19. OTHER EXPERIMENTAL TECHNIQUES

debates in the community over standards for model assessment. As the field is now beginning to achieve resolutions where protein side chains can be visualized, reliable new assessments based on methods from X-ray crystallography are emerging, but at lower resolutions robust assessment remains elusive.

19.10.4.1. Model accuracy

Before assessing the resolution of a reconstruction, the fundamental question of whether the model even qualitatively represents the original data must be addressed. Regardless of the reconstruction methodology used, the fundamental questions to be asked are whether computed projections of the reconstruction match both the raw particles and class averages, and whether all of the particle views are represented by the three-dimensional model in some orientation. The 'Eulers' option in the workflow provides a number of tools for making such model assessments both qualitatively and quantitatively.

19.10.4.2. Measures of resolution and resolvability

Resolution in single-particle processing is distinct from the related concept of resolvability. Resolvability is a measure of the level of detail visible in a model, in terms of the shortest separation distance over which two objects can be identified as being distinct. However, in structural biology, 'resolution' is a statement of the spatial frequency at which the noise level exceeds a threshold. It is important to recognize that a model with 4 Å resolution could be low-pass filtered to a resolvability of only 20 Å, and yet cryo-EM resolution measures would still (properly) show it to have 4 Å resolution. To help bring the resolvability in line with the resolution, it is typical to apply an appropriate filter to the three-dimensional reconstruction such that the resolvability is in reasonable agreement with the resolution, but there remains no consensus in the community over the optimal filter and/or filtration level appropriate for this task. *EMAN2* provides a signal-to-noise-ratio- and structure-factor-based technique, as well as two mechanisms for assessing the resolution of a reconstruction.

19.10.4.3. Model/noise bias

The final issue to consider in a single-particle reconstruction is the well known model/noise bias problem (Stewart & Grigorieff, 2004). With a traditional iterative refinement strategy, and very high noise levels in the raw particle images, it is possible to produce a reconstruction including features derived from the initial model or from systematic algorithmic artifacts which are not represented in the raw data. There are relatively few techniques for assessing this sort of bias, and each single-particle reconstruction package handles this issue differently. In *EMAN*, the use of iterative class averaging during the iterative refinement process permits this bias to be greatly reduced or eliminated, when used as suggested. Ensuring that the reference-free class averages agree well with projections of the reconstruction can at least place some limits on the extent of such artifacts.

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