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A simple Fourier filter for suppression of the missing wedge ray artefacts in single-axis electron tomographic reconstructions

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ABSTRACT

The limited specimen tilting range that is typically available in electron tomography gives rise to a region in the Fourier space of the reconstructed object where experimental data are unavailable – the missing wedge. Since this region is sharply delimited from the area of available data, the reconstructed signal is typically hampered by convolution with its impulse response, which gives rise to the well-known missing wedge artefacts in 3D reconstructions. Despite the recent progress in the field of reconstruction and regularization techniques, the missing wedge artefacts remain untreated in most current reconstruction workflows in structural biology. Therefore we have designed a simple Fourier angular filter that effectively suppresses the ray artefacts in the single-axis tilting projection acquisition scheme, making single-axis tomographic reconstructions easier to interpret in particular at low signal-to-noise ratio in acquired projections. The proposed filter can be easily incorporated into current electron tomographic reconstruction schemes.

1. Introduction

Three-dimensional electron tomographic reconstructions are produced by the acquisition of a set of tilted projections that are subsequently aligned and subjected to a reconstruction algorithm. Unfortunately, the computed reconstructions typically suffer from a number of artefacts arising from an imprecise projection alignment, from the structural instability of specimens during tomogram acquisition, and from the presence of the missing wedge region in the Fourier space of reconstructions, which remains free of experimental data due to the limited possibility of specimen tilting in transmission electron microscopes (TEMs). The utilizable range of specimen tilting is restricted not only by physical constraints imposed by the design of TEMs, specimen holders and grids, but also by the increasing effective thickness of specimen sections at tilting (Koster and Barcena, 2006; Penczek and Frank, 2006; Turner and Valdre, 1992). Therefore, reconstructions without the artefacts caused by the missing wedge can only be obtained when imaging needle-shaped (or other narrowly shaped specimens) mounted in dedicated holders that allow tilting in TEMs without restrictions (Kawase et al., 2007).

At the single-axis tilting tomographic acquisition scheme, with a tilting range bounded by the highest-tilt angles Φ_{min} and Φ_{max}, the missing wedge has a form of a sharply delimited double V-shaped region. Consequently, the region of available data is represented by a cylinder with the missing wedge (area marked $\Omega_3(R,\Phi,Z)$ in Fig.1A), supposing that Fourier transform values at all spatial frequencies within the angular range $<\Phi_{min}, \Phi_{max}>$ up to some maximum spatial frequency R_{max} can be estimated. In two-dimensional reconstructions, $\Omega_3(R,\Phi,Z)$ reduces to a butterfly-shaped area $\Omega_2(R,\Phi)$ (Fig.1B) defined as

$$\Omega_2(R,\Phi) = \begin{cases} 1 & \text{if } |R| \le R_{max},\ \Phi_{min} \le \Phi \le \Phi_{max}, \text{and} \\ 0 & \text{otherwise.} \end{cases} \quad (1)$$

Its Fourier transformation leads to the well-known impulse response of the missing wedge $W_2(r,\varphi)$ (Fig.1C) (Carazo, 1992; Radermacher, 1988; Tam and Perez-Mendez, 1981a,b), which convolves with the reconstructed signal, giving rise to three kinds of distinctive artefacts: (i) the ray artefacts of relatively low intensity but infinite in length, which are perpendicular to each highest-tilt projection, (ii) the elongation of reconstructed features in the direction of the axis of the missing wedge ($\equiv z$ axis in most 3D

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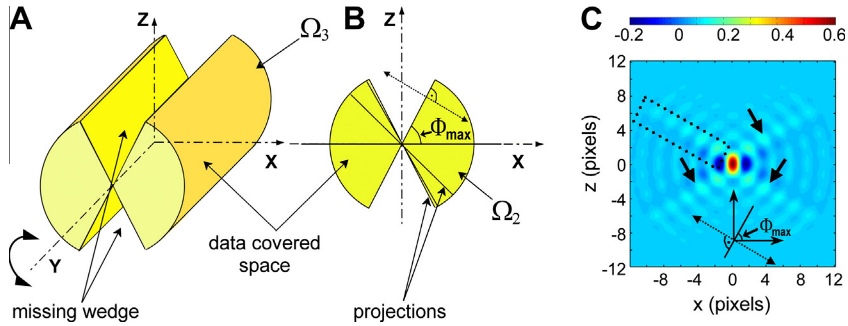

Fig.1. The geometric shape of areas of available data at the single axis tilting scheme with a tilting range limited to $\pm\Phi_{max}$ in Cartesian coordinates. (A) Area $\Omega_3(X,Y,Z)$ in 3D space, (B) $\Omega_2(X,Z)$ in 2 dimensions. (C) Impulse response $W_2(x,z)$ of $\Omega_2(X,Z)$ shown in (B) computed according to (Komrska, 1983). Values of $W_2(x,z)$ were divided by the magnitude in the central maximum of the impulse response of an unobstructed (full) circle with the same diameter. Dotted rectangle encloses one of the four side rays pairs perpendicular to the highest-tilt projections (light blue areas correspond to the positive line, dark blue to the negative line), arrows indicate the remaining three pairs of side rays.

reconstructions), and (iii) the side minima joining the central spot in the direction of the *x*-axis.

The standard reconstruction methods currently used in electron tomography in structural/cell biology – the weighted back-projection (WBP), (Radermacher, 1992), the direct Fourier methods (DFM) (Crowther et al., 1970; Lanzavecchia et al., 1993; Natterer, 1985), and the iterative methods ART (Gordon et al., 1970) and SIRT (Gilbert, 1972) – can determine only a small amount of Fourier coefficients in the missing wedge in close proximity of the tilting axis, see e.g. (Lee et al., 2008). Therefore, a major portion of the missing wedge region remains empty, which gives rise to the missing wedge artefacts that make the interpretation of reconstructions difficult (e.g. Quinto et al., 2009; Radermacher, 2006), especially at low signal-to-noise ratios in projections of crowded environments of in situ specimens (Forster et al., 2005; Frangakis and Hegerl, 2006; Frangakis et al., 2002; Grünewald et al., 2003).

The missing wedge artefacts can be mitigated if regularization terms are incorporated into the reconstruction schemes (Carazo, 1992; Fanelli and Oktem, 2008; Norlen et al., 2009; Penczek and Frank, 2006; Quinto et al., 2009). While the standard reconstruction methods already regularize the solution of the severely ill-posed reconstruction problem (Fanelli and Oktem, 2008; Norlen et al., 2009), further improvement of the reconstruction quality can be achieved if it is possible to introduce some kind of *a priori* knowledge about specimens under study into the reconstruction schemes. Methods like the total variation minimization (TV) (Lu et al., 2010; Persson et al., 2001; Aganj et al., 2007), constrained maximum entropy tomography (Skoglund et al., 1996), discrete tomography (Batenburg et al., 2009), the HECT reconstruction method (Jarisch, 2010), and especially the equally-sloped tomography (EST) (Miao et al., 2005) have demonstrated their efficiency for a variety of specimen types, including cryo-tomograms of cells (Aganj et al., 2007; Lee et al., 2008). Unlike the regularization methods, the electron lambda-tomography reconstruction method (ELT) (Quinto et al., 2009) does not require any specific knowledge about specimens but reduces in particular the background clutter in reconstructions through minimization of the interference of information from structures outside the reconstructed region of interest.

Indeed, the missing wedge artefacts can be reduced or even completely avoided if the missing wedge is filled with the appropriate projection data. A full elimination of the missing wedge can be achieved by symmetry operations if the reconstructed object has a symmetry, or by sub-tomogram averaging of repetitive cellular structures or of multiple copies of randomly oriented identical particles (Bartesaghi et al., 2008; Forster and Hegerl, 2007; Frangakis et al., 2002; Ofverstedt et al., 1997). Reduction of the missing wedge area is being routinely achieved by recording of two or more tilt series of projections around different tilting axes in double-axis tilting tomography (e.g. (Mastronarde, 1997; Penczek et al., 1995)) or multiple-axis tilting tomography (Messaoudii et al., 2007). In conical tomography (Lanzavecchia et al., 2005; Zampighi et al., 2005), the missing wedge is reduced to a missing cone after specimen tilting followed by in-plane rotations. All these techniques lead to suppression of the missing wedge artefacts in reconstructions (Mastronarde, 1997), however, specimens might be exposed to higher electron doses.

In low-dose cryo-electron tomography in structural/cell biology, reconstructions are nowadays commonly computed by WBP, ART or SIRT and then subjected to various denoising procedures in order to facilitate their interpretation (Fernandez, 2012; Frangakis and Hegerl, 2006; Narasimha et al., 2008). These methods, including the efficient non-linear anisotropic diffusion (NAD) (Frangakis and Hegerl, 2001), however, do not specifically aim at suppression of the missing wedge artefacts. Therefore we propose a simple angular filter for single-axis tilting tomographic reconstructions, which efficiently suppresses the missing wedge ray artefacts by damping the sharp transition of the non-zero data region to the zero-filled missing wedge region in the Fourier space of the reconstructed object. The removal of the rays simplifies the interpretation of reconstructed volumes, in particular in sections perpendicular to the tilting axis.

2. Theory

In the theory of Fraunhofer diffraction, the Abbe theorem (Komrska, 1983; Straubel, 1895) states that each straight edge of a diffraction shade gives rise to an intensity line in the Fraunhofer diffraction pattern, which is perpendicular to this edge and passes through the central spot of the diffraction pattern. In the case of a tomographic reconstruction of a two-dimensional object from an angularly limited set of projections, the data and the missing wedge regions are in its Fourier space sharply separated by the lines of both highest-tilt projections (Fig.1B). The data area $\Omega_2(R, \Phi)$ has a geometric shape of a 2-fold rotationally symmetric section star as defined in (Komrska, 1983), except that the angular range $<\Phi_{min}, \Phi_{max}>$ of the transparent sectors of $\Omega_2(R, \Phi)$ is generally not limited to $<-\pi/4, \pi/4>$ in electron tomographic experiments. Komrska also showed that the diffraction pattern of 2-fold section stars is real and contains four pairs of arms, which are perpendicular to the straight edges of the section stars. Each pair is composed of two adjacent intensity lines passing over the primary diffraction spot at a small distance. The analytic computation of