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July 9, 2012

Cryo-EM Workshop@NUS

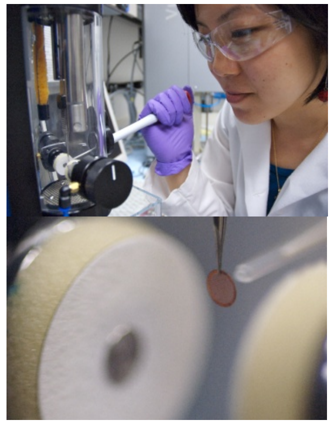
General Cryo-EM References

- Glaeser, R. M., Downing, K, DeRosier, D., Chiu, W. and Frank, J. (2007) Electron Crystallography of Biological Macromolecules, Oxford University Press.
- Chang J, Liu X, Rochat RH, Baker ML, Chiu W. (2012). Reconstructing virus structures from nanometer to near-atomic resolutions with cryo-electron microscopy and tomography. *Adv Exp Med Biol.* **726**:49-90.
- Jensen, G. (2010) Methods in Enzymology Volume 481. Many review papers

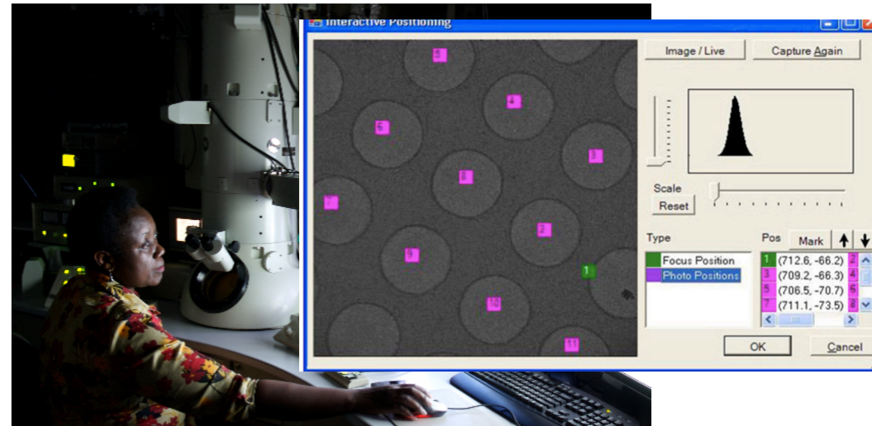
Pipeline in Single Particle Cryo-EM

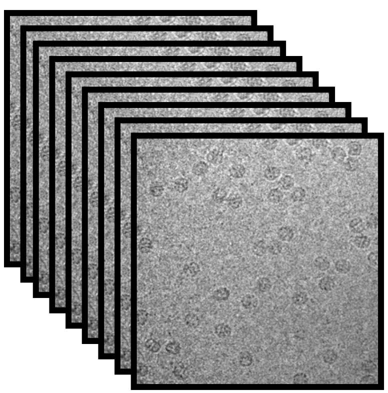
Biochemical Preparation

Cryo-EM Sample Preparation

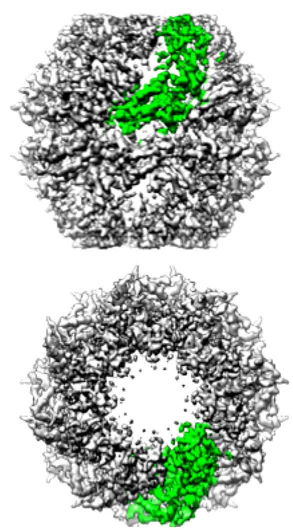


High Resolution Automated Data Collection
JADAS

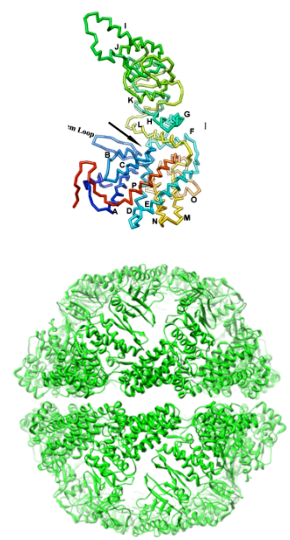




Data Archiving & Processing
EMEN



3D Reconstruction
EMAN



Model Building & Validation
Gorgon

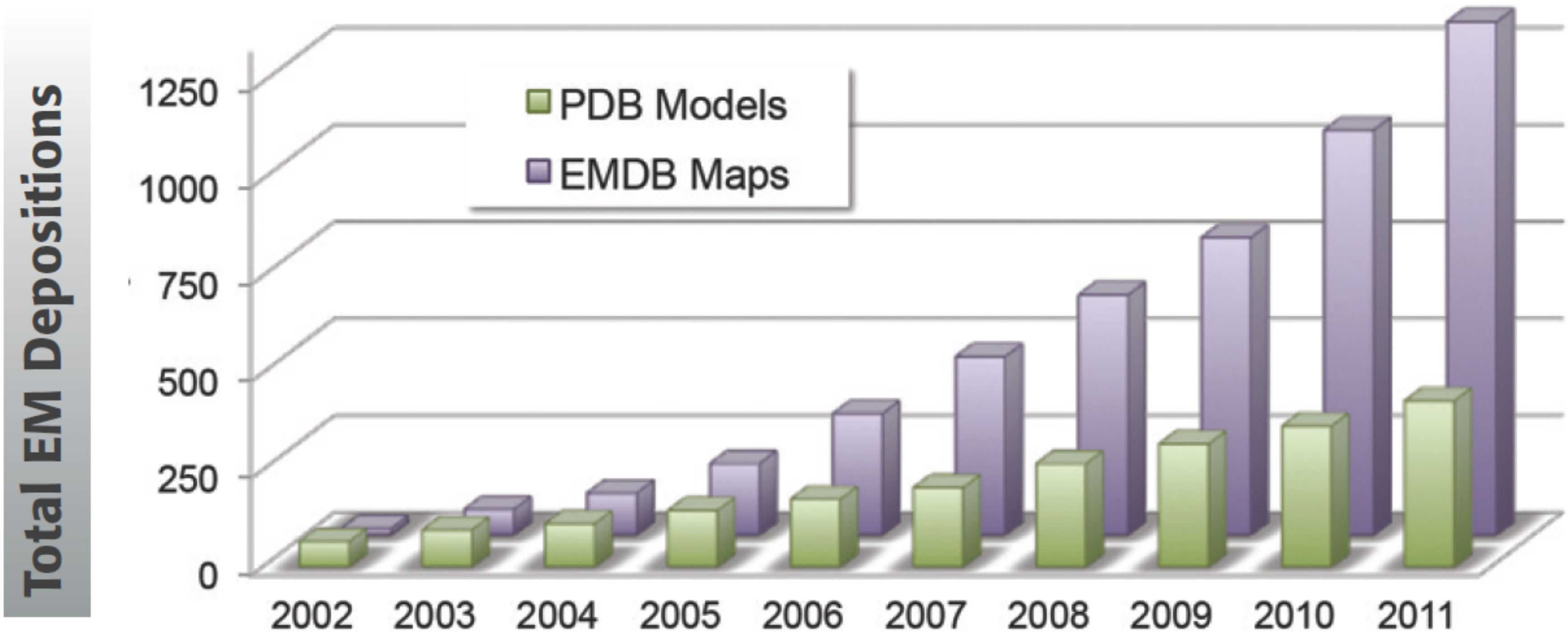
WORLDWIDE PDB PROTEIN DATA BANK

Structure Deposition

Why CryoEM?

- 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

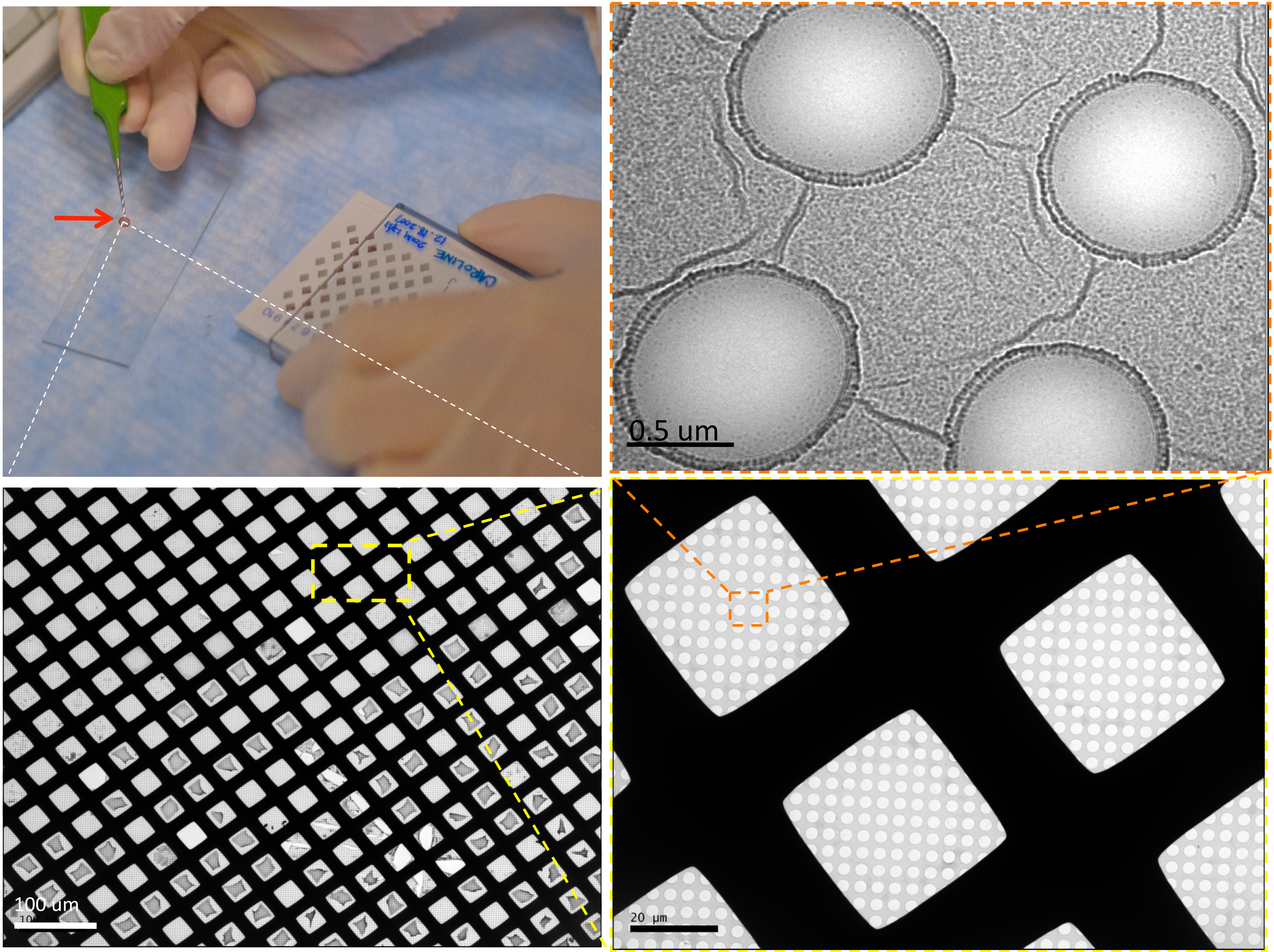
Growth of EM Entries



Courtesy of Cathy Lawson, PDB, Rutgers University

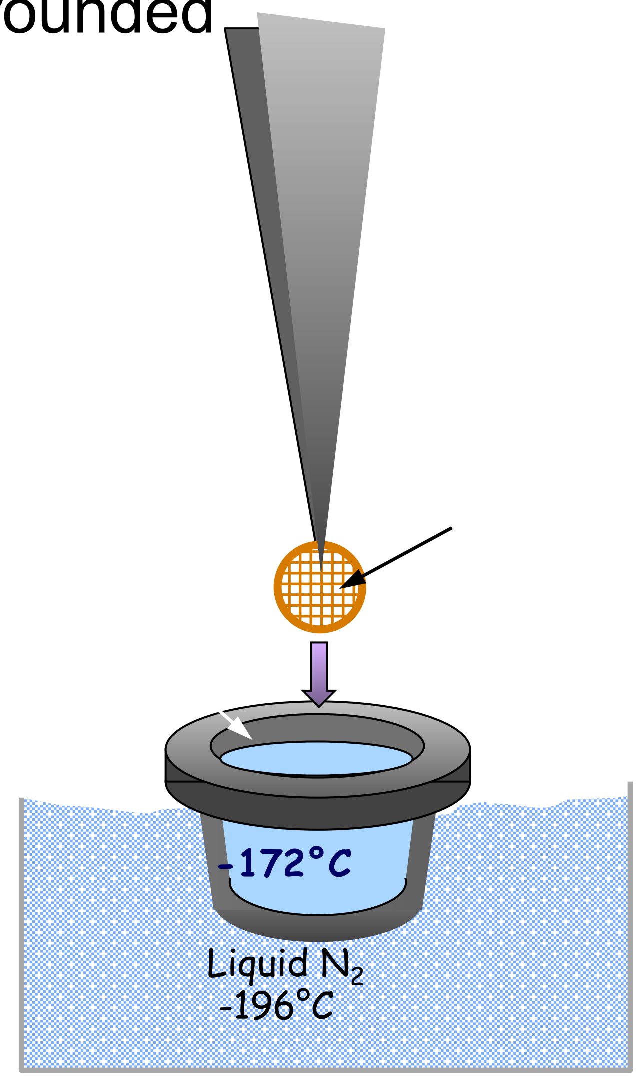
Cryo-Specimen Preparation

- Dubochet, J., Adrian, M., Chang, J.J., Homo, J.C., Lepault, J., McDowall, A.W. & Schultz, P. (1988). *Q Rev Biophys* **21**, pp. 129-228.
- Taylor, K.A., and Glaeser, R.M. (2008). Retrospective on the early development of cryoelectron microscopy of macromolecules and a prospective on opportunities for the future. *J Struct Biol 163,* 214-223*.*
- Megan J. Dobro, Linda A. Melanson, Grant J. Jensen, Alasdair W. McDowall (2010) Plunge Freezing for Electron Cryomicroscopy. *Methods in Enzymology* 481, 63-82.

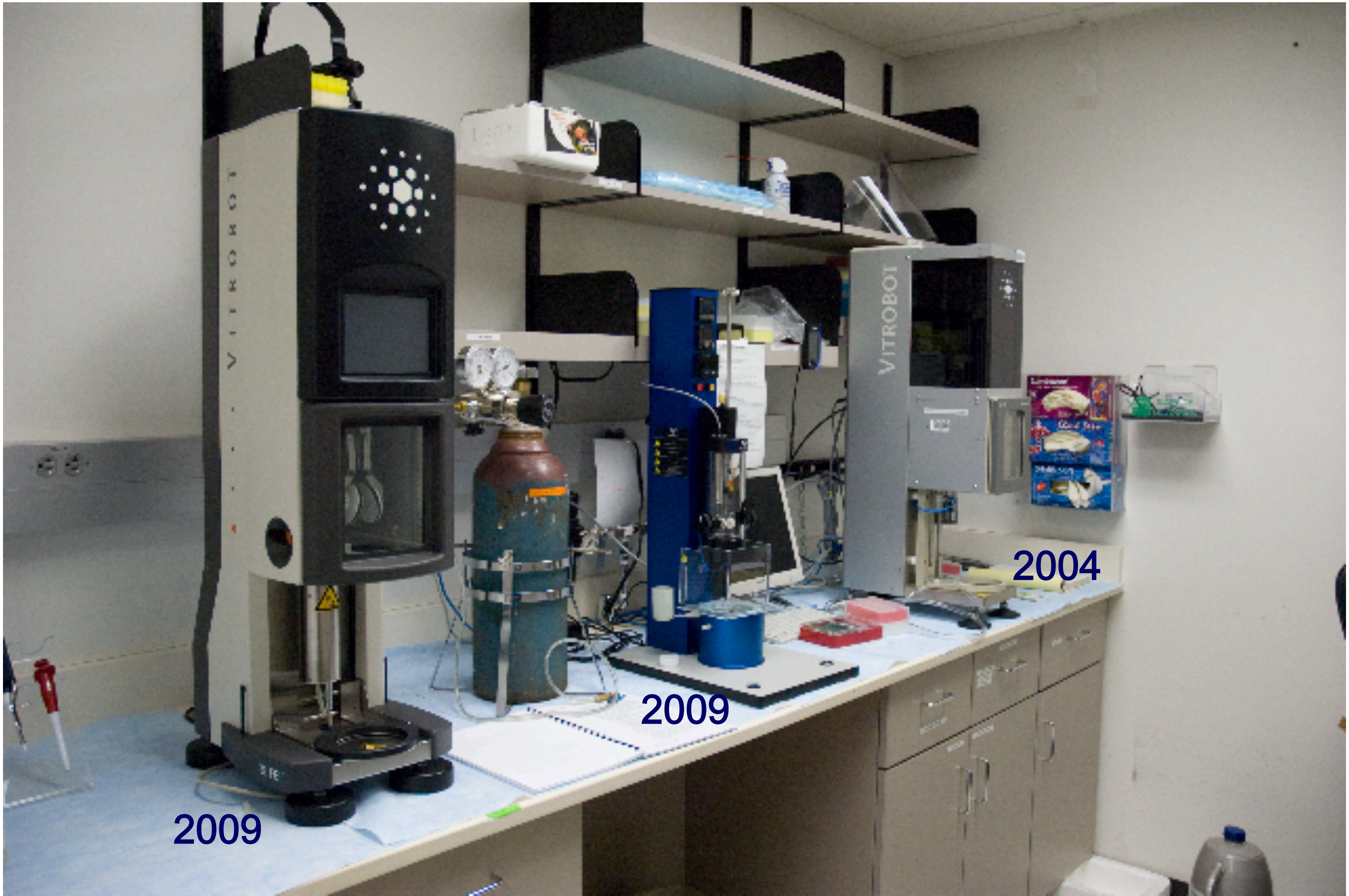

0.5 um
100 um
20 µm

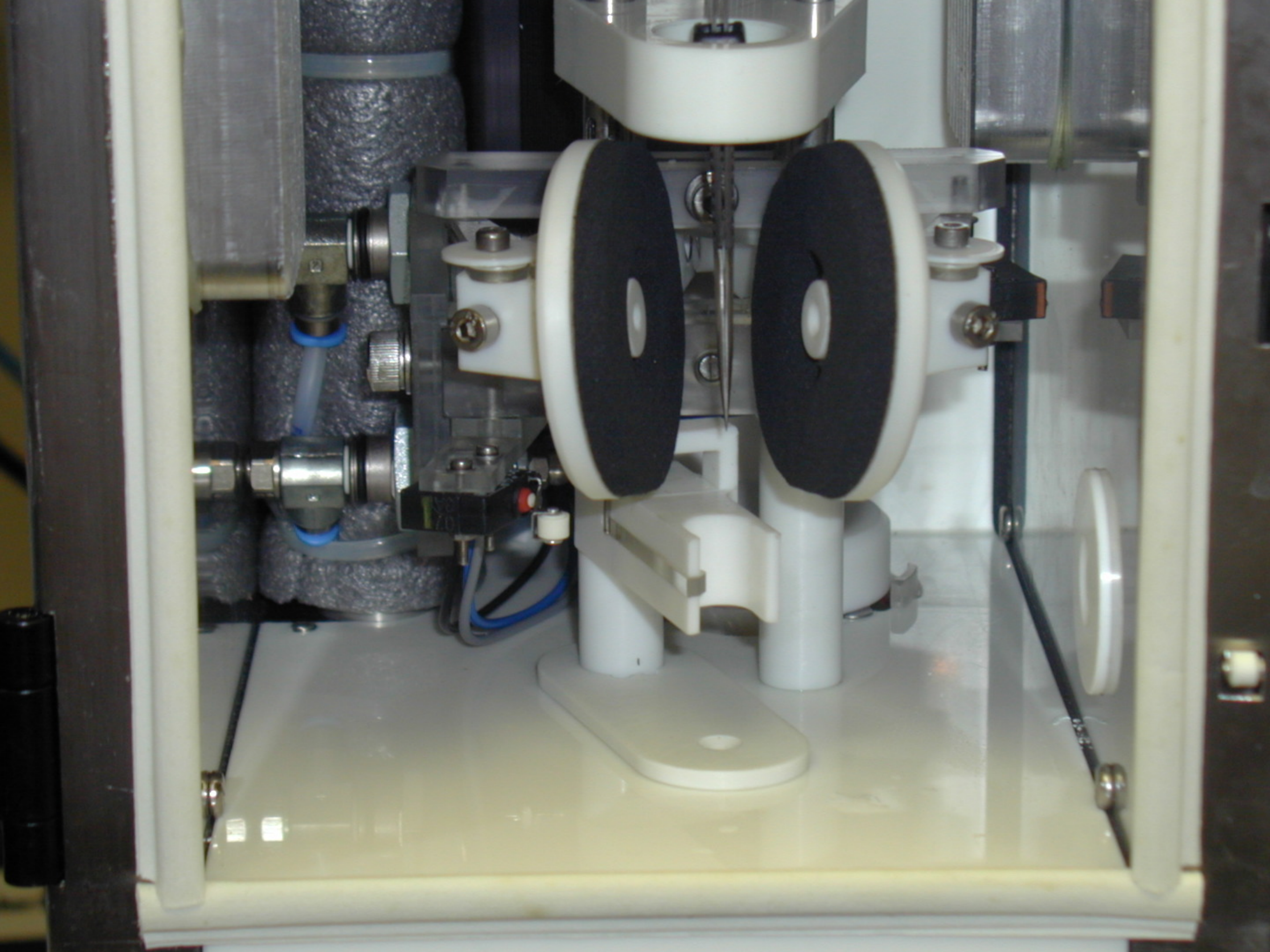
Basic Requirements for Vitrification Apparatus

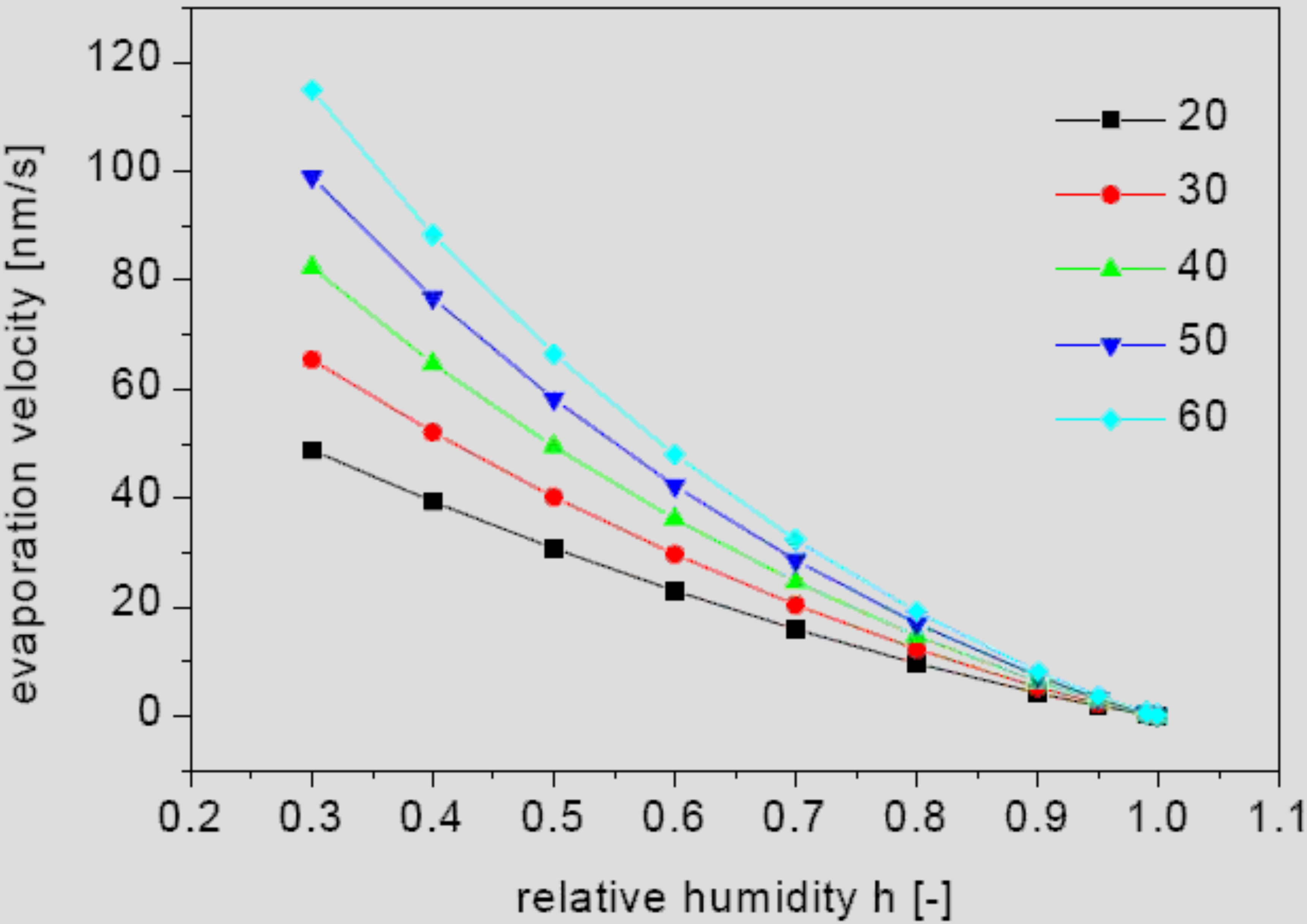
1. Ambient
   - low humidity & proper electrical grounded
2. Blotting mechanism
   - manual, pneumatic or electronic
3. Blotting chamber
   - high humidity
4. Plunging mechanism
   - high entrance velocity
5. Cryogen
   - high cooling efficiency



Cryo-Specimen Preparation



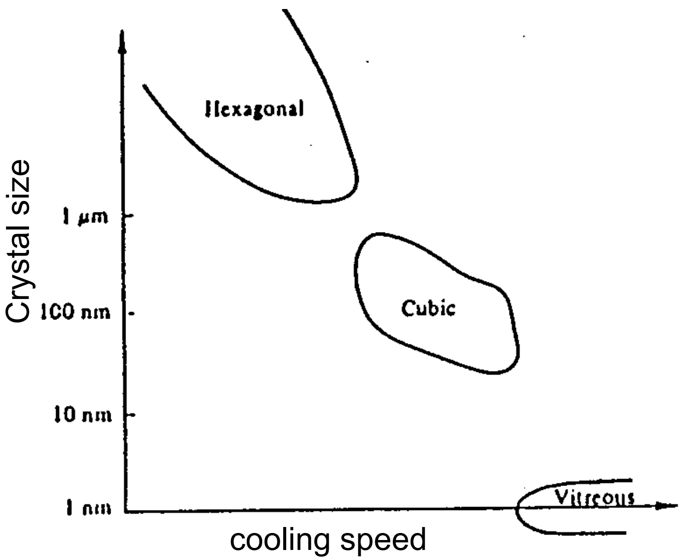




Courtesy Dr. Peter Frederik, University of Masstricht

Vitrification

Vitrification – immobilization of H_2O is achieved before its crystallization



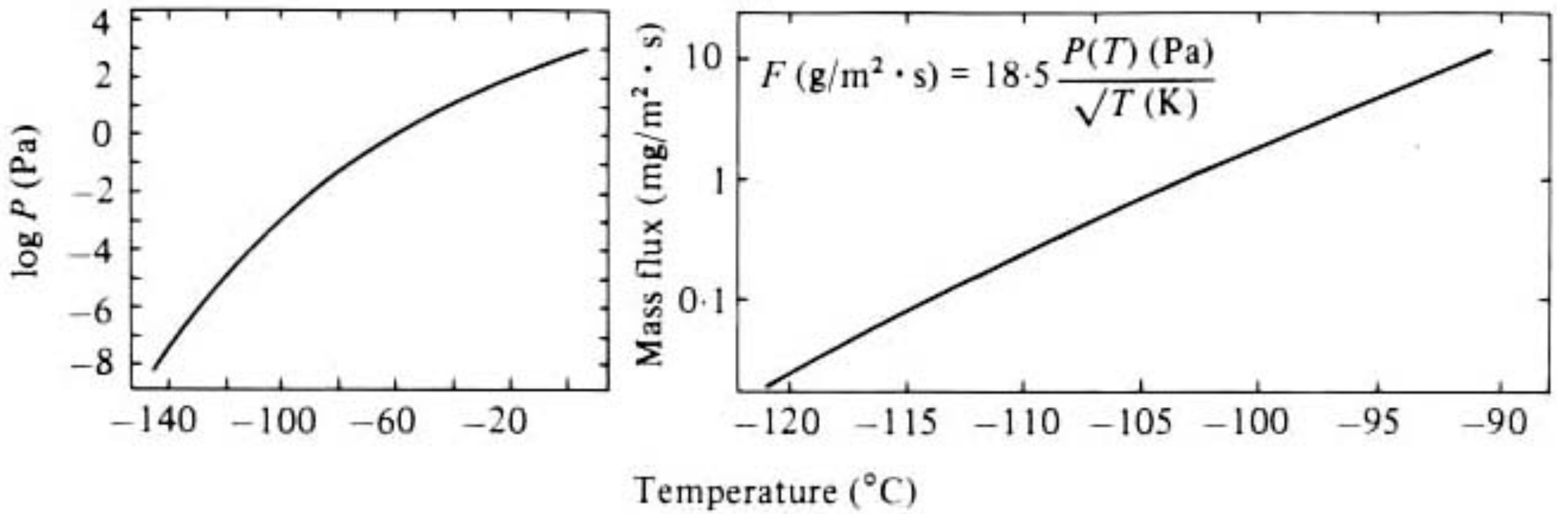
- rapid cooling rate
- small mass of specimen

<u>For pure water</u>:

cooling rate: **10^6 °C/sec** (for 1 μm thick layer)

vitrification time: t_V **= 10^{-4} sec**

Sublimation of ice in an electron microscope ?



(a) Vapour pressure *P* in Pa as function of the temperature in °C. (133Pa = 1 Torr; 10^5 Pa = 1 bar = 0.987 atm)

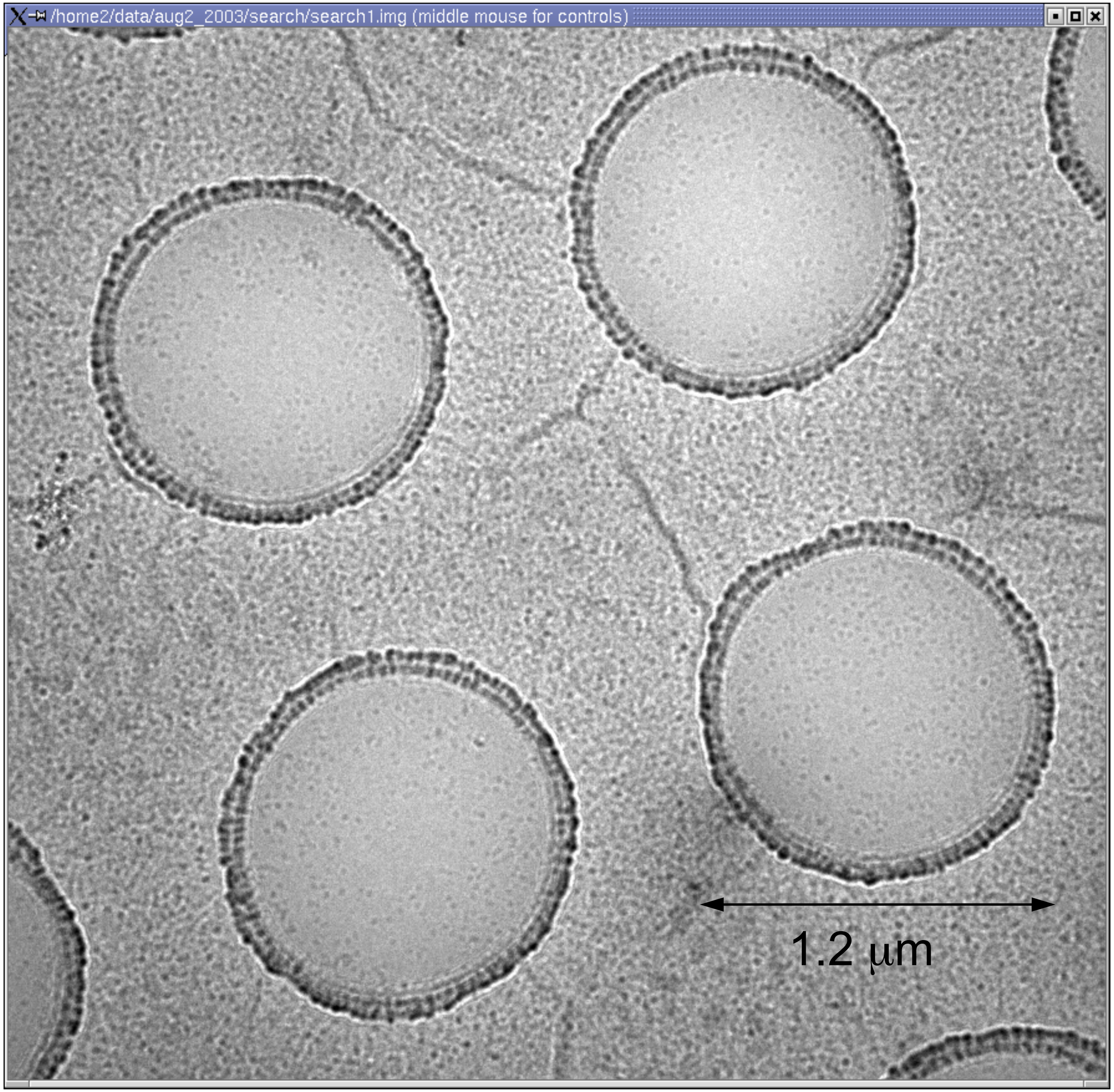
(b) Sublimation of ice in perfect vacuum as a function of the temperature in °C. (1 mg/m^2 corresponds to 1 nm thickness at a density of 1 g/cm^3).

** Sublimation of vitreous ice is very small at $T \leq T_v$

Courtesy of Dr. J. Dubochet

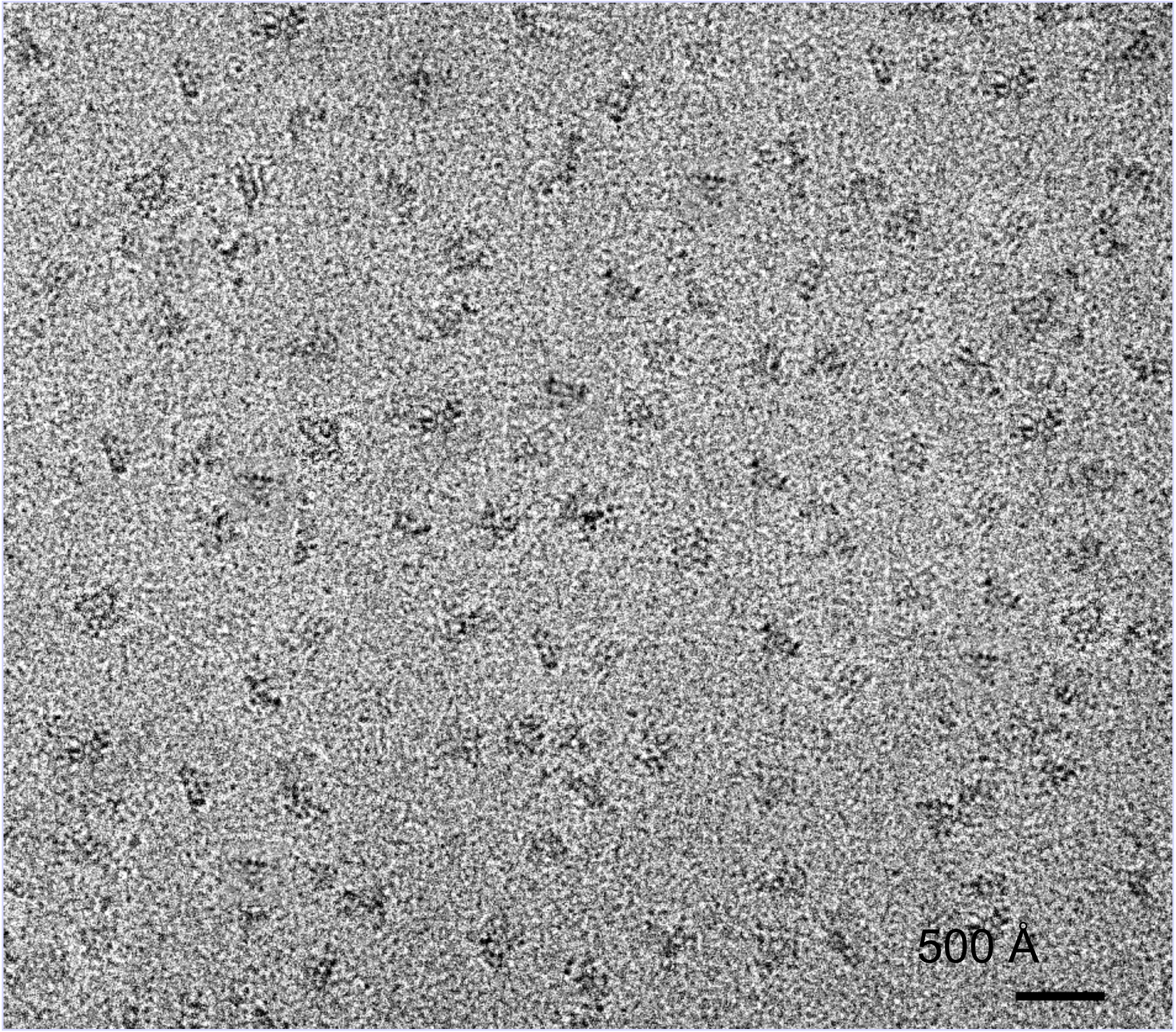
Vitrified Sample

in 20 mM Mops (pH 7.4), 300 mM KCl, 1 mM DTT, 0.4% CHAPS, 5% sucrose



Courtesy of
Dr. I Serysheva

200 kV image of ice-embedded Ion Channel



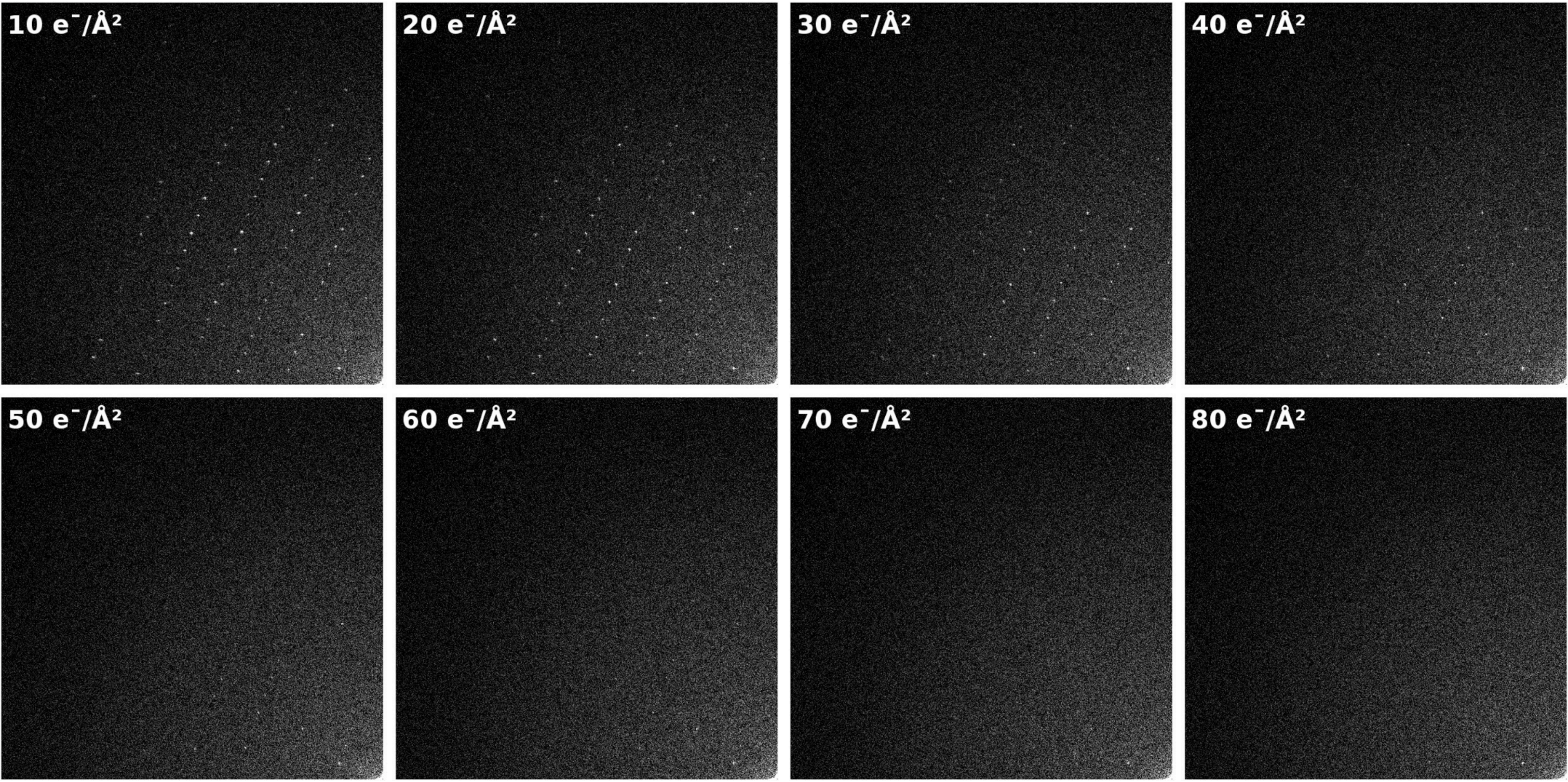
Courtesy of
Dr. I Serysheva

Radiation Damage Assessment of Protein Crystals

Record a series of 9-10 electron images or electron diffraction patterns from a single thin protein crystal (catalase crystal)

Measure quantitatively the fading of the diffraction spot intensities as a function of cumulative exposure (also known as dose)

Radiation Damage Studies of Ice Embedded Catalase Crystal



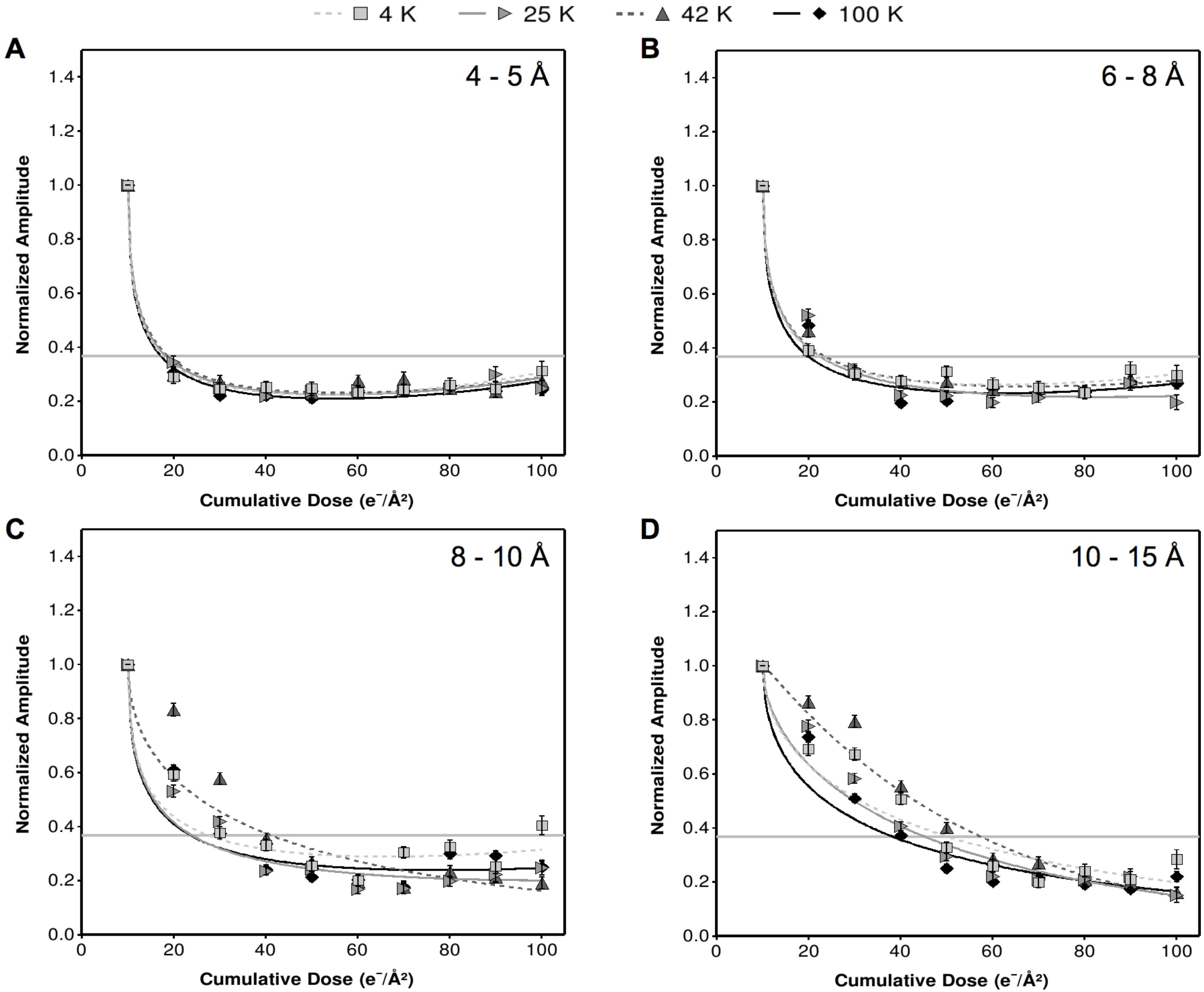
B Bammes, J Jakana, M Schmid, W Chiu *JSB* **169:** 331-341 (2010)

Quantification of Damage

N_e (1/e) decay dose

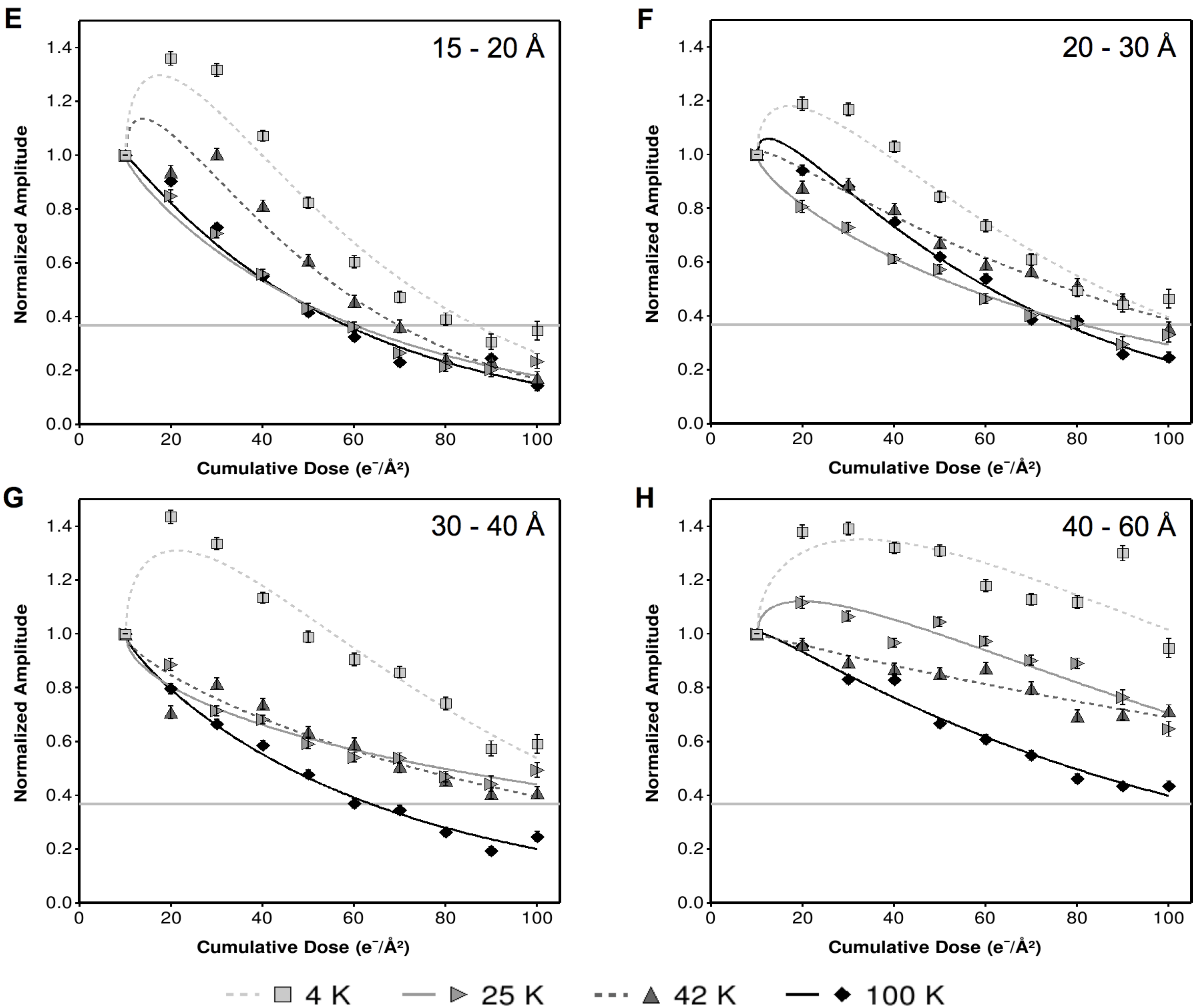
Dissimilarity factor

Fading of Fourier Amplitudes at different temperatures



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

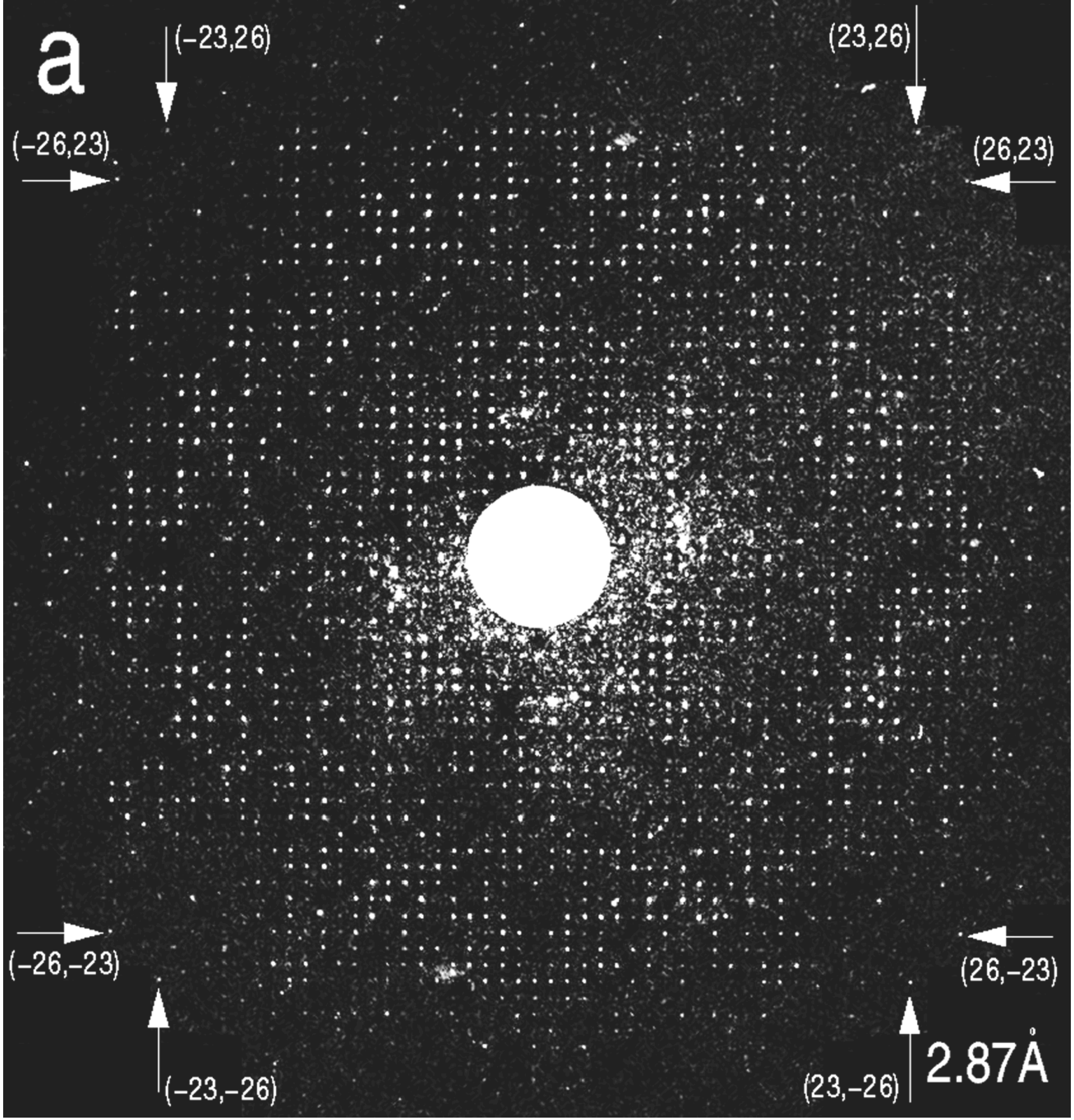
Fading of Fourier Amplitudes at different temperatures



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

Image of a 2D protein crystal

G Ren

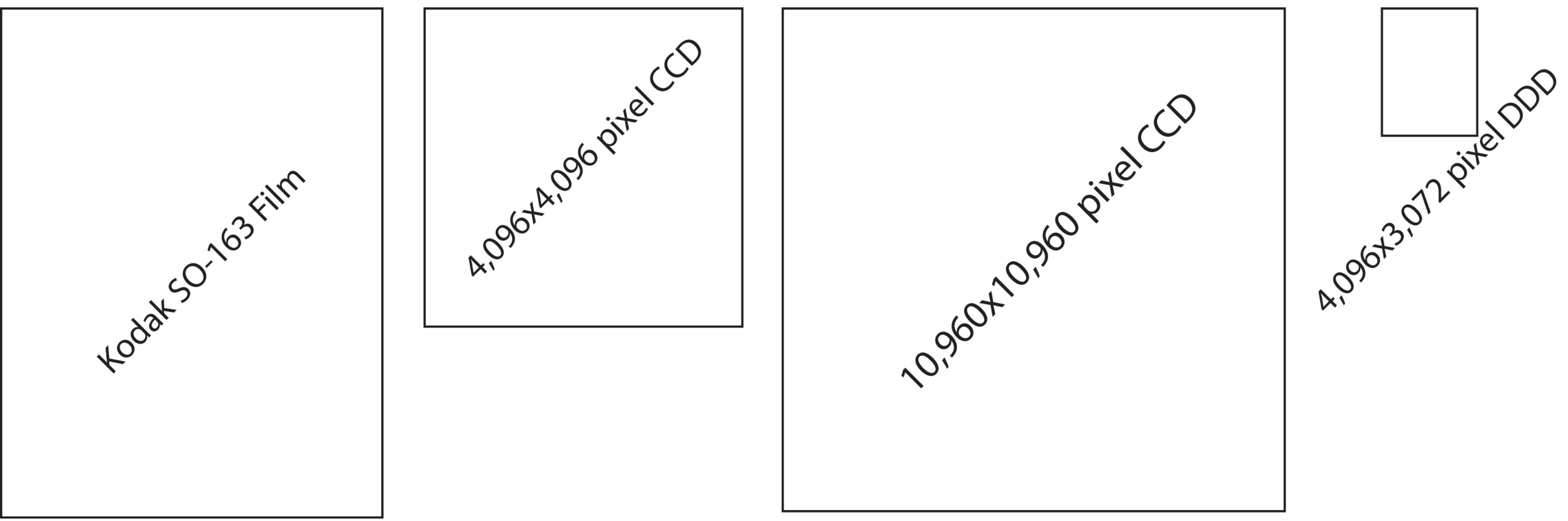


Electron Diffraction Pattern of a Protein 2-D Crystal

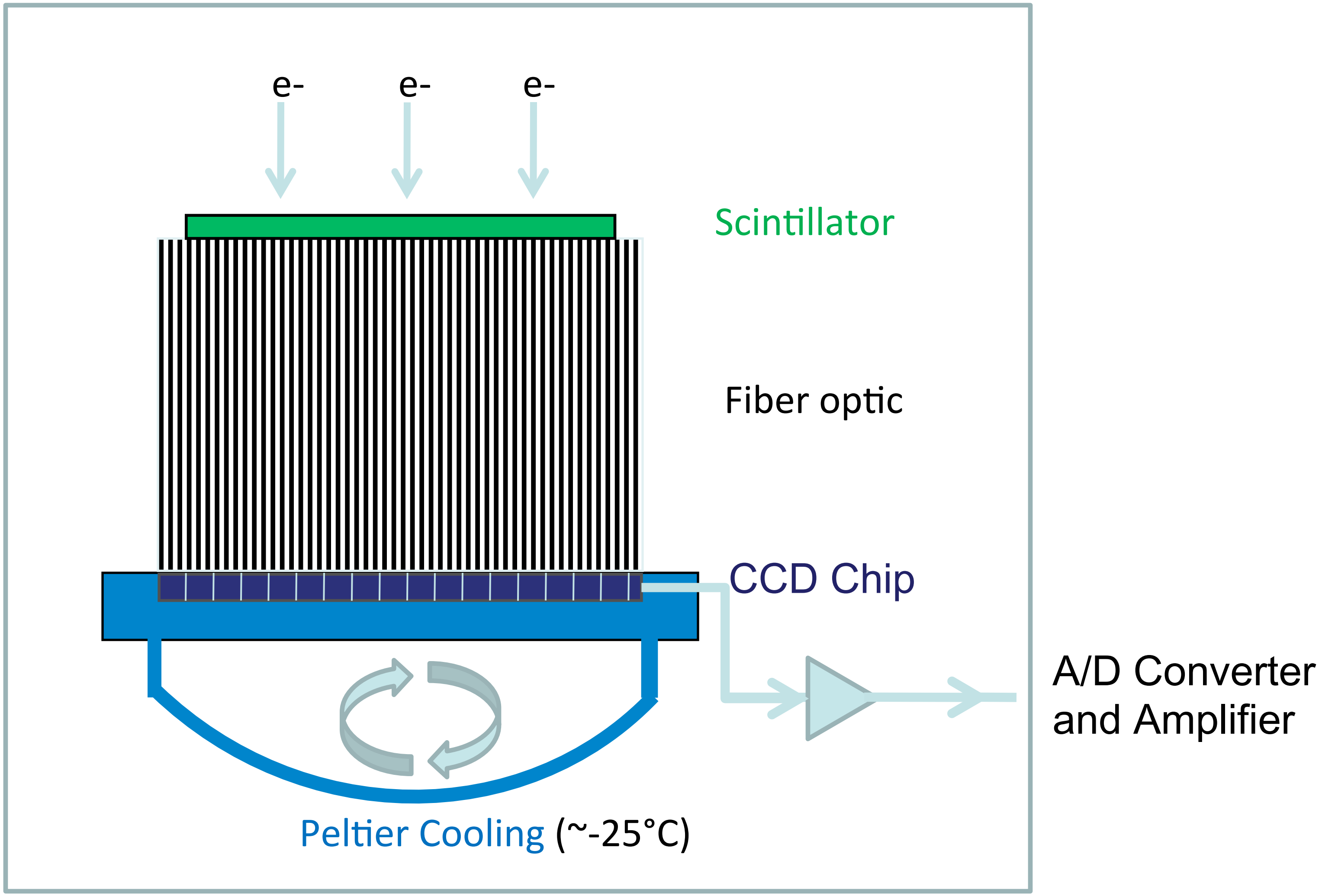
Ren & Mitra

Detector Physical Sizes

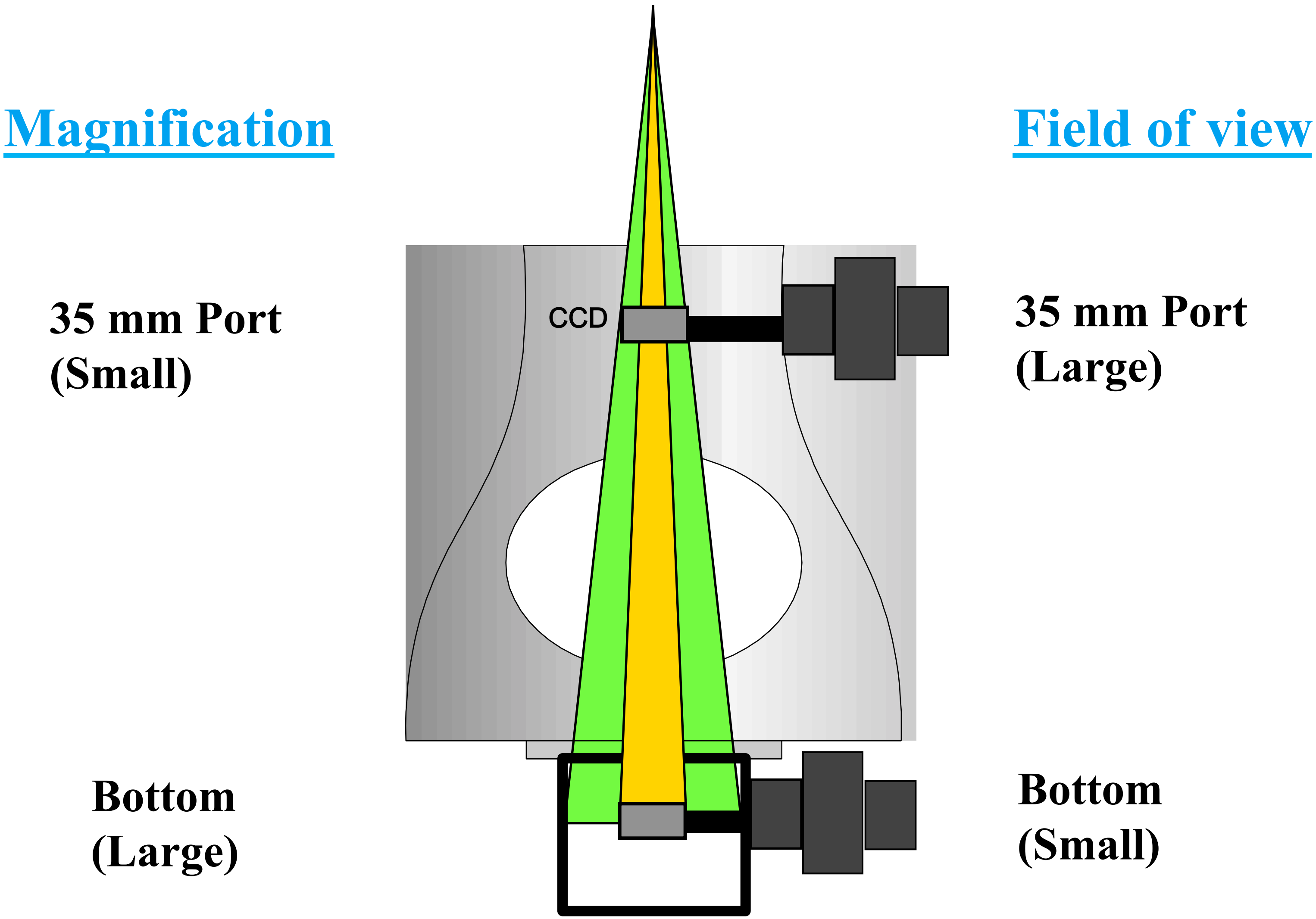
- 4x4 K CCD (16 megapixels with 15 µm pixel size)
- 10x10 K CCD (111 megapixel with 9 µm pixel size)
- 3x4 K DDD (12.6 megapixel with 6 µm pixel size)
- Photographic film



TEM CCD Architecture



Field of View and Microscope Ports

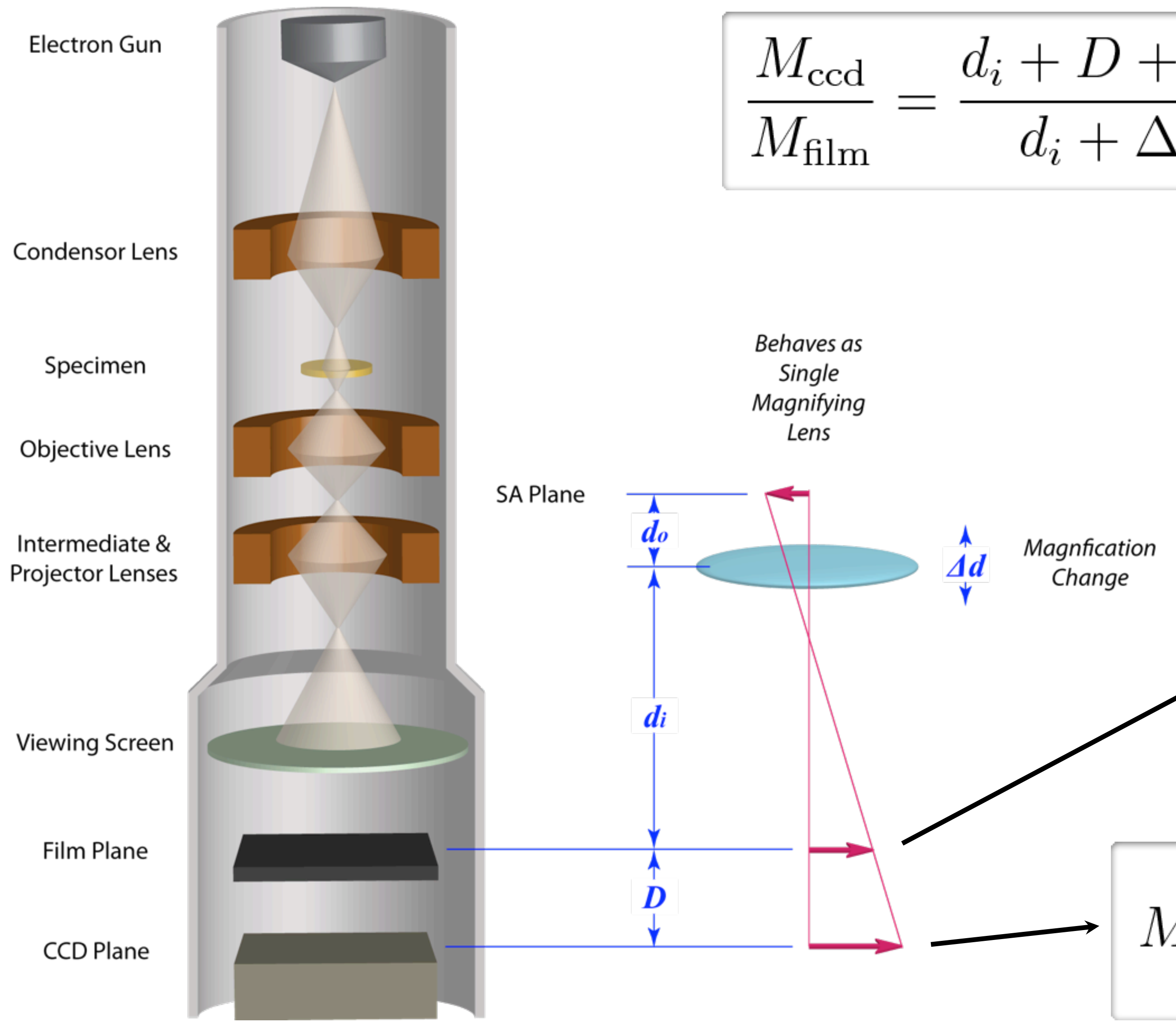


C Booth, Gatan

Magnification Factor

$$\frac{M_{\rm ccd}}{M_{\rm film}} = \frac{d_i + D + \Delta d}{d_i + \Delta d} = 1 + \frac{D}{d_i + \Delta d}$$

Depends on MAG



$$M_{\rm film} = \frac{d_i + \Delta d}{d_o - \Delta d}$$

$$M_{\rm ccd} = \frac{d_i + D + \Delta d}{d_o - \Delta d}$$

Δd varies in different magnification of the scope

- Resolution: Simply, the ability to distinguish contrast (usually fine detail.) Often expressed in conjunction with MTF.
- Sensitivity: The level of (incoming) signal required to produce an intensity (output) change.
- Point Spread Function (PSF): Distribution of photons from incoming electron (i.e., cloud due to random motion of electron.)
- Dynamic Range: Simply, the range of values that can be distinguished between a maximum level and zero (noise.) Driven by combination of full well (affected by pixel size) and noise baseline with no exposure. Can be increased with binning.
- DQE (Detection Quantum Efficiency): Best "overall" measure of a camera's ability to transfer signal accounting for noise from sensor to output. Expressed over a range of spatial frequencies. Noise-free image DQE = 1.

C Booth, Gatan

Resolution (d) in object (real) space and Frequency (s) in Fourier (diffraction) space

Object space resolution: d (nm)

Total sampling points in an image: N

Fourier space sampling distance: Δs (1/nm) = 1/(Nd)

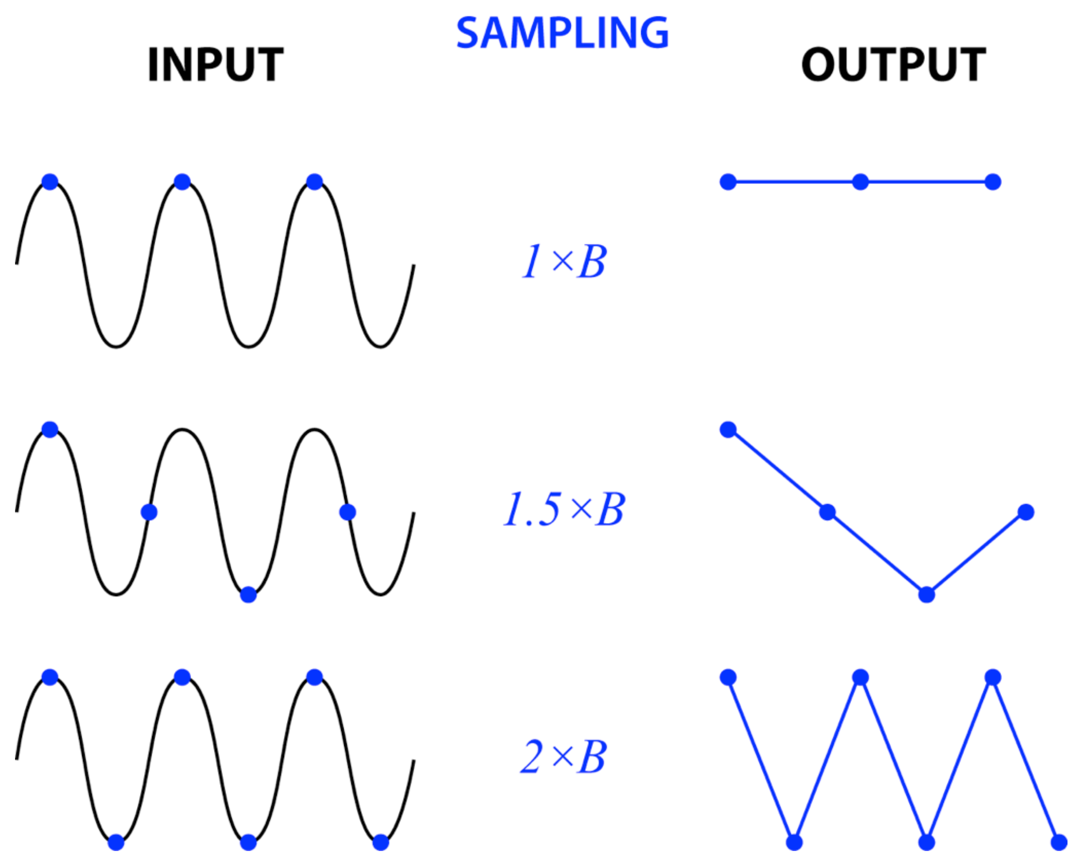
Maximum frequency (Nyquist) in Fourier space

$s_{max} = ½ d$

Nyquist Frequency

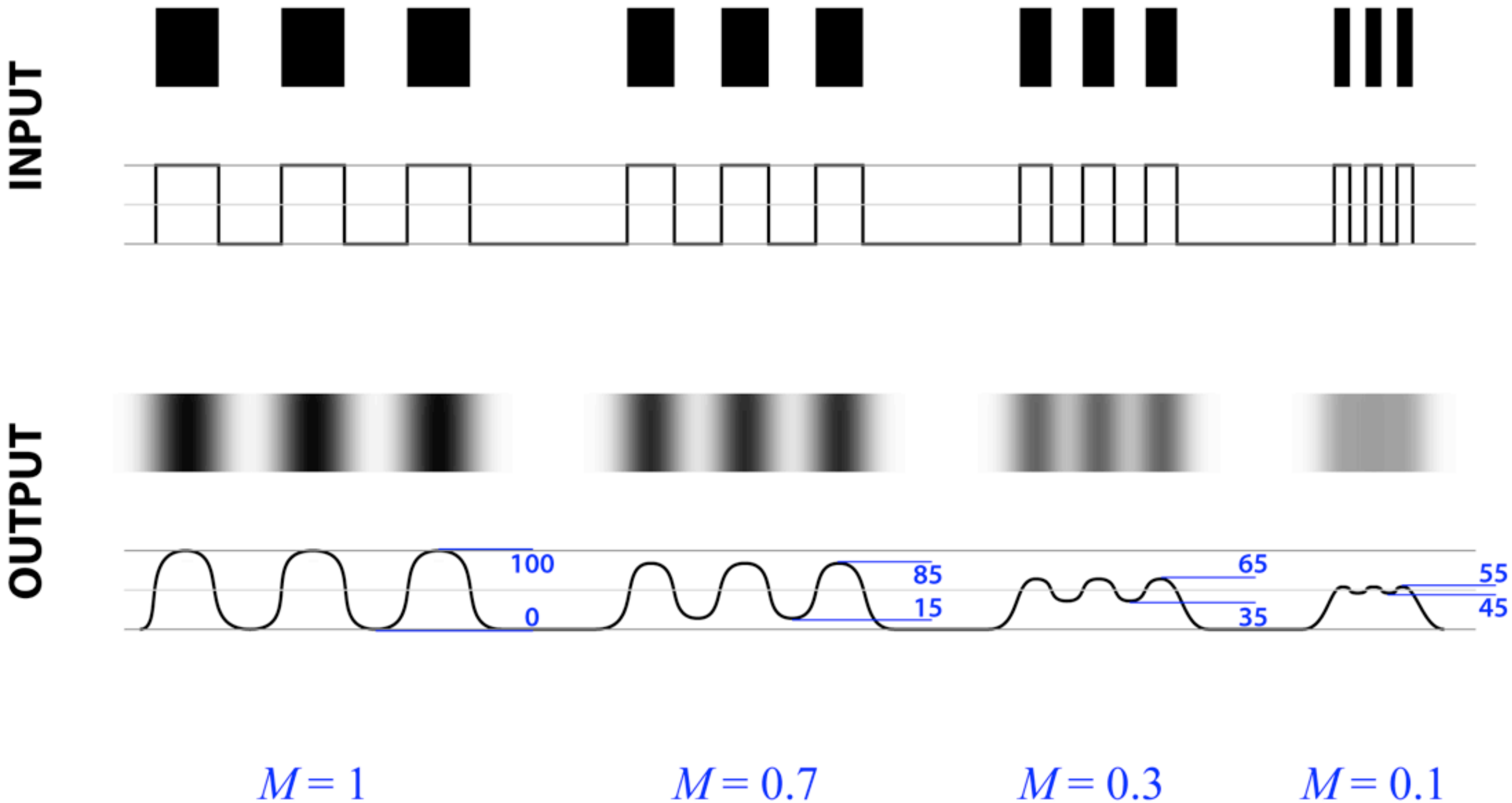
Nyquist-Shannon Sampling Theorem

If a signal contains no information at frequencies higher than S_{max}=B, then it can be reconstructed by sampling with a frequency of $2S_{max}$ or higher

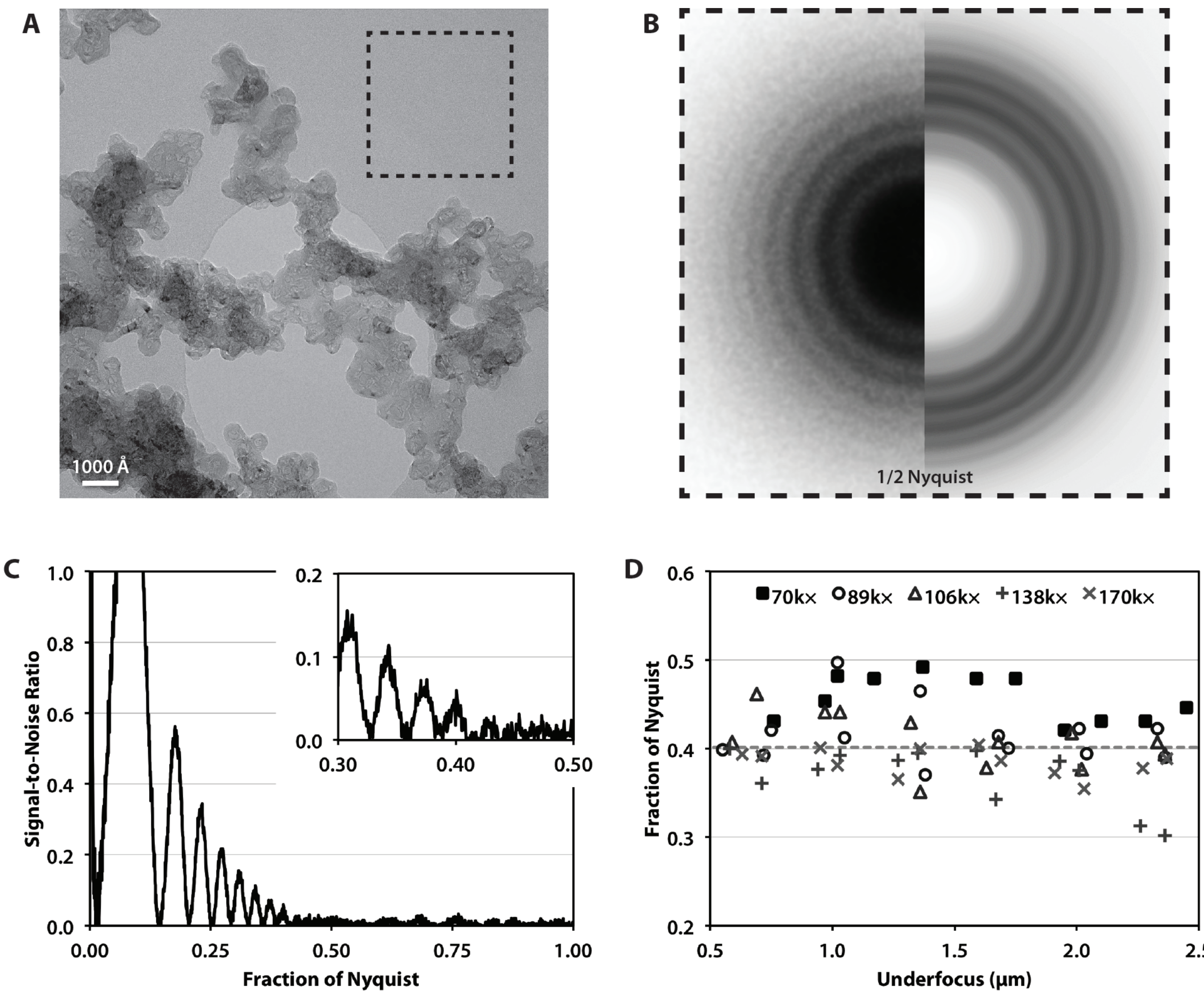


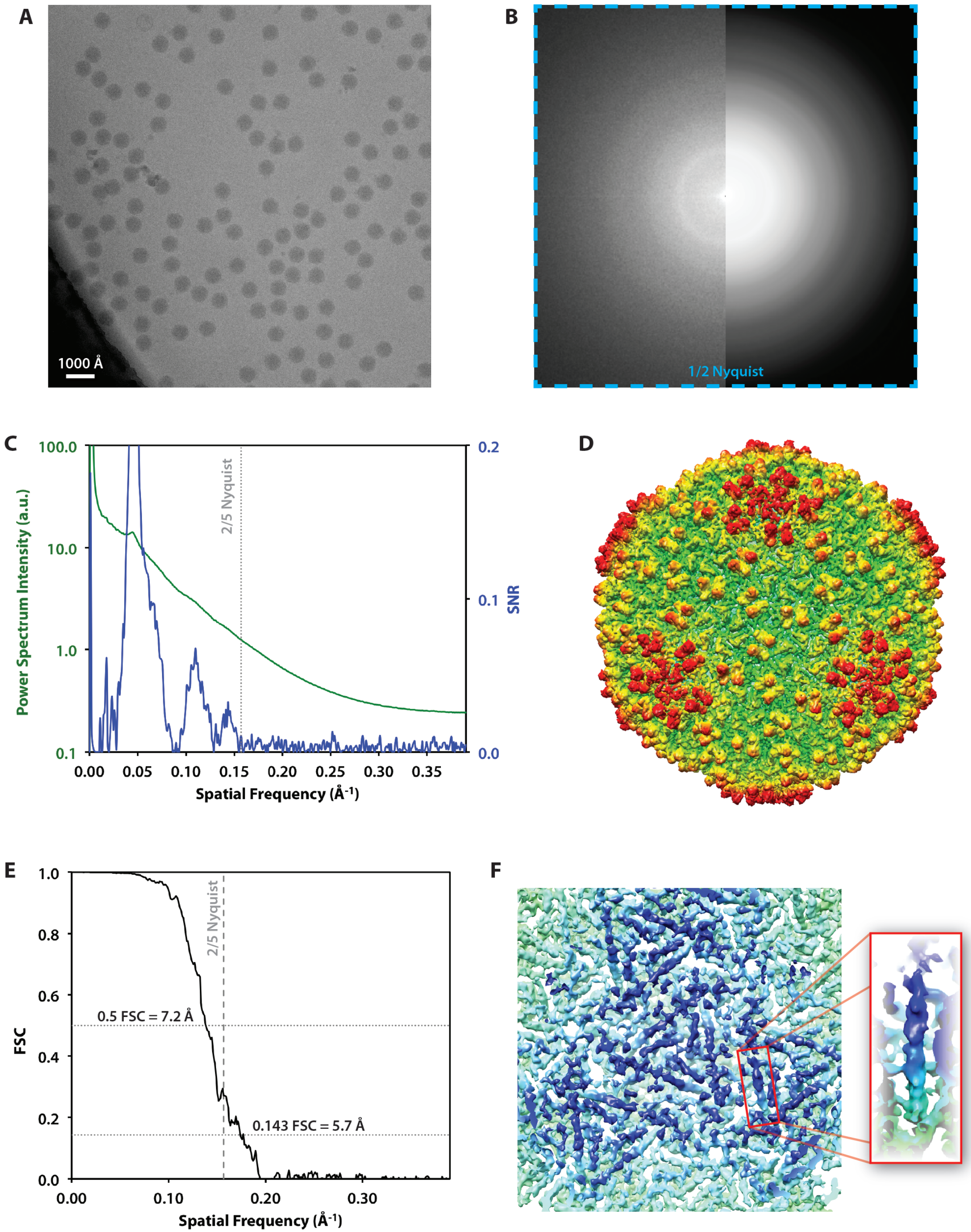
Modulation Transfer a Function of Frequency

$$M = (I_{max} - I_{min})/(I_{max} + I_{min})$$



Practical Assessment of CCD Using C-film Image





Gatan 10 K CCD for 300kV at ~70K x mag

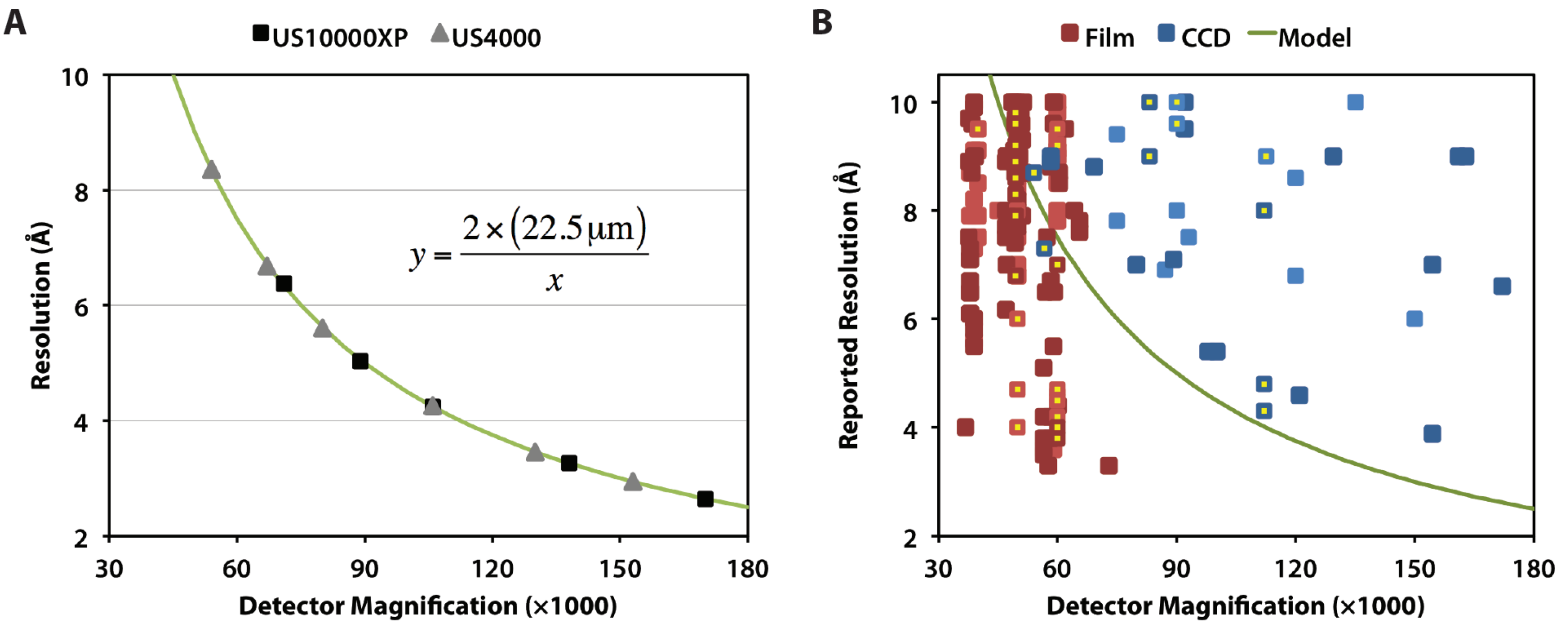
Bammes *JSB* 175(3): 384-393 (2011)

Gatan 4k CCD Performance w/ 300kV

Scope Mag	Detector Mag	Pixel size	$(\text{Nyquist})^{-1}$ (Å)	$(1/2\text{Nyquist})^{-1}$ (Å)	$(2/3\text{Nyquist})^{-1}$ (Å)
30,000	42,000	3.57	7.14	14.29	10.71
40,000	56,000	2.68	5.36	10.71	8.04
50,000	70,000	2.14	4.29	8.57	6.42
60,000	84,000	1.79	3.57	7.14	5.37
80,000	112,000	1.34	2.68	5.36	4.02
100,000	140,000	1.07	2.14	4.28	3.21
120,000	168,000	0.89	1.79	3.57	2.67
150,000	210,000	0.71	1.43	2.86	2.13

Donghua Chen et al (2008) *JSB*

CCD Detector Resolution vs Magnification

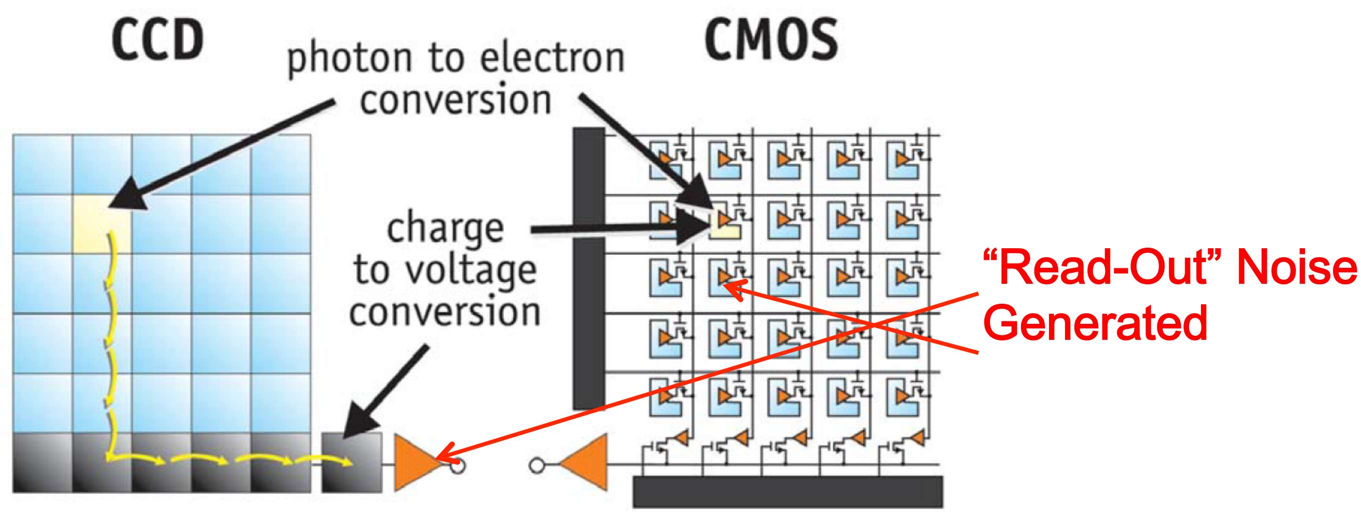


Bammes et al. *JSB* 175(3): 384-393 (2011)

Sensor type plays major role in camera performance and optimization:

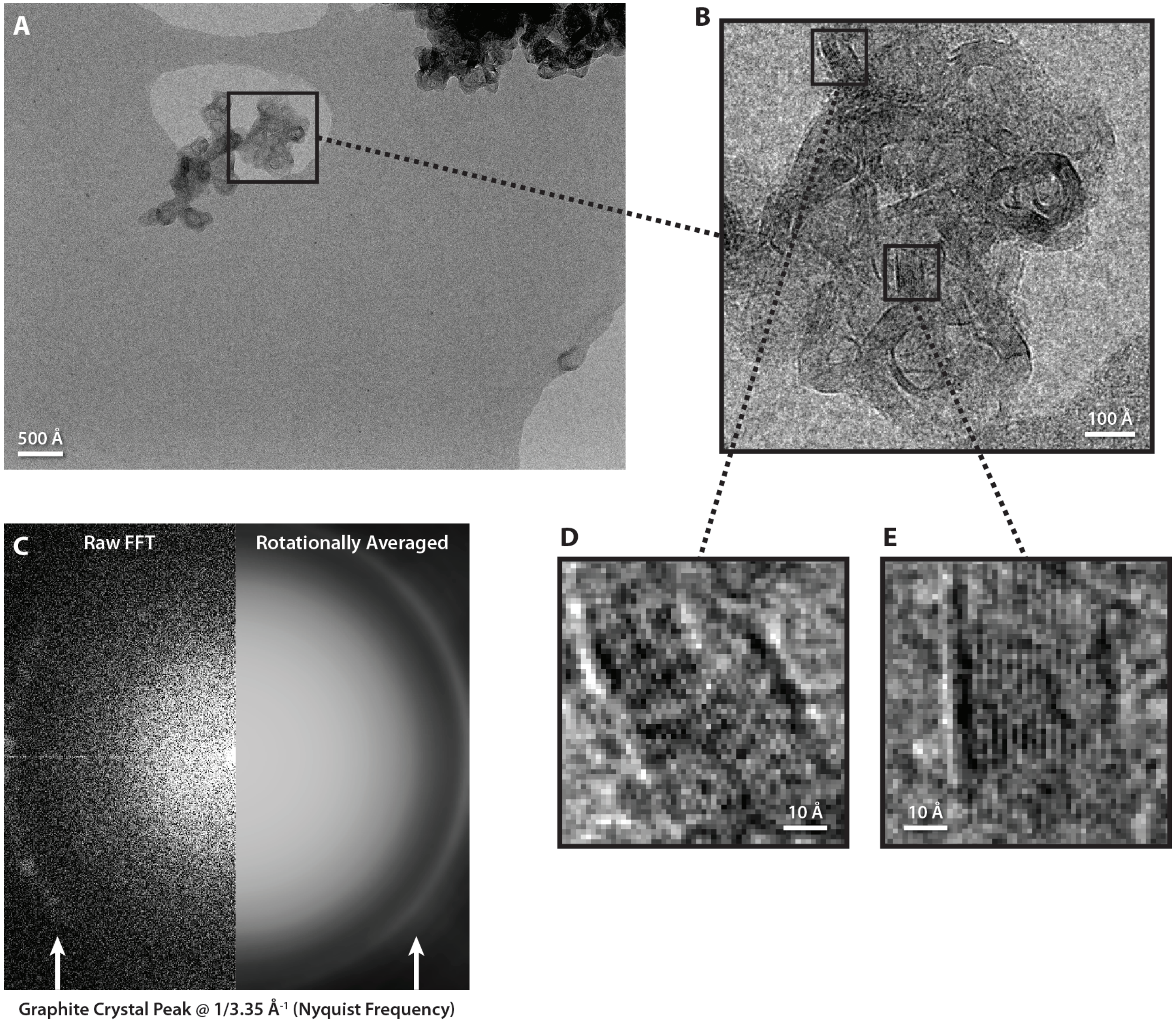
- CCD: Charge is transferred between neighboring cells, and "read-out" (i.e., noise) is seen at final stage: Binning minimizes impact of read-out noise.
- CMOS: Charge immediately converted to voltage (read out with digital output): Supports high frame rates, low overall electronics noise.

http://en.wikipedia.org/wiki/Active_pixel_sensor



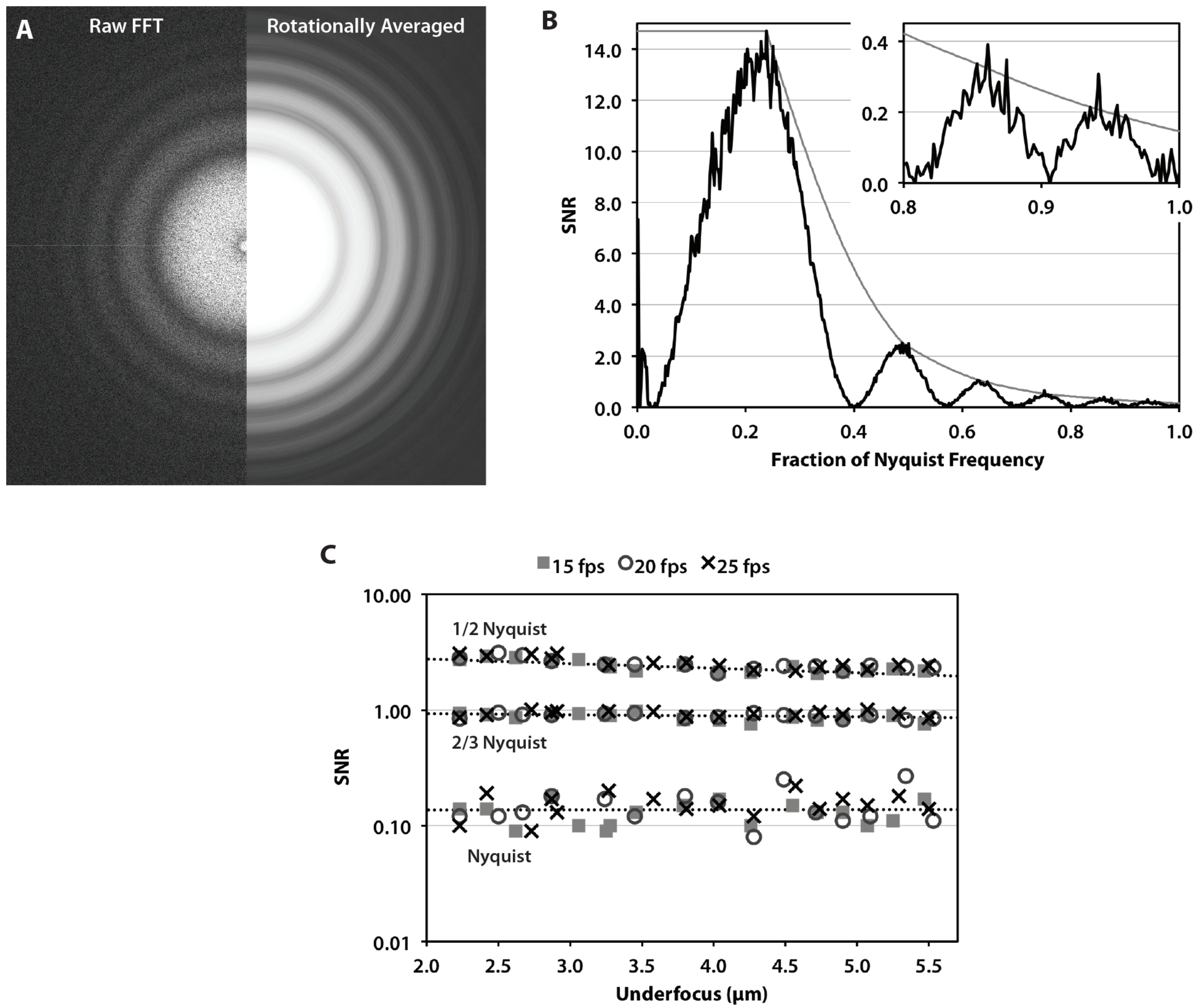
CCDs move photogenerated charge from pixel to pixel and convert it to voltage at an output node. CMOS imagers convert charge to voltage inside each pixel.

DDD Performance with Graphite

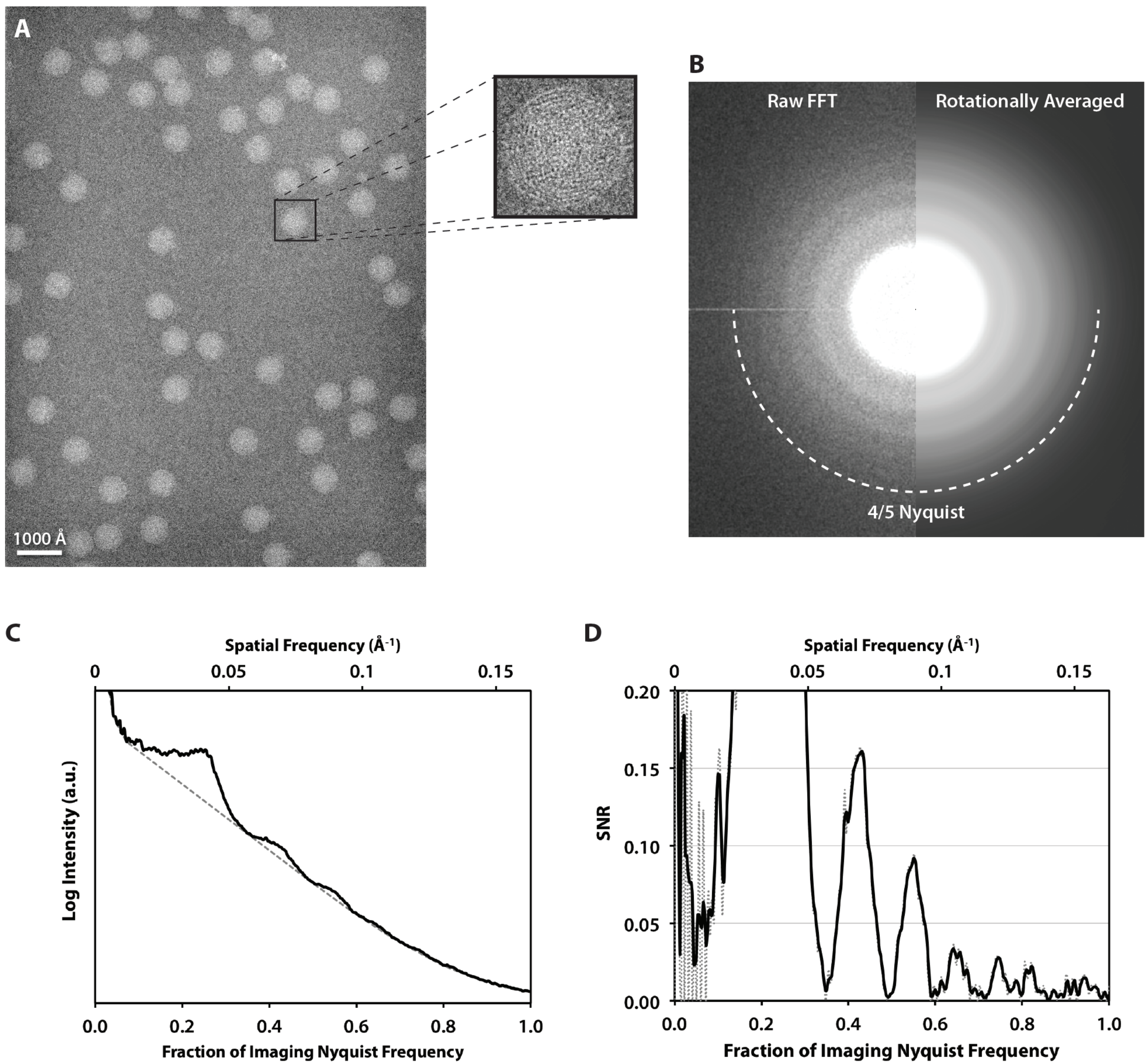


Bammes et al (2012) *JSB* **177**:589-601.

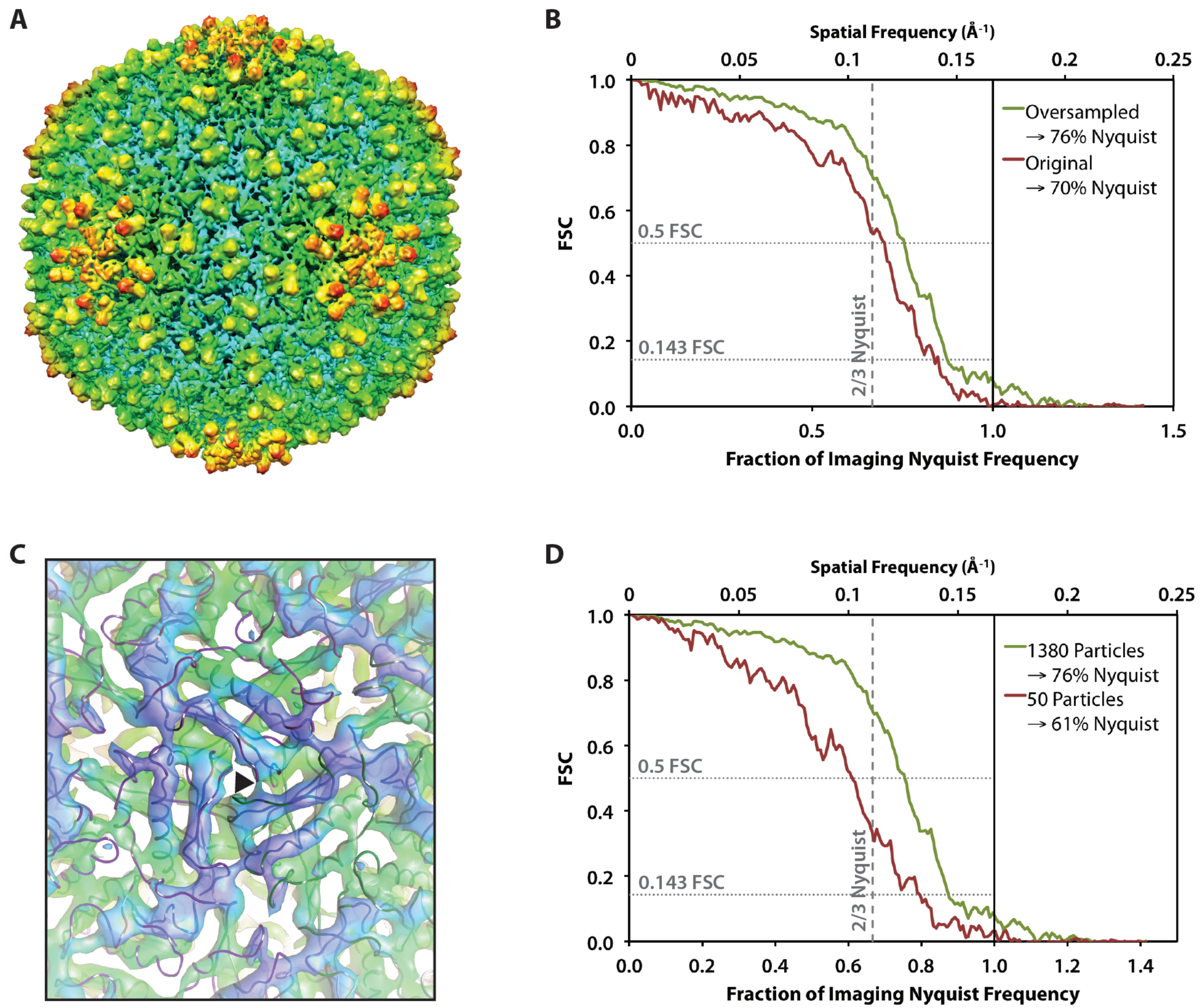
DDD performance test with C-film

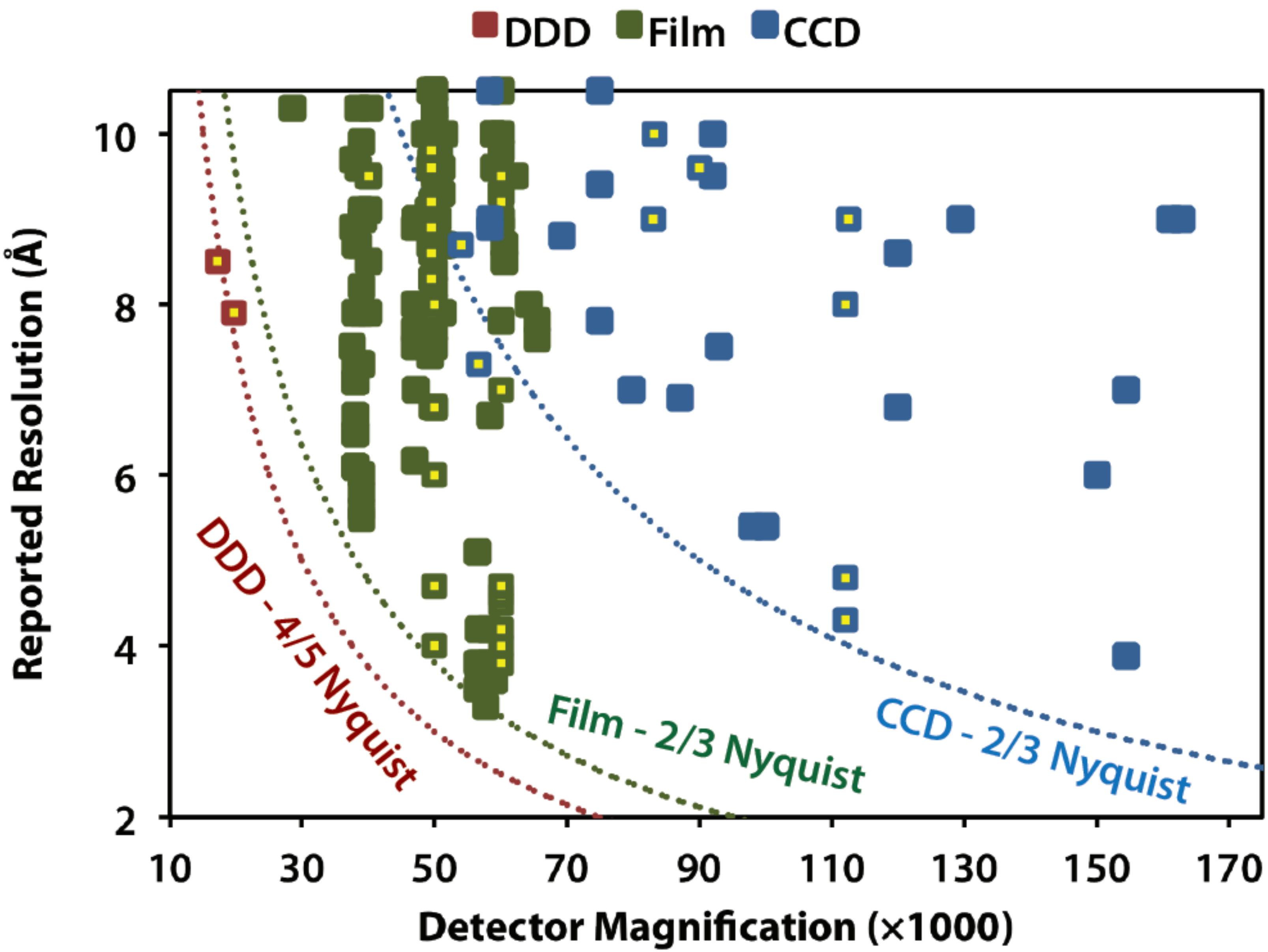


DDD performance with virus particle



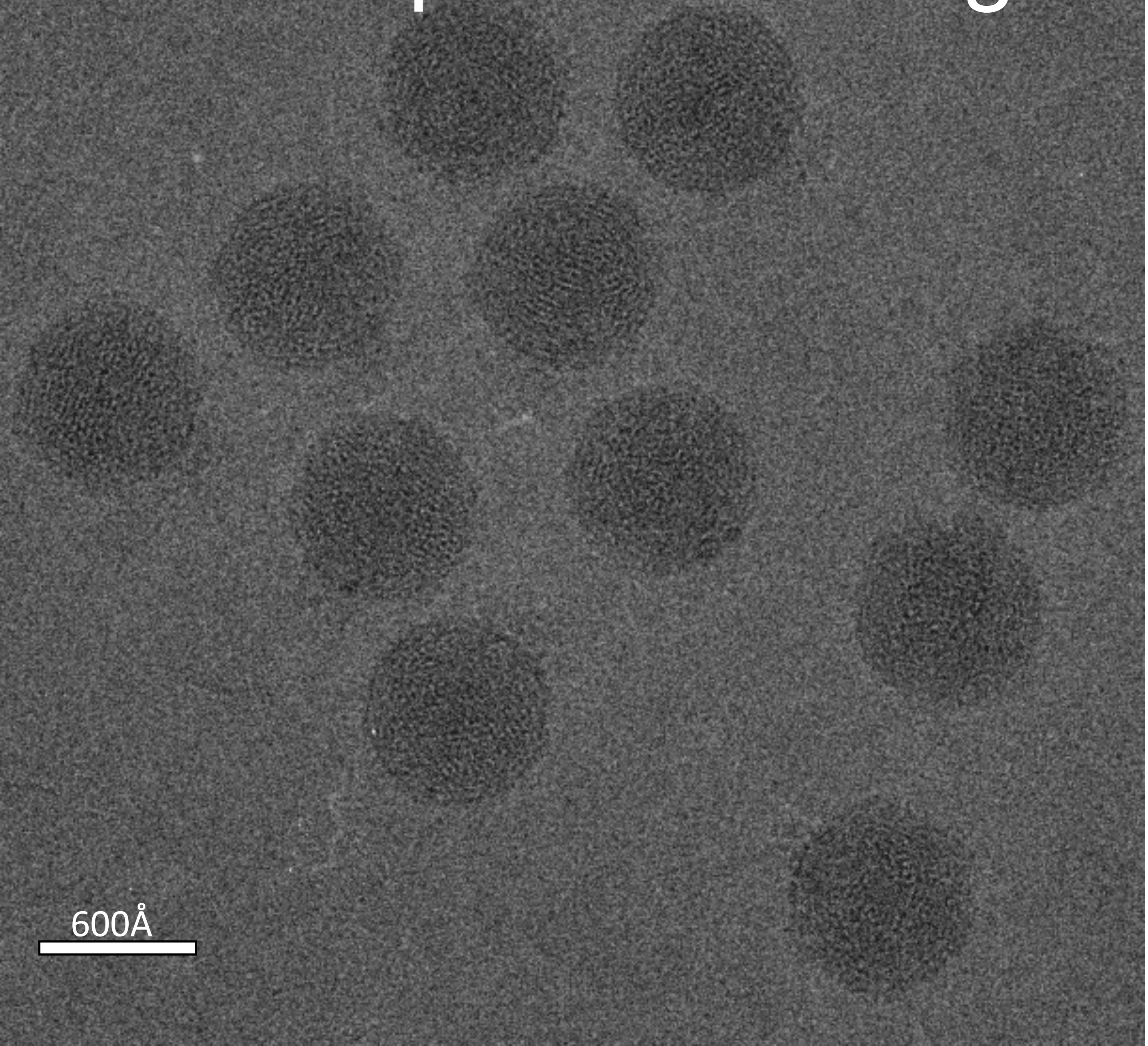
DDD performance with virus particle





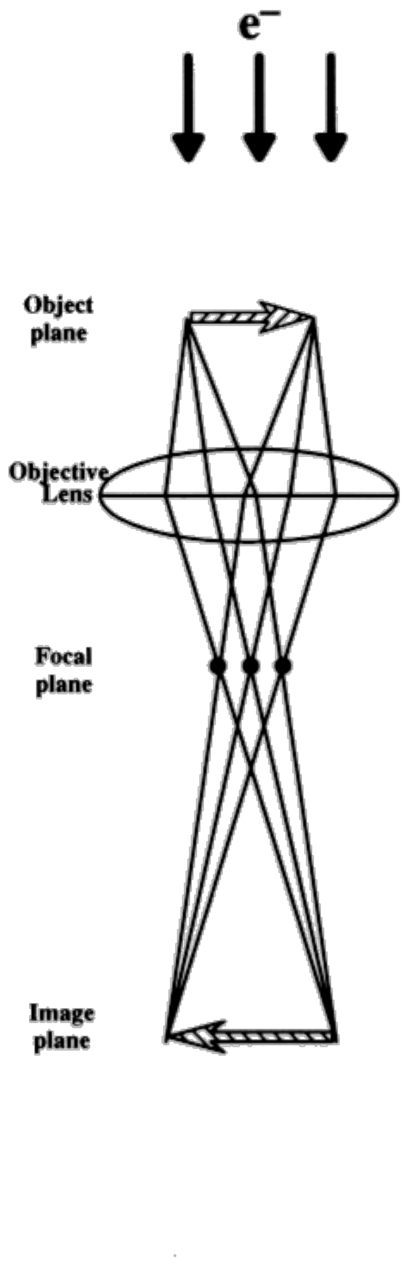
DDD
Film
CCD
Reported Resolution (Å)
10
8
6
4
2
10
30
50
70
90
110
130
150
170
Detector Magnification (×1000)
DDD - 4/5 Nyquist
Film - 2/3 Nyquist
CCD - 2/3 Nyquist

Epsilon15 Phage



- Vitrobot
- JEM3200FSC
- 300 kV
- In column energy filter
- Recorded on films
- Scanner: Nikon

Joanita Jakana at NCMI, Houston



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

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

$$\Psi_o(x_o, y_o) \simeq 1 + i\sigma\nu(x_o, y_o)$$

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

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

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

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

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

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

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

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

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

Image intensity $I_i(x_i, y_i)$

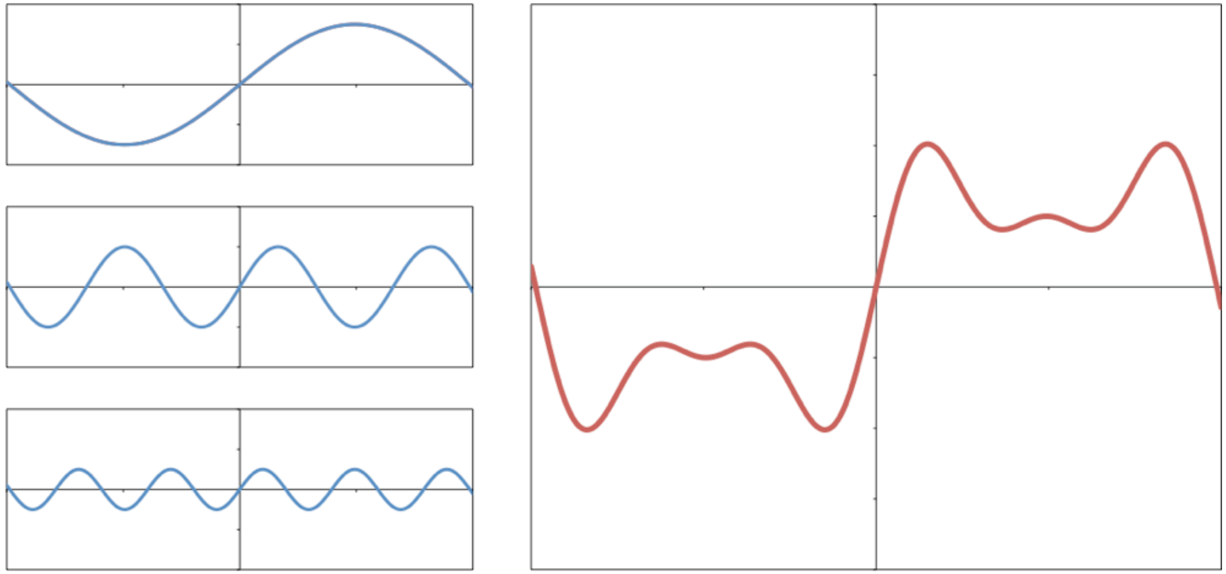
$$I_i(x_i, y_i) = \delta(0,0) - 2\sigma\nu(x_i, y_i) * \mathfrak{F}^{-1}\left[\sin \gamma(S)\right]$$

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

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

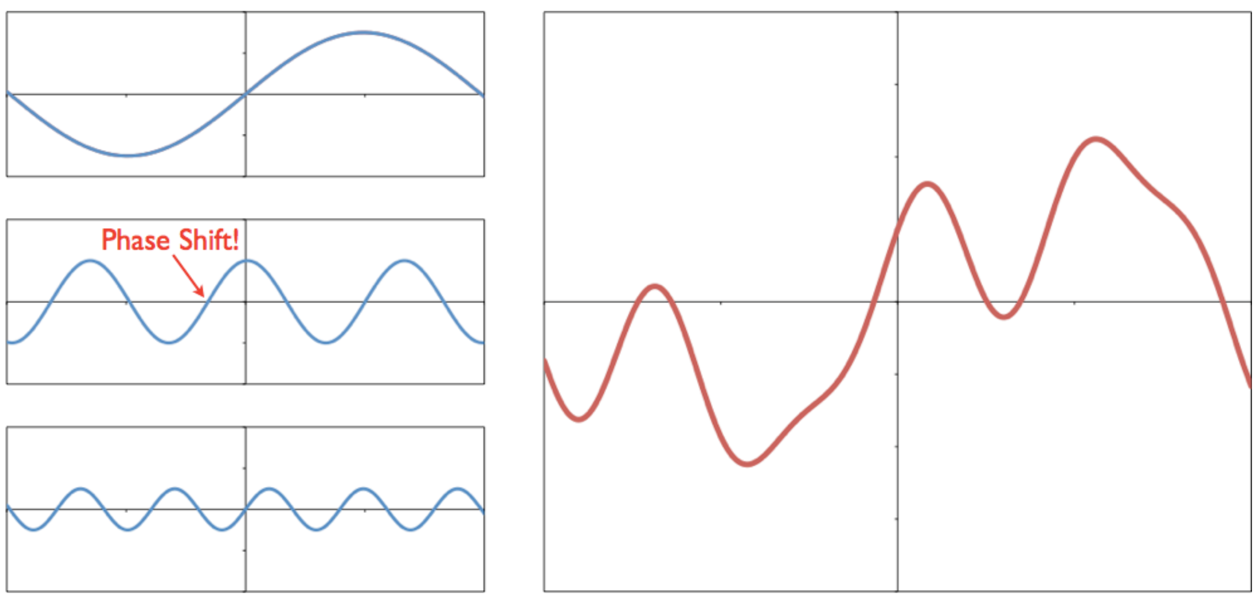
Individual Sine Curves

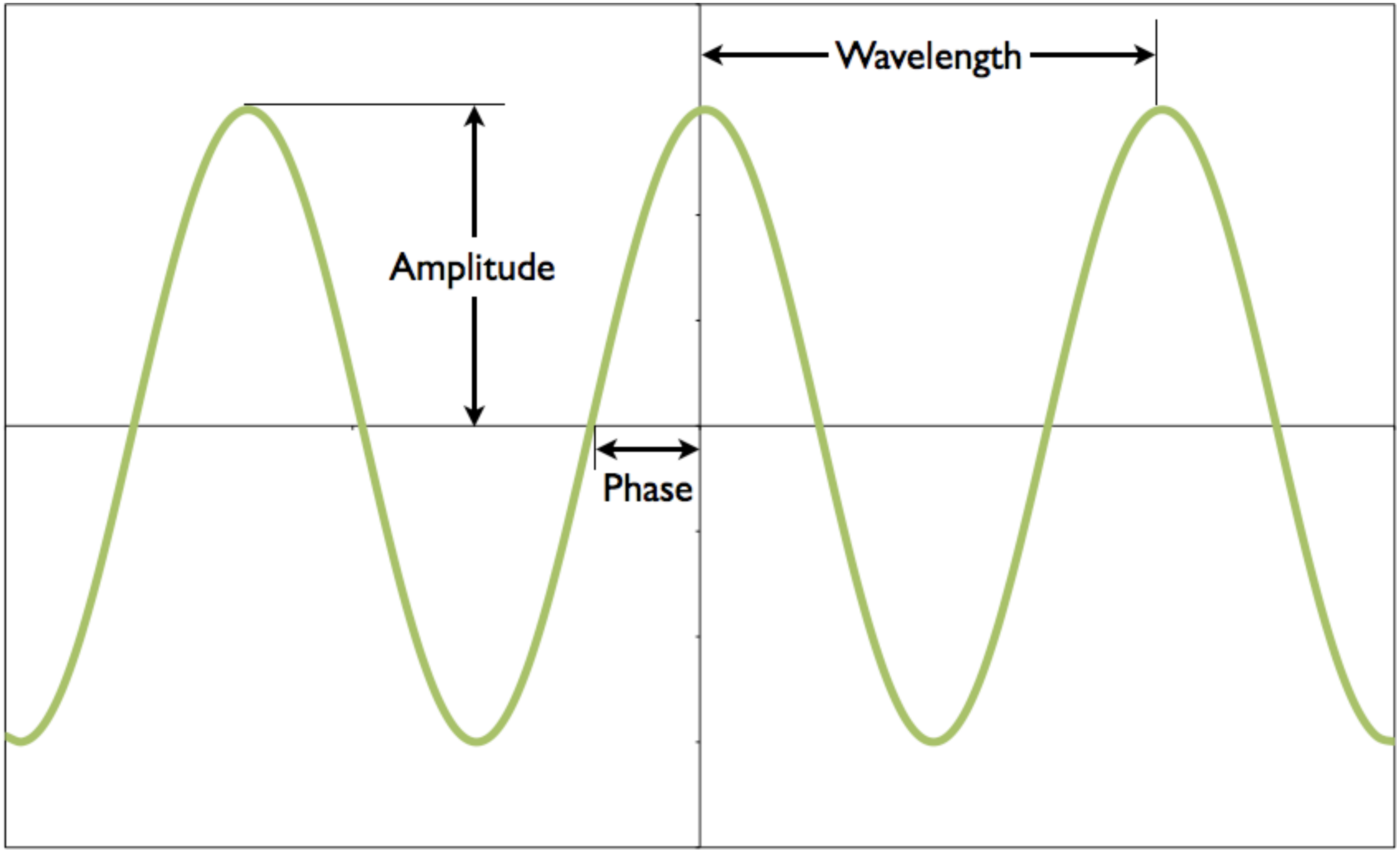
Sum of All Three Curves



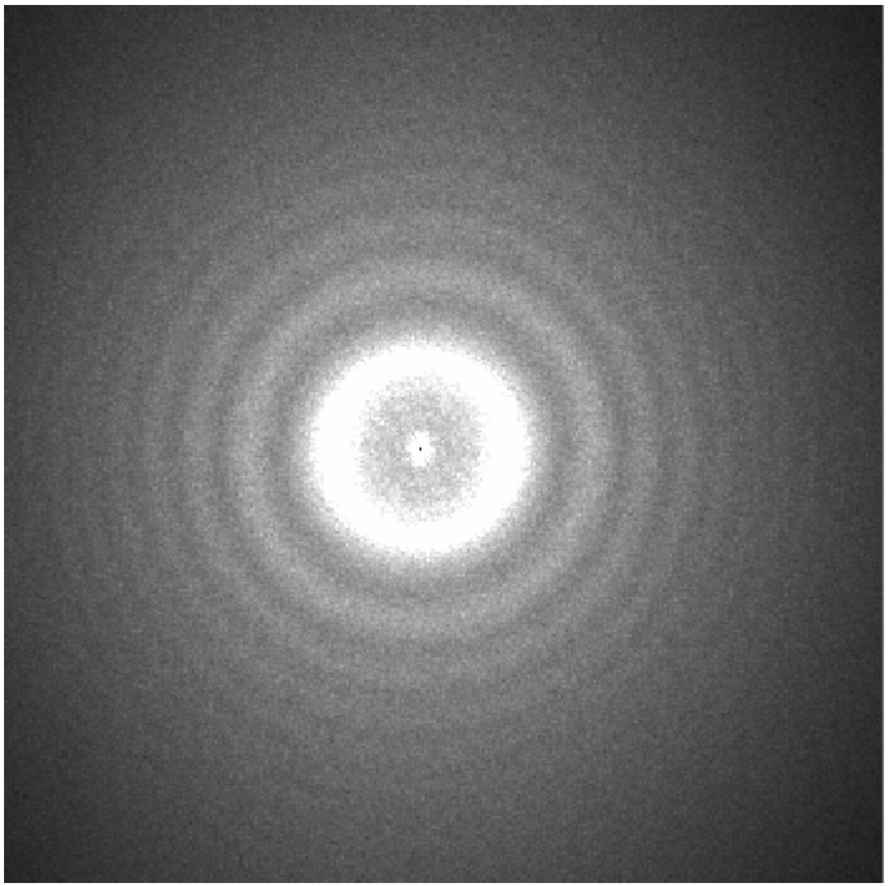
Individual Sine Curves

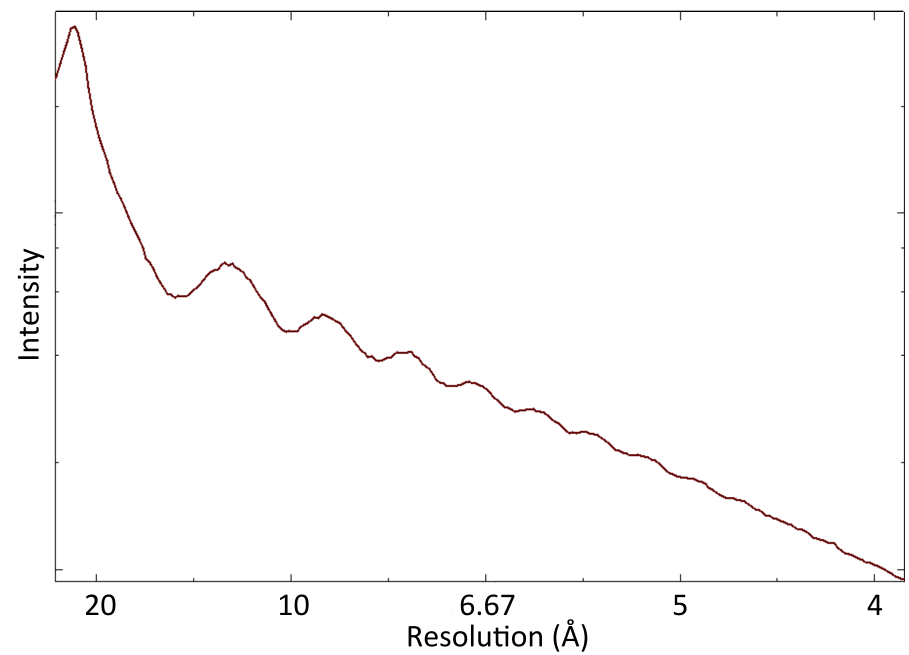
Sum of All Three Curves




Wavelength
Amplitude
Phase

Data Quality Assessment: Computed FFT of Particle Images





1.04μm defocus

Joanita Jakana

Computed Diffraction Pattern

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

Structure factor

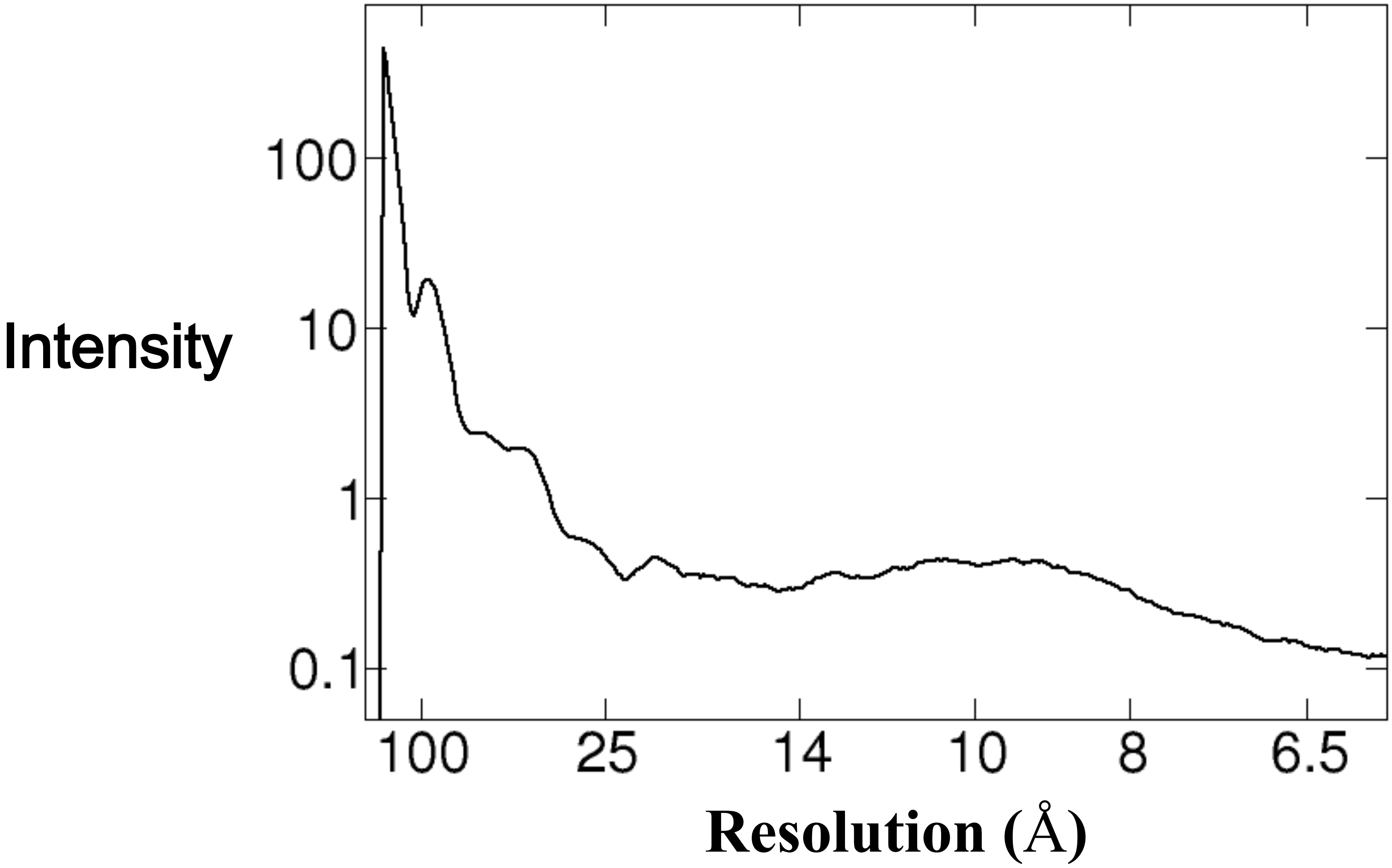
Contrast transfer function

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

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

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

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

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.

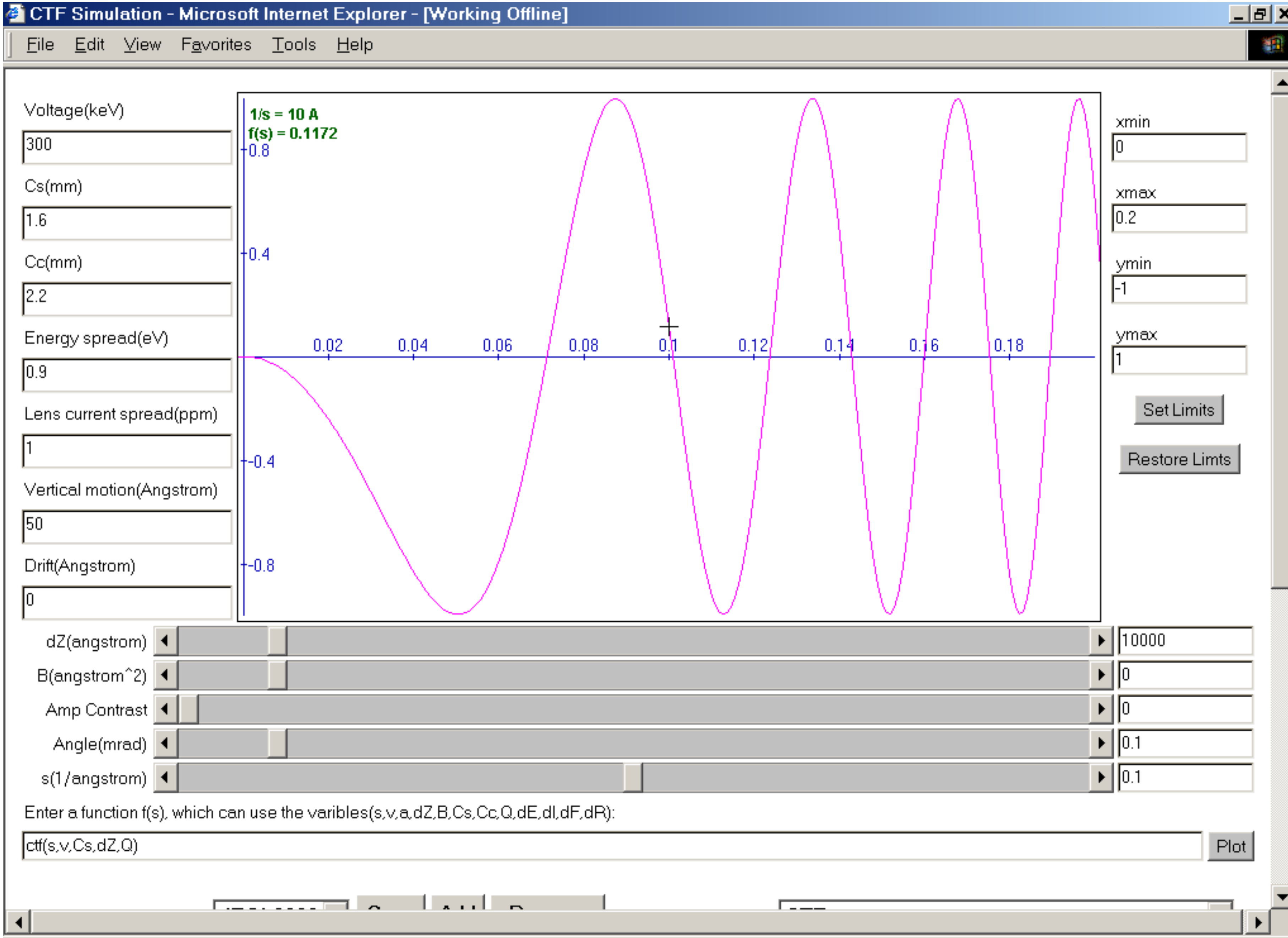
This applet is known to run on Windows (Netscape and Internet Explorer), Linux (i386) (Netscape), SGI IRIX (Netscape), OS/2 Warp and MacOS X. Please inform me if you found that this applet runs or has problems to run on other platforms.

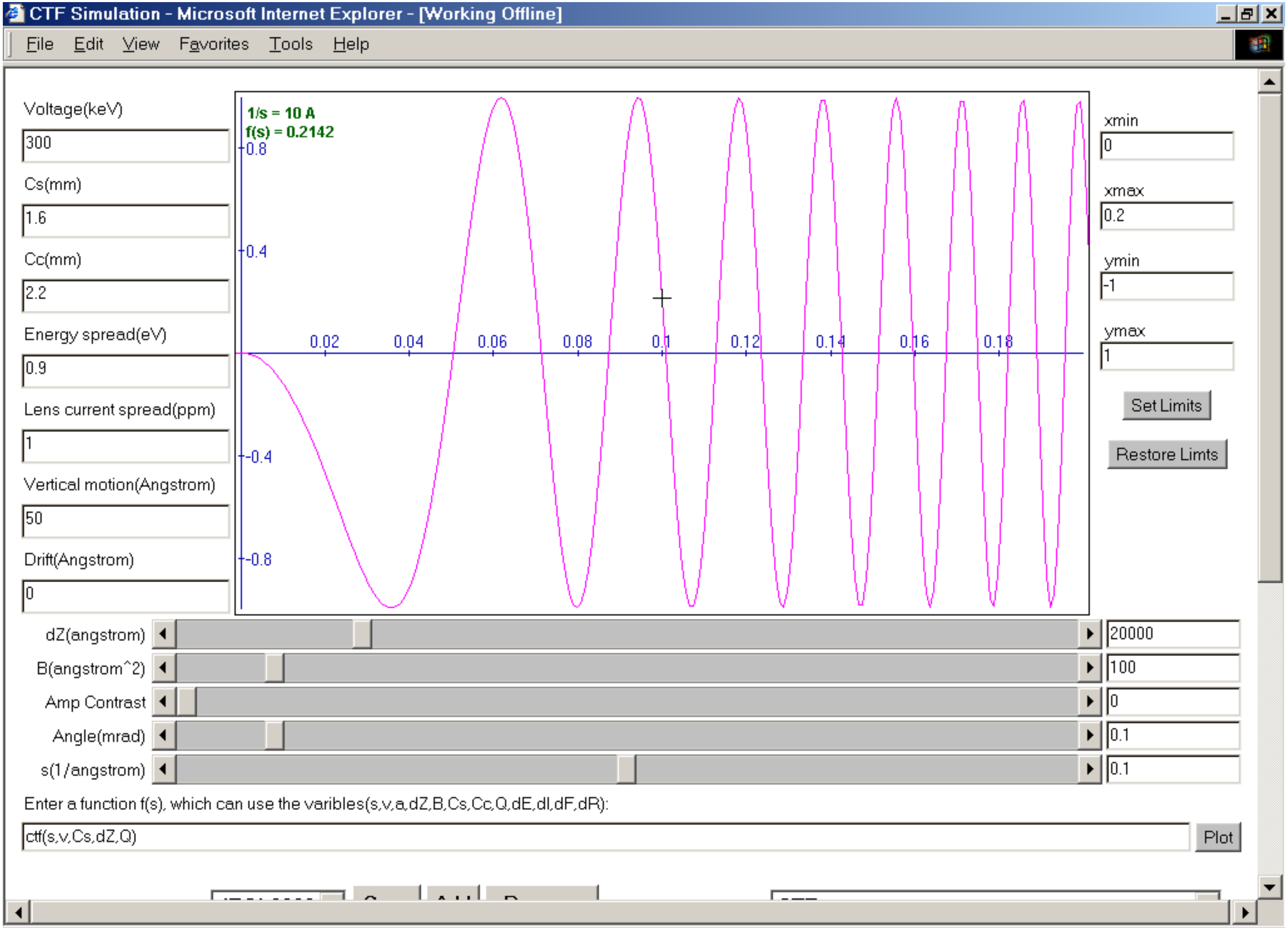
The following is the detailed descriptions for some aspects of the applet page.

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

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

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:

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

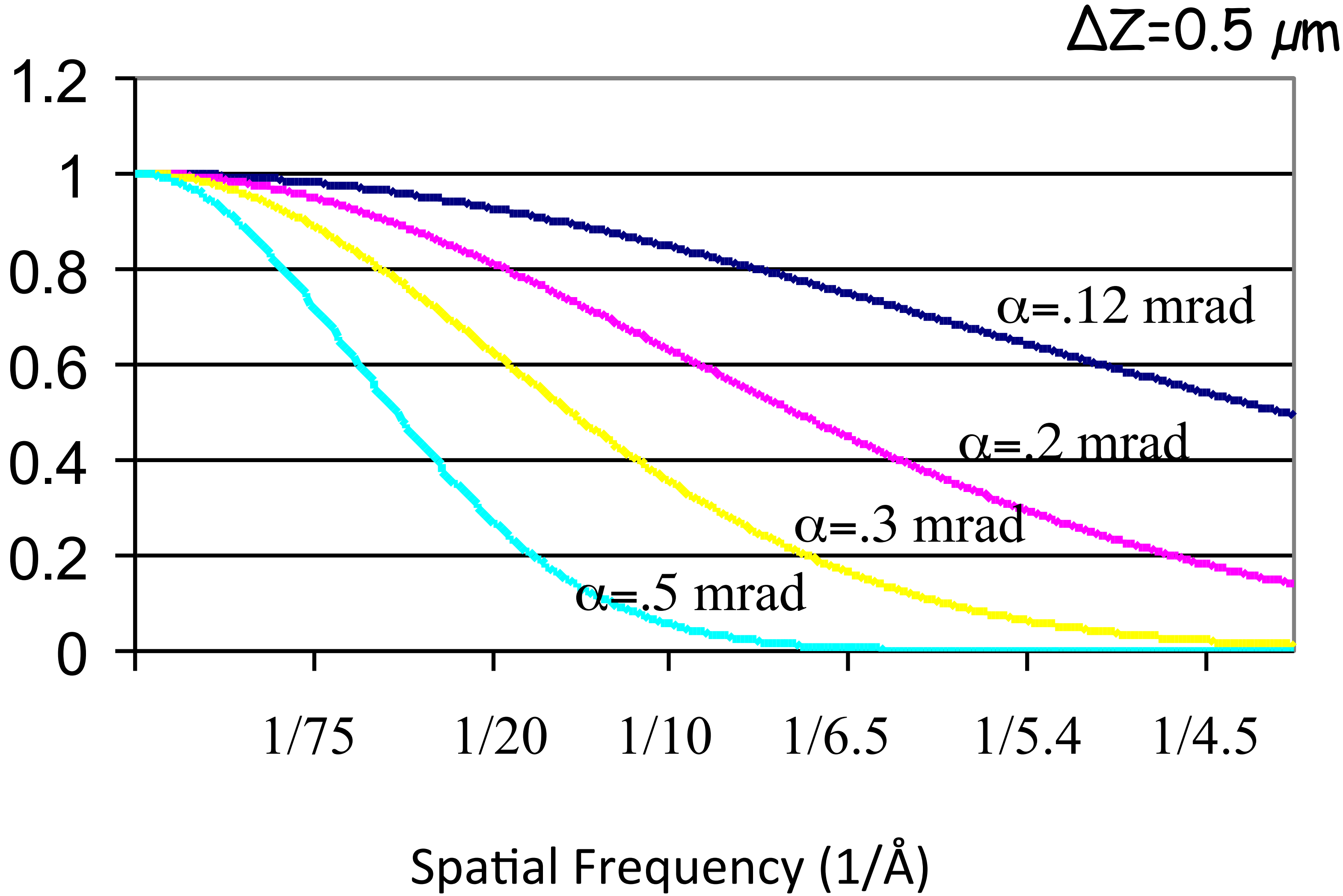
Sinusoidal type fluctuation:

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

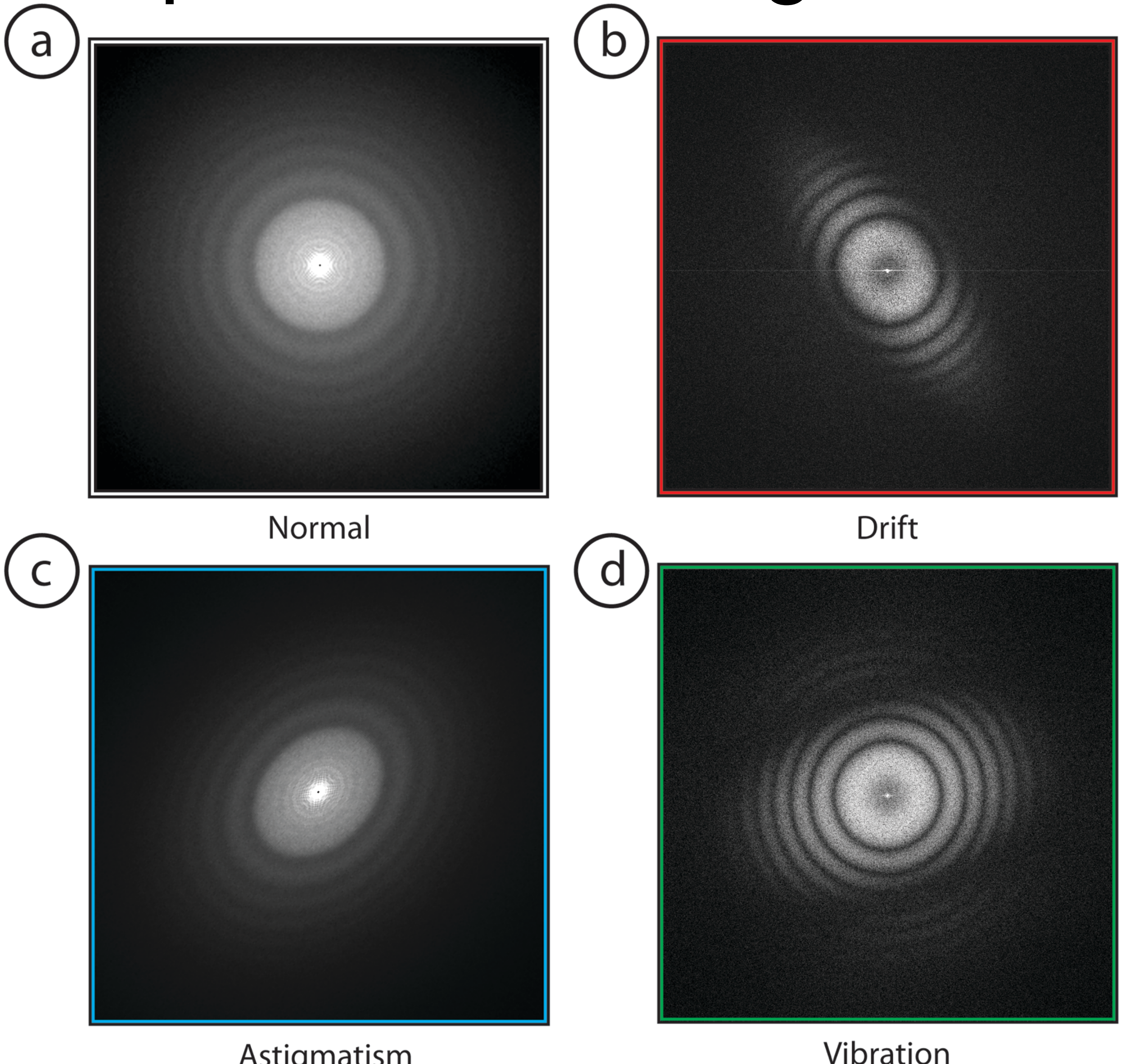
Drift:

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

Spatial Coherence Envelope Function



Power Spectrum of Images of C-Film



R Rochat
J Jakana

Gaussian Approximation for Cumulative Envelope Function

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

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

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

CTF Simulation - Microsoft Internet Explorer

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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

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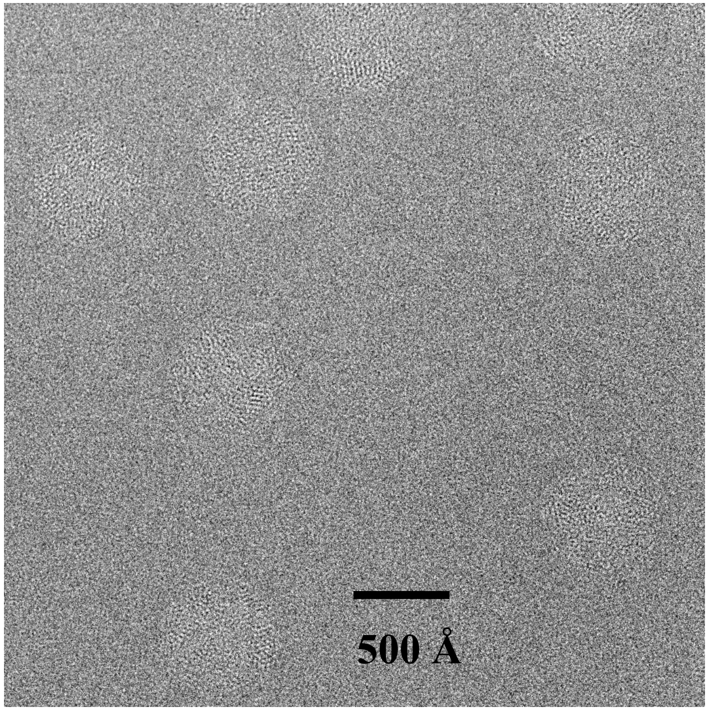
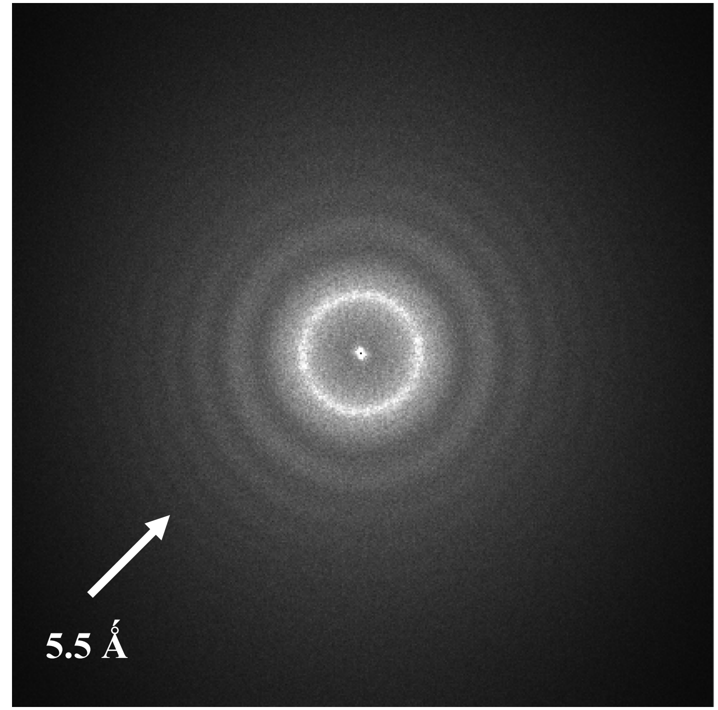
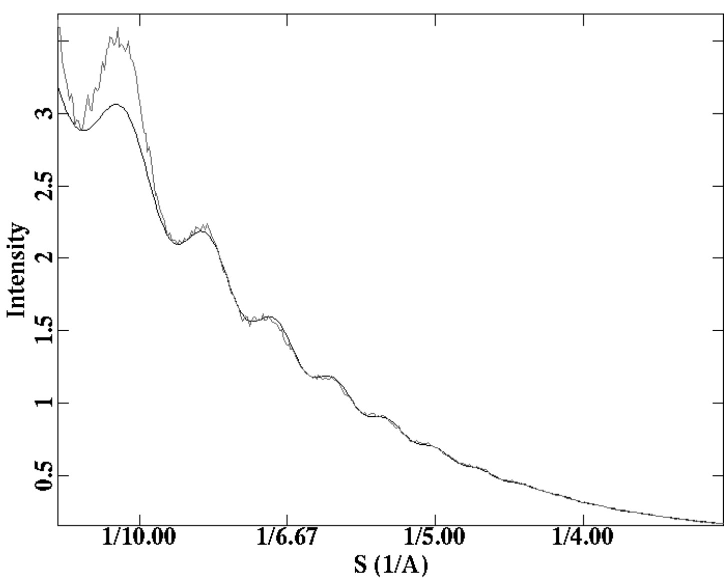
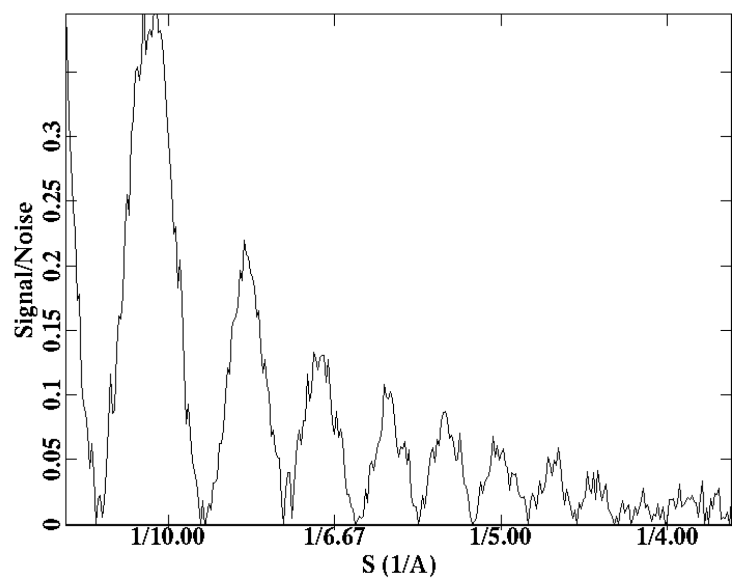
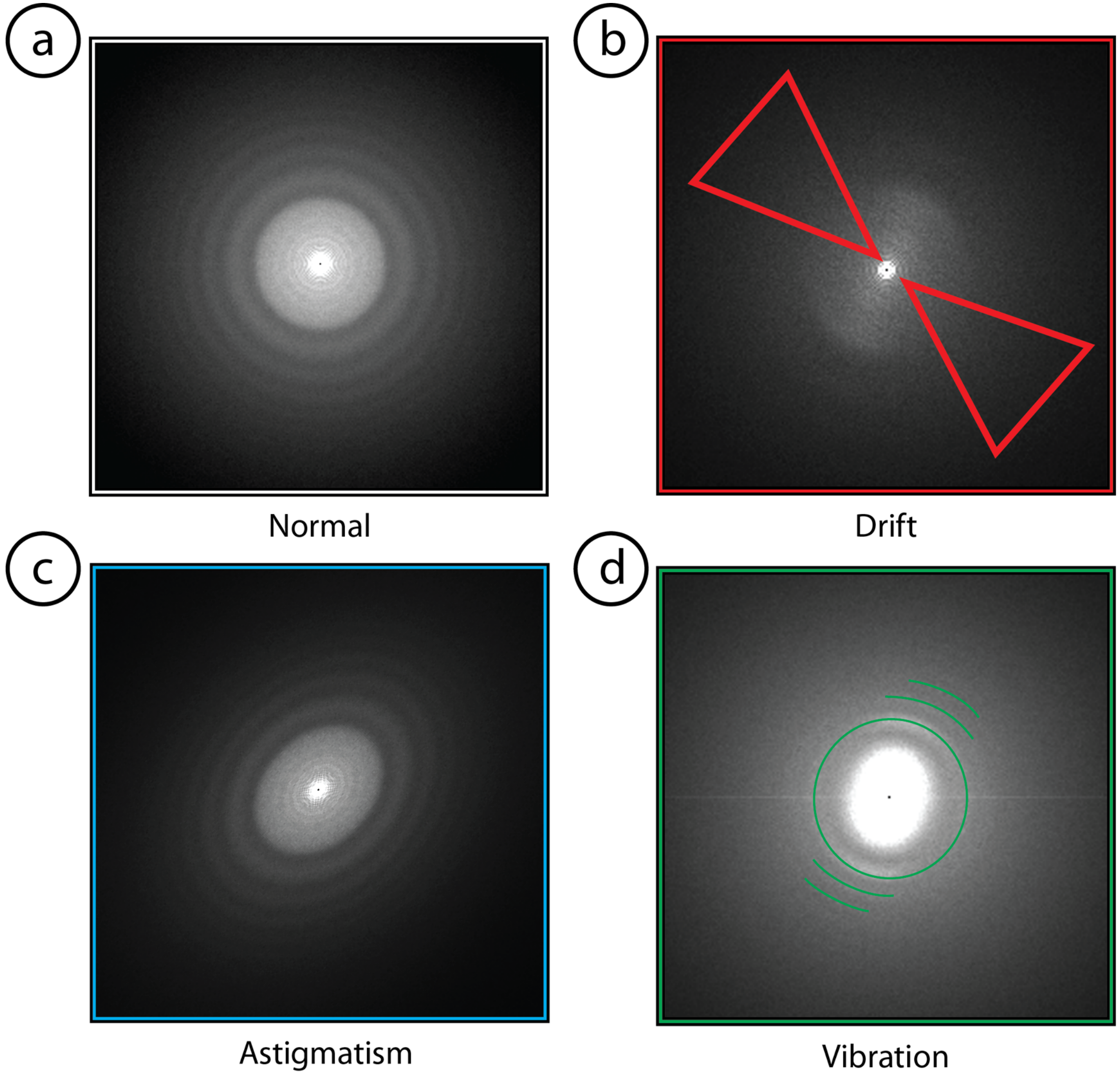
Application











a
b
Normal
Drift
c
d
Astigmatism
Vibration