Resolution Assessment in Electron Tomography: a Cross-Validation Approach

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Although resolution assessment in Single Particle Analysis (SPA) remains short of consensus, several measures – such as the Fourier Shell Correlation and the Differential Phase Residual - are widely used. FSC and DPR exploit redundancy in the data set by measuring the consistency, as a function of spatial frequency, of two density maps calculated from an arbitrary division of the data into half sets. Resolution assessment in electron tomography poses additional problems: first, there is generally only one density map; second, limits are posed by number of the projections, and anisotropy arises from the "missing wedge" effect. To date, only one criterion has been proposed for tomography, and its applicability is confined to a particular class of reconstruction algorithms [1]. In practice, resolution assessment in electron tomography has usually relied on the visibility (subjectively decided) of certain details or, if a component of known structure is present in the tomogram, it can be used as reference in an FSC calculation (FSC_{ref}) [2]. We now propose a criterion for the resolution of tomograms, based on cross-validation. It operates by calculating, as a function of the spatial frequency, the consistency between each projection and the corresponding reprojection of a tomogram calculated from *all other* projections. We call it the "leave one out" (LOO) method. As adapted to compensate for the differing noise statistics of the data compared, and averaged over all projections, we obtain the 3D Noise-compensated LOO measure (3DNLOO).

A tilt series consists of N projections. $X^{(i)}$ is the Fourier transform of projection i. The tomogram generated from the full tilt series produces reprojections whose Fourier transforms are $\tilde{X}^{(i)}$. Tomograms generated by excluding the corresponding input projections yield reprojections with Fourier transforms, $\tilde{X}^{-(i)}$. At a given radial frequency k, we define the 2DNLOO for projection i as

$$2DNLOO^{(i)}(k) = \frac{FRC^{(i)}_{X\tilde{X}^-}(k)}{FRC^{(i)}_{X\tilde{X}}(k)},$$

i.e. the ratio between two Fourier Ring Correlations [3]. The difference in the noise statistics of the projection and reprojection is compensated by including, as denominator, the FRC calculated between the input projection and the corresponding reprojection of the *full* tomogram. According to the Central Section Theorem, 2DNLOO can be interpreted as a resolution estimate related to a slice of the 3D Fourier transform of the reconstructed volume. The 3DNLOO measure of resolution for the full reconstruction is derived from 2DNLOO, by integrating separately, both at the numerator and the denominator, over the tilt series. Since 3DNLOO is basically a (normalized) FSC coefficient, resolution is specified as the frequency at which this measure falls below a given threshold, as in SPA, where values of 0.5, 0.3, 3σ, and 0.142 are in use. Resolution as given by 3DNLOO is linked to the signal-to-noise ratio (SNR) of the tomogram by $3DNLOO \cong SNR/(SNR+1)$. This relationship allows us to relate the threshold value used to determine resolution from the 3DNLOO curve to a specific SNR. The NLOO approach also

provides a straightforward means to assess the anisotropy of resolution in a tomogram, i.e. by plotting resolution, as given by 2DNLOO for a specified threshold, as a function of tilt-angle.

In order to assess the consistency of 3DNLOO with the previously used FSC$_{ref}$, which is intuitively appealing but is not applicable unless the tomographic volume happens to contain a known reference structure, we performed the following simulation experiment. Tomograms were calculated from a tilt series that was generated computationally from a cryo-EM density map of the Hepatitis B Virus capsid, a round particle of 320 Å diameter [4]. This map has a resolution of 9Å and, for present purposes, is essentially noise-free. In a first experiment, the tilt series covered the range, -72° to $+72^{o}$ at 1° intervals, and white noise was added to the projections at a SNR of 0.5. We compared the 3DNLOO results to those of a FSCref, calculated between the tomogram and the original density map. The two measures show generally good agreement (Figure 1a), and the tomogram resolution, at a threshold of 0.5, is ~ 17 Å in each case. Its resolution is reduced relative to that of the original density map by the discrete angular sampling and the introduced noise. Different levels of noise were tested with a larger angular step of 2° (Figure 1b) and also gave good agreement between the two measures, indicating that 3DNLOO provides reliable estimates of resolution under a variety of conditions. Preliminary applications of the method to real (experimental) sets of tomographic data, from both vitrified specimens and plastic sections, have yielded similarly encouraging results. A program implementing 3DNLOO, called ELECTRA, has been written and is available on request.

References

[1] P. A. Penczek, J. Struct. Biol. 138 (2002) 34.
[2] K. Grünewald et al., Science 302 (2003) 1396.
[3] W. O. Saxton and W. Baumeister, J. Microsc. 127 (Pt 2) (1982) 127.
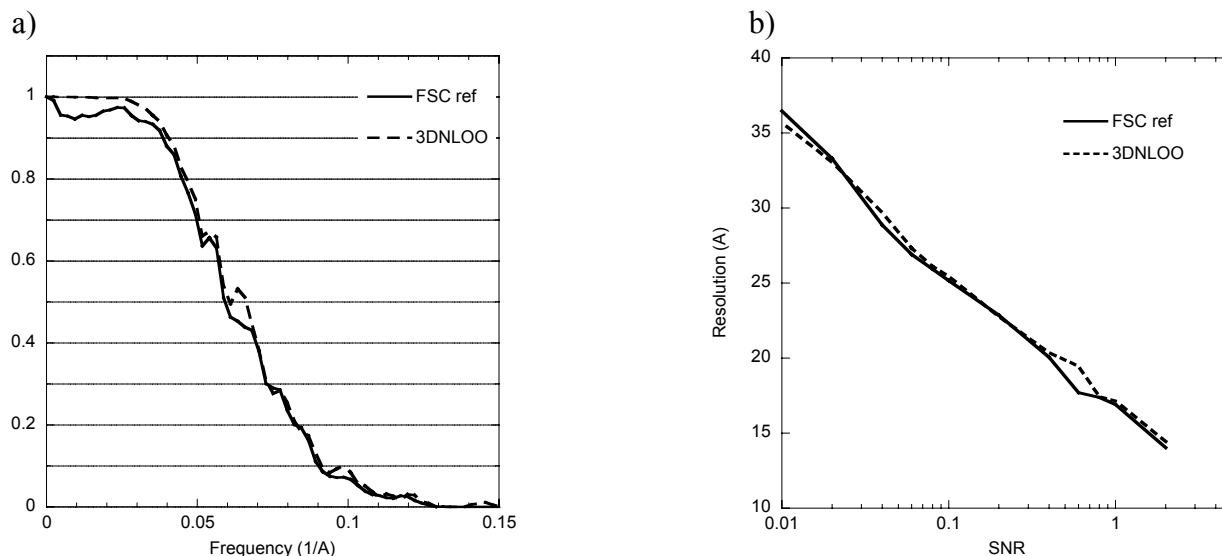[4] J. F. Conway et al., Nature 386 (1997) 91.

Fig. 1. Resolution curves were calculated for synthetic tomograms of the HBV capsid. a) for an angular step of 1° (145 images) and SNR=0.5. b) Resolution versus SNR for angular step of 2° (73 images). Threshold value=0.5.