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Fully three-dimensional defocus-gradient corrected backprojection in cryoelectron microscopy

Ivan G. Kazantsev [a,1], Joanna Klukowska [b], Gabor T. Herman [b,*], Laslo Cernetic [c]

[a] RISØ, Materials Research Department, Technical University of Denmark, DK-4000, Roskilde, Denmark
[b] Department of Computer Science, Graduate Center, City University of New York, New York, NY 10016, USA
[c] Department of Image Processing and Computer Graphics, University of Szeged, Szeged, Hungary

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ABSTRACT

Recognizing that the microscope depth of field is a significant resolution-limiting factor in 3D cryoelectron microscopy, Jensen and Kornberg proposed a concept they called defocus-gradient corrected backprojection (DGCBP) and illustrated by computer simulations that DGCBP can effectively eliminate the depth of field limitation. They did not provide a mathematical justification for their concept. Our paper provides this, by showing (in the idealized case of noiseless data being available for all projection directions) that the reconstructions obtained based on DGCBP from data produced with distance-dependent blurring are essentially the same as what is obtained by a classical method of reconstruction of a 3D object from its line integrals. The approach is general enough to be applicable for correcting for any distance-dependent blurring during projection data collection. We present a new implementation of the DGCBP concept, one that closely follows the mathematics of its justifications, and illustrate it using mathematically described phantoms and their reconstructions from finitely many distance-dependently blurred projections.

1. Introduction

Three-dimensional cryoelectron microscopy (3D cryoEM) is an increasingly powerful tool for solving the structure of macromolecular complexes, providing resolution on the order of a nanometer. To increase resolution to subnanometer scale, reconstruction methods have to take further image formation model features into account. In 3D cryoEM, 2D projection images, called *micrographs*, of a 3D mass distribution (e.g., a macromolecule) are affected by many factors that modify the amplitudes and phases of the image of the specimen and which must be corrected for in order to reconstruct the true object [1]. One of the most important among these factors is the contrast transfer function of the microscope. The *contrast transfer function* (CTF) is the Fourier transform of a point spread function that describes the response of the imaging system to a point object. It affects various frequencies by modulating the magnitude and sign of their amplitude. CTF depends on many parameters of the imaging system, among them defocus. In electron microscopes, the defocus varies with the distance from the electron source. Thus, given a 3D specimen, each layer (defined as a plane perpendicular to the electron beam) is blurred by a slightly different transfer function. Most of the methods of correction for CTF ignore this dependence on distance from the electron source. As the technology of electron microscopy improves (as achievable resolution increases) and the need for imaging larger specimens emerges, this imperfection of electron microscopes, which has not been considered important in the past, is likely to become an essential limitation. The difference between two reconstructions, one that uses the same CTF function for each layer of the specimen and one that takes distance dependence into consideration is illustrated in Fig. 1. An extensive discussion of how Fig. 1 was obtained is presented in Section 6.

There are several approaches in the literature that address the distance-dependent CTF issue [2–8]. Jensen and Kornberg's [4] approach makes CTF correction an integral part of the reconstruction procedure. In this paper we revisit the concept behind their method in order to provide a mathematical justification for it and to put it into the context of traditional computerized tomography techniques.

The method proposed by Jensen and Kornberg [4], based on the concept of defocus-gradient corrected backprojection (DGCBP), is an approach that operates on micrographs taken from arbitrary directions. The method exploits features of the forward model for 3D cryoEM together with the general structure

* Corresponding author. Tel.: +1 212 817 8193; fax: +1 212 817 1510.
E-mail addresses: kazantsev.ivan6@gmail.com (I.G. Kazantsev), jklukowska.gc@gmail.com (J. Klukowska), gaborherman@yahoo.com (G.T. Herman), cslaszlo@inf.u-szeged.hu (L. Cernetic).
[1] Present address: Institute of Computational Mathematics and Mathematical Geophysics, Lavrentieva 6, Novosibirsk 630090, Russia.

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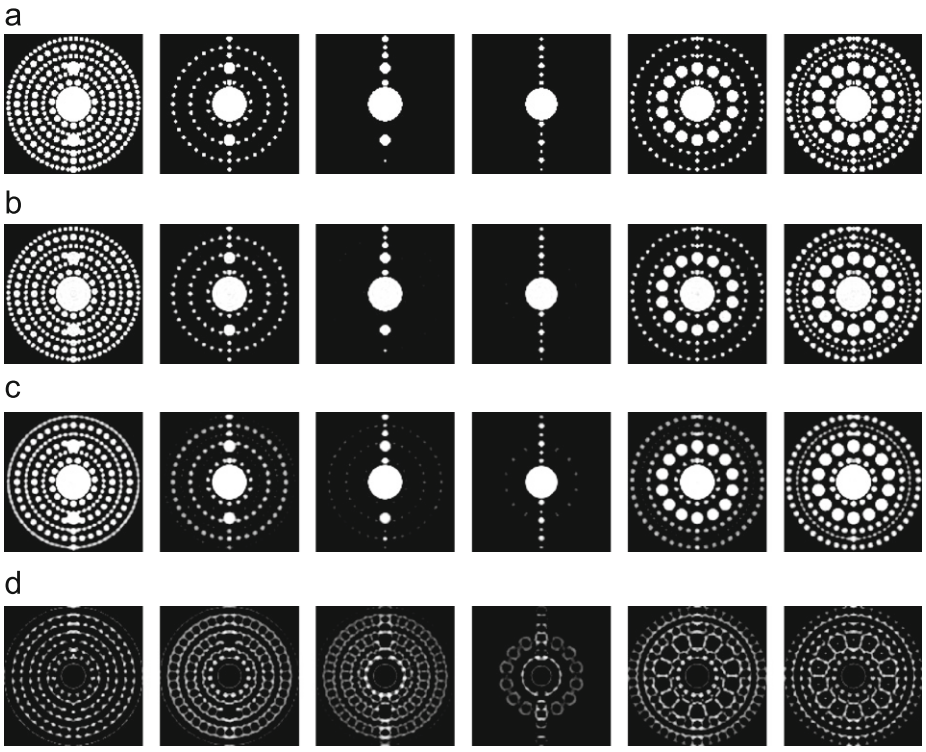

Fig. 1. Cross-sections of a phantom (a) and of three reconstructions from projection data that were calculated with distance-dependent blurring: with correction for the distance-dependent contrast transfer function (b), with correction appropriate for the central layer of the specimen (c), and with no correction for the contrast transfer function (d).

of the weighted backprojection technique [9]. However, the authors of [4] did not elaborate how their method relates to other reconstruction and/or correction approaches, and they provided only a heuristic (rather than mathematical) justification as to why the method should work.

In our recent work [8] we provided a mathematical verification of the DGCBP concept for the case in which projections are obtained from a single axis rotation mode of data collection. We demonstrated that, for that geometry, DGCBP and the frequency distance relation method described by Dubowy and Herman [6] are equivalent in the sense that the mathematical formulas that describe a 2D object reconstructed by the two methods from its distance-dependently distorted 1D projections from all directions around the axis of rotation are in fact the same.

In this paper we generalize that proof to 3D objects to be reconstructed from 2D projections taken from arbitrary directions, again with the assumption that data for all directions are available. We make use of stationary phase approximation, which was introduced to the field by Edholm and Lewitt [10] and Xia et al. [11] and then used by Dubowy and Herman [6] in the frequency distance relation method mentioned above. (For a brief review of stationary phase approximation, see Appendix C.) We show that results obtained using the DGCBP concept are equivalent to the results produced by a classical reconstruction method from ideal projection data. This completes the mathematical justification of the DGCBP approach.

Our paper is organized as follows. In the next section we present a nonmathematical overview of our ideas. The rest of the paper contains mathematical discussions (including proofs of our claims) and simulation results. In Section 3 we provide the background and introduce the notation used throughout the paper. In Section 4 we review the principles of image formation in an electron microscope and the model for the CTF. In Section 5 we outline the DGCBP concept in terms of integral equations and explore its effect on the 3D delta function as a test object. We also derive the main result of our work regarding the equivalence of the DGCBP concept and deblurred (weighted) backprojection applied to undistorted projection data. In Section 6 we present numerical test results. For clarity, only essential mathematical formulas are included in the text. Details of mathematics and implementation are delayed until the appendices, so as not to interrupt the flow of the main ideas.

2. Overview

This section provides an overview of the material presented in this paper. We describe operators that are defined in the following sections and their use in modeling of and in correcting for distance-dependent CTF blurring in cryoEM, without any mathematical derivations. All claims made here are proven in the following sections and the appendices.

We first model mathematically the process of projection taking in cryoEM. Such a model needs to incorporate the distance-dependent nature of the CTF. We then mathematically model the correction for distance-dependent CTF that Jensen and Kornberg [4] incorporated into the weighted backprojection reconstruction algorithm.

We start by defining several operators which we first use to describe ideal projections (line integrals with no blurring at all). This projection operator $\mathcal{P}$ is composed of a rotation operator $\mathcal{R}$ and a compression operator $\mathcal{C}$. The imaged molecule is represented by a function of three variables v, $v(x_1,x_2,x_3)$ is the density of this molecule at the point $(x_1,x_2,x_3)^T$. Given a function v and two angles θ and ϕ, the operator $\mathcal{R}$ gives us a function $[\mathcal{R}v](\theta,\phi,x_1,x_2,x_3)$ that represents the density value of the molecule at a point (x_1,x_2,x_3) after it has been rotated by the angle θ around X_3-axis and then by the angle ϕ around X_2-axis. Note that the coordinate system is attached to the microscope, so that the values of the molecule at a point $(x_1,x_2,x_3)^T$ before and after the rotation are different. To obtain the projection of