CTF determination and correction in electron cryotomography

J.J. Fernández\textsuperscript{a,b,*}, S. Li\textsuperscript{a}, R.A. Crowther\textsuperscript{a}

\textsuperscript{a}MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK
\textsuperscript{b}Department of Computer Architecture and Electronics, University of Almería, Almería 04120, Spain

Received 8 December 2005; received in revised form 27 February 2006; accepted 28 February 2006

Abstract

Electron cryotomography (cryoET) has the potential to elucidate the structure of complex biological specimens at molecular resolution but technical and computational improvements are still needed. This work addresses the determination and correction of the contrast transfer function (CTF) of the electron microscope in cryoET. Our approach to CTF detection and defocus determination depends on strip-based periodogram averaging, extended throughout the tilt series to overcome the low contrast conditions found in cryoET. A method for CTF correction that deals with the defocus gradient in images of tilted specimens is also proposed. These approaches to CTF determination and correction have been applied here to several examples of cryoET of pleomorphic specimens and of single particles. CTF correction is essential for improving the resolution, particularly in those studies that combine cryoET with single particle averaging techniques.

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PACS: 07.05.P; 42.30.W

Keywords: Contrast transfer function; CTF; Electron cryotomography; Image processing algorithms; Image reconstruction

1. Introduction

Electron cryotomography (cryoET) has a unique potential to elucidate the structure of large biological specimens at molecular resolution [1]. However, technical and computational advances are required to realize this potential [2]. This article addresses one of the current computational limitations in cryoET: determination and correction of the contrast transfer function (CTF) of the electron microscope.

CTF determination has been a subject of intensive study in the last decade in three dimensional electron microscopy (3DEM) [3–11]. An approach based on periodogram averaging [5,6,8] has become well established. CTF correction is a crucial stage in any high resolution structural analysis by 3DEM. Phase flipping ensures contrast to be consistent at all spatial frequencies [12]. Amplitude correction is usually carried out by a Wiener-like weighted combination of the data, either projection images [12] or 3D maps [13], and a sharpening by an inverse temperature factor [14].

In cryoET, there has not been pressing need of CTF correction so far, because typical nominal defocus values are in the range 8–15 μm underfocus [15–20], which allow a resolution up to 4.0–5.5 nm. However, if molecular resolution is to become attainable, CTF correction will be critical [21]. This article presents an approach that overcomes the extremely low signal-to-noise ratio (SNR) in tomographic data and the defocus gradient in images of tilted specimens.

2. CTF determination

2.1. Background on CTF determination

The CTF models the linear image formation system of the microscope [22,23]. The CTF gives rise to oscillations in
the power spectrum of the image. The location of these oscillations is essential for accurate determination of defocus and astigmatism, but it is difficult in cryomicroscopy due to the extremely low SNR. The strategies devised to facilitate their detection try to smooth the spectrum by azimuthal averaging [3,4,10,11] or by pure spectral estimation methods [5–8].

Periodogram averaging subdivides the image into tiles whose power spectrum is computed by means of the periodogram, i.e. the squared magnitude of the Fourier transform [5,6,8]. All the power spectra are then averaged to yield the “averaged periodogram”. Periodogram averaging allows a better estimate of the true power spectrum of the image, as the noise is significantly reduced [5]. There is a tradeoff between number and size of tiles, i.e. noise reduction vs. resolution of the averaged spectrum [5,8].

Background subtraction then aims to extract the oscillatory component of the power spectrum. Usually it consists of fitting a curve to the positions of the local minima of the smoothed power spectrum [5,6,10,11]. Finally, the values of defocus and astigmatism are determined by maximization of a correlation coefficient between the background-subtracted smoothed power spectrum and a calculated CTF [4,8,10,11].

Our approach to reliable determination of the CTF parameters in the untilted plane depends on extensive periodogram averaging of areas with similar defocus throughout the tilt series. This significantly increases the number of tiles in the averaging, with a substantial noise reduction, thereby yielding a better estimate of the true power spectrum. In order to smooth the power spectrum further, rotational averaging is also applied [5], assuming negligible astigmatism in automated data collection in cryoET [24]. Finally, we have developed a new way, embedded in the optimization itself, to remove the background from the smoothed power spectrum. We have adopted CTFFIND3 [8] as the software base to implement our approach.

2.2. Strip-based periodogram averaging

The method consists of the following steps:

1. For each image in the tilt series, a strip is extracted where the CTF is similar to that in the untilted plane. Assuming an eucentric tilt series, this strip is located around the tilt axis, running parallel to it. Its width depends on a parameter $\Delta D$ that represents the maximum difference in defocus for which the CTF can be assumed invariant. The relationship between the strip width $w$ and $\Delta D$ is given by $\tan \theta = \Delta D / w$, where $\theta$ denotes the tilt angle (Fig. 1). Note that the strip equals the image width in the case of the untilted specimen.

2. For each image, the strip is subjected to a tiling process. The tiles are overlapping with an optimal overlap of half the tile size [5]. For every tile, its power spectrum is computed.

3. The power spectrum at mean defocus is estimated as the average of the spectra of all the tiles in the tilt series.

4. Finally, rotational averaging is applied to further smooth the power spectrum.

The total change in focus $T$ across the defined strip (Fig. 1) is the change in focus along the center line set by $\Delta D$, plus the change in focus due to the thickness $t$ of the specimen itself:

$$T = \Delta D + \frac{t}{\cos \theta}.$$  

$\Delta D$ should be chosen to keep $T$ within the bound set by the projection approximation [25]. For typical specimens, setting $\Delta D$ to the approximate thickness of the sample is usually appropriate.

Table 1 shows the number of tiles involved in the strip-based periodogram averaging as a function of $\Delta D$ and using typical values for tile sizes [5,8]. Fig. 2 illustrates the performance of strip-based periodogram averaging in terms of noise reduction and detection of the CTF oscillations. As a proof of concept, a highly underfocused tilt series (61 images of 2K x 2K pixels, tilt range...
Table 1

<table>
<thead>
<tr>
<th>Tile size</th>
<th>ΔD (nm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>96 × 96</td>
<td>1681</td>
</tr>
<tr>
<td>128 × 128</td>
<td>961</td>
</tr>
<tr>
<td>160 × 160</td>
<td>576</td>
</tr>
</tbody>
</table>

Fig. 2. Performance of strip-based periodogram averaging: (a) the power spectrum estimate resulting from periodogram averaging applied to the image from the untilted specimen; (b) estimate from the strip-based approach with ΔD = 200 nm. The tile size was 128 × 128 and the number of tiles was 961 and 17 577, respectively. The pixel size was 10 Å.

[-60°, 60°], 10 Å/pixel) was collected and the power spectrum was estimated (a) based on only the image of the untilted specimen and (b) based on the strip-based approach described here with ΔD = 200 nm, with tiles of 128 × 128 in both cases. The improved SNR and detection of the CTF zeros is evident (see Fig. 3), thanks to the substantial increase (around 18 ×) of the number of tiles.

2.3. Determination of defocus from the power spectrum

We have embedded background subtraction into the fitting itself. The background curve is modeled by least-squares cubic spline fitting [26] to the set of samples of the power spectrum located at the positions of the zeros of the theoretical CTF. The defocus is determined by maximizing a correlation coefficient [8] between the background-subtracted smoothed power spectrum and the squared theoretical CTF:

\[ CC(D) = \frac{\sum (P(\rho_i) - B(\rho_i, D))^2 C^2(\rho_i, D)}{\sum (P(\rho_i) - B(\rho_i, D))^2 C^2(\rho_i, D)}. \]

where \( D \) denotes the defocus, \( P(\rho_i) \) is the power spectrum estimate, \( B(\rho_i, D) \) represents the spline function modeling the background, \( C(\rho_i, D) \) is the CTF function as in CTFFIND3 [8] and \( \rho_i \) is the discrete spatial frequency. The set of knots used for the spline-based background fit is limited to the positions of the zeros of the theoretical CTF. As a consequence, it will not give a reliable fit at low resolution, specially up to the position of the first zero of the CTF. Thus, this region is excluded from the fitting. Fig. 3 illustrates background subtraction and defocus estimation for the highly underfocused tilt series used in Fig. 2. The attenuating envelope in the experimental background-subtracted power spectrum introduces an implicit weighting in the correlation function, effectively weighting down the contribution of the higher resolution terms and leading to a more reliable determination of the defocus.

Once an estimate of the defocus \( D_0 \) at the untilted plane is available, the defocus at any point of any image in the tilt series can be determined by simple geometric rules. This can be analytically expressed, under the assumption of an eucentric tilt series and the tilt axis located along the Y-axis, as:

\[ D(x) = D_0 + d(x) \tan \theta, \]

where \( x \) is the coordinate along the X-axis, \( D(x) \) denotes the defocus for the pixels along the x-line, \( d(x) \) is the distance to the tilt axis and \( \theta \) is the tilt angle.

3. CTF correction

3.1. An approach to CTF correction in cryoET

To deal with the defocus gradient in images of tilted specimens (Eq. (3)), we exploit a similar concept of strip to that described for CTF determination, except that now the strip is not restricted to be around the tilt axis (Fig. 4). Considering the parameter ΔD as described before, a strip can be extracted along any x-line of the image. A single constant defocus value can be considered for the strip, being computed by Eq. (3), where \( x \) is the index of the center line of the strip.

The algorithm for CTF correction of an image of a tilted specimen can be outlined as follows:

1. Extract a strip around the x-line of the original image.
2. Compute the Fourier transform of the strip.
3. Correct for the CTF assuming the defocus given by Eq. (3).
4. Compute the inverse Fourier transform.
5. Extract the corrected x-line from the corrected strip and store it into the x-line of the output corrected image.

The CTF correction in Step (3) is merely a restoration problem with a spatially invariant CTF, as usual in 3DEM. Phase correction can be done by standard phase flipping. Amplitude correction can be carried out by means of a Wiener-like filter. The overall effect is that each part of each projected image is locally corrected as well as possible for the local value of defocus, prior to the tomogram being computed. For consistency, the value for the parameter ΔD
that controls the width strip should be lower than or equal to that used for CTF determination.

3.2. A filter for amplitude restoration

The standard Wiener filter used in 3DEM to estimate the true value of a frequency component from the observed values is [12]:

$$\frac{|C|}{C^2 + f^2},$$

where $C$ denotes the value of the CTF and $f$ prevents overamplification of noise when the denominator is small. When applied to restore single images, this filter presents the weakness that it does not provide sufficient inverse filtering (see Fig. 5, dotted line).

We have devised a new filter for amplitude correction. This filter restores those frequency components with high CTF magnitudes by an inverse filter (i.e. division by the CTF); those components where the noise is dominant are attenuated with the standard filter used in 3DEM (Eq. (4)); in between, a transition function is used:

$$\begin{cases}
\frac{|C|}{C^2 + f_2} & \text{for } |C| \in [0, f_2], \\
w|C| + (1 - w)\frac{|C|}{C^2 + f_2} & \text{for } |C| \in [f_2, f_1], \\
\frac{|C| - f_2}{f_1 - f_2} & \text{for } |C| \in [f_1, 1],
\end{cases}$$

where $f_1$ and $f_2$ define the ranges to turn on/off the different functions in the filter. Fig. 5 shows the filter with $f_1 = 0.5$ and $f_2 = 0.25$. The maximum restoration factor given by the filter is $2\times$ for $|C| = 0.5$.

We apply amplitude correction to the spatial frequencies beyond the first maximum of the CTF.
4. Results

The evaluation of CTF determination and correction has been carried out using experimental cryoET datasets of yeast spindle pole body (SPB) [27], vaccinia virus (VV) [18] and hepatitis B virus (HBV) core [28].

4.1. CTF determination

We have determined the CTF in a number of tilt series from SPB, VV and HBV core, covering a range of defocus from around 27 to 5 μm. All data sets were acquired on an FEI Tecnai F30 TEM (300 kV, Cs = 2 mm) and an amplitude contrast of 7% was assumed [29]. The images were collected on CCD with 2K×2K pixels and the number of images in a tilt series was around 60–80, covering the ranges \(-60^\circ, 60^\circ\) or \([-70^\circ, 70^\circ]\) at intervals of 1.5° or 2°. The tilt series of HBV core had 52 images with a tilt range of \(-46^\circ, 56^\circ\) at 2° interval. The pixel size for the SPB dataset was 10 Å/pixel, 8.2 Å for VV and 5 Å/pixel for HBV. The tilt series were aligned with IMOD [30] using gold markers. The search for the defocus was carried out in the range of ±10 μm around the nominal defocus in steps of 50 nm.

In order to evaluate the influence of the parameter \(\Delta D\) on CTF determination, we carried out several tests using different values of \(\Delta D\) from 0 to 0.5 μm, in intervals of 50 nm. In these tests, we used three different values for the tile size following the guidelines in [8]: 96 × 96, 128 × 128, 160 × 160. For every value of \(\Delta D\), the three tile sizes were tested, resulting in three independent defocus estimates. An average defocus and a standard deviation were then computed from the three estimates. Fig. 6 summarizes the results obtained for five tilt series with estimated defoci around 26.6, 21.6, 15.5, 10.2 and 5.7 μm, respectively. The figure shows steady estimates using values of \(\Delta D\) larger than 100–200 nm, with standard deviations lower than 1% of the average defocus. However, CTF determination based on low values of \(\Delta D\), and particularly that based solely on the untilted specimen (i.e. \(\Delta D = 0\) μm), produces inaccurate estimates with larger deviations. As a further test, each tilt series was divided in half, assigning neighbouring images alternately to each half, and the two half data sets were analyzed separately to see how well the
defocus estimates agreed. For the SPB and VV data sets, using values of \( \Delta D \) larger than 200–250 nm, the estimates of defocus from the half data sets agreed with each other and with the estimate from the whole data set, demonstrating the robustness of the approach. For HBV core, the half data sets did not give consistent defocus estimates, because of the small amount of scattering material and the limited number of images in the tilt series.

Fig. 7 shows the results of the defocus estimation for six tilt series. Each montage shows the fitted two-dimensional squared CTF (left half) and the observed, background-subtracted power spectrum (right half). These results were obtained with the parameter \( \Delta D \) set up to 200 nm and a tile size of 128 × 128.

4.2. CTF correction

CryoET is currently used for structural studies of large pleomorphic structures or, combined with single particle averaging techniques, of macromolecular assemblies at medium resolution [1]. SPB was tested as a representative example of pleomorphic structures [27]. HBV core was used to assess CTF correction in cryoET with single particle averaging. The known structure of HBV core at high resolution [28] makes it suitable to test our approach to CTF correction.

4.2.1. Spindle Pole Body

Two tilt series of SPB were selected for this test. First, a highly underfocused tilt series, where the defocus \( D_0 \) was found to be 26.65 \( \mu \)m (hereinafter referred as the 26 \( \mu \)m tilt series), was used as a proof of concept. Second, we tested a closer-to-focus tilt series (\( D_0 = 15.45 \mu m \), referred as 15 \( \mu \)m tilt series in the sequel), which approaches the defocus used in standard cryoET studies of pleomorphic structures. In both cases, correction of only the phases and of amplitudes and phases were tested, with the parameter \( \Delta D \) set to 200 nm and the filter parameters \( f_1 = 0.5 \) and \( f_2 = 0.25 \).

Fig. 7. Diagnostic output obtained from the defocus estimation for six tilt series. Every montage shows the fitted CTF in the left half, and the computed background-subtracted power spectrum in the right half. (a–d) SPB, (e) VV, (f) HBV. The defocus was found to be (a) 26.65 \( \mu \)m, (b) 21.65 \( \mu \)m, (c) 15.8 \( \mu \)m, (d) 15.45 \( \mu \)m, (e) 10.2 \( \mu \)m, and (f) 5.7 \( \mu \)m. The pixel size for (a–d) was 10 Å, for (e) was 8.2 Å and for (f) was 5 Å.

Fig. 8. Effect of CTF correction on tomograms. An area of the tomograms obtained from the original 26 \( \mu \)m tilt series (left), and from the tilt series with amplitudes and phases corrected (right) is shown. To increase the contrast, ten slices were summed together to generate these images.
The 26 μm tilt series consisted of 61 images (tilt range \([-60^\circ, 60^\circ]\), 2° interval) whereas the 15 μm tilt series had 82 images (tilt range \([-60^\circ, 60^\circ]\), 1.5° interval). For this test, the original images were binned, resulting in 1K × 1K images with a pixel size of 20 Å. The range of defocus in the 26 μm tilt series was \([24.91 \mu m, 28.39 \mu m]\), corresponding to the defocus at the extremes of the highest tilt images, whereas for the 15 μm tilt series it was \([13.71 \mu m, 17.19 \mu m]\).

From the 26 μm tilt series, tomograms were computed with weighted backprojection using IMOD [30]. The effects of the CTF correction that can be seen in the tomograms (see Fig. 8) are similar to those found in the projection images. The gold particles look sharper with a smaller black fringe around. In general, the whole specimen also looks sharper and less blurred and, in particular, the microtubules (MT) have thinner and better delineated walls. An artifact due to the CTF in the tomogram computed from the original tilt series is a thin white line in the middle of the MTs (best seen by viewing the image obliquely along the MT). This artifact is substantially attenuated in the CTF-corrected tomograms. No significant differences were found between the methods for CTF correction. In the 15 μm tilt series the effects of the CTF correction could hardly be detected in the images and the tomograms (results not shown here).

To better identify the effects of the CTF correction, we derived an average MT motif from the tomograms. As MTs reconstructed by cryoET look like pairs of parallel walls because of the missing wedge [31], we extracted central segments of MTs from the 2D slices of the tomograms using FindEM [32]. As a template, we used a central slice of a MT at 20 Å/pixel computed from the PDB coordinates (entry 1JFF). A total of 1200 segments with a length of 48 nm were extracted from the tomograms, and were then aligned to the template with Spider [33]. The average MT motifs generated are shown in Fig. 9. In the case of the 26 μm tilt series, there are clear differences before and after CTF correction. The original average MT had blurred walls and a sharp artifact running along the center of the MT. As a result of CTF correction, the walls of the MT are thinner and sharper, and the artifact is substantially attenuated. In the case of the 15 μm tilt series, the MT walls on the corrected tomograms are again sharper but the difference is less marked than in the 26 μm series.

As a final assessment, the Fourier ring correlation (FRC) [34] was computed between the average MT motifs in the 26 μm tilt series and the corresponding ones in 15 μm tilt series (Fig. 10). The FRC with the original motifs exhibits several contrast reversals in those frequency regions where the CTFs of the two tilt series have opposite signs. However, the FRC with the motifs obtained from the CTF-corrected tomograms clearly shows consistent contrast through the entire frequency range.

4.2.2. Hepatitis B virus core

The tilt series of HBV core selected for this test had 52 images (tilt range \([-46^\circ, 56^\circ]\), 2° interval) of size 2K × 2K with a pixel size of 5 Å. The defocus \(D_0\) was found to be 5.7 μm using a \(\Delta D = 200 \mu m\). CTF correction based on phases only and on amplitudes and phases were carried out with the same value for the parameter \(\Delta D\) and the filter parameters \(f_1 = 0.5\) and \(f_2 = 0.25\).

![Fig. 9. Effect of CTF correction on the average MT motif: (a) 26 μm series; (b) 15 μm series. The average motifs from the tomogram obtained from the original tilt series (left) and from the tilt series with amplitudes and phases corrected (right) are shown. The size of the motif was 48 nm long.](image)

![Fig. 10. Fourier ring correlation between the average MT motifs in the 26 μm tilt series and the corresponding ones in 15 μm tilt series. The effect of CTF correction is here shown to data out to the 3rd zero of the 26 μm CTF.](image)
From each of the three tilt series (original, phase-corrected and corrected amplitudes and phases), eighty reconstructed HBV particles were extracted from tomosgrams, after reconstruction with weighted backprojection at a 20 Å limit. Using Bsoft [35], these 3D volumes were then aligned to a previous map of HBV core [28] that was filtered to 20 Å and sampled at 5 Å. The aligned particles were then summed to yield the average HBV core in each tomogram. To increase the SNR, icosahedral symmetry was then applied to the 3D maps. Figs. 11 and 12 show some slices and 3D views of these symmetrized average maps. The maps from the corrected tomograms show an evident improvement over the original map. The map with amplitudes and phases corrected exhibits more detail than the one based only on phase correction.

The Fourier shell correlation (FSC) [36] curves in Fig. 13(a) show quantitatively the resolution improvement after CTF correction. The maximum resolution attained in the original map is around 34 Å (according to the 0.5 FSC criterion), whereas the maps corrected for the CTF reach 23 Å. The FSC brings out the phase reversal around 30 Å of the 3D map from the original tilt series with respect to the template. A plot of the amplitudes of the Fourier components (Fig. 13(b)) demonstrates the effect of amplitude correction, which is particularly evident beyond 45 Å (approximately, the location of the first peak of the CTF).

We also corrected for the CTF with other values of $\Delta D$, 50 and 500 nm. However, the results showed negligible differences, in terms of FSC and amplitude amplification,
with those just presented. On the other hand, for comparison purposes, we also corrected for the CTF with the frequently used Wiener filter in 3DEM with $f_2 = 0.25$. The results were no better than those with only phase correction presented above.

5. Discussion

CTF determination and correction are becoming necessary as structural studies by cryoET approach molecular resolution \[1,2,21\]. We have presented here an approach to CTF determination and correction that deals with the low SNR and the defocus gradient in images of tilted specimens. It is based on the assumption of eucentric tilt series. It also assumes the same defocus along the projection path through the specimen, since the projection approximation [25] is valid for the thickness of specimens and the limiting resolution of 2 nm expected.

5.1. CTF determination

CTF determination is based upon an accurate estimation of the defocus on the untilted plane by strip-based periodogram averaging. The defocus is found by an optimization process where background subtraction has been embedded into the CTF fitting. The approach has been applied to a number of cryoET tilt series and the results exhibit good levels of accuracy and stability over a wide range of defocus.

The critical parameter is $\Delta D$, which represents the height over which the same defocus can be assumed. An extensive study of defocus determination as a function of $\Delta D$ has been carried out, showing that values greater than 100 or 200 nm are necessary for an accurate estimation (with standard deviation less than 1% of the average defocus). Lower values of $\Delta D$ yielded inaccurate estimates, particularly in the case with $\Delta D = 0$, i.e. defocus estimation based solely on the image of the untilted specimen. Therefore, strip-based periodogram averaging is useful to reduce the noise in the power spectrum and allow accurate defocus estimation. On the other hand, the results do not exhibit significant changes with high values of $\Delta D$, but beyond $\Delta D = 500$ nm the projection approximation [25] may not apply.

5.2. CTF correction

CTF correction in CryoET is difficult because of the defocus gradient for tilted specimens. Winkler and Taylor [37] presented a restoration method for electron tomography of stained specimens that worked with a spatially variant CTF. The method required regularization of the solution due to its sensitiveness to noise, and its application to cryoET still remains to be tested.

Our approach to CTF correction essentially decomposes the global spatially variant restoration problem into multiple local spatially invariant problems that are confined to the width of a strip. Every strip is corrected for the CTF according to standard techniques. For amplitude correction a new filter has been devised.

The effects of CTF correction on pleomorphic structures are marginal unless the dataset is highly underfocused. Even at high underfocus, the effects on the tomograms are difficult to observe. Only when averaging of repeating structures is performed, do the effects become distinguishable. For the 26 $\mu$m SPB dataset, it was possible to detect the differences before and after CTF correction on the images and the tomogram, which were specially evident around the gold particles. When pieces of MTs were summed up, the averaged MT clearly showed the restoration done. However, for the SPB tilt series acquired at relatively standard defocus conditions (around 15 $\mu$m), the effects of CTF correction were minor. Despite this, the FRC between the averaged MTs computed from different tomograms clearly showed the benefits of CTF correction: consistent contrast throughout the frequency range. Regarding the VV dataset (D$_0$ = 10.2 $\mu$m), the results (not shown here) showed little difference before and after CTF correction.

CryoET combined with single particle averaging techniques allows structural studies of macromolecular complexes at medium resolution \[1,16,20,21\]. We have used HBV core as an example of such studies. The combination of tens of particles in conjunction with the icosahedral symmetry made medium resolution achievable. CTF correction made it possible to improve the resolution from 34 to 23 $\AA$, allowing us to clearly discern features of the HBV core, such as the spikes. The improvement due to amplitude correction can be seen on the 2D slices and the 3D views, and is clearly shown up on the plot of the amplitudes compared to just phase flipping. The filter designed in this work has been key for this enhancement.

CTF correction opens up the possibility of combining multiple particles from different tomograms having consistent contrast to reach resolutions approaching 2 nm or even farther. So far, the lack of approaches to CTF correction has restricted single particle cryoET studies to the resolution of the first zero of the CTF [21].

5.3. Implementation

Our implementation turns out to be very efficient as it relies on fast Fourier transform algorithms that exploit the computational redundancy over multiple transforms of the same size (FFTW). Computation times for CTF determination and for CTF correction of single images depend on the strip size, and range in the order of minutes in standard computers. Several examples of computation times of our implementation on a Pentium IV 3.40 GHz follow. The time for estimating the power spectrum from 17577 tiles of 1024 × 1024 takes around 1 m. The correction of an untilted image of 1024 × 1024 takes around 1 s. The correction of a high tilt image of 1024 × 1024 based on 1024 FFTs of 60 × 1024 takes around 25 s. The correction of a low tilt image
of 1024 × 1024 based on 1024 FFTs of 960 × 1024 takes around 10 m. Our software is freely available on request.

6. Conclusion

This work has addressed CTF determination and correction, one of the current computational problems in cryoET that limits the resolution attainable. We have proposed an approach to CTF determination that overcomes the low SNR in cryoET by strip-based periodogram averaging extended throughout the tilt series and a spline-based strategy for background subtraction. The method of CTF correction deals with the defocus gradient in images of tilted specimens by decomposing the global restoration problem into multiple local spatially-invariant problems. The approach has been applied to examples of cryoET of pleomorphic specimens and of single particles. CTF correction has shown little effect on pleomorphic specimens due to the inherent resolution limits. However, CTF correction is essential for improving the resolution in those studies that combine cryoET with single particle averaging techniques, if molecular resolution is to be achieved.

Acknowledgements

The authors wish to thank A. Roseman for fruitful discussions, help with FindEM and revising the manuscript, N. Grigorieff for kindly providing CTFFIND3, S. Wynne for HBV core samples, J.L. Carrascosa for vaccinia data, L. Amos for the PDB of the microtubule model and T. Horsnell for his support and help with the computer farm. This work has been partially supported by the UK Medical Research Council and HFSP, EMBO, Spanish MEC and EU (Grants: MEC-TIC2002-00228, HFSP2003-ST00107, MEC-PR2004-0367, EMBO-ASTF-323-2005, MEC-TIN2005-00447, EU-FP6-LSHG-CT-2004-502828).

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