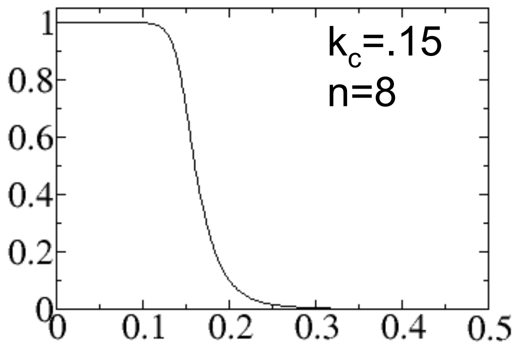
$k<k_c$ -> 1.0
else -> 0

Image Filtration
Sharp Lowpass



$k<k_c$ -> 1.0
else -> 0

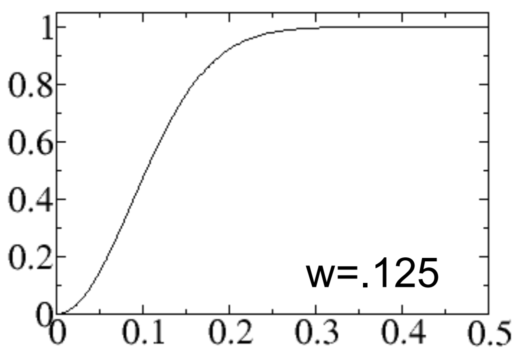
Image Filtration
Butterworth Lowpass



$$\frac{1}{\sqrt{1+\left(\frac{k}{k_c}\right)^{2n}}}$$

$$\bar{F}'(k_x, k_y) = \left(1 + \frac{(k_x^2 + k_y^2)^n}{k_c^{2n}}\right)^{-2} \bar{F}(k_x, k_y)$$

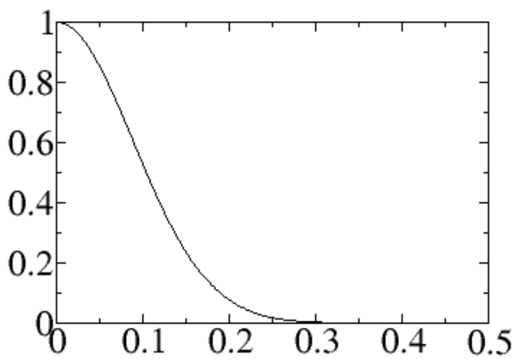
Image Filtration
Gaussian Highpass



$$1.0 - e^{-\left(\frac{k}{w}\right)^2}$$

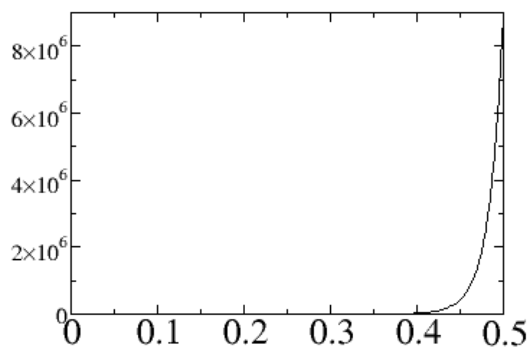
Test Image

Deconvolution



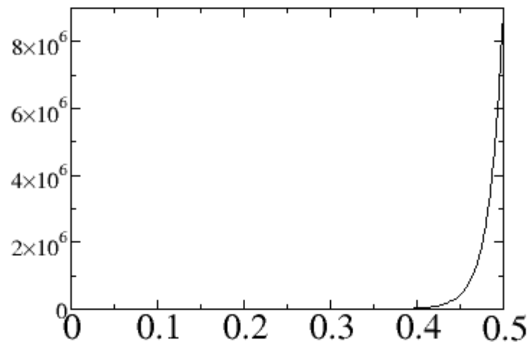
$$e^{-\left(\frac{x}{w}\right)^2}$$

Deconvolution



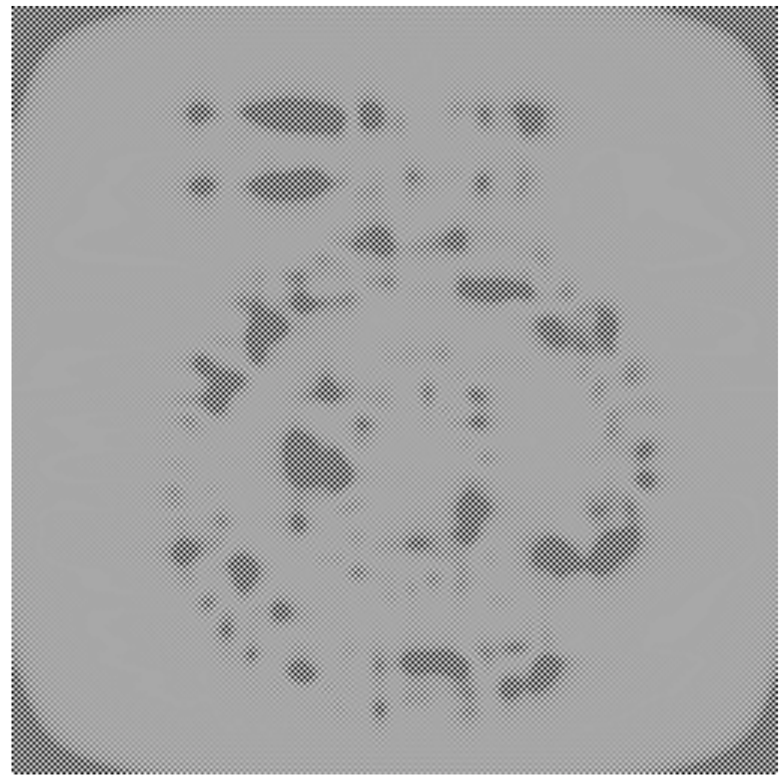
$$e^{\left(\frac{x}{w}\right)^2}$$

Deconvolution

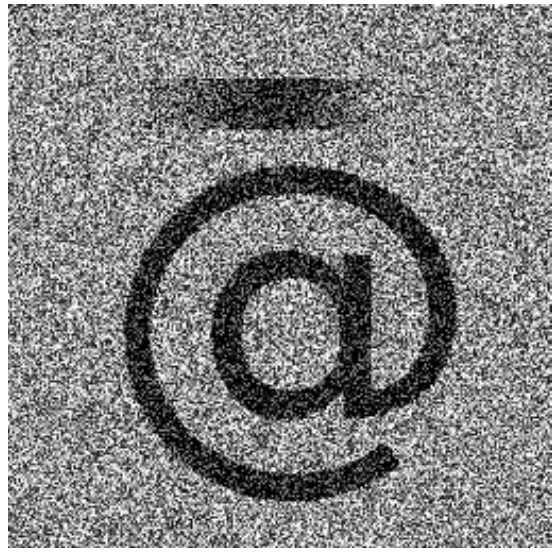


$$e^{\left(\frac{x}{w}\right)^2}$$

From Discrete valued image

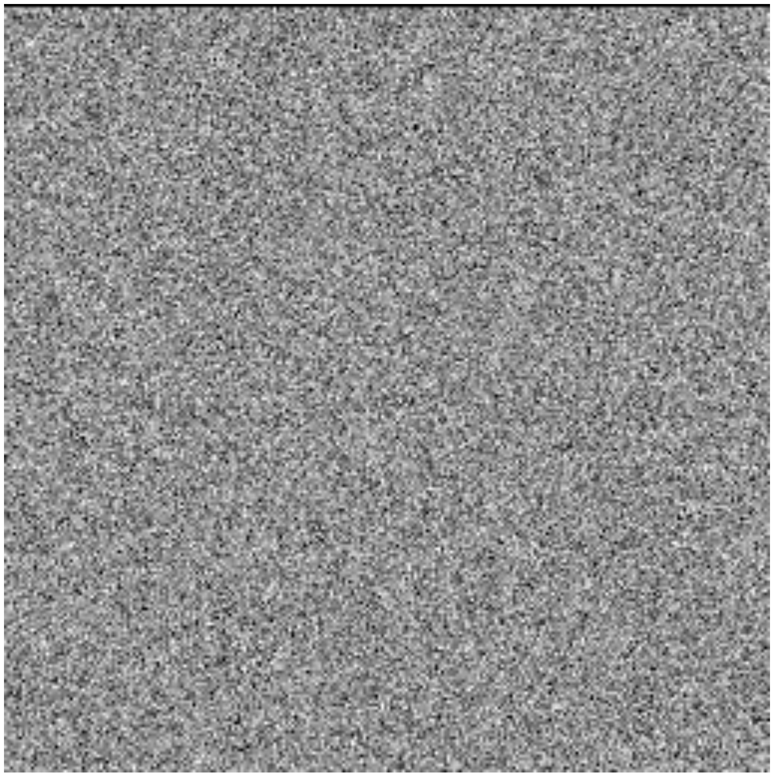


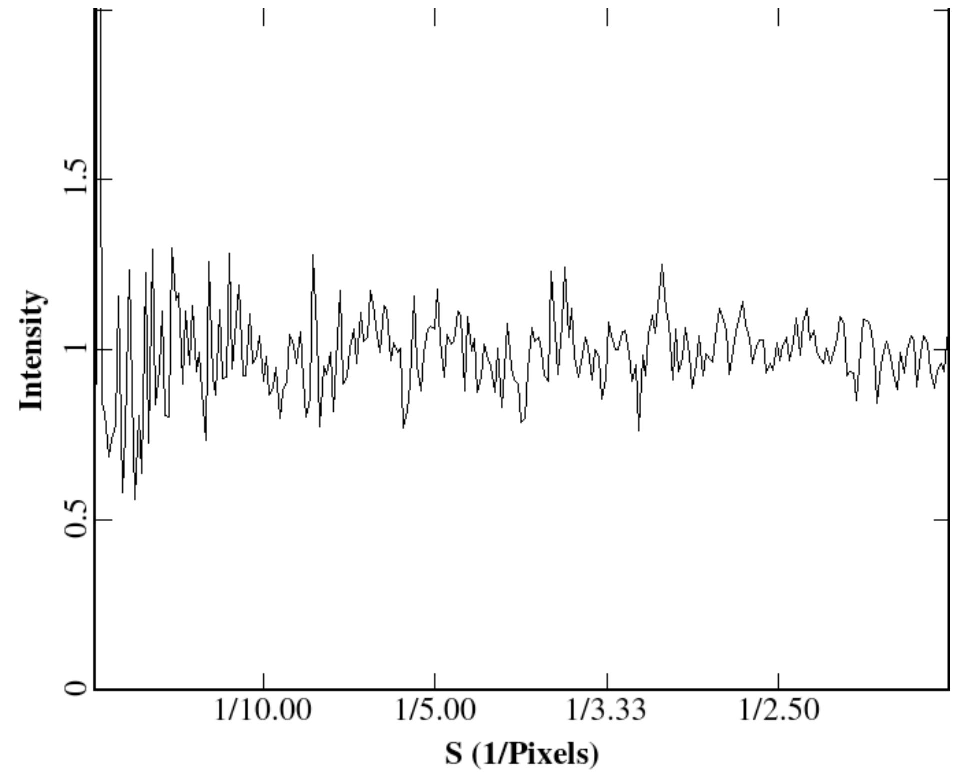
Noise



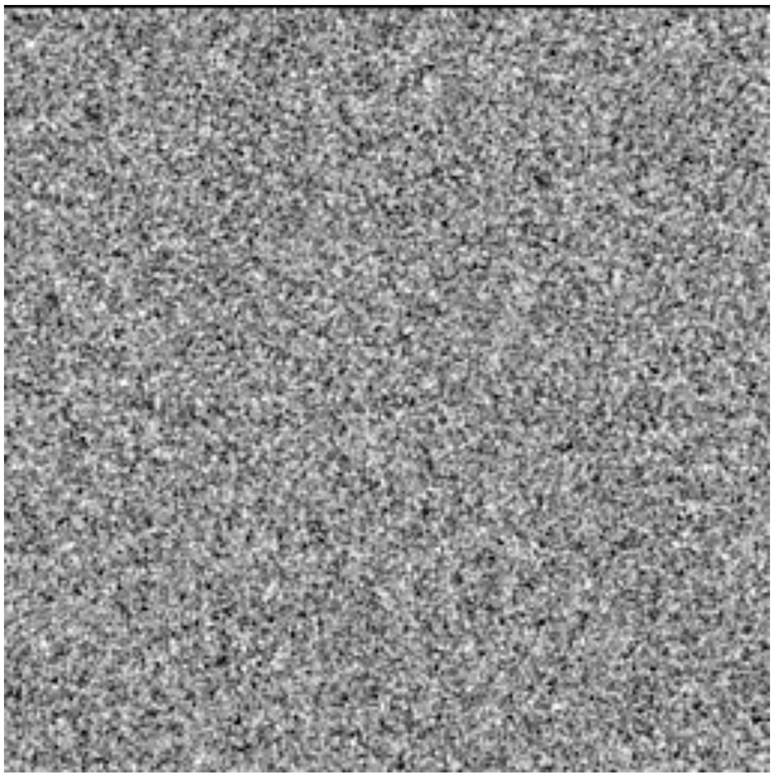
How do we characterize the noise ?

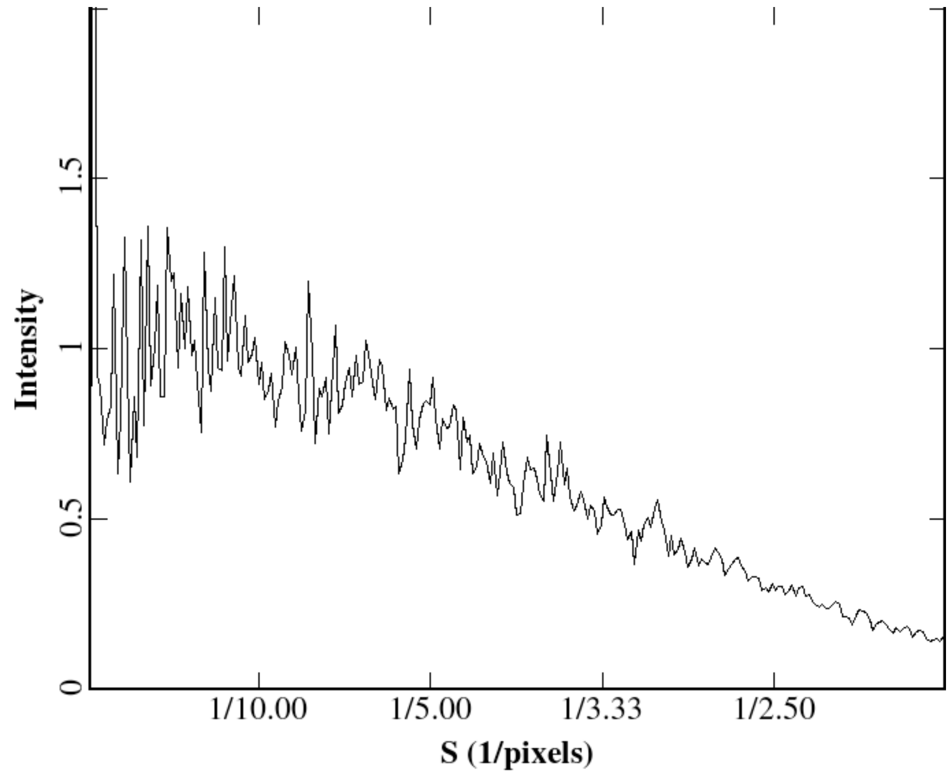
White Noise



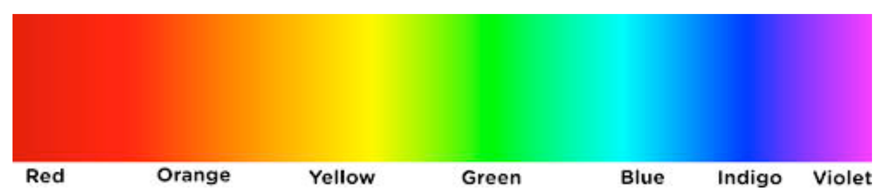


Pink Noise





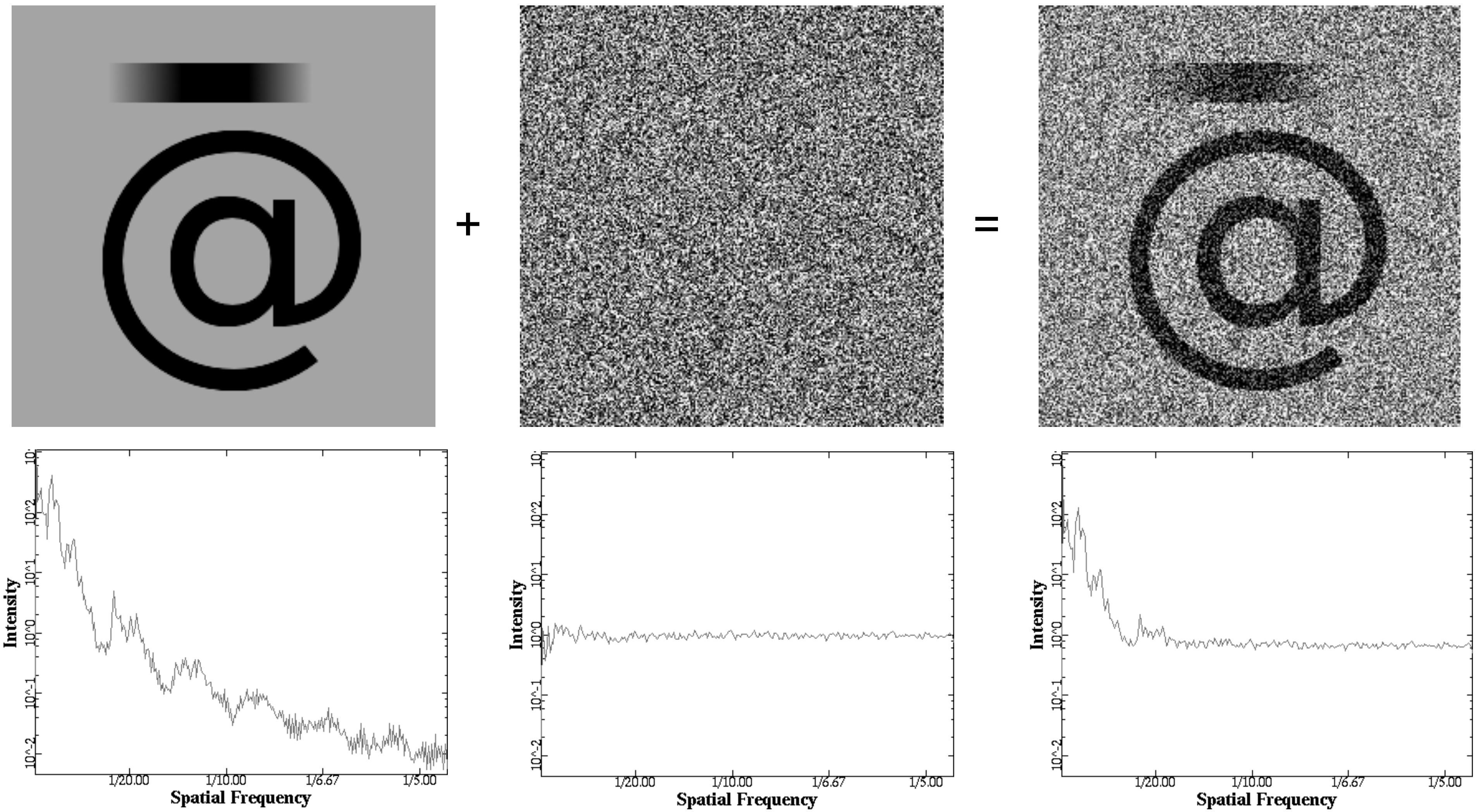
Intensity
0
0.5
1
1.5
1/10.00
1/5.00
1/3.33
1/2.50
S (1/pixels)



Red
Orange
Yellow
Green
Blue
Indigo
Violet

Optimal Filtration

$a_{x,y}$ $b_{x,y}$ $c_{x,y}$



Optimal Filtration

$$W(s_x, s_y) = F(s_x, s_y)\, C(s_x, s_y)$$

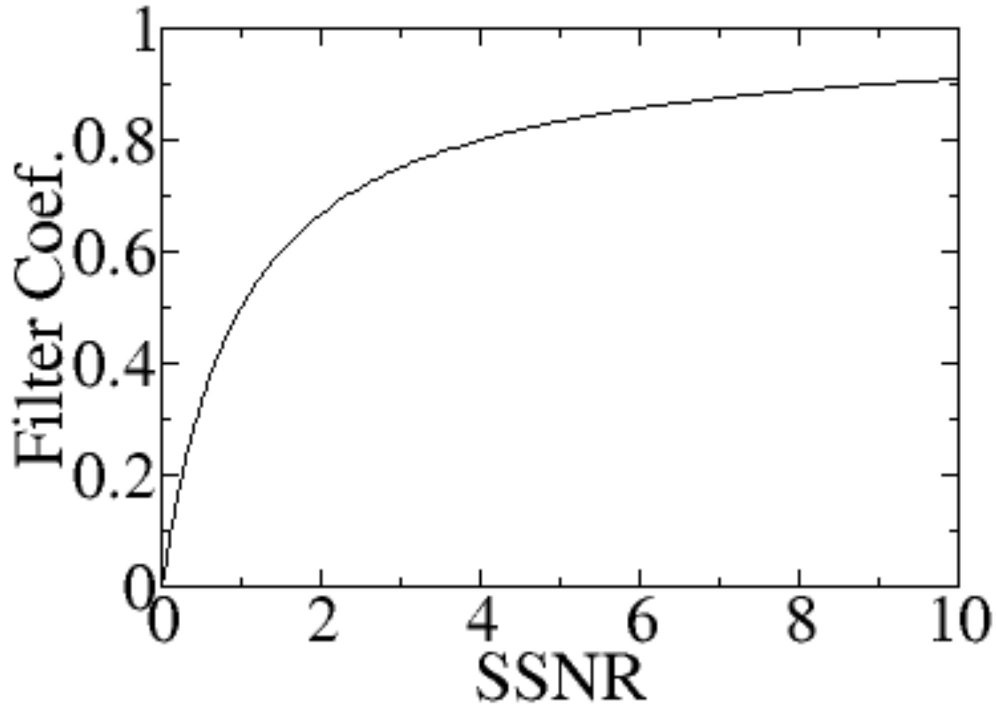
where

$$\sum_{x,y} (c_{x,y} - a_{x,y})^2$$

Answer is a Wiener Filter:

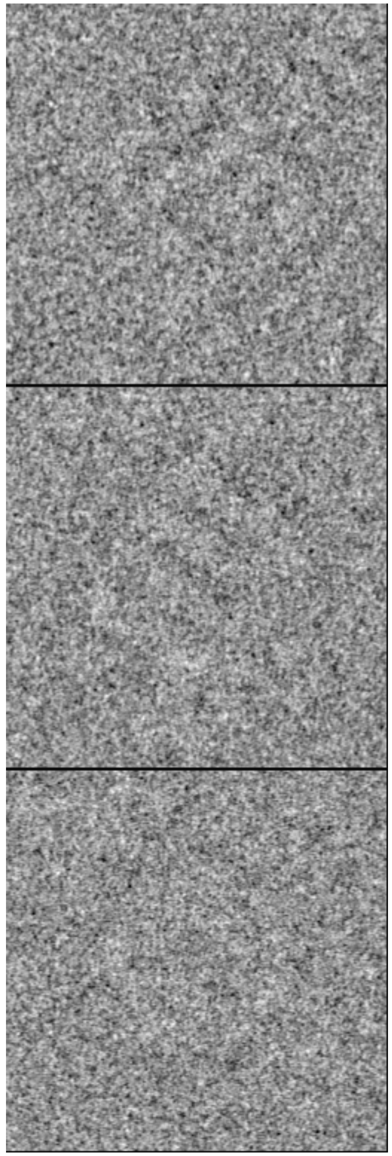
$$F(s_x, s_y) = \frac{1}{1 + \frac{1}{SNR(s_x, s_y)}}$$

Wiener Filter

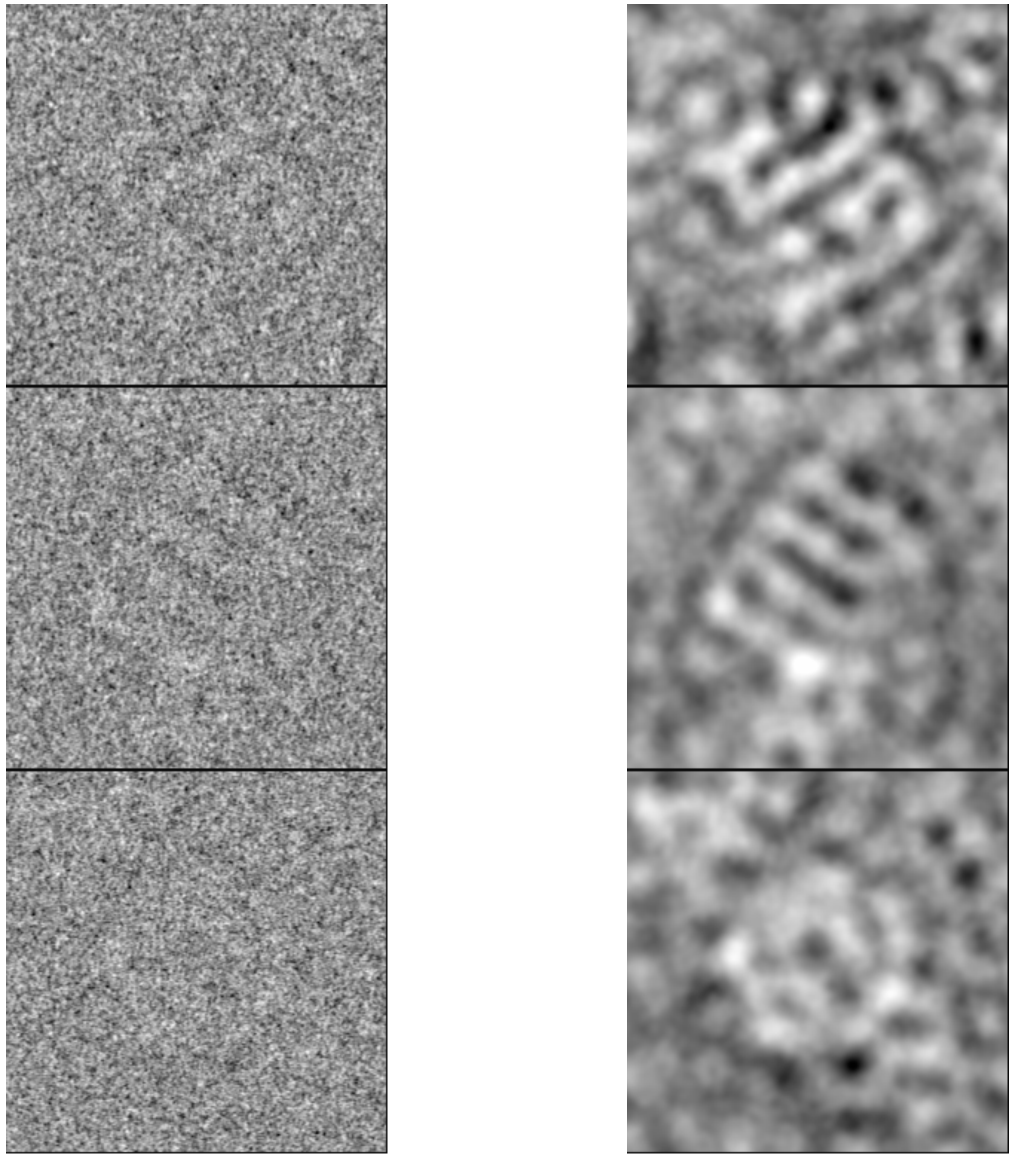


$$F(s_x, s_y) = \frac{1}{1 + \frac{1}{SNR(s_x, s_y)}}$$

Wiener Filter



Wiener Filter



CTF & MTF

- CTF - Contrast Transfer Function
    - Weak phase approximation - sin(gamma)
- MTF - Modulation Transfer Function
    - Specimen motion
    - Detector issues
    - Envelope function of Optics

Image Evaluation

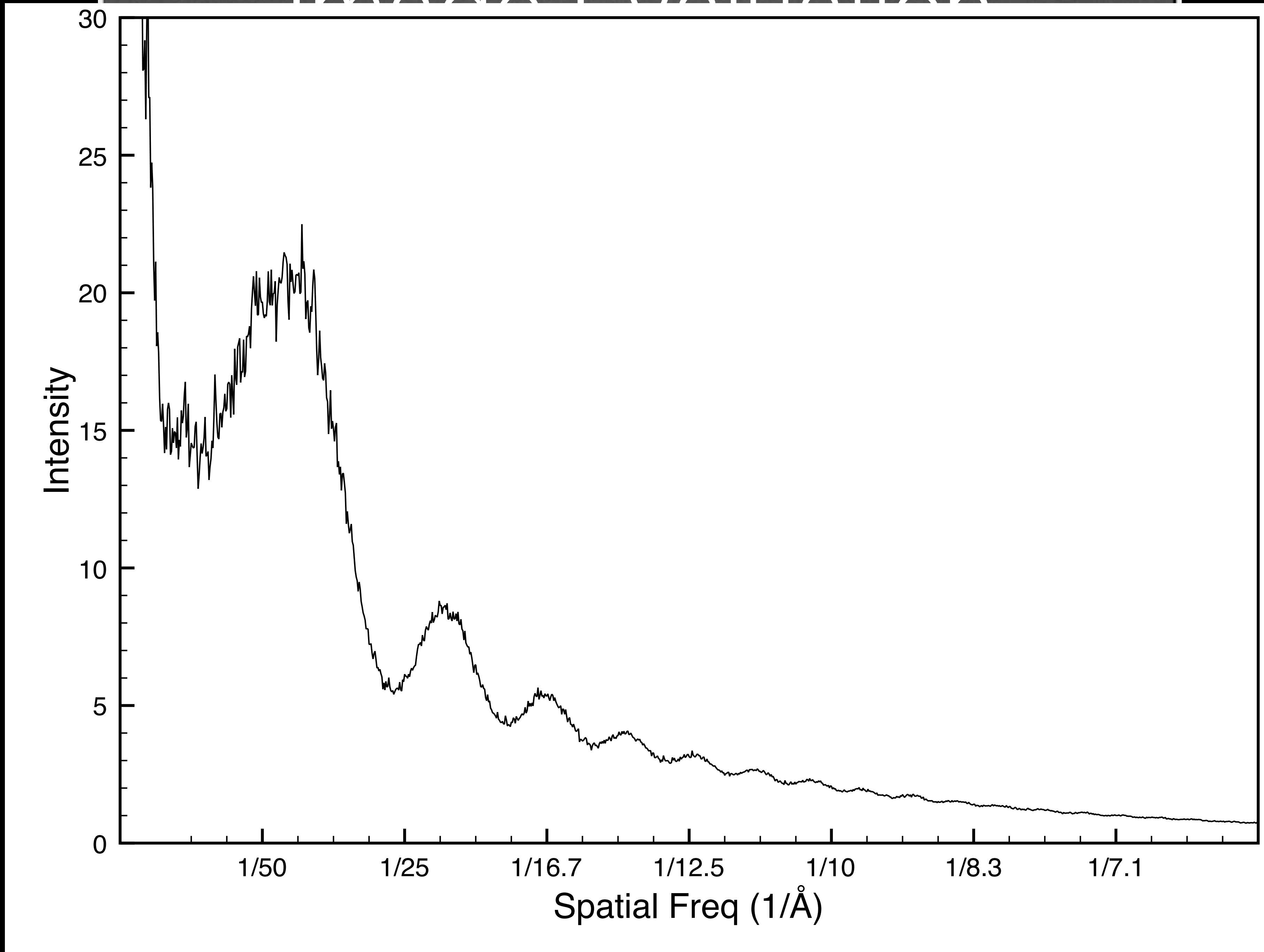
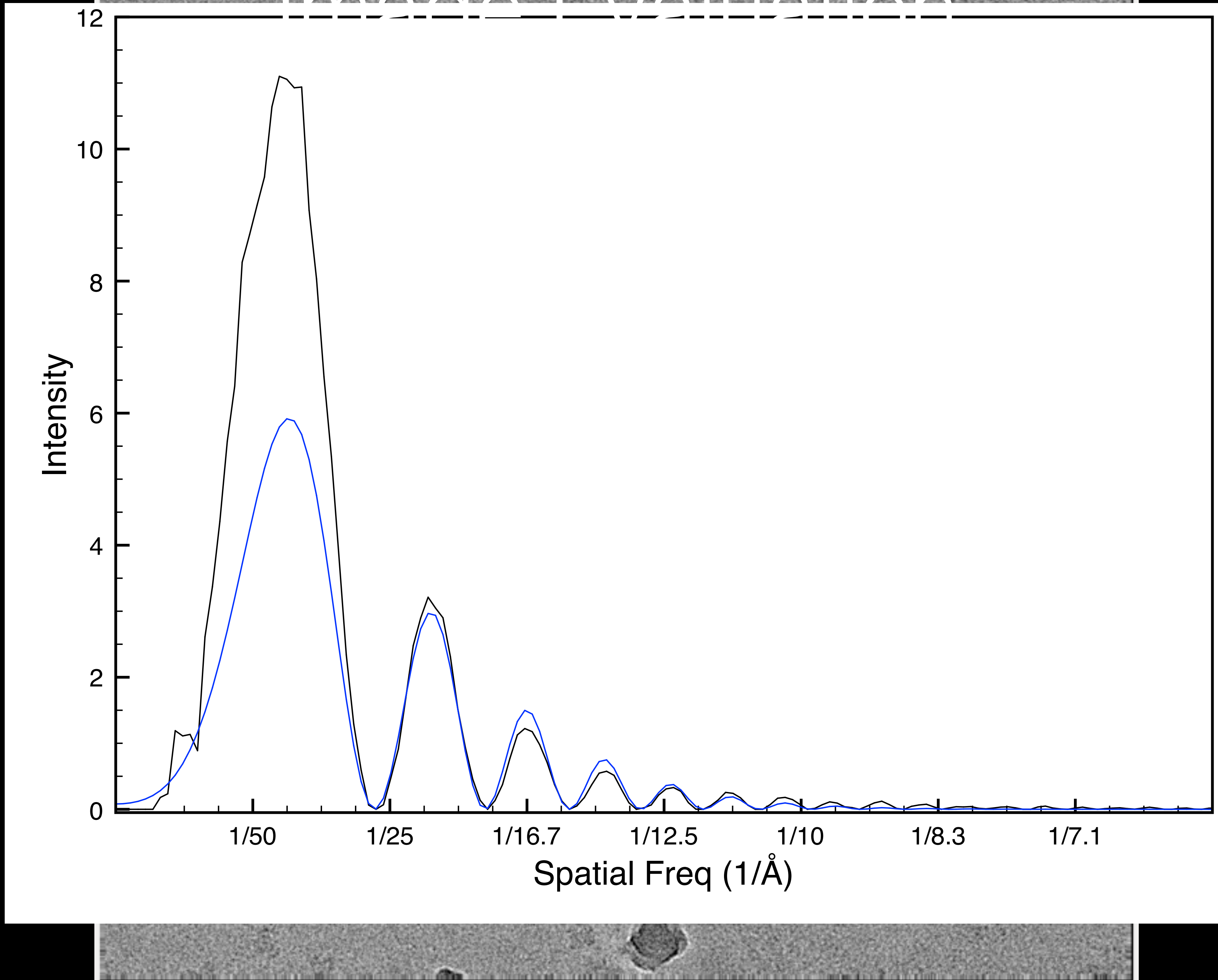
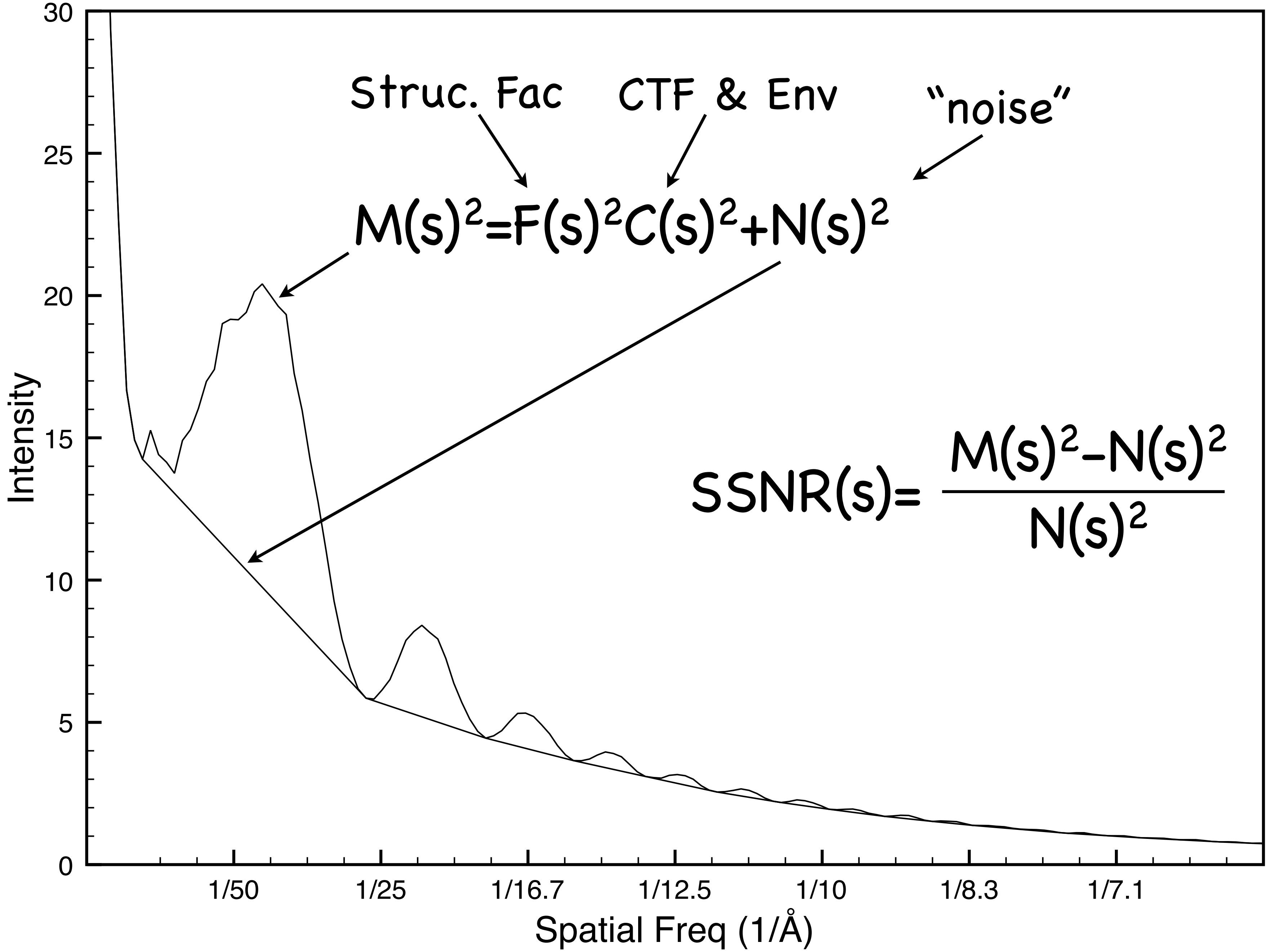


Image Evaluation





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

$$SSNR(s)=\frac{M(s)^2-N(s)^2}{N(s)^2}$$

CTF Correction

Measured Image Ideal Particle Random Noise

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

CTF Envelope

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

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

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

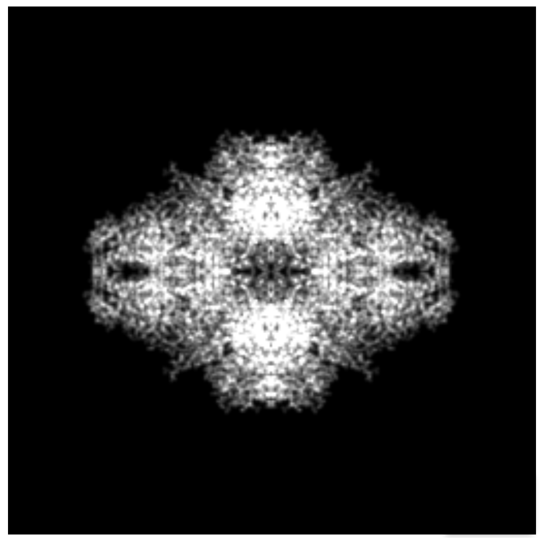
MTF

Q : Amplitude Contrast
C_s : Spherical Aberration
ΔZ : Defocus
λ : Electron Wavelength

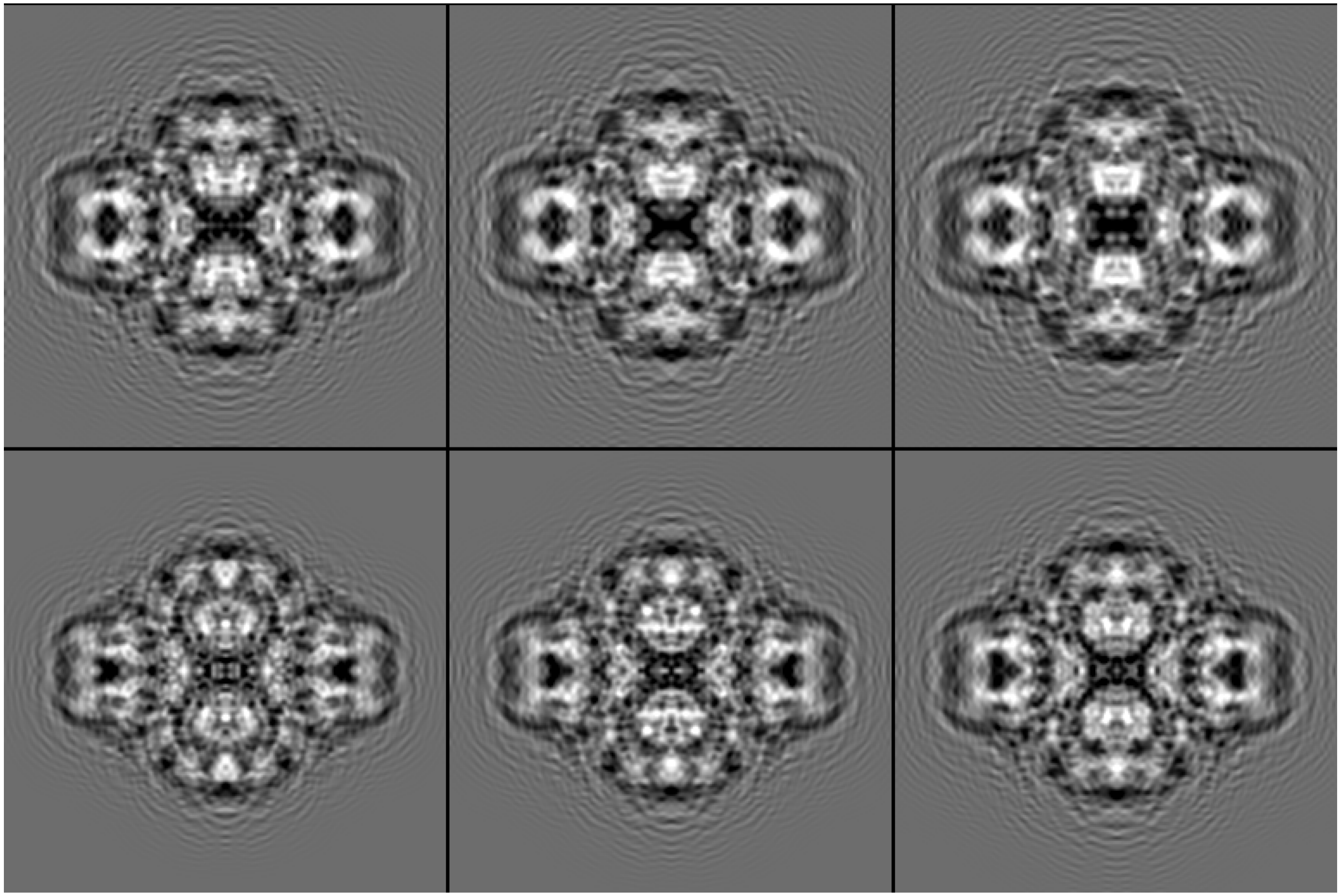
CTF Demo

What if we don't fix it ?

(and we only want very low resolution)

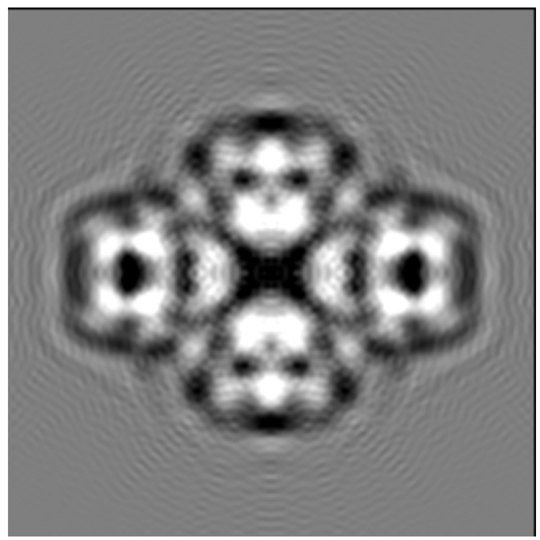


1.6 μm　　1.8 μm　　2.0 μm

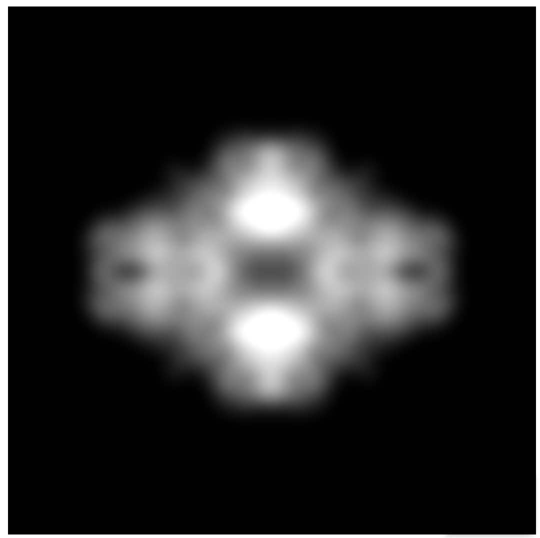


1.0 μm　　1.2 μm　　1.4 μm

Average no correction



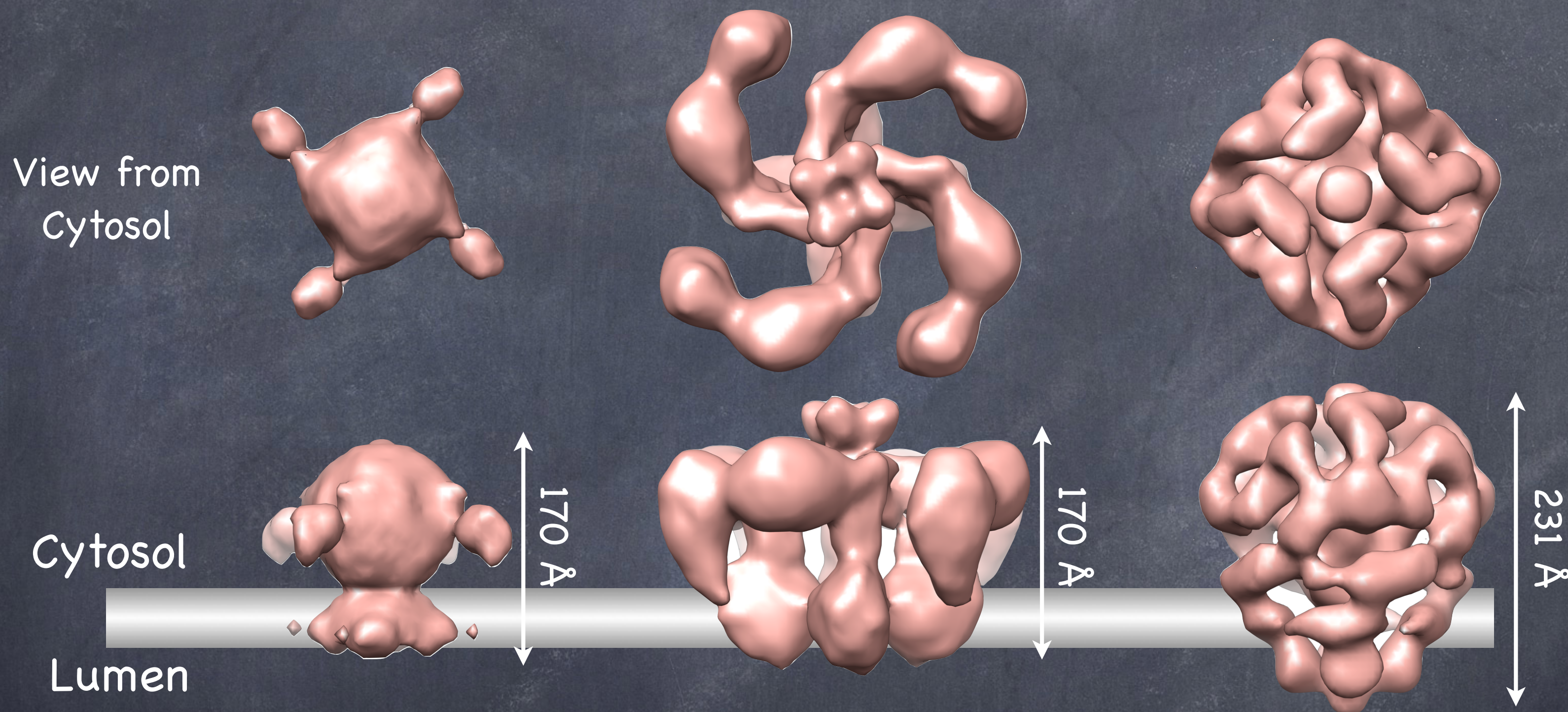
20 Å Lowpass Filter



20 Å Lowpass Filter Avg

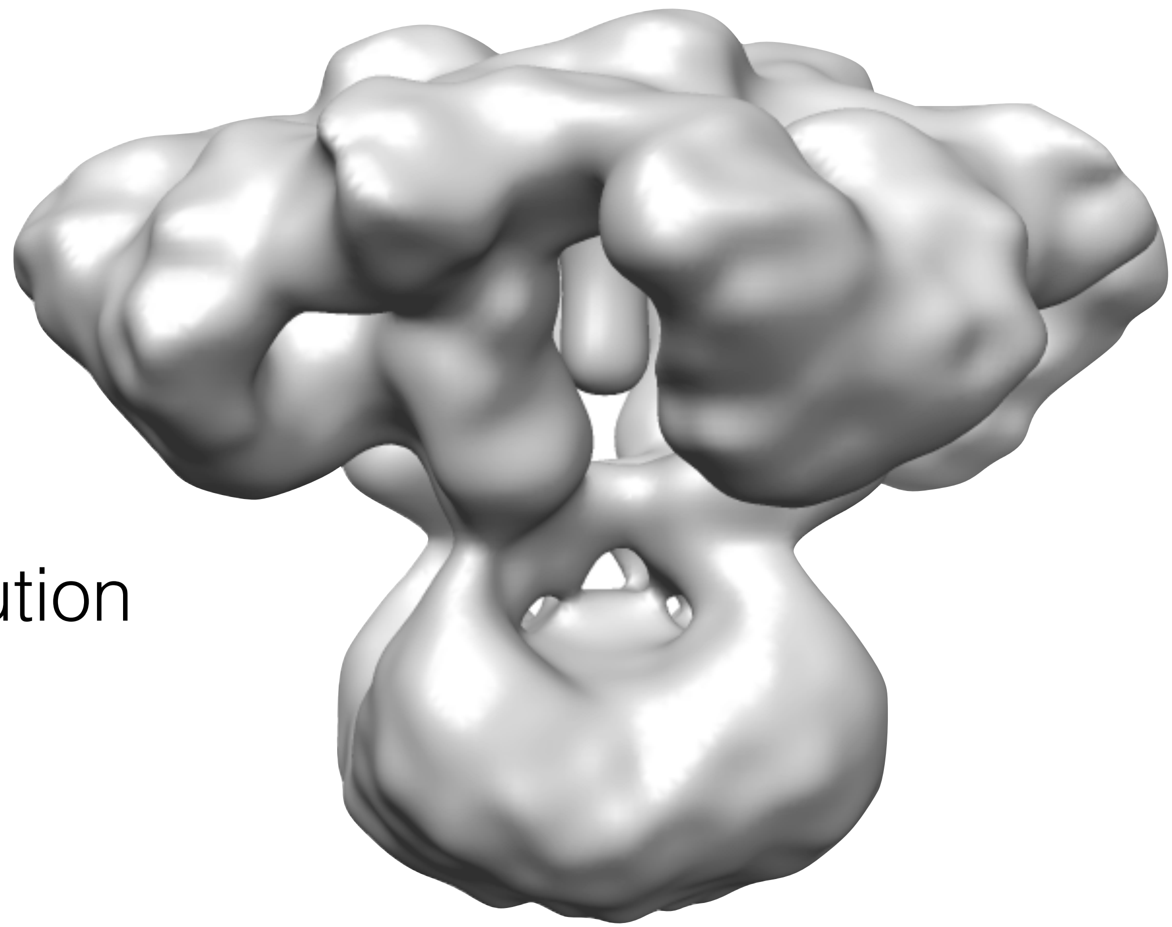
Particle-based CTF and assessment

Cryo-EM Maps of IP_3R



Q.-X. Jiang et al. (2003) *Serysheva et al. (2003)* *C. Sato et al. (2004)*

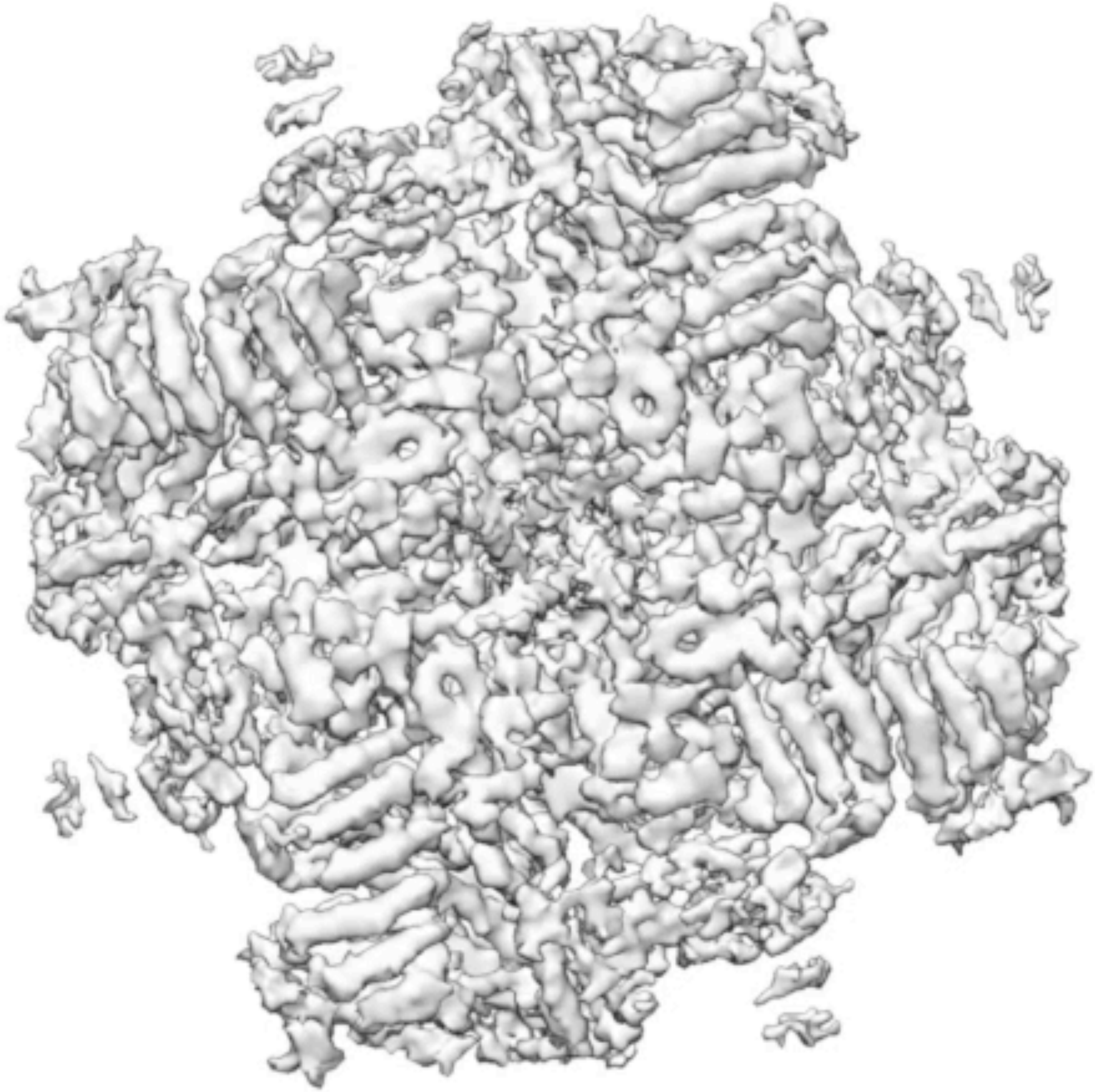
IP3R (2011)



~10 Å Resolution
→ ~17 Å
→ ~14 Å

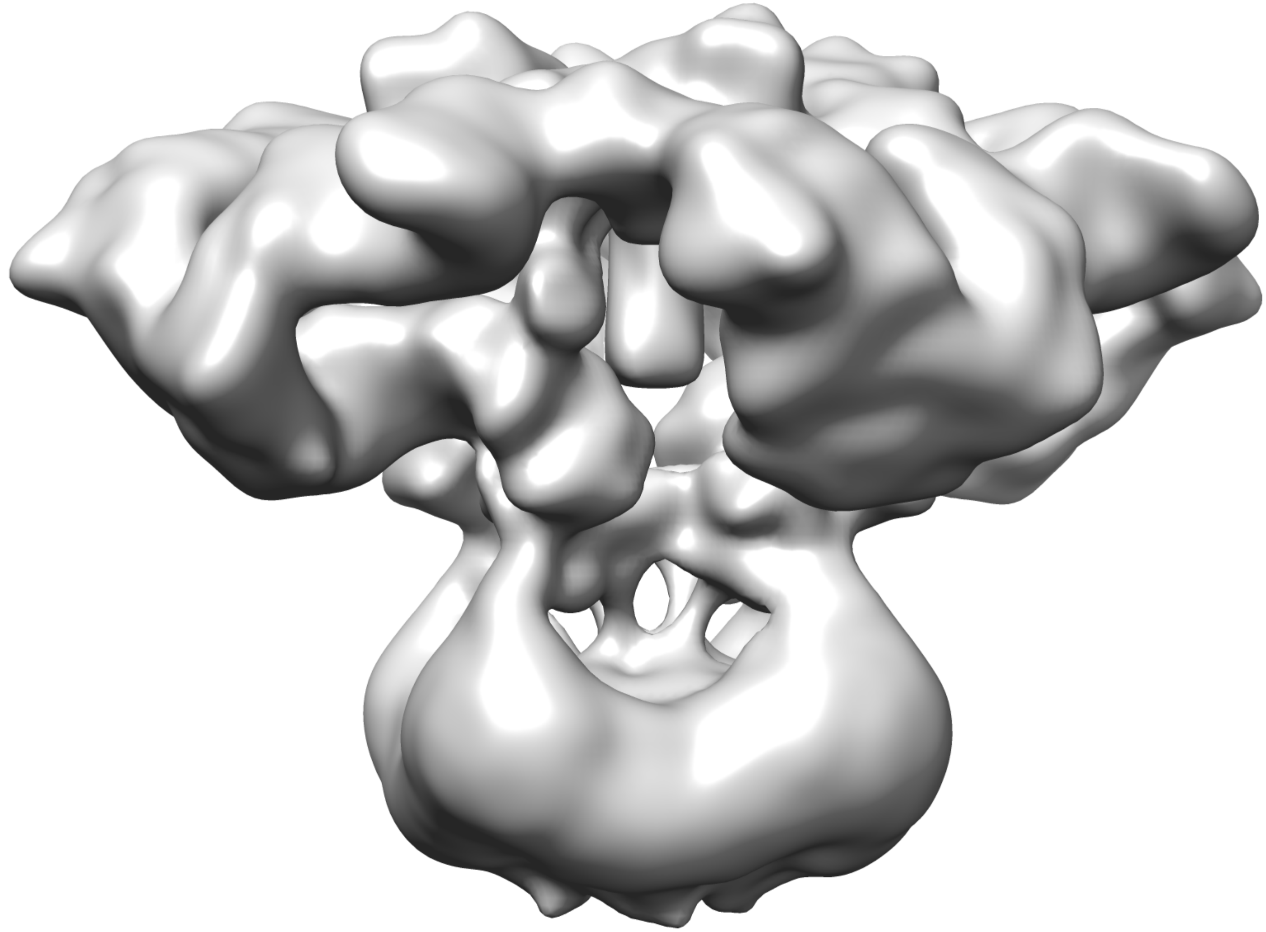
220 Å

CryoEM Density Map of IP3R1



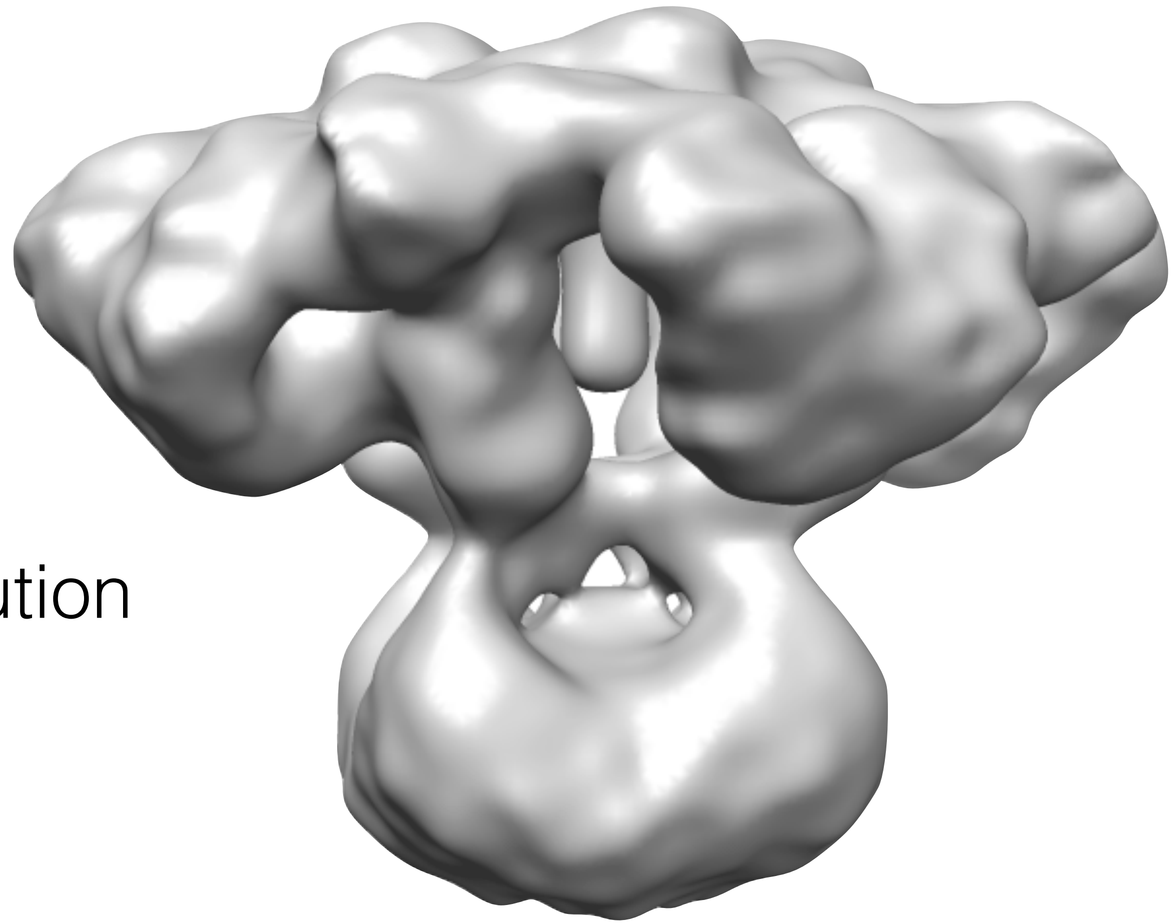
4.7 Å Resolution

IP3R (2015 - Filtered)



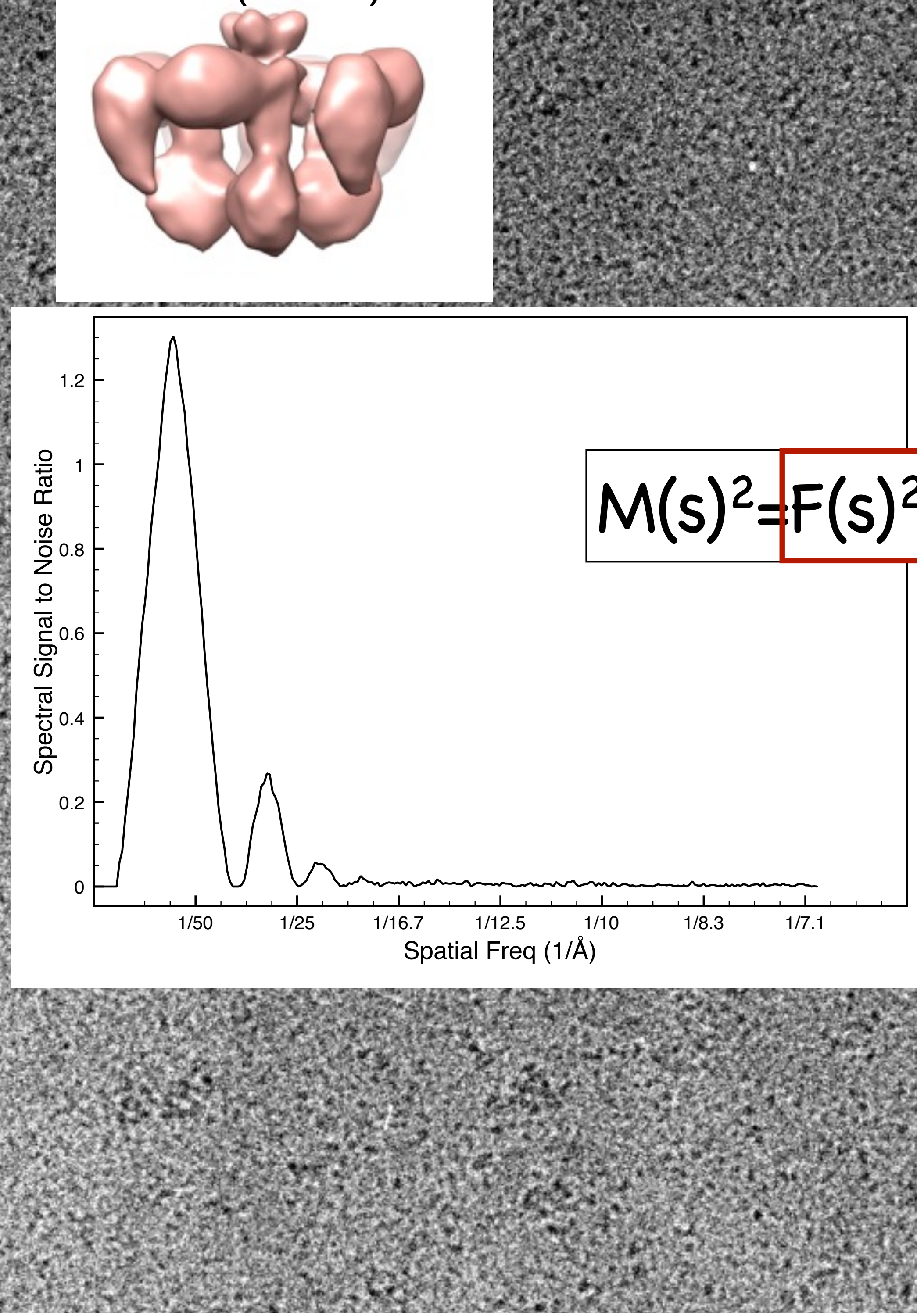
IP3R (2011)

~10 Å Resolution
→ ~17 Å
→ ~14 Å

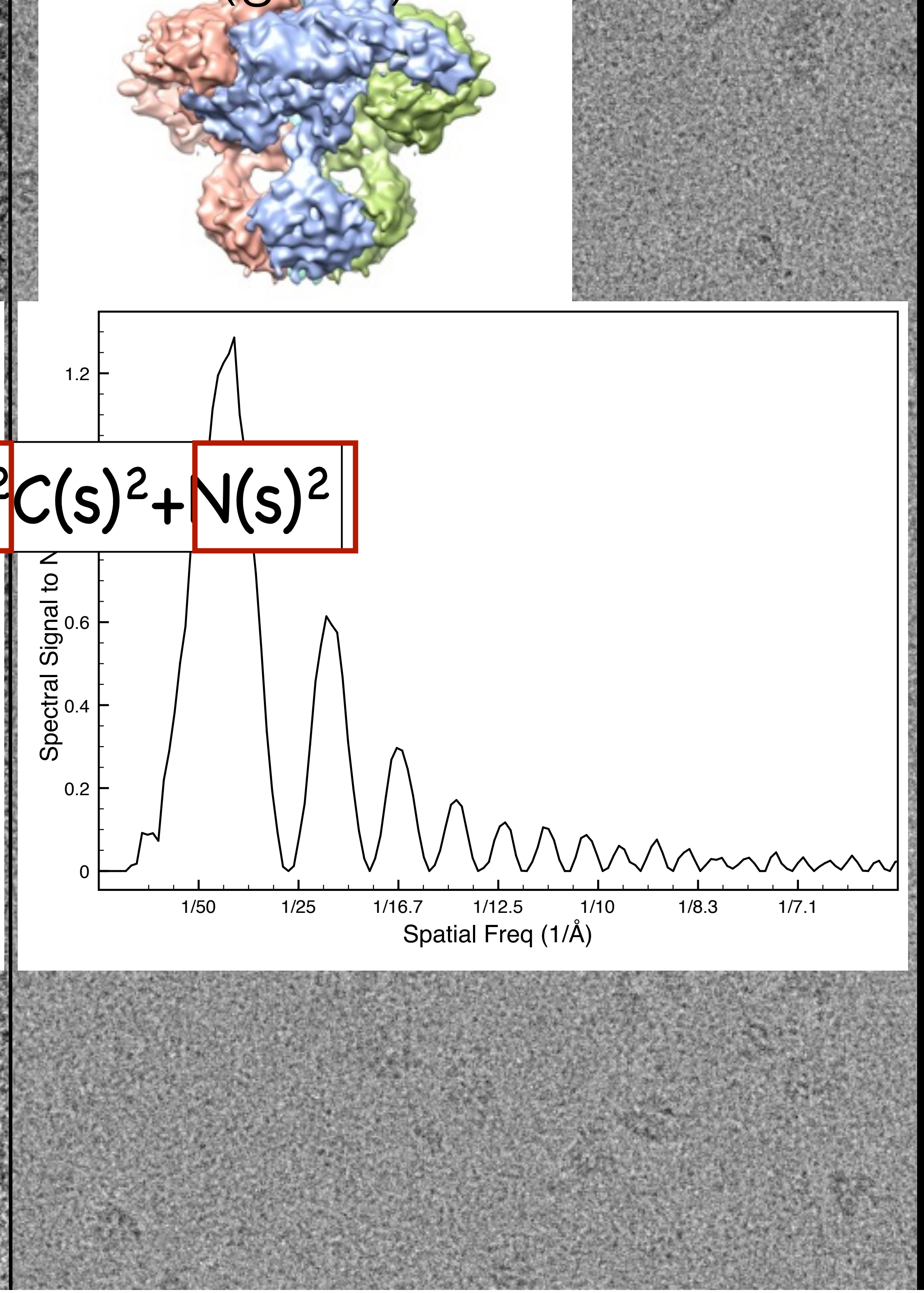


220 Å

2002 (bad)

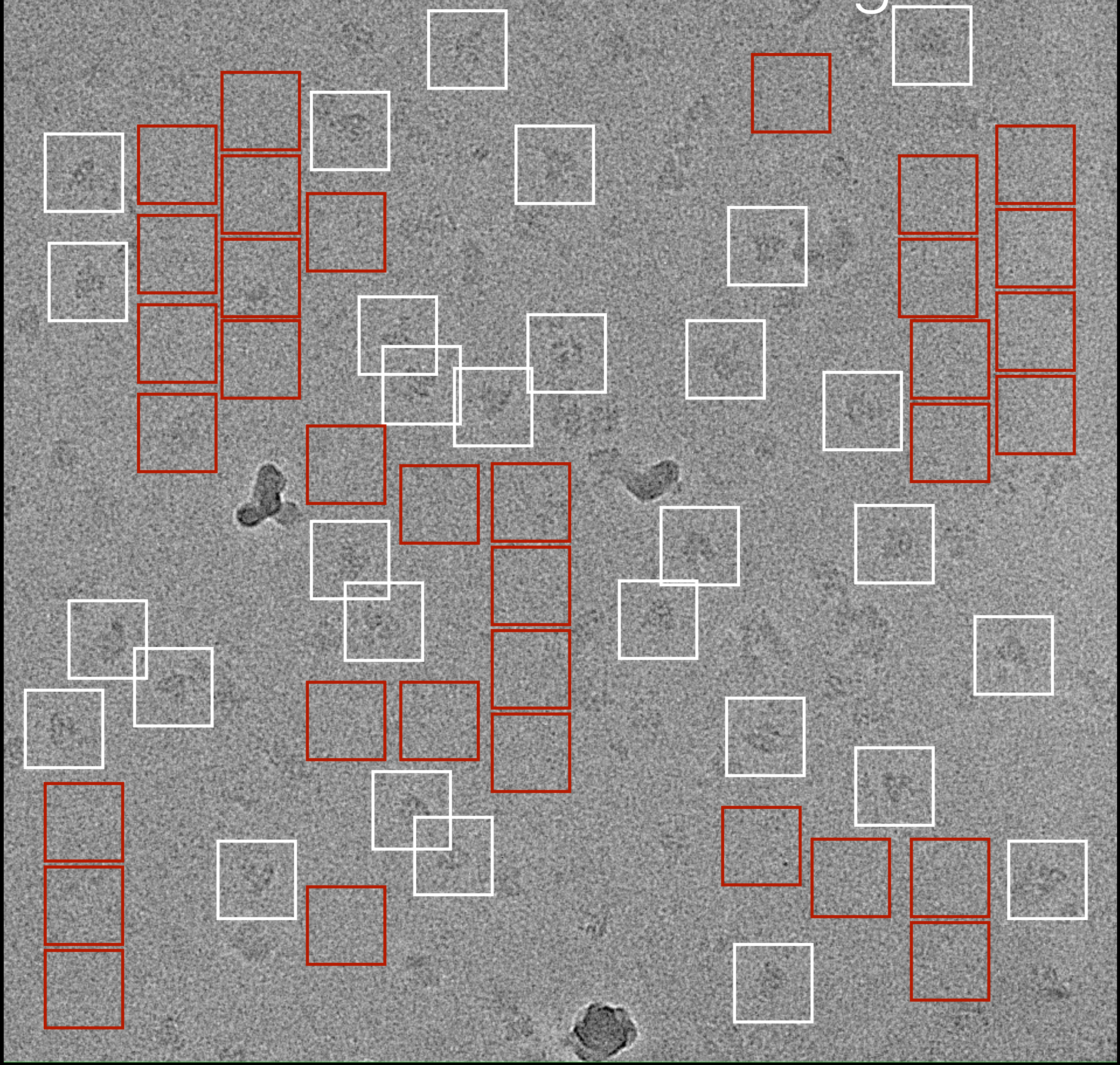


2011 (good)



$$M(s)^2 = \boxed{F(s)^2} C(s)^2 + \boxed{N(s)^2}$$

Particles Rather than Regions



Density Problems

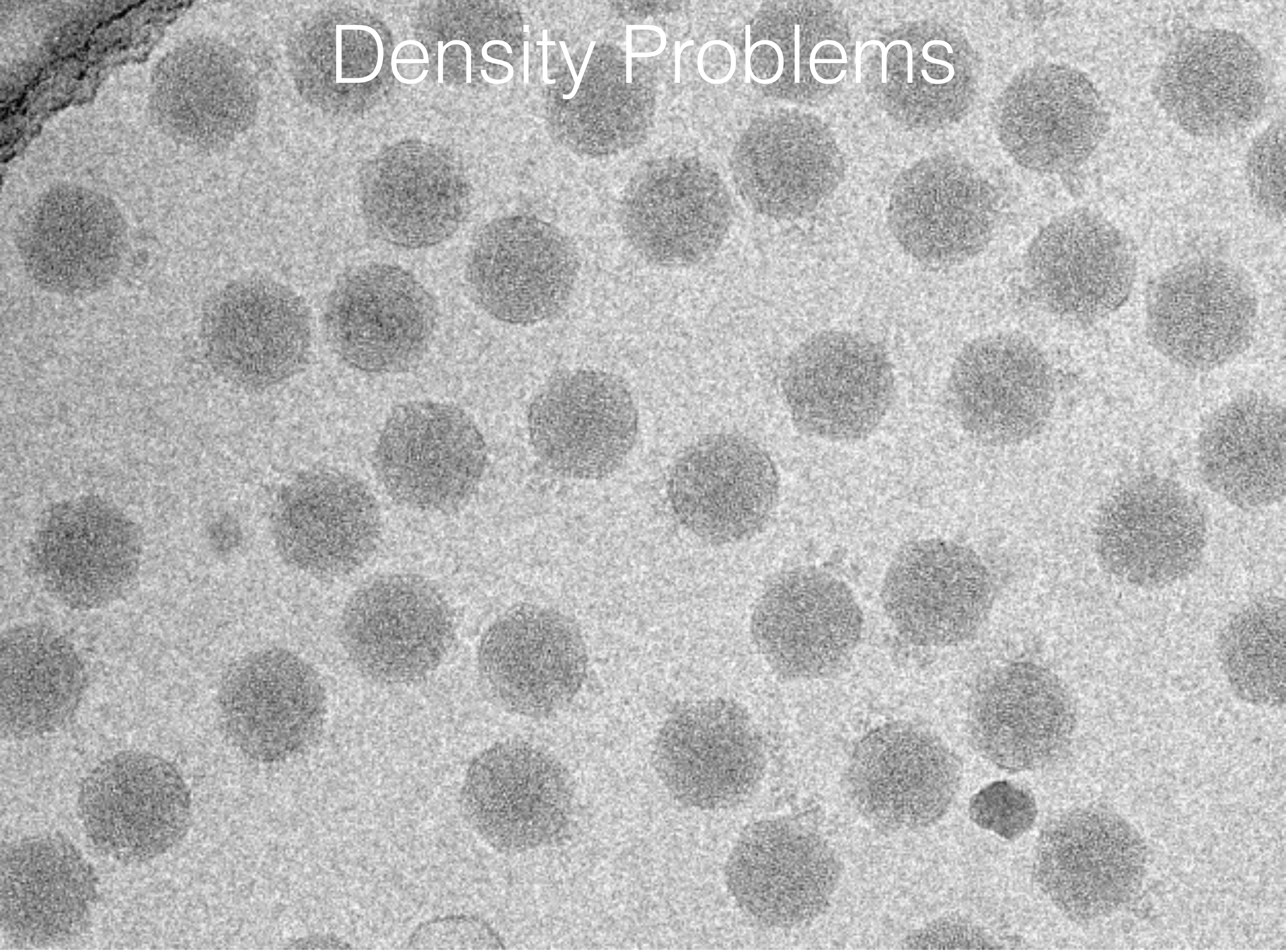
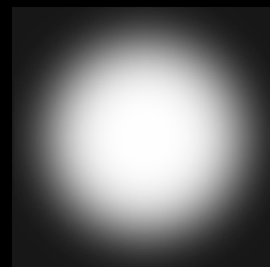
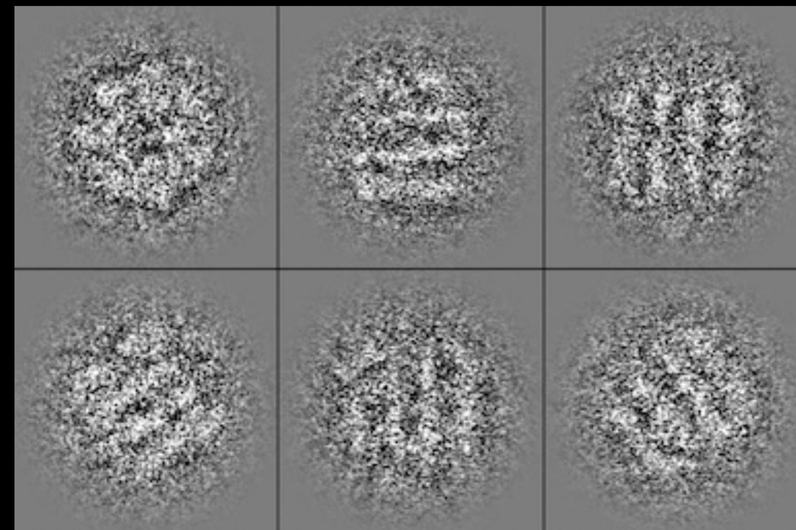
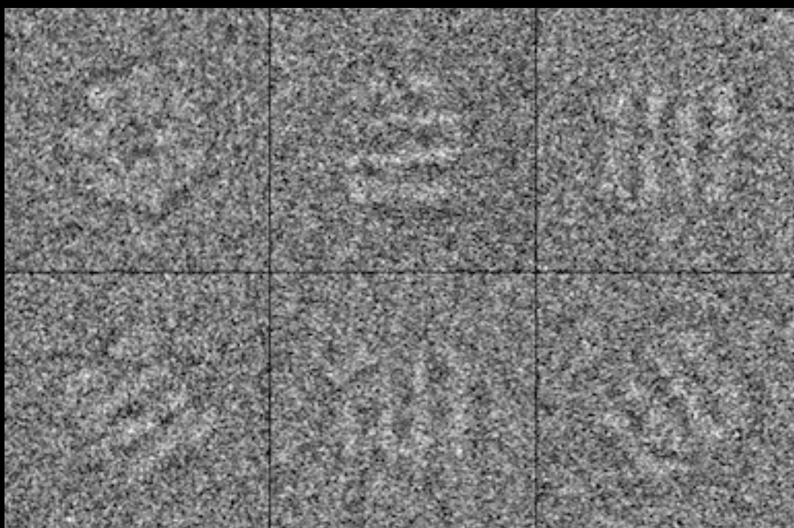
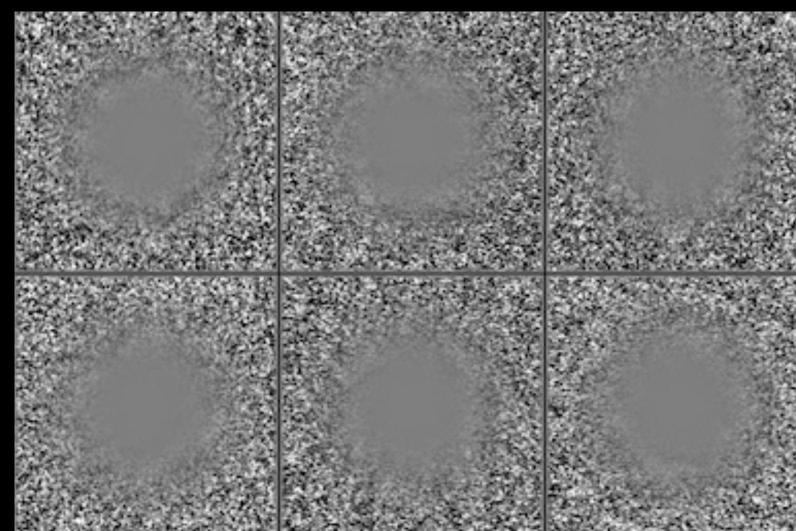


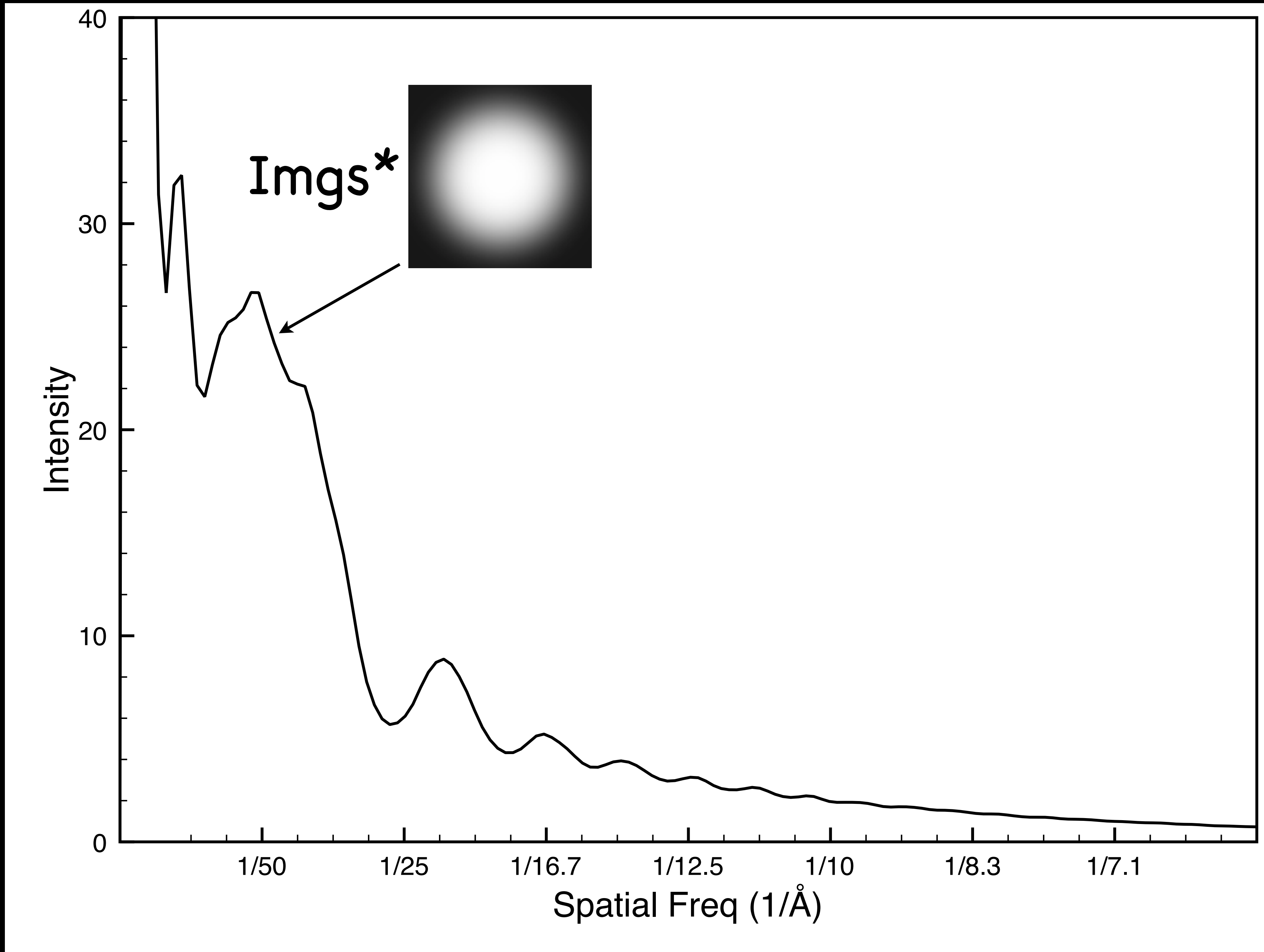
Image Evaluation



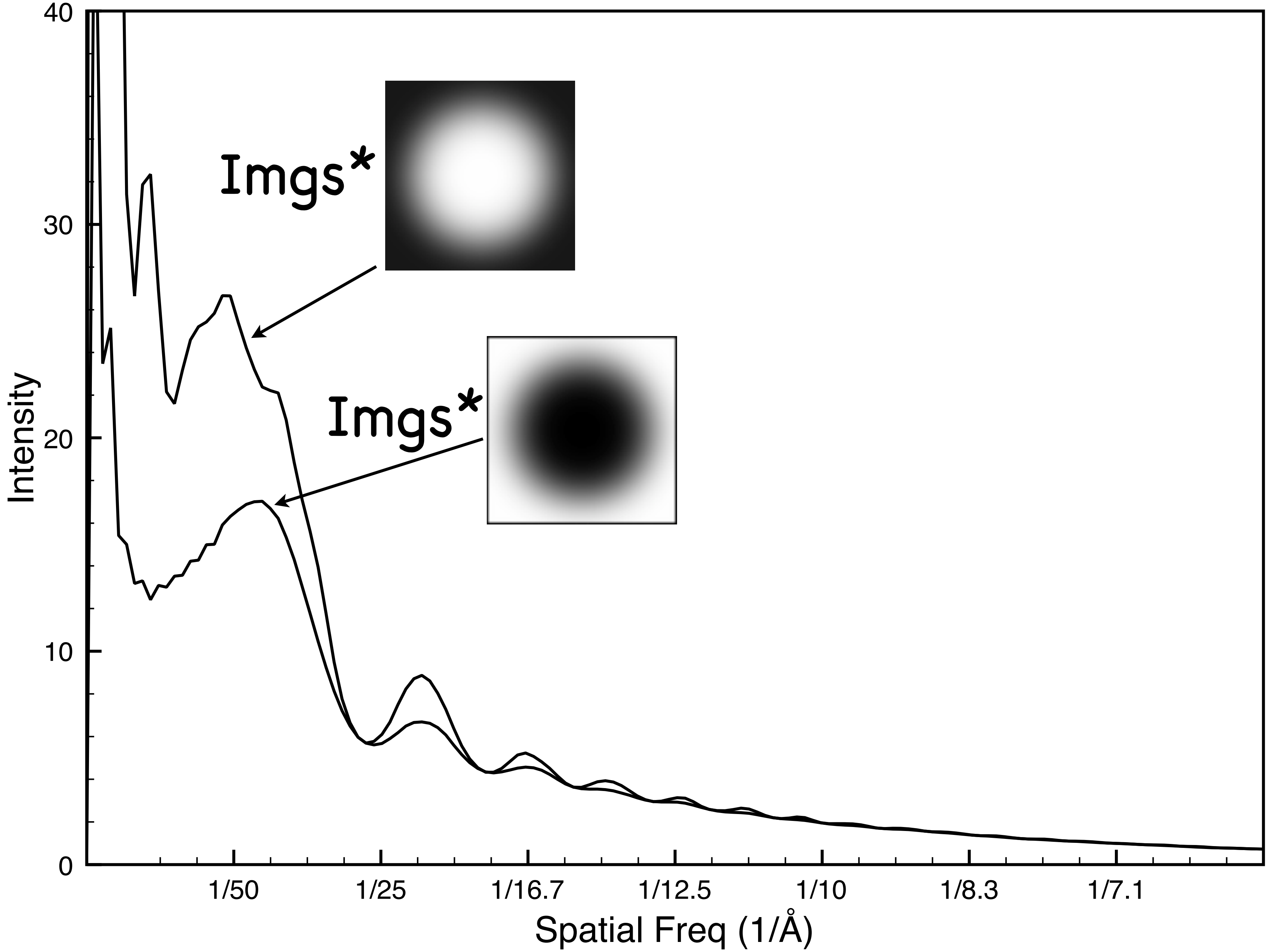








40
30
20
10
0
Intensity
Imgs*
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)


40
30
20
10
0
Intensity
Imgs*
Imgs*
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
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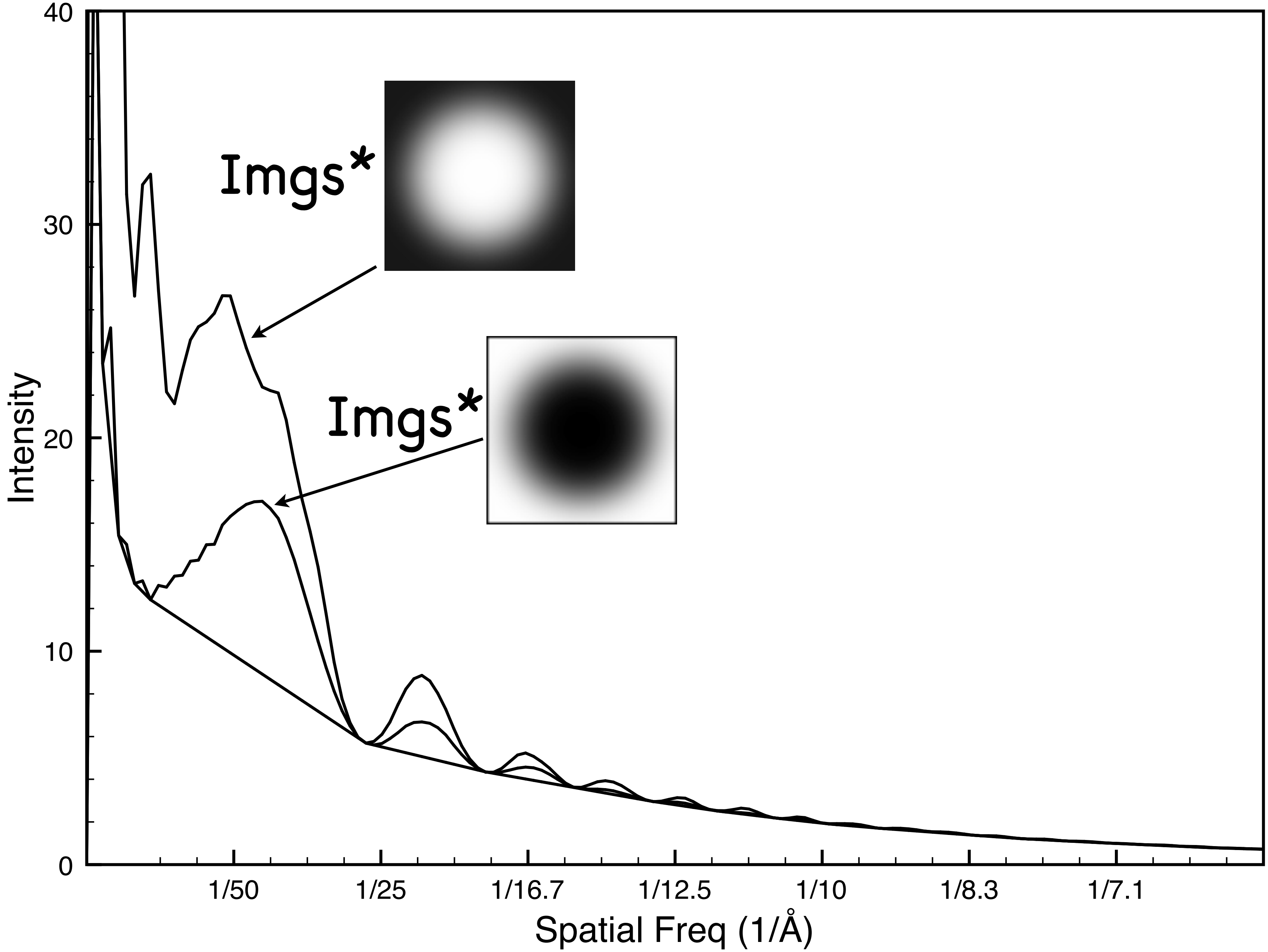
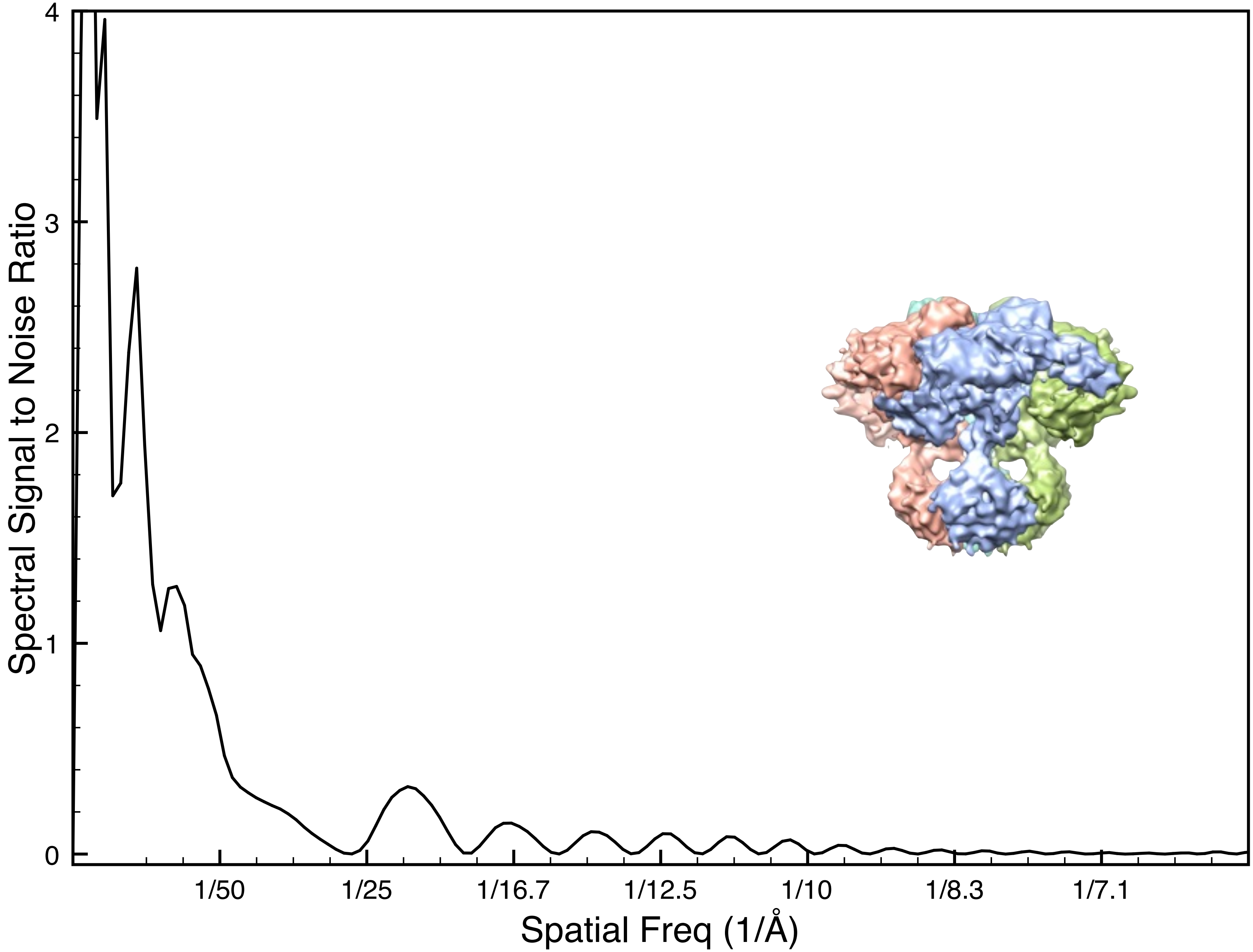
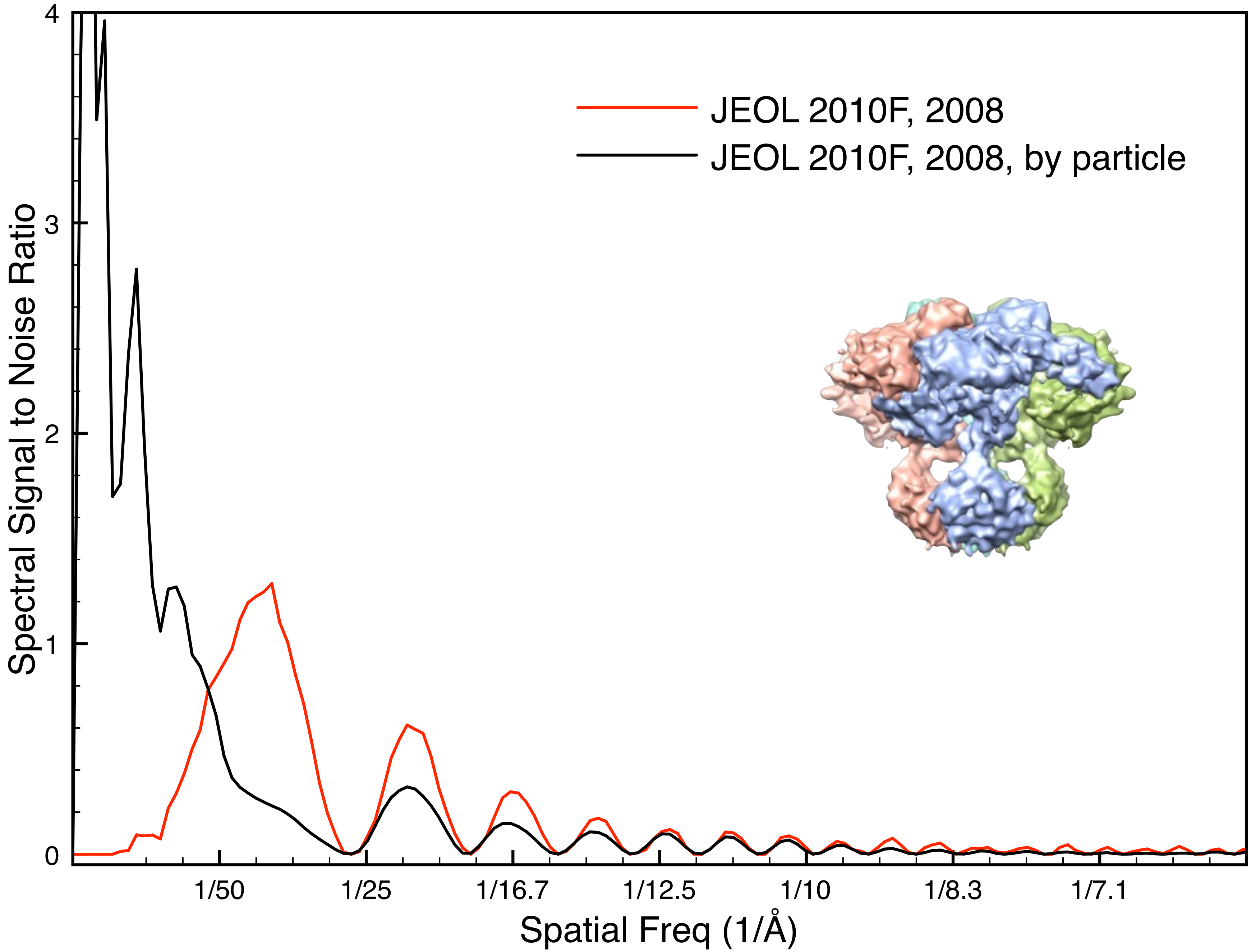
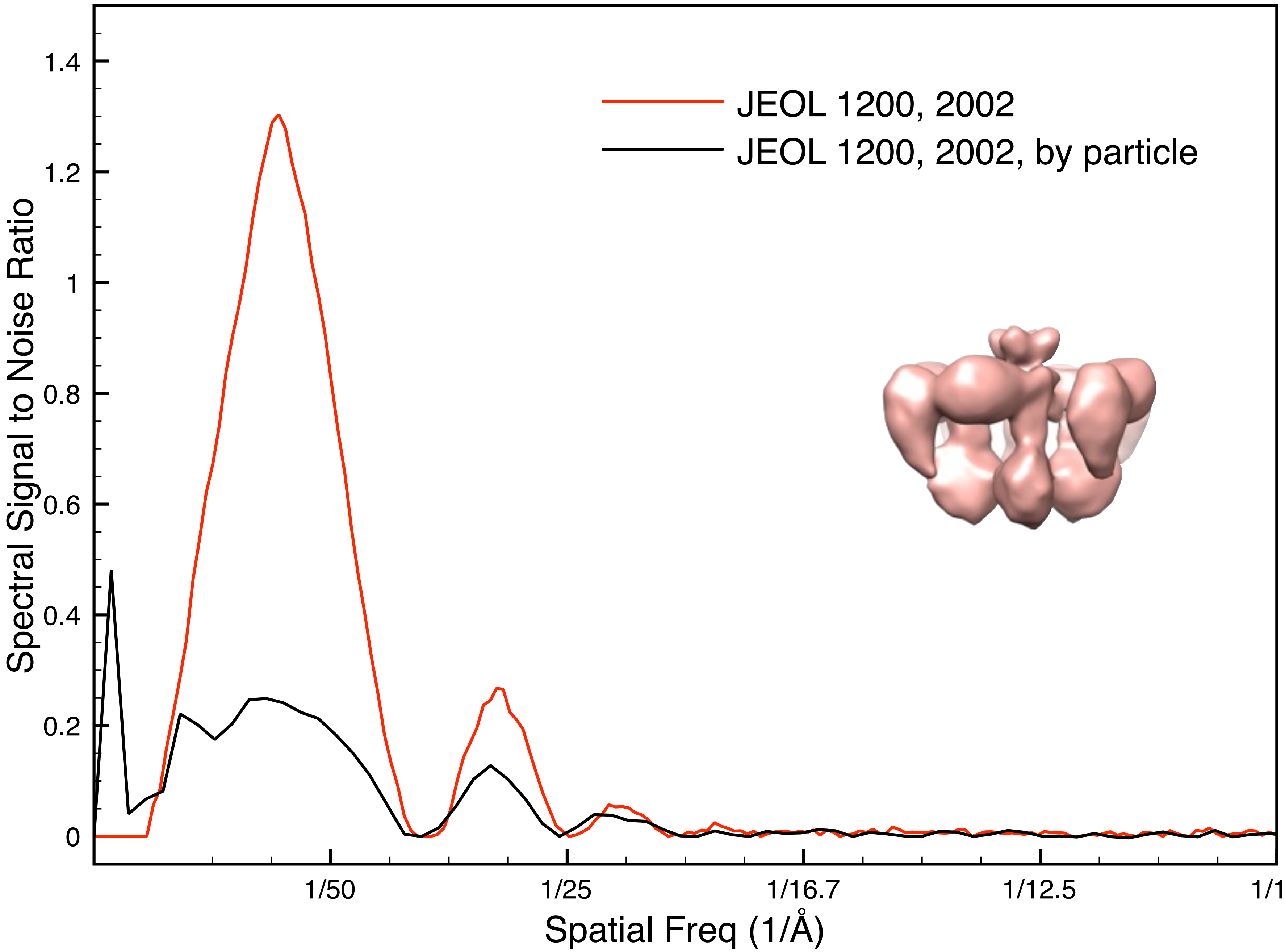
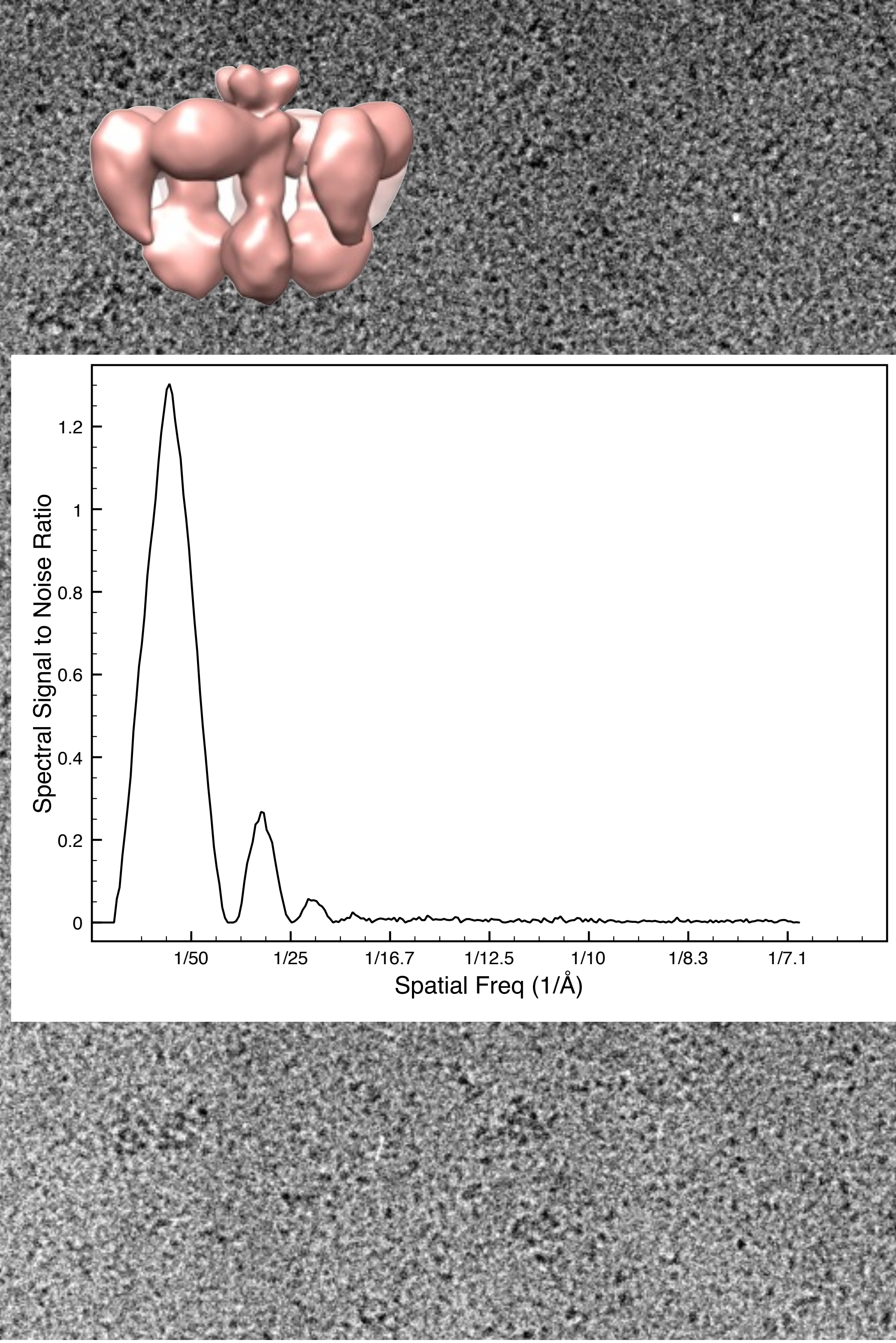
Spatial Freq (1/Å)

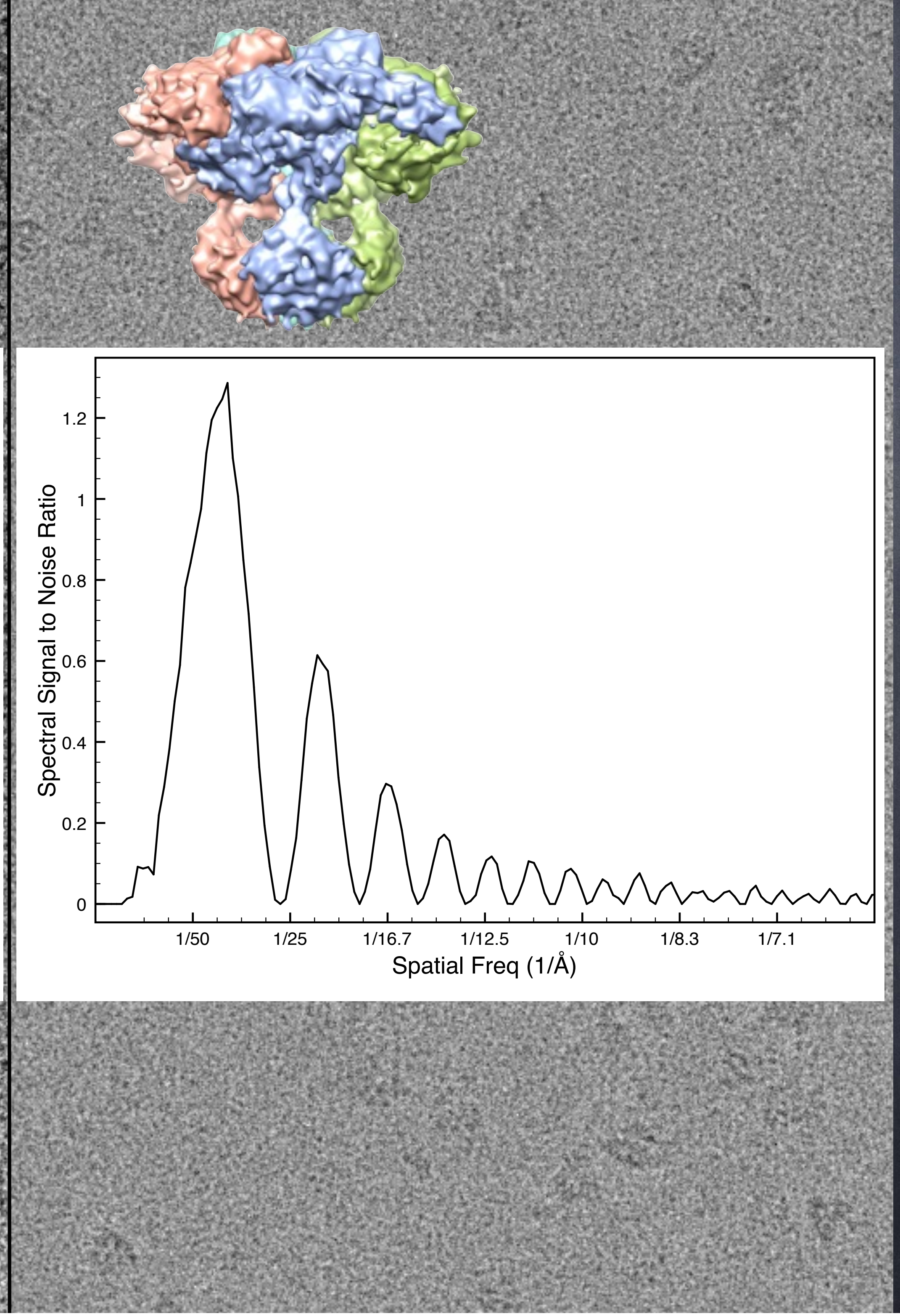

Imgs*
Imgs*
40
30
20
10
0
Intensity
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)

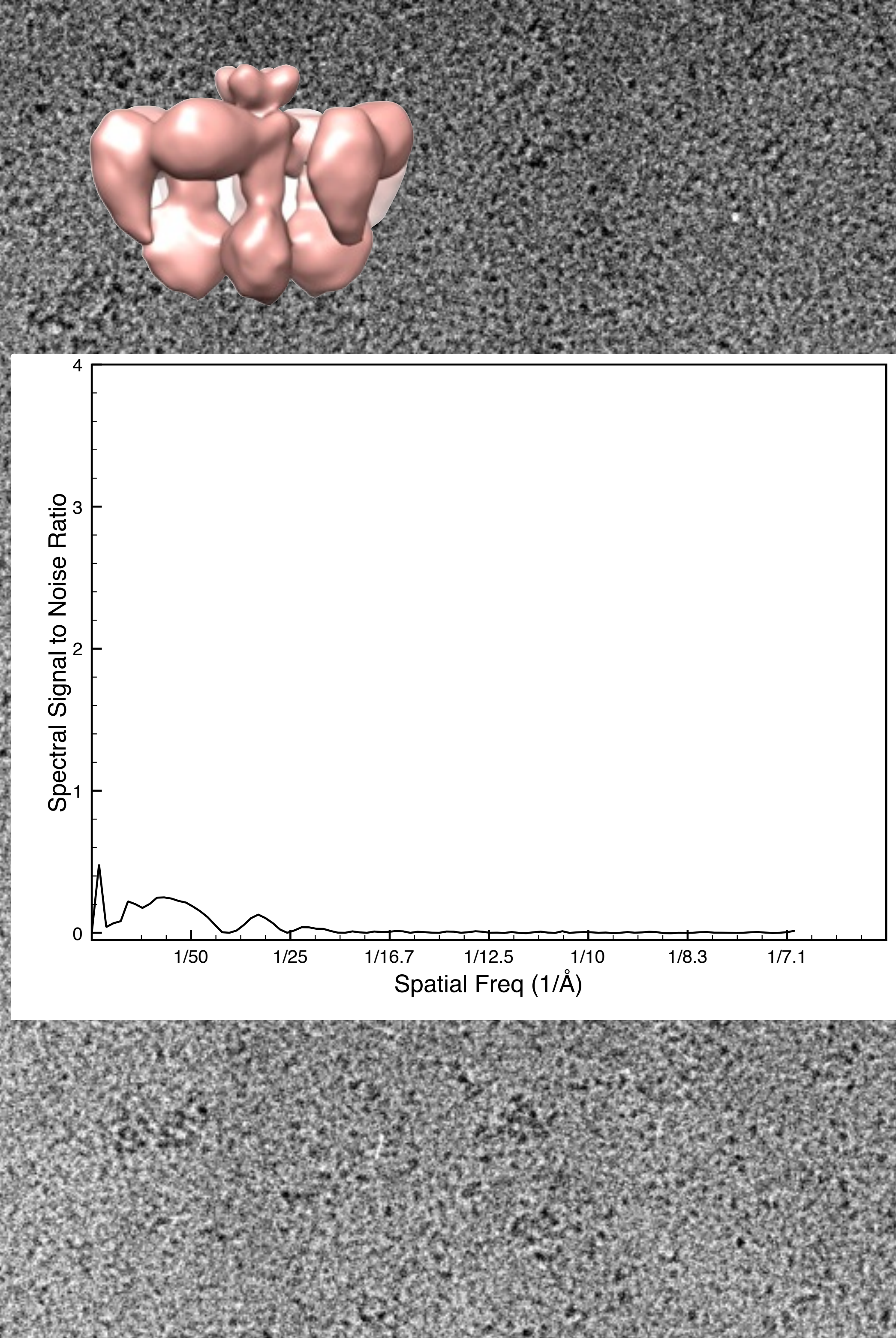

Spectral Signal to Noise Ratio
4
3
2
1
0
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)


JEOL 2010F, 2008
JEOL 2010F, 2008, by particle
Spectral Signal to Noise Ratio
Spatial Freq (1/Å)
0
1
2
3
4
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1

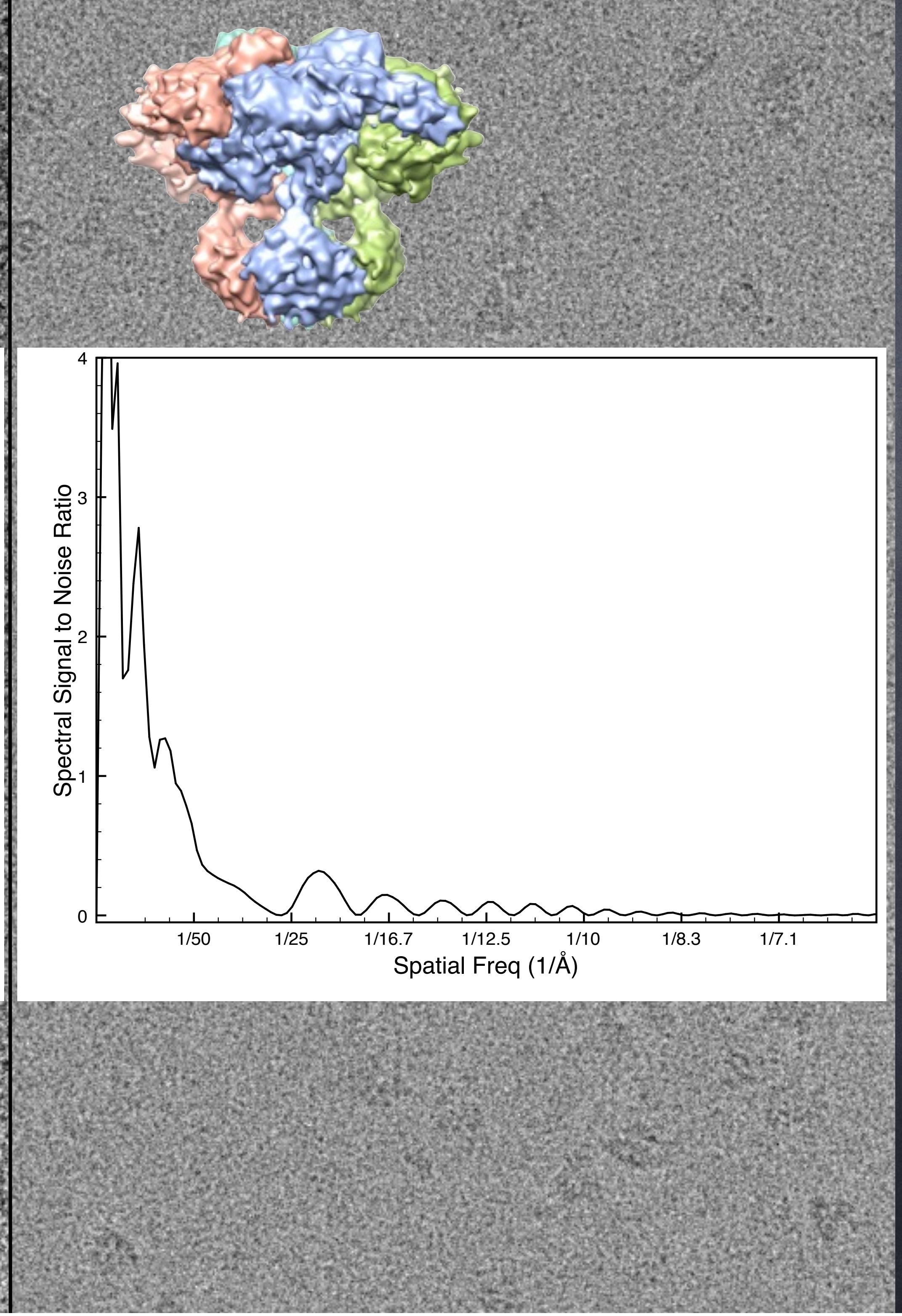

JEOL 1200, 2002
JEOL 1200, 2002, by particle
Spectral Signal to Noise Ratio
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1/50
1/25
1/16.7
1/12.5
Spatial Freq (1/Å)


Spectral Signal to Noise Ratio
1.2
1
0.8
0.6
0.4
0.2
0
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)


Spectral Signal to Noise Ratio
1.2
1
0.8
0.6
0.4
0.2
0
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1
Spatial Freq (1/Å)



Spectral Signal to Noise Ratio
Spatial Freq (1/Å)
4
3
2
1
0
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1



Spectral Signal to Noise Ratio
Spatial Freq (1/Å)
4
3
2
1
0
1/50
1/25
1/16.7
1/12.5
1/10
1/8.3
1/7.1

CTF Correction

Average

Weight

Measured image

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

$$k_i = ?$$

- Maximize SNR of $T(s,\theta)$
- Minimize RMSD between T and F

$$\sqrt{\sum_{x,y}(t(x,y)-f(x,y))^2}$$

CTF Correction

Wiener Filter	CTF Correction	SNR Weight

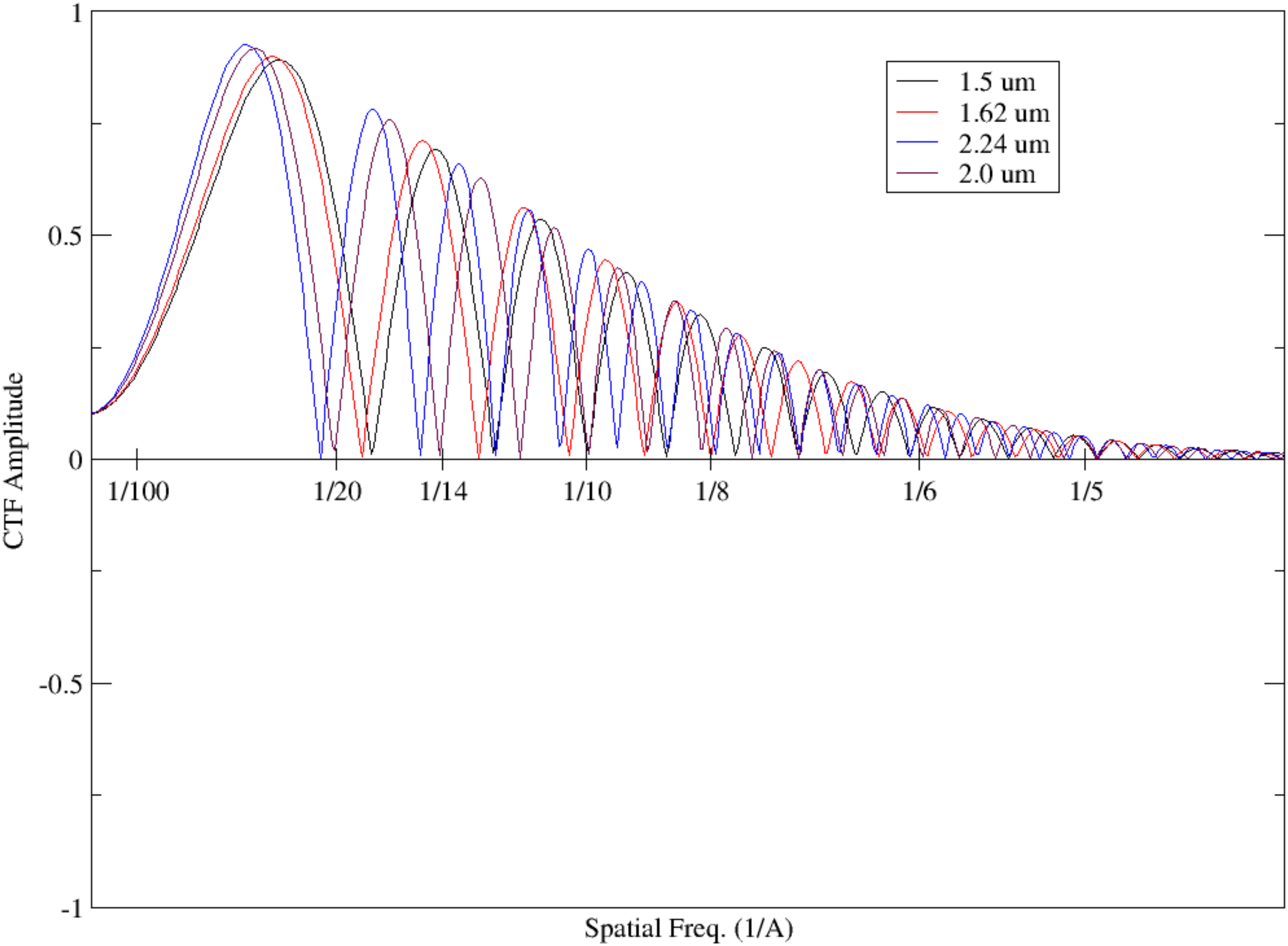
$$\overline{T}(s,\theta) = \frac{F^2(s)R(s)}{1+F^2(s)R(s)} \sum_i \frac{1}{C_i(s)E_i(s)} \frac{R_i(s)}{R(s)} \overline{M}_i(s,\theta)$$

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

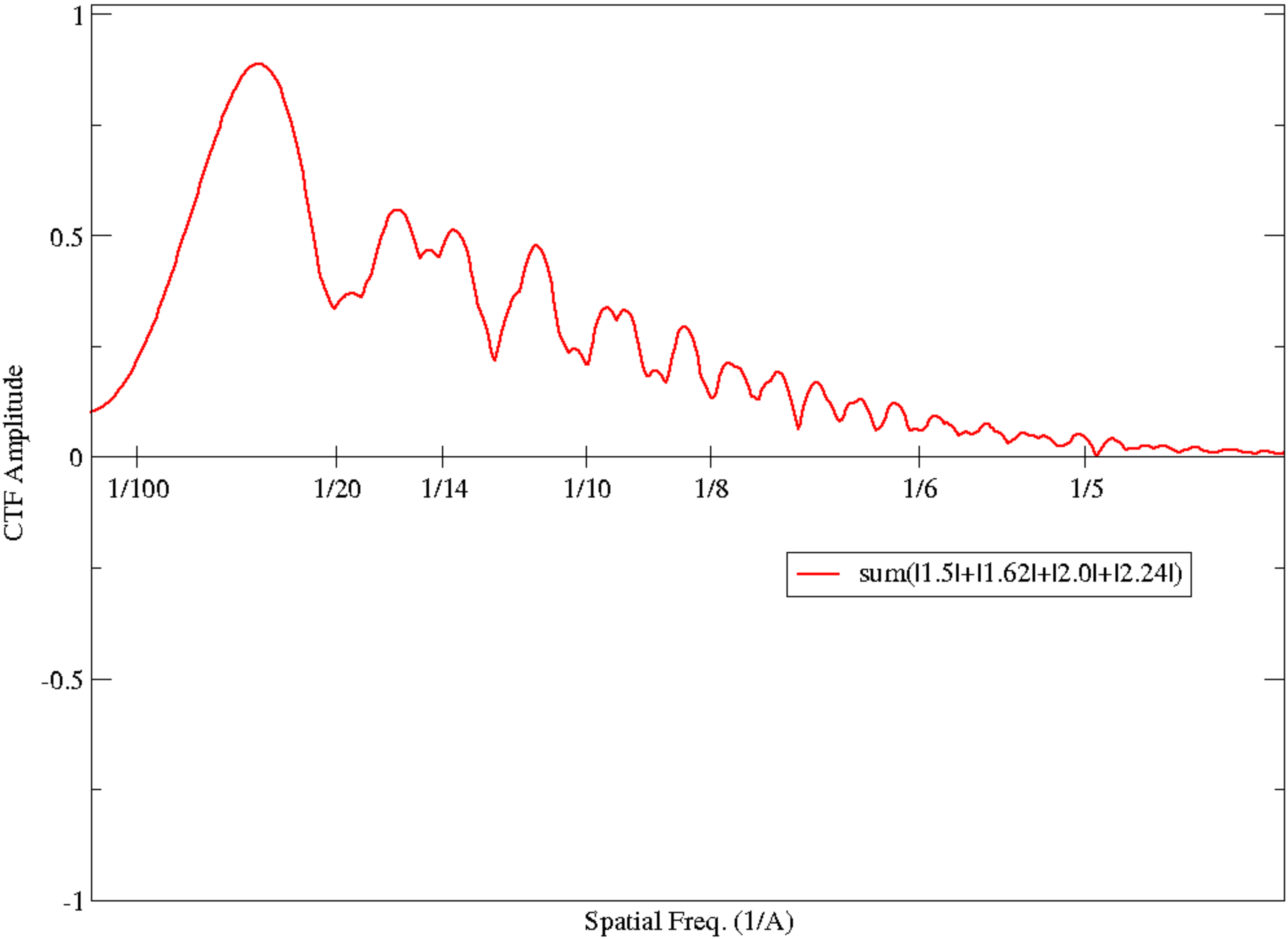
SSNR of particle i

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

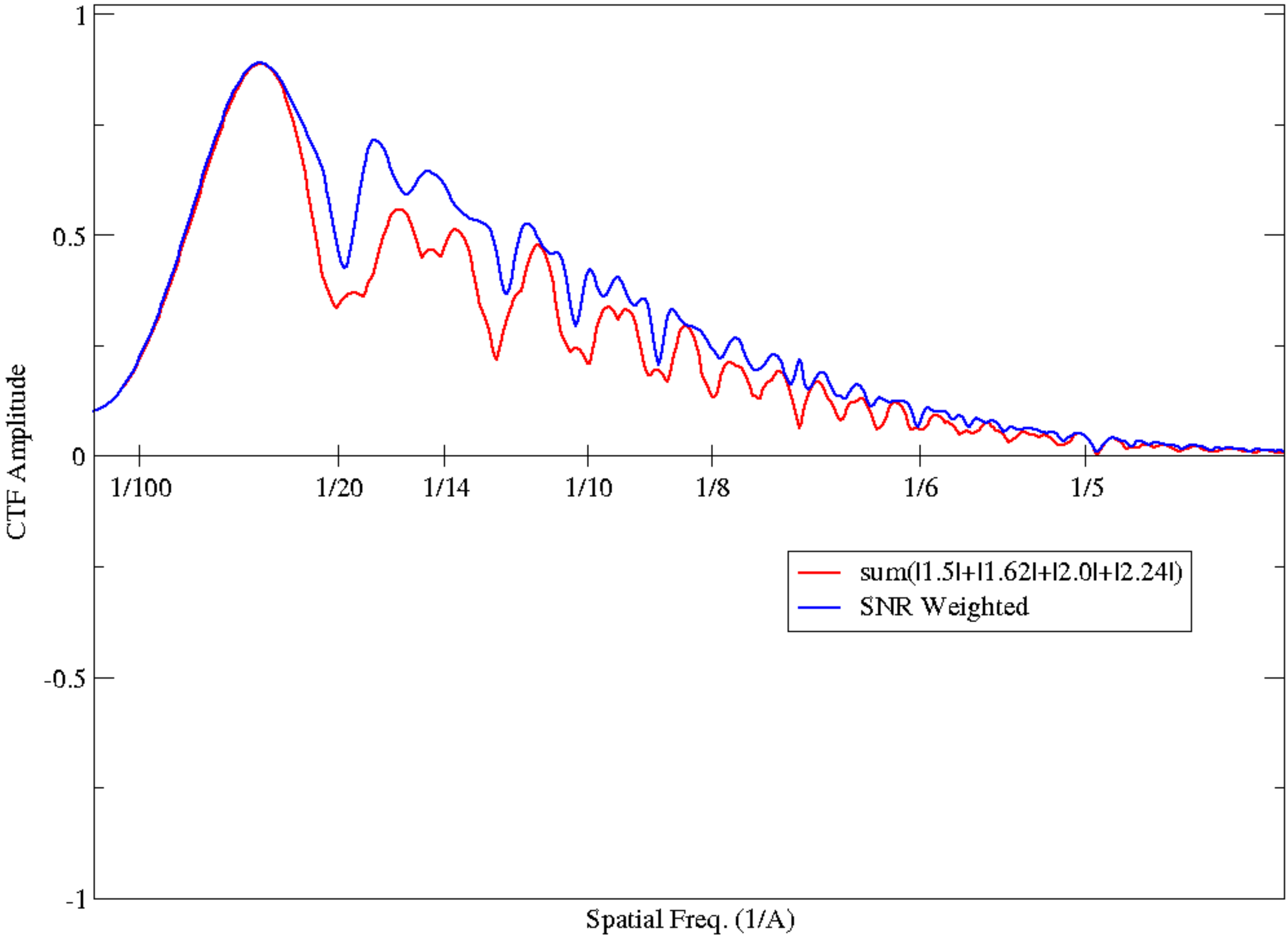
SSNR sum of all particles i

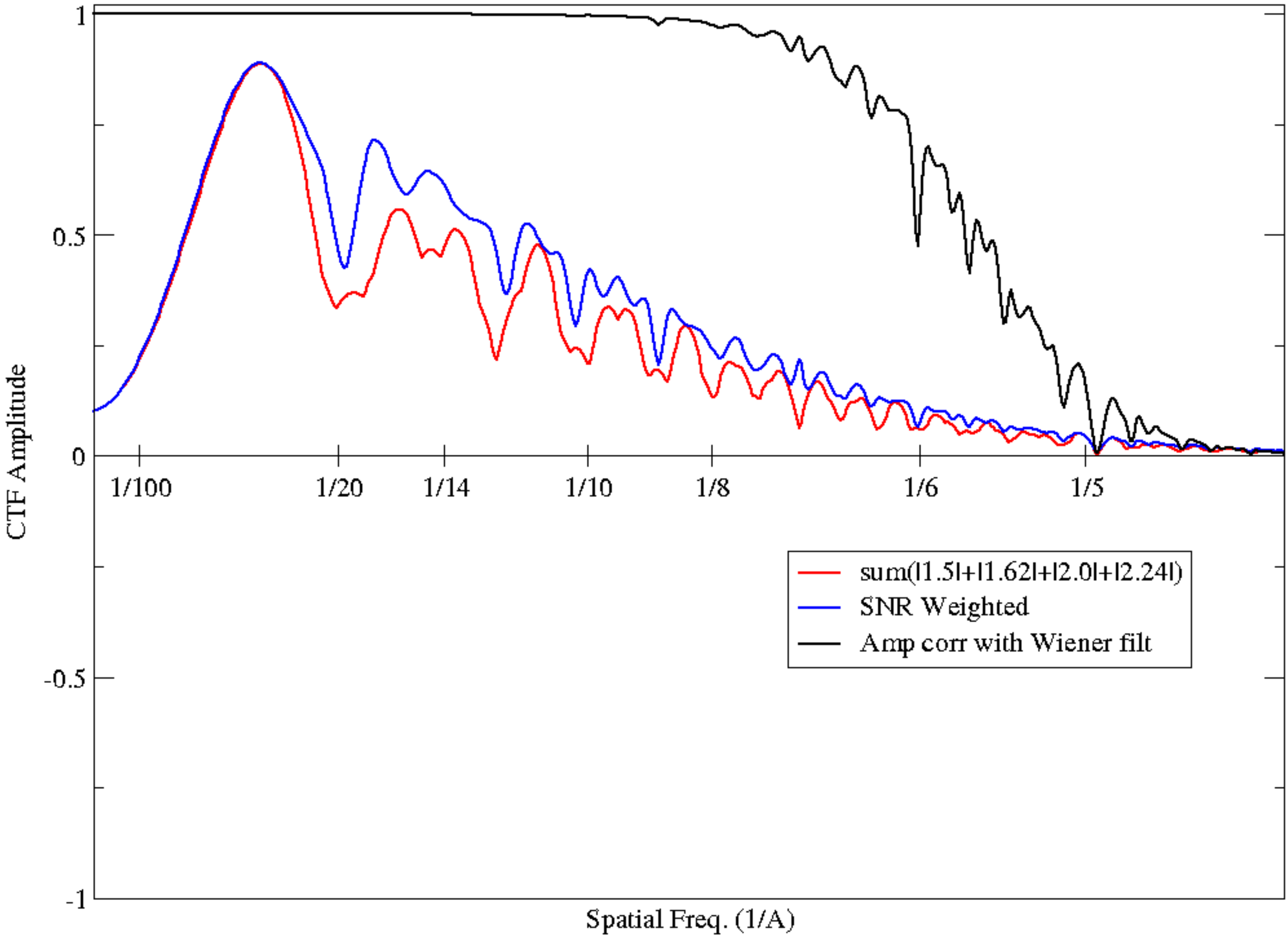


1
0.5
0
-0.5
-1
CTF Amplitude
1/100
1/20
1/14
1/10
1/8
1/6
1/5
Spatial Freq. (1/A)
1.5 um
1.62 um
2.24 um
2.0 um



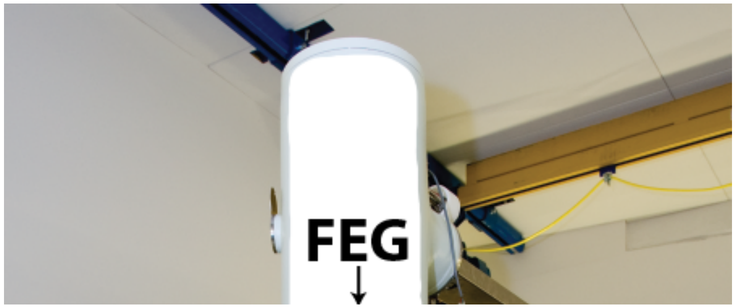
1
0.5
0
-0.5
-1
CTF Amplitude
1/100
1/20
1/14
1/10
1/8
1/6
1/5
sum(|1.5|+|1.62|+|2.0|+|2.24|)
Spatial Freq. (1/A)

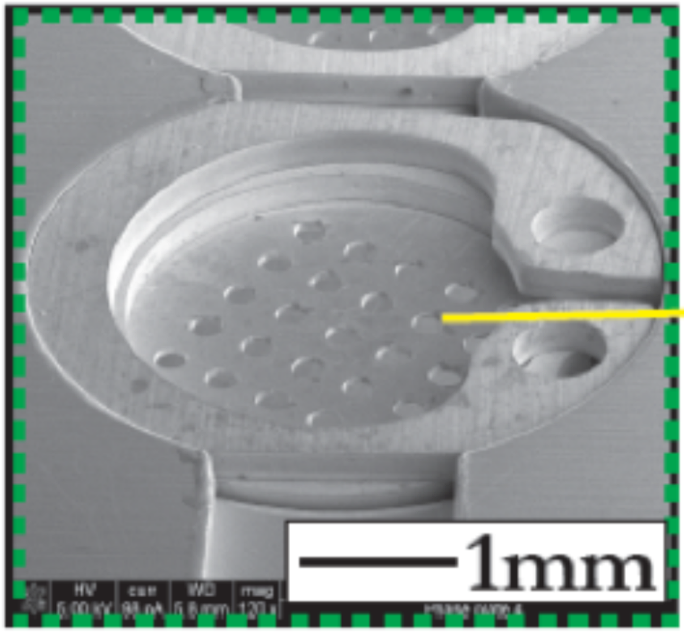

1
0.5
0
-0.5
-1
CTF Amplitude
1/100
1/20
1/14
1/10
1/8
1/6
1/5
sum(|1.5|+|1.62|+|2.0|+|2.24|)
SNR Weighted
Spatial Freq. (1/A)



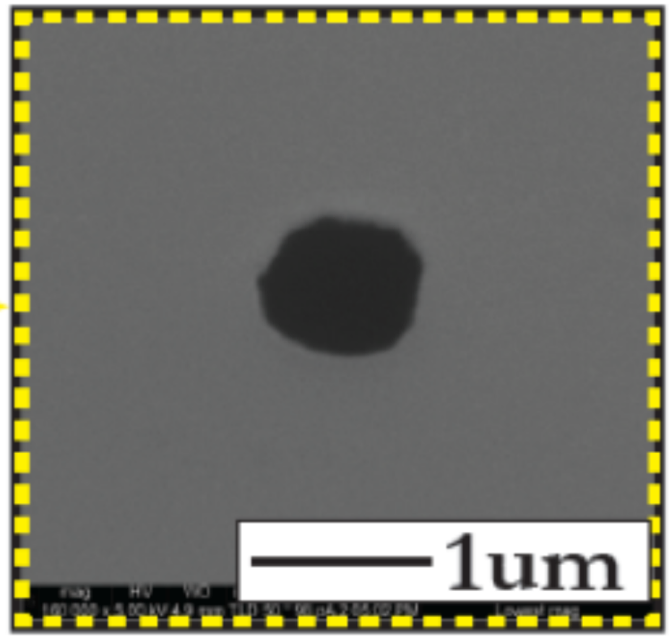
1
0.5
0
-0.5
-1
CTF Amplitude
1/100
1/20
1/14
1/10
1/8
1/6
1/5
Spatial Freq. (1/A)
sum(|1.5|+|1.62|+|2.0|+|2.24|)
SNR Weighted
Amp corr with Wiener filt

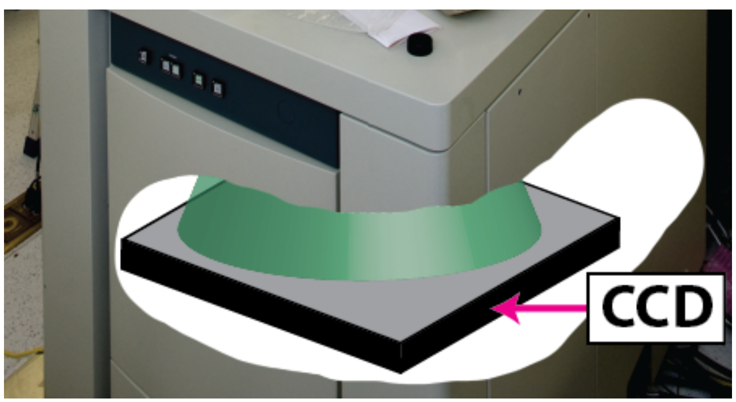
Zernike Phase Plate





Phase Plate Hole



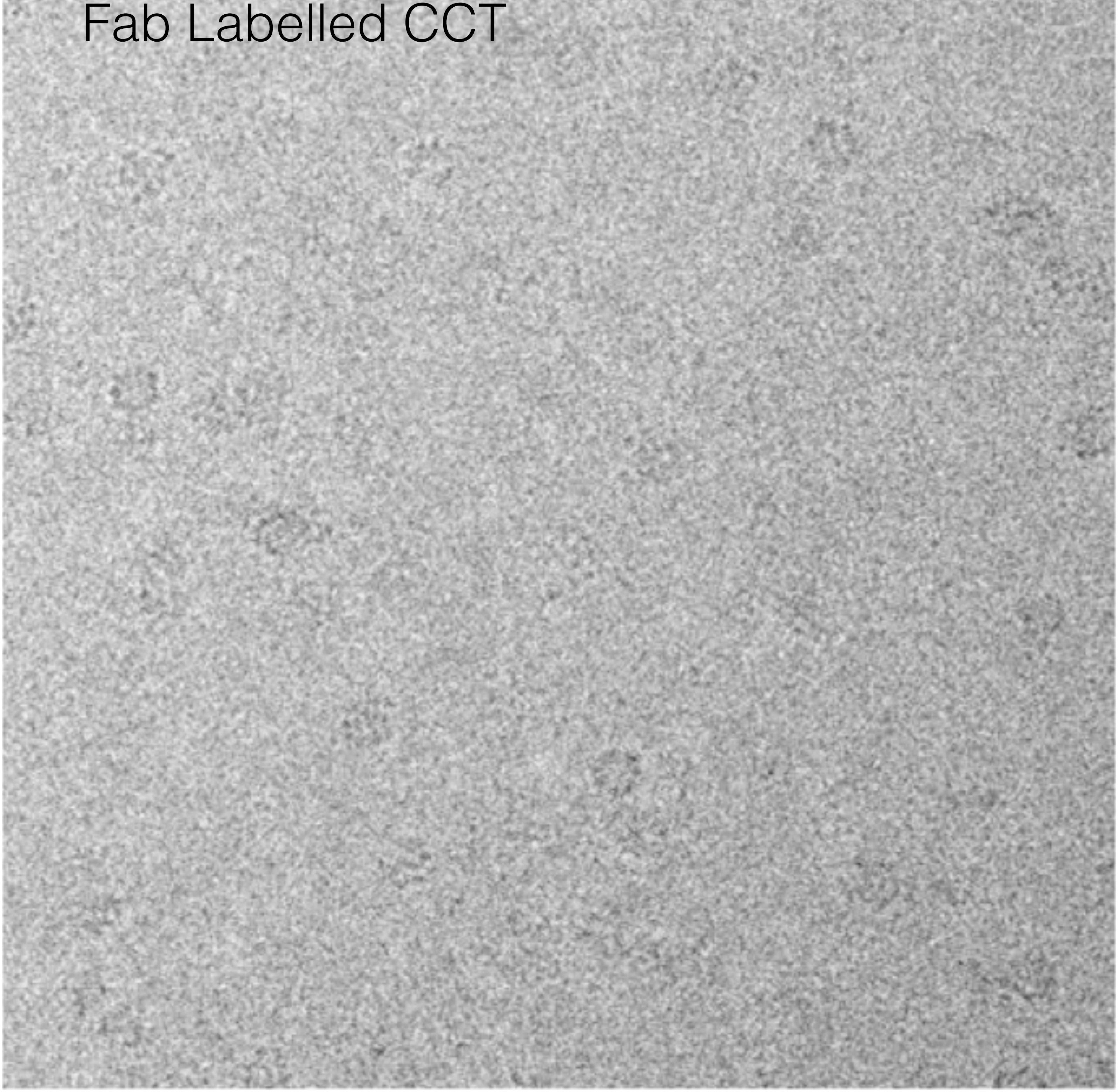


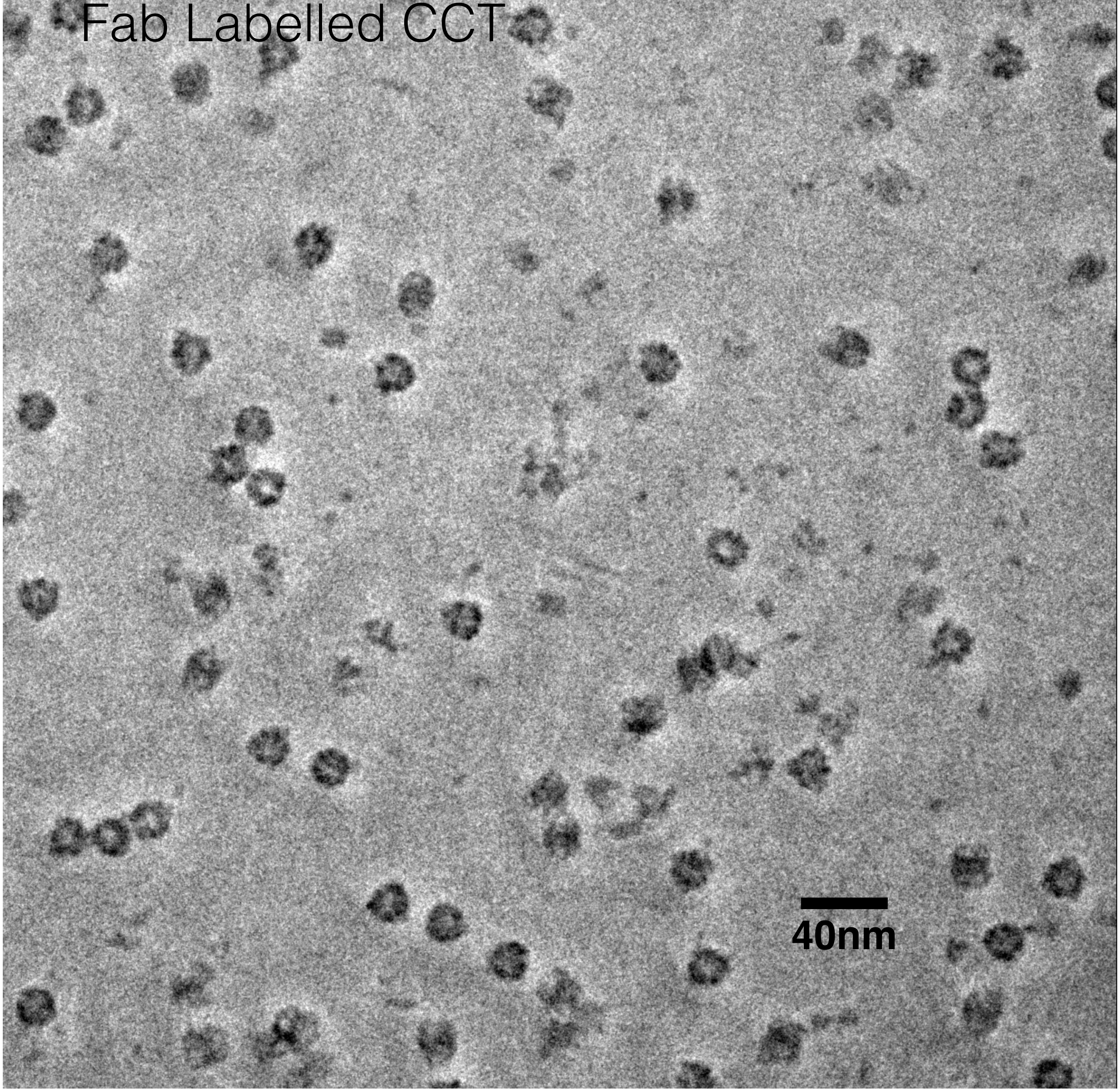
Demo

What are they Good for ?

- Small things
  - 2-5x low resolution contrast boost
- Whole Cells
  - Can identify much more of the cytosol
- Dynamics
  - Better per-particle contrast -> better classification

Fab Labelled CCT




Fab Labelled CCT
40nm