Contrast Transfer Function estimation for tilted, non-thin, weak-phase objects

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Currently, the resolution in reconstructed 3D volumes from (unstained) biological specimens is about an order of magnitude lower than the point resolution in a conventional transmission image. For spatial frequencies beyond the point resolution, i.e. the first zerocrossing of the contrast transfer function (CTF), phase contrast images cannot be interpreted and volumetric reconstructed without an accurate forward model is not possible. Here we present some the main optical ingredients for the full recipe that is necessary to measure all relevant parameters for a forward model. Where a full description includes incorporation of finite sample thickness, amplitude contrast, inelastic scattering, detector MTF and DQE and all aberrations, we focus on how accurately we can automatically measure the CTF assuming only a weak phase object in the absence of any aberrations other than a known C_s.

With this assumption the image formation is governed by the action of the CTF $T(q) = -\sin(\phi(q))$ which is multiplied to the object wave function, where the phase term is given by $\phi(q) = 2\pi(\frac{1}{4}C_s\lambda^3 q^4 - \frac{1}{2}\Delta f q^2)$. For a tilted specimen the CTF is not space invariant anymore in the sample plane due to change of focus. This variation in defocus is large (~1 μm) for high tilt angles around 70° and a field-of-view on the order of micrometers. First we developed a method to automatically estimate the CTF from an image of an amorphous sample and the minima in its angularly averaged power spectral density (PSD). In Figure 1 we plot the square of the oscillating part of the CTF where dimensionless units are used $q^* = q_0 C_s^{1/4}\lambda^{3/4}$ and $\Delta f^* = \Delta f\,(C_s\lambda)^{-1/2}$. We can incorporate any number of zero crossings $\phi(q) = k\pi, k \in \mathbf{Z}$ of the CTF for the estimate of the true defocus and at the same time obtain an intrinsic uncertainty estimation. We have tested the robustness of the algorithm by imaging carbon 30 times at three different nominal defocii, compare Figure 2. The drift of the stage in the beginning of the measurement can be clearly seen. If the sample is not exactly perpendicular to the optical axis, lateral drift will change the effective defocus. The spread in the estimation is only 2-3 nm at set defocii of > 1 μm. The estimated uncertainty from one image (grey area in Fig.2) is larger than the actual measured uncertainty. We compared the performance of our algorithm on 100 simulated test images (with background and noise) and against the ACE toolbox [1]. The defocus was set to 1 μm and estimated to 999±0.5 nm from the first 9 minima. Estimation from the first minima only was substantially more uncertain than minima 2-7 and of course the combined estimation. The ACE toolbox showed 2 clusters of estimation at 920nm and 960 nm. This deviation is only evident at higher minima, starting from the fourth, compare Figure 3. For tilted samples the CTF becomes space variant and estimation from a whole field-of-view results in an “average” defocus value. Partitioning the image into small strips of constant defocus helps to prevent this, compare Figure 4. We will report on further efforts to include the effect of amplitude contrast and finite sample thickness that do play a role under realistic experimental conditions in cryo-TEM and how these parameters influence the statistical estimation error.

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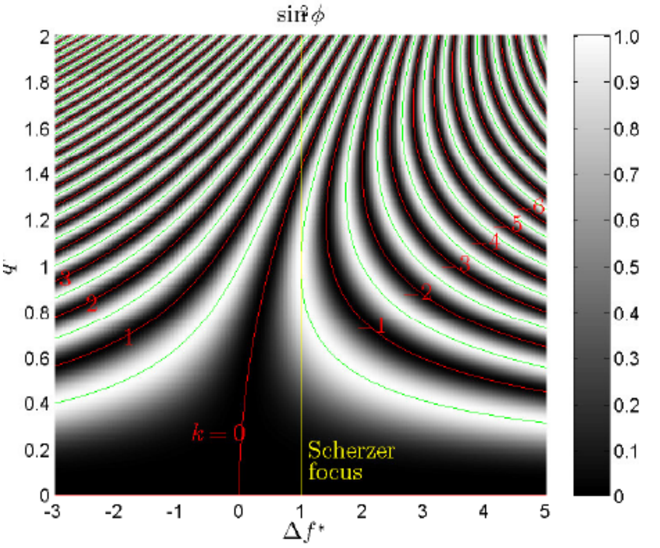

Figure 1. Square of the oscillating part of the CTF. The red line indicates minima and the green line maxima.

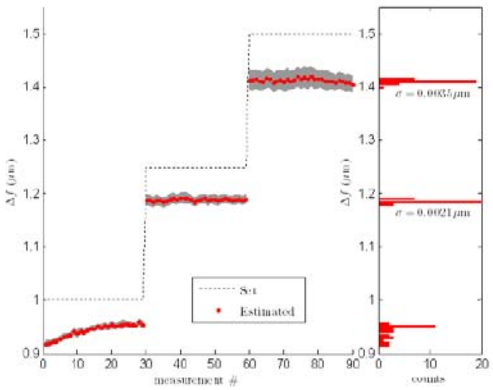

Figure 2. Repeated measurements of defocus, the right parts show the spread in estimation.

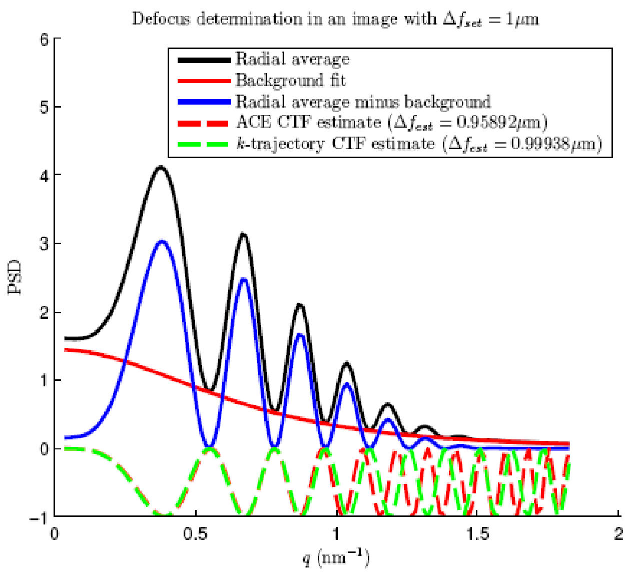

Figure 3. CTF estimation from simulations and comparison with the ACE toolbox.

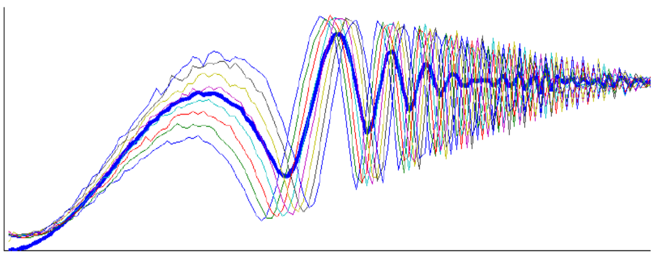

Figure 4. Influence of a tilt angle of only 17° on the measured PSD (from simulations). Thick blue line is the average over the whole field-of-view and the thin line correspond to the strip base

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