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Visualization and quality assessment of the contrast transfer function estimation

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ABSTRACT

The contrast transfer function (CTF) describes an undesirable distortion of image data from a transmission electron microscope. Many users of full-featured processing packages are often new to electron microscopy and are unfamiliar with the CTF concept. Here we present a common graphical output to clearly demonstrate the CTF fit quality independent of estimation software. Separately, many software programs exist to estimate the four CTF parameters, but their results are difficult to compare across multiple runs and it is all but impossible to select the best parameters to use for further processing. A new measurement is presented based on the correlation falloff of the calculated CTF oscillations against the normalized oscillating signal of the data, called the CTF resolution. It was devised to provide a robust numerical quality metric of every CTF estimation for high-throughput screening of micrographs and to select the best parameters for each micrograph. These new CTF visualizations and quantitative measures will help users better assess the quality of their CTF parameters and provide a mechanism to choose the best CTF tool for their data.

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1. Introduction

1.1. Background

The technique of three-dimensional electron microscopy (3DEM) enables the determination of a 3D structure from multiple 2D images collected by a transmission electron microscope. One of the many complications when collecting electron microscopy data is the effect of aberrations on a micrograph. Contrast is needed to differentiate particles from the background and is critical to align particles to produce highly detailed averages. To create this contrast, the microscope is focused just beyond the sample, in a practice known as underfocus. This underfocus increases the oscillating aberrations and distortion of the image data. The contrast transfer function (CTF) describes this delocalization of the density in the sample particles, which obscures the high-resolution information needed for a 3D reconstruction. These distortions described by the CTF are caused by inherent properties of the lens from both defocus and spherical aberrations. In Fourier space, characteristic Thon rings can be seen due to this density delocalization (Thon, 1966). These rings are often used for estimating the CTF parameters needed for correction of the aberrations. The CTF is formulated in Fourier space using the following equation:

$$\mathrm{PSD}(\vec{s}) = E^2(s) \cdot F^2(\vec{s}) \cdot \mathrm{CTF}^2(\vec{s}) + N^2(s) \quad (1)$$

where $\vec{s}$ is the 2D spatial frequency, PSD(s) is the calculated 2D power spectral density (PSD) of the micrograph, E^2 is the envelope, F^2 represents the structure factors of the sample, CTF^2 is the square of the contrast transfer function, and N^2 describes the additive background noise. Derivation of this equation from weak-phase object approximation theory has been addressed several times in the past (Mallick et al., 2005; Yang et al., 2009; Lander et al., 2009; Fernández et al., 2006; Saad et al., 2001; Sander et al., 2003; Huang et al., 2003; Ludike and Chiu, 2002; Angert et al., 2000). In this study, both $N^2(s)$ and $E^2(s)$ are assumed to depend only on the radial spatial frequency, s, and have no angular component. The structure factors, F^2, can have a large effect on the PSD, thereby affecting the oscillation behavior of the CTF (especially at low frequencies). For this study, any potential contributions from these structure factors are ignored. The CTF equation itself is defined as:

$$\mathrm{CTF}(\vec{s}) = B \cdot \sin(\gamma(\vec{s})) + A \cdot \cos(\gamma(\vec{s})) \propto \sin(\gamma(\vec{s}) + \varphi) \quad (2)$$

where $\gamma(\vec{s})$ is the wave aberration equation (discussed later), B is the phase contrast, and A is the amplitude contrast. The value of the

Abbreviations: 3DEM, three-dimensional electron microscopy; CTF, contrast transfer function; PSD, power spectrum density; CCD, charge-coupled device; DDD, direct detection device; FSC, Fourier shell correlation; FBC, Fourier band correlation; STIV, Sulfolobus turreted icosahedral virus.

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amplitude contrast, A, is defined to be constrained between 0 and 1, but under most circumstances it is closer to 0. The CTFFIND online documentation recommends values of 0.07 for vitreous ice samples and 0.15 for negatively-stained samples (Mindell and Grigorieff, 2003). The value of B is generally calculated from the amplitude contrast value, A, and varies with the definition of the amplitude contrast (Fernando and Fuller, 2007). Amplitude contrast can be defined in various ways which differ in the method of normalization and the effective shift of the oscillating signal that they each produce. We prefer the equation:

$$\begin{aligned}\mathrm{CTF}(\vec{s}) &= \sqrt{1-A^2}\cdot\sin(\gamma(\vec{s})) + A\cdot\cos(\gamma(\vec{s})) \\ &= \sin(\gamma(\vec{s}) + \arcsin(A))\end{aligned} \tag{3}$$

where B is defined as a variable dependent on A, with $B=\sqrt{1-A^2}$ in Eq. (2). By allowing only A to vary, the number of free parameters is reduced and the CTF equation always produces a normalized sine function. Inside the trigonometric functions of the CTF equation is the wave aberration function, $\gamma(\vec{s})$ given by the following formula:

$$\gamma(\vec{s}) = \gamma(s,\ \theta) = \frac{\pi}{2}C_s\lambda^3 s^4 + \pi\lambda z(\theta)s^2 \tag{4}$$

where s and θ are respectively the radial and angular component of spatial frequency, $z(\theta)$ is the angular-dependent defocus with a positive underfocus, and C_s is the spherical aberration constant. Lastly, λ is the wavelength of the electrons; it is given by a separate equation dependent on the electrical potential difference of the microscope in volts, V:

$$\lambda = \frac{h}{\sqrt{2m_e e_c\ V}}\cdot\left(1+\frac{e_c\ V}{2m_e c^2}\right)^{-1} \tag{5}$$

where c is the speed of light, e_c is the charge of the electron, m_e is the mass of the electron, and h is Planck's constant. The first term is the classical expression for the wavelength and the second term in parentheses is a relativistic correction factor. For example, a microscope with a potential difference of 300 kV produces electrons with a wavelength of 1.97 pm.

The wave aberration equation (Eq. (4)) indicates where the effects of both the underfocus and the spherical lens contribute to the CTF. The amount of defocus, $z(\theta)$, is set by the microscope operator and is dependent on the angular spatial frequency, θ, only in the presence of astigmatism. Before any CTF estimation process begins, three fixed microscope settings must be input: the potential difference voltage (V), the spherical aberration (C_s), and the pixel size at the specimen level of the micrograph. The spherical aberration is caused by off-axis electrons near the edge of the lens that focus closer to the lens than the central electrons, leading to an imperfection in the produced image. Each microscope has a characteristic spherical aberration determined by the manufacturer, and the provided value is rarely adjusted during CTF estimation. The image pixel size and C_s terms can be systematically adjusted to produce an identical fit with only a change to the defocus term (see Supplemental text for example). Because of interchangeability of the C_s term and the pixel size, there is no reason to adjust the C_s term when the pixel size is often less accurate and thus can account for any smaller C_s term inaccuracies. If changing the spherical aberration term provides an improved CTF fit, in our experience it is more likely that C_s term is correct and the real problem is with an inaccurate pixel size. The opposite sign of the spherical aberration relative to the positive underfocus value in Eq. (4) causes an expansion or reduced number of oscillations at higher frequencies in the CTF function. Together the spherical and defocus aberrations must both be corrected after image collection.

1.2. CTF estimation parameters

There are several stand-alone programs for measuring the effects of the CTF (Mindell and Grigorieff, 2003; Mallick et al., 2005; Jiang et al., 2012; Yang et al., 2009; Sidorov, 2002) and a system for CTF estimation is included in every single particle processing package (Lander et al., 2009; Ludtke et al., 1999; Heymann and Belnap, 2007; Frank et al., 1996; Tang et al., 2007; Hohn et al., 2007; Van Heel et al., 2012; Sorzano et al., 2004). Tomographic approaches have recently been integrating CTF estimation and correction for their data (Voortman et al., 2011; Philippsen et al., 2007; Mariani et al., 2011; Xiong et al., 2009; Zanetti et al., 2009; Fernández et al., 2006; Winkler and Taylor, 2003). Due to the additional complications introduced by tilted images and significantly lower signal-to-noise levels, tomographic approaches require a completely different approach than the one discussed here.

Four key CTF parameters are required for each micrograph in order to correct or reduce the effects of the CTF in the final 3D reconstruction. Any CTF estimation program will output these four CTF parameters: two defocus parameters, the amplitude contrast, and the astigmatism angle. In some programs, such as CTFFIND (Mindell and Grigorieff, 2003), the amplitude contrast is a user-supplied input. The two defocus and angle parameters specify the astigmatism. If no astigmatism is present only two parameters are required: the amplitude contrast and a single defocus value.

1.3. CTF estimation quality and interoperability

In some labs, each micrograph is thoroughly scrutinized for its quality and researchers will visually reject a micrograph for which the CTF Thon rings do not surpass a particular frequency resolution cutoff (Fotin et al., 2004, 2006; Unwin, 1993; Yu et al., 2011; Zhang et al., 2010; Zhou, 2008). Each automated CTF estimation program has a built-in metric to assess the CTF fit quality. CTFFIND uses the correlation coefficient of the CTF against a background-subtracted PSD (Mindell and Grigorieff, 2003). (As demonstrated later, a better correlation coefficient may not always provide a better CTF estimate.) ACE1 made an attempt to improve this by normalizing the PSD envelope and converting it to a 1D profile via elliptical averaging before taking the correlation (Mallick et al., 2005). ACE1 called this revised metric the *confidence* and it was considered that a value above 0.8 indicated an acceptable CTF fit for use in a 3D reconstruction (Stagg et al., 2006). It was shown that if input variables to the program are carefully maintained, better ACE confidences produce better 3D reconstruction resolutions (Stagg et al., 2014). The general applicability of these metrics will be addressed below.

Many CTF programs have several custom input variables, such as pixel binning, and may require users to run them multiple times on the same micrograph to get a satisfactory estimate. Additionally, some CTF programs do not provide correlations, instead supplying vastly different quality metrics (e.g., confidence), and therefore do not permit comparison of their output with that of other programs. This leads to the common conundrum that almost all 3DEM structures are solved using a single CTF estimation program.

The intention of the present study is to take any CTF estimation parameters, independent of program, and provide a common means for assessing them. Such an assessment would also permit the creation of a method for direct comparison, as is necessary for high-throughput CTF analysis. To this end, a method was developed for measuring the resolution of the CTF signal. The process of obtaining the resolution of the CTF required the creation of improved methodology for extracting the oscillating signal from the raw micrograph. The resulting software is directly integrated within the Appion EM processing suite (Lander et al., 2009), which

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