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March 14, 2011

Cryo-EM Essentials Lecture

EMAN Workshop

NCMI
National Center for Macromolecular Imaging

National Center for Research Resources, NIH

National Center for Research Resources

Research Focus at NCMI: Cryo-EM

- Develop Cryo-Electron Microscopy for structure determination of molecular machines in solution states without crystals at atomic resolution; and of frozen, hydrated cells/organelles at molecular resolution

- Collaborate with biological investigators on projects to drive the technology

- Share our experimental and computational methodologies and facilities freely with the global academic community

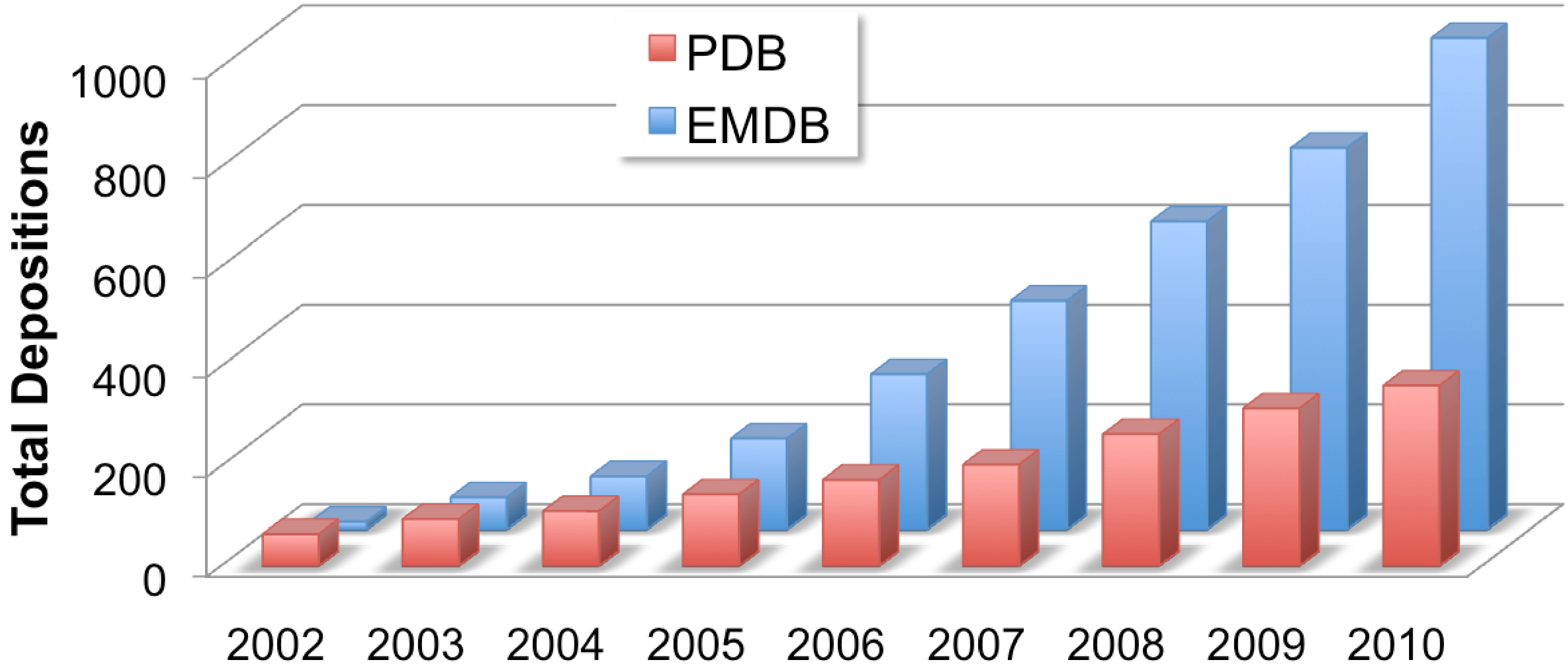
NCMI Cryo-EM Collaborators in USA

NCMI Cryo-EM Global Collaborators

Why Cryo-EM ?

- Can determine structures at different chemical or biological conformation states
- Can work with large complex of mixed/dynamic conformations
- Can determine Structure that cannot be tackled readily by NMR or crystallography
- Need only low concentration (<1mg/ml) in less than 100 µl sample
- Resolution can be reached to ~3.5 Å in favorable cases

Growth of Cryo-EM Entries of Maps & Models

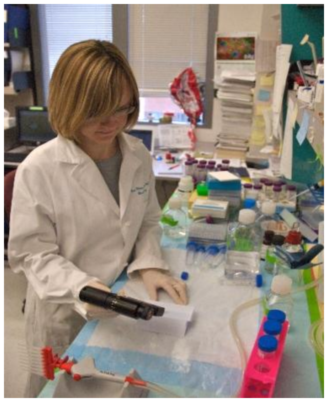


EMDataBank.org
Unified Data Resource for CryoEM

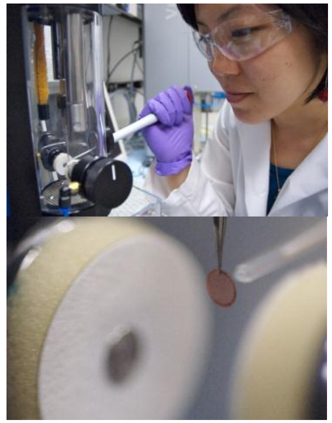
http://emdb-info.rutgers.edu/growth-chart

Pipeline in Single Particle Cryo-EM

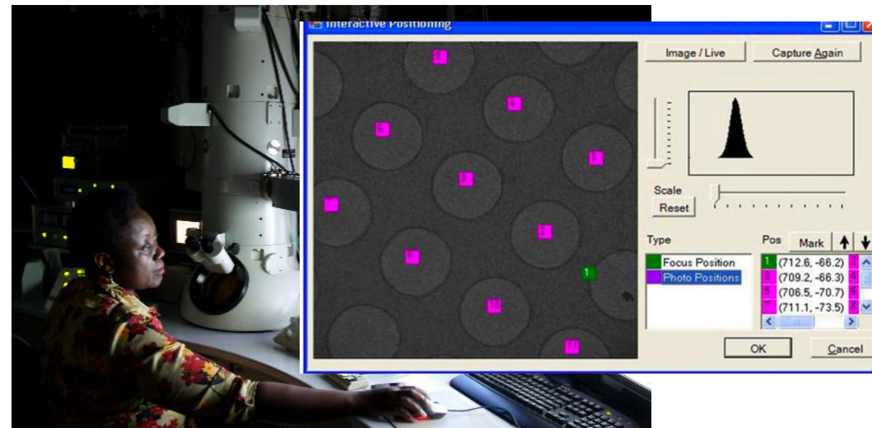
Biochemical Preparation

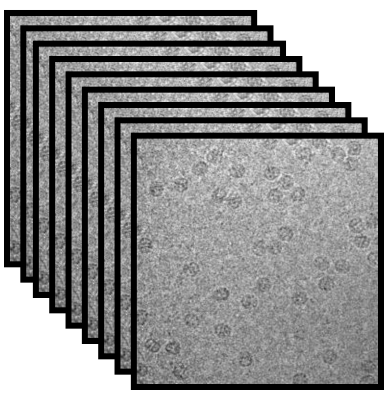


Cryo-EM Sample Preparation

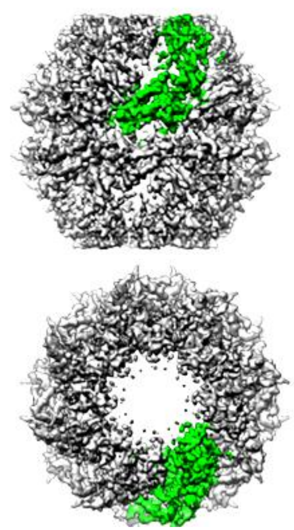


High Resolution Automated Data Collection
JADAS

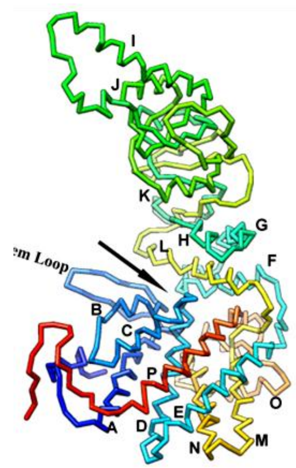




Data Archiving & Processing
EMEN



3D Reconstruction
EMAN



Model Building & Validation
Gorgon

WORLDWIDE PDB PROTEIN DATA BANK

Structure Deposition

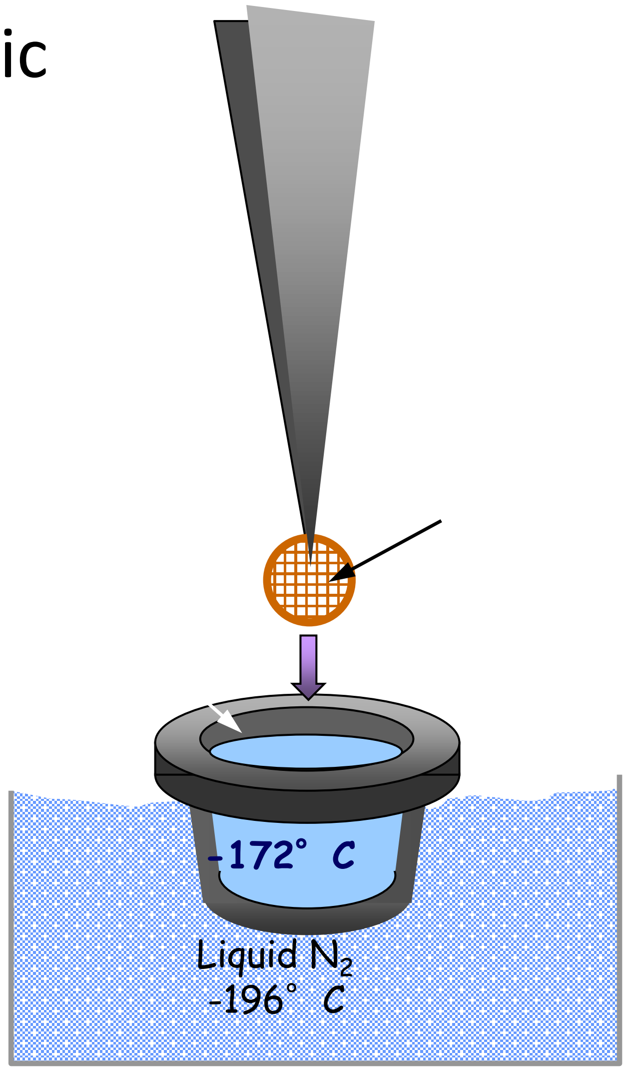
Basic Requirements for Vitrification Apparatus

1. Blotting mechanism
   - manual, pneumatic or electronic

1. Blotting chamber
   - high humidity

2. Plunging mechanism
   - high entrance velocity

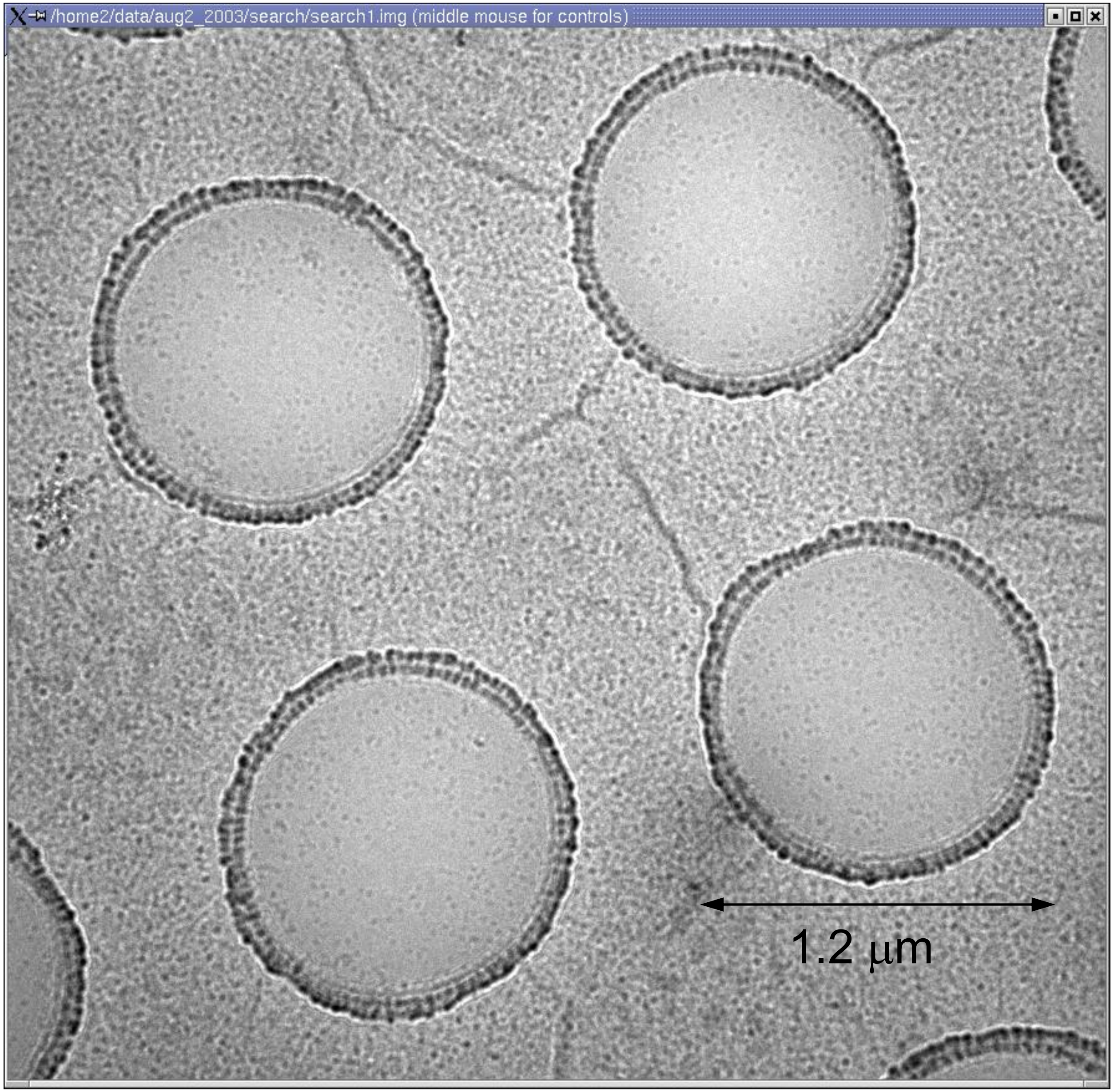
3. Cryogen
   - high cooling efficiency



Dubochet et al. (1988) *Q Rev Biophys* **21**: 129-228

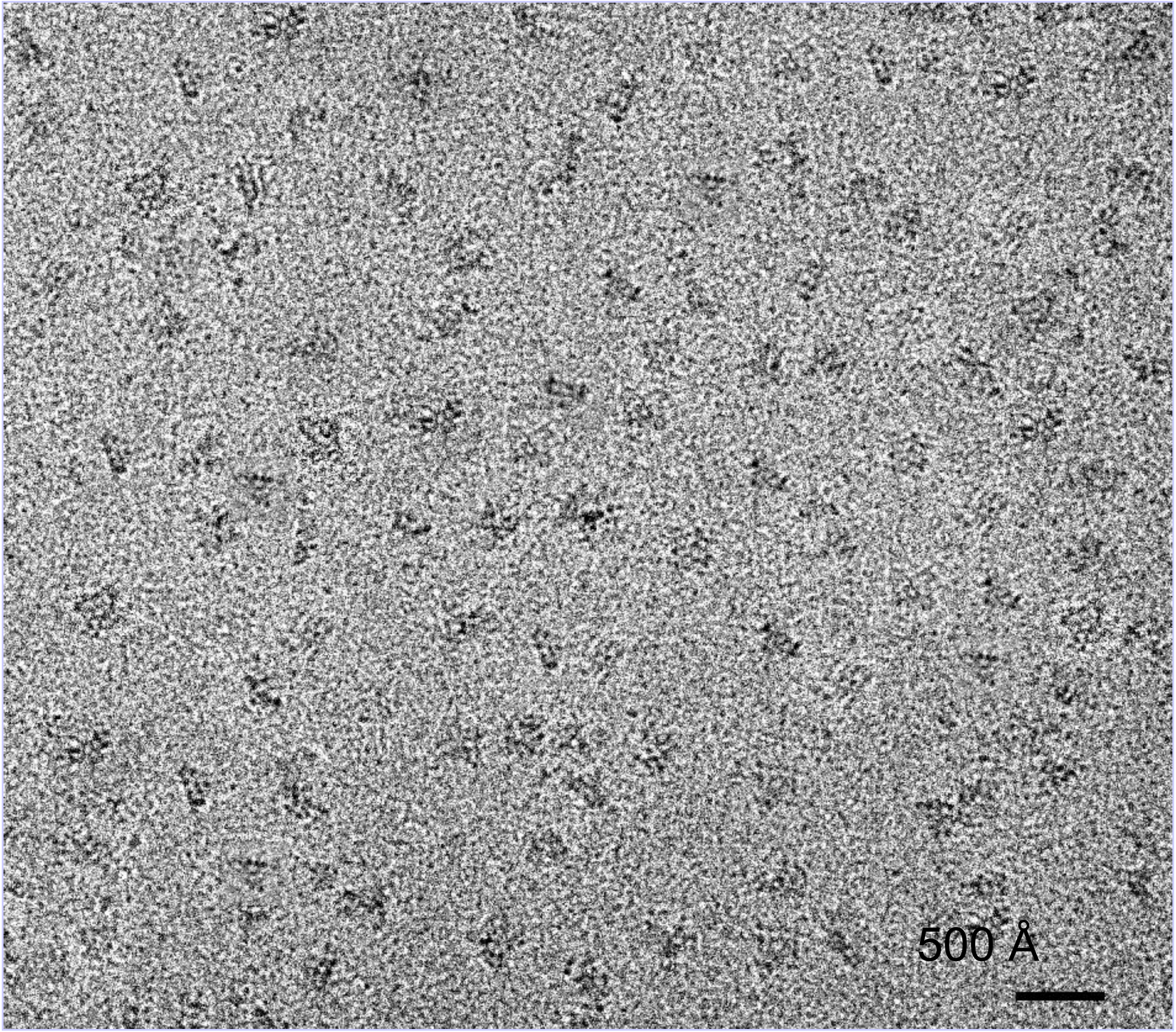
Cryo-Specimen Preparation

Vitrified Sample in Search Mode



Courtesy of
Dr. I Serysheva

200kV Image of Ice-Embedded Ion Channel



Courtesy of
Dr. I Serysheva

Electron Cryo-Microscopes at NCMI

JEM 2100

200 kV
4k CCD Camera

2007

JEM 2010 F

200 kV
4k CCD Camera

2002

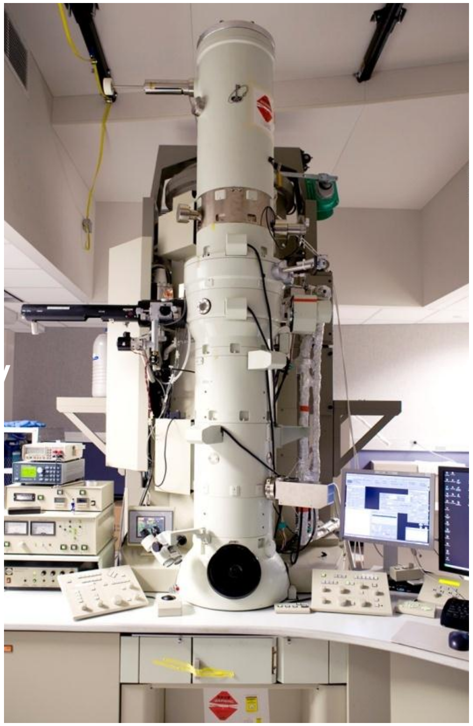
JEM 2200 FS

200 kV
4k CCD Camera
Energy Filter
Zernike Phase Plate

2009

JEM 3200 FSC

300 kV
10k CCD Camera
Energy filter



2006

Electron Microscope Detectors

- Photographic film
- CCD camera: scintillator-photomultiplier detector
- CMOS direct detection

Gatan 4k CCD Performance at 300keV

Scope Mag	Detector Mag	Pixel size	Nyquist (Å)	2/3Nyquist (Å)
30,000	42,000	3.57	7.14	10.71
40,000	56,000	2.68	5.36	8.04
50,000	70,000	2.14	4.29	6.42
60,000	84,000	1.79	3.57	5.37
80,000	112,000	1.34	2.68	4.02
100,000	140,000	1.07	2.14	3.21
120,000	168,000	0.89	1.79	2.67
150,000	210,000	0.71	1.43	2.13

Chen et al (2008) *JSB* **163**:45-52

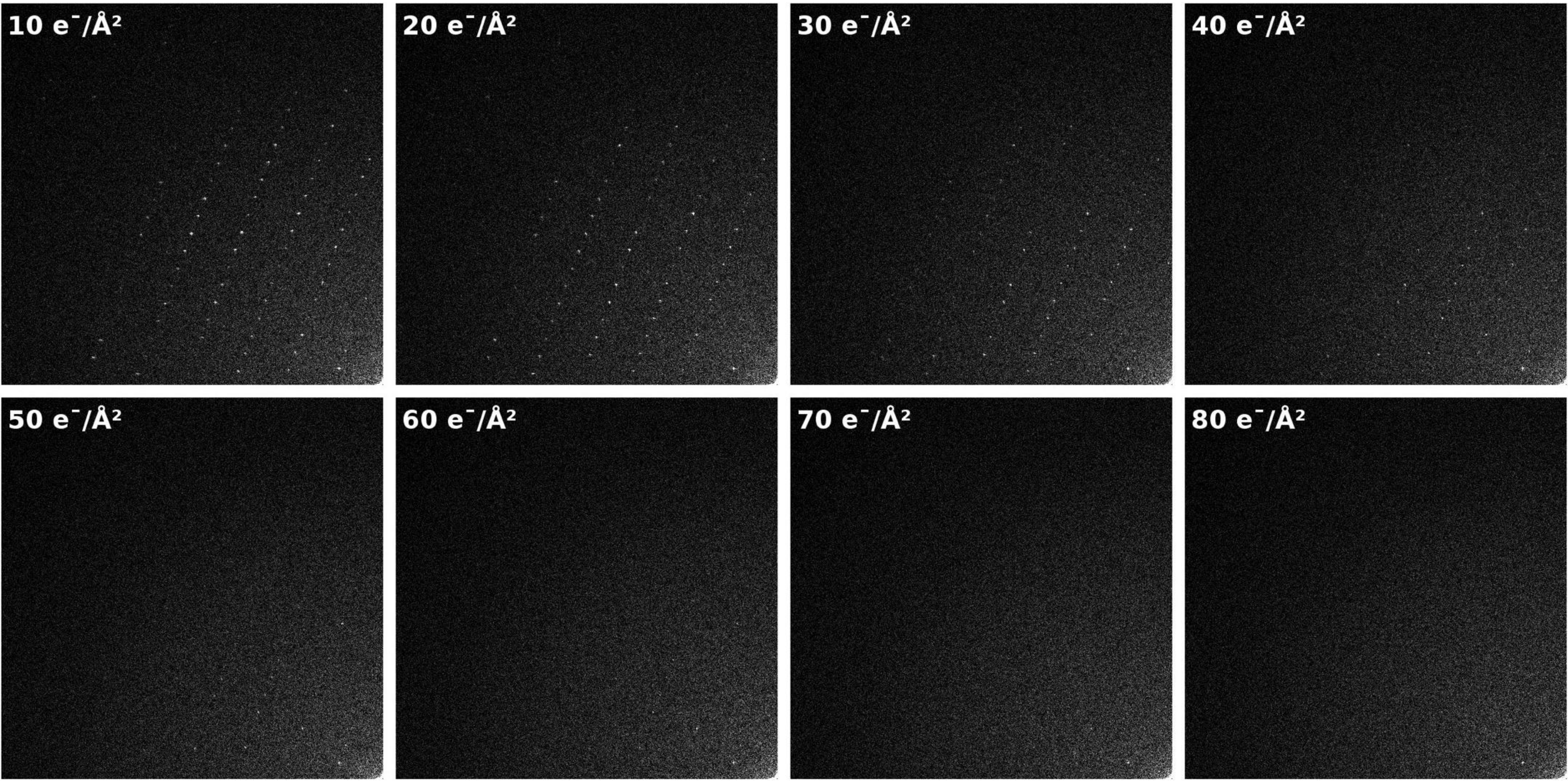
Radiation Damage Assessment of Protein Crystals

- Record a series of 9-10 electron images or electron diffraction patterns from a single crystal

- Measure quantitatively the fading of the diffraction spot intensities as a function of cumulative exposure

P. N. Unwin and R. Henderson (1975) *JMB* **94**: 425-40.

Radiation Damage Studies of Ice Embedded Catalase Crystal



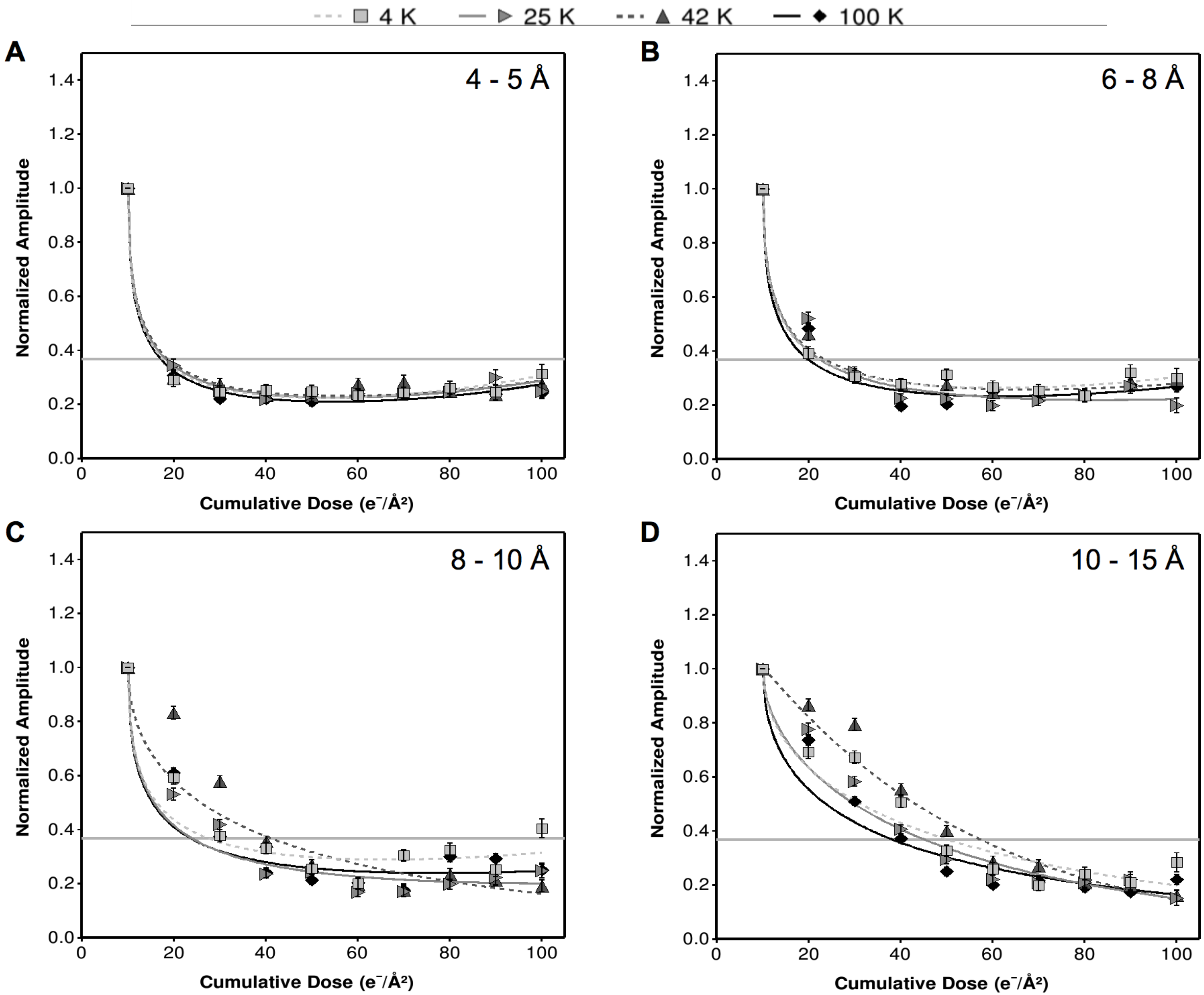
B Bammes, J Jakana, M Schmid, W Chiu *JSB* **169:** 331-341 (2010)

Quantification of Damage

N_e (1/e) decay dose

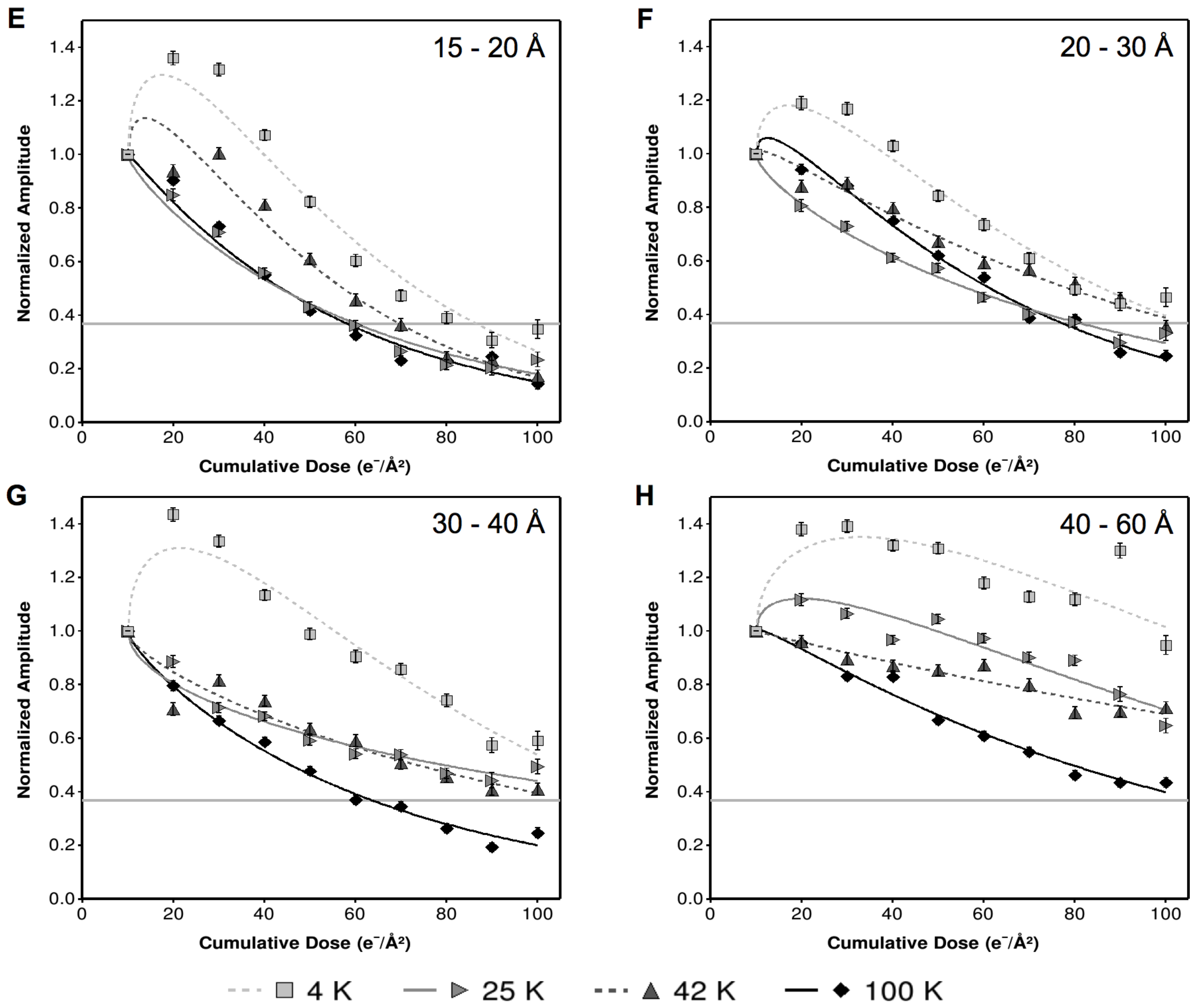
Dissimilarity factor

Fading of Fourier Amplitudes at Different Temperatures

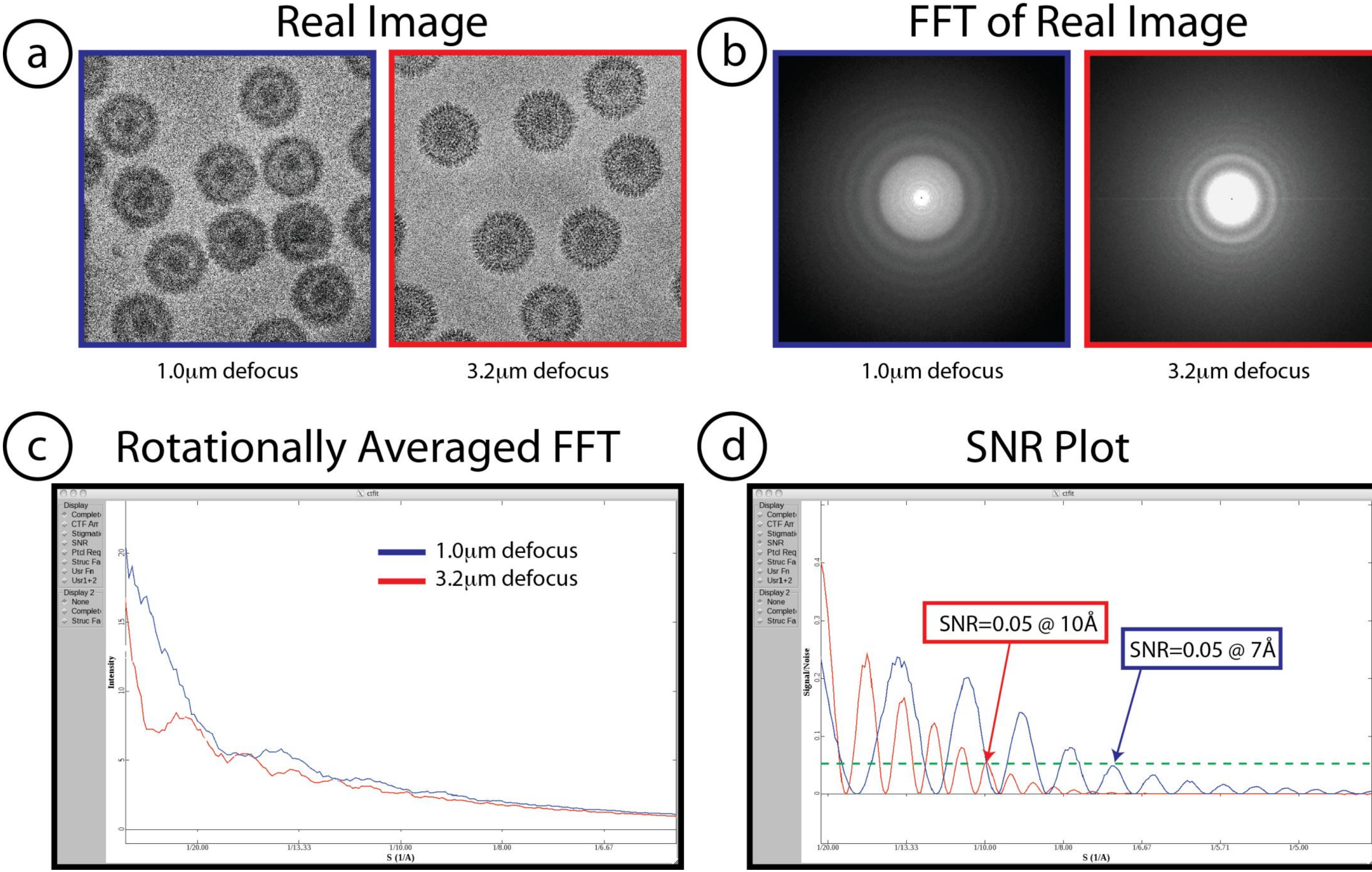


B Bammes, J Jakana, M Schmid, W Chiu *JSB* **169:** 331-341 (2010)

Fading of Fourier Amplitudes at Different Temperatures

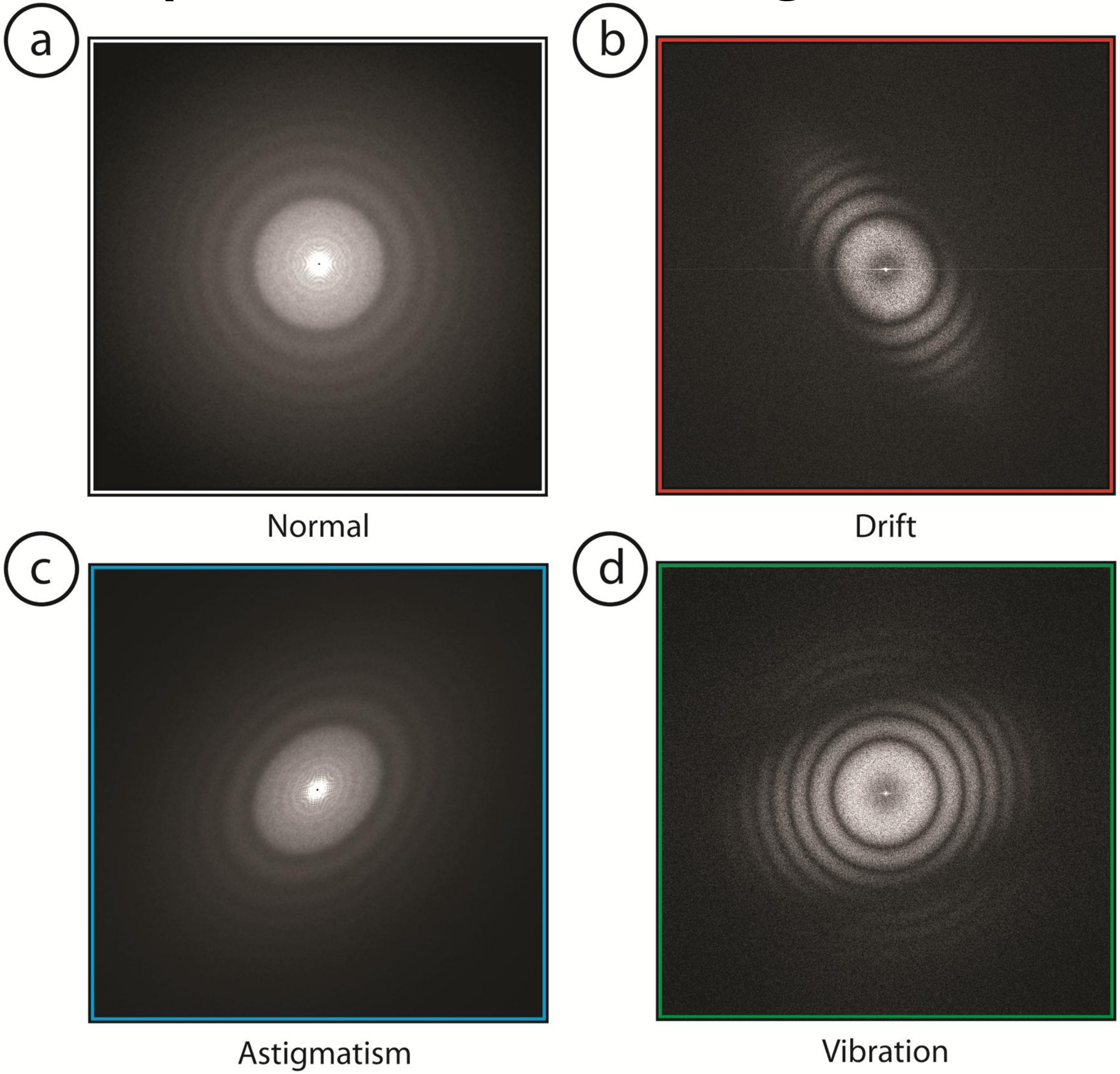


B Bammes, J Jakana, M Schmid, W Chiu *JSB* **169:** 331-341 (2010)



Courtesy of Ryan Rochat

Power Spectrum of Images of C-Film



R Rochat
J Jakana

Computed Diffraction Pattern

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

Structure factor

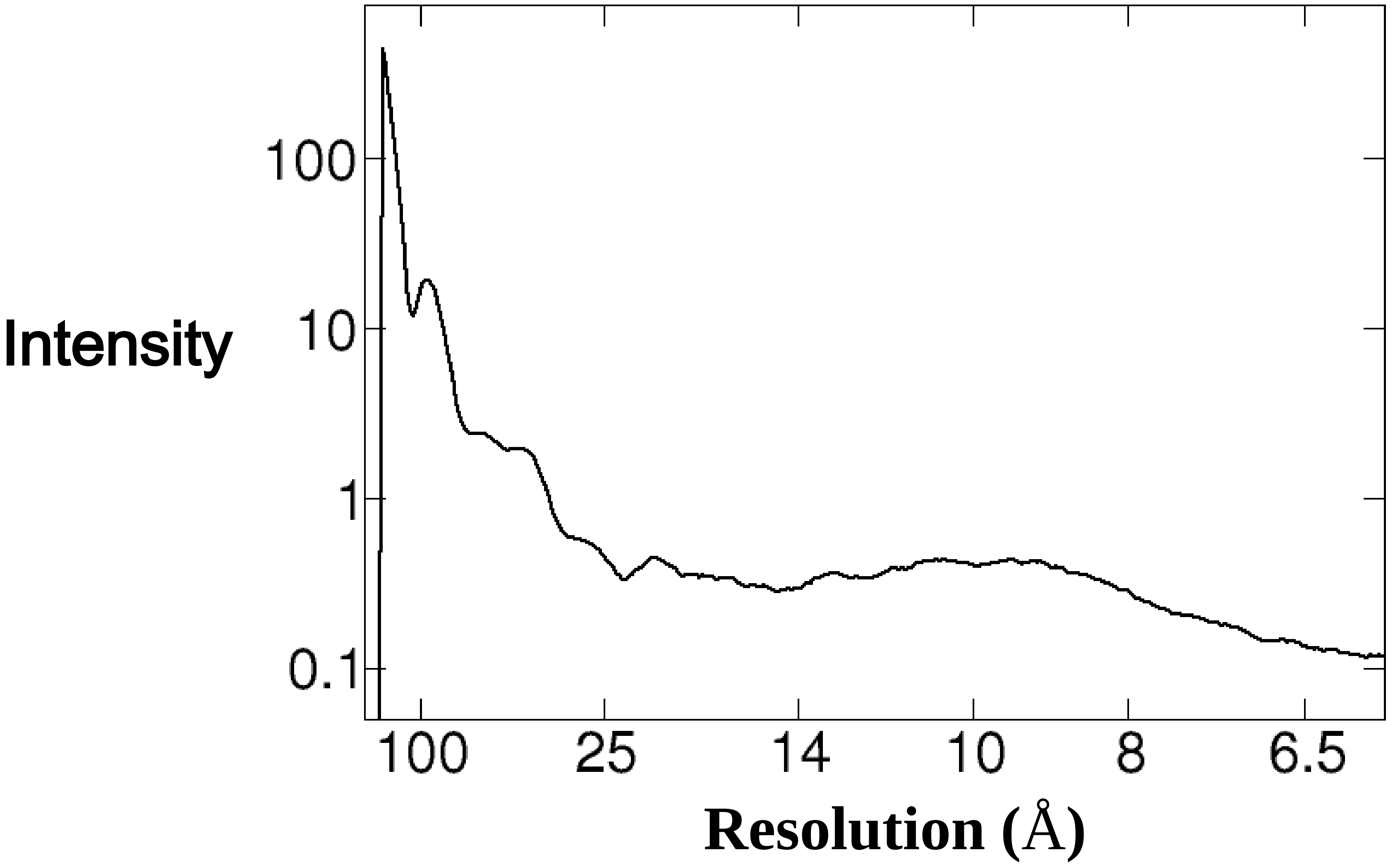
Contrast transfer function

Envelope function

Background

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

X ray Scattering Intensity of GroEL



Ludtke et al (2001) J Mol Biol 314, 253-262

Computed diffraction pattern

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

Structure factor

Contrast transfer function

Envelope function

Background

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 - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Back Search Favorites History

Address http://ncmi.bcm.tmc.edu/~wjiang/ctf Go

Home Research Photo

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.

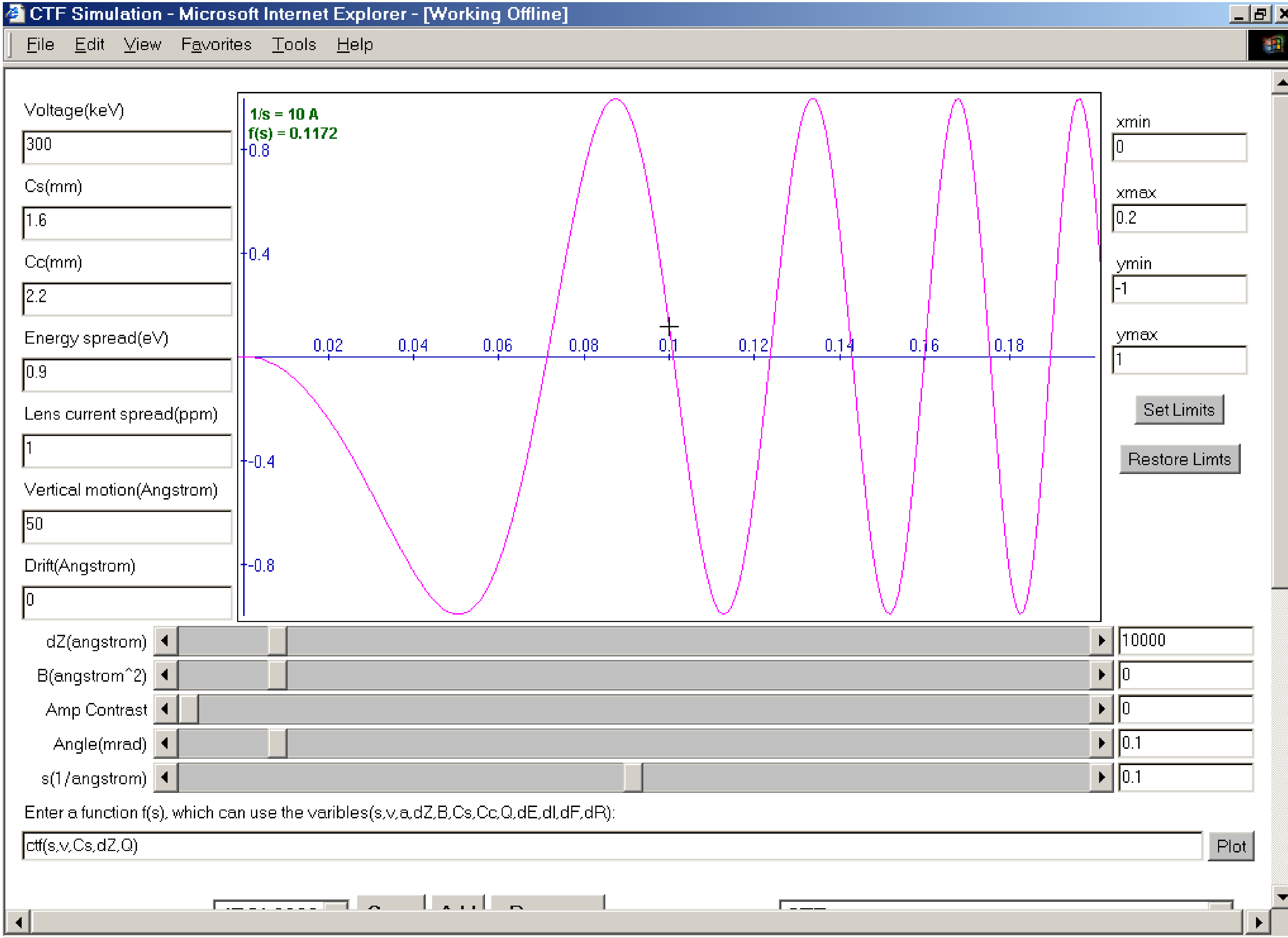
This applet is known to run on Windows (Netscape and Internet Explorer), Linux (i386) (Netscape), SGI IRIX (Netscape), OS/2 Warp and MacOS X. Please inform me if you found that this applet runs or has problems to run on other platforms.

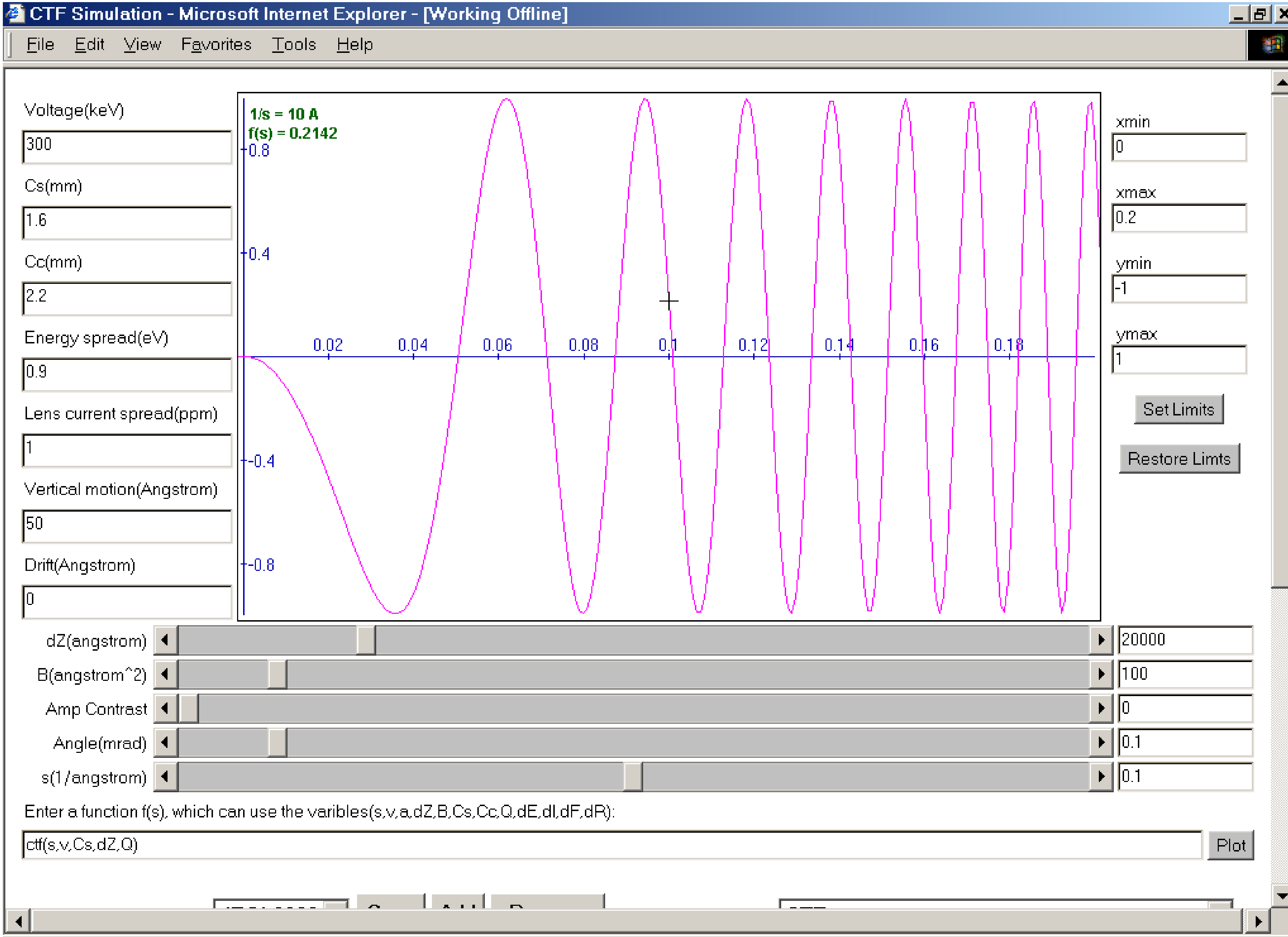
The following is the detailed descriptions for some aspects of the applet page.

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

m bcm148-147.alkek.dhcp.bcm.tmc.edu Welcome! friend fro Internet


CTF Simulation - Microsoft Internet Explorer - [Working Offline]
File Edit View Favorites Tools Help
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
1/s = 10 A
f(s) = 0.1172
0.8
0.4
-0.4
-0.8
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
xmin
0
xmax
0.2
ymin
-1
ymax
1
Set Limits
Restore Limts
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)
Plot



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

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:

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

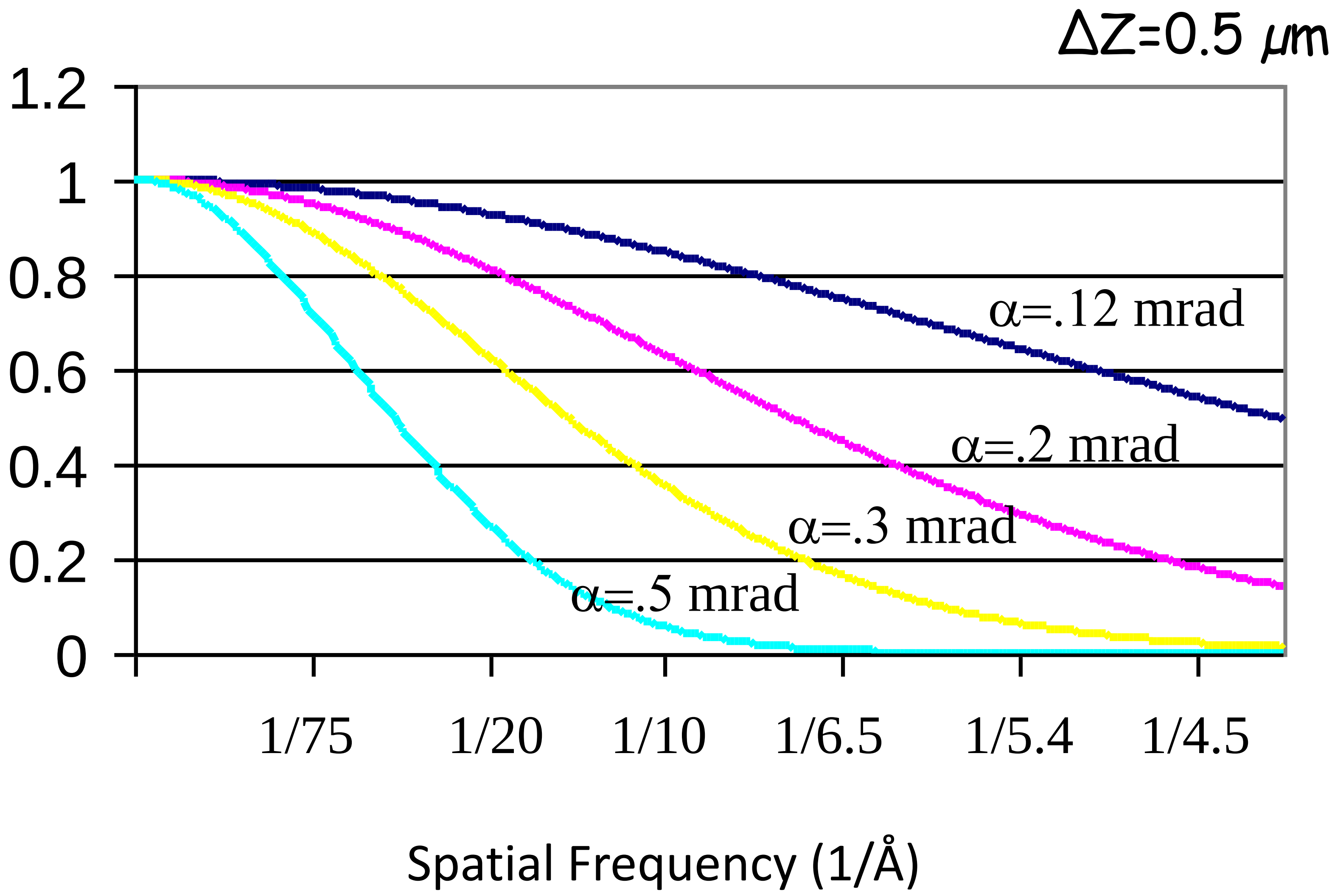
Sinusoidal type fluctuation:

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

Drift:

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

Spatial Coherence Envelope Function



Gaussian Approximation for Cumulative Envelope Function

$$Env^2(s) \sim \exp(-2Bs^2)$$

$$B = \frac{1}{4} B_{cryst}$$

B = wah B factor = EMAN 1 B factor

Henderson B factor = B_{cryst} = EMAN 2 B factor

Computed diffraction pattern

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

Structure factor

Contrast transfer function

Envelope function

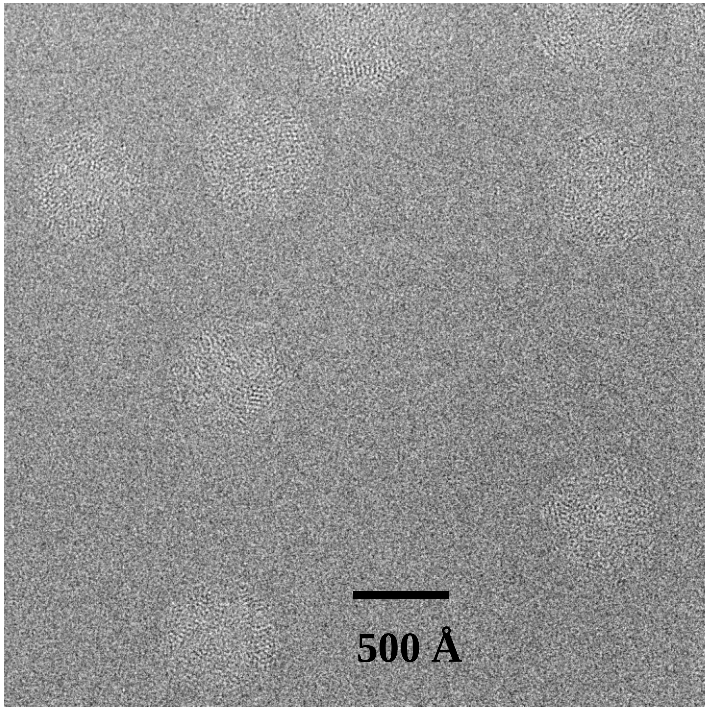
Background

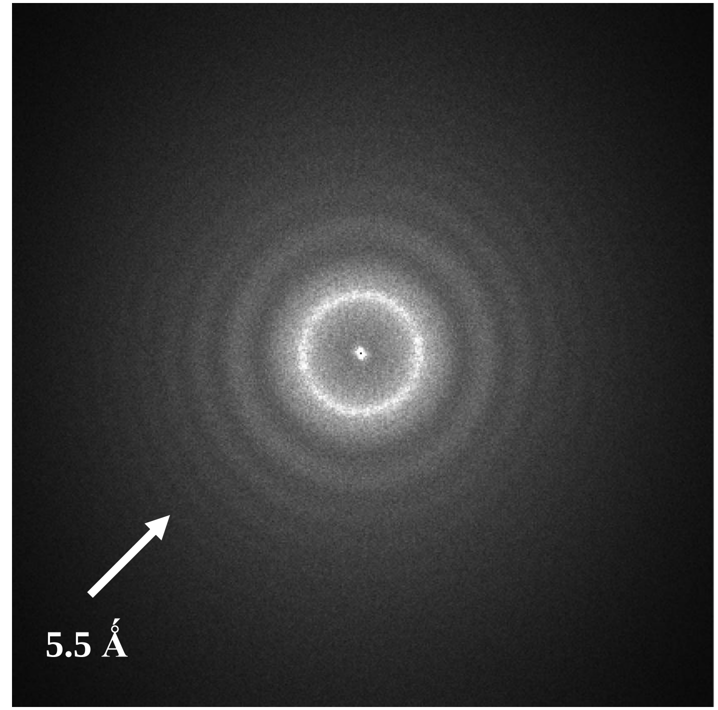
Noise Function

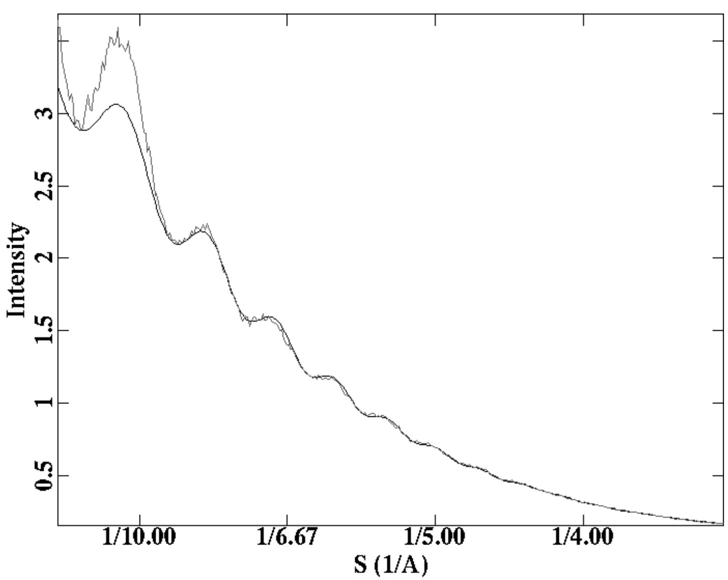
$$N^2(s) = n_1 \exp(n_2 s + n_3 s^2 + n_4 s^{1/2})$$

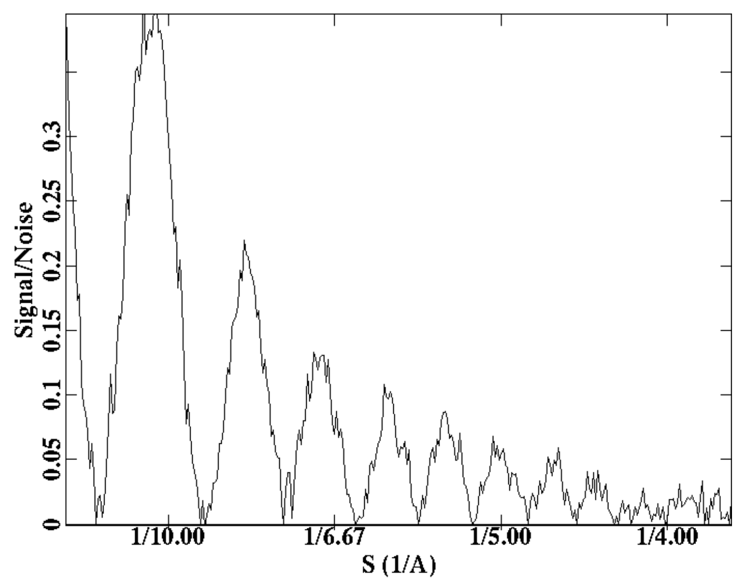
$$\text{Contrast} = (F^2 \ \ CTF^2 \ \ E^2) / N^2$$

Application









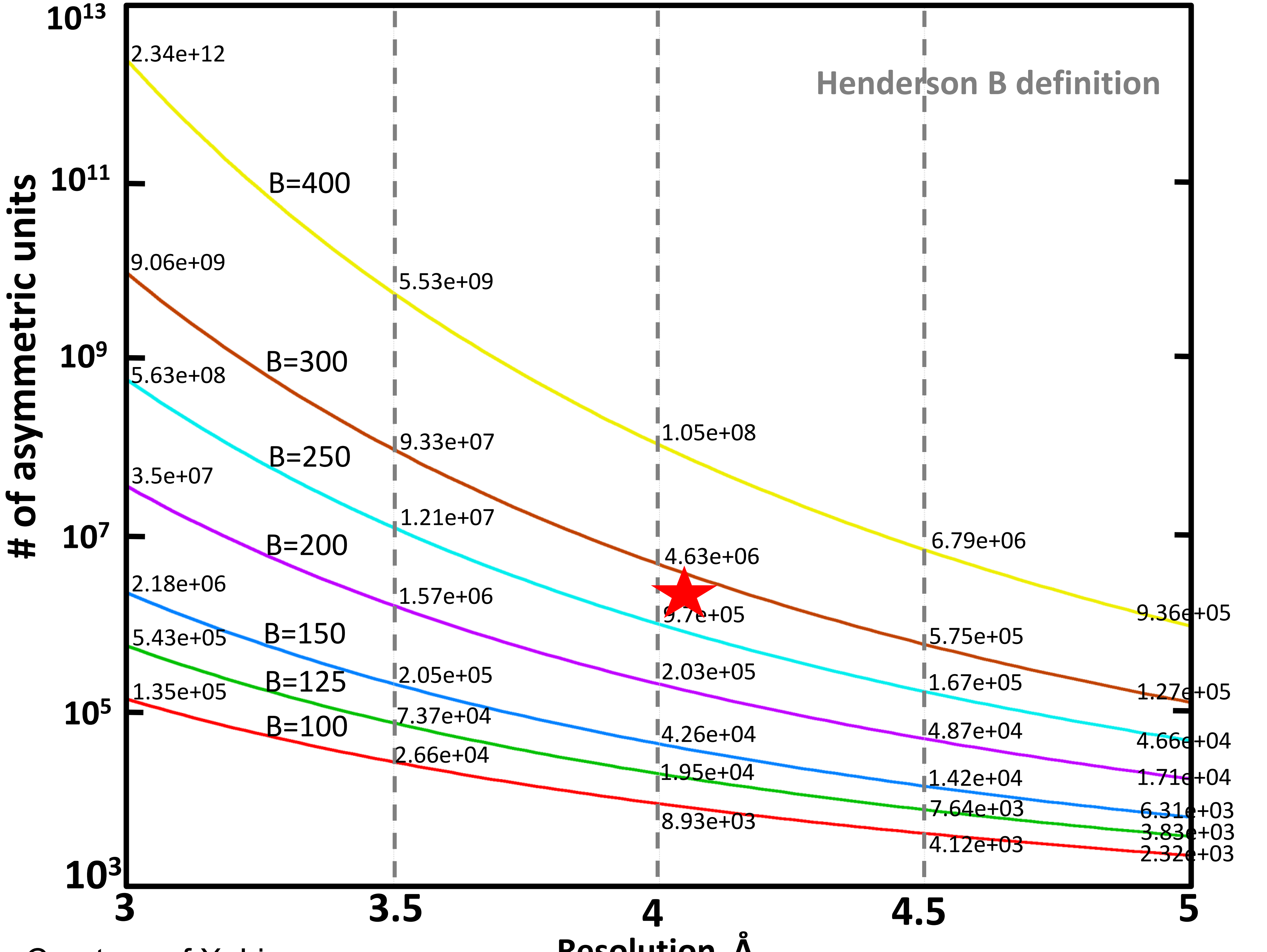
Number of particles required for a 3-D reconstruction

$$N_{asym-unit} = N_{ptcl} * N_{asym} = \frac{<S>^2}{<N>^2} \frac{30\pi}{N_e \sigma_e d} e^{B/2d^2}$$

Where $<S>^2/<N>^2 = 1/3$, $N_e = 20\ e^-/\text{Å}^2$, $\sigma_e = 0.04\ \text{Å}^2$

Rosenthal and Henderson (2003) *JMB* **333**, 721-745

Liu et al (2007) *J Struct Biol* **160**:11-27
Murata et al. (2000) *Structure* **18**:903-912

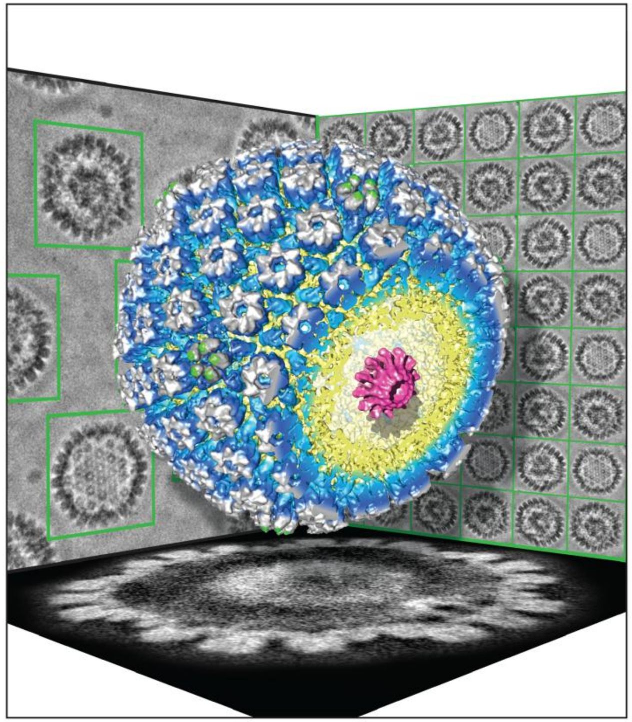


Courtesy of X. Liu

Future Cryo-EM Technology

- Elimination of specimen movement
- Higher contrast image (phase plate)
- Improved detector (CMOS)
- Extend to smaller and larger particles
- Study particles of mixed conformations
- Map validation
- Model validation
- Standard for data deposition to PDB

Zernike Phase Contrast Cryo-EM



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General Cryo-EM References

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