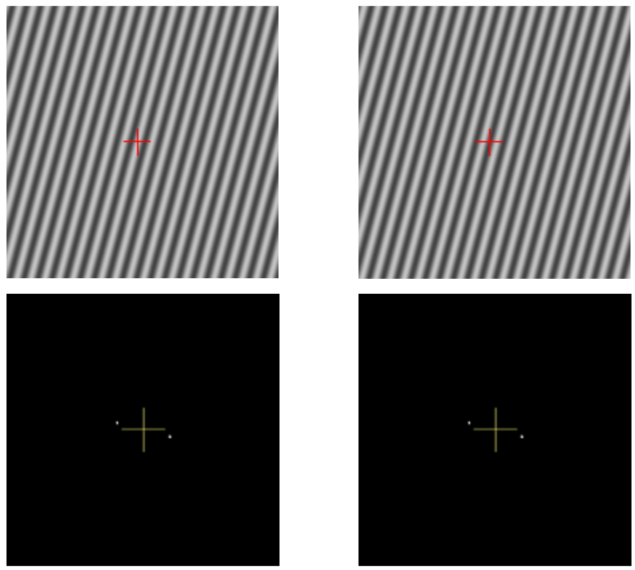
CTF Correction with IMOD

CTF Correction

- When microscope is operated in underfocus to produce phase contrast, the contrast is inverted in some spatial frequency ranges

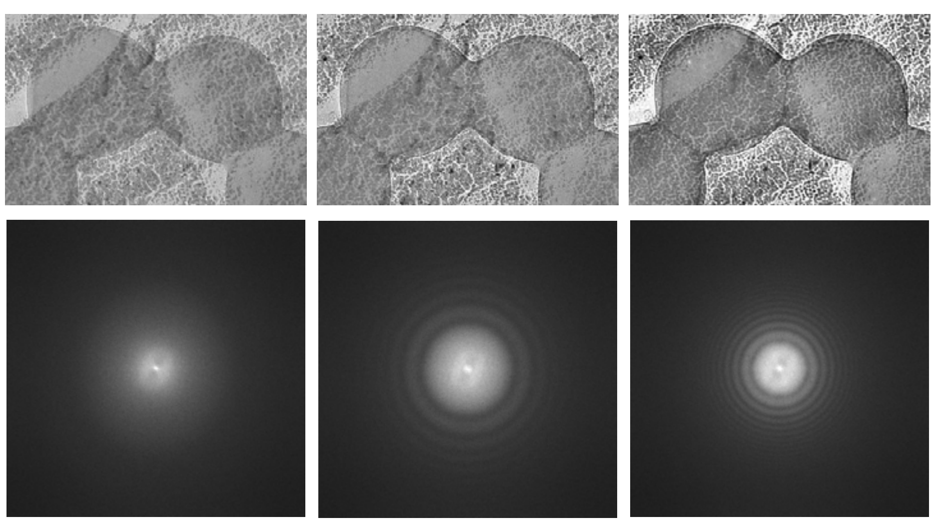
We See Only Amplitudes, Not Phases, in these Transforms



Phase-shifted images with the same power spectrum

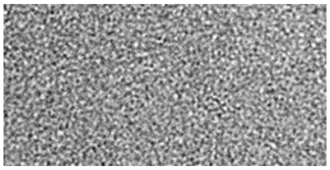
Underfocus Enhances Phase Contrast of Low Frequencies

- Most of what we perceive in an image consists of low frequencies
- Thus underfocus enhances the contrast that we see

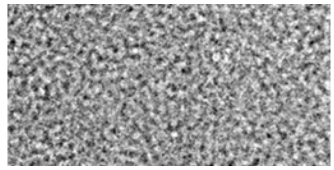


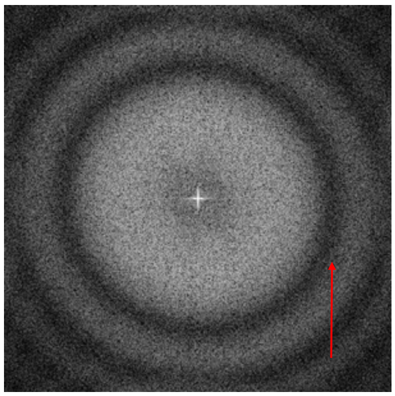
0.4 µm underfocus | 2 µm underfocus | 6 µm underfocus

Underfocus Also Produces Thon Rings in Power Spectra

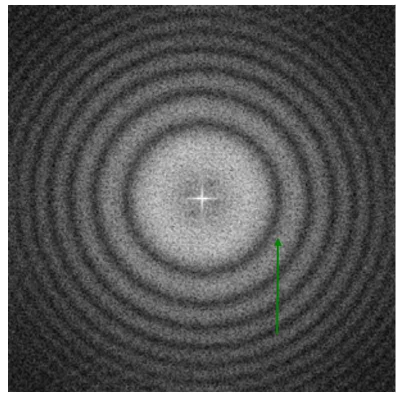


Carbon film





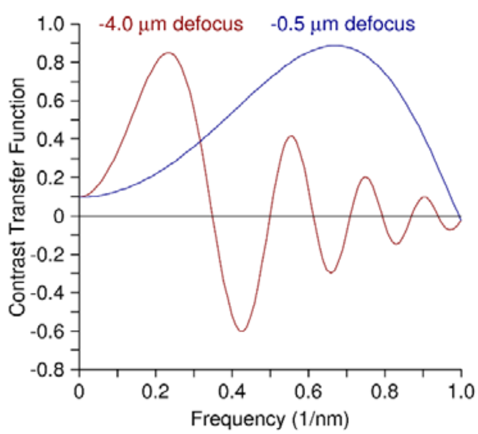
1.5 µm underfocus
First zero at 0.56/nm (1/1.8 nm)



5 µm underfocus
First zero at 0.32/nm (1/3.1 nm)

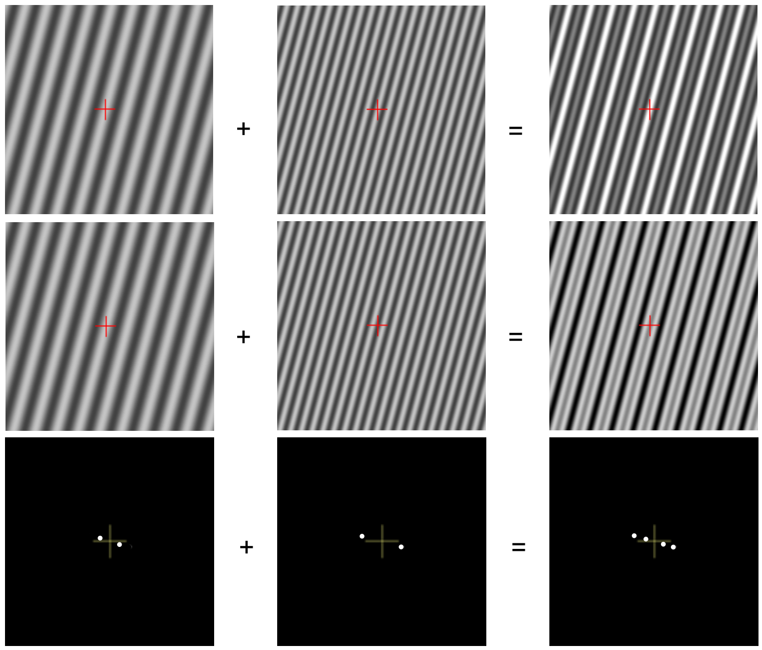
The CTF Produces Contrast Inversions

- Thon rings from oscillations in the CTF are an intrinsic result of the physics of phase image formation with underfocus
- In fact the CTF goes THROUGH zero, so contrast is inverted for frequencies between the first and second zero, third and fourth zero, etc.



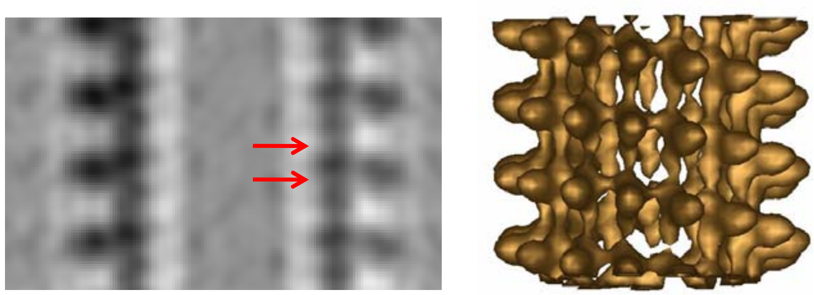
Example:
300 kV FEG
0.5 nm pixel size
Amplitude contrast 0.1
First inversion for -4 µm:
1/2.8 nm to 1/2.0 nm

Phases Must Be Right to See a Correct Image



CTF Correction Matters for Subvolume Averaging

- Without CTF correction, information past the first zero is incorrect and will give the wrong structure
- Example from a microtubule decorated with Eg5, taken at -8 µm defocus; the 4-nm tubulin repeat is between 1st and 2nd zero
- Without correction, Eg5 heads have the wrong shape and tubulin is almost 180º out of phase from where it should be



Average from Eg5-decorated microtubule, not corrected

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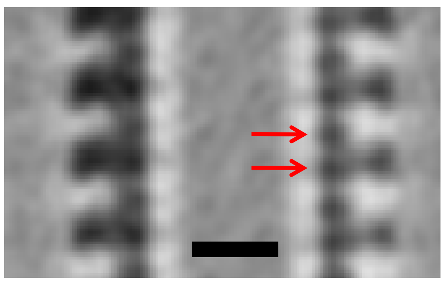
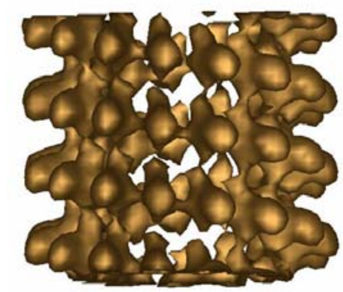


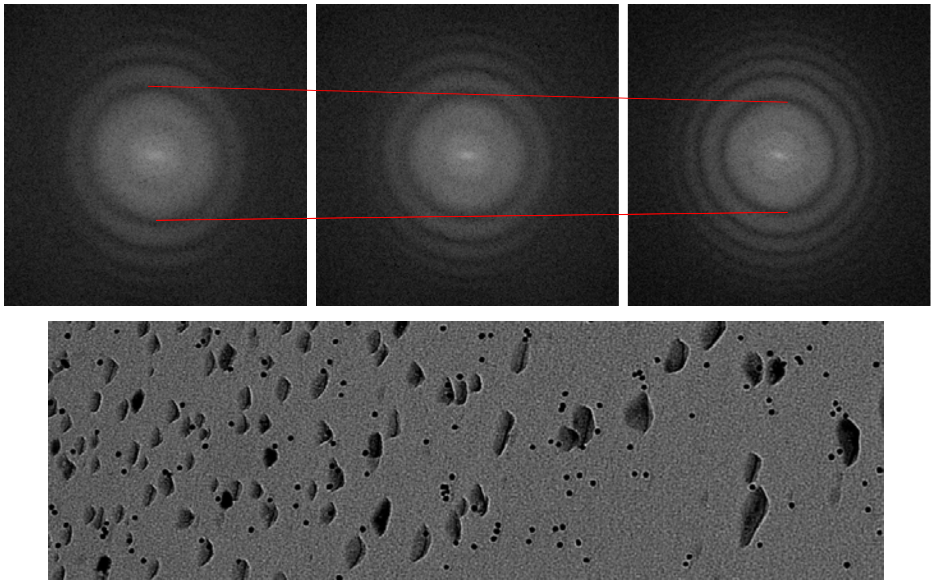


Average from Eg5-decorated microtubule, CTF corrected

What Makes CTF Correction of Tilt Series Hard?

- There is a different defocus gradient across each tilt series image
  - Not straightforward to invert phases in an FFT
  - Adding together data from different defoci will blur the power spectrum, make it hard to detect zeros

Defocus and Thon Ring Variation in Tilted Sample



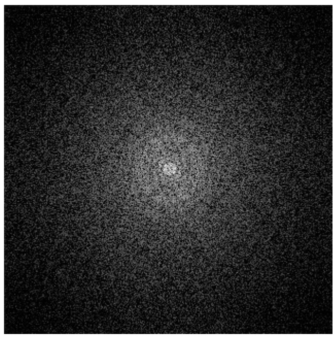
5 nm gold on carbon film, tilted to 65°

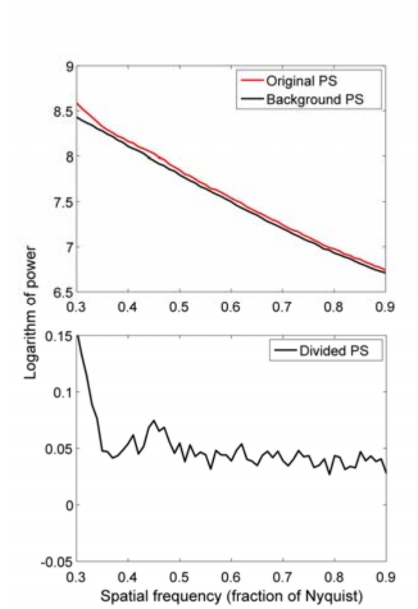
4.9 µm underfocus 6 µm underfocus 7.1 µm underfocus

What Makes CTF Correction of Tilt Series Hard?

- There is a different defocus gradient across each tilt series image
  - Not straightforward to invert phases in an FFT
  - Adding together data from different defocuses will blur the power spectrum, make it hard to detect zeros
- The dose is ~10-20 fold lower per micrograph than for single-particle averaging
  - Data must be averaged from multiple images to see CTF effect in power spectrum, unless camera is very efficient or defocus is large
- There may be little information past the second zero (or even the first zero with a CCD camera)
  - This depends on relationship between pixel size and defocus
  - For cryoET with relatively low defocus, there may be few zeros in spectrum, unless very high resolution is being targeted

Rotational Averaging and Background Subtraction Are Essential for Seeing CTF Zeros in Tilt Series



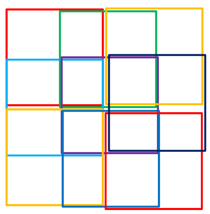


Rotational Averaging and Background Subtraction Are Essential for Seeing CTF Zeros in Tilt Series

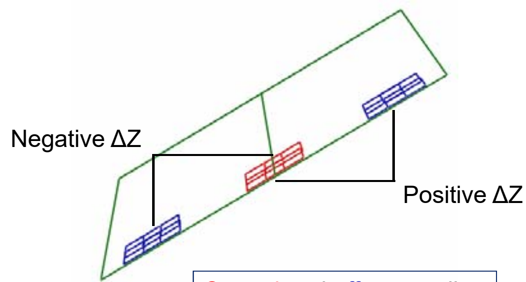
- The Ctfplotter program uses noise images to estimate the background
  - These are specific to camera, microscope, KV, binning.
  - Take series of blank images increasing in counts by factor of 1.5-2
  - Place these in /usr/local/ImodCalib/CTFnoise directory
  - See Ctfplotter man page for instructions
  - Do this once, use images on many data sets
- Ctfplotter interpolates between the nearest pair of noise images to find the noise background for a given image

To Compute Power Spectra, Each Image Is Divided into Overlapping Tiles

- This is periodogram averaging, standard method used to get power spectra for CTF correction
  - Tiles are typically 256 pixels square and overlap by 50%
  - Fourier transforms are taken separately and averaged
- For tilt series images, tiles are classified by distance from tilt axis in center:
  - Close to axis (Z height within 200 nm of center): central tiles
  - Farther from axis (Z height difference > 200 nm): off-center tiles



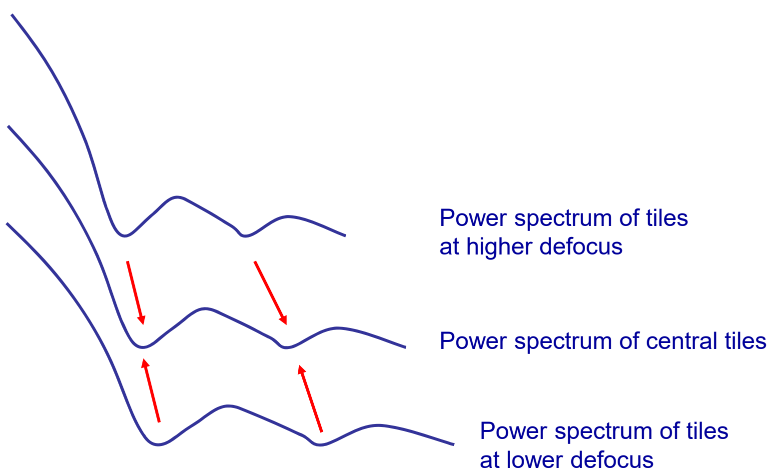
Overlapping tiles



Central and off-center tiles

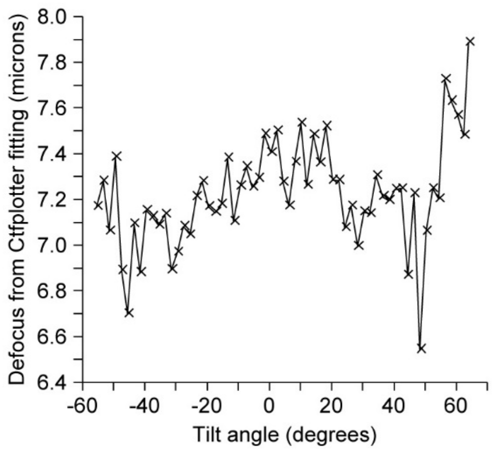
Adding Together Spectra from Different Defoci

- Power spectra from off-center tiles are scaled and shifted so that the first and second zeros are reinforced when they are added into spectra from central tiles



Goals in CTF Correction

- Ultimate goal is to find defocus for as many individual views as possible
  - Defocus can vary 0.2-0.4 μm from one tilt to next
  - Reliable fitting to single views is possible for data from direct detectors, especially K2; may be possible with CCD data too.



Defocus measured from single views
- Tilt series of Giardia ventral disk
- Acquired on Krios with energy filter and K2 camera
- Total dose 27 $e^-/Å^2$

Goals in CTF Correction

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  - Defocus can vary 0.2-0.4 μm from one tilt to next
  - Reliable fitting to single views is possible for data from direct detectors, especially K2; may be possible with CCD data too.
  - If signal is not strong enough to allow reliable fitting, fitting to every view will do more harm than good
- More modest goal, almost always achievable, is to find systematic change in defocus through series
  - Defocus can vary by up to ~1 μm from one end of series to other
  - Fitting to ranges of 10º to 40º can be useful for this
- Fallback goal: fit to whole series and find better estimate than the nominal defocus

Starting Ctfplotter

- Be sure to check these parameters when you reach CTF correction panel:
  - Microscope voltage
  - Spherical aberration – a constant for model of microscope and pole piece. 0 is allowed
  - Expected defocus
- Select the noise configuration file (it lists individual noise files)
- Start Ctfplotter and zoom the graph
  - Vertical axis is log of power; horizontal is frequency in 1/pixel
  - Magenta curve is power spectrum, green is a fitted curve
  - Use left mouse button to draw rubber band around region to magnify
  - Place rubber band inside desired region – e.g., to get X to range from 0.1 to 0.4 (usually good), draw band from 0.11 to 0.39
  - Zoom again vertically if necessary to visualize hump after first zero
  - Don’t be misled by a big hump before the first zero when there is lots of gold

Ctfplotter Steps 1

- Make sure expected defocus is correct to within 10%
  - Click on minimum of dip with left mouse if the fit is not close
  - If necessary, either adjust expected defocus or switch to use “Current defocus estimate”
- Add in the off-center tiles (select “All tiles”)
- Open Fitting Range dialog and adjust fitting parameters
  - Adjust start of range to where fit deviates from falling part of PS
  - Adjust end of range to wherever is appropriate
  - Turn on baseline fitting if curvature of the baseline interferes with the region that you are trying to fit
  - Select “Vary exponent” if fit is stable and it makes the curve fit better
- Switch to use “Current defocus estimate” to get PS that are most consistent with the defocus

Ctfplotter Steps 2

- Determine an angular range that can be fit through the whole series
  - Reduce the angular range and go to high tilt at both ends of series: see what works
  - Set the step size between ranges to half of the angular range
- Step through the series, storing values in the table as you go
- Or, autofit to all steps, then check the fits by double-clicking each line in the table