Transfer Function Restoration in 3D Electron Microscopy via Iterative Data Refinement

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5th April 2001

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Abstract

Three-dimensional electron microscopy (3D-EM) aims at obtaining structural information of macromolecular complexes within a typical resolution range of between 2 to 0.3 nm from the projection images produced by an electron microscope. As any other imaging device, the electron microscope introduces a transfer function (called in this field Contrast Transfer Function, CTF) into the image acquisition process which modulates the different frequencies of the projection signal. Thus, the 3D reconstructions performed with these CTF-affected projections is also affected by an implicit 3D transfer function. Depending on the preparation procedure, the effect of this CTF is quite dramatic limiting severely the achievable resolution. In this work we make use of the *Iterative Data Refinement* technique to obtain CTF-free reconstructions. It is shown that the approach can be successfully applied to noiseless as well as to noisy data.

1 Introduction

The structural information of biological complexes, i.e., their shape and spatial conformation, is vital in molecular biology as complementary information to biochemical studies. This knowledge can help, for example, in new drug development and in the understanding of many diseases. Nowadays, the 3D structure of a protein can be addressed using two different approaches: one predicts the conformational state of the complex based on its biochemical properties and the possible similarity with other proteins whose structure is known; and the other applies 3D reconstruction algorithms to data collected by some experimental technique. There exist several possible biophysical techniques for visualizing a protein, such as X-rays, NMR or electron microscopy (EM). The main drawbacks of the first two are that they are very restrictive with respect to the range of proteins that can be studied. 3D-EM is a technique which provides only medium-low resolution structural information of macromolecules, however, it does not need special conditions of the specimens and it has been established as a useful technique in the field of structural biology.

One of the limiting factors of 3D electron microscopy is that it is difficult to obtain high resolution structural details due to the strong effect of the microscope transfer function on the experimental projections. Particularly, as will be shown later, the CTF introduces severe phase shifts and eliminates all information at certain frequencies. One way of obtaining higher resolution results is by compensating for the effect of the microscope transfer function. Several such methods have been proposed

[1, 6, 7, 12, 14, 16], but they usually apply some kind of Wiener-like division by the transfer function in the Fourier space, and also amplify noise at those frequencies where the transfer function has small magnitude.

In this work we apply the Iterative Data Refinement (IDR) technique introduced by Censor, Elfving and Herman [2] and further studied by Herman and co-workers [8, 9, 11] to remove the effect of the microscope imperfection and, thus, obtain high resolution structural information about the macromolecules under study. Although in this work only simulations with phantoms have been carried out, the results are encouraging enough to justify future tests on experimental data sets.

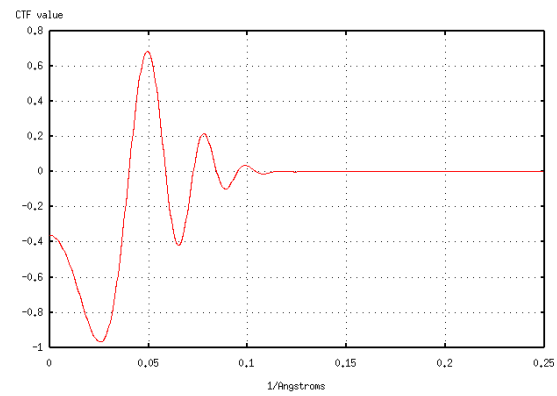

Figure 1: CTF used in the cryomicroscopy simulations.

2 Materials and methods

Contrast Transfer Function

Image formation by an the electron microscope is due to two different physical processes during the electron interaction with the specimen: first, a shift in the electrons phase, and second, an electron direction change. Both effects are combined to produce a single modulation transfer function called Contrast Transfer Function [5, Chapter 2.II]. A cross-sectional plot of such a modulating function, typical for cryomicroscopy, can be seen in Figure 1. Notice that the sign changes and the crossings of 0 are responsible for a contrast inversion in the projection image and for the complete elimination of the information at certain frequencies. A parametric model of this transfer function is given in [15], and it is used in the simulations presented in this work.

Iterative Data Refinement

This technique was first introduced in Censor [2] in the context of 2D medical tomography, see Section 10.5 in [3]. The underlying idea is to change iteratively the projection images so that the process converges to the ideal (without transfer function) projections. Calling g_i^k the projection in the direction i at the iterative step k, g^k the set of projections at step k, P_i the projection operator in the direction i, C_i the contrast transfer function operator for that image, and R the reconstruction operator, the IDR algorithm is formulated as $g_i^{k+1} = \mu^{k+1} g_i^0 + (P_i - \mu^{k+1} C_i P_i) R g^k$ where μ^{k+1} is a relaxation parameter applied at iteration $k+1$ and g^0 represents the set of experimental images. Basically, the procedure proceeds as follows: a first reconstruction is done with the experimental images g^0, then every projection is modified following a mixture of the original projections, the reprojection of the just reconstructed volume and this same reprojection after the CTF. The new set of images is again used for reconstruction and so on. ART+blobs [10] has been used as the reconstruction operator R.

Simulations

For the sake of objective assesment of quality, simulations with a phantom have been run following the Figures of Merit approach described in Sorzano [13]. The phantom corresponds to bacteriorhodopsin, whose structure is known at atomic resolution (3.5Å) [4]. The surface rendering of this structure, calculated from a volume sampled at 2Å/pixel, can be seen on Figure 2.

Computational projections have been done simulating the cryomicroscopy conditions. In cryomicroscopy the specimens are embedded in ice, and are then imaged at very low electron doses to preserve structural details**.** As a consequence, the projection images that are obtained with this technique are extremely noisy, with signal-to-noise ratios lower than 1.0. The CTF that has been used for the projections is shown in Figure 1. 2000 images were taken all over the projection space, a selection of these projections is shown in Figure 3. Angular Gaus-

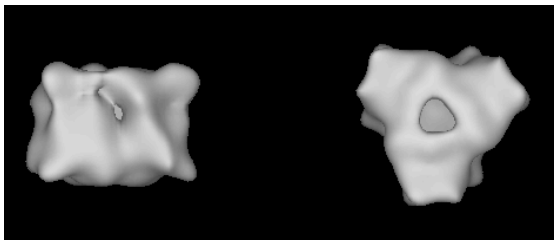

Figure 2: Bacteriorhodopsin phantom filtered at 17Å (maximum resolution permitted by the simulated CTF).

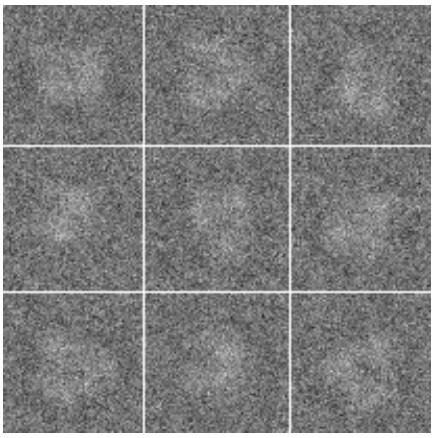

Figure 3: A selection of projections simulating cryomicroscopy images from the bacteriorhodopsin phantom.

sian noise (N(0,5)) and shift Gaussian noise (N(0,2)) have been added to simulate the positional uncertainty in 3D electron microscopy.

3 Results

Three different reconstructions have been performed to test the efficacy of the IDR algorithm: the first one without any CTF correction, the second one with phase correction, and the third with amplitude and phase correction via IDR. Figure 4 shows the results for each case while Table 1 shows the L2 and L1 measures of the error [13] between each reconstruction and the original phantom and the maximum resolution [5, Chapter 5.V] achieved with each method. Table 1, as well as the 3D reconstructions performed with the different degrees of CTF correction, show that the IDR technique achieves better reconstructions with higher resolution.

Corrective action	L2error	L1 error	Resolution
No action	0.995844	0.961378	28Å
Phase corrected	0.996125	0.963414	20Å
IDR	0.997908	0.967625	17Å

Table 1: Reconstruction similarity measure after different corrective actions.

4 Conclusions

The IDR's ability of removing the transfer function effect with noiseless data has been proved in [9]. In this work we have extended those results to extremely noisy data. At the same time, we have shown the importance of applying CTF correction to 3D electron microscopy in order to obtain high resolution reconstructions. Further work must be done to tune the IDR free parameters to the specific cases of cryomicroscopy and negative staining. However, these preliminary results encourage us to proceed further on experimental data sets with this CTF correction technique which allows the application of particular transfer functions to each projection and which avoids the noise amplification effect caused by most other correction methods used so far.

Acknowledgements

Dr. Marabini is partially supported by a grant from the Spanish "Ministerio de Educación y Ciencia". Partial support is acknowledged to the "Comisión Interministerial de Ciencia y Tecnología" of Spain through project BIO980761 and TIC990361. The work of Y. Censor was supported by Research Grant 592/00 from the Israel Science Foundation founded by the Israel Academy of Sciences and Humanities.

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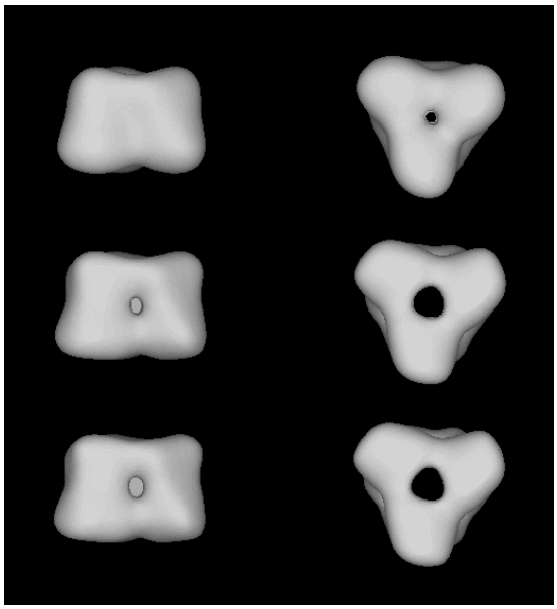

Figure 4: From top to bottom: Reconstruction without CTF correction, reconstruction with phase correction, reconstruction with amplitude and phase correction.

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