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Iterative reconstruction of cryo-electron tomograms using nonuniform fast Fourier transforms

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ABSTRACT

Algorithms for three-dimensional (3D) reconstruction of objects based on their projections are essential in various biological and medical imaging modalities. In cryo-electron tomography (CET) a major challenge for reconstruction is the limited range of projection angles, which manifests itself as a "missing wedge" of data in Fourier space making the reconstruction problem ill-posed. Here, we apply an iterative reconstruction method that makes use of nonuniform fast Fourier transform (NUFFT) to the reconstruction of cryo-electron tomograms. According to several measures the reconstructions are superior to those obtained using conventional methods, most notably weighted backprojection. Most importantly, we show that it is possible to fill in partially the unsampled region in Fourier space with meaningful information without making assumptions about the data or applying prior knowledge. As a consequence, particles of known structure can be localized with higher confidence in cryotomograms and subtomogram averaging yields higher resolution densities.

1. Introduction

In cryo-electron tomography (CET) a three-dimensional (3D) image of the sample is reconstructed from transmission electron microscope (TEM) images, which are approximately parallel 2D projections of the object (Hawkes, 2006). Micrographs of the sequentially tilted sample are obtained, typically a single axis tilt series. From the projection data it is in principle possible to retrieve 3D information according to the projection-slice theorem (Radon, 1917). In practice, the performance of the reconstruction algorithm, which inverts the projection process, determines the accuracy of the 3D reconstruction of the sample. In CET, major challenges for the reconstruction process are the low signal-to-noise ratio (SNR) of the micrographs and the limitation of the projection angle, typically from -60° to 60° due to the geometry of sample and sample holder. The tilt restriction leaves a wedge-shaped area in 3D Fourier space unsampled making the reconstruction task ill-posed ("missing wedge problem") (Lucić et al., 2005).

The most common and best-known reconstruction algorithm in CET is weighted backprojection (WBP), which has been introduced several decades ago (Harauz and van Heel, 1986; Radermacher et al., 1986; Ramachandran, 1971). In the 3D electron microscopy (EM) field development of reconstruction techniques has mostly focused on single particle analysis. Algebraic reconstruction methods such as the Algebraic Reconstruction Technique (ART) have been introduced almost as early as WBP (Gordon et al., 1970). Common iterative reconstruction methods in the 3D EM field are ART with blobs (Marabini et al., 1998), simultaneous iterative reconstruction technique (SIRT) (Gilbert, 1972; Penczek et al., 1992) and Simultaneous Algebraic Reconstruction technique (SART) (Wan et al., 2011).

Another major category of reconstruction algorithms is based on Fourier transformation. The representatives are the fast Fourier summation algorithm (Sandberg et al., 2003), the gridding method (Penczek et al., 2004) and the nearest neighbor (NN) direct inversion method (Grigorieff, 1998; Zhang et al., 2008). These Fourier based methods have been shown to result in more accurate reconstructions than the algebraic methods (without imposing constraints) in terms of Fourier Shell Correlation (FSC) (Penczek et al., 2004).

On the other hand, considerable advances have been made to solve the inverse problem of reconstructing an object from projections, especially in the medical imaging field. There is a trend towards iterative reconstruction algorithms. It is attractive to use Fourier-based interpolation methods in such iterative schemes due to their high accuracy and speed compared to real-space based approaches. For instance, (Fessler and Sutton, 2003) introduced the min–max interpolation for nonuniform fast Fourier transform and later combined it into an iterative procedure for 2D tomographic

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reconstruction (Matej et al., 2004). Potts and co-workers (Knopp et al., 2007) introduced a method, which we refer to as Iterative Nonuniform fast Fourier transform (NUFFT) based Reconstruction method (INFR) in the following. In this method the reconstruction is formulated as an algebraic optimization problem, which is solved using the conjugate gradient method and NUFFT. INFR has been shown to result in excellent reconstructions when applied to magnetic resonance imaging data, but it has not been applied to cryo-EM data to our knowledge. In particular, it has not been characterized to what extent the excellent interpolation characteristics of INFR are beneficial to obtain meaningful information in parts of the missing wedge.

Here, we apply INFR to the reconstruction of tomograms from cryo-electron micrographs and compare the reconstruction quality to the state-of-the art methods. Specially, we devised a computationally efficient implementation of INFR for single-axis CET in our software. Simulations show that the reconstructions obtained by INFR are more accurate than reconstructions using NN direct inversion method and WBP for tilt series covering the complete angular range. For restricted angular sampling, INFR is capable of retrieving meaningful information in some regions of the missing wedge in Fourier space, in particular in the low frequency regime. When applied to experimental CET data, the improved reconstruction accuracy of INFR in the low frequencies has important consequences: sensitivity and accuracy of particle localization by template matching are increased considerably and subtomogram averaging yields higher resolution results due to more accurate subtomogram alignment.

2. Material and methods

2.1. Nonuniform fast Fourier transform (NUFFT)

For reconstruction of cryo-electron tomograms we implemented INFR. In the following, we explain the method and our specific implementation.

First, we briefly discuss the NUFFT (Keiner et al., 2009), which is the basis of the reconstruction algorithm described here. Given the function $f(x), x \in I_N$ and $I_N := \{x = (x_t)_{t=0,\dots,d-1} \in Z^d : -\frac{N}{2} \le x_t < \frac{N}{2}\}$ (the equispaced grid) as the input, NUFFT tries to evaluate the following trigonometric polynomial efficiently at the reciprocal points $k_j \in \left[-\frac{1}{2}, \frac{1}{2}\right)^d$, $j = 0, \dots M-1$:

$$\hat{f}(k_j) := \sum_{x \in I_N} f(x) e^{-2\pi i x k_j}. \tag{1}$$

In contrast to the regular discrete Fourier transforms, k_j can be on an arbitrary nonuniform grid. In matrix vector notation, Eq. (1) can be rewritten as

$$\hat{f} = Af \tag{2}$$

with the nonequispaced Fourier matrix $A := e^{-2\pi i x k_j}, x \in I_N$, $j = 0, \dots, M-1$.

One approach for fast computation of Eq. (2) is based on the factorization $A \approx BFD$ (Potts et al., 2001), where D is the inverse Fourier transform of a window function w, F is the oversampling Fourier matrix and B is a sparse matrix of the window function w with the cut-off parameter m, which contains at most $(2m+1)^d$ non-zero entries per row (Fig.S1). The basic idea of this factorization, which resembles the reverse gridding method (Penczek et al., 2004), is the following: the accurate interpolation in Fourier space to a different grid is achieved by convolution with an appropriate window function w, which is compensated for by prior multiplication with the inverse of the real space transform of w. The accuracy of this approach depends on the oversampling factor and the choice of window function w and its cut-off parameter m. It has been suggested that the Kaiser-Bessel window function provides high accuracy and a typical choice of m would be 3 for an oversampling factor 2 (Fessler and Sutton, 2003; Jackson et al., 1991).

The adjoint (or conjugate transpose) NUFFT is defined as the sum

$$f'(x) := \sum_{j=0}^{M-1} \hat{f}(k_j) e^{2\pi i x k_j}, \quad x \in I_N, \tag{3}$$

or in matrix vector notation

$$f' = A^H \hat{f}. \tag{4}$$

Its efficient computation can be analogously achieved by the factorization $A^H \approx D^T F^T B^T$. It has been shown that the gridding method can be seen as an efficient algorithm to compute A^H (Potts et al., 2001).

2.2. Reconstruction as an optimization problem

Without loss of generality, we consider the reconstruction of a 2D image from 1D projections for the sake of simplicity. According to the projection-slice theorem the Fourier transform of a projection corresponds to a slice in the Fourier space of the object (Radon, 1917). Given M observations b as the Fourier transforms of projections, we define the matrix A as a nonuniform Fourier transform matrix depending on the sampling geometry:

$$A := e^{-2\pi i x k_j}, k_j \in \left[-\frac{1}{2}, \frac{1}{2}\right)^2, \quad j \in [0, \dots, M). \tag{5}$$

The reconstruction problem is to recover $f(x), x \in \{-\frac{N_1}{2}, \dots, \frac{N_1}{2} - 1\} \times \{-\frac{N_2}{2}, \dots, \frac{N_2}{2} - 1\}$ defined on a regular grid, such that:

$$Af = b \tag{6}$$

Solving Eq. (6) can be formulated as an optimization problem:

$$f = \arg\min \|b - Af\|. \tag{7}$$

This is a least square problem and when the sampling density compensation (Pipe and Menon, 1999) is considered its solution requires solving the following equation:

$$A^H W A f = A^H W b. \tag{8}$$

Here $W := diag(w_m)$ is the density compensation matrix, which account for the nonuniform distribution of the sampling in Fourier space (Fig.1b). For example, in CET the sampling is very dense towards zero frequency and thus the projections contain to some degree redundant information in these frequencies (Crowther et al., 1970). It is important to weigh the information in Fourier space according to the overall sampling pattern because low frequencies would be artificially enhanced otherwise.

Eq. (8) can be solved using the conjugate gradient method on the normal equations (Saad, 2003), in which the matrix vector multiplications in this optimization algorithm are substituted by the (adjoint) nonuniform Fourier transform operators. Throughout the iterative optimization the residual decreases and agreement of the reconstruction with the observations increases. Interestingly, it can be shown (Knopp et al., 2007) that the result of the first iteration of the conjugate gradient optimization starting from a void volume is similar to the solution of the gridding method. When optimization is continued, the iterative approach becomes more accurate than the gridding method (Bronstein et al., 2002).

Excessive optimization nevertheless has the risk to overfit to the noise if the observations are noisy. In order to be noise robust, the procedure should be terminated when the residual is below the noise level ε:

$$\|A^H W b - A^H W A f\| < \varepsilon. \tag{9}$$