Study Meeting 4: Introduction to the CTF

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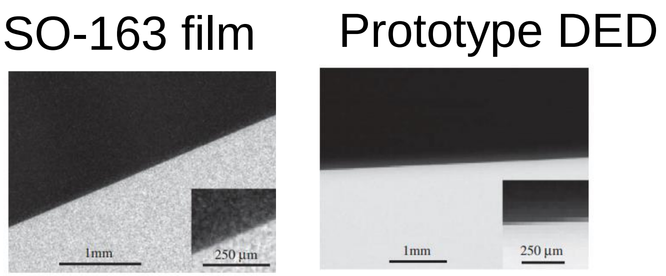
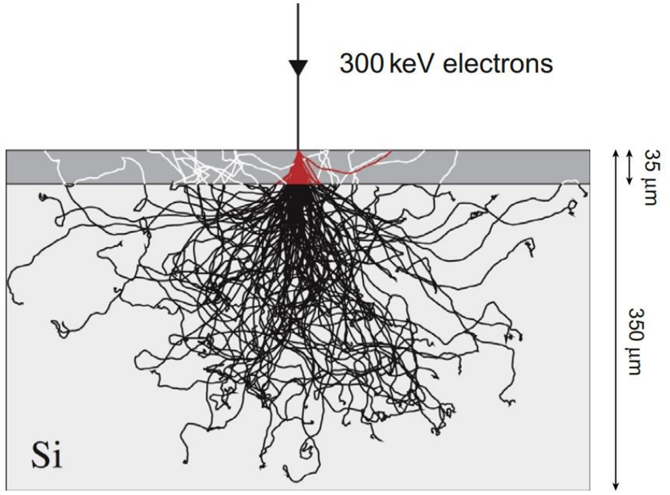
Direct Electron Detector

- DED are better
  - Fog noise (dark gain reference)
  - Back thinning
  - Motion correction
  - Reduced coincidence loss
- MTF: the camera envelope function
- DQE: Input SNR to output SNR

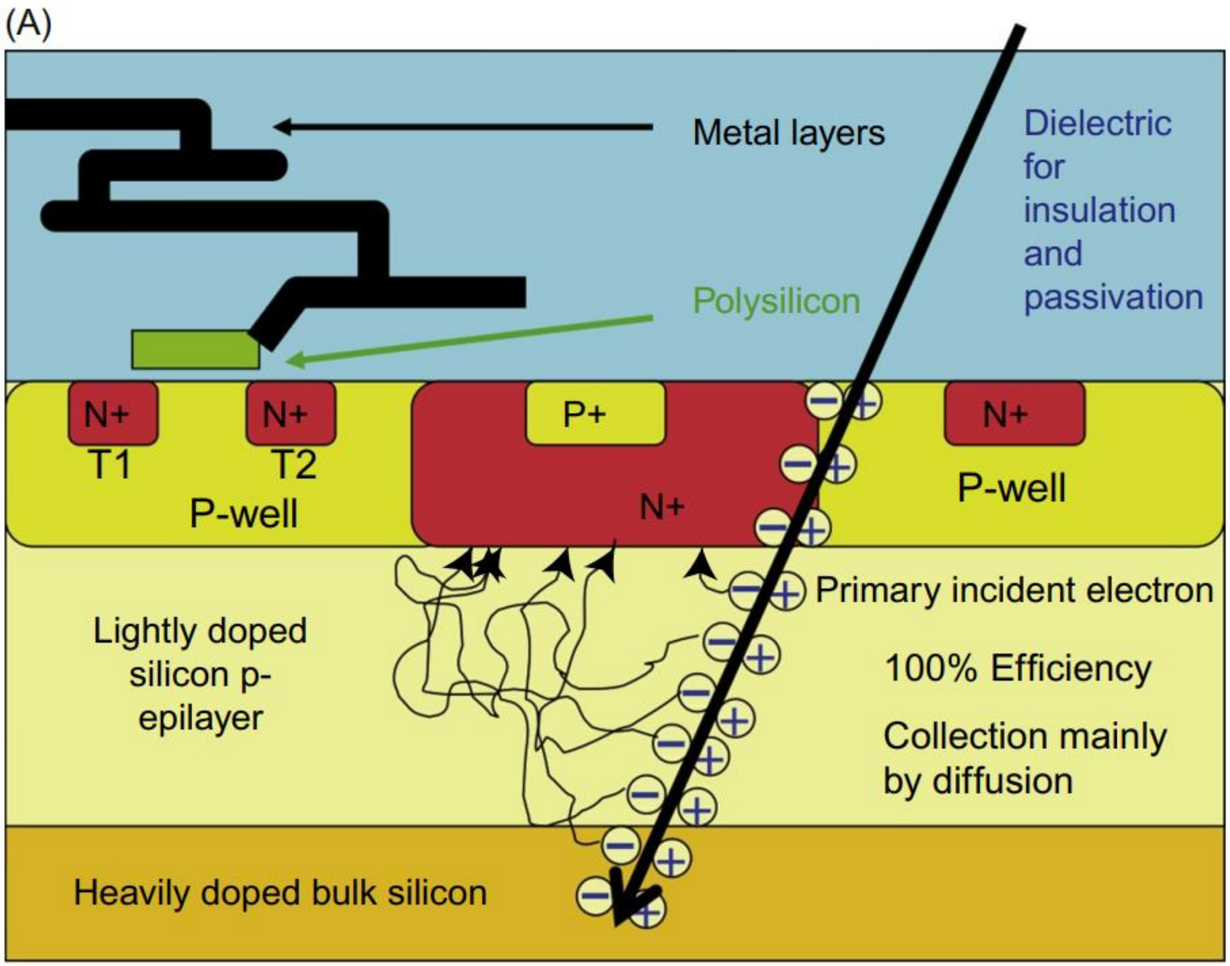




Unanswered question

- Last time: Francisco asked about the effect of acceleration voltage on DED imaging:

Direct Electron Detector



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McMullan, Faruqi, Henderson (2016)
Methods in Enzymology, Vol. 579

Effect of electron voltage on DED

Interacts less with the sample

- Inelastic scattering lowered
- Reduced contrast

- Effect on back scattering?
- Interacts less with the camera
  - Shorter time to interact with camera
  - Higher chance of coincidence loss (no, may have voltage difference below the cut off inherent to the camera)

Goals for today

- Image formation in EM
- The contrast transfer theory
- CTF equation
- Effect of various parameters on the CTF
- Why CTF estimation matters
- Envelope functions

Diffraction

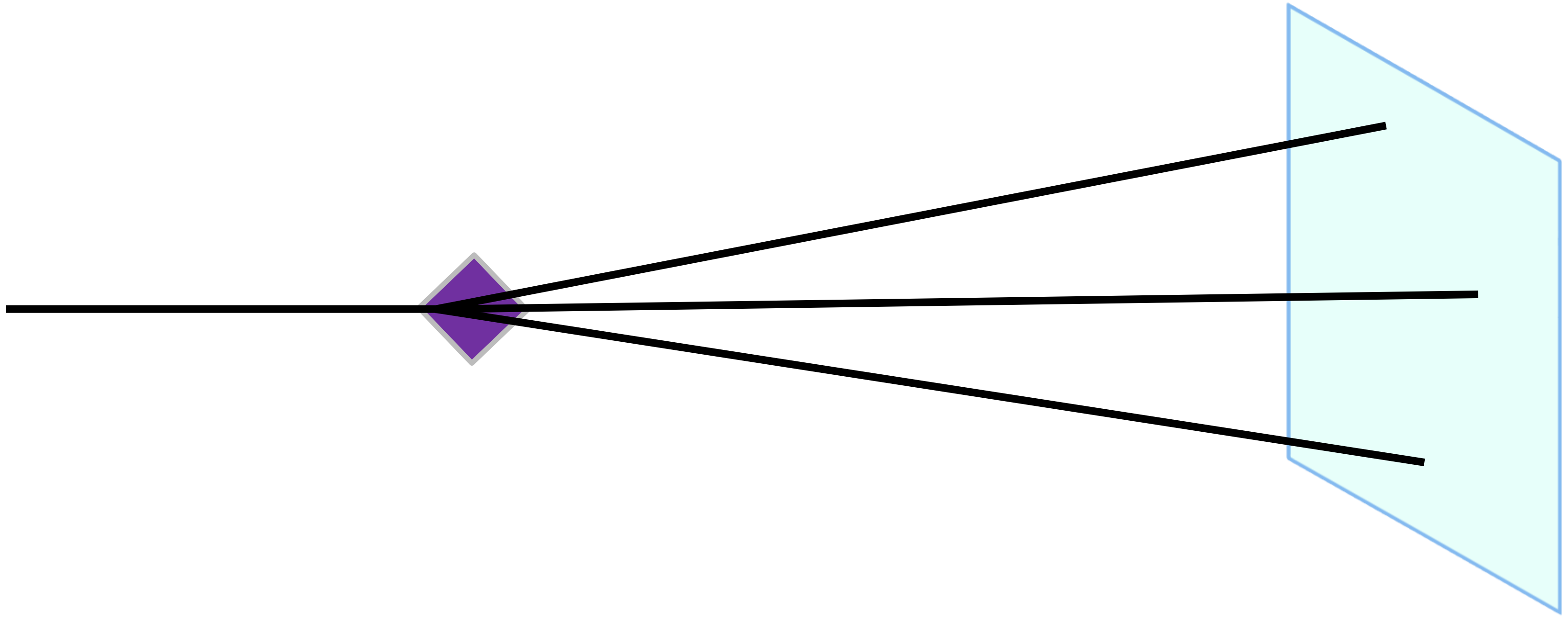
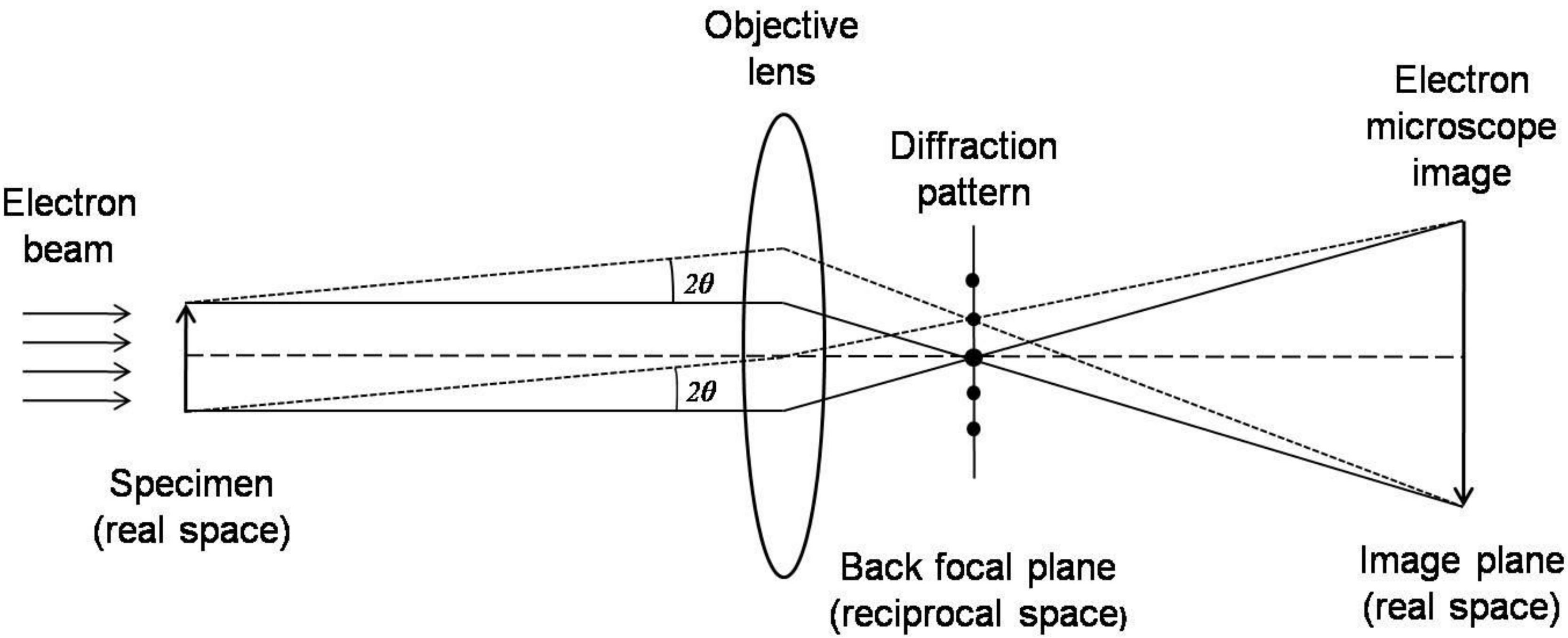
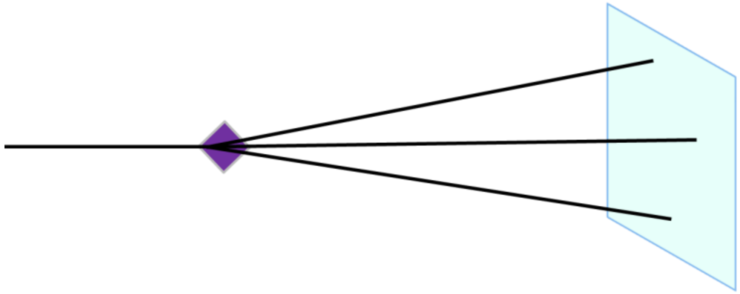
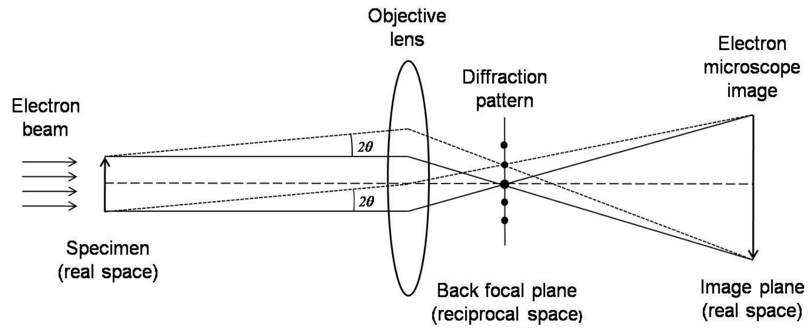


Image formation in EM



X-ray vs. EM image formation





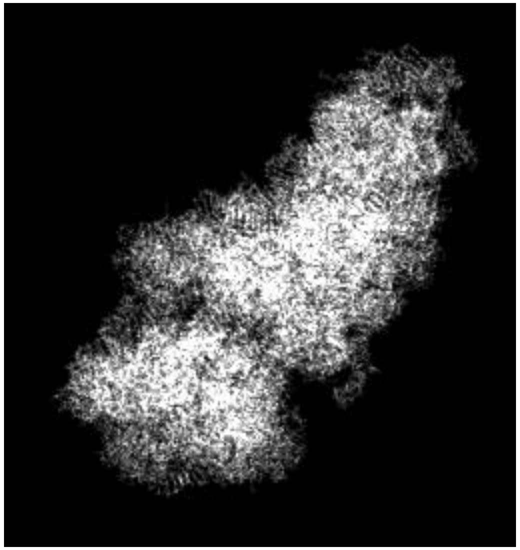
- Amplitude contrast
- Phase contrast
- EM uses lenses so we also get phase information
  - Interaction between the scattered and unscattered beam

Projection of 3D density

- Projection of a 3D object into 2D
- Each pixel contains the integral of the Coulomb potential along the z-direction

Projection along z

Take the integral of each value along the z axis



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```
e2project3d.py [map.mrc] --outfile=[output.mrcs] --orientgen=eman:delta=[whatever] --projector=standard --verbose=3
```

Contrast Transfer Function

- (in Fourier space)

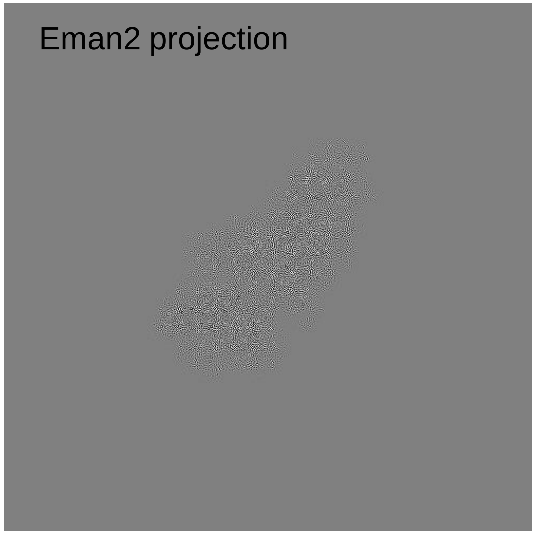
$$H(k) = 1[\sin\gamma(k) - W\cos\gamma(k)]$$

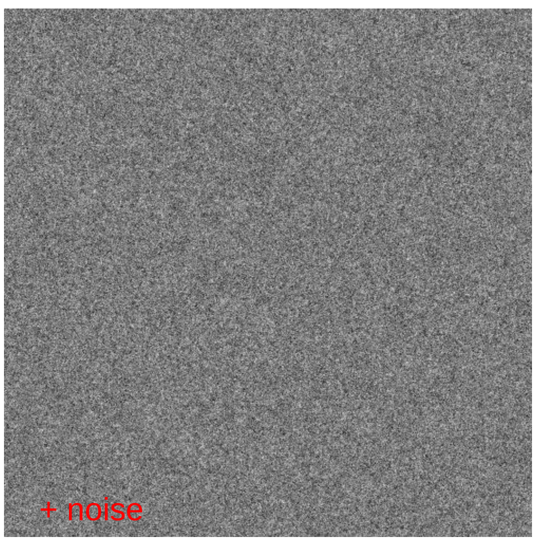
$$\gamma(k) = 2\pi(-0.5\Delta z\lambda k^2 + 0.25 Cs\lambda^3 k^4)$$

W	amplitude contrast ratio
Δz	defocus: underfocus is positive
λ	electron wavelength
Cs	spherical aberration
k	spatial frequency

Zhu *et al.* 1997
J. Struct. Bio. 118:197-219

300 kV, all zero





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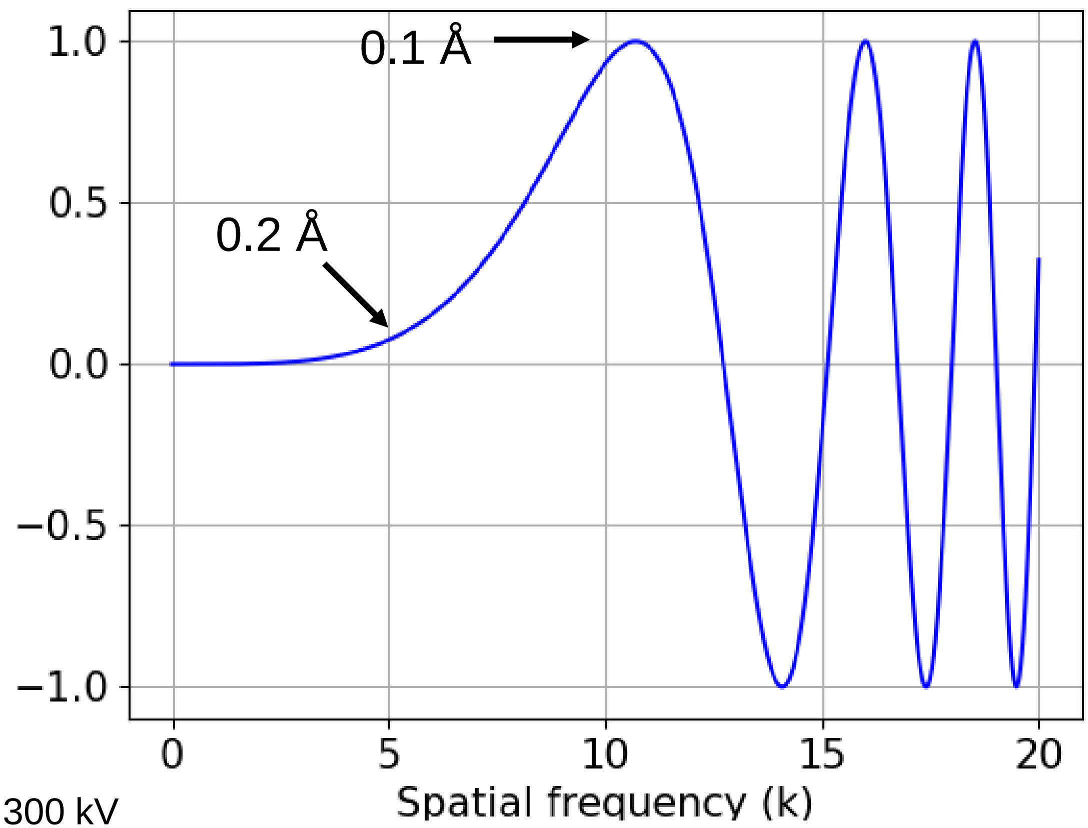
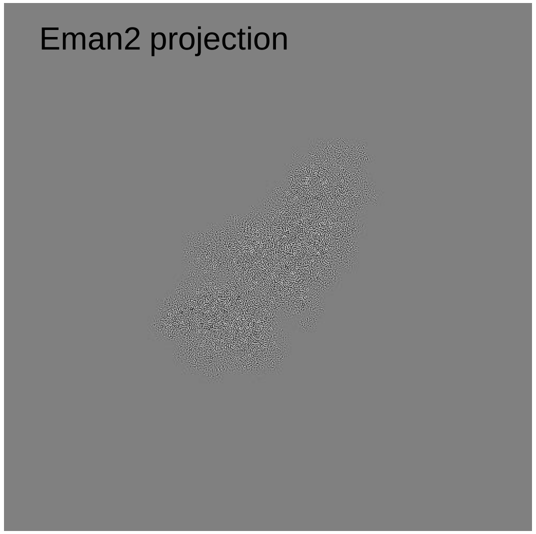
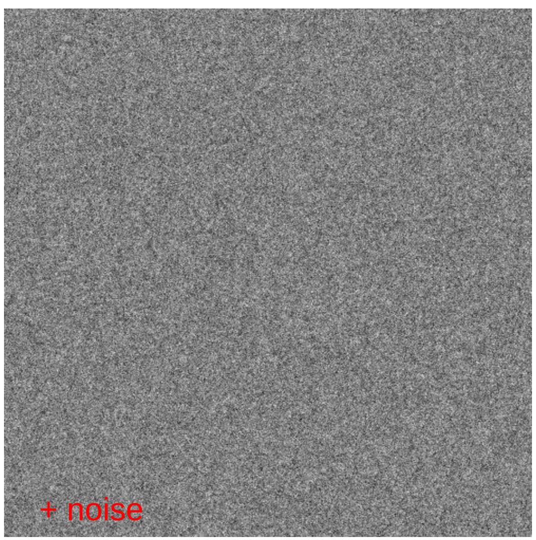


300 kV
A=0
B=0 Å^2
Cs=0 mm
Def=0 µm

e2proc2d.py Input.mrcs Output_CTF.mrcs --process=math.simulatectf:ampcont=0:bfactor=0:cs=0.000001:defocus=0:voltage=300:noiseamp=0.3:noiseampwhite=0.8

300 kV, 0.1 amp. contrast





$$H(k) = 1[\sin\gamma(k) - W\cos\gamma(k)]$$
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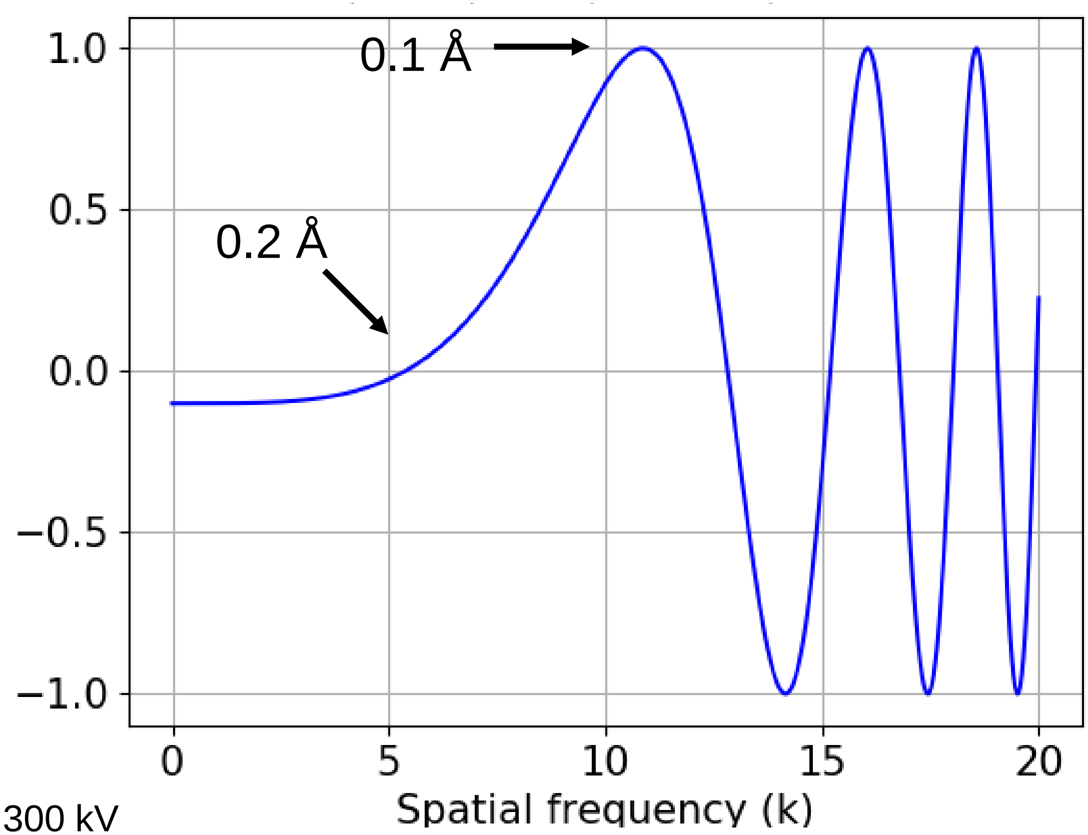

300 kV
A=0.1
B=0 Å²
Cs=0 mm
Def=0 µm

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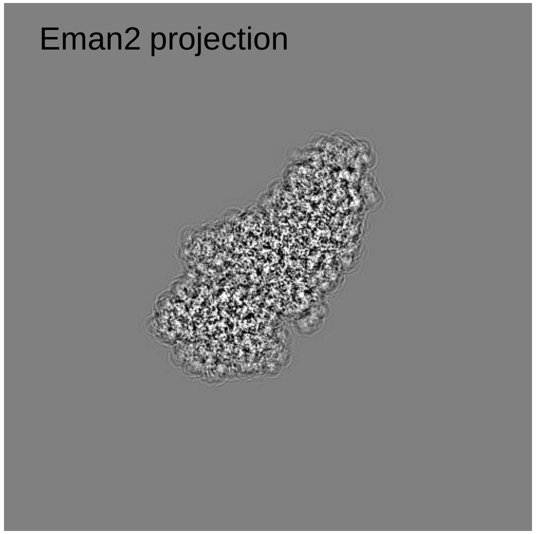
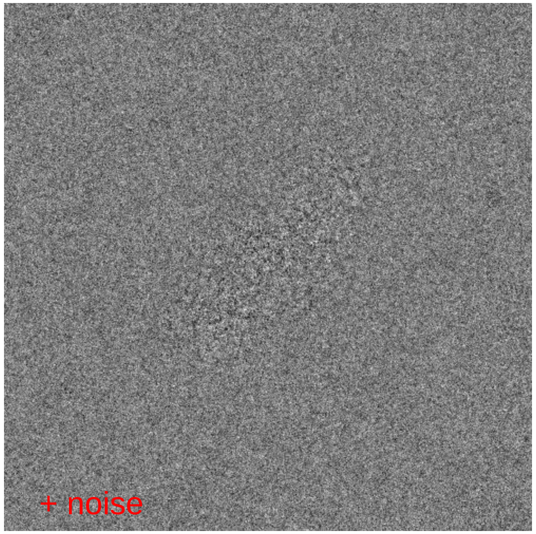
e2proc2d.py Input.mrcs Output_CTF.mrcs --process=math.simulatectf:ampcont=0.1:bfactor=0:cs=0.000001:defocus=0:voltage=300:noiseamp=0.3:noiseampwhite=0.8

0.25 μm defocus





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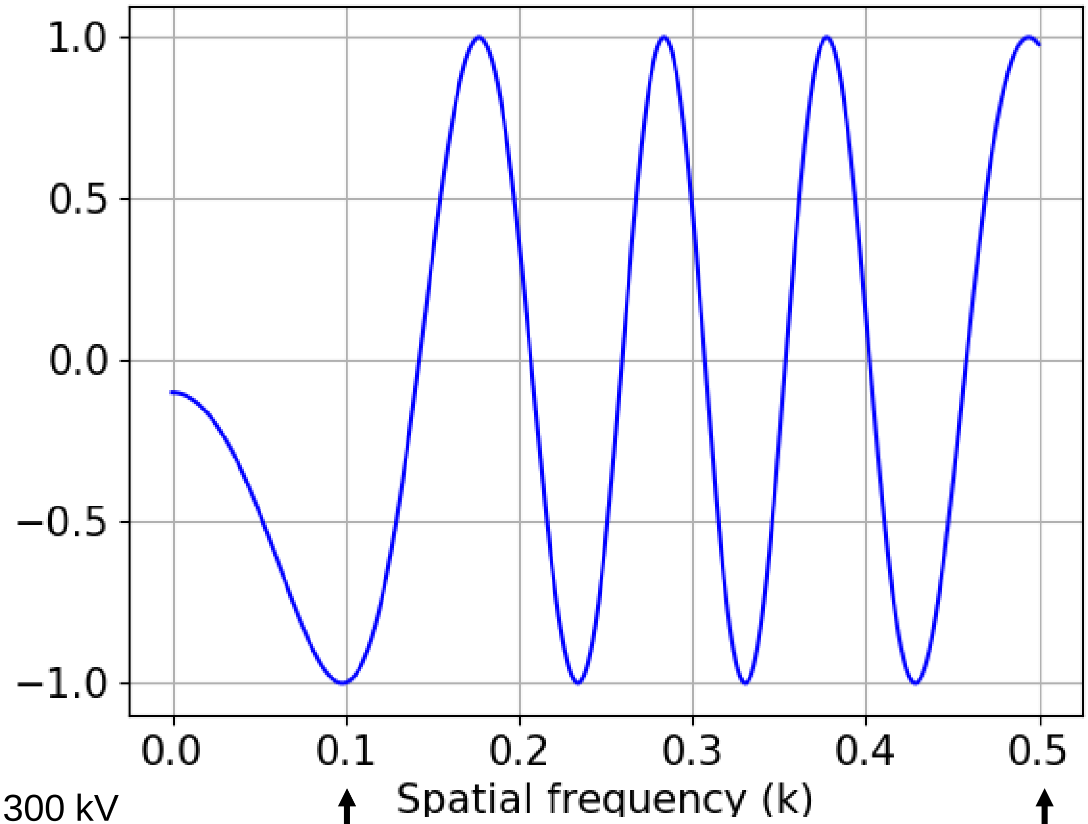
$$\gamma(k) = 2\pi(-0.5\Delta z \lambda k^2 + 0.25 Cs \lambda^3 k^4)$$



300 kV
A=0.1
B=0 Å^2
Cs=2 mm
Def=0.25 μm

10 Å

2 Å

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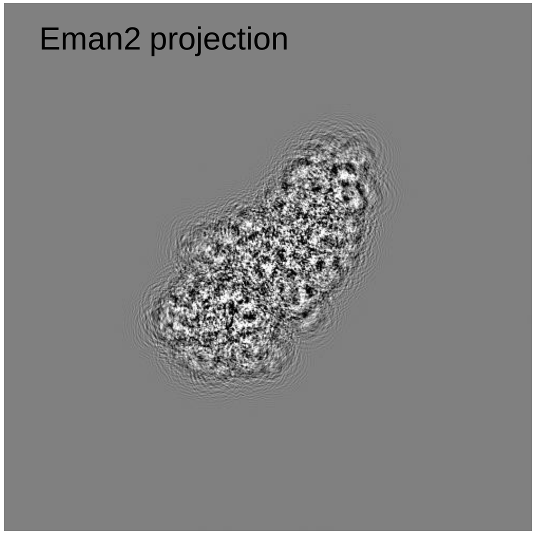
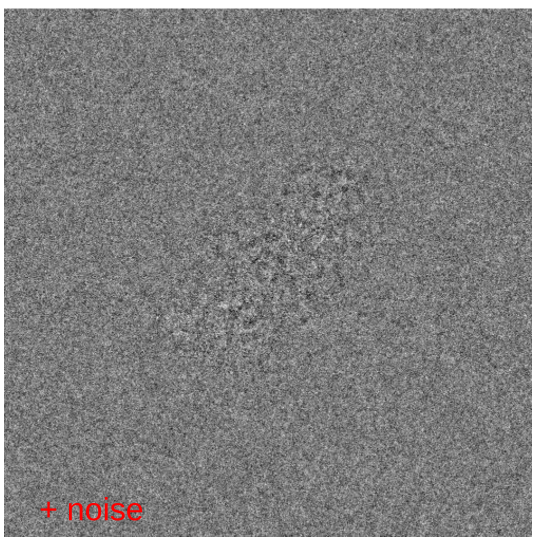
e2proc2d.py Input.mrcs Output_CTF.mrcs --process=math.simulatectf:ampcont=0.1:bfactor=0:cs=2.0:defocus=0.25:voltage=300:noiseamp=0.3:noiseampwhite=0.8

0.5 µm defocus





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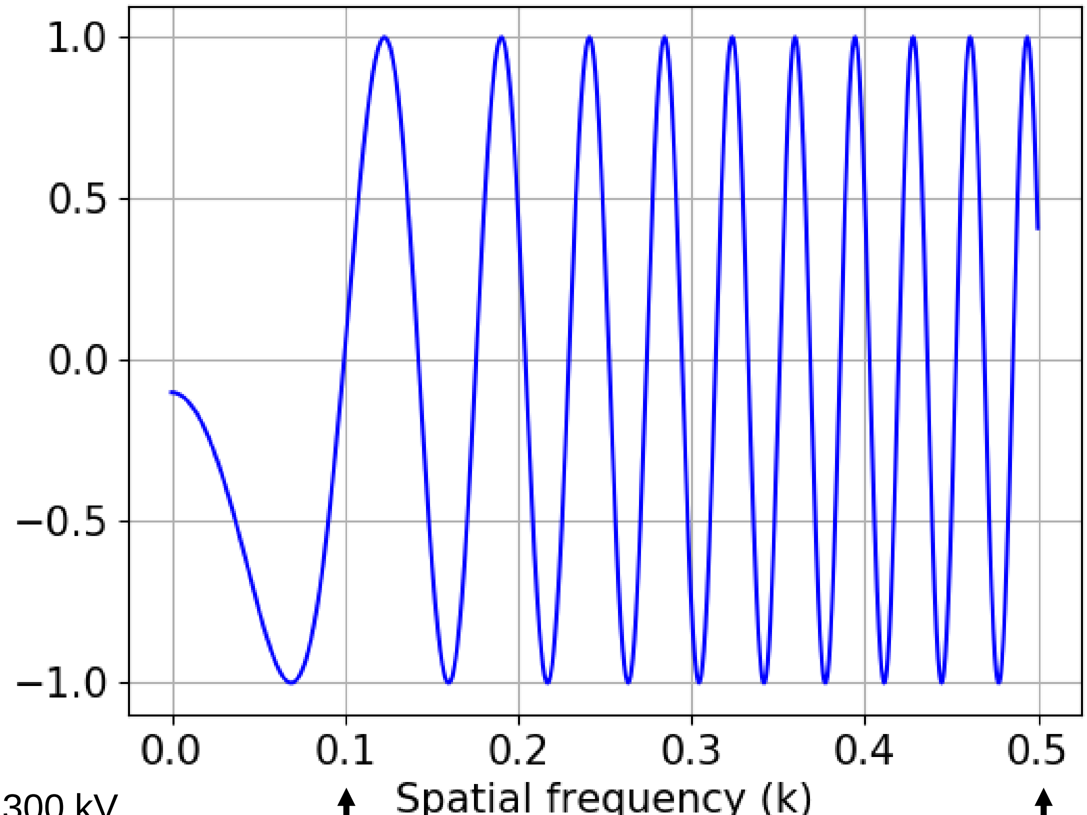

300 kV
A=0.1
B=0 Å^2
Cs=2 mm
Def=0.5 µm

10 Å

2 Å

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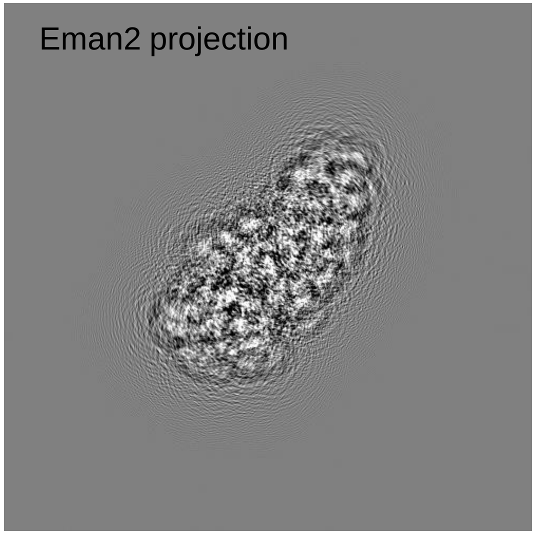
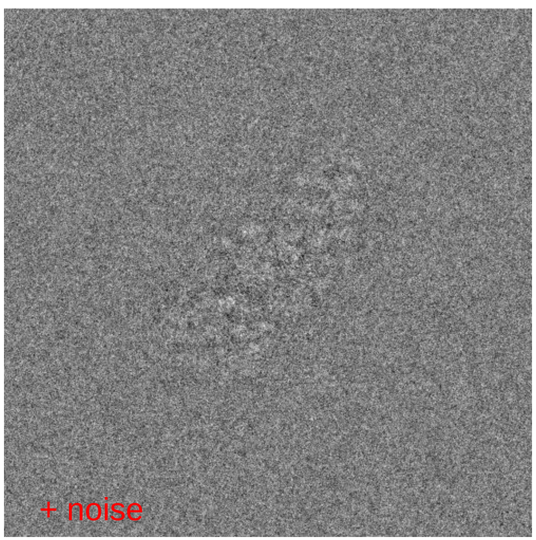
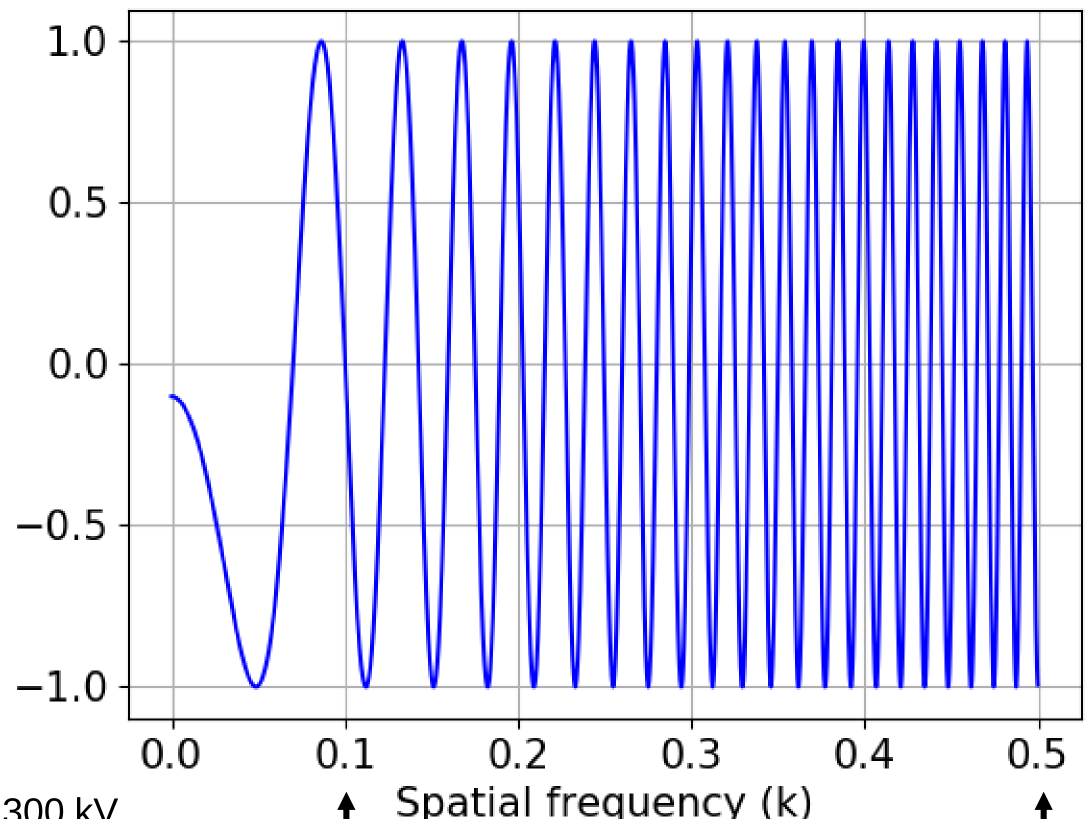
e2proc2d.py Input.mrcs Output_CTF.mrcs --process=math.simulatectf:ampcont=0.1:bfactor=0:cs=2.0:defocus=0.5:voltage=300:noiseamp=0.3:noiseampwhite=0.8

1 µm defocus





$$H(k) = 1[\sin\gamma(k) - W\cos\gamma(k)]$$
$$\gamma(k) = 2\pi(-0.5\Delta z\lambda k^2 + 0.25 Cs\lambda^3 k^4)$$



300 kV
A=0.1
B=0 Å^2
Cs=2 mm
Def=1 µm

10 Å

2 Å

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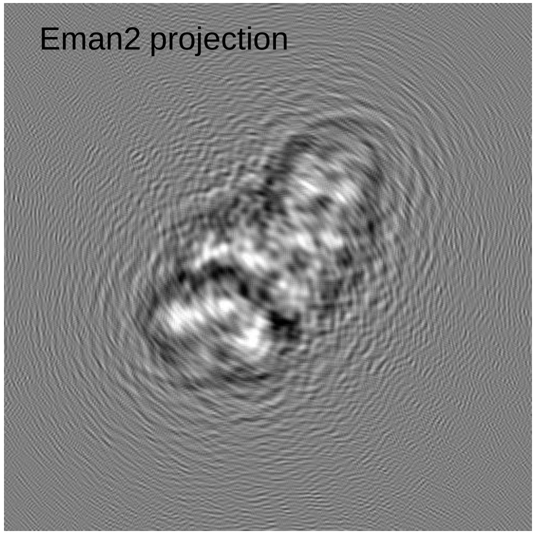
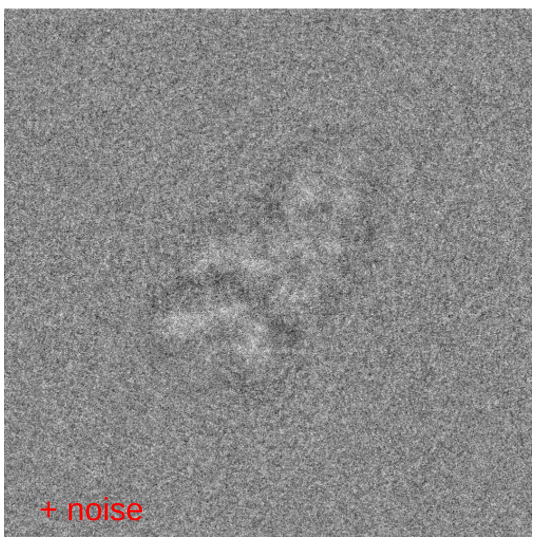
e2proc2d.py Input.mrcs Output_CTF.mrcs --process=math.simulatectf:ampcont=0.1:bfactor=0:cs=2.0:defocus=1.0:voltage=300:noiseamp=0.3:noiseampwhite=0.8

5 µm defocus





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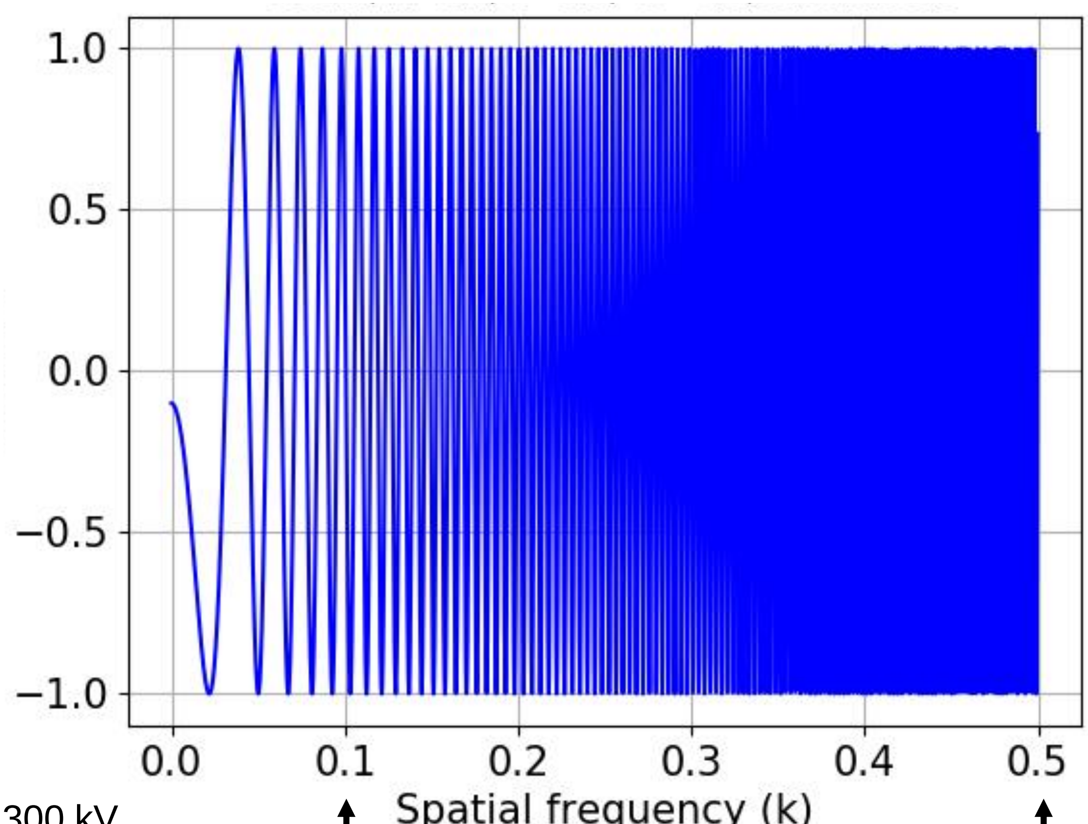
$$\gamma(k) = 2\pi(-0.5\Delta z\lambda k^2 + 0.25Cs\lambda^3k^4)$$



10 Å

2 Å

300 kV
A=0.1
B=0 Å^2
Cs=2 mm
Def=5 µm

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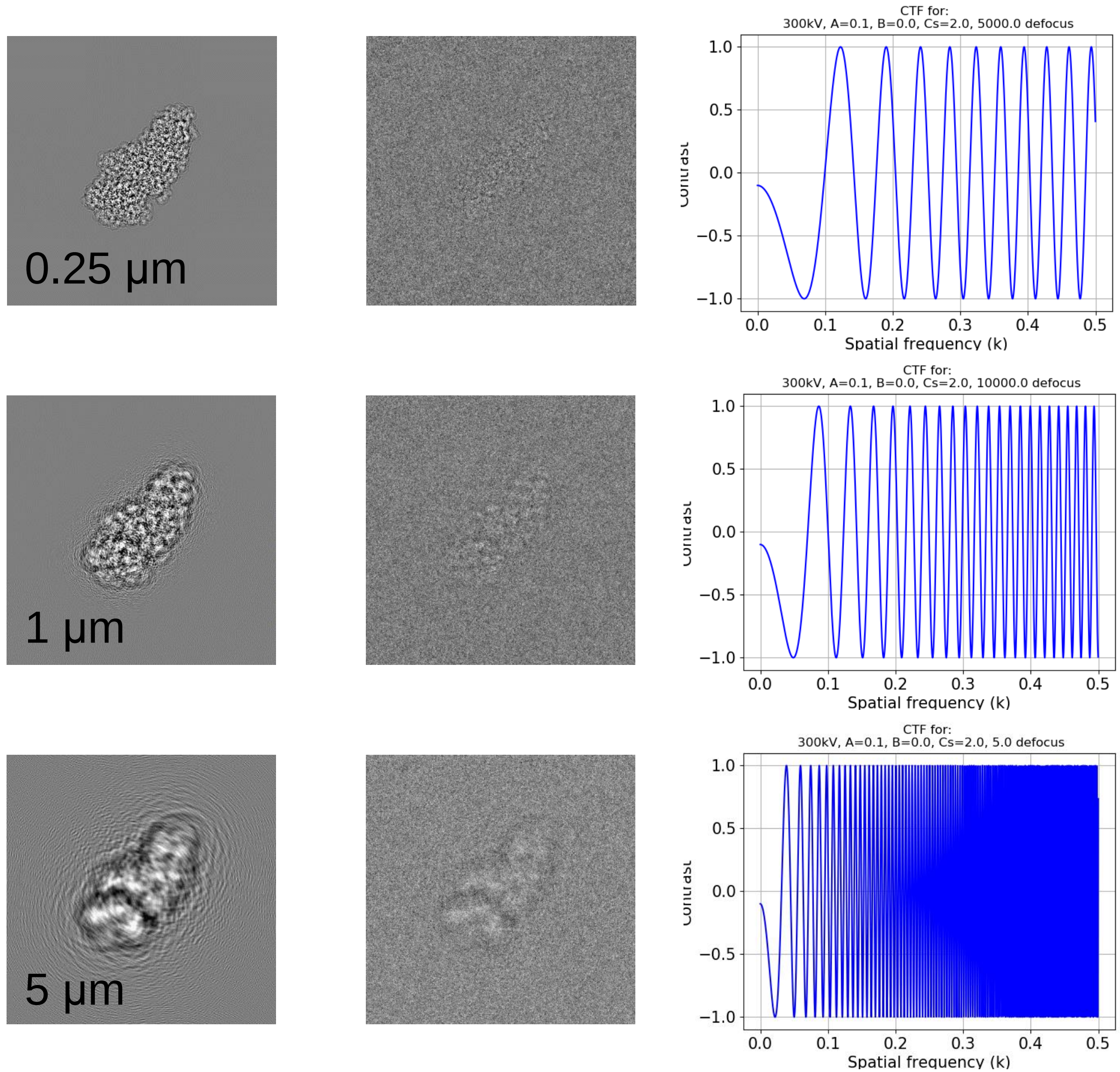
e2proc2d.py Input.mrcs Output_CTF.mrcs --process=math.simulatectf:ampcont=0.1:bfactor=0:cs=2.0:defocus=5.0:voltage=300:noiseamp=0.3:noiseampwhite=0.8

Change of defocus

Higher defocus improves contrast

The CTF changes more rapidly



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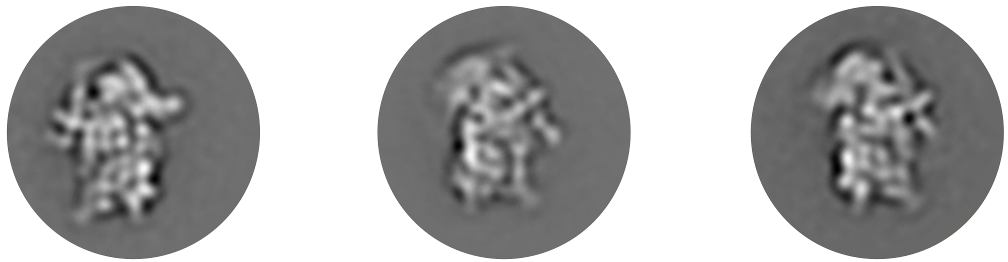
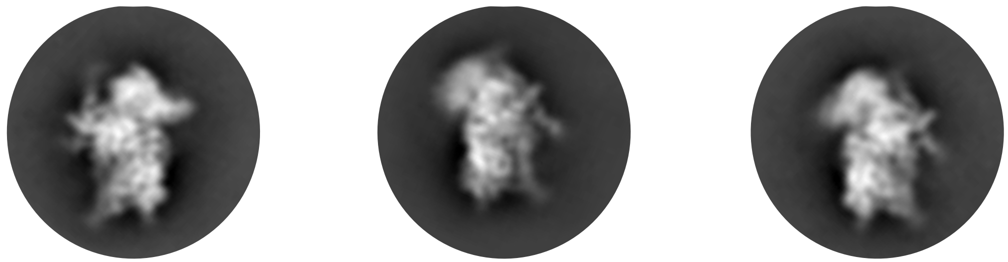
Why estimating the CTF matters

No correction



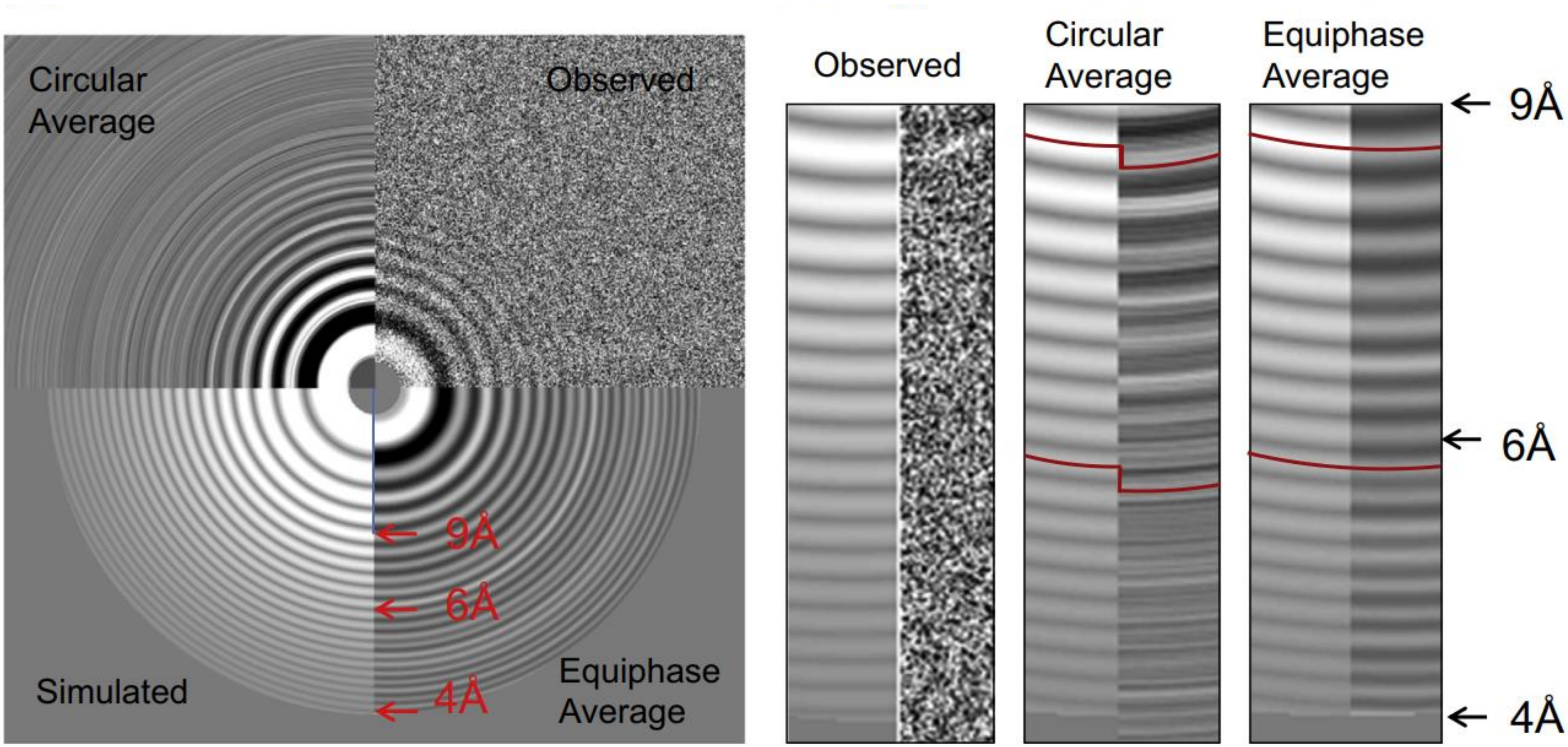
CTF corrected



- Various programs estimate the CTF
- CTF estimation is inherently inaccurate

CTF estimation

- More detail in gCTF, CTFFind4, and Zhu *et al. 1997*



Zhang, (2016)
J. Struc. Bio. 193:1-12

Effects of estimation error

- Errors of greater than $\pi/2$ flip phases incorrectly

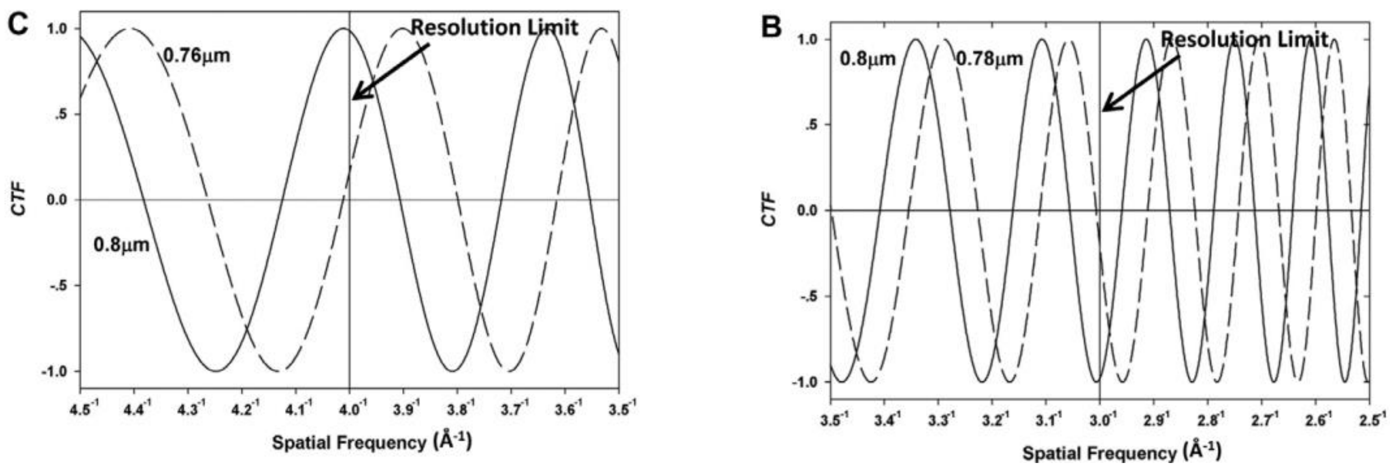


Table 3
Resolution Limit imposed by inaccuracy of defocus determination.

Res. (Å)	100 kV	200 kV	300 kV	400 kV
2.0	54 Å	80 Å	102 Å	122 Å
3.0	122 Å	179 Å	228 Å	274 Å
4.0	216 Å	319 Å	406 Å	488 Å
7.0	662 Å	976 Å	1244 Å	1494 Å

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Zhang & Zhou (2011)
J. Struc. Bio. 175:253-263

Goals for today

- Image formation in EM
- The contrast transfer theory
- CTF equation
- Effect of various parameters on the CTF
- Why CTF estimation matters
- Envelope functions

Envelope functions

Finite source size
(source: q)

$$E_{pc}(k) = \exp\left[-\pi^2 q^2 (k^3 C_s \lambda^3 - \Delta z k \lambda)^2\right],$$

Energy spread
(δz defocus variation)

$$E_{es}(k) = \exp\left[-\frac{1}{16 \ln 2} \pi^2 \delta z^2 k^4 \lambda^2\right],$$

MTF of film

$$E_f(k) = 1/[1 + (k/k_f)^2],$$

Generic envelope func.
(drift, specimen charging, multiple scattering)

$$E_g(k) = \exp\left[-(k/k_g)^2\right],$$

$$I(\mathbf{k}) = E_{pc}(k) E_{es}(k) E_f(k) E_g(k) H(k) \Phi(\mathbf{k}) + N(\mathbf{k}).$$

B-factor: the generic envelope function

$$I(\mathbf{k}) = E_{pc}(k)E_{es}(k)E_f(k)E_g(k)H(k)\Phi(\mathbf{k}) + N(\mathbf{k}).$$

$$e^{-Bk^2}$$

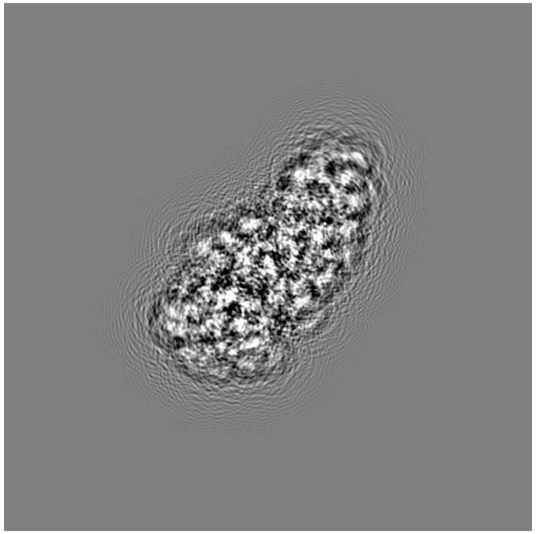
"[this] formulation is in conflict with the theory of partial coherence, according to which the damping term due to finite source size is defocus dependent" (Frank, 2006)

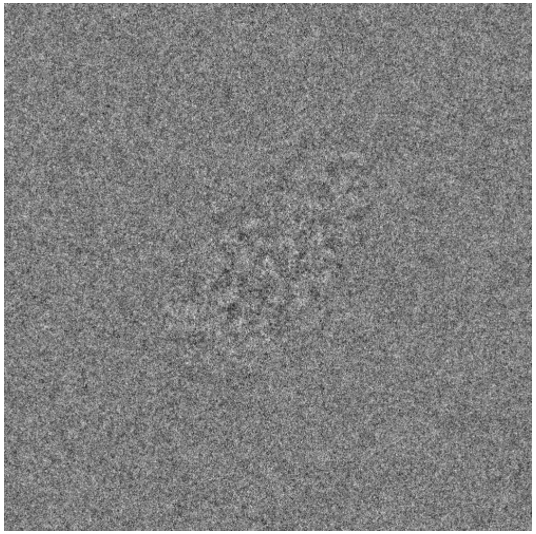
Finite source size

(source: q)

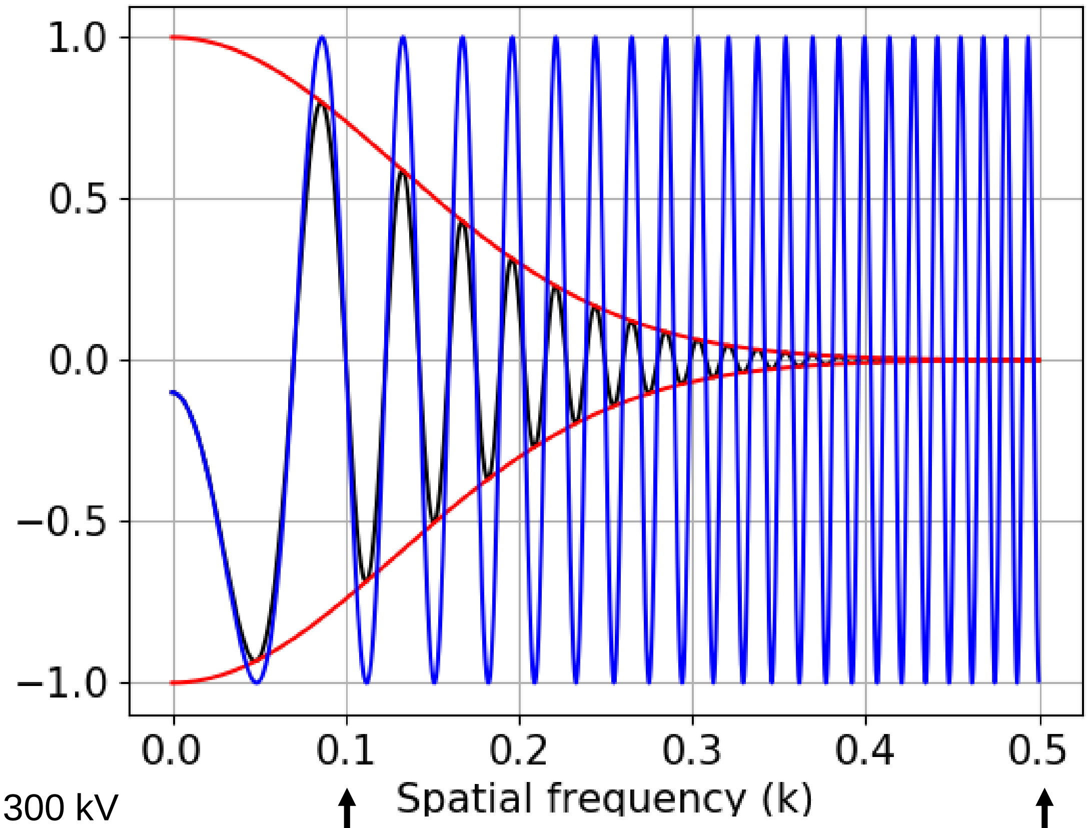
$$E_{pc}(k) = \exp\left[-\pi^2 q^2 (k^3 C_s \lambda^3 - \Delta z k \lambda)^2\right],$$

B-factor: 30 Å^2





$$H(k) = 1[\sin\gamma(k) - W\cos\gamma(k)]$$
$$\gamma(k) = 2\pi(-0.5\Delta z\lambda k^2 + 0.25 Cs\lambda^3 k^4)$$



300 kV
A=0.1
B=30 Å^2
Cs=2 mm
Def=0.25 μm

10 Å

2 Å

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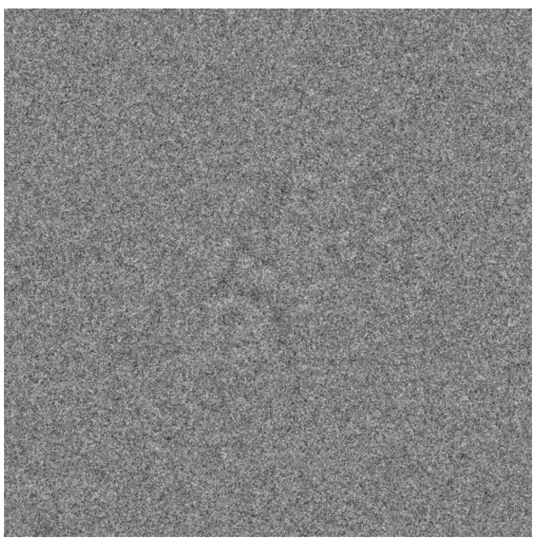
e2proc2d.py Input.mrcs Output_CTF.mrcs --process=math.simulatectf:ampcont=0.1:bfactor=30:cs=2.0:defocus=1.0:voltage=300:noiseamp=0.3:noiseampwhite=0.8

B-factor: 1500 Å^2



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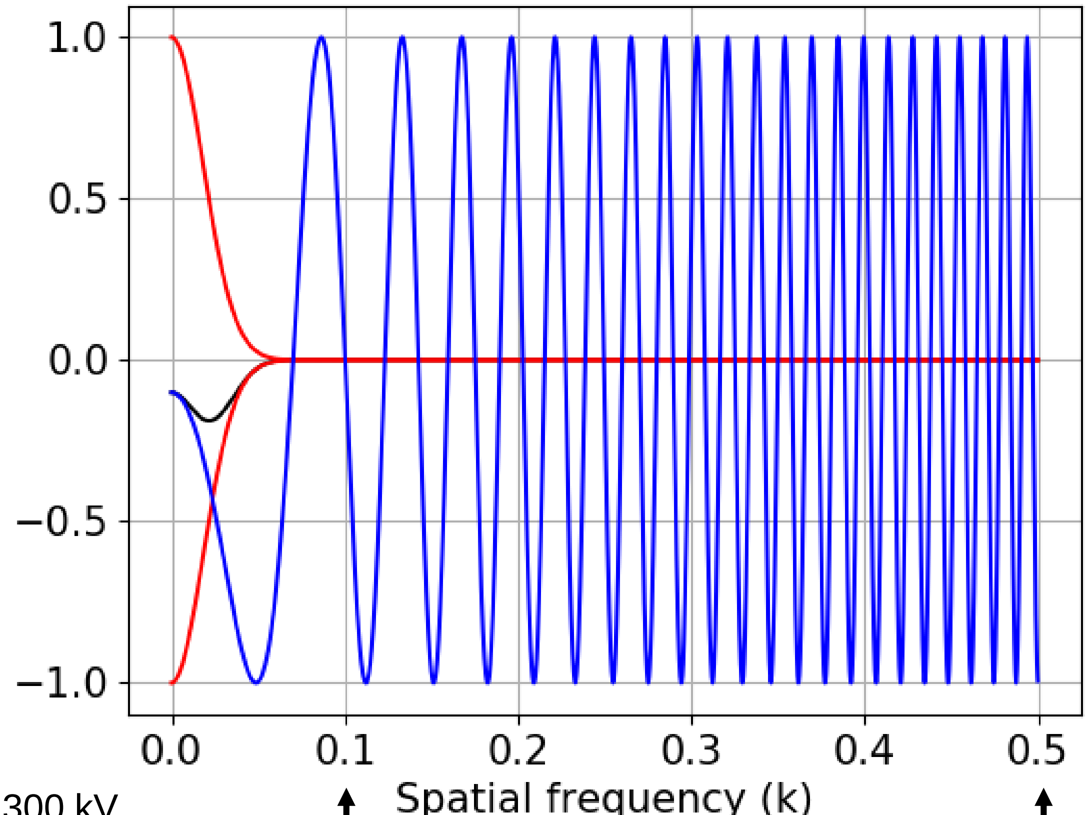

300 kV
A=0.1
B=1500 Å^2
Cs=2 mm
Def=0.25 μm

10 Å

2 Å

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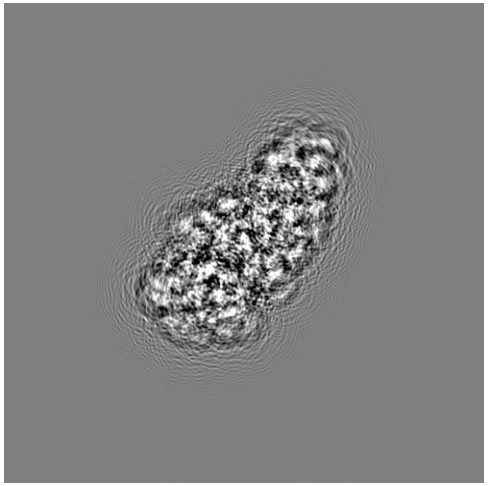
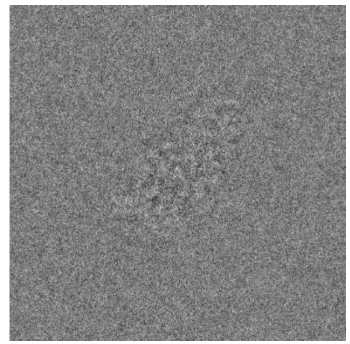
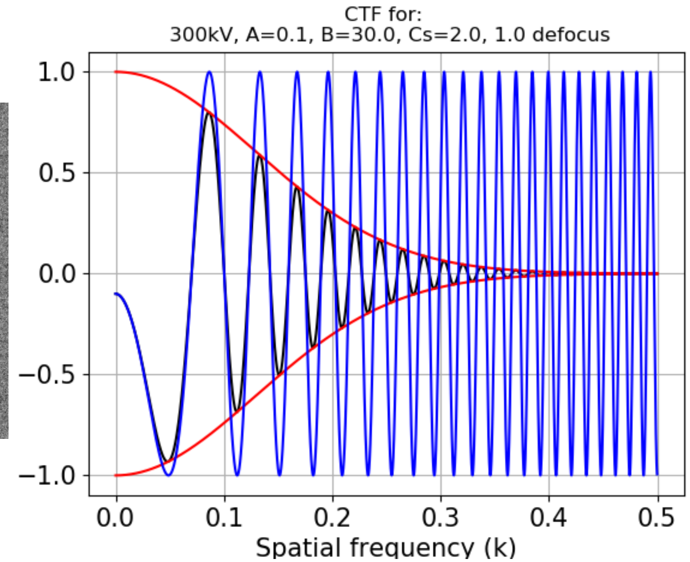
e2proc2d.py Input.mrcs Output_CTF.mrcs --process=math.simulatectf:ampcont=0.1:bfactor=1500:cs=2.0:defocus=1.0:voltage=300:noiseamp=0.3:noiseampwhite=0.8

B-factor

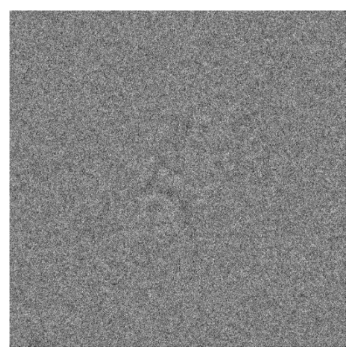
30 Å²

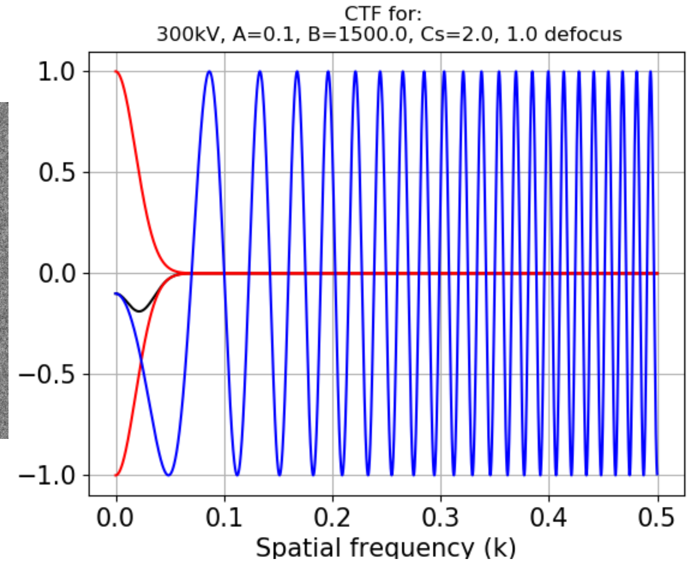






1500 Å²





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Effect of different acceleration voltage

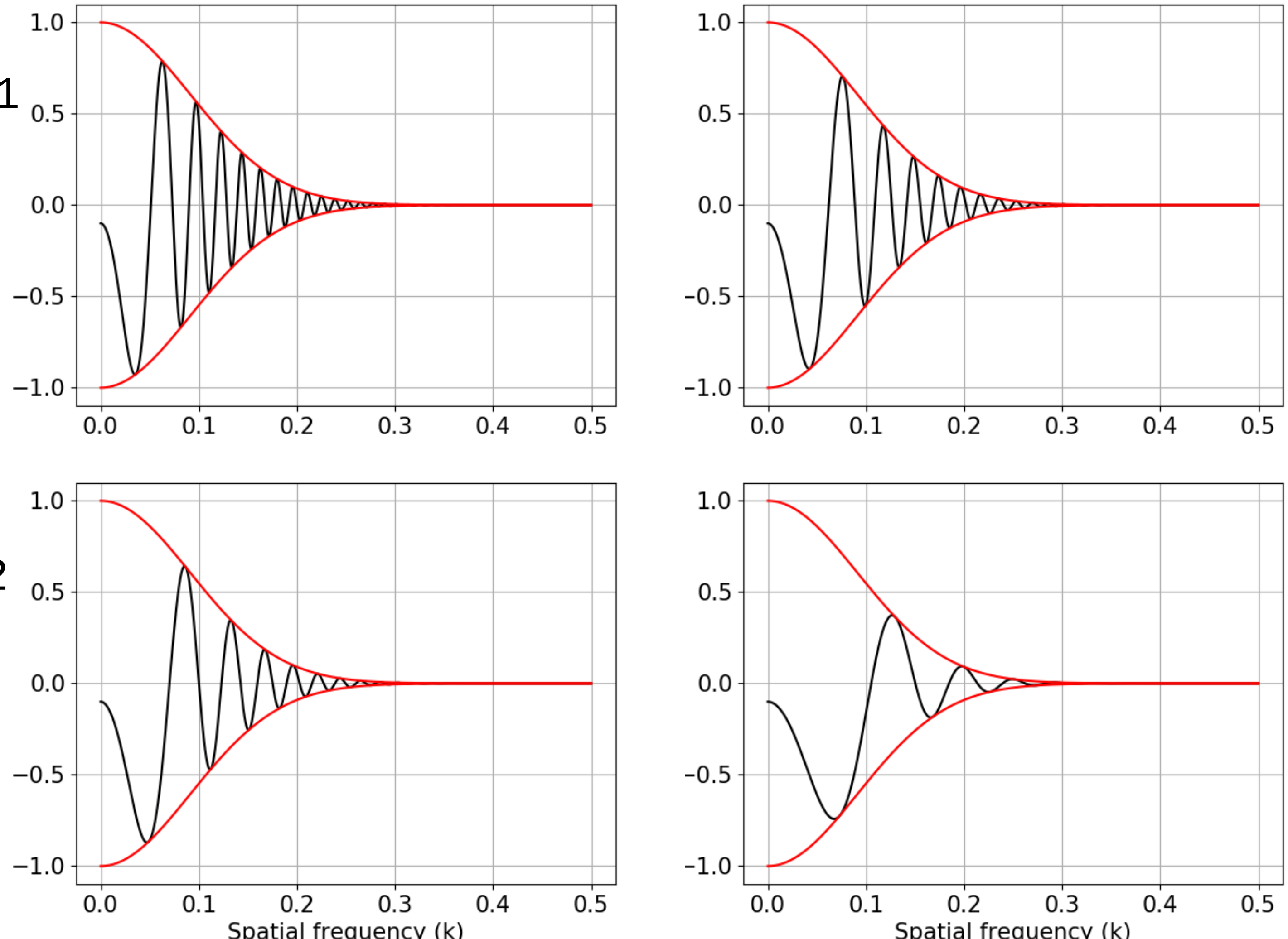
$$H(k) = 1[\sin\gamma(k) - W\cos\gamma(k)] \qquad \gamma(k) = 2\pi(-0.5\Delta z\lambda k^2 + 0.25Cs\lambda^3 k^4)$$

100 kV
Area= 681

200 kV
Area=670

300 kV
Area=662

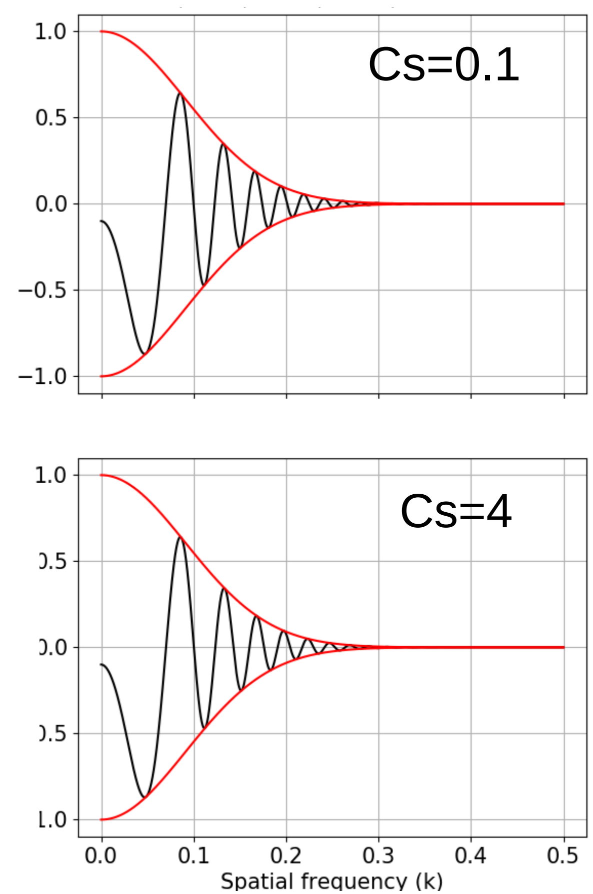
1000 kV
Area=650



Conclusion

$$H(k) = 1[\sin\gamma(k) - W\cos\gamma(k)]$$
$$\gamma(k) = 2\pi(-0.5\Delta z\lambda k^2 + 0.25Cs\lambda^3k^4)$$

- Everything is a trade off
- Defocus increases contrast but makes CTF estimation more difficult (error prone)[*citation needed!*]
- Δz, λ, Cs change the CTF
  – NB: Why Cs doesn't matter much…



How to investigate further

- Eman2

e2pdb2mrc.py	PDB → MRC
e2project3d.py	Make projections
e2filtertool.py	math.simultatectf
e2proc2d.py	apply in bulk

- Script got CTF examples

  ctf_simulation_v0.4.py

  github.com/zubengithub/CTF

Next Three sessions

- Grids
- Electron-specimen interactions
- Data processing strategies (MC2, gCTF, CTFFind4, particle polishing)