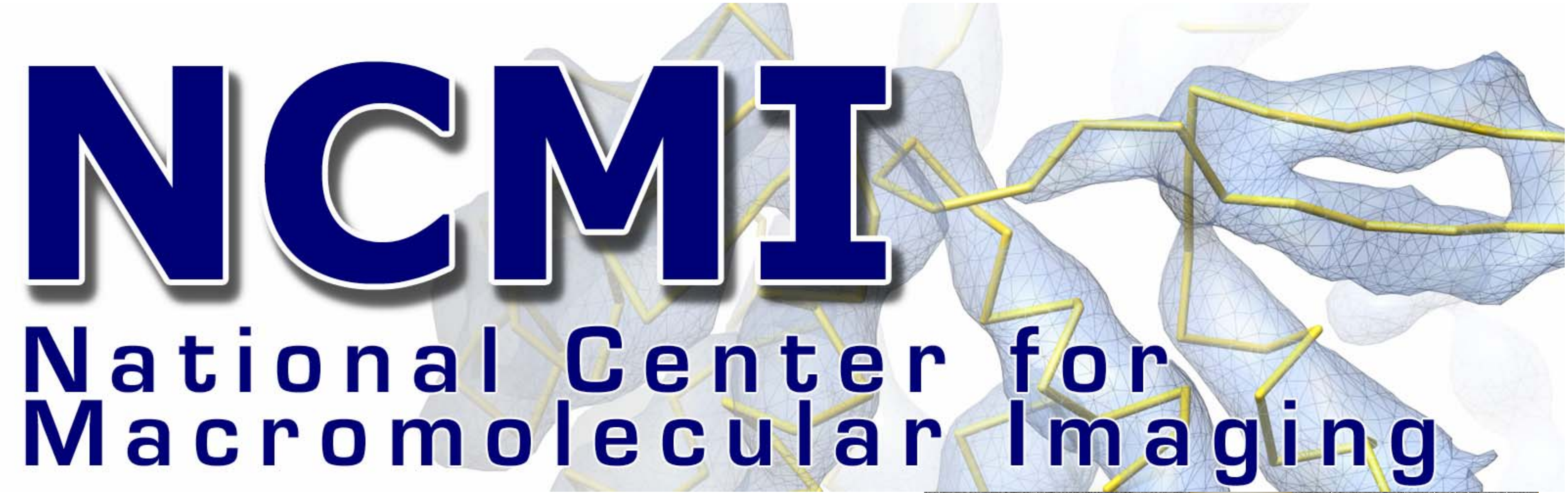
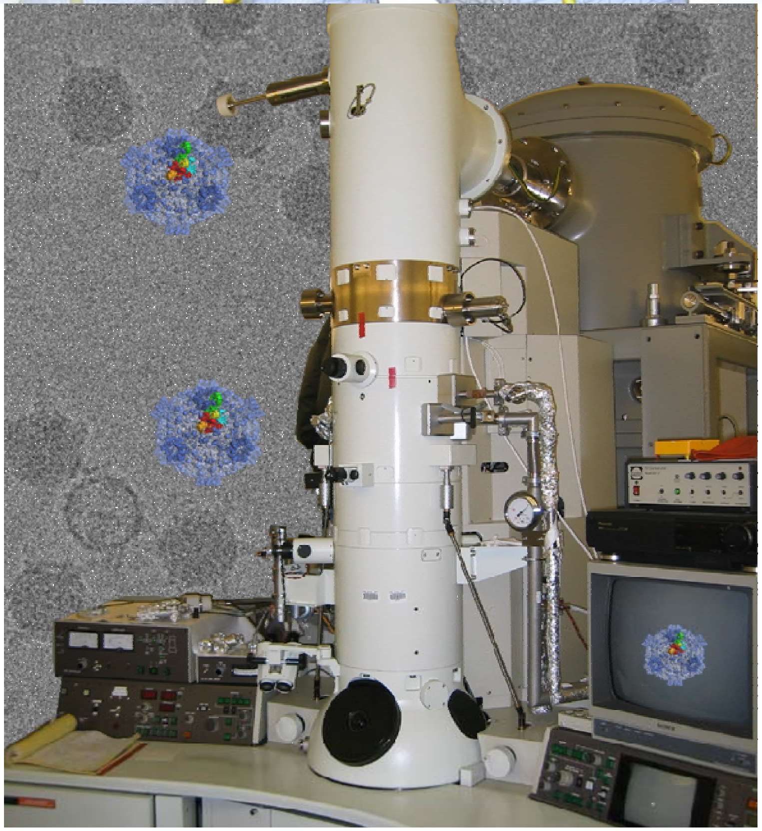


NCMI

National Center for Macromolecular Imaging

Wah Chiu
wah@bcm.edu
Baylor College of Medicine

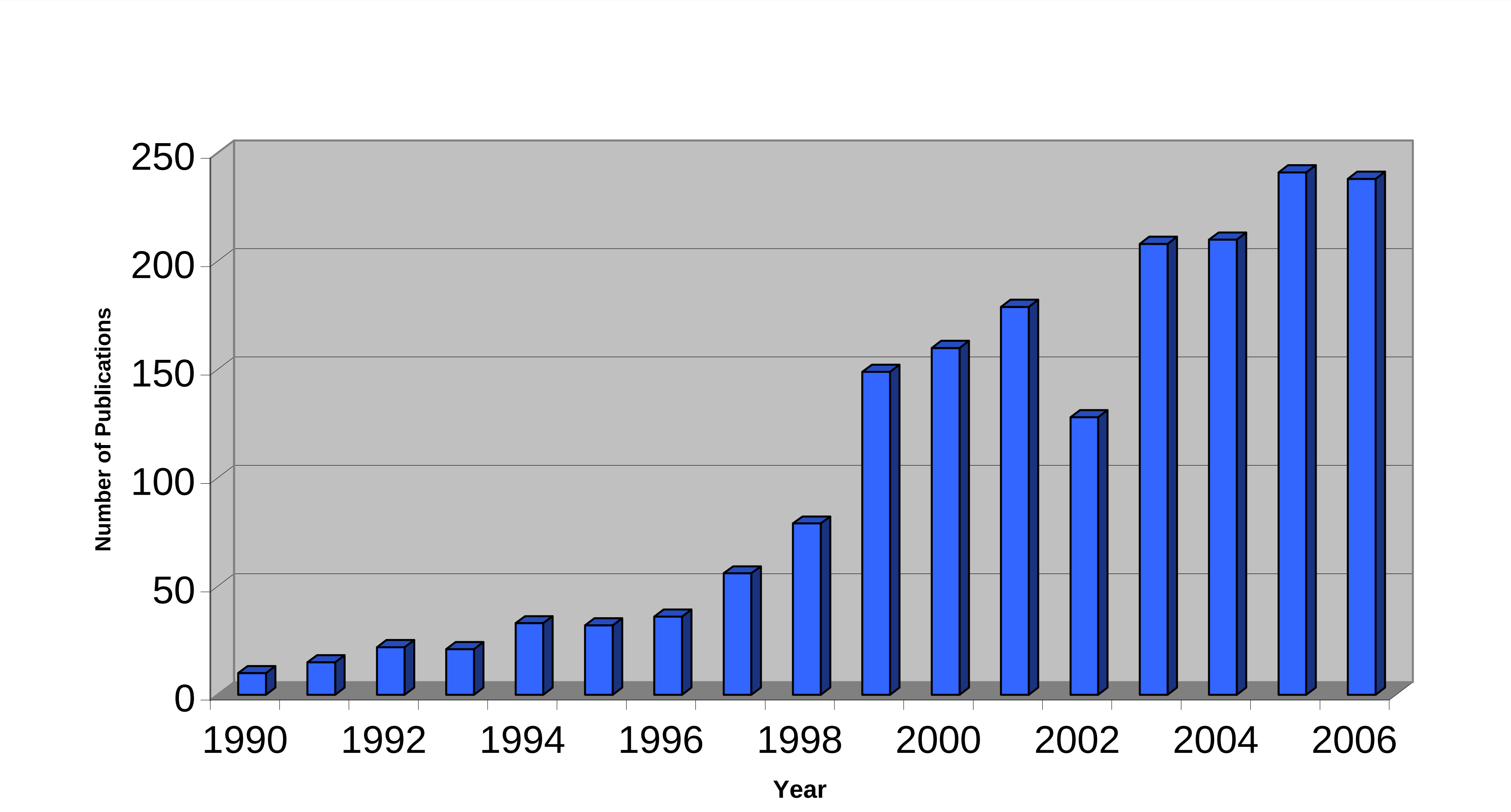


National Center for Research Resources

NSF

NIGMS

Trends in Macromolecular Cryo-EM



Matthew Baker (2007)

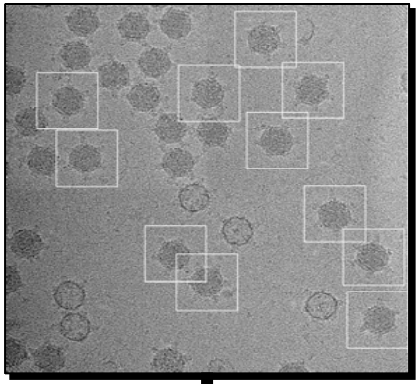
From Sample to Structure

biochemical preparation

cryo-em sample preparation

imaging

data collection



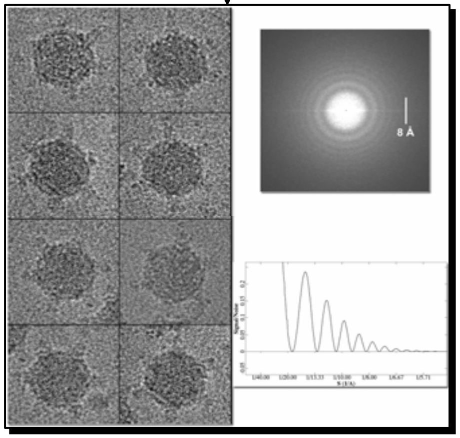
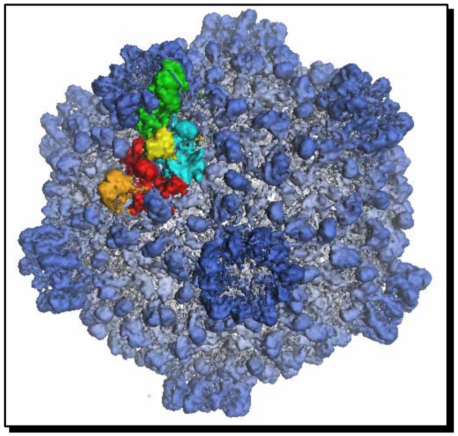
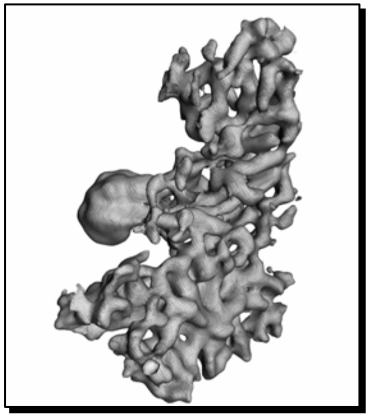


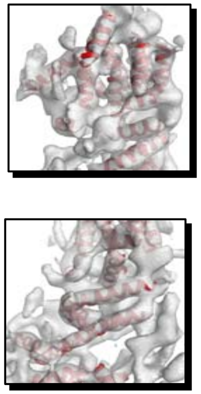
image processing



reconstruction



structural analysis



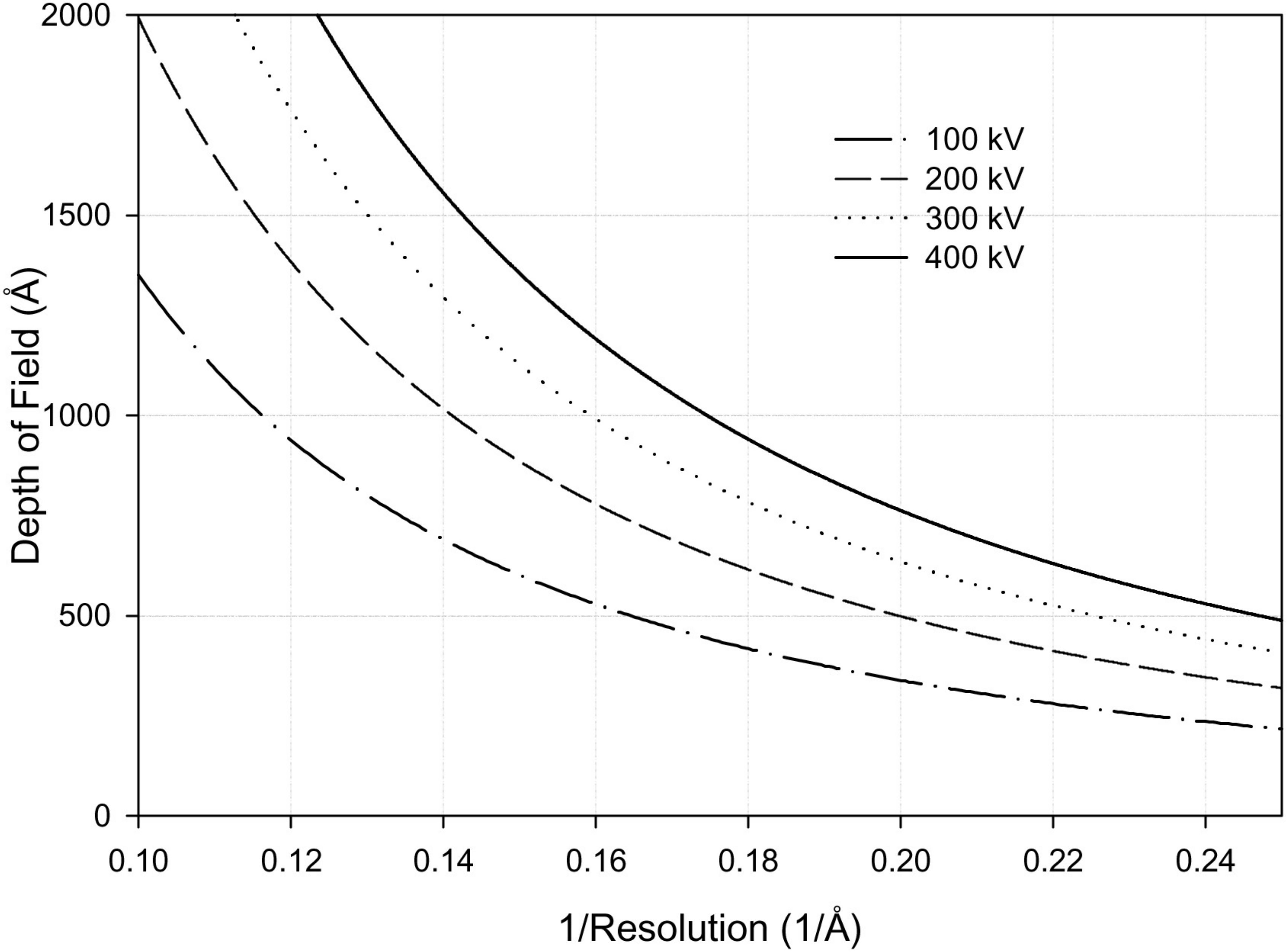
annotation

Chiu et al *JEOL News* (2006)

Image Contrast Theory

- Image is a true 2-D projection of the 3-D object with the same focus throughout
- There is only elastically scattered electron in forming the image

Depth of Field Dependence on Resolution and Sample Thickness

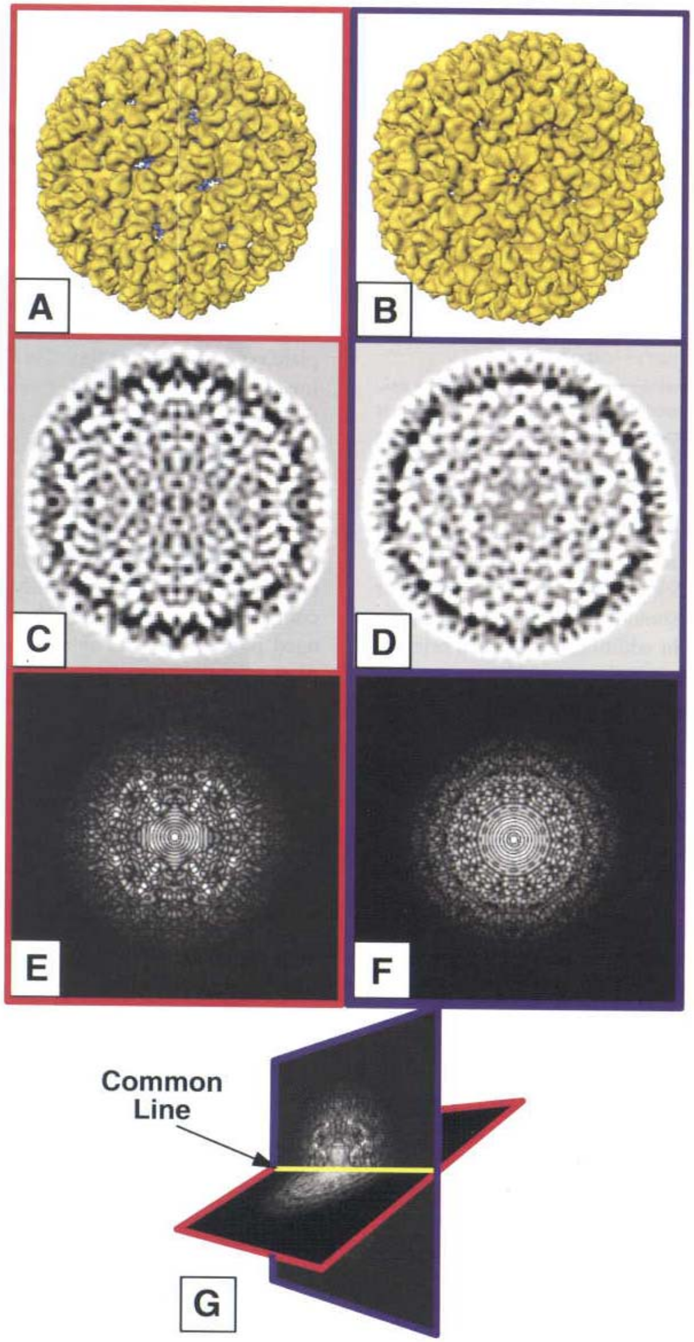


Zhou and Chiu, *Adv Prot Chem* **64**: 93-130 (2003)

3D Object

Projection Image

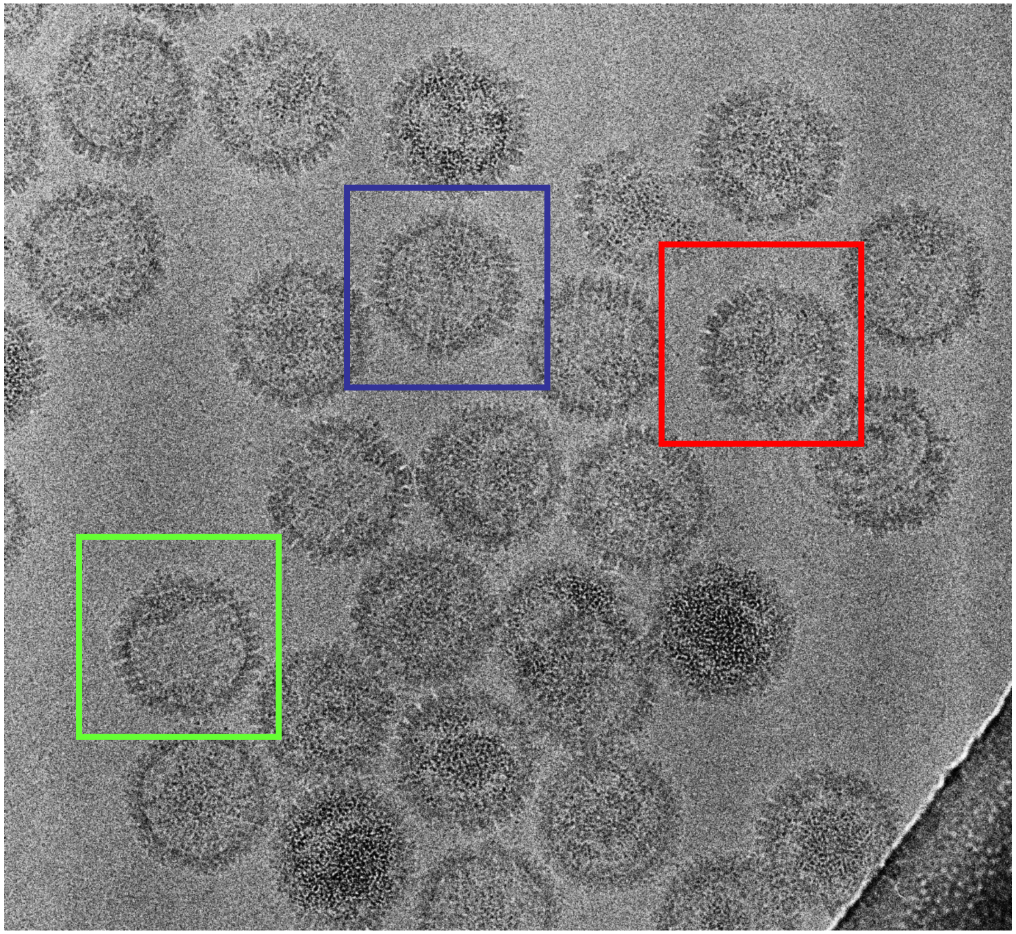
Fourier Transform

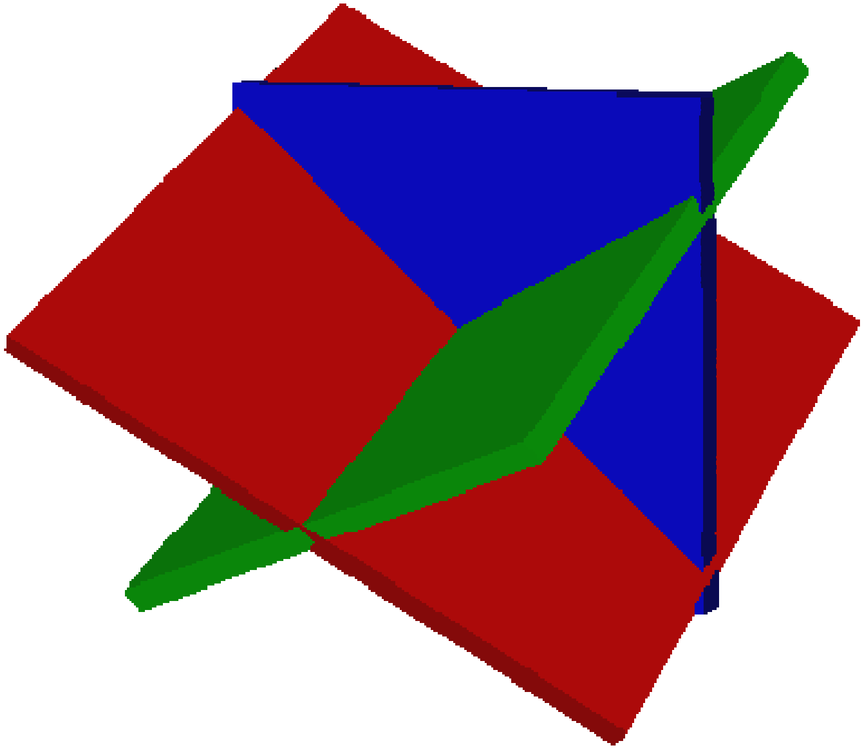


Thuman-Commike & Chiu,
Micron **31**: 687-711 (2000)

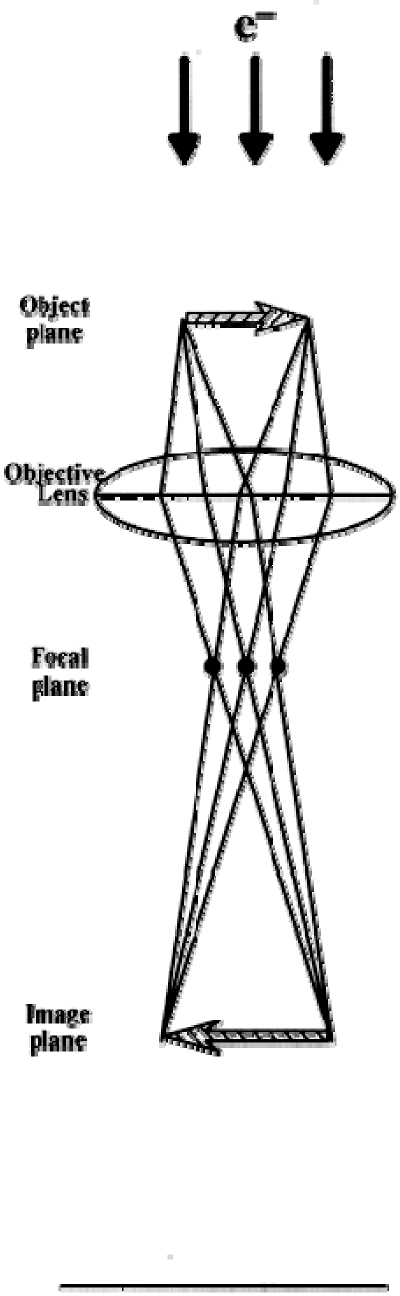
Single Particle Images

Equivalent data in Fourier space





Hong Zhou



Object Coulomb potential function $V(x_o, y_o, z_o)$

Object transmitted wave function $\Psi_o(x_o, y_o)$

$$\Psi_o(x_o, y_o) \approx 1 + i\sigma v(x_o, y_o)$$

$$v(x_o, y_o) = \int V(x_o, y_o, z_o)\, dz_o$$

Phase shift $\gamma(S)$ *introduced by objective lens*

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

Diffraction wave function $\Psi_d(S_x, S_y)$

$$\Psi_d(S_x, S_y) = F(S_x, S_y) \exp\left(i\gamma(S)\right)$$

$$F(S_x, S_y) = \mathcal{F}\left[\Psi_o(x_o, y_o)\right]$$

Diffraction intensity $I_d(S_x, S_y) = \Psi_d(S_x, S_y)\ \Psi_d^*(S_x, S_y)$

Image wave function $\Psi_i(x_i, y_i)$

$$\Psi_i(x_i, y_i) = \mathcal{F}^{-1}\left[\Psi_d(S_x, S_y)\right]$$

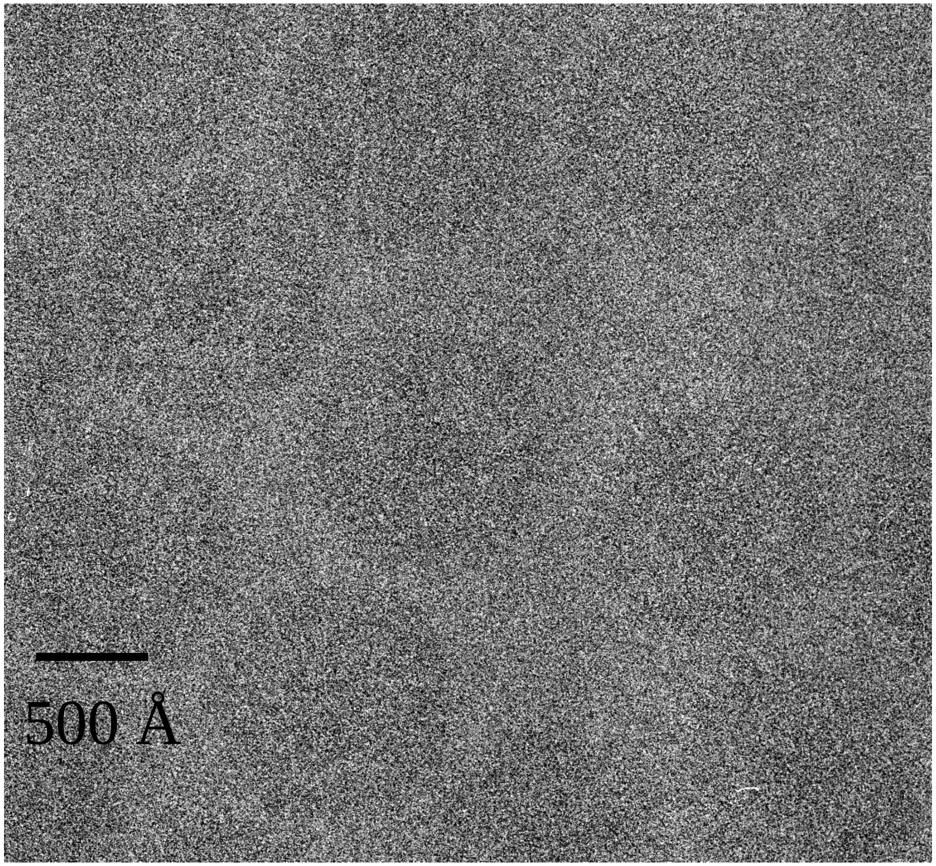
Image intensity $I_i(x_i, y_i)$

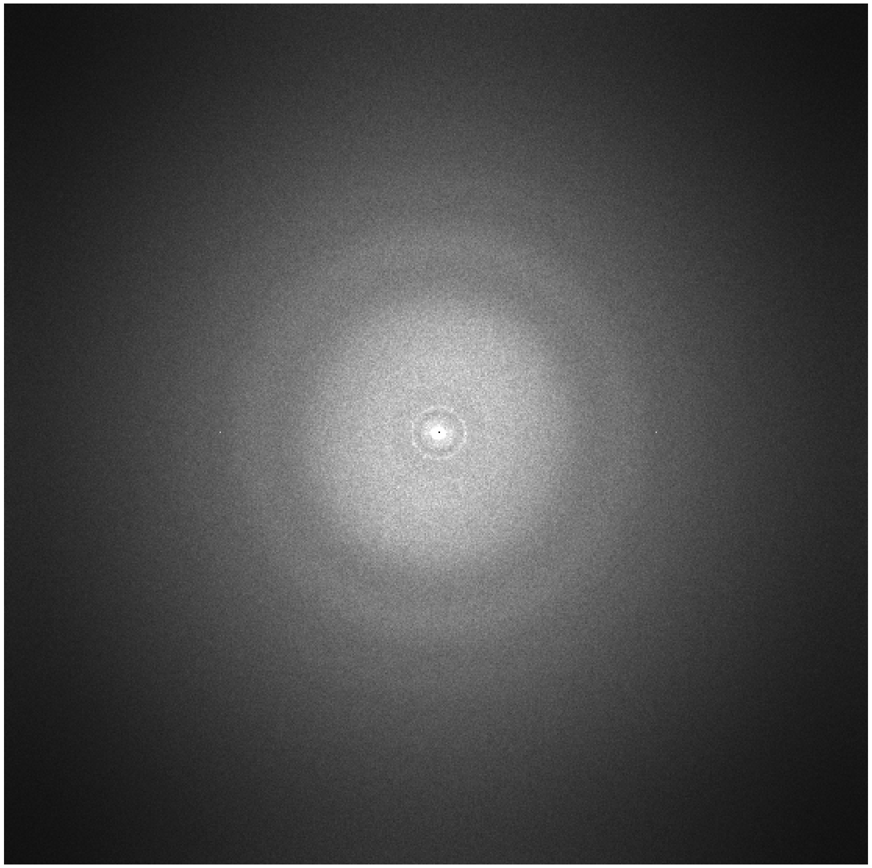
$$I_i(x_i, y_i) = \delta(0, 0) - 2\sigma v(x_i, y_i) * \mathcal{F}^{-1}\left[\sin \gamma(S)\right]$$

Computed diffraction wave function $T(S_x, S_y)$

$$\begin{aligned} T(S_x, S_y) &= \mathcal{F}\left[I_i(x_i, y_i)\right] \\ &= \delta(0, 0) - 2\, F(S_x, S_y) \sin \gamma(S) \end{aligned}$$

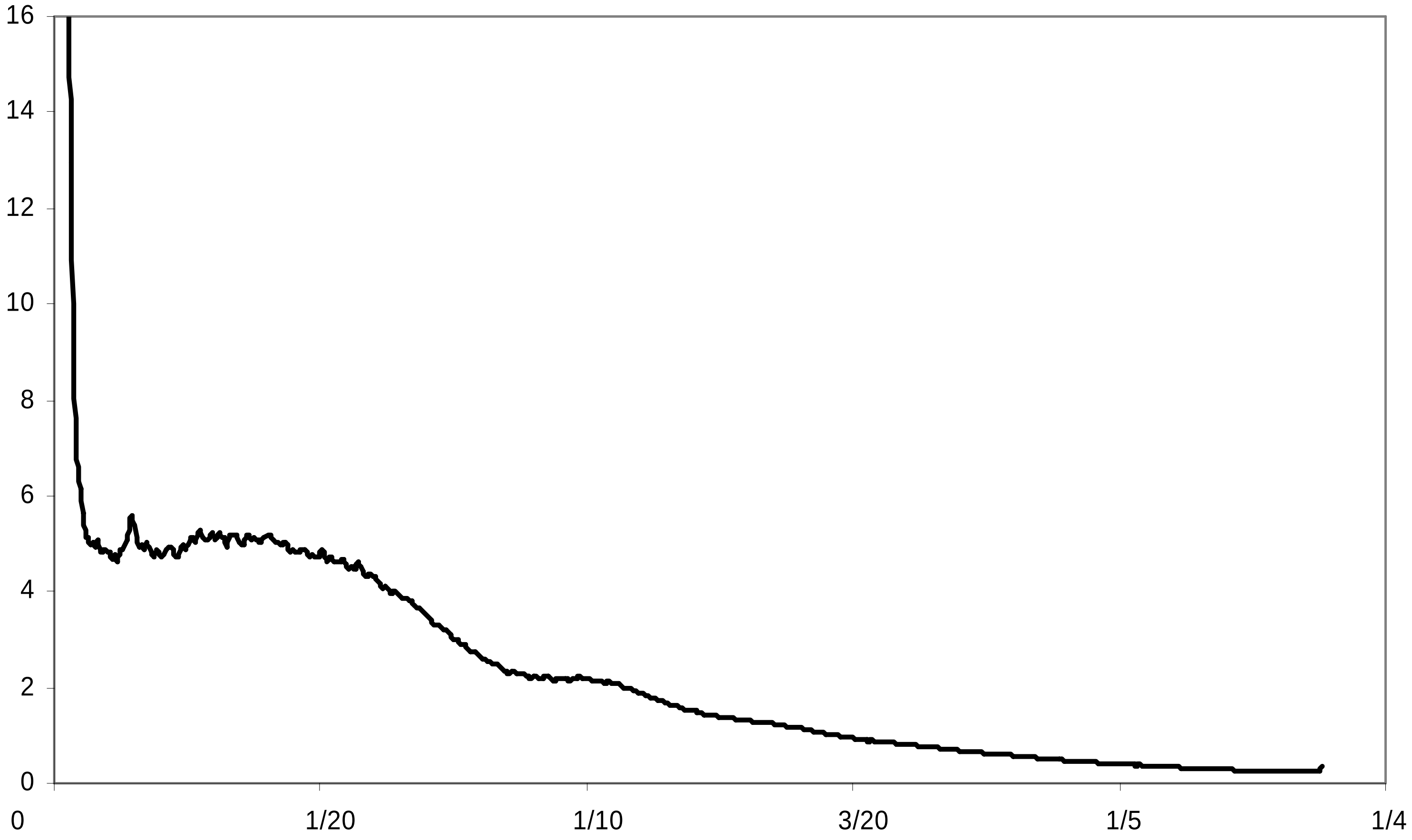
400 kV image data of HSV-1 capsids





Joanita Jakana

Circularly Averaged Power Spectrum



Spatial frequency (1/Å)

Joanita Jakana

Computed diffraction pattern

$$\boxed{F^2(s)}\ CTF^2(s)\ Env^2(s) + N^2(s)$$

Structure factor

Contrast transfer function

Envelope function

Background

Saad et al *JSB* **133**: 32-42 (2001)

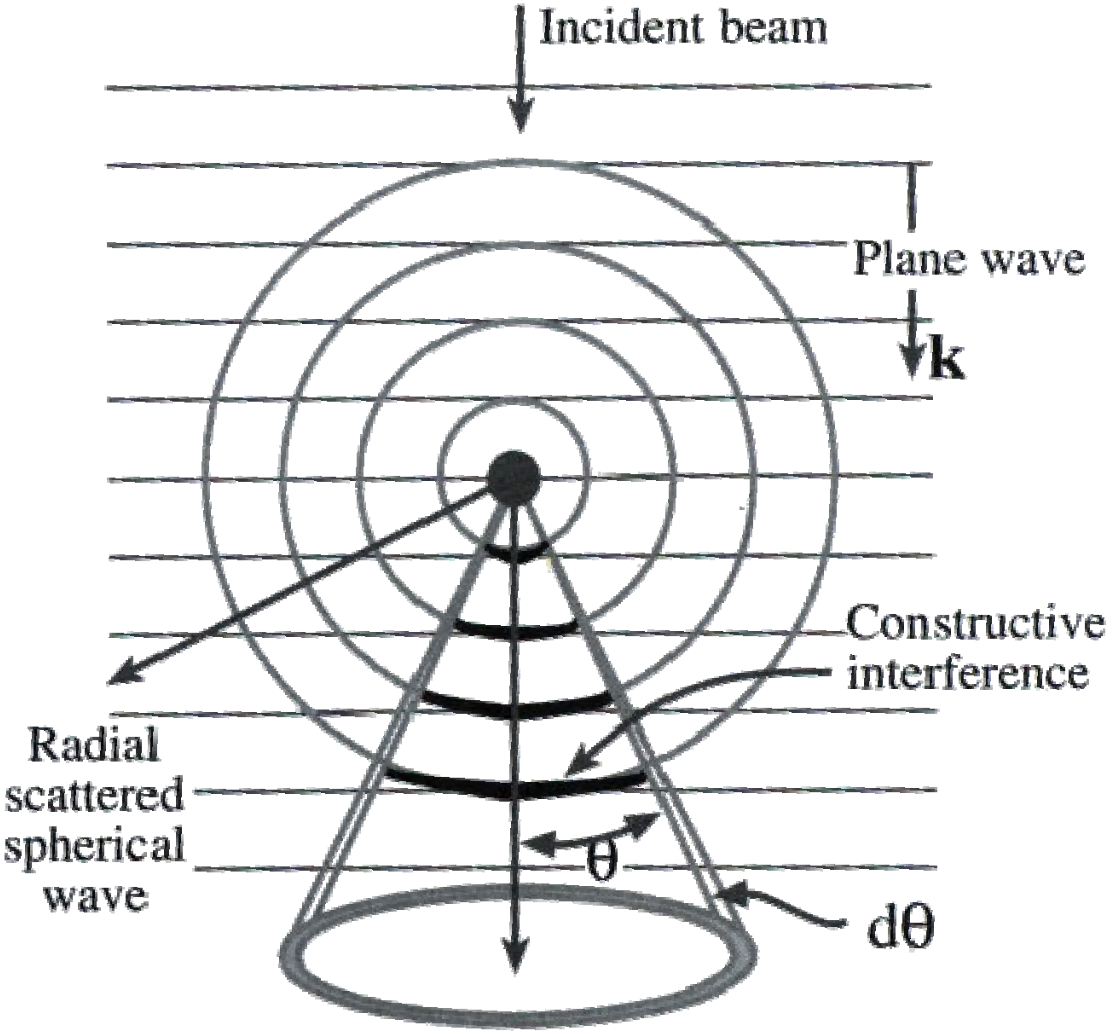


Figure 3.6. The generation of a scattered wave by the interaction of a plane wave (horizontal lines) with a point charge. The circles represent the scattered spherical wavefronts which are in phase (same λ). The in-phase constructive interference between the plane and spherical waves is shown by the dark arcs. The angles θ and *d*θ are the same as in Figure 2.3

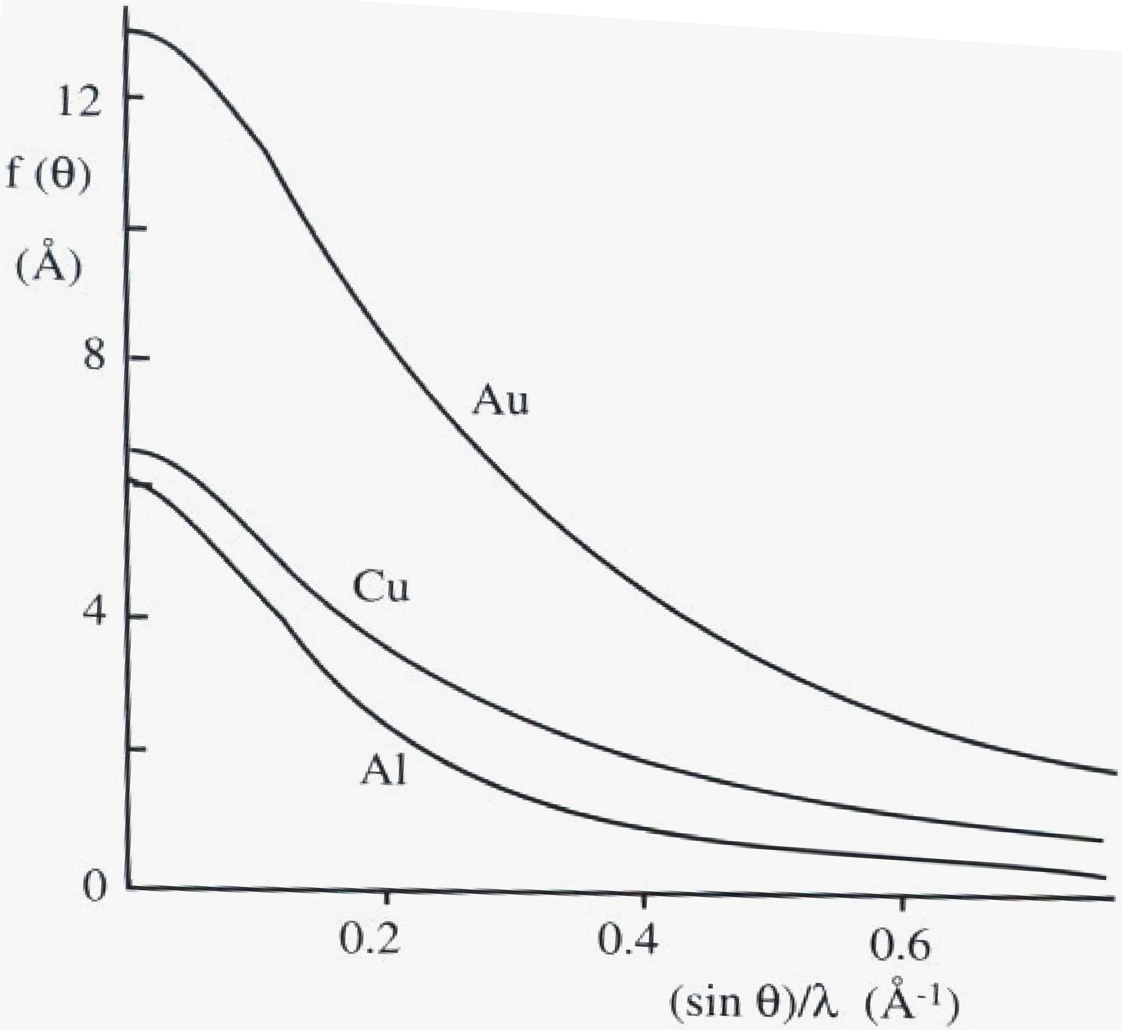
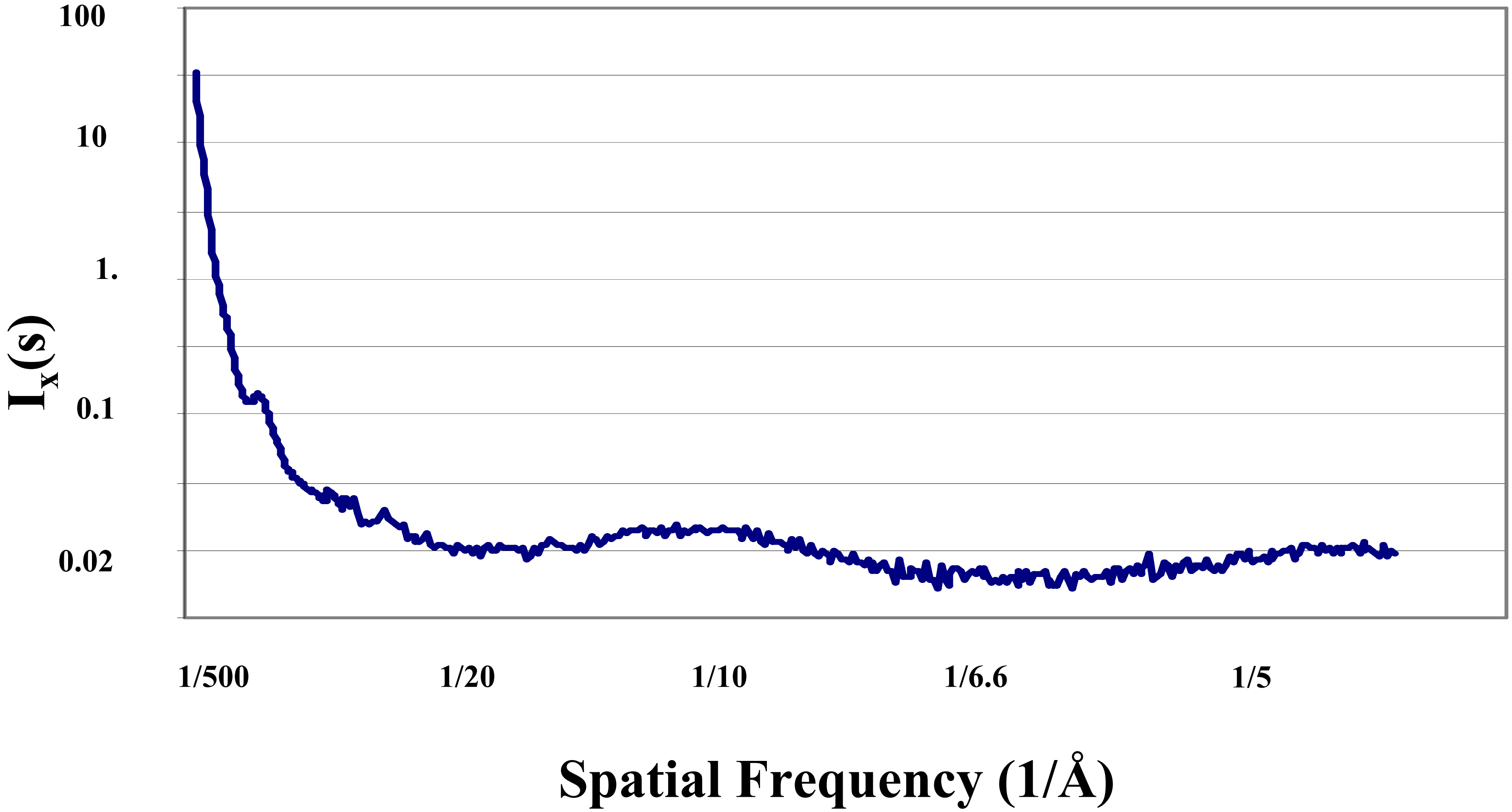


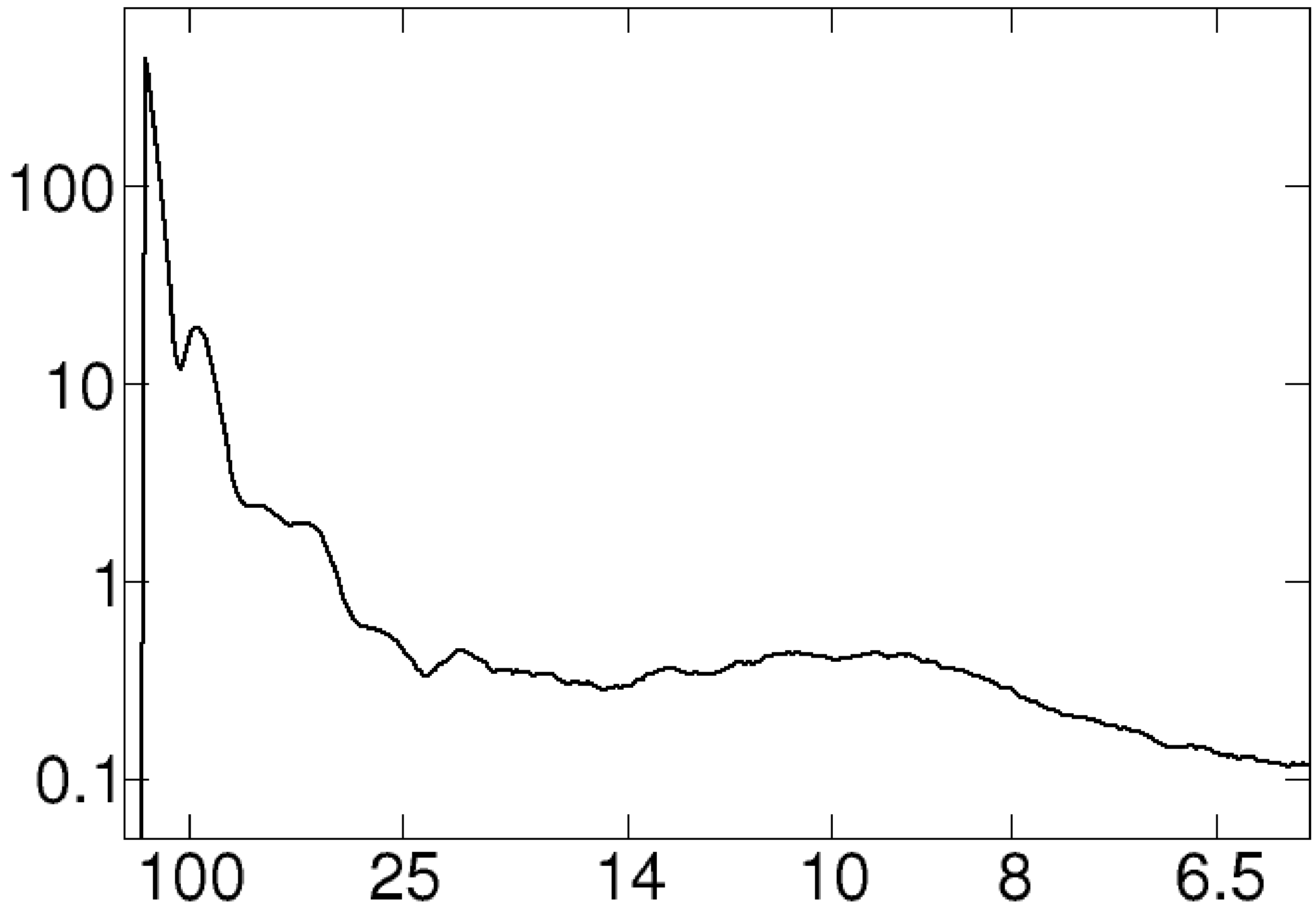
Figure 3.5. Change in the atomic scattering factor $f(\theta)$ with scattering angle θ (calculated from equation 3.10) showing that elastic scattering decreases with angle away from the incident beam direction (θ = 0°) and increases with Z.

X-ray Scattering Intensity of HSV-1 Capsids



Dr. Hiro Tsuruta at SLAC

X ray Scattering Intensity of GroEL



Resolution (Å)

Computed diffraction pattern

$$F^2(s) \quad CTF^2(s) \quad Env^2(s) \quad + \; N^2(s)$$

Structure factor

Envelope function

Background

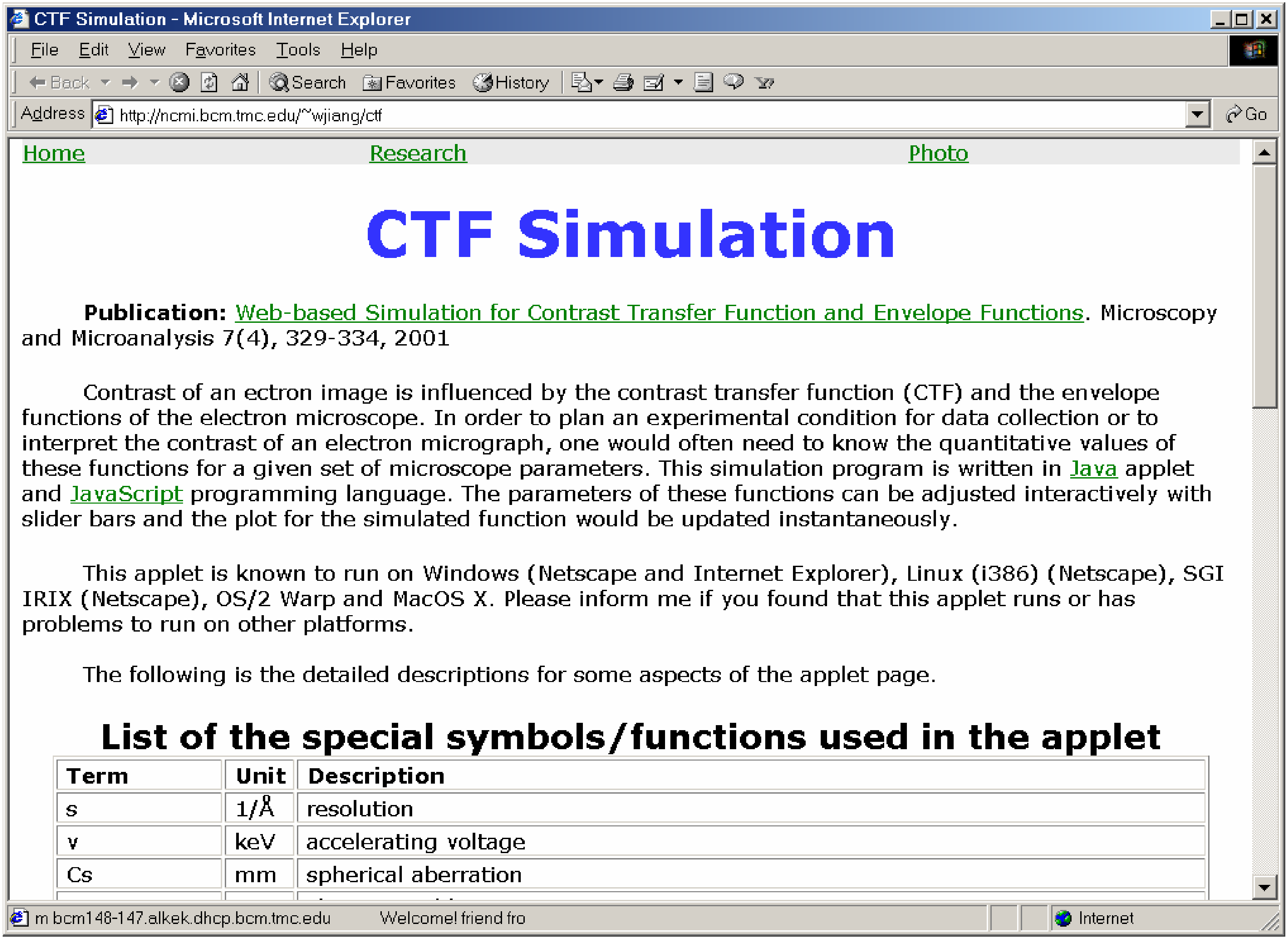
Contrast transfer function

Contrast Transfer Function

$$CTF(s) = -A\,[(1-Q^2)^{1/2}\sin(\gamma) + Q\cos(\gamma)]$$

$$\gamma(s) = -2\pi\,(C_s\lambda^3 s^4/4 - \Delta Z\,\lambda s^2/2)$$

ΔZ is vector dependent if there is an astigmatism



CTF Simulation

Publication: Web-based Simulation for Contrast Transfer Function and Envelope Functions. Microscopy and Microanalysis 7(4), 329-334, 2001

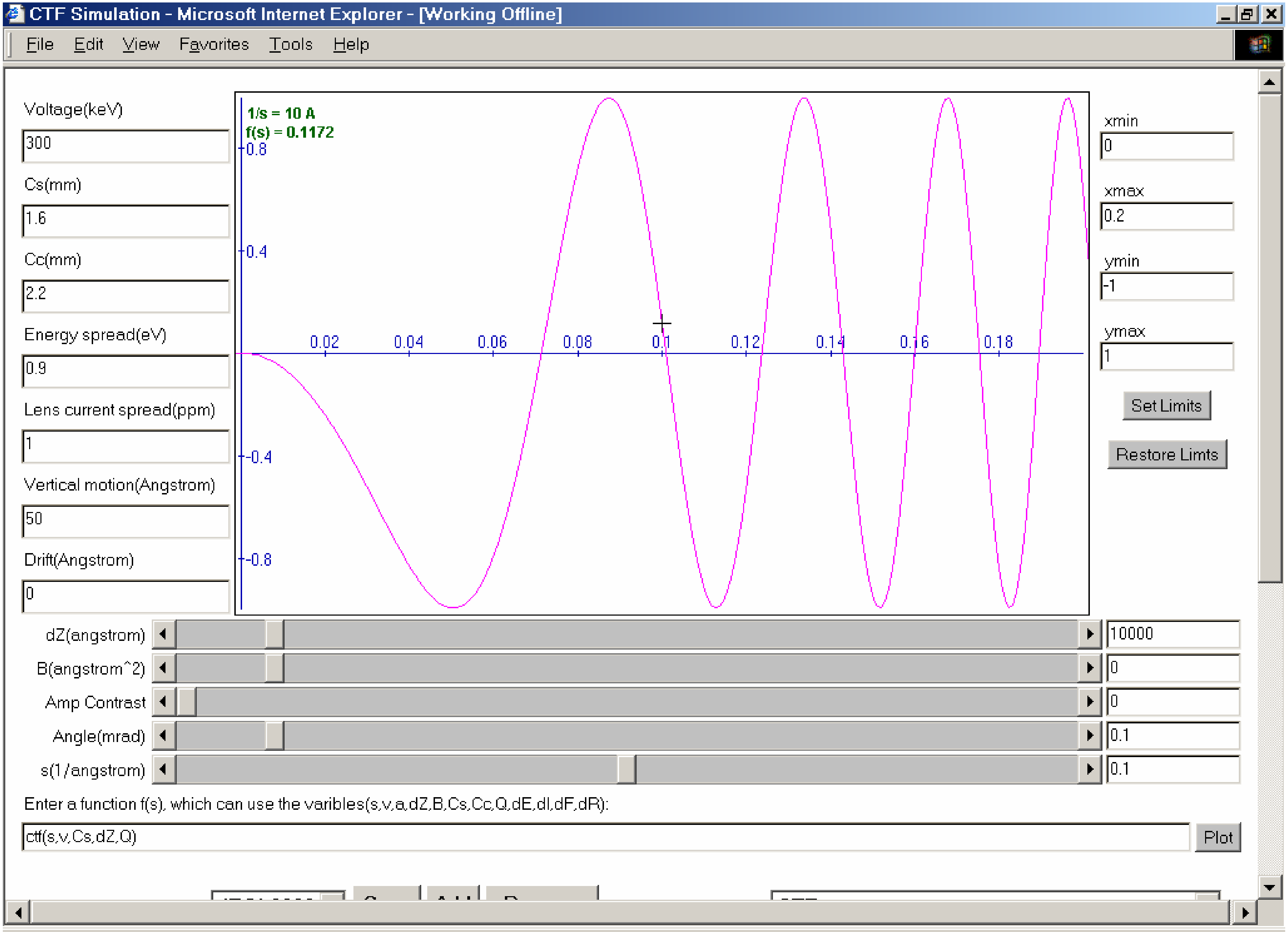
Contrast of an ectron image is influenced by the contrast transfer function (CTF) and the envelope functions of the electron microscope. In order to plan an experimental condition for data collection or to interpret the contrast of an electron micrograph, one would often need to know the quantitative values of these functions for a given set of microscope parameters. This simulation program is written in Java applet and JavaScript programming language. The parameters of these functions can be adjusted interactively with slider bars and the plot for the simulated function would be updated instantaneously.

This applet is known to run on Windows (Netscape and Internet Explorer), Linux (i386) (Netscape), SGI IRIX (Netscape), OS/2 Warp and MacOS X. Please inform me if you found that this applet runs or has problems to run on other platforms.

The following is the detailed descriptions for some aspects of the applet page.

List of the special symbols/functions used in the applet

Term	Unit	Description
s	1/Å	resolution
v	keV	accelerating voltage
Cs	mm	spherical aberration



Voltage(keV) 300

Cs(mm) 1.6

Cc(mm) 2.2

Energy spread(eV) 0.9

Lens current spread(ppm) 1

Vertical motion(Angstrom) 50

Drift(Angstrom) 0

xmin 0

xmax 0.2

ymin -1

ymax 1

dZ(angstrom) 10000

B(angstrom^2) 0

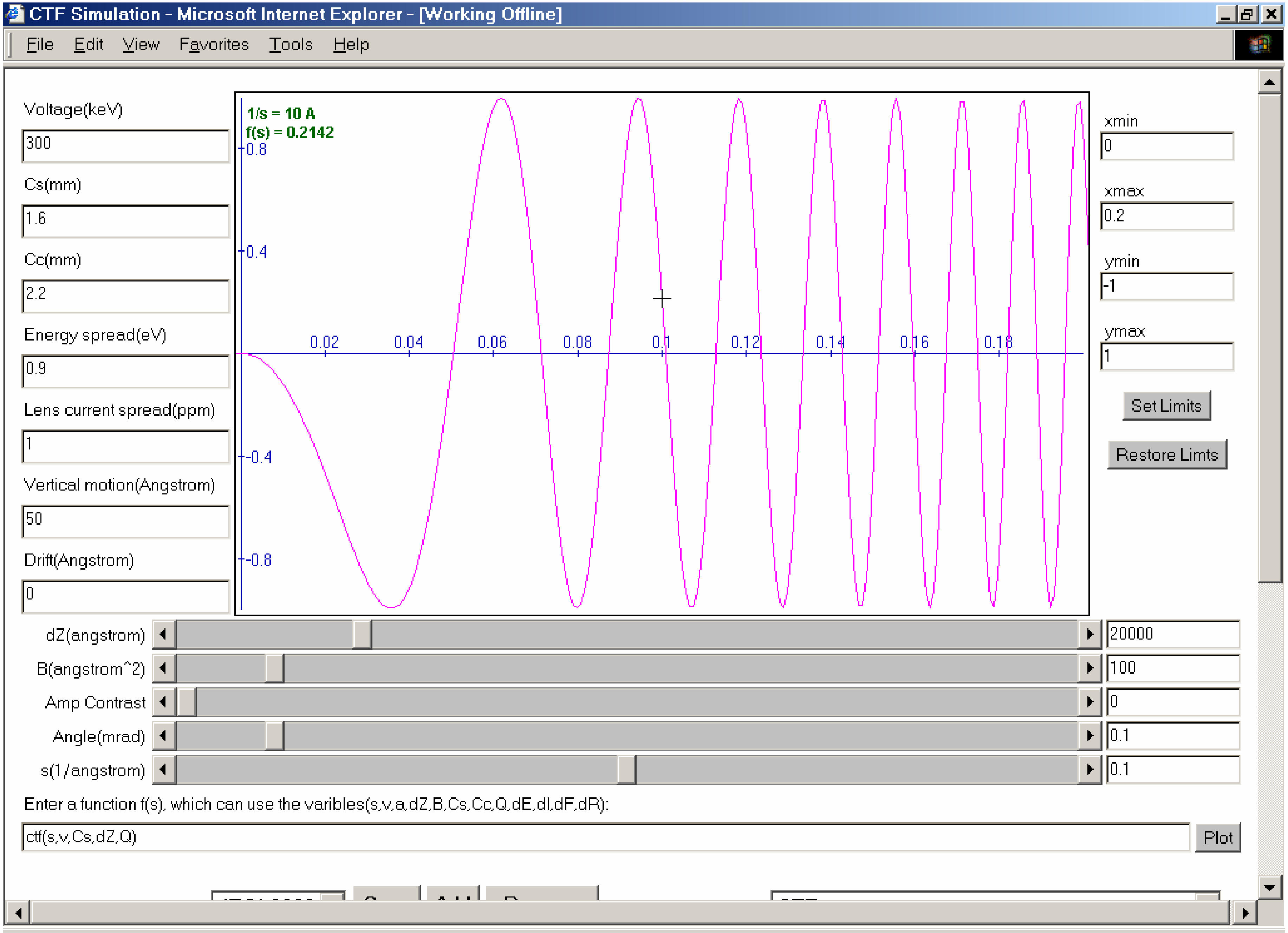
Amp Contrast 0

Angle(mrad) 0.1

s(1/angstrom) 0.1

Enter a function f(s), which can use the varibles(s,v,a,dZ,B,Cs,Cc,Q,dE,dI,dF,dR):

ctf(s,v,Cs,dZ,Q)



Voltage(keV) 300

Cs(mm) 1.6

Cc(mm) 2.2

Energy spread(eV) 0.9

Lens current spread(ppm) 1

Vertical motion(Angstrom) 50

Drift(Angstrom) 0

xmin 0

xmax 0.2

ymin -1

ymax 1

dZ(angstrom) 20000

B(angstrom^2) 100

Amp Contrast 0

Angle(mrad) 0.1

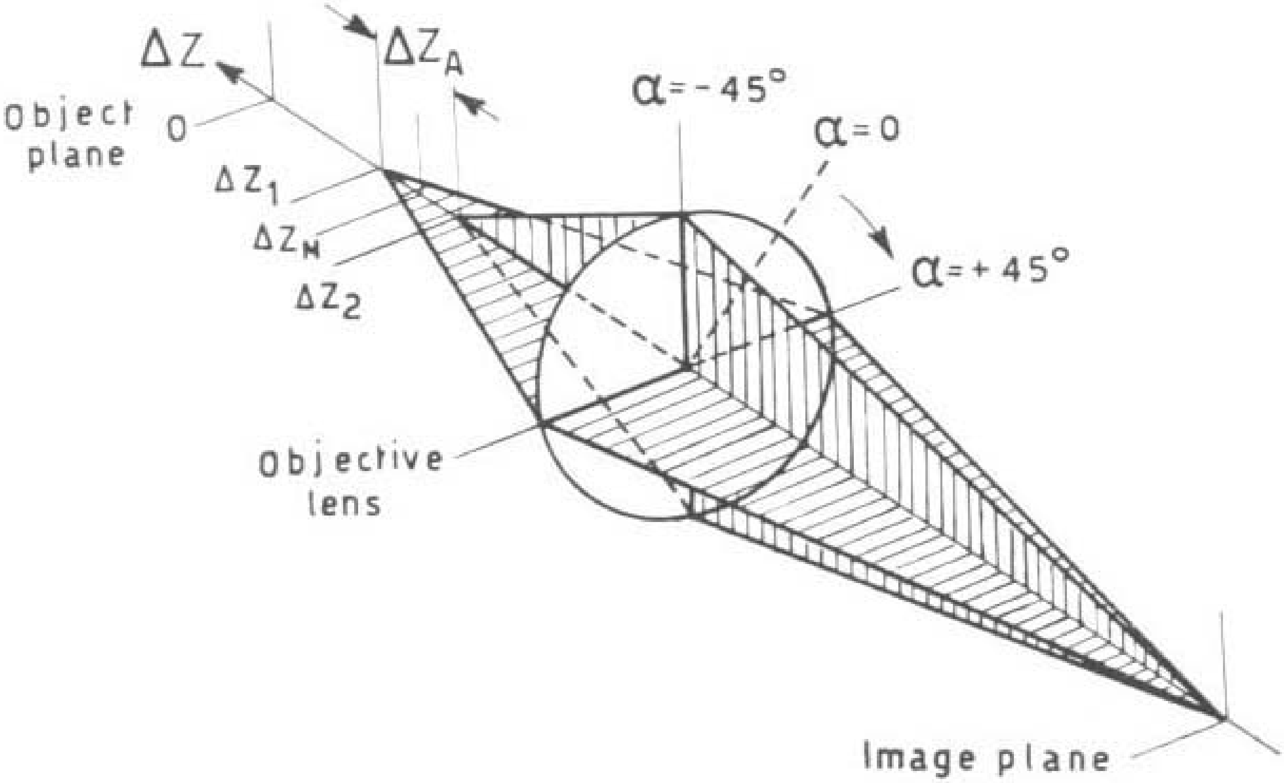
s(1/angstrom) 0.1

Enter a function f(s), which can use the varibles(s,v,a,dZ,B,Cs,Cc,Q,dE,dI,dF,dR):

ctf(s,v,Cs,dZ,Q)

Jiang & Chiu *Microsc. and Microanal.* **7**:329-334 (2001)

Astigmatism



From F. Thon

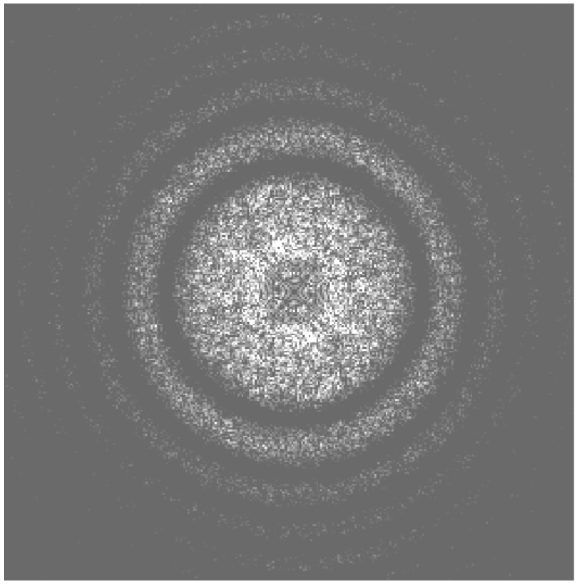
Astigmatism

$$\Delta Z_{eff}(\alpha) = \Delta Z_m + (\Delta Z_a \sin 2\alpha)/2$$

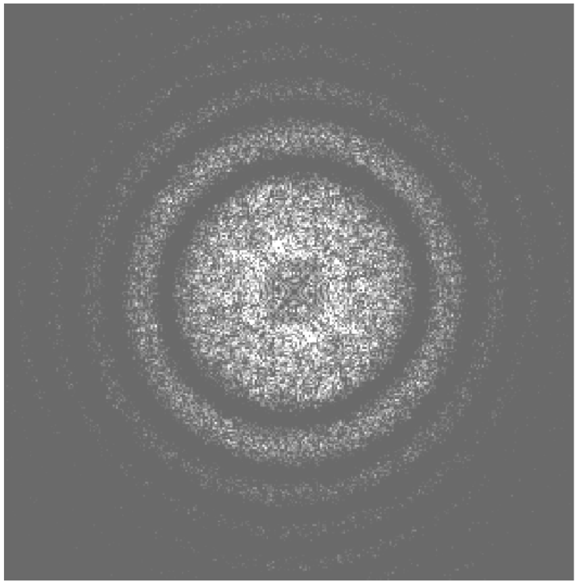
$$\Delta Z_m = (\Delta Z_1 + \Delta Z_2)/2$$

$$\Delta Z_a = \Delta Z_1 - \Delta Z_2$$

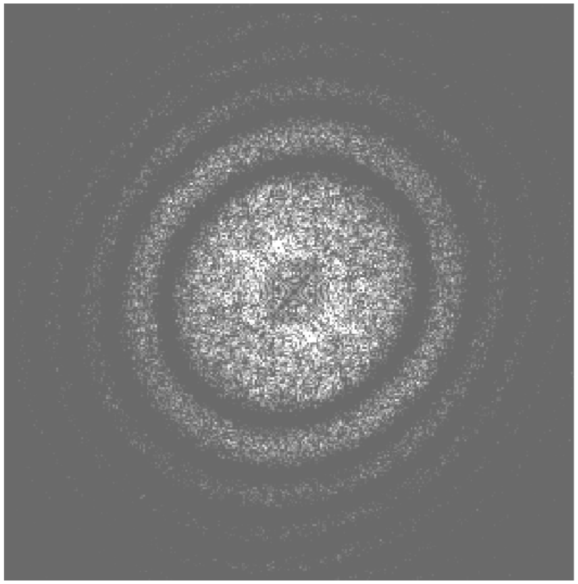
Synthetic Power Spectrum $\Delta Z = 0.8\mu m$



Astigmatism amplitude = $0.0\mu m$



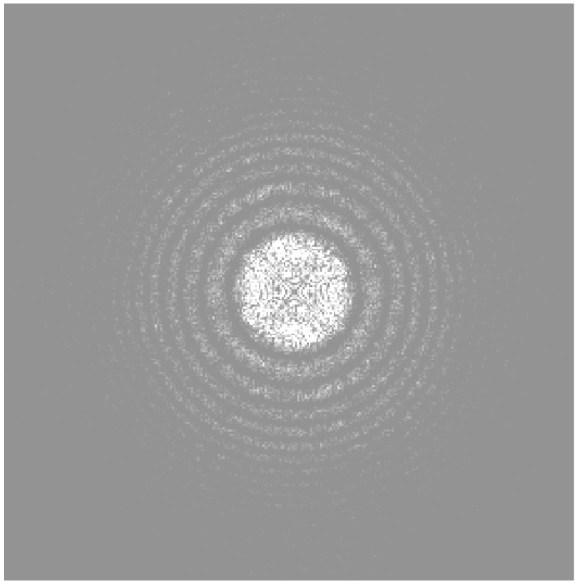
Astigmatism amplitude = $0.0267\mu m$



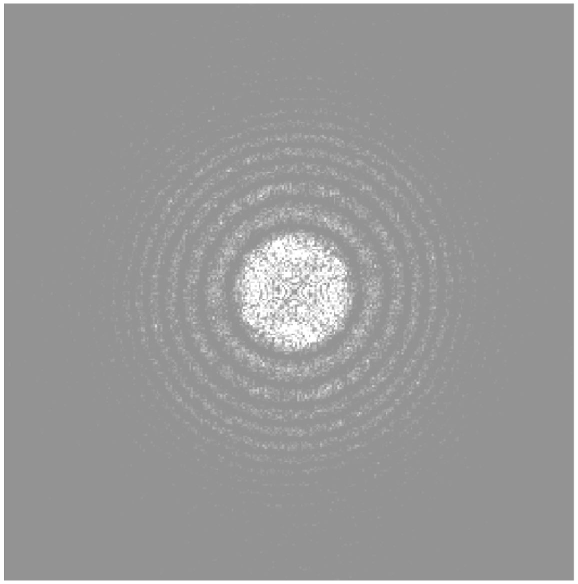
Astigmatism amplitude = $0.1\mu m$

From Wen Jiang

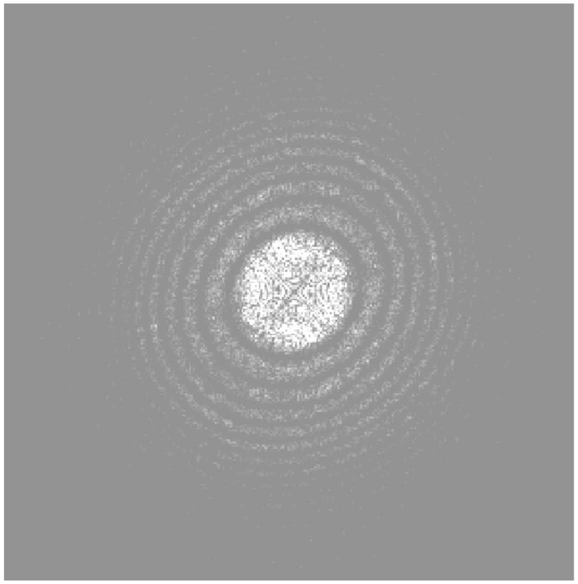
Synthetic Power Spectrum ΔZ = 3 μm



Astigmatism
amplitude =
0.0μm



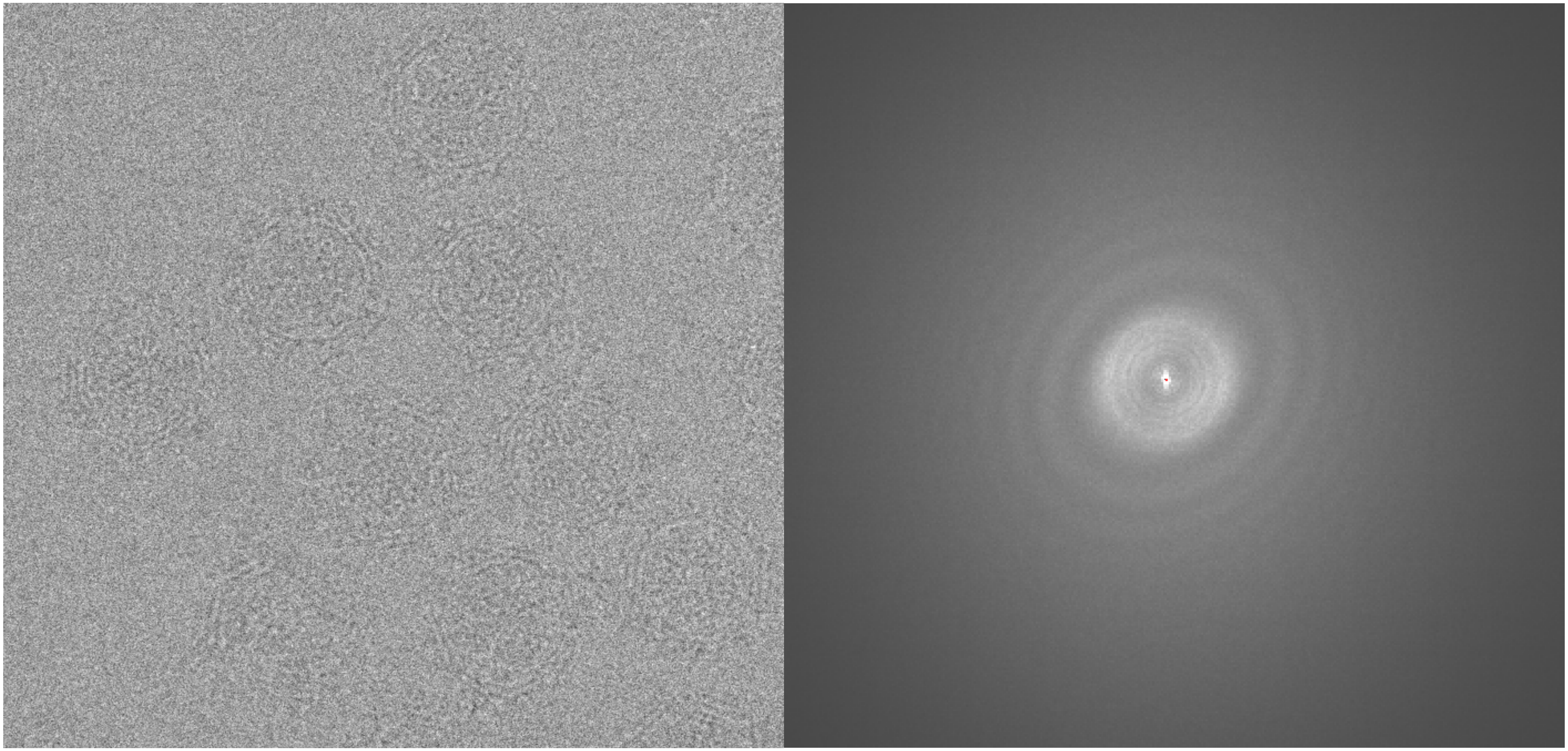
Astigmatism
amplitude =
0.1μm



Astigmatism
amplitude =
0.375μm

From Wen Jiang

Astigmatism in Single Particle Image



From Dr. Angel Paredes

Computed diffraction pattern

$$F^2(s)\ \ CTF^2(s)\ \ Env^2(s)\ \ +\ N^2(s)$$

Structure factor

Contrast transfer function

Envelope function

Background

EM Envelope Functions : Env(s)

Gaussian type source:

$$G_{sc}(s) = \exp[-\pi^2 \alpha^2 (C_S \lambda^2 s^3 - \Delta Z s)^2]$$

Gaussian type fluctuation:

$$G_{tc}(s) = \exp[-\frac{\pi^2}{16 \ln 2} C_C^2 \lambda^2 (\frac{\Delta E}{E})^2 s^4]$$

Gaussian type fluctuation:

$$G_{ol}(s) = \exp[-\frac{\pi^2}{4 \ln 2} C_C^2 \lambda^2 (\frac{\Delta I}{I})^2 s^4]$$

Sinusoidal type fluctuation:

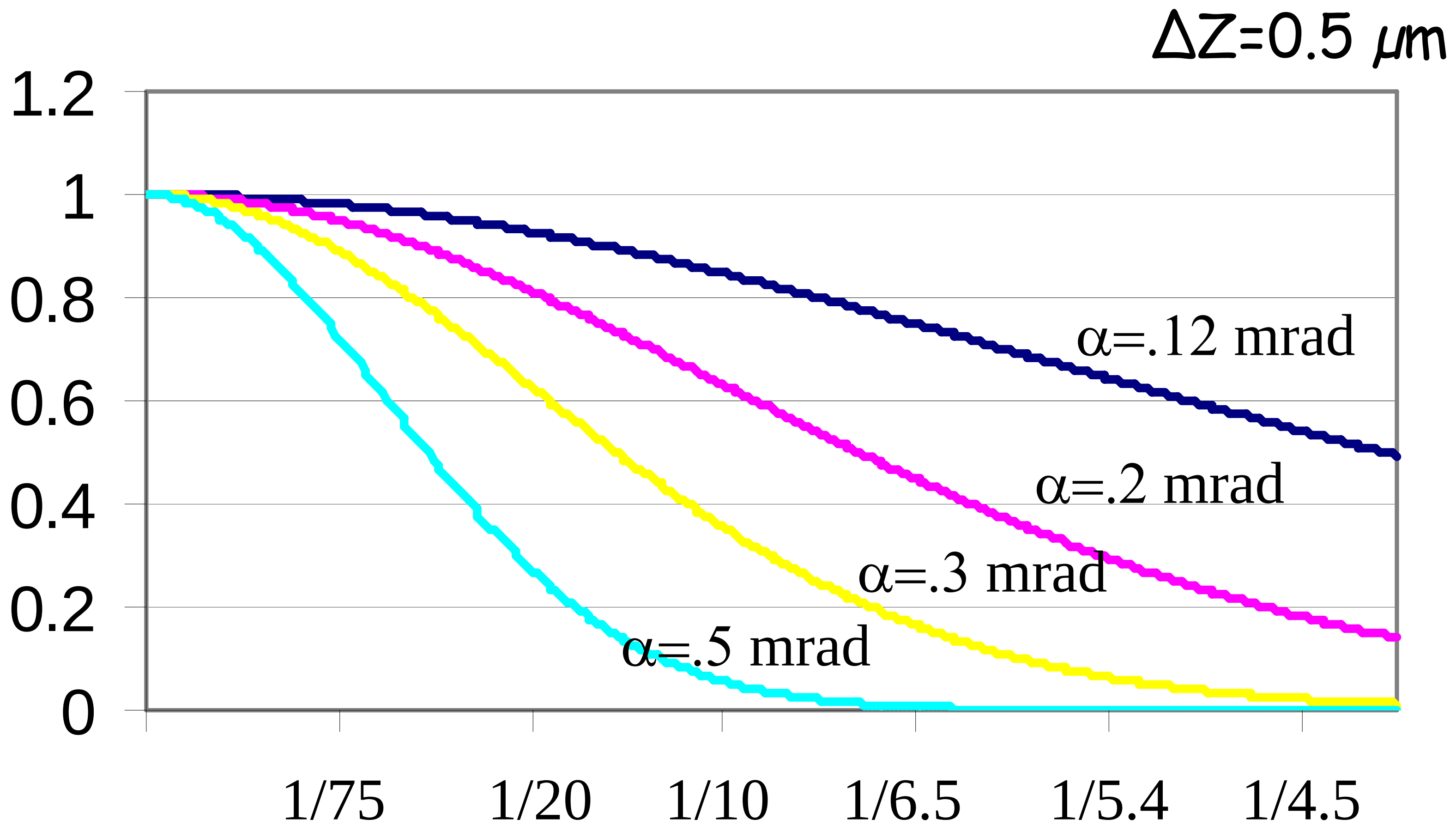
$$G_{lm}(s) = J_O(\pi \Delta f \lambda s^2)$$

Drift:

$$G_{tm}(s) = \frac{\sin(\pi s \Delta r)}{\pi s \Delta r}$$

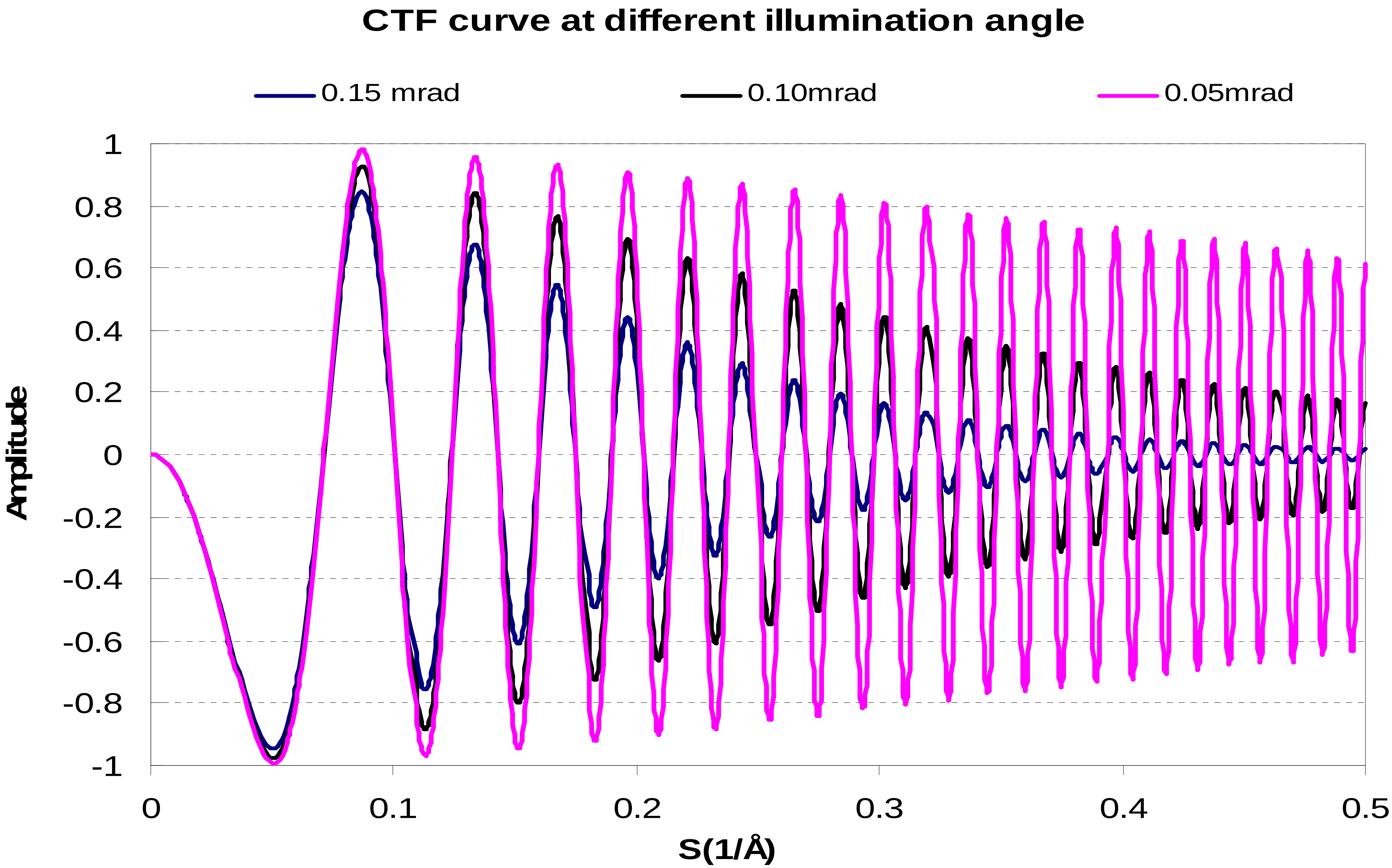
Chiu *Scanning Electron Microsc.* **1:**569-580 (1978)

Spatial coherence envelope function



Spatial Frequency (1/Å)

300 keV, Cs = 1.6 mm, defocus = 1 μM



EM Envelope Functions : Env(s)

Gaussian type source:

$$\mathrm{G}_{sc}(s) = \exp[-\pi^2\alpha^2(C_S\lambda^2 s^3 - \Delta Z s)^2]$$

Gaussian type fluctuation:

$$G_{tc}(s) = \exp[-\frac{\pi^2}{16\ln 2}C_C^2\lambda^2(\frac{\Delta E}{E})^2 s^4]$$

Gaussian type fluctuation:

$$G_{ol}(s) = \exp[-\frac{\pi^2}{4\ln 2}C_C^2\lambda^2(\frac{\Delta I}{I})^2 s^4]$$

Sinusoidal type fluctuation:

$$G_{lm}(s) = J_O(\pi\Delta f\lambda s^2)$$

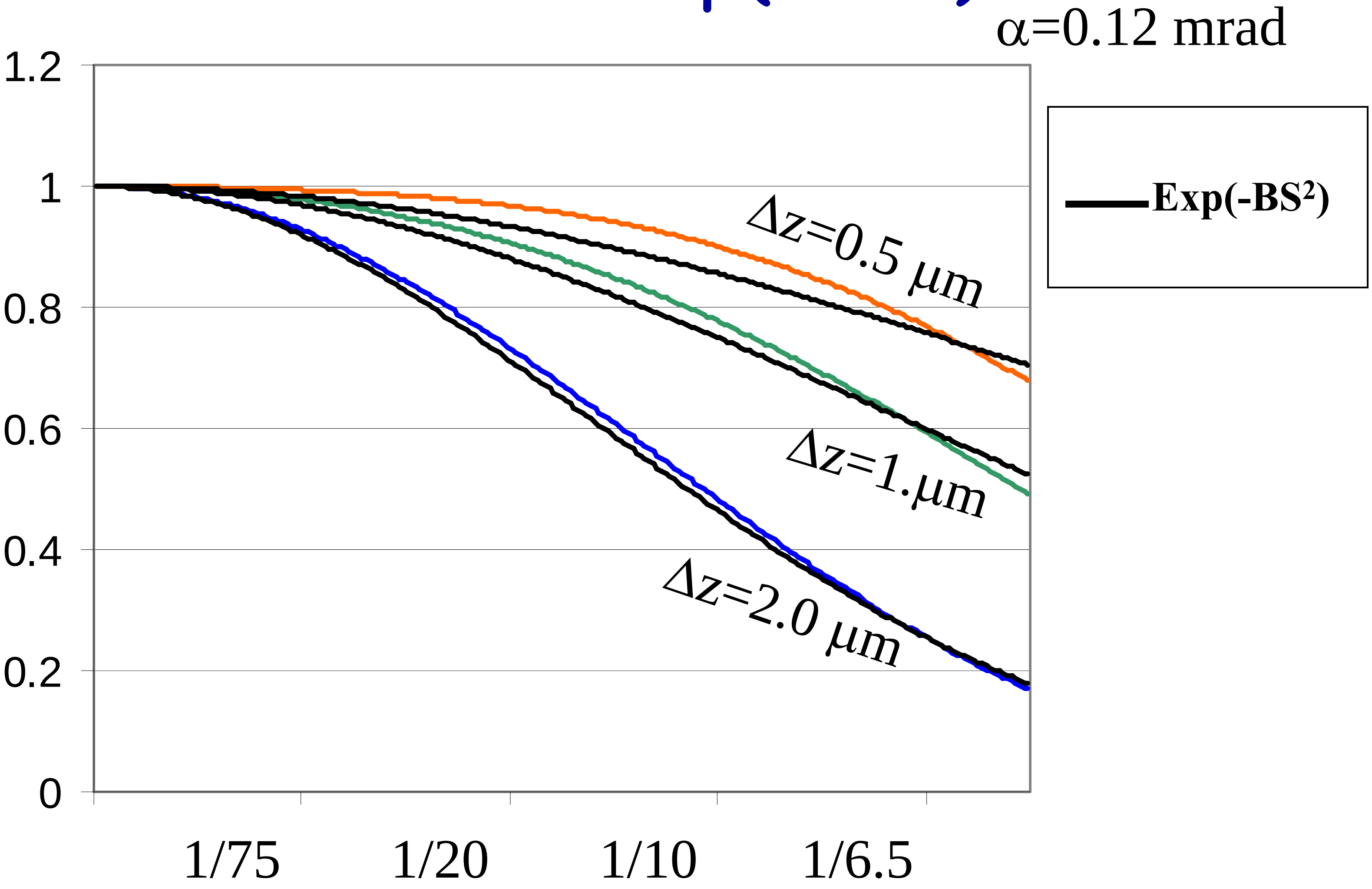
Drift:

$$G_{tm}(s) = \frac{\sin(\pi s\Delta r)}{\pi s\Delta r}$$

Gaussian Approximation for Cumulative Envelope Function

$\mathrm{Env}^2(s) \sim \exp(-2\mathbf{B}S^2)$

Fitting the spatial coherence envelope function with exp($-BS^2$)



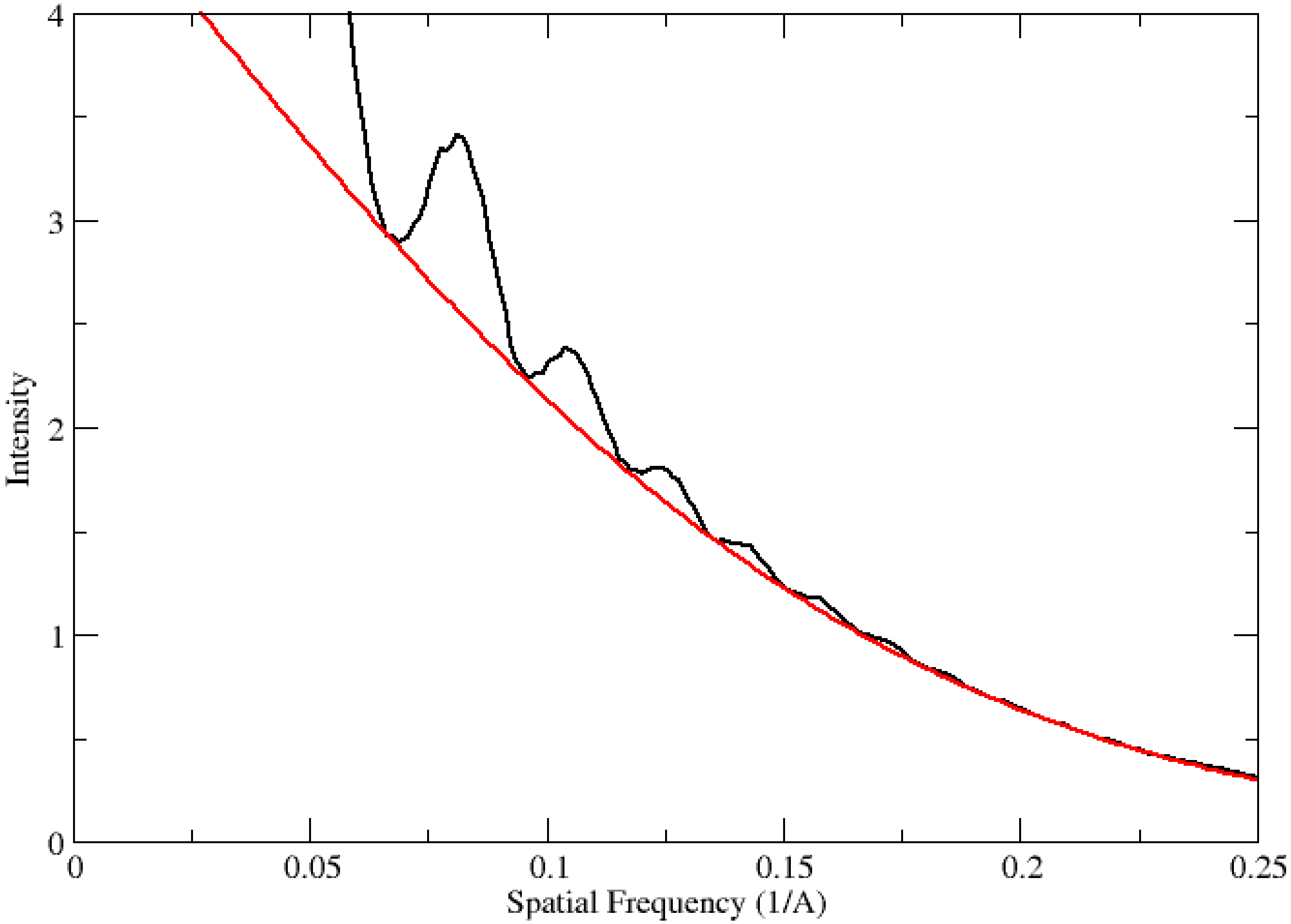
Spatial Frequency (1/Å)

Computed diffraction pattern

$$\underbrace{F^2(s)}_{\text{Structure factor}}\ \underbrace{CTF^2(s)}_{\text{Contrast transfer function}}\ \underbrace{Env^2(s)}_{\text{Envelope function}} + \underbrace{N^2(s)}_{\text{Background}}$$

Noise Function

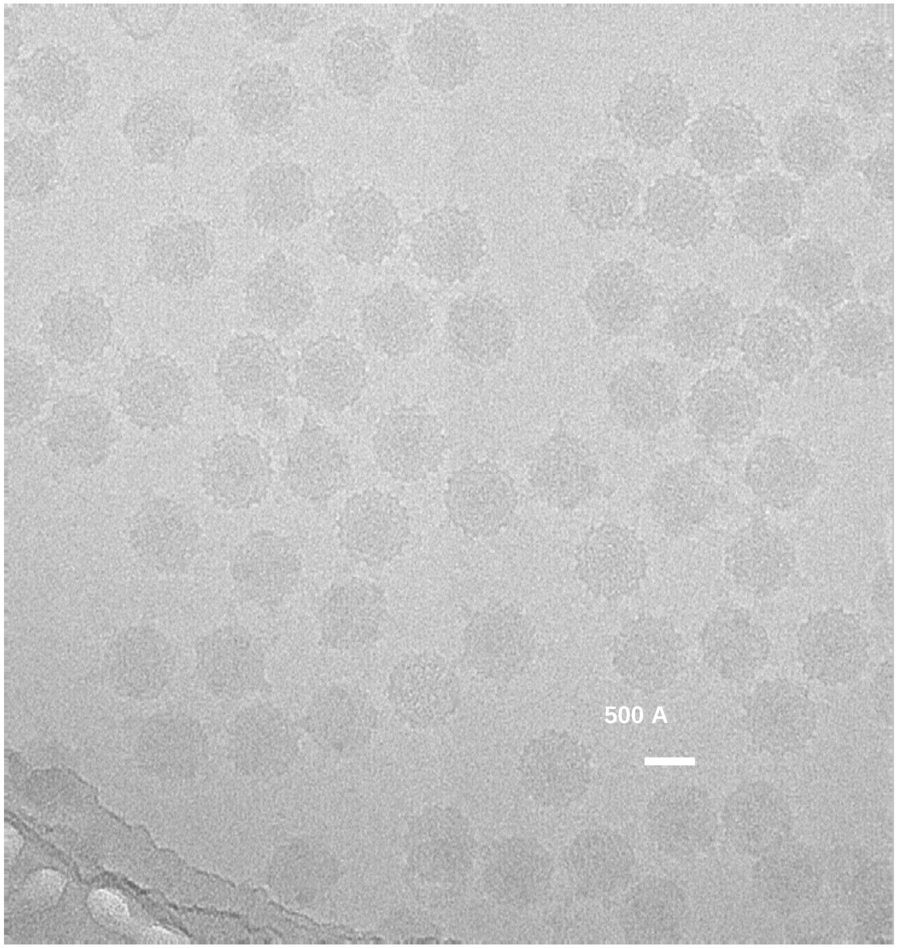
$$N^2\,(s) = n_1 \exp\,(n_2 s + n_3 s^2 + n_4 s^{1/2})$$

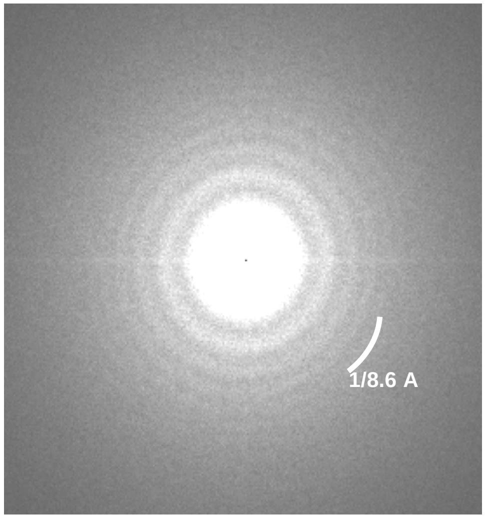


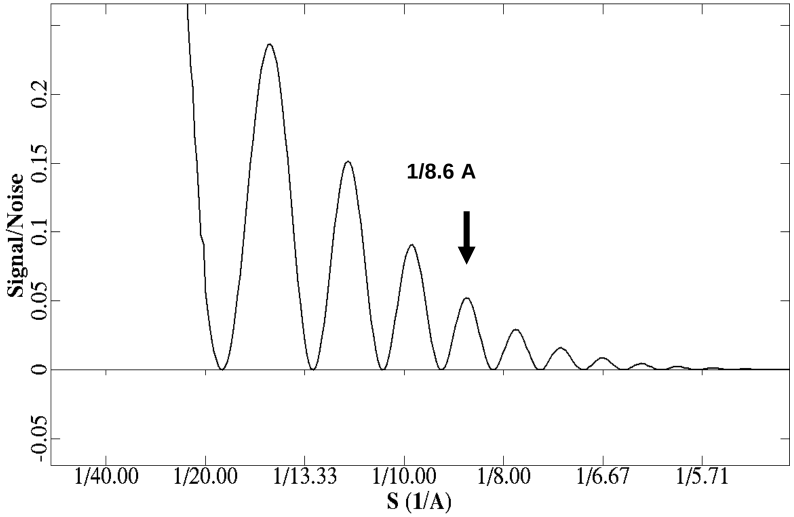
From Dr. Z. Li

$$\text{Contrast} = (F^2\ \ CTF^2\ \ E^2\,) \,/\, N^2$$

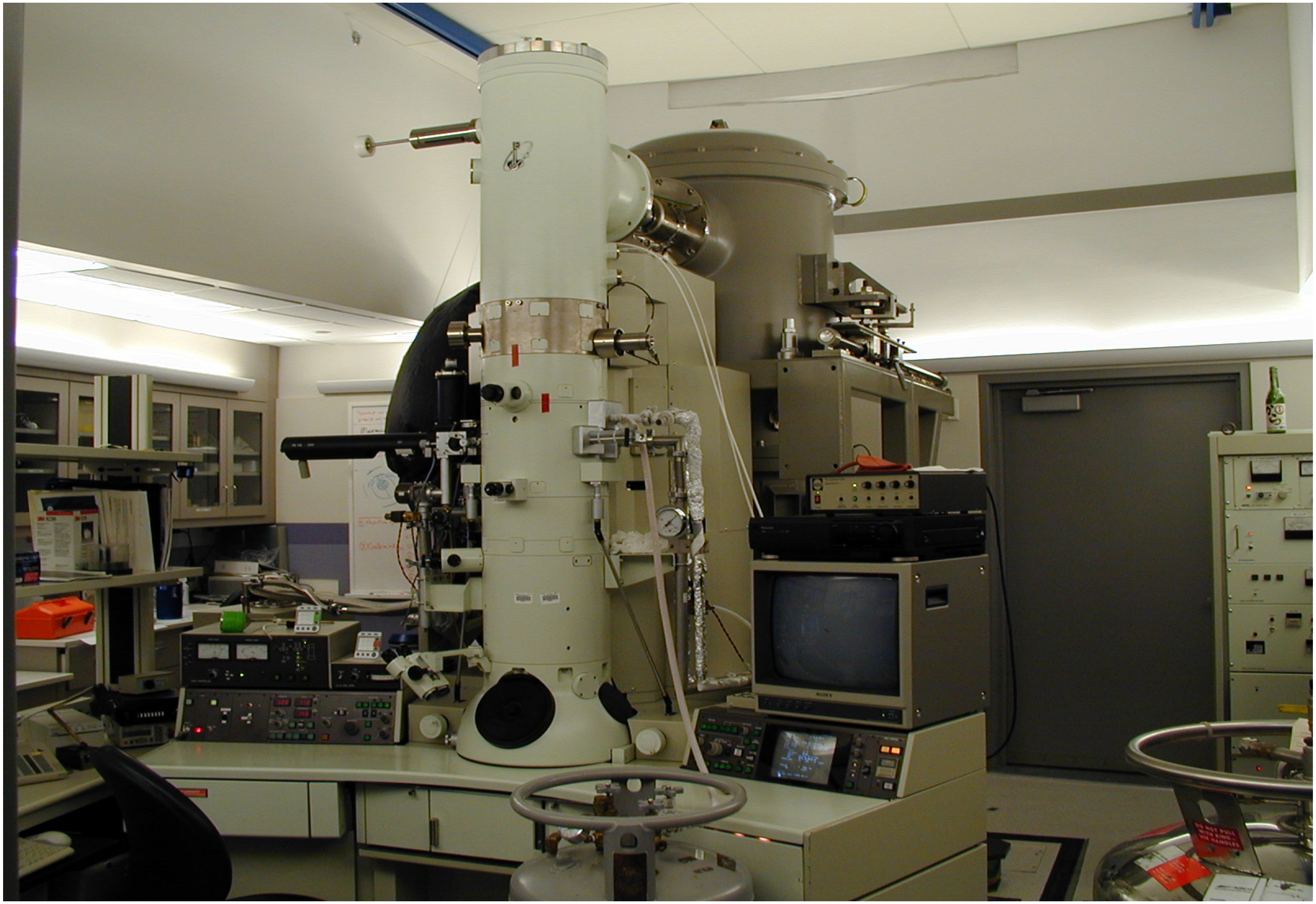
200kV Image and Power Spectrum of CPV





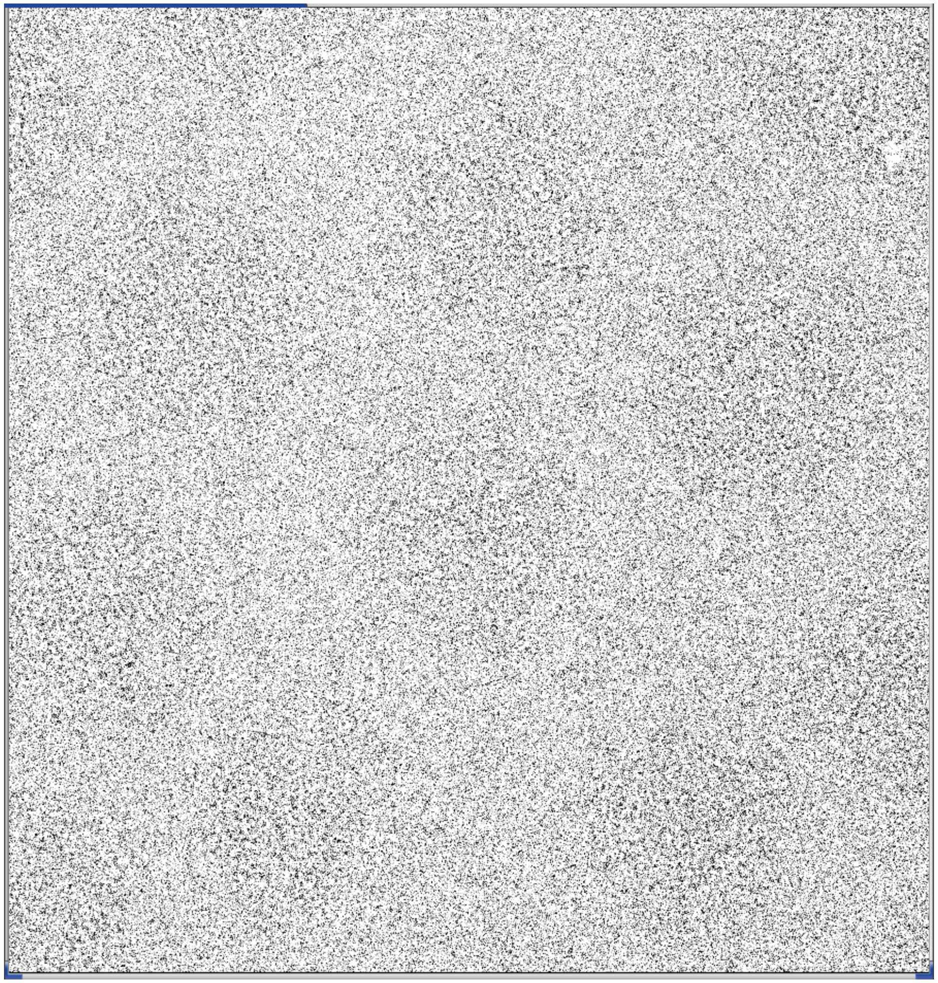


Booth JSB 2004

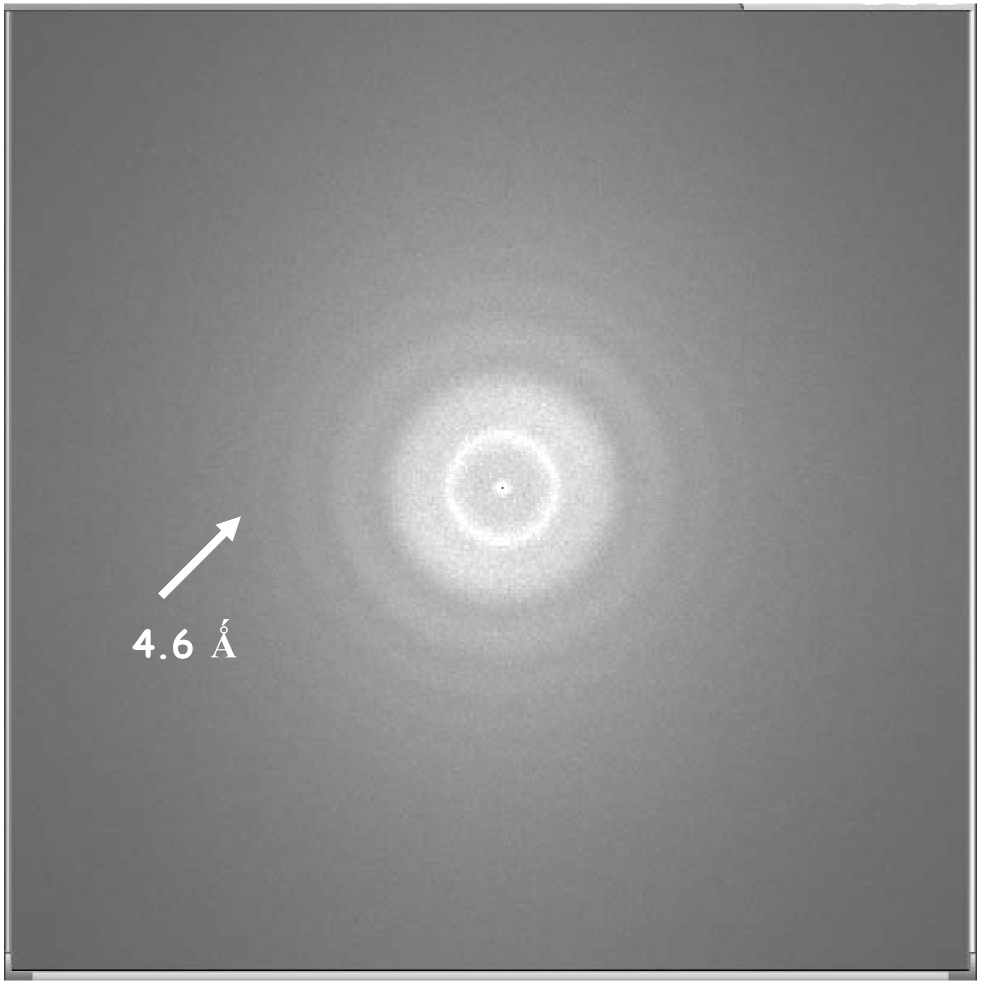


JEOL 3000SFF electron cryomicroscope at NCMI
equipped with liquid helium stage and field emission gun

300kV Image

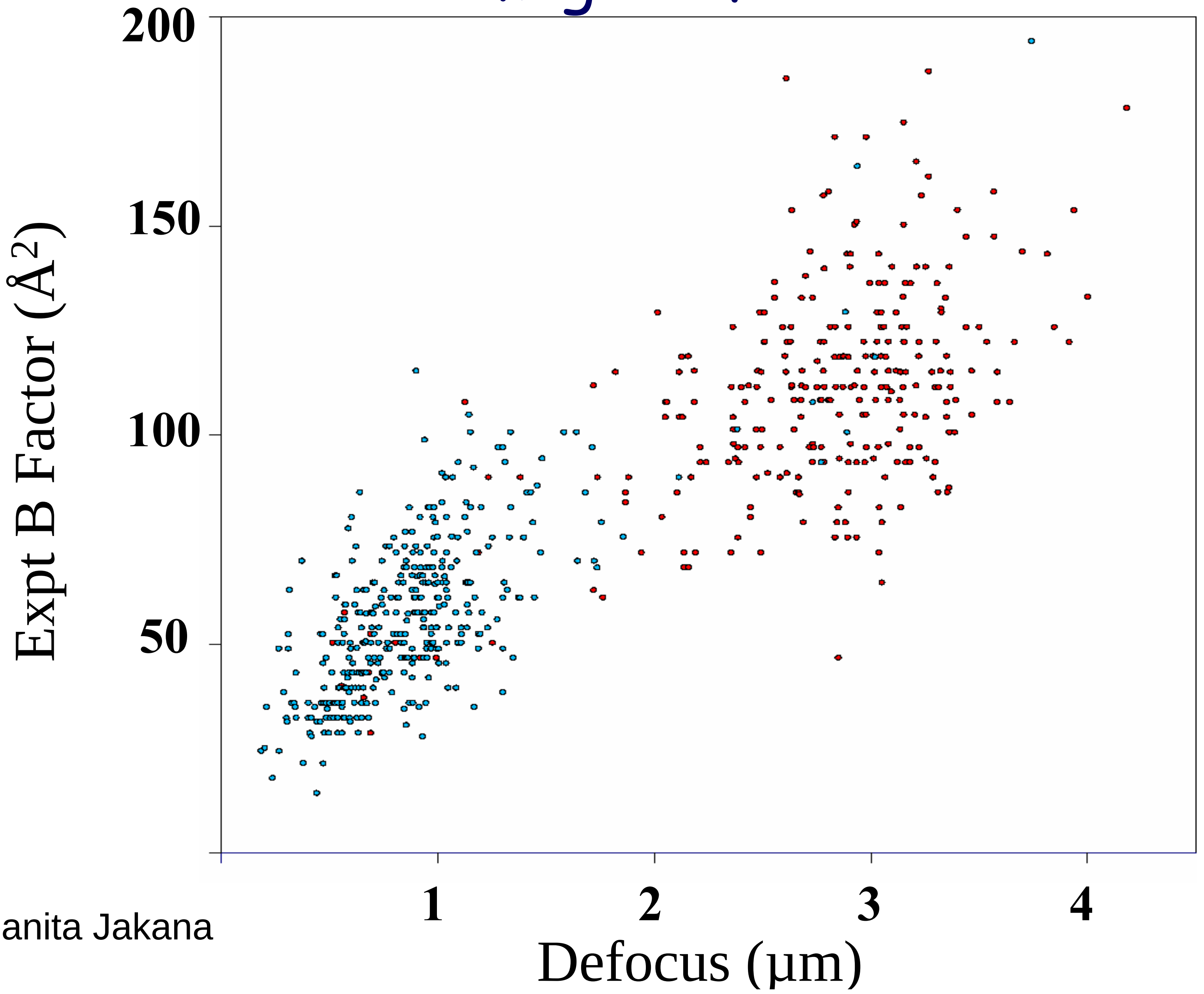


Power Spectrum



156 particles, $\Delta Z = 0.60\ \mu m$
Joanita Jakana, MSA Proceeding, 2004

Experimental B factor vs defocus for 300 kV Images of CPV

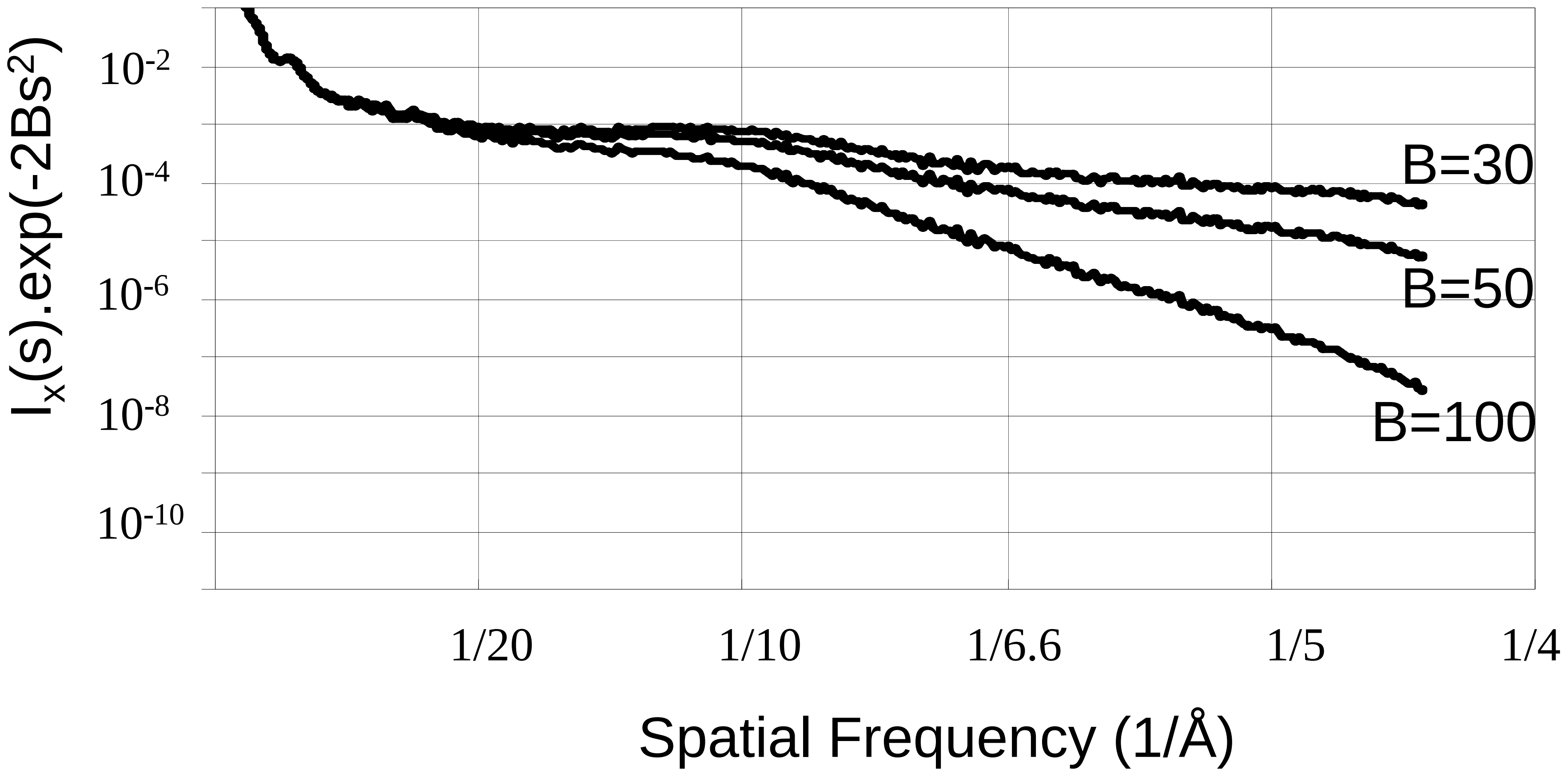


Joanita Jakana

Number of particles

required for a 3-D reconstruction is inversely proportional to

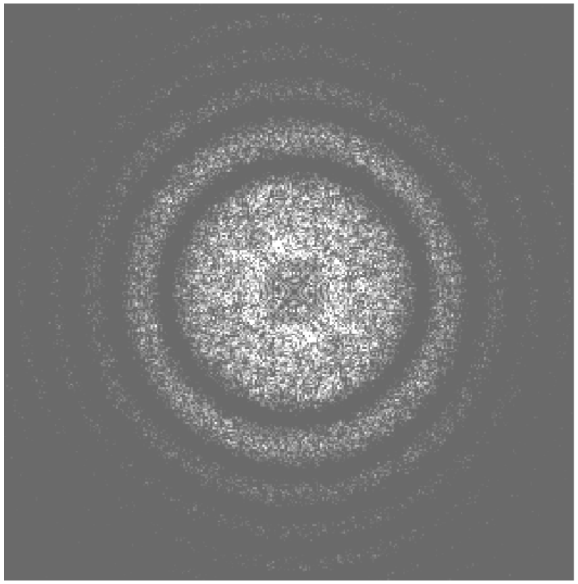
$$F^2(S)\exp(-2BS^2)$$


$I_x(s).\exp(-2Bs^2)$
10^{-2}
10^{-4}
10^{-6}
10^{-8}
10^{-10}
B=30
B=50
B=100
1/20
1/10
1/6.6
1/5
1/4
Spatial Frequency (1/Å)

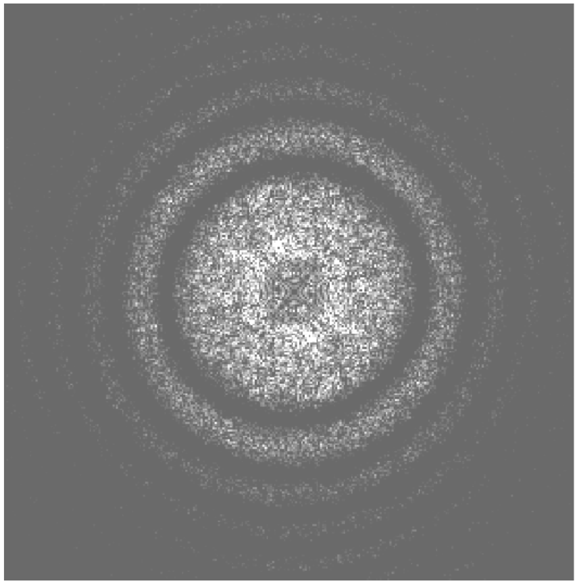
Causes of High B-Factor

- Large angle of illumination (defocus dependent)
- Astigmatism (defocus independent)
- Local specimen movement (defocus independent)
- Insufficient sampling (defocus independent)

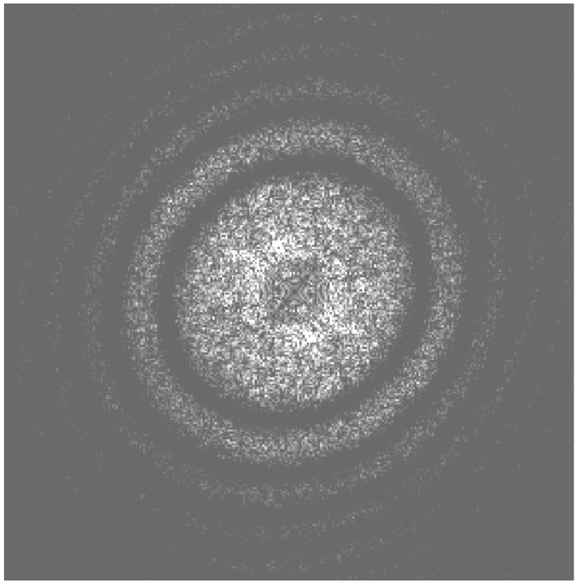
Synthetic Power Spectrum ΔZ = 0.8μm



Astigmatism amplitude = 0.0μm



Astigmatism amplitude = 0.0267μm

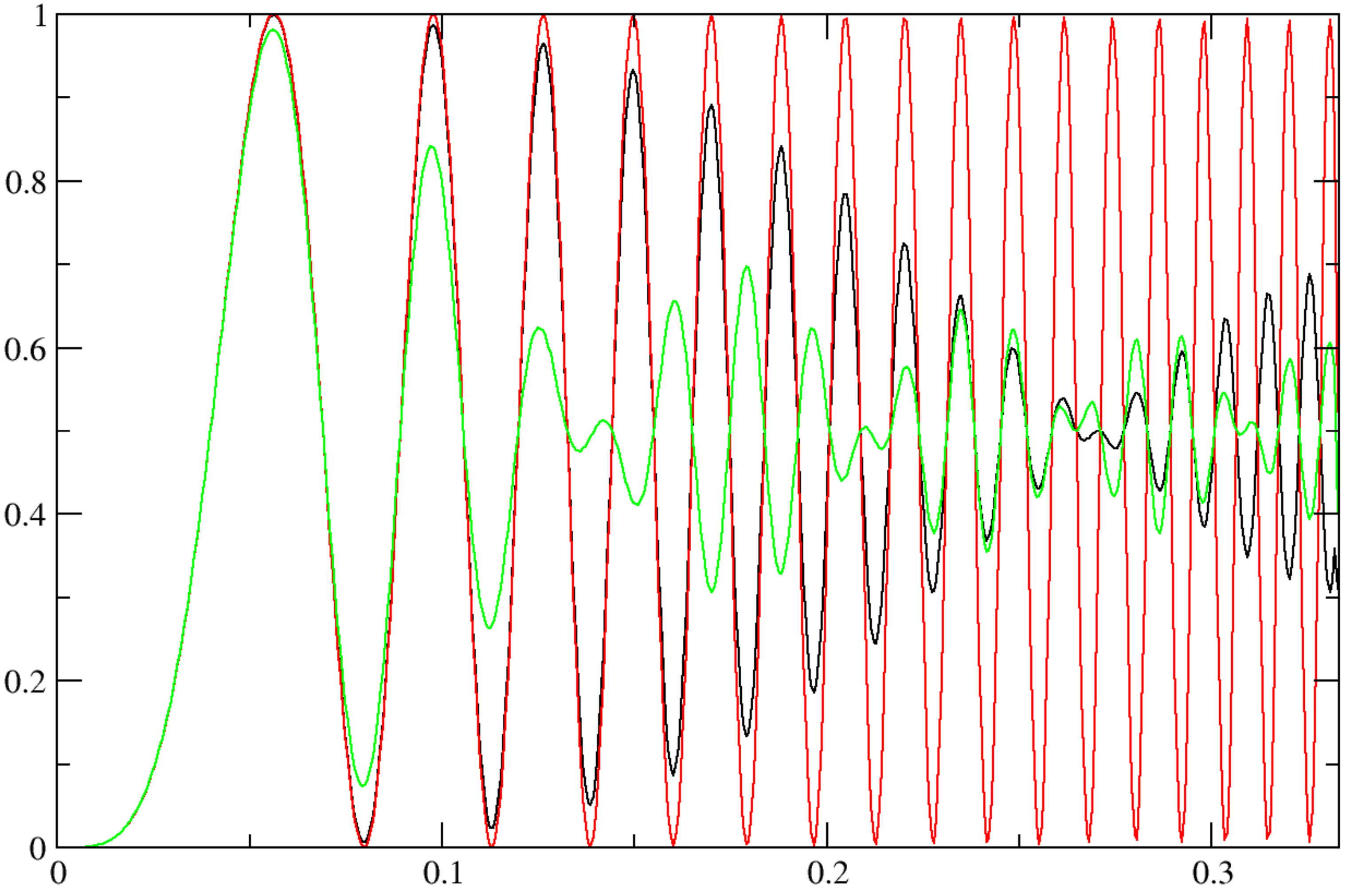


Astigmatism amplitude = 0.1μm

From Wen Jiang

300kV, Cs=1.6mm

ΔZ=0.8 μm   astigmatism = 0.0 μm, 0.0267 μm, 0.1μm



Sampling

- Sampling distance in real space :

  $\Delta x = \frac{1}{2} - 1/3$ expected resolution

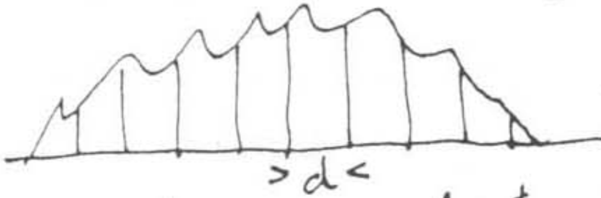
- Sampling distance in Fourier space

  $\Delta S = 1/(N\ \Delta x)$

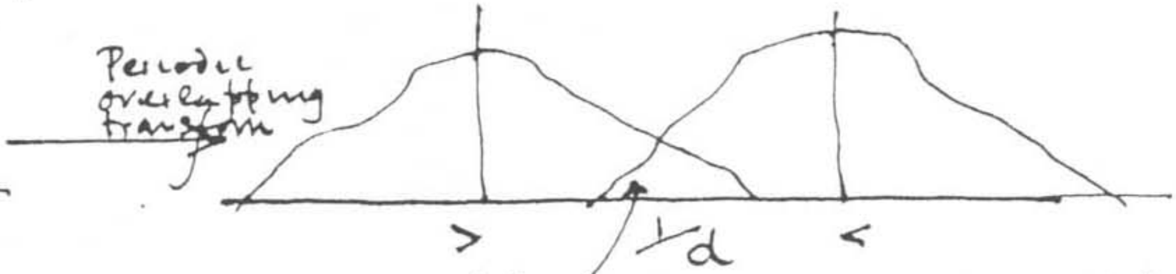
- Choice of sampling (Δx) and box size (N) depends on expected resolution and the defocus used

Shannon - Nyquist sampling and aliasing.

Hence if object density contains fine details out to a spatial frequency $\frac{1}{2d}$ (ie spatial period 2d) then sampling must not be coarser than d, otherwise neighbouring copies of the periodic transform begin to overlap. If the overlap were severe, high frequency terms from one copy of the transform overlap and contaminate low frequencies of the next. ie high frequencies would masquerade as low ones. This is known as <u>aliasing</u>.



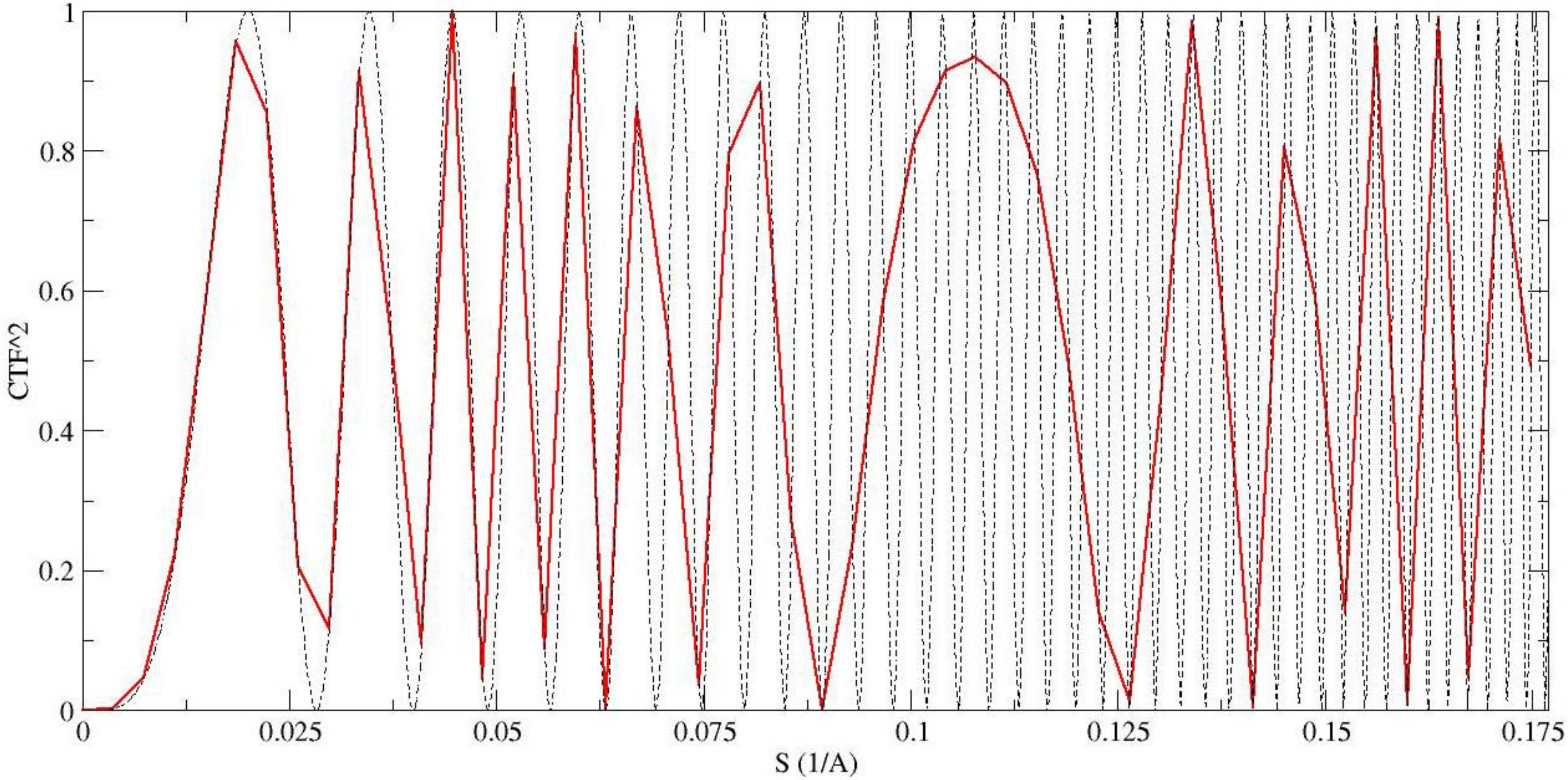
Coarsely sampled object



hi frequency components overlap lower frequency components.

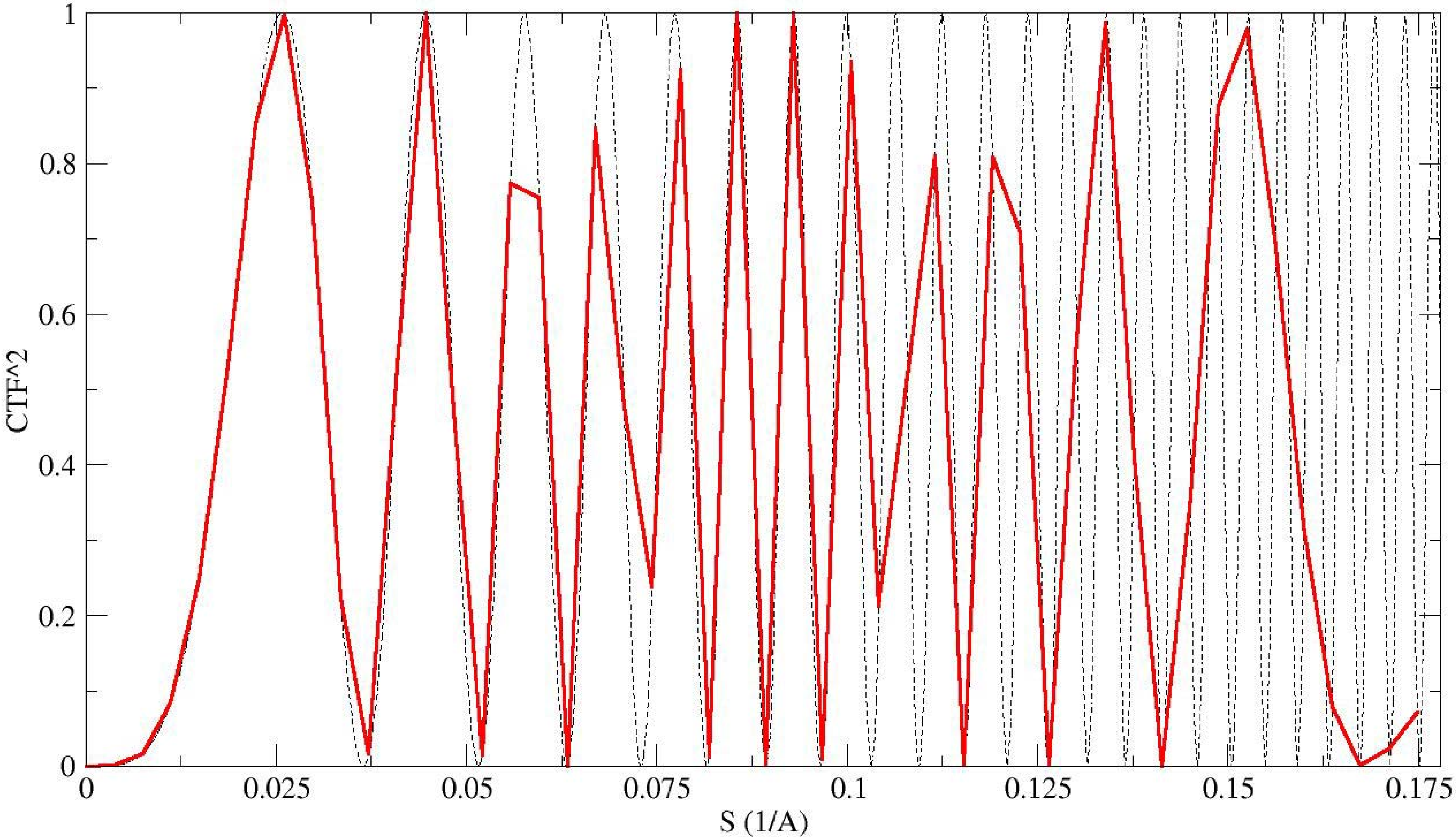
From Tony Crowther, MRC

Effect of Box Size on CTF Appearance



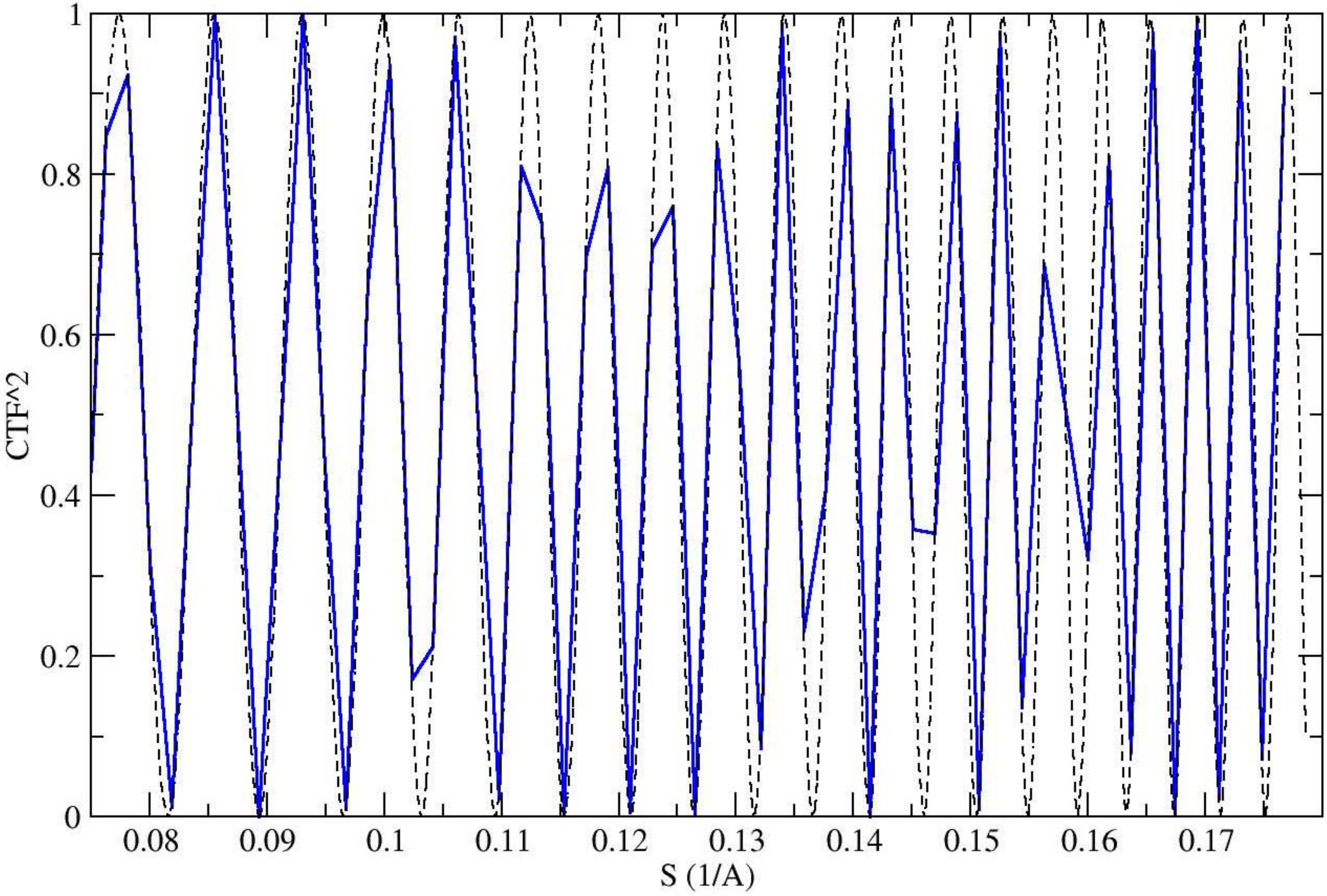
200 kV, *Cs* = 1.2mm, 2.8 *A*/pixel. 96x96 pixels box, ΔZ=5 μm

Effect of Box Size on CTF Appearance



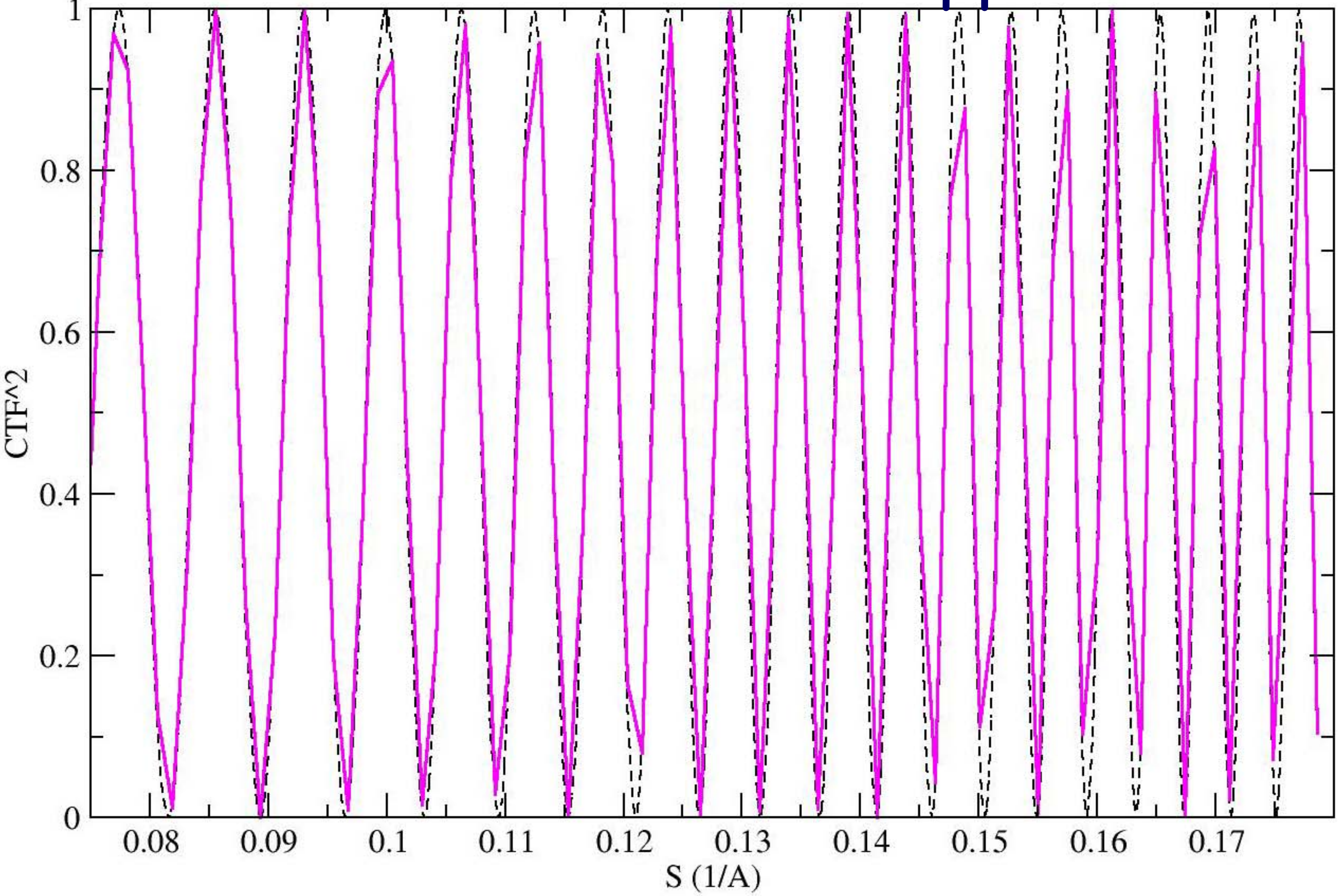
200 kV, *Cs* = 1.2mm, 2.8 *A*/pixel. 96x96 pixels box, ΔZ=3 μm

Effect of Box Size on CTF Appearance

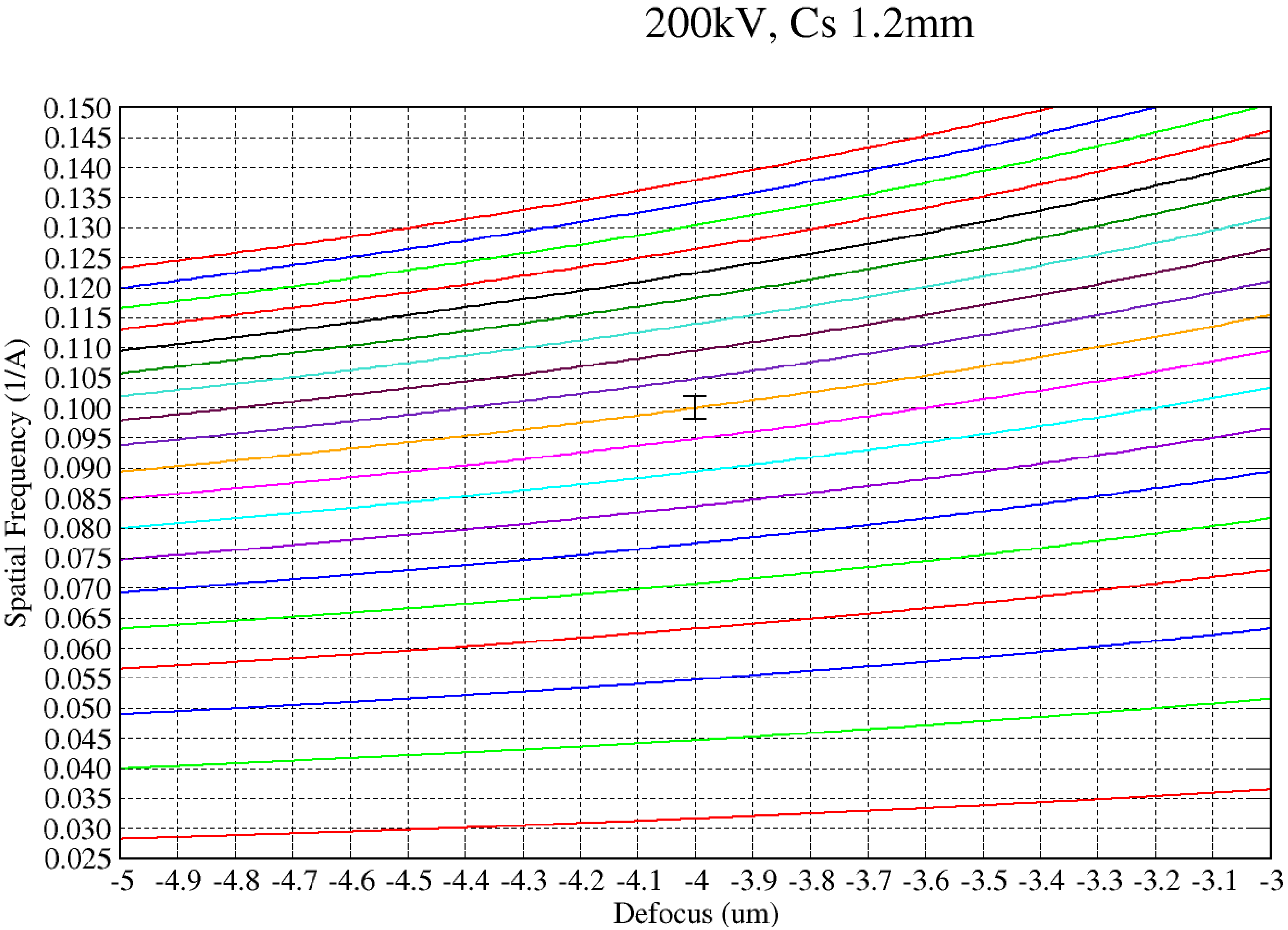


200 kV, *Cs* = 1.2mm, 2.8 *A*/pixel. 192x192 pixels box, ΔZ=3 μm

Effect of Box Size on CTF Appearance

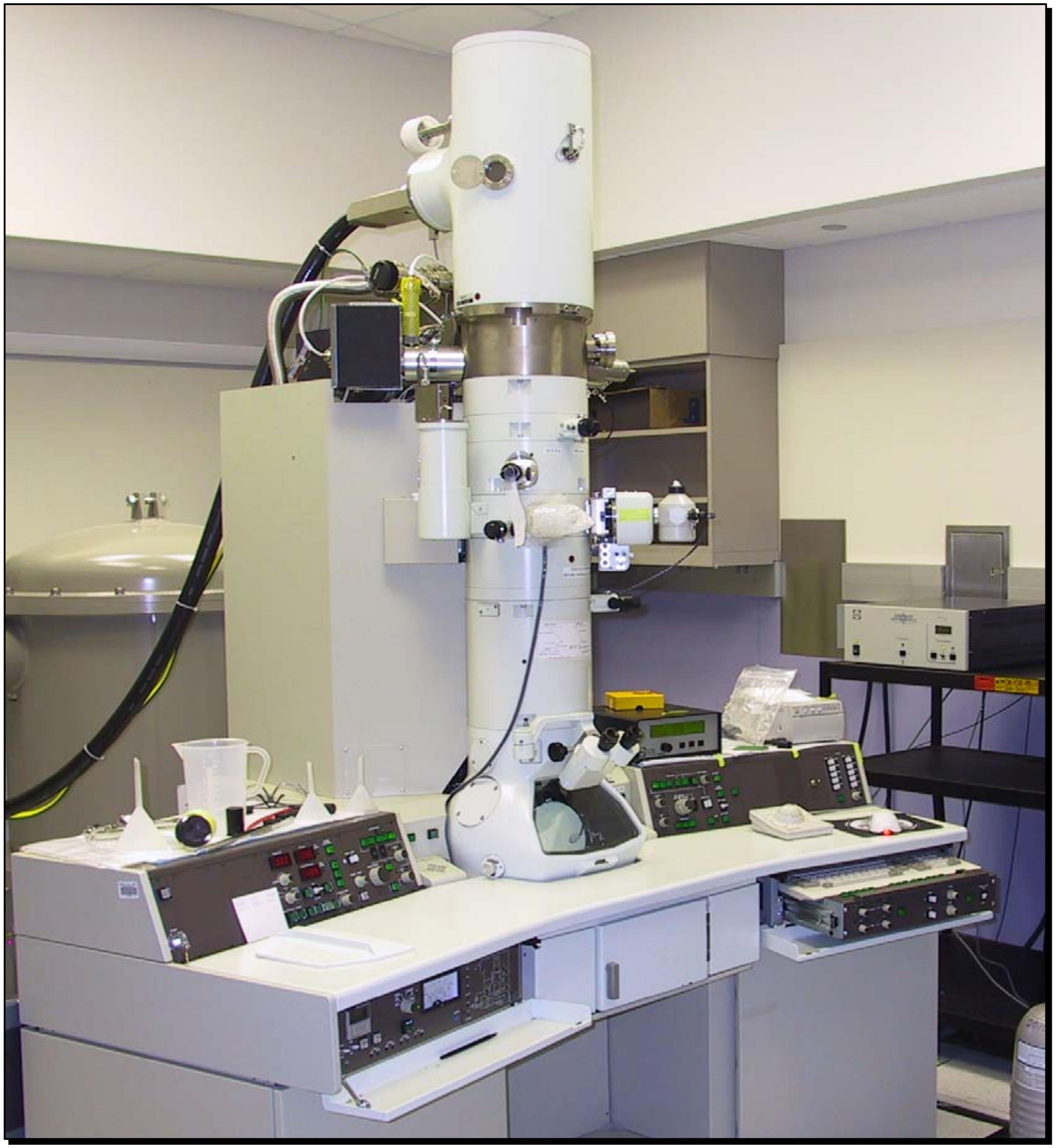


200 kV, *Cs* = 1.2mm, 2.8 *A*/pixel. 288x288 pixels box, ΔZ=3 µm

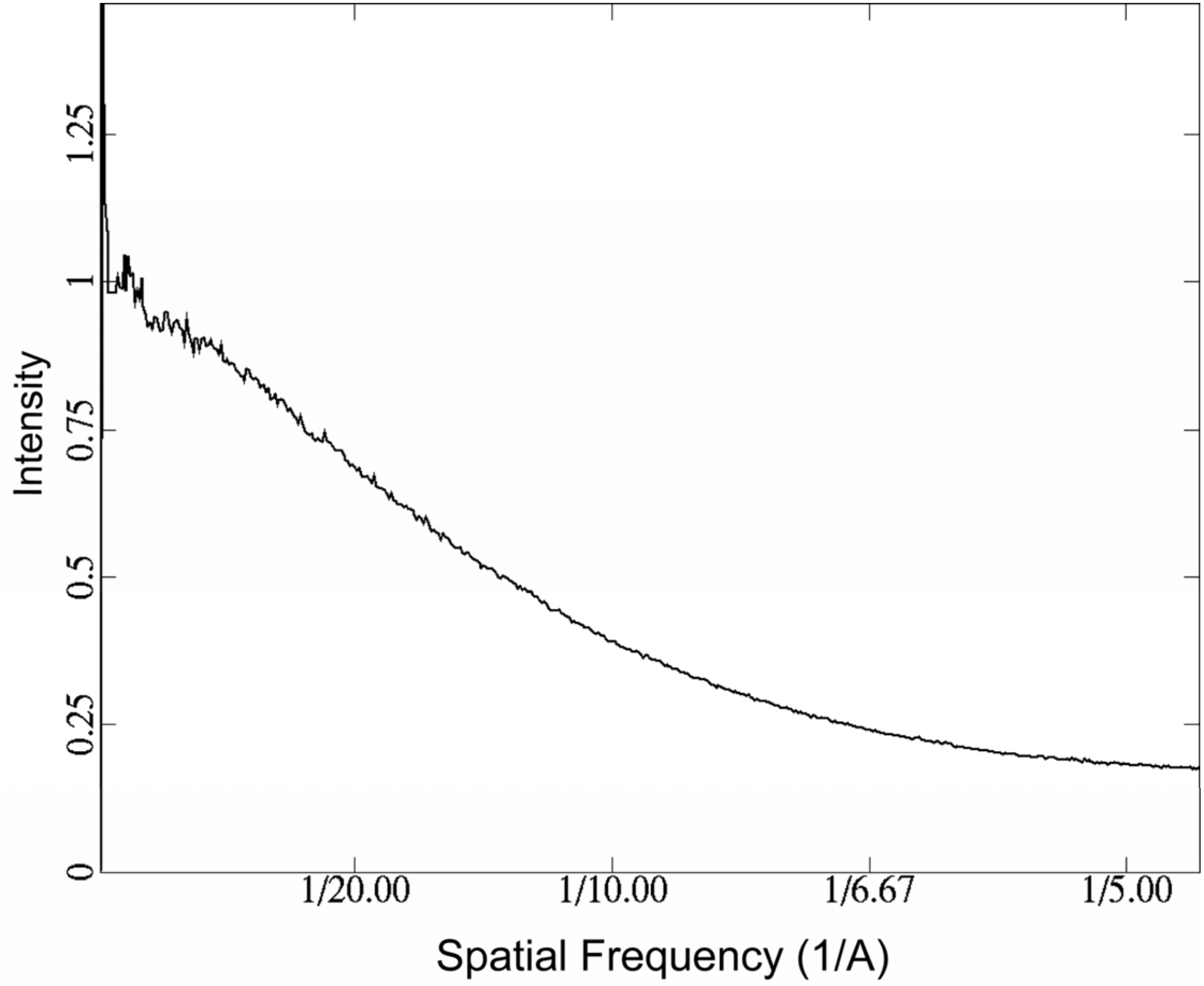

200kV, Cs 1.2mm
Spatial Frequency (1/A)
0.150
0.145
0.140
0.135
0.130
0.125
0.120
0.115
0.110
0.105
0.100
0.095
0.090
0.085
0.080
0.075
0.070
0.065
0.060
0.055
0.050
0.045
0.040
0.035
0.030
0.025
-5
-4.9
-4.8
-4.7
-4.6
-4.5
-4.4
-4.3
-4.2
-4.1
-4
-3.9
-3.8
-3.7
-3.6
-3.5
-3.4
-3.3
-3.2
-3.1
-3
Defocus (um)

CCD Camera for Single Particle Imaging

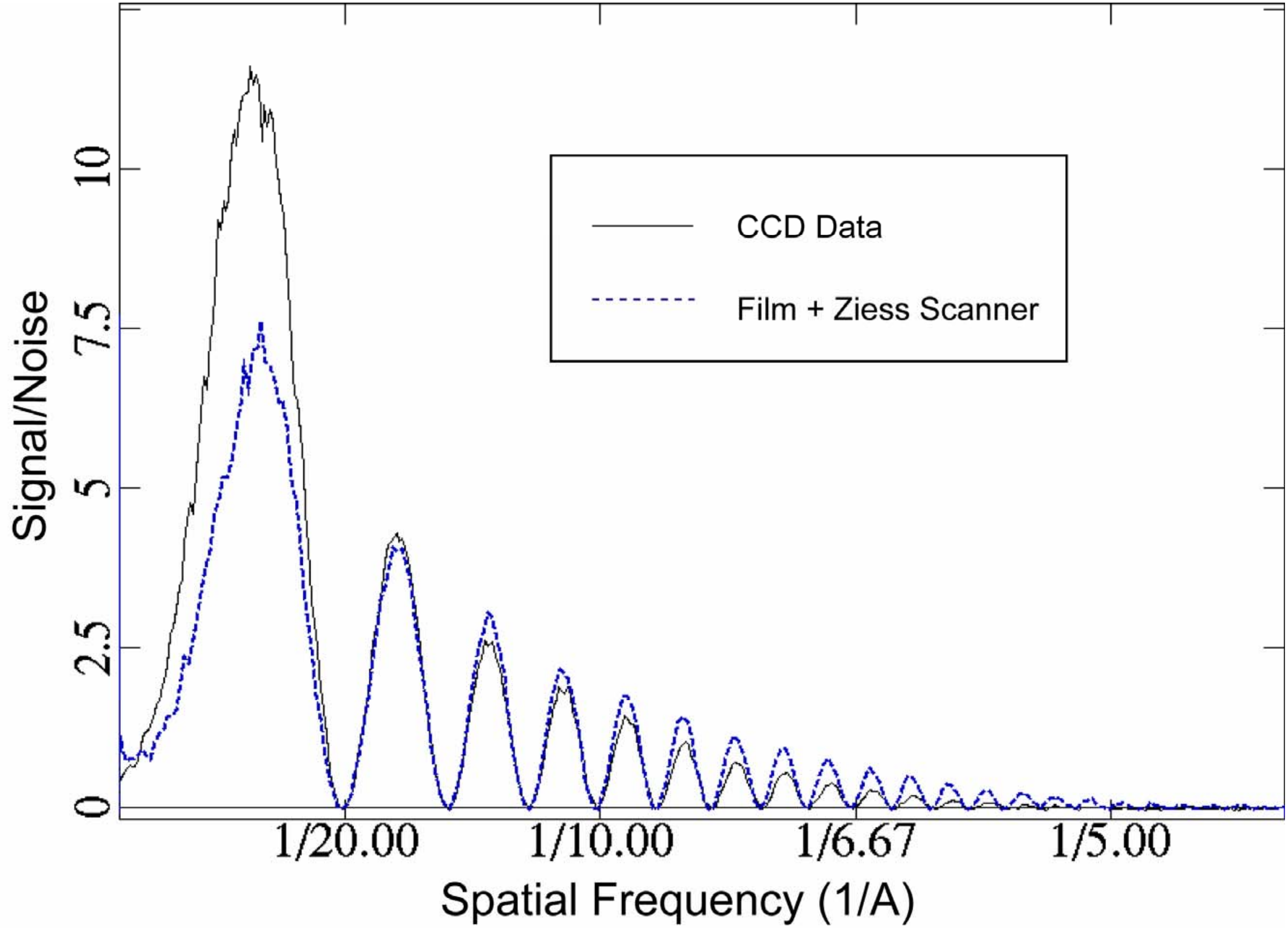
JEM2010F with a Gatan 4k CCD



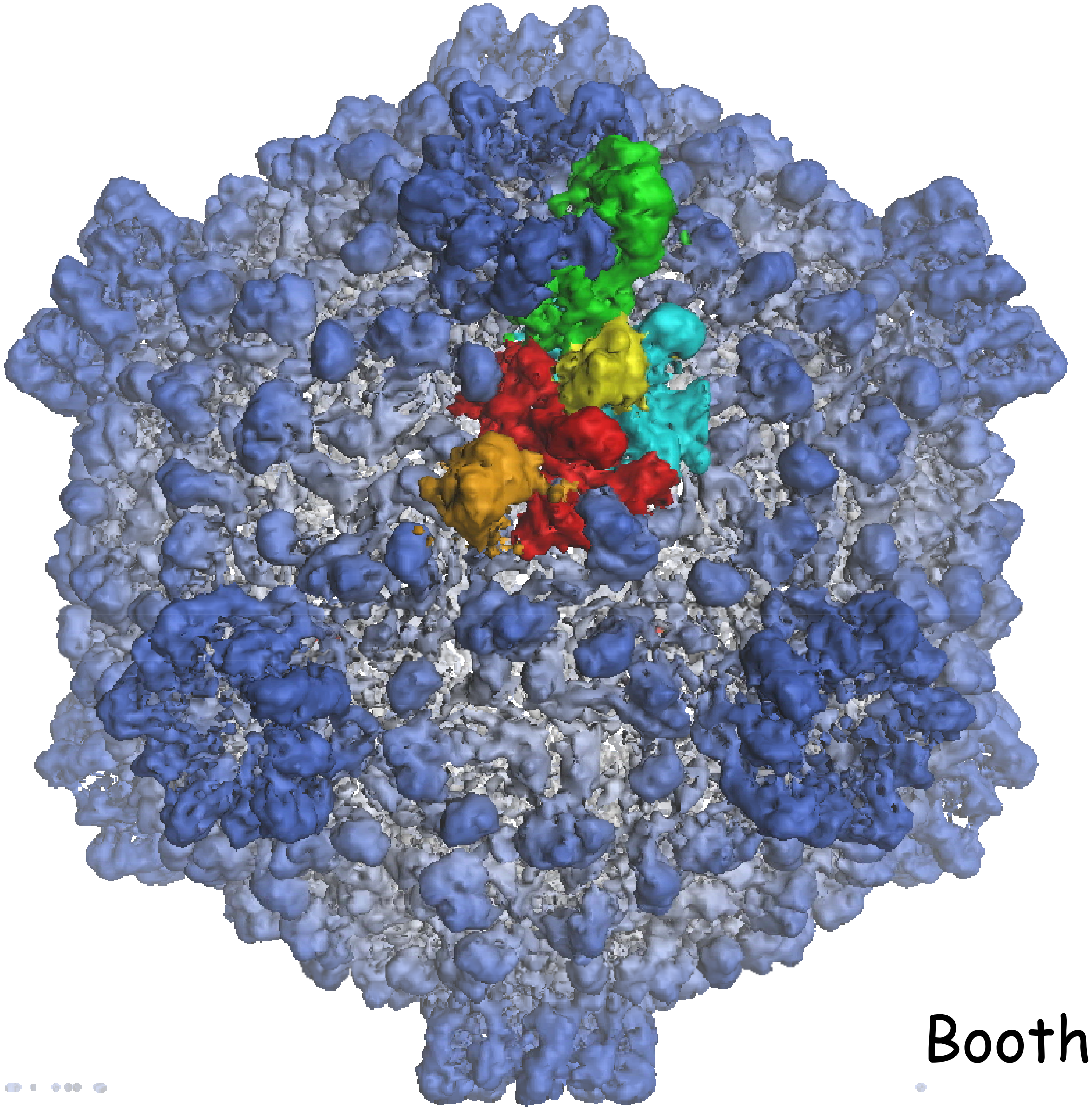
MTF of 4k CCD at 200 kV



S/N of 200 kV Carbon Film Image

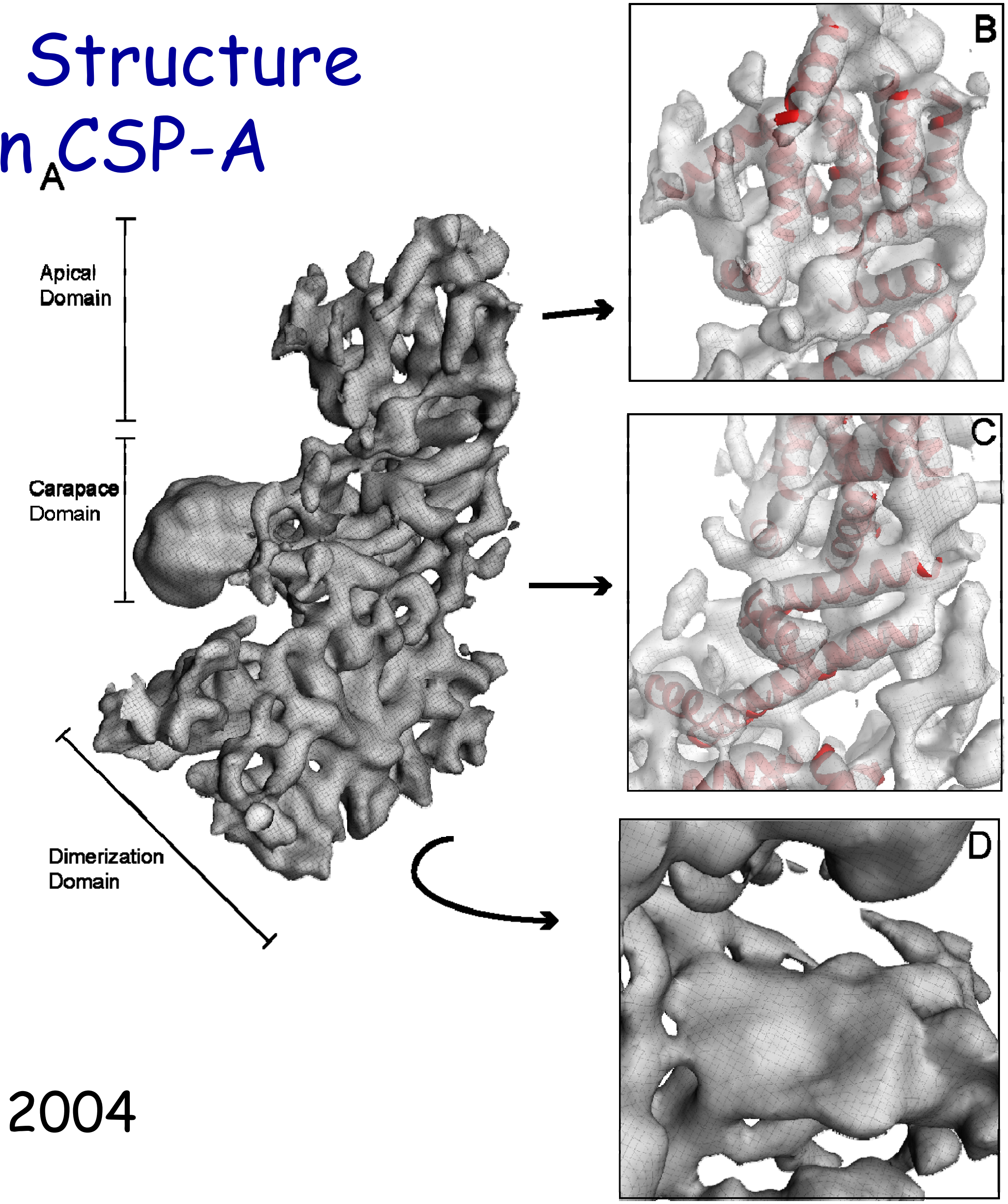


9Å Map of CPV from CCD Images



Booth et al, JSB 2004

Secondary Structure Elements in CSP-A



Booth, JSB, 2004

Magnification To Use For Higher Resolution Structure Study

Effective Microscope Magnification	Å/pix	Dimension of CCD Frame on Specimen (nm)	% CCD Frame Area wrt 82800 x	2/5 Nyquist (Å)
55,200	2.71	1,110	225	13.55
69,000	2.17	886	144	10.84
82,800	1.81	738	100	9.03
110,400	1.35	554	56	6.77
138,000	1.08	443	36	5.42
207,000	0.72	295	16	3.61

15 microns/pixel Gatan 4k CCD

References

Jiang, W. & Chiu, W. Web-based simulation for contrast transfer function and envelope functions. *Microsc & Microanal* **7**, 329-334 (2001).

Ludtke, S.J., Jakana, J., Song, J.-L., Chuang, D. & Chiu, W. A 11.5 Å single particle reconstruction of GroEL using EMAN. *J Mol Biol* **314**, 253-262 (2001).

Saad, A. et al. Fourier amplitude decay of electron cryomicroscopic images of single particles and effects on structure determination. *J Struct Biol* **133**, 32-42 (2001)

Zhou, Z.H. et al. CTF determination of images of ice-embedded single particles using a graphics interface. *J Struct Biol* **116**, 216-22 (1996).

Zhu, J., Penczek, P.A., Schroder, R. & Frank, J. Three-dimensional reconstruction with contrast transfer function correction from energy-filtered cryoelectron micrographs: procedure and application to the 70S E coli ribosome. *J Struct Biol* **118**, 197-219 (1997).

Toyoshima, C. & Unwin, P.N.T. Contrast transfer for frozen-hydrated specimens: Determination from pairs of images. *Ultramicroscopy* **25**, 279-292 (1988).

References

Chiu, W. Factors in high resolution biological structure analysis by conventional transmission electron microscopy. *Scanning Electron Micros* **1**, 569-580 (1978).

Wade, R.H. & Frank, J. Electron microscope transfer functions for partially coherent axial illumination and chromatic defocus spread. *Optik* **49**, 81-92 (1977).

Frank, J. Determination of source size and energy spread from electron micrographs using the method of Young's fringes. *Optik* **44**, 379-91 (1976).

Frank, J. The envelope of electron microscopic transfer functions for partially coherent illumination. *Optik* **38**, 519-36 (1973).

Thon, F. Phase contrast electron microscopy. in *Electron Microscopy in Material Sciences* (ed. Valdre, U.) 571-625 (Academic Press, Inc., New York, 1971).

Erickson, H.P. & Klug, A. The Fourier transform of an electron micrograph: effects of defocussing and aberrations, and implications for the use of underfocus contrast enhancement. *Phil. Trans. Roy. Soc. Lond. B* **261**, 105-18 (1970).