Contrast transfer and CTF correction

The weak phase approximation
Contrast transfer function
Determining defocus
CTF correction methods

Why do we need to bother with defocus?


The weak phase approximation

Review of Lecture 3:

EM image = projected electron scattering density of object modified by the CTF
If the object is thin and weakly scattering (i.e., made of light atoms), a simplified form of the CTF function can be derived.

The phase shift \( \Phi(r) \) from a weak phase object is small, and the wave expression \( \psi \exp[i \Phi(r)] \) can be approximated by the series

\[
\psi \approx [1 + \frac{i}{2} \Phi(r)^2 + \frac{1}{3} \Phi(r)^3 - \ldots]
\]

Because the phase shift is small, the 3rd order and higher terms can be ignored.

This approximation, combined with the phase shift introduction by spherical aberration, leads to the expression for the phase contrast transfer function, given on the next slide.
Phase CTF formula from the weak phase approximation

Phase CTF = \(-2 \sin [\pi (\Delta Z \lambda q^2 - C_s \lambda^3 q^4/2)]\)

- \(C_s\) – spherical aberration coefficient
- \(\Delta Z\) – defocus
- \(q\) – spatial frequency
- \(\lambda\) – electron wavelength

Ideal CTF curves

FEG images of carbon film

Causes of CTF decay

- Loss of spatial coherence - source size
- Image drift
- Thick ice
- Specimen charging
- Chromatic aberration - variation in voltage
- Variation of lens current
Decay caused by loss of spatial coherence

defocus = 4 µm
Beam divergence = 0.09 mrad

Drift and jumping

Effect of drift on the CTF

No drift

10 Å/sec drift
The CTF is the FT of the Point Spread Function

Diffraction patterns

PSF

FT

CTF

Tilt geometry and defocus

Effects of CTF on 2D projections

Effects of CTF on a 3D map

For 60° tilt in a typical tomogram recorded on a 4k CCD, the defocus will vary by roughly ±1 µm around the mean value, which is normally 4-15 µm.

from Fernandez, Li & Crowther (2006) CTF determination and correction in electron cryotomography. Ultramicrosc. 106, 587-596. Strip CTF correction is implemented in IMOD
Why don't I see Thon rings???

- Ice too thick
- No carbon in image
- Too little specimen – vitreous ice alone does not give Thon rings! (and too thin ice excludes sample)
- Too close to focus on a non-FEG source

CTF ripples are superposed on a large background of incoherent scattering, noise and other features

Rotationally averaged total sum of image power spectra, band-pass filtered

Profile of the averaged spectrum

Background fitting and subtraction give a more accurate view of the CTF ripples
Comparison of the line profile of the rotationally averaged spectrum with the calculated contrast transfer function of the microscope.

Acceleration VOLTAGE    :    200  kV
CHROMATIC aberration   :    None
SPHERICAL aberration : 2.1     mm
FOCAL length of objective :  1.6    mm
APERTURE :  50.0 micrometer
DEFOCUS values           :   28600.00  A
PIXEL size in curve             :   2.52 A

Procedures for measuring defocus

**SPIDER/WEB** - graphical interface for overlaying experimental and theoretical curves
http://spider.wadsworth.org/spider_doc/spider/docs/spider.html

**EMAN2** - evalimage graphical interface
http://blake.bcm.edu/emanwiki/EMAN2/Programs/e2evalimage

**CTFFIND4** – graphical/automated
Chops up areas into boxes
Uses estimate of starting defocus
Searches over a specified range of defocus
Estimates astigmatism
Gives split display output for verification of result
http://grigoriefflab.janelia.org/ctffind4

**BSOFT** graphical/automated
http://lsbr.niams.nih.gov/bsoft/

CTFFIND4 output

Astigmatism

Defocus 2.405, 2.442 μm
Defocus 1.146, 1.219 μm
Fig. 1. Simulated power spectra with increasing astigmatism are shown. The caption shows the two defocus values corresponding to the power spectrum. The Thou rings can distort to an ellipse, a parabola or a hyperbola.

How to measure an astigmatic CTF

The ellipse must be fitted or measured in sectors to get the degree and angle of astigmatism so that the zeroes can be correctly determined for all directions.

What range of defocus is needed?

Angle of astigmatism, \( \theta \)

(does not depend on convention used by your program)

Minimum defocus

Maximum defocus
CTF curves from different images in a dataset

Methods of CTF correction

1. Phase flipping - can be done on raw images

2. Full restoration of amplitudes: Multiply each image FT by its own CTF, then add up all the equivalent views and divide the sum by the sum of all the CTF’s squared, plus a constant related to the signal:noise ratio (Wiener factor) to avoid division by zero.

\[
\text{FT}_{\text{Merged}_\text{class}} = \frac{\sum_{i=1}^{N} \text{FT}_{\text{class}_i} \cdot \text{CTF}_i}{\sum_{i=1}^{N} (\text{CTF}_i^2) + w}
\]

Effect of Wiener filtering

The larger the value of w, the more small fluctuations are suppressed - similar to low pass filtering.
Steps in full amplitude restoration

This can only be done by combining images of different defocus

References