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

McMullan, Faruqi, Henderson (2016)
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

Electron beam

Specimen (real space)

Objective lens

Diffraction pattern

Back focal plane (reciprocal space)

Electron microscope image

Image plane (real space)
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

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

- (in Fourier space)

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

- \( W \): amplitude contrast ratio
- \( \Delta z \): defocus: underfocus is positive
- \( \lambda \): electron wavelength
- \( Cs \): spherical aberration
- \( k \): spatial frequency
300 kV, all zero

\[ H(k) = 1[\sin\gamma(k) - W\cos\gamma(k)] \]
\[ \gamma(k) = 2\pi(-0.5\Delta z\lambda k^2 + 0.25Cs\lambda^3 k^4) \]
300 kV, 0.1 amp. contrast

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

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

Eman2 projection

Spatial frequency (k)

- 0.1 Å
- 0.2 Å

300 kV
A = 0.1
B = 0 Å²
Cs = 0 mm
Def = 0 μm

+ noise
0.25 μm defocus

H(k) = 1[sinγ(k) − Wcosγ(k)]
γ(k) = 2π(−0.5Δzλk^2 + 0.25Csλ^3k^4)

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

Eman2 projection

+ noise
0.5 μm defocus

\[ H(k) = 1[\text{siny}(k) - W\cos y(k)] \]
\[ y(k) = 2\pi(-0.5\Delta z\lambda k^2 + 0.25Cs\lambda^3k^4) \]

300 kV
A=0.1
B=0 Å²
Cs=2 mm
Def=0.5 μm

Eman2 projection + noise

Noisy signal
1 μm defocus

Eman2 projection

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

\[ \gamma(k) = 2\pi \left(-0.5 \Delta z \lambda k^2 + 0.25 Cs \lambda^3 k^4 \right) \]

300 kV
A=0.1
B=0 Å²
Cs=2 mm
Def=1 μm

Input.mrcs
Process=math.simulatectf:ampcont=0.1:bfactor=0:cs=2.0:defocus=1.0:voltage=300:noiseamp=0.3:noiseampwhite=0.8
Output_CTF.mrcs
$H(k) = 1[\sin \gamma(k) - W \cos \gamma(k)]$

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

300 kV
A=0.1
B=0 Å²
Cs=2 mm
Def=5 μm
Change of defocus

Higher defocus improves contrast

The CTF changes more rapidly

- 0.25 μm
- 1 μm
- 5 μm
Why estimating the CTF matters

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

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

Zhang, (2016)
Effects of estimation error

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

Table 3
Resolution Limit imposed by inaccuracy of defocus determination.

<table>
<thead>
<tr>
<th>Res. (Å)</th>
<th>100 kV</th>
<th>200 kV</th>
<th>300 kV</th>
<th>400 kV</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>54 Å</td>
<td>80 Å</td>
<td>102 Å</td>
<td>122 Å</td>
</tr>
<tr>
<td>3.0</td>
<td>122 Å</td>
<td>179 Å</td>
<td>228 Å</td>
<td>274 Å</td>
</tr>
<tr>
<td>4.0</td>
<td>216 Å</td>
<td>319 Å</td>
<td>406 Å</td>
<td>488 Å</td>
</tr>
<tr>
<td>7.0</td>
<td>662 Å</td>
<td>976 Å</td>
<td>1244 Å</td>
<td>1494 Å</td>
</tr>
</tbody>
</table>
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- CTF equation
- Effect of various parameters on the CTF
- Why CTF estimation matters
- Envelope functions
Envelope functions

Finite source size
(source: q)

Energy spread
(δz defocus variation)

MTF of film

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

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

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

\[ E_f(k) = \frac{1}{1 + (k/k_f)^2}, \]

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

\[ I(k) = E_{pc}(k) E_{es}(k) E_f(k) E_g(k) H(k) \Phi(k) + N(k). \]
B-factor: the generic envelope function

\[ I(k) = E_{pc}(k)E_{es}(k)E_{f}(k)E_{g}(k)H(k)\Phi(k) + N(k). \]

\[ e^{-Bk^2} \]

“[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

\[ E_{pc}(k) = \exp \left[ -\pi^2 q^2 (k^3 C_s \lambda^3 - \Delta z k \lambda)^2 \right]. \]
**B-factor: 30 Å²**

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

- **300 kV**
- **A=0.1**
- **B=30 Å²**
- **Cs=2 mm**
- **Def=0.25 μm**

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**Output:**

```
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 Å²

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

300 kV
A=0.1
B=1500 Å²
Cs=2 mm
Def=0.25 μm
B-factor

30 Å²

1500 Å²
Effect of different acceleration voltage

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

\[ \gamma(k) = 2\pi \left( -0.5 \Delta z \lambda k^2 + 0.25 C s \lambda^3 k^4 \right) \]

100 kV
Area = 681

200 kV
Area = 670

300 kV
Area = 662

1000 kV
Area = 650
Conclusion

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

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

• Everything is a trade off
• Defocus increases contrast but makes CTF estimation more difficult (error prone) [citation needed!]
• $\Delta z$, $\lambda$, 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)