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Resolution in Electron Tomography

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1. INTRODUCTION

Traditionally, in computed tomography practiced in radiology, the resolution of the reconstruction is expressed in terms of the number of evenly spaced projections required for the faithful reconstruction of an object that has a given diameter (see equation (10) below). The tacit assumption is that projection data have a sufficient spectral signal-to-noise ratio (SSNR) in the whole frequency range in order to reproduce the object faithfully. In electron microscopy, the situation is dramatically different, as the electron dose limitations result in very low SSNR in the individual projections. The suppression of signal is particularly severe in high spatial frequencies, where the signal is affected by the envelope function of the microscope and the high amount of ambient noise, as well as in some low spatial frequency

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regions (due to the influence of the contrast transfer function (CTF) of the electron microscope). In single-particle reconstruction, a satisfactory level of the SSNR in the 3D reconstruction is achieved by including a large number of 2D projections (tens to hundreds of thousands) that are averaged during the reconstruction process. Except for rare cases (Boisset *et al.*, 1998), the angular distribution of projections is not an issue, as the large number of molecules and the randomness of their orientations on the support grid all but guarantee uniform coverage of angular space. The concern is whether the number of projections per angular direction is sufficient to yield the desired SSNR or whether the angular distribution of projections is such that the oversampling of the 3D Fourier space achieved during the reconstruction process will yield the desired SSNR. The resolution measures used in single-particle reconstruction are designed to evaluate the SSNR in the reconstruction as a function of spatial frequency (Penczek, 2002). The 'resolution' of the reconstruction is reported as a spatial frequency limit beyond which the SSNR drops below a selected level, for example below one.

The most commonly used resolution measure in single-particle reconstruction is the Fourier shell correlation (FSC), the 3D equivalent of the Fourier ring correlation (Saxton and Baumeister, 1982). Its advantages are the ease of use and its direct relationship to the SSNR in the data, thus leading to straightforward interpretation of the results. The FSC is evaluated by taking advantage of the large number of single-particle images available: the total data set is randomly split into halves; for each subset a 3D reconstruction is calculated (in 2D, a simple average); and two objects are compared in Fourier space:

$$\mathrm{FSC}(f,g;r)=\frac{\sum\limits_{|\|\mathbf{y}_n\|-r|\le\varepsilon}\hat{f}(\mathbf{y}_n)\hat{g}^*(\mathbf{y}_n)}{\left\{\left(\sum\limits_{|\|\mathbf{y}_n\|-r|\le\varepsilon}\left|\hat{f}(\mathbf{y}_n)\right|^2\right)\left(\sum\limits_{|\|\mathbf{y}_n\|-r|\le\varepsilon}\left|\hat{g}(\mathbf{y}_n)\right|^2\right)\right\}^{1/2}} \tag{1}$$

In equation (1), $\hat{f}$ is the Fourier transform of f, the asterisk indicates complex conjugation, 2ε is a pre-selected shell thickness, the $\mathbf{y}_n$ form a uniform grid in Fourier space and $r = \|\mathbf{y}_n\|$ is the magnitude of the spatial frequency. The FSC yields a 1D curve of correlation coefficients as a function of r. Note that the FSC is insensitive to linear transformations of the objects' densities. An FSC curve everywhere close to one reflects strong similarity between f and g; while an FSC curve with values close to zero indicates the lack of similarity between f and g. Particularly convenient for the interpretation of the results in terms of 'resolution' is the relationship between the FSC and SSNR (Frank and Al-Ali, 1975):

$$SSNR=2\frac{FSC}{1-FSC}. \tag{2}$$