Volumetric spectral signal-to-noise ratio

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Measuring the quality of biological macromolecules that were reconstructed in three dimensions (3D) out of electron-microscopy images is still an open problem. We propose a method to compute the 3D frequency distribution of the resolution based on the 3D Spectral Signal-to-Noise Ratio (SSNR 3D) [1]. This had already been done in [2] for the class of reconstruction algorithms operating in Fourier space. Here, we broaden the measure to encompass all reconstruction algorithms. The basis of the method is to measure the consistency between the data and the corresponding set of reprojections computed for the reconstructed 3D map. The idiosyncracies of the reconstruction algorithm are taken explicitly into account by performing a noise-only reconstruction. The information to build the SSNR can be used to produce a Volumetric SSNR (VSSNR). Our method also overcomes the need to partition the data in two sets. Therefore, our measure yields a true resolution estimate and not only a reproducibility measure. The methodology described in this work is integrated in the software package Xmipp (http://www.cnb.uam.es/~bioinfo).

Let $X^{(i)}_{K,L}$, denote the 2D discrete Fourier transform of the input image indexed by $i=1,...,I$. By convention, we use K,L as the spatial frequency indices. After determination of the relative orientations of the individual views, the tomographic reconstruction algorithm produces a 3D map of the underlying specimen. This 3D map is then used to generate the corresponding set of reprojected images with Fourier transforms $\tilde{X}^{(i)}_{K,L}$. We define the Input SSNR for an image i as $ISSNR^{(i)}_{K,L}=\frac{\left\|\tilde{X}^{(i)}_{K,L}\right\|^2}{\left\|\tilde{X}^{(i)}_{K,L}-X^{(i)}_{K,L}\right\|^2}$, i.e., the ratio between the signal (the part of the experimental images that is explained by the reconstructed map) and the noise (the unexplained part). An advantage of this measure is that it explicitly measures the performance of the reconstruction algorithm. The unexplained part of the experimental images may be caused not only by random noise but also by structural heterogeneities, angular assignment errors, … The ISSNR reflects the SSNR at the input of the reconstruction algorithm. However, the latter performs "an averaging job" with other projections from similar projection directions and, therefore, the SSNR at the output is higher. We explicitly measure how the reconstruction algorithm attenuates the noise at each frequency by injecting white Gaussian noise and by performing a reconstruction under exactly the same conditions as for the reconstruction performed for the macromolecule. In this way, we compute the noise-

reduction factor at each frequency of the image i as $\alpha_{K,L}^{(i)} = \frac{\left\|\tilde{N}_{K,L}^{(i)}\right\|^2}{\left\|N_{K,L}^{(i)}\right\|^2}$ where N now represents noise-only images, and $\tilde{N}$ denotes the corresponding reprojection calculated from the noise-only 3D reconstruction map. Finally, we define the output SSNR for the image at hand as $SSNR_{K,L}^{(i)} = \max\left\{0, \frac{ISSNR_{K,L}^{(i)}}{\alpha_{K,L}^{(i)}} - 1\right\}$. The SSNR has a simple intuitive interpretation and leads to a very natural threshold-based definition of the resolution limit: we will only trust those frequency components whose energy is above what would be obtained if the algorithm was applied to noise only. Notice that $SSNR^{(i)}$ is a real-valued image defined in the Fourier space. We make use of the Central-Slice theorem to fit the set of images $\{SSNR^{(i)} : i = 1,...,I\}$ by a real-valued volume in the Fourier space $VSSNR(\omega)$ such that $VSSNR(\omega_{K,L}^{(i)}) = SSNR_{K,L}^{(i)}$. This latter operation is performed using Volumetric ART+blobs [3]. This volume provides an estimate of the SNR frequency distribution. The information provided by this volume is very helpful to understand the anisotropy of the reconstructed volume and its relationship to the angular distribution. (Some directions might have been better represented or completely ignored in the projection set.) This results in an uneven SSNR distribution that is easily detected by our measure. This anisotropy can be later used to build tailored lowpass filters as proposed in [2].

We applied this methodology to compute the volumetric SSNR of a 3D reconstruction of GroEL. We used the same data as described in [4]. Figure 1 shows the histogram of the tilting angle of the experimental data set and the corresponding VSSNR where the anisotropic resolution achieved due to the uneven angular distribution of the data is clearly identified.

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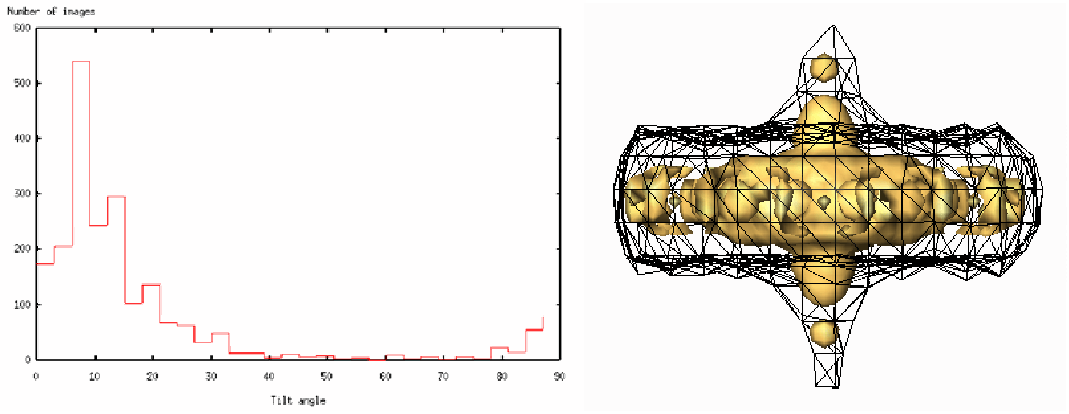

Figure 1. Histogram of the tilting angle of 2610 cryo-negative images of GroEL [4] and corresponding VSSNR (isosurfaces at VSSNR=1 (mesh) and VSSNR=4 (solid)) of their reconstruction when all 14 symmetries are taken into account. The SSNR=1 [1] is achieved at a frequency of 1/25Å^{-1}.