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Radiation dose reduction and image enhancement in biological imaging through equally-sloped tomography

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ABSTRACT

Electron tomography is currently the highest resolution imaging modality available to study the 3D structures of pleomorphic macromolecular assemblies, viruses, organelles and cells. Unfortunately, the resolution is currently limited to 3–5 nm by several factors including the dose tolerance of biological specimens and the inaccessibility of certain tilt angles. Here we report the first experimental demonstration of equally-sloped tomography (EST) to alleviate these problems. As a proof of principle, we applied EST to reconstructing frozen-hydrated keyhole limpet hemocyanin molecules from a tilt-series taken with constant slope increments. In comparison with weighted back-projection (WBP), the algebraic reconstruction technique (ART) and the simultaneous algebraic reconstruction technique (SART), EST reconstructions exhibited higher contrast, less peripheral noise, more easily detectable molecular boundaries and reduced missing wedge effects. More importantly, EST reconstructions including only two-thirds the original images appeared to have the same resolution as full WBP reconstructions, suggesting that EST can either reduce the dose required to reach a given resolution or allow higher resolutions to be achieved with a given dose. EST was also applied to reconstructing a frozen-hydrated bacterial cell from a tilt-series taken with constant angular increments. The results confirmed similar benefits when standard tilts are utilized.

1. Introduction

It has been four decades since DeRosier and Klug first reconstructed a negatively stained bacteriophage T4 tail from a single electron microscope (EM) projection (De Rosier and Klug, 1968). Since then a number of important methodological advances have been made. The development of rapid freezing techniques has allowed biological samples to be preserved in a near-native state and decreased their sensitivity to radiation damage (Taylor and Glaeser, 1974; Dubochet et al., 1998). The use of electronic detectors and the implementation of automated low-dose data acquisition schemes have made it possible to record multiple projections quickly without damaging the specimen unnecessarily (Dierksen et al., 1993). Although electron tomography has already emerged as the most powerful method for obtaining the 3D pleomorphic structure of macromolecular assemblies, organelles and thin cells (Lučić et al., 2005; Jensen and Briegel, 2007; McIntosh, 2001), it still faces two irreconcilable requirements. To obtain high-quality and high-resolution 3D images, both the tilt range and the number of projections have to be as large as possible. On the other hand, the total dose used must be minimized, since biological specimens are gradually destroyed by the high-energy electrons (Henderson, 1995), which limits the number of projections that can be acquired. Furthermore, electron tomography also suffers from the missing wedge problem (i.e. specimens can not be tilted beyond ±70° and hence the data in the remaining ±20° projections is missing), low contrast and low signal to noise ratios (Lučić et al., 2005; Jensen and Briegel, 2007; McIntosh, 2001).

Here we report the first experimental demonstration of EST (equally-sloped tomography) to alleviate these limitations. As a proof of principle, we used EST to reconstruct single keyhole limpet hemocyanin (KLH) particles, a 7.9 MDa macromolecule consisting of a double-layered and hollow barrel complex about 30 nm in diameter and 35 nm in length (Mouche et al., 2003). KLH was chosen for the study because a model of this molecule's structure to 12 Å resolution is available. The structural model, obtained by averaging hundreds of projection images (Mouche et al., 2003), allowed us to perform various quantitative tests. In comparison

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with the standard WBP (Frank, 1992; Radermacher, 1992; Harauz and van Heel, 1986), ART and SART (Marabini et al., 1998; Kak and Slaney, 2001; Natterer and Wubbeling, 2001), EST produces reconstructions with apparently equal resolutions with only two-thirds the dose. Furthermore, EST reconstructions exhibited higher contrast, less peripheral noise, more easily detectable boundaries and reduced missing wedge effects. We also used EST to reconstruct a frozen-hydrated spirillum cell from a tilt-series taken with the traditional constant angular increments, with similar increases in contrast and clarity.

2. Equally-sloped tomography

2.1. The pseudo-polar fast Fourier transform

Conventional tomography reconstructs a 3D object from a tilt-series of projections with constant angular increments. Since the set of projections are on a polar grid and the object on a Cartesian grid, interpolations have to be performed during the reconstruction process. This is due to the fact that no direct and exact fast Fourier transform exists between the polar and Cartesian grids (Briggs and Henson, 1995). Presently, the most popular 3D image reconstruction method in electron tomography is WBP, in which the interpolations are done in object space (Frank, 1992; Radermacher, 1992; Harauz and van Heel, 1986). However, if the projections are obtained with constant slope increments, it has been shown that there exists a direct and exact fast Fourier transform called the pseudo-polar fast Fourier transform (PPFFT) between a pseudo-polar grid and the Cartesian grid (Mersereau and Oppenheim, 1974; Averbuch et al., 2008). Fig. 1 shows a pseudo-polar grid and its relationship to the Cartesian grid. For an $N \times N$ Cartesian grid, the corresponding pseudo-polar grid is defined by a set of $2N$ lines, each line consisting of $2N$ grid points mapped out on N concentric squares. The $2N$ lines are subdivided into a horizontal group (in blue) defined by $y = sx$, where s is the slope and $|s| \leqslant 1$, and a vertical group (in red) defined by $x = sy$, where $|s| \leqslant 1$; the horizontal and vertical groups are symmetric under the interchange of x and y, and $\triangle s = 2/N$. When these conditions are met, there exists the PPFFT and its inverse algorithm between a pseudo-polar grid and a Cartesian grid that is mathematically exact and geometrically faithful (Averbuch et al., 2008). Note that The PPFFT and its inverse algorithm was originally developed to interpolate tomographic projections from the polar to the Cartesian grid in Fourier space (Mersereau and Oppenheim, 1974; Averbuch et al., 2008). The idea of acquiring tomographic tilt-series at equal slope increments and then combining PPFFT with iterative algorithms for 3D image reconstructions was first suggested by Miao et al. (2005).

2.2. The EST reconstruction algorithm

Compared to other data acquisition schemes (Saxton et al., 1984; Leszczynski et al., 1988), the pseudo-polar grid acquires projections with constant slope increments, and allows the use of the mathematically exact PPFFT. The implementation of the PPFFT, however, requires two stringent conditions: (i) the tilt range has to be from $-90°$ to $+90°$ and (ii) the number of projections needs to be $2N$ for an $N \times N$ object. These conditions make it impossible to directly apply PPFFT to electron tomography. We overcame these limitations by combining PPFFT with an iterative algorithm (Miao et al., 2005), which was inspired by the iterative phase recovery algorithms in coherent diffraction microscopy (Miao et al., 1999, 2008). Fig. 2 shows the schematic layout of the algorithm. We first converted the electron micrograph projections to Fourier slices in the pseudo-polar grid. As illustrated in Fig. 1, the distance between the sampling points on the individual $2N$ lines of the pseudo-polar grid varies from line to line. In order to calcu-

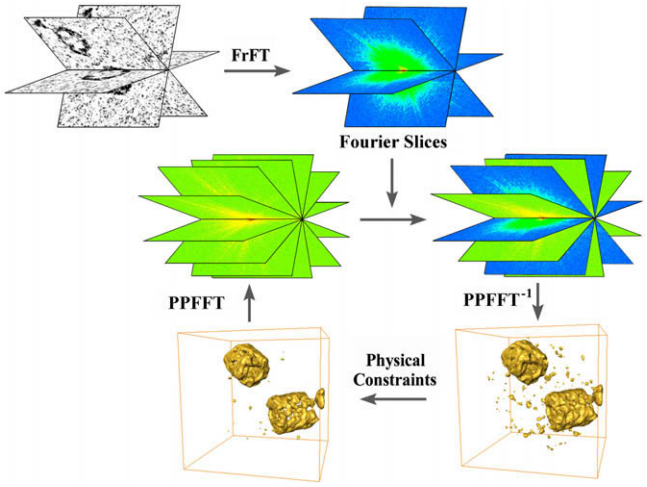

Fig. 2. Schematic layout of the iterative EST method. The algorithm iterates back and forth between Fourier and object space. In each iteration, the calculated slices are updated with the measured (experimental) slices in Fourier space and the physical constraints are enforced in object space.

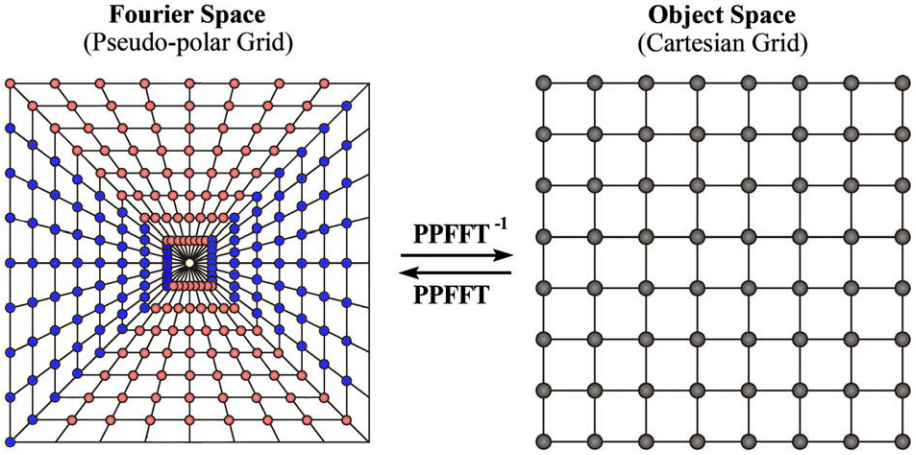

Fig. 1. Pseudo-polar grid and pseudo-polar fast Fourier transform. For an $N \times N$ Cartesian grid where $N = 8$ in this case, the corresponding pseudo-polar grid is defined by a set of $2N$ lines, each line consisting of $2N$ grid points mapped out on N concentric squares. The $2N$ lines are subdivided into a horizontal group (in blue) and a vertical group (in red) with constant slope increments in each group. (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)