Resolution assessment of 3D reconstructions by spectral signal-to-noise ratio

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One approach for assessing the resolution limit for 3D reconstructions calculated from electron micrograph data has been to compute two independent density maps and to test for the concordance of their 3D Fourier components, for example, by differential phase residuals (DPR) [1], or Fourier ring correlations (FRC) [2]. While this approach provides a good test for the reproducibility of the experiment, it constrains us to calculate resolution limits based on subsets of the available data. In addition, it does not explicitly test for the validity of the reconstruction process – reproducibility alone does not guarantee that the calculated density maps accurately represent the raw data. These problems are avoided in the method developed by Conway *et al* [3] in which an FRC-based resolution assessment is made between each constituent raw image of the 3D density map and its corresponding reprojection from that map, and these are then averaged over all such image/reprojection pairs for the map. However, use of the FRC in this way raises the difficulty of determining a statistically sound resolution threshold. This motivated us to develop an alternative criterion to FRC within the framework of comparing all image/reprojection pairs. This new criterion, the Spectral Signal-to-Noise Ratio (SSNR), is based on previous work in 2D [4,5].

3D spectral signal-to-noise ratio. Let us consider a data set X, which consists of I independent projections $\{x_{k,l}^{(i)}\}$, $i=1,\ldots,I$ with corresponding 2D Fourier transforms $\{X_{m,n}^{(i)}\}$, $i=1,\ldots,I$. After determination of the orientations of the views, the icosahedral reconstruction algorithm produces a 3D density map of the underlying specimen. This 3D map (or model) is then used to generate a corresponding set of reprojections $\{p_{k,l}^{(i)}\}$, $i=1,\ldots,I$ with Fourier transforms $\{P_{m,n}^{(i)}\}$, $i=1,\ldots,I$; these provide an estimate of the signal that is initially present in our data. We then define the input spectral signal-to-noise ratio (ISSNR) as follows:

$$\mathrm{ISSNR}(X;\omega)=\frac{\sum_{i=1}^{I}\sum_{(m,n)\in R(\omega)}\left|P_{m,n}^{(i)}\right|^2}{\sum_{i=1}^{I}\sum_{(m,n)\in R(\omega)}\left|X_{m,n}^{(i)}-P_{m,n}^{(i)}\right|^2} \qquad (1)$$

where $R(\omega)$ is a Fourier domain annulus with radial frequency ω. The numerator in (1) is an estimate of the signal power at frequency ω, while the denominator is a measure of the corresponding input noise energy (unexplained portion of the data). We choose to characterize the noise-reducing effect of the 3D reconstruction procedure by introducing the noise reduction factor $\alpha(\omega)$ which is typically a function of ω, but which also depends on the type of algorithm used, the imaging parameters (angles), the type of symmetry (e.g., icosahedral), and the number of views. By definition, $\alpha(\omega)$ is the proportion by which the noise variance is reduced by the reconstruction algorithm. An unbiased estimate of the true spectral signal-to-noise ratio on the reconstructed signal can then be obtained as:

$$\mathrm{SSNR}(X;\omega)=\left(\frac{\mathrm{ISSNR}(X;\omega)}{\alpha(\omega)}\right)-1 \qquad (2)$$

This is the 3D extension of the 2D SSNR criterion for correlation averaging (in this former simpler case, $\alpha(\omega)=1/(I-1)$). An operational resolution limit is specified as the spatial frequency at which the SSNR falls below an acceptable baseline. The difficulty with (2) is that the quantity $\alpha(\omega)$, which depends on many application-specific parameters, cannot in general be determined analytically. Our solution is to take a black box approach and determine $\alpha(\omega)$ empirically by injecting noise into the reconstruction algorithm with all parameters being the same as for X. Practically, this is equivalent to setting $\alpha(\omega)\cong\mathrm{ISSNR}(N;\omega)$ where N denotes a corresponding series of I noise-only images (either background or computer generated). Hence, we can assess the quality of our 3D reconstruction by comparing the ISSNR curves for the two modalities: X (data=signal+noise) and N (noise only) (cf. Fig. 1).

In the estimation of ISSNR, each input image is rescaled with respect to its reprojected view using a regression formula of the type $x_{k,l}^{(i)}=a\cdot x_{k,l}^{(i)}-b$. The regression parameters are determined by minimizing the difference between two Gaussian lowpass filtered versions of $x_{k,l}^{(i)}$ and $p_{k,l}^{(i)}$, which tends to produce more robust parameters.

Properties of the 3D SSNR. The proposed criterion provides an objective assessment of the quality of the reconstruction algorithm itself by looking at the consistency between the result and the input data. In addition, comparison of the two ISSNR curves provides an intuitive and sound assessment of the quality of the reconstruction. In short, we will trust only those signal frequency components whose energy is significantly above what would have been obtained if the algorithm was applied to noise only. Although the computation is performed in 2D, the SSNR criterion (2) also has a direct interpretation in 3D because of the central slice theorem. Specifically, the $\mathrm{SSNR}(X;\omega)$ will provide a valid estimate of the SSNR in 3D whenever the average across views (index i) can be assimilated to an average across angles in the 3D Fourier space, that is, when the views are evenly distributed with respect to orientation.

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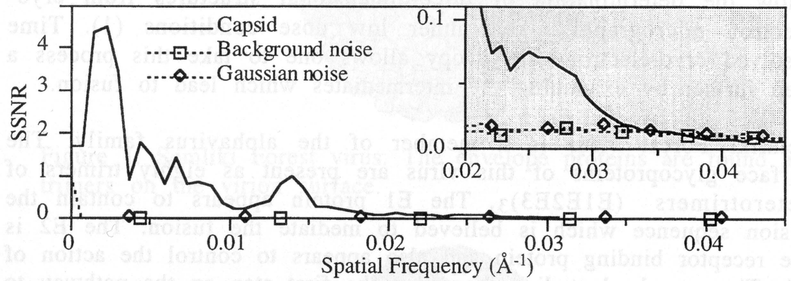

Figure 1. An ISSNR curve is shown for viral capsids versus two estimates of noise. Background images from the same micrograph is used to estimate $\alpha(\omega)$ in the noise-only case (boxes). The intersection between the two curves provides us with an estimate of the resolution limit (28Å^{-1} – see inset). Computer generated Gaussian noise (diamonds) is equally applicable as a noise estimate.

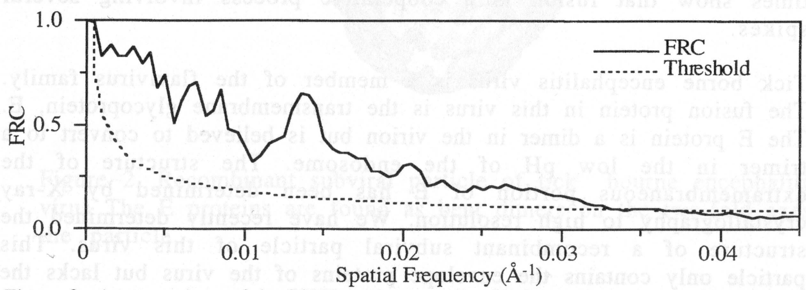

Figure 2. A comparison of the SSNR with our earlier FRC-based resolution (boxes) is shown together with a standard deviation-based threshold (diamonds). The estimated resolution is the same for both methods.