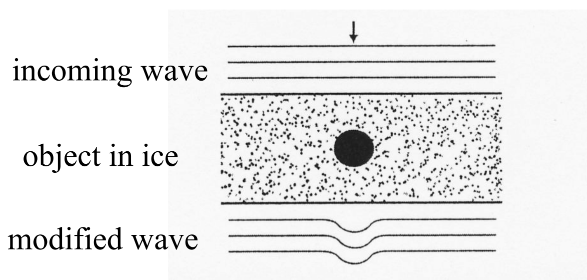
Image Formation

Jan. 30 2017

Image formation

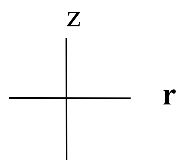
- Object is modeled as a weak-phase object
- Consider only phase contrast at first
- Will add amplitude contrast later

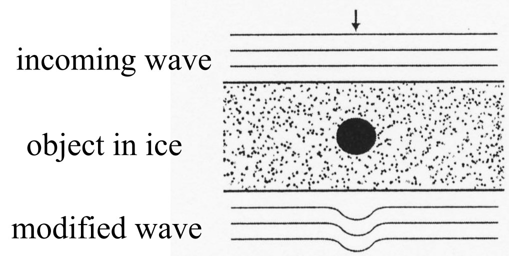


Observe: projected density of object

Frank, 2006

Image Formation





$\psi_0 = \exp(ikz)$

$$\Phi(\mathbf{r}) = \int C(\mathbf{r}, z)\, \mathrm{d}z$$

$$\Psi(\mathbf{r}) = \Psi_0 \exp[i\Phi(\mathbf{r})]$$

Taylor Expansion of modified wave equation: $\Psi(\mathbf{r}) = \Psi_0 \left[1 + i\Phi(\mathbf{r}) - \frac{1}{2}\Phi(\mathbf{r})^2 + \cdots \right]$

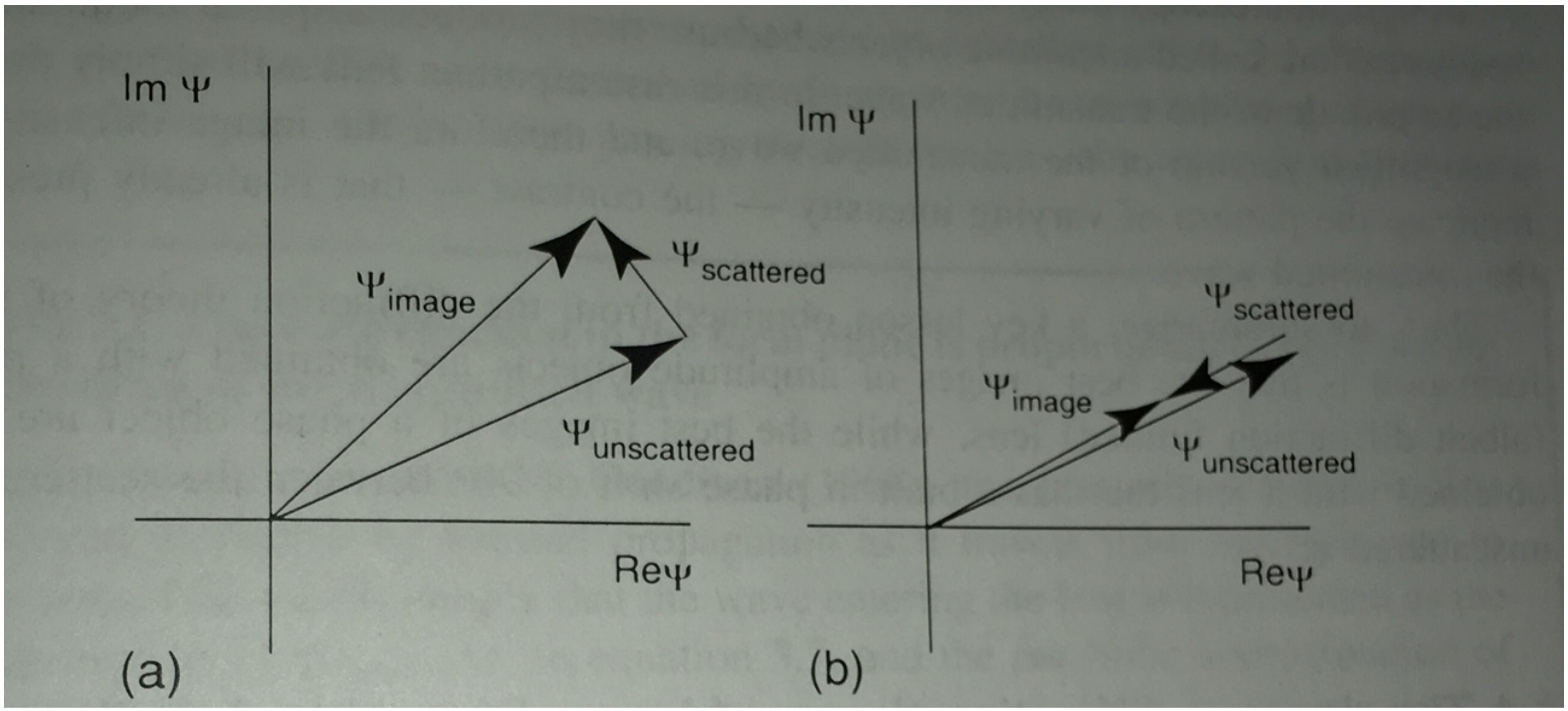
Weak phase approximation: $\phi(\mathbf{r}) \ll 1$ $\quad \Psi(\mathbf{r}) = \Psi_0 \left[1 + i\Phi(\mathbf{r}) \right]$

unmodified wave

scattered wave
(90º phase shift)

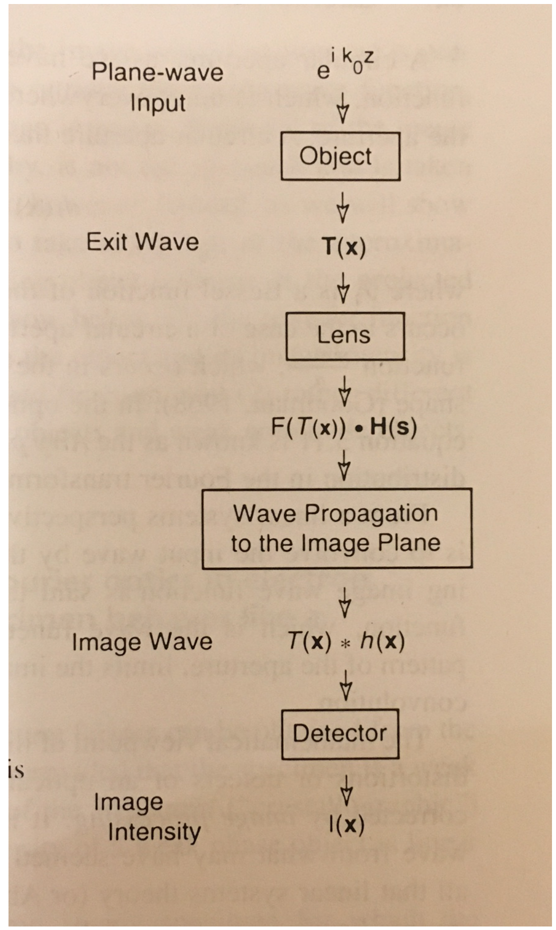
Frank, 2006

Perfect Lens: No Contrast



Glaeser et al, 2007

Flow Diagram: Actual Lens

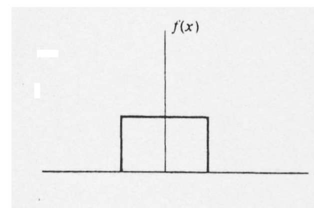


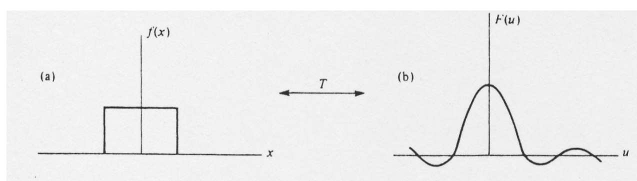
Glaeser et al, 2007

Image Formation

Insert an objective aperture in back-focal plane

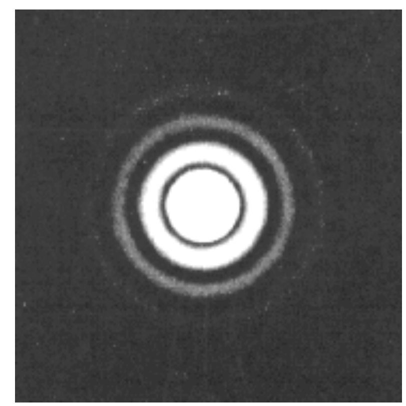
$$A(\mathbf{k}) = \begin{cases} 1 & \text{for } |k| = \theta/\lambda \le \theta_1/\lambda \\ 0 & \text{elsewhere} \end{cases}$$





sinc function

Airy Disk
(point spread function)



Contrast Transfer Function (CTF)

- Observed image:
    - $f(\mathbf{x}) = o(\mathbf{x}) * psf(\mathbf{x}) * env(\mathbf{x}) + n(\mathbf{x})$
        - f(**x**) : observed projection of image
        - o(**x**): true projection of image
        - psf(**x**): point-spread function
        - env(**x**): envelope function of microscope
        - n(**x**): noise
  - FT:
    - $F(\mathbf{k}) = O(\mathbf{k}) \times CTF(\mathbf{k}) \times ENV(\mathbf{k}) + N(\mathbf{k})$

Image Formation

Lens aberrations and defocusing shift the phase of the scattered wave, as described by γ(k)

$$\gamma(k) = 2\pi\left\{-\lambda\left[\frac{\Delta z}{2} + \frac{z_{astg}}{2}\sin 2(\phi - \phi_0)\right]k^2 + \frac{1}{4}\lambda^3 C_s k^4\right\}$$

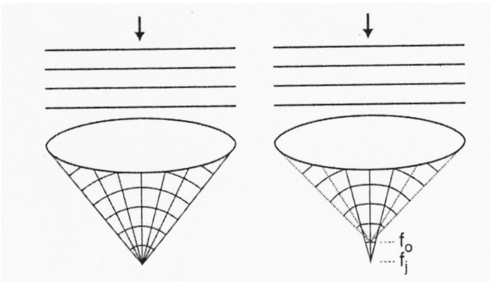
Defocus dependent

Defocus independent

λ: wavelength of electron
Δz: defocus (underfocus positive)

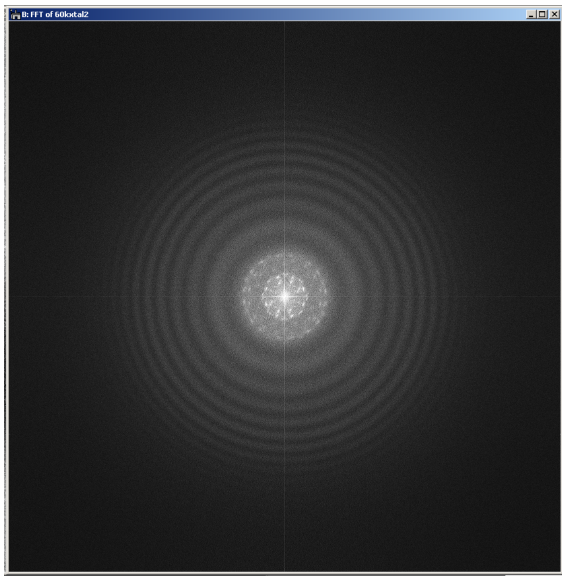
$\gamma(\mathbf{k})$ is called the wave aberration function
At back focal plane, FT of image is multiplied by $\sin(\gamma)$

C_s: Spherical Aberration

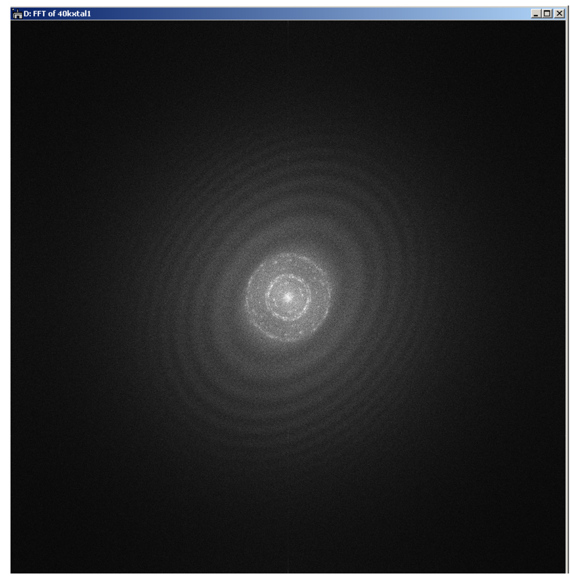


Frank, 2006

CTF Visualization: FFT of Image

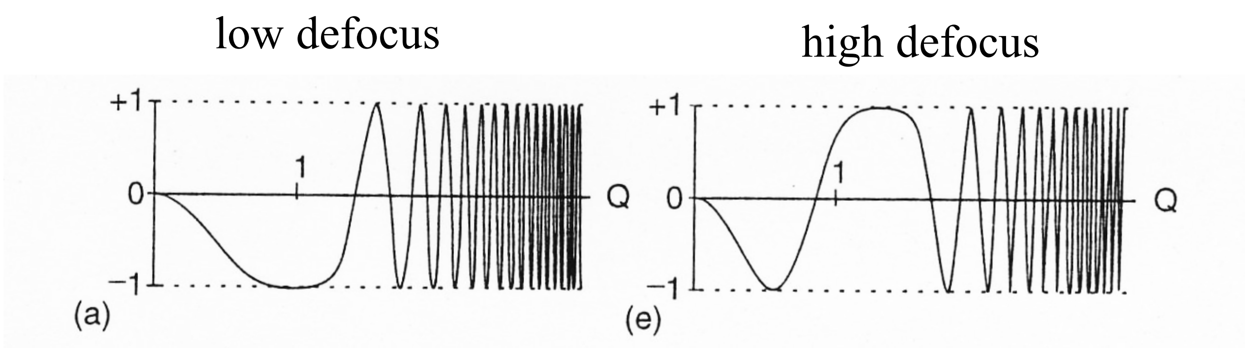


good image



Astigmatism

Contrast Transfer Function radial plots



- phase starts off negative
- phases alternately reversed
- oscillates faster at higher resolution areas
- very low-resolution region is high-pass filtered
- higher resolution is also filtered at nodes

Coherence and Envelope Functions

CTF is dampened because of partial coherence of beam

a) finite source size of beam

$$E_i(k) = \exp\left[-\pi^2 q_0^2 (C_s \lambda^3 k^3 - \Delta z \lambda k)^2\right]$$

defocus dependent

b) energy spread of beam

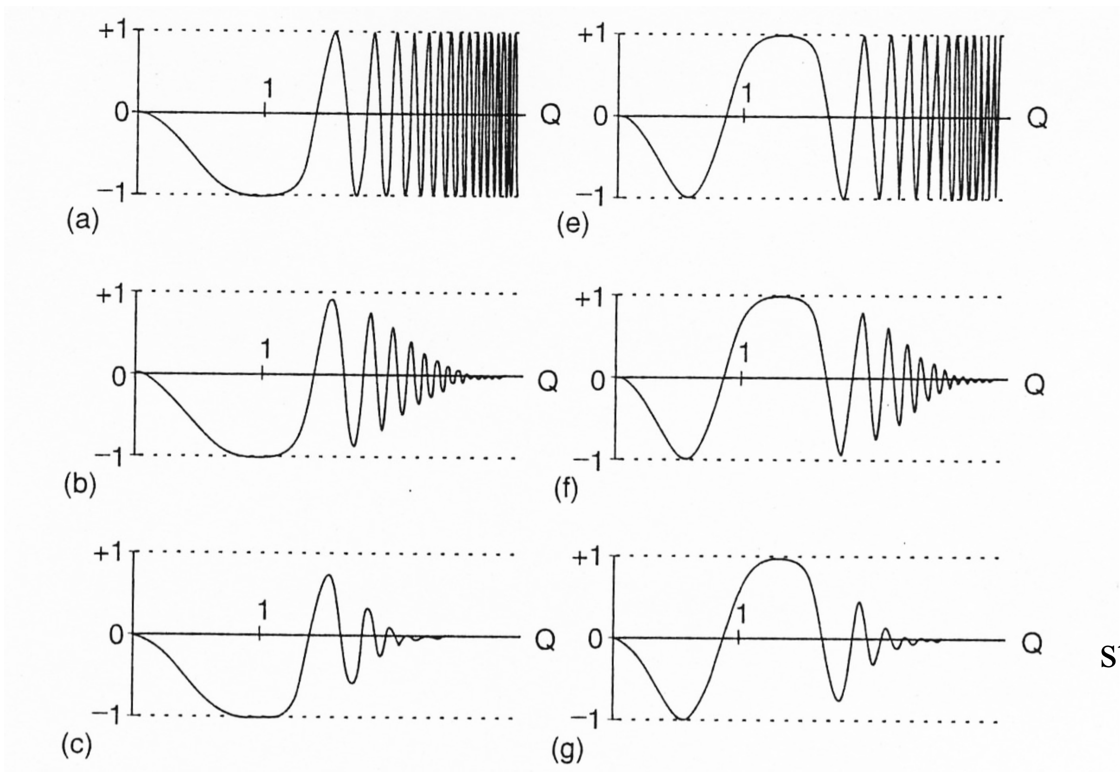
$$E_e(k) = \exp\left[-(\pi \delta z \lambda k^2/2)^2\right]$$

defocus independent

c) other effects: energy fluctuations, coolant fluctuations, mechanical movement, ... approximate as Gaussian B factor $\exp(-Bk^2)$

$$ENV(k)=E_i(k)E_e(k)E_B(k)$$

Coherence and Envelope Functions



no envelope

strong envelope

Amplitude Contrast

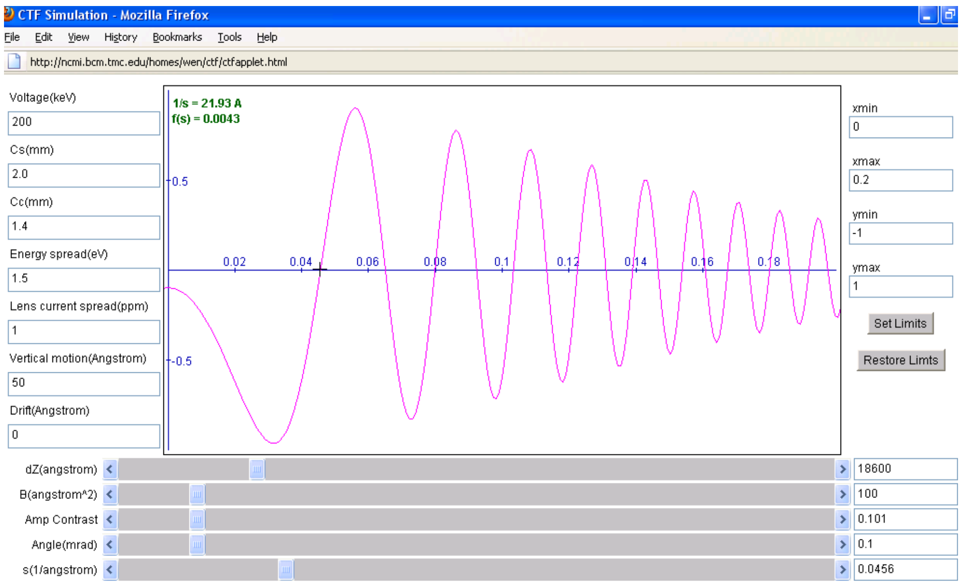
- Due to loss of electrons due to
  - scattering outside of aperture
  - removal by inelastic scattering
- ratio of amplitude to phase contrast depends on atomic weight
- Assuming homogeneous specimen, get modified CTF:

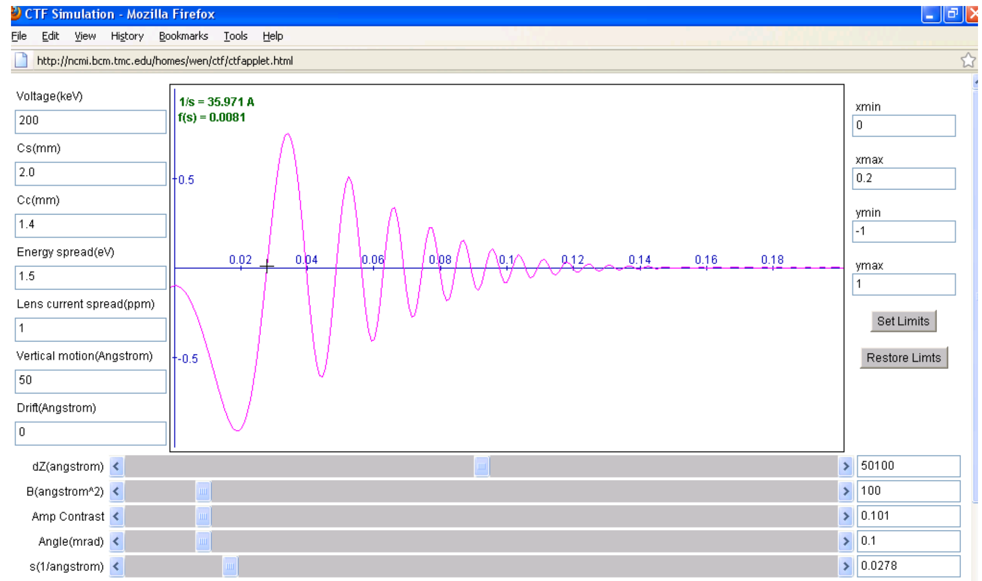
$$H'(k) = A\cos\gamma(k) + \sqrt{1 - A^2}\sin\gamma(k)$$

A: % amplitude contrast (~7% for cryo)

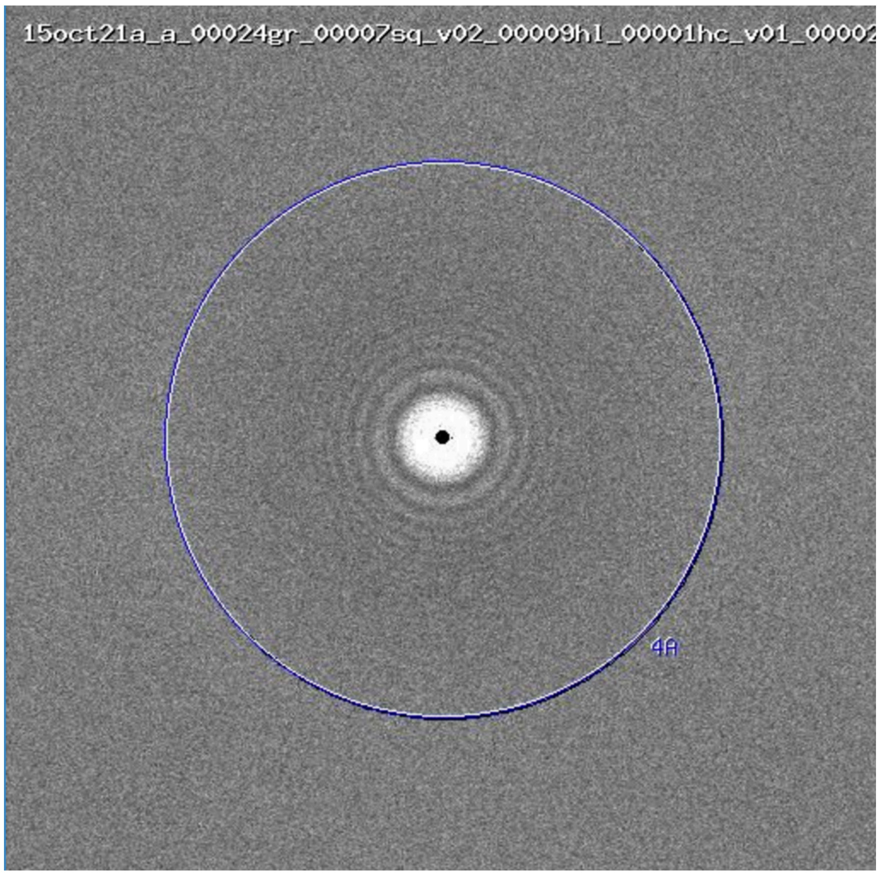
Complete CTF Simulator

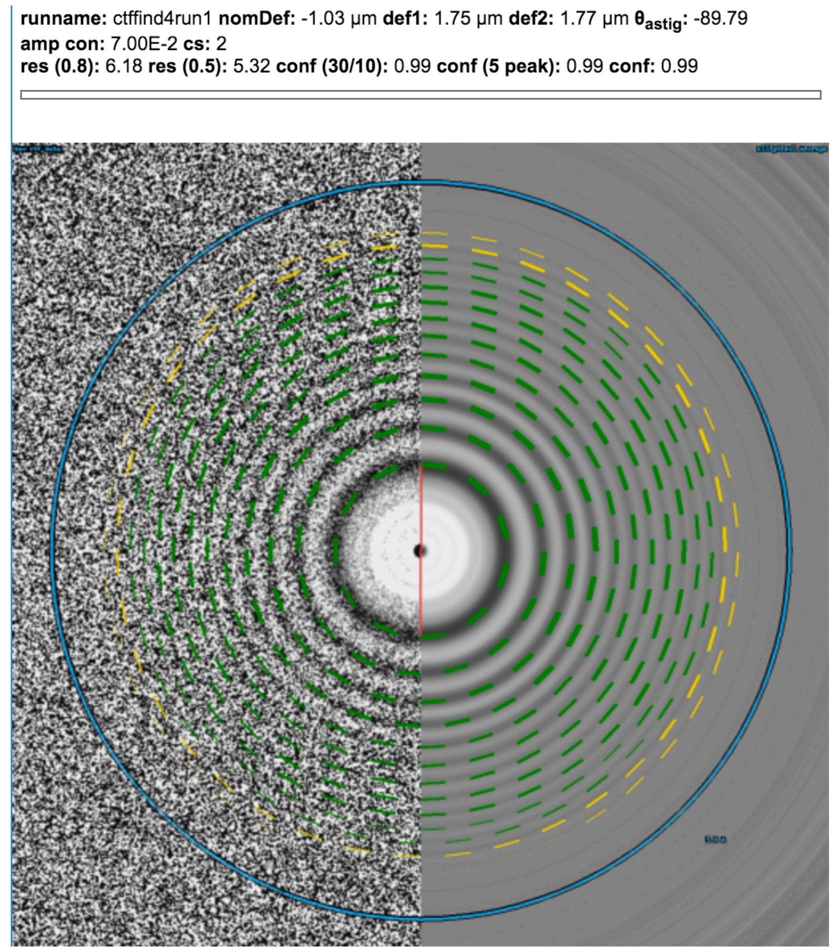
http://jiang.bio.purdue.edu/ctfsimu



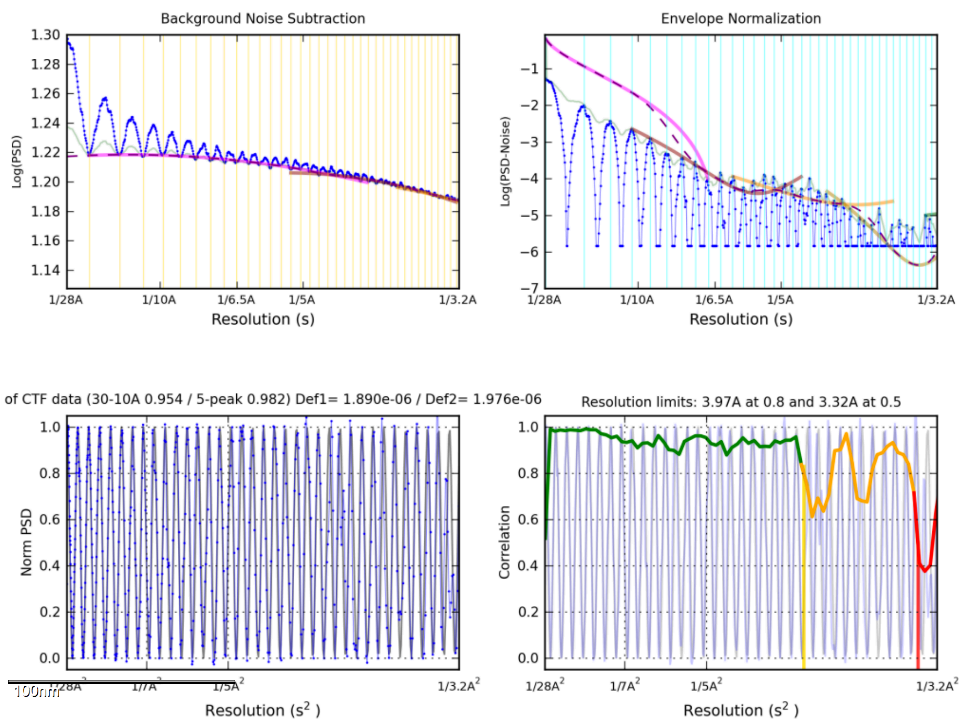


CTF Determination: CTFFIND3/4, gCTF

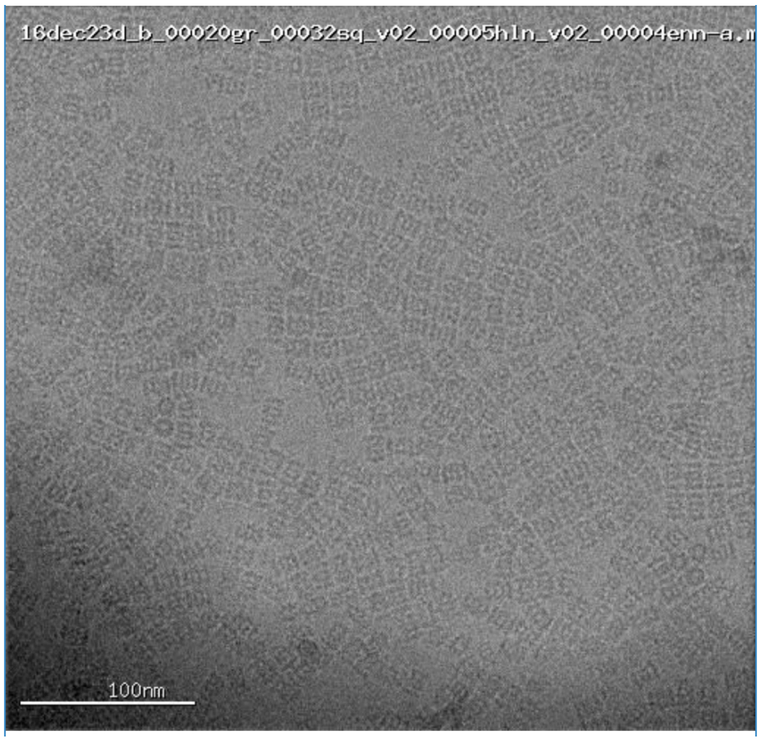


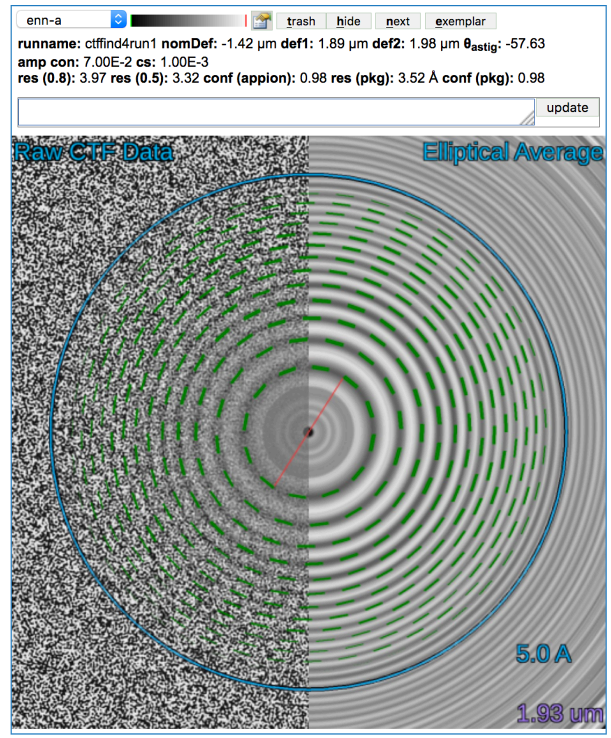


CTF Determination: 1D Plots (Appion)

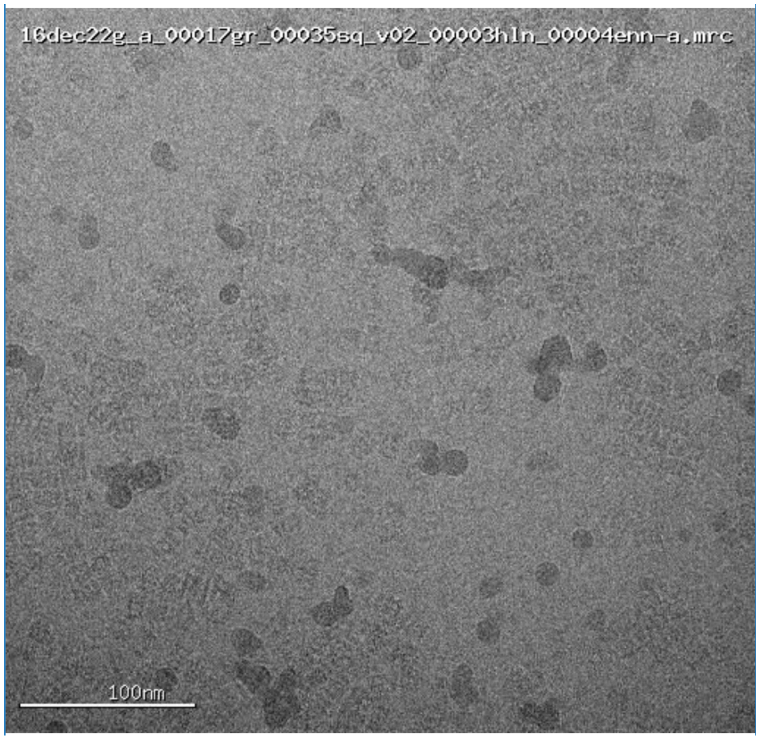


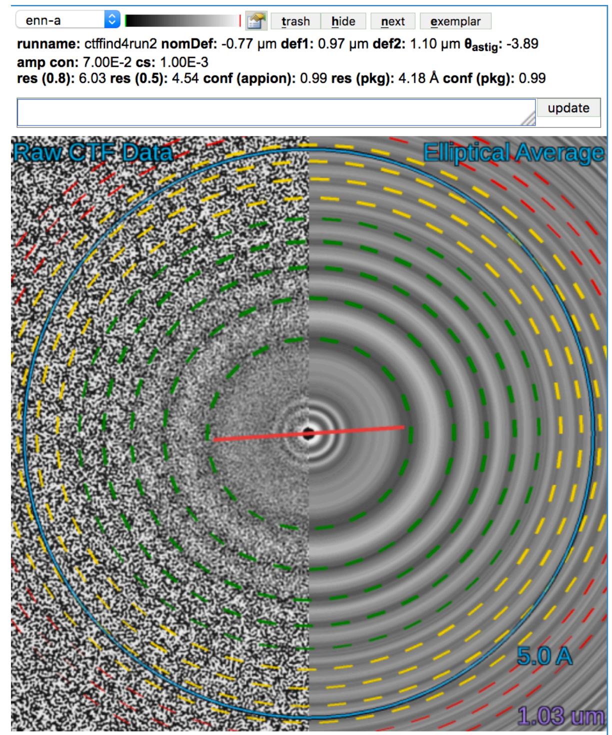
2 um Underfocus



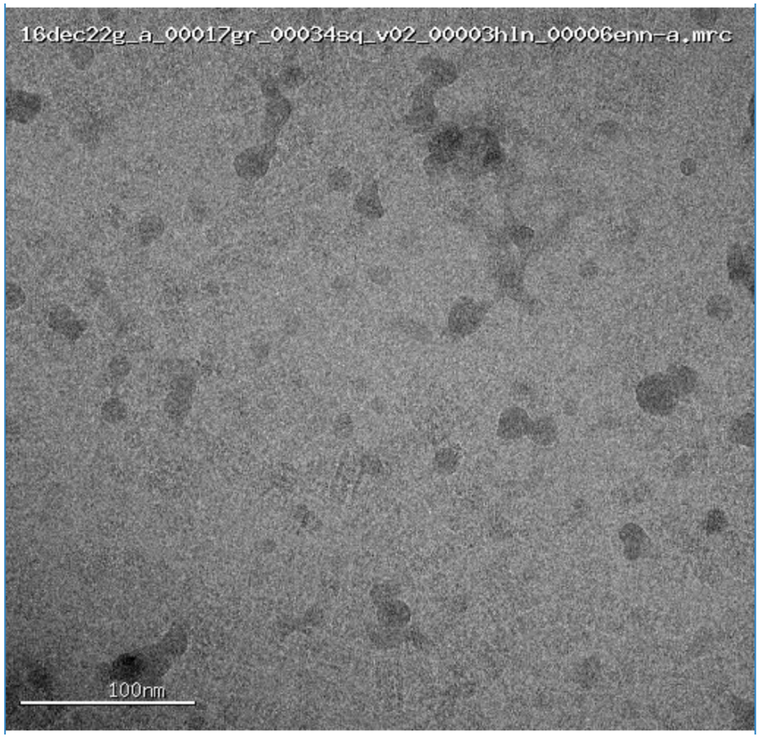


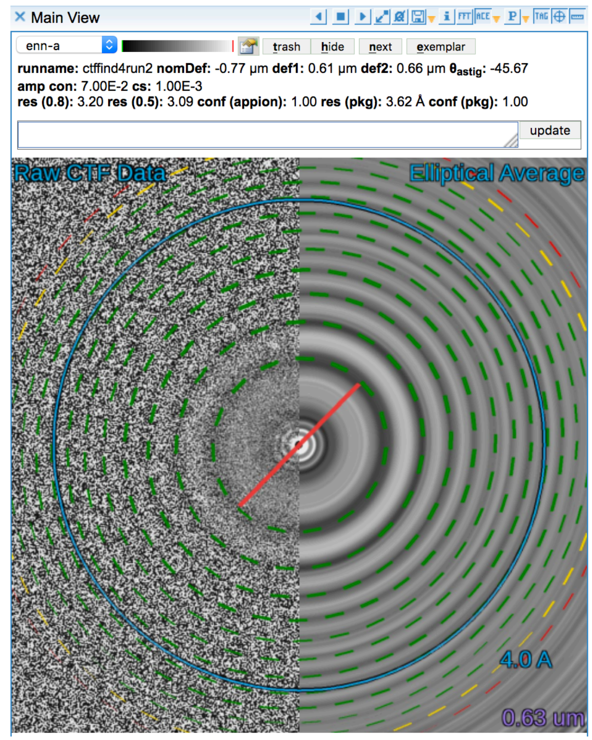
1 um underfocus



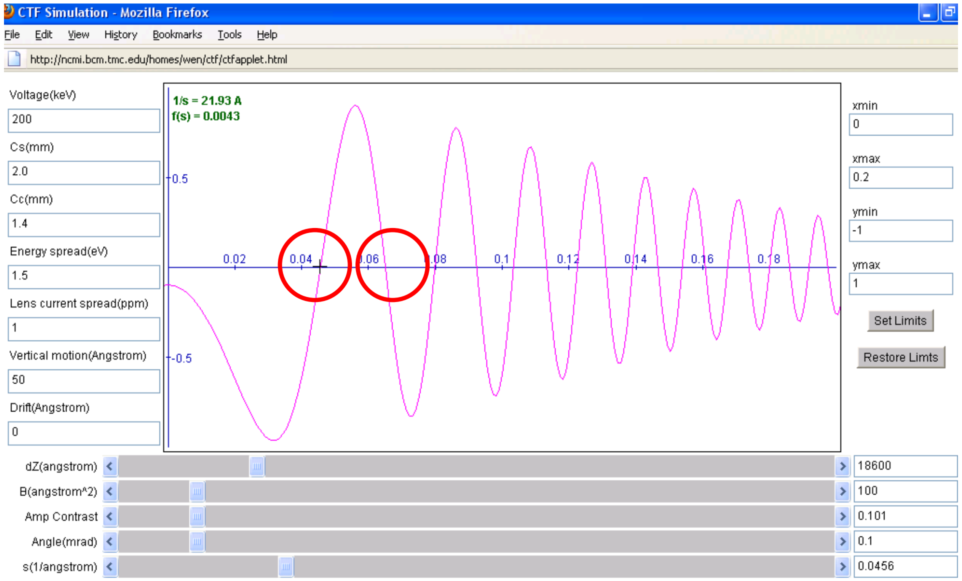


0.6 um Underfocus





Contrast Transfer Function Correction



Need to avoid dividing by zero!!

Contrast Transfer Function Correction

- low-pass filter – within first CTF0
  - negative stain, tomography
  - negative stain – high amplitude contrast, limited resolution
- phase flipping
- phase flipping plus amplitude correction
  - Wiener Filter

$$I'(\mathbf{k}) = I(\mathbf{k})\, S(\mathbf{k})$$

$$S(\boldsymbol{k}) = \frac{H^*(\boldsymbol{k})}{|H(\boldsymbol{k})|^2 + SNR(\boldsymbol{k})}$$

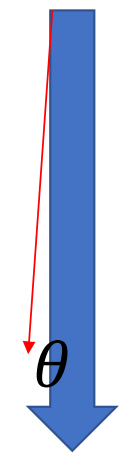
H(k): CTF
I(k): FFT of image

Importance of Beam Tilt

- Beam should be parallel to optical axis of microscope
- Otherwise, CTF equation is modified and phase error is introduced
- Needs to be accurate within 1 mrad for high resolution

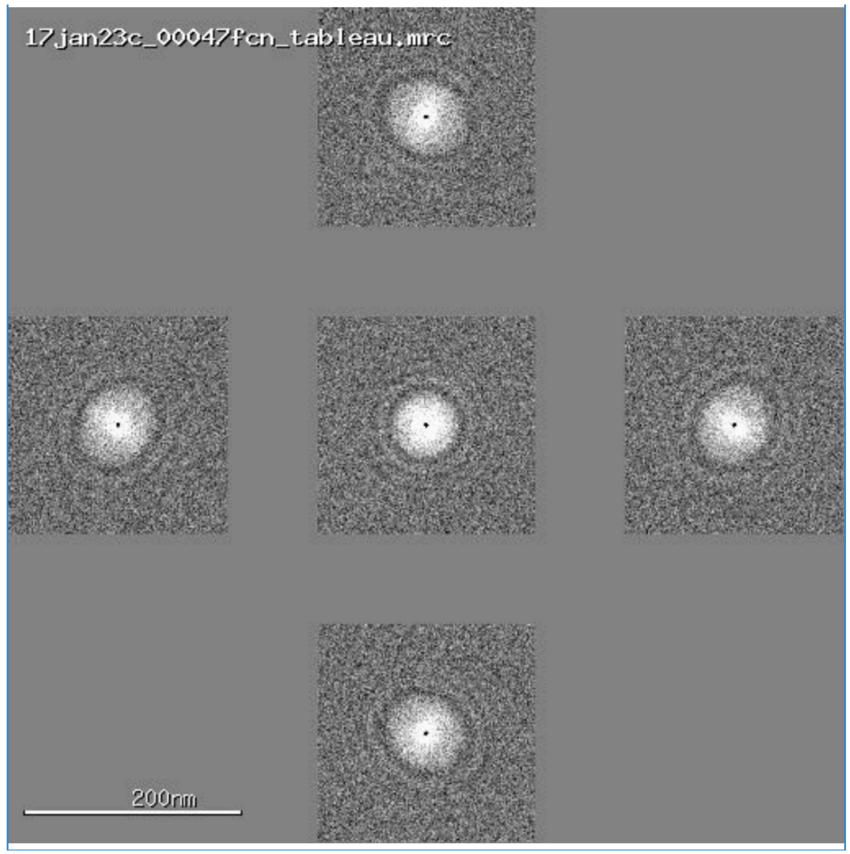
$$\Delta\alpha(\boldsymbol{k}) = -2\pi\theta C_s\lambda^2 k^3\widehat{\boldsymbol{k}} \cdot \Delta\hat{\boldsymbol{s}}$$

s: unit vector in direction of beam tilt
K: unit vector in direction of diffraction spot
θ: Amount of beam tilt from optical axis
α: phase error introduced



Beam Tilt Correction

Tilt +/- 10 mrad in X and Y
Pattern should be symmetric



Further Reading

- Frank, J. (2006). Three Dimensional Electron Microscopy of Macromolecular Assemblies (Chapter 2). Oxford University Press: New York (2006).
- Glaeser, R.M., Downing, K., DeRosier, D., Chiu, W., and Frank, J. (2007). Electron Crystallography of Biological Macromolecules. Oxford University Press: New York (2007)
- Steward, E.G. (2011) Fourier Optics: An Introduction (2nd Ed)