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Conical Fourier shell correlation applied to electron tomograms

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ABSTRACT

The resolution of electron tomograms is anisotropic due to geometrical constraints during data collection, such as the limited tilt range and single axis tilt series acquisition. Acquisition of dual axis tilt series can decrease these effects. However, in cryo-electron tomography, to limit the electron radiation damage that occurs during imaging, the total dose should not increase and must be fractionated over the two tilt series. Here we set out to determine whether it is beneficial fractionate electron dose for recording dual axis cryo electron tilt series or whether it is better to perform single axis acquisition. To assess the quality of tomographic reconstructions in different directions here we introduce conical Fourier shell correlation ($cFSC_{e/o}$). Employing $cFSC_{e/o}$, we compared the resolution isotropy of single-axis and dual-axis (cryo-)-electron tomograms using even/odd split data sets. We show that the resolution of dual-axis simulated and cryo-electron tomograms in the plane orthogonal to the electron beam becomes more isotropic compared to single-axis tomograms and high resolution peaks along the tilt axis disappear. $cFSC_{e/o}$ also allowed us to compare different methods for the alignment of dual-axis tomograms. We show that different tomographic reconstruction programs produce different anisotropic resolution in dual axis tomograms. We anticipate that $cFSC_{e/o}$ can also be useful for comparisons of acquisition and reconstruction parameters, and different hardware implementations.

1. Introduction

1.1. Electron beam damage and total electron dose are limiting the resolution in cryo-electron tomography

Cryo-electron microscopy (cryo-EM) has the primary advantage that vitrification, the fixation technique being used for specimen preparation, excellently preserves biological structures, enabling atomic resolution data to be obtained (Zhang et al., 2010; Amunts et al., 2014). Though preservation is excellent, the main limitation is that vitrified biological specimens are highly sensitive to electron beam exposure, which increases radiation damage leading to loss of structural integrity. Images are therefore recorded using a limited total electron dose, resulting in data with low signal-to-noise ratio (SNR). The total dose used during data collection ultimately limits the resolution of cryo-EM reconstructions. In single particle analysis (SPA) as well as in electron crystallographic techniques data resulting from a large number identical objects are averaged which increases the resolution of the final reconstructed object without increasing electron beam radiation damage. Electron tomography (ET) generally targets uniquely shaped objects for which averaging is not possible. In practice, the total electron dose used to expose the targeted object in a tomographic tilt series is ~5 times higher than that of an individual image used in SPA. Therefore, electron radiation damage is often the main resolution limiting factor (Diebolder et al., 2012).

1.2. Fourier shell correlation for resolution estimation in cryo-EM

In SPA, Fourier shell correlation (FSC) is often used as a measure for the resolution. In short, datasets are split in two, and subsequent shells in Fourier space from each normalized half are correlated with each other (van Heel and Harauz, 1986; Harauz and van Heel, 1986). FSC resolution measurement, which is rather an

Abbreviations: cFSC, conical Fourier shell correlation; $cFSC_{e/o}$, conical Fourier shell correlation of even/odd split datasets; EM, electron microscopy; ET, electron tomography; FFT, Fast Fourier Transform; FRC, Fourier ring correlation; FSC, Fourier shell correlation; SNR, signal-to-noise ratio; SPA, single particle analysis; SSNR, spectral signal-to-noise ratio.

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internal consistency assessments of reconstructions, is the 3D equivalent of the Fourier Ring correlation (FRC; Saxton and Baumeister, 1982; van Heel, 1982):

$$FSC_{12}(r_i) = \frac{\sum_{r_i} F_1(r_i) * F_2(r_i)^*}{\sqrt{\sum_{r_i} F_1^2(r_i) * \sum_{r_i} F_2^2(r_i)}} \quad (1)$$

where r_i is the voxel element in Fourier space at radius r, $F_1(r_i)$ the complex structure factors of volume 1, and $F_2(r_i)^*$ the complex conjugate of the structure factor of volume 2. FSC is directly linked to the spectral signal-to noise ratio (SSNR) (Frank and Al-Ali, 1975)

$$SSNR \cong \frac{FSC}{1 - FSC} \quad (2)$$

and widely used for resolution estimation of SPA reconstructions (Malhotra et al., 1998). For the FSC measurement, the reconstructed volumes from two half sets are compared. Assuming a 50% decrease of the SNR for each half set compared to the full data set, it can be compensated for the resulting underestimation of resolution by defining a modified "ideal" FSC′ (Rosenthal and Henderson, 2003) which has following relation to the measured $FSC_{measured}$:

$$FSC' = \frac{2\,FSC_{measured}}{FSC_{measured} + 1} \quad (3)$$

In practice, a number for the attained resolution of a reconstructed volume is often defined as the highest frequency where the correlation coefficient remains above a certain threshold value. For instance, SSNR = 2.0, according to (2) and (3), would correspond to a measured $FSC_{measured}$ = 0.5 and a FSC′ = 0.66, although other criteria are also used and discussed (Boettcher et al., 1997; van Heel and Schatz, 2005). This FSC was implemented for quality estimation of single-axis cryo-electron tomograms in the software package *ELECTRA* (Cardone et al., 2005), assuming that Eq. (3) stays valid when splitting a tomographic tilt series into two (even and odd) half series:

$$FSC_{e/o} = \frac{2\,FSC_{measured}}{FSC_{measured} + 1}. \quad (4)$$

However, due to high noise levels and restricted geometry of data acquisition, interpreting measurements on tomograms is challenging.

1.3. Electron tomography data acquisition geometry influences resolution isotropy

In electron tomography the angular range for collecting images of the object of interest is inherently limited. The restricted data acquisition geometry leads to anisotropic resolution, as it causes inhomogeneous sampling of the object in the frequency domain. In many cases, the restricted geometry is due to the slab-like shape of the transmission electron microscope specimen that contains the object which limits the maximal angular tilt range for which images can be acquired. The number of views within that tilt range is limited due to the maximal allowable electron dose. For practical reasons, cryo-ET is usually performed by restricted tilting along a single fixed rotation axis, resulting in a missing data wedge in Fourier space (Fig. 1A). As a consequence, the resolution that can be obtained by single-axis tilt electron tomography is not isotropic and deteriorates in the direction of the electron beam and, to a lesser extent, in the direction orthogonal to the tilt axis and the electron beam (Diebolder et al., 2012). Dual-axis tomography (Penczek et al., 1995) and conical tomography (Lanzavecchia et al., 2005) were proposed to increase isotropy by reducing the missing wedge of a single tilt tomogram to a missing pyramid or a missing cone, respectively (Fig. 1B and C). The latest generation of transmission electron microscopes and sample grid holders allow recording of tilt series with dual-axis tomographic geometries (Iancu et al., 2005), but these methods are rarely applied to vitrified samples (Diebolder et al., 2014; Dudkina et al., 2010; Nickell et al., 2003; Myasnikov et al., 2013). One of the reasons is closely linked to the electron dose sensitivity of the specimen. For vitrified specimen the total electron dose to which a specimen can be exposed without inducing radiation damage in the resolution range aimed for by the experiment is limited. Therefore, when recording a second perpendicular tilt series, the total electron dose has to be divided over two tilt series. For choosing the optimal data collection geometry a number of questions then arise: given that dual-axis reconstruction requires additional alignment of the two halve datasets, is recording a dual-axis tilt series more advantageous than recording on a single tilt tomographic series (Chen et al., 2014; Guesdon et al., 2013)? Also, what is the most advantageous way to reconstruct dual-axis tilt series into a single tomogram? Should one use schemes based on independent single-axis tilt series alignments followed by merging of reconstructed single-axis tomograms (Mastronarde, 1997; Schoenmakers et al., 2005), or is common alignment of all projection images of both tilt series prior to back projection more advantageous (Winkler and Taylor, 2013; Cantele et al., 2010)?

1.4. Estimation of resolution isotropy

Several methods are described that provide a means to assess the quality of cryo-electron tomograms. In the *ELECTRA* software suite a method, called *NLOO*, is implemented which is based on FRC between a projection image of a tilt series and the corresponding re-projection that is obtained from a reconstruction omitting this projection. This results in resolution measurements for the individual tilt angles in a data set. Two other methods exist that are based on measurement of the three-dimensional spectral signal-to-noise ratio (3D SSNR, Penczek, 2002; Unser et al., 2005). Real space estimation of the point spread function has also been implemented (Heidari Mezerji et al., 2011). Nevertheless, FSC is still used for comparison of overall resolution for the different tomography geometries (Cardone et al., 2005; Lanzavecchia et al., 2005; Cantele et al., 2010).

In order to assess resolution in a directional way, segmentation of the Fourier space has been introduced. Fourier space can be split into "sectors" as a function of elevation from the horizontal plane (Fourier sector correlation, Mastronarde, 1997), and Penczek and Frank (2006) defined a "resolution cone" along the tilt axis which contains redundant information and thus can be used for SSNR measurement.

In this study, we investigate to what extent the use of dual-axis geometry increases the isotropy and the resolution of cryo-electron tomograms. Therefore, we developed "conical Fourier shell correlation of even-odd split tomograms" ($cFSC_{e/o}$). $cFSC_{e/o}$ is based on a modified even-odd Fourier shell correlation ($FSC_{e/o}$) and – besides general resolution estimation – allows directional resolution assessment in an electron tomogram. The $cFSC_{e/o}$ is computed within double sided cones that are directed in different directions, sampling the whole Fourier space (Fig. 1D–F). We applied $cFSC_{e/o}$ as a directional measure for the resolution in single- and dual-axis electron tomograms of vitrified lipid vesicles with bound antibodies, and compare these with simulations of similar data acquisition geometries. We also used dual-axis tomograms of stained plastic sections to evaluate different reconstruction programs. Our results show that the isotropy of the resolution in the *XY*-plane (in directions orthogonal to the electron beam) in dual-axis tomograms is clearly enhanced compared to single-axis counterparts, recorded using the same total electron dose. Comparison of various commonly used software