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Restoration of weak phase-contrast images recorded with a high degree of defocus: The "twin image" problem associated with CTF correction

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ABSTRACT

Relatively large values of objective-lens defocus must normally be used to produce detectable levels of image contrast for unstained biological specimens, which are generally weak phase objects. As a result, a subsequent restoration operation must be used to correct for oscillations in the contrast transfer function (CTF) at higher resolution. Currently used methods of CTF correction assume the ideal case in which Friedel mates in the scattered wave have contributed pairs of Fourier components that overlap with one another in the image plane. This "ideal" situation may be only poorly satisfied, or not satisfied at all, as the particle size gets smaller, the defocus value gets larger, and the resolution gets higher. We have therefore investigated whether currently used methods of CTF correction are also effective in restoring the single-sideband image information that becomes displaced (delocalized) by half (or more) the diameter of a particle of finite size. Computer simulations are used to show that restoration either by "phase flipping" or by multiplying by the CTF recovers only about half of the delocalized information. The other half of the delocalized information goes into a doubly defocused "twin" image of the type produced during optical reconstruction of an in-line hologram. Restoration with a Wiener filter is effective in recovering the delocalized information only when the signal-to-noise ratio (S/N) is orders of magnitude higher than that which exists in low-dose images of biological specimens, in which case the Wiener filter approaches division by the CTF (i.e. the formal inverse). For realistic values of the S/N, however, the "twin image" problem seen with a Wiener filter is very similar to that seen when either phase flipping or multiplying by the CTF is used for restoration. The results of these simulations suggest that CTF correction is a poor alternative to using a Zernike-type phase plate when imaging biological specimens, in which case the images can be recorded in a close-to-focus condition, and delocalization of high-resolution information is thus minimized.

1. Introduction

Unstained biological specimens are often well approximated as being weak phase objects. As Zernike emphasized in his Nobel lecture, images of phase objects show no contrast in a perfectly corrected microscope [1]. In order to overcome this problem, the objective lens is normally defocused by an amount that is large enough to produce sufficient contrast to see the specimen. As an example, a defocus value of 1 μm or more might be used in order to see particles with a molecular weight of ~1 MDa.

Although the low-resolution features of a phase object are made visible by introducing a substantial amount of defocus, the higher-resolution features then become badly corrupted due to oscillations in the contrast transfer function (CTF). This adverse consequence of using high amounts of defocus can be overcome to a substantial degree by computational "image restoration". Applying a computational CTF correction to an out-of-focus image is, in fact, not unlike Gabor's original concept of optical restoration of the object from an in-line hologram—which is nothing other than a highly defocused image [2].

It is well known that optical reconstruction of an object from an in-line hologram suffers from a substantial artifact, however, an effect that is referred to as the "twin image problem". As Gabor explained in his Nobel acceptance speech [3], optical reconstruction of an in-line hologram produces two images superimposed on each other, one of which is in sharp focus and the second of which is defocused by twice the amount of that in the original hologram. The issue that is addressed here, therefore, is the extent to which computational CTF correction also suffers from a similar "twin image" problem.

Our original purpose in simulating CTF correction was to understand how effectively it deals with the fact that a portion of the scattered wave produces an interference pattern in the region of the image that is adjacent to, but outside the geometric shadow

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of a small particle, for example a multiprotein complex. As is explained below, this delocalized information is not modulated by the usual CTF. Instead, the delocalized information can be described as a sum of interference fringes, each with a different spatial frequency, that are shifted in phase by an amount proportional to the product of the defocus and the spatial frequency.

We have investigated this question by applying three commonly used restoration techniques to the Fourier transforms of various simulated images. The results show that "phase flipping" and multiplying by the CTF both restore only about half of the original signal, the second half going into a doubly-defocused twin image (background). Although the results obtained by these two methods are similar, phase flipping results in a slightly better restoration of signal than does multiplying by the CTF. In light of these first results, it is not surprising that we also found that the ability of a Wiener filter to restore the object depends upon the value of the parameter that is used to estimate the signal-to-noise ratio (SNR). When the SNR is high, using a Wiener filter approaches the operation of dividing by the CTF, which is algebraically guaranteed to produce perfect restoration (but only in the absence of noise-amplification at the zeros of the CTF). When the SNR is low, however, as it is in low-dose cryo-EM images, use of a Wiener filter approaches the operation of multiplying by the CTF.

One of the advantages of recording images of weak phase objects with a phase-contrast aperture [4–8] is that defocus is no longer required in order to produce adequate contrast, and thus no information-delocalization occurs. On the other hand, this advantage would not be as important as it first sounds, if it were also true that no information-delocalization remained after the appropriate CTF correction had been applied. Since both numerical simulations and analytical theory show that CTF correction can be only partially effective in restoring the initially delocalized information, however, we conclude that CTF correction is a poor alternative to the use of in-focus phase-contrast imaging.

2. Simulation methods

Image simulations were carried out using scripts written in DigitalMicrograph (Gatan Inc., Pleasanton, CA). For calculation of single-sideband images, the original image was defined as a complex array so that the full Fourier transform would be computed and one half could then be set to zero. Fourier transforms of the images were modified either by multiplying by an appropriately defined function or by separating the modulus and phase components and modifying these as appropriate.

A modified spoke pattern was generated using the function "sin(n*itheta)" in DigitalMicrograph that makes a full-circle pattern with n spokes. The full-circle pattern was then masked to produce a narrow wedge, after which the test pattern was low-pass filtered to smooth the edges of the pattern.

A two-dimensional, sinusoidal cross-grating pattern was defined in a 512×512 pixel array as the product of one-dimensional sine functions that are parallel to the x-axis and the y-axis, respectively. The strongest Fourier components in this test pattern therefore run diagonally with respect to the x- and y-axes. This full pattern was masked with a square box whose edge-length was equal to 6.5 cycles of the sine functions.

An image of the 50S (large) subunit of the *Escherichia coli* ribosome was calculated using atomic coordinates given in the PDB file 1VOR [9]. A molecular model was generated using the "copy from pdb" command in SPIDER [10] to calculate a 3D density at a resolution of 0.1 nm/pixel. Functions in Bsoft [11] were then used to project the density and output a file in TIFF format as a 512×512 array.

Noise was not included in the simulations shown here. We assume that (1) the signal and the electron shot-noise that are present in experimental image intensities are additive, and (2) these two terms remain additive during the CTF-correction operations that are applied during data analysis. It is true that CTF correction of just the intensity pattern corresponding to the electron shot-noise itself will result in a texture whose amplitude spectrum is no longer "white" but whose phases are still random. Even so, this texture will be uniformly the same within the envelope of a particle and in the area outside the particle. Since an appropriate level (and texture) of "CTF-corrected" noise can be added to the results shown here, there is no loss of generality in computing and displaying only the effects that delocalization and subsequent restoration have on the signal. The purpose of NOT including noise in the simulations is to avoid confusion between the effects that are due to delocalization of the signal and those that are added by noise. In practice, the delocalized signal is largely masked by the noise, but because of the additivity of the signal and the noise, both the delocalization of signal and its partial restoration will be well described by our noise-free simulations.

3. Background and theory

The CTF that is used for image restoration in cryo-EM is given, in the simplest case, by

$$\mathrm{CTF}(s) = \sin \gamma(s),$$
$$\gamma(s) = 2\pi\left[\frac{C_s\lambda^3}{4}s^4 - \frac{\Delta z}{2}s^2\right], \quad (1)$$

where s is the spatial frequency (resolution); $\gamma(s)$ is the wave aberration associated with spherical aberration and defocus; C_s is the coefficient of spherical aberration; λ is the electron wavelength; and Δz is the defocus of the objective lens.

We have ignored the wave aberration due to spherical aberration in this paper, in order to emphasize solely the effect of defocus. For typical electron microscopes, the wave aberration due to spherical aberration makes a significant contribution to delocalization for only the highest-resolution features, for example Fourier components with a wavelength shorter than ~0.5 nm. The addition of a spherical aberration term in the simulations shown here would have had no visible effect, and in any case it would not contribute new principles to what is learned from the simulations presented here.

If the specimen is a weak phase object, then the Fourier transform of the experimental image intensity, $\hat{I}_{\mathrm{exp}}(s)$, is related to the Fourier transform of the shielded Coulomb potential of the object, $F(s)$, by

$$\hat{I}_{\mathrm{exp}}(s) = \delta(s) - 2F(s)\mathrm{CTF}(s). \quad (2)$$

The derivation of Eq. (2) assumes that the Fourier transform of the object satisfies Friedel's law and that the sinusoidal Fourier components of the object are spatially unbounded, as they are for a two-dimensional crystal [12]. Under these conditions, pairs of sinusoidal "fringes" in the image that are produced by interference of one diffracted beam with the unscattered beam and by the interference of its Friedel mate with the unscattered beam are shifted in opposite directions. The amount of their respective phase shifts corresponds to the magnitude of the resolution-dependent phase distortion, $\gamma(s)$. Depending upon the amount of defocus, these individual pairs of fringes thus vary from being in phase with one another to being completely out of phase with one