particles within a 'box' and normalizing the density to a standard variation), contrast-transfer-function correction, 2D alignment and 3D reconstruction. Many independent groups have developed various software packages for 3D EM reconstruction such as *SPIDER* (see Chapter 19.8), *IMAGIC 4D* (Chapter 19.9), *EMAN* (see Chapter 19.10), *AUTO3DEM* and *FREALIGN* (Ludtke *et al.*, 1999; Shaikh *et al.*, 2008; Yan *et al.*, 2007; Grigorieff, 2007). Numerous aspects of the reconstruction procedure are still being developed to achieve higher resolution, such as individual-particle contrast-transfer-function correction, magnification correction, local refinement in 2D alignment, and averaging among quasi-equivalent subunits within the icosahedral asymmetric unit after the 3D reconstruction. The highest resolution structure obtained for a virus is the 3.1 Å resolution structure of the cytoplasmic polyhedrosis virus.

The definition of resolution of a single-particle cryo-EM reconstruction is different from that used for assessing a crystal structure. The resolution of an EM reconstruction is usually estimated using the 'Fourier shell correlation' method (van Heel & Schatz, 2005). This requires dividing the randomly chosen images into two separate groups and computing reconstructions assuming the previously determined orientations. The resultant maps are then Fourier transformed to permit a comparison of the Fourier amplitudes and phases. The resolution at which the correlation between the amplitudes falls below 0.5 or the average phase difference is greater than 45° is usually a conservative estimate of resolution used by many groups, although other definitions are also in use (Henderson, 2004). Just as is the case for crystallographic maps, the quality of maps at the same resolution can vary depending on the degree of accuracy of the phase determination. Therefore, a visual inspection of a 3D EM map should serve as a useful check.

In contrast to single-particle cryo-EM, cryo-ET can be used to study biological samples that lack homogeneity (Milne & Subramaniam, 2009; Li & Jensen, 2009; Hoenger & McIntosh, 2009; Subramaniam *et al.*, 2007). In this technique, images of the same sample oriented by a succession of tilt angles are taken about a common axis and are subsequently combined into a three-dimensional map. Because the same sample is repeatedly exposed to the electron beam, the allowable electron dose has to be spread over all the tilted images, making the exposure for each image much less than for single-particle cryo-EM. This significantly reduces the limit of achievable resolution. In addition, the physical limitation on the tilt angles results in a 'missing wedge' problem. Dual-axis tomography has been developed to alleviate this problem to some extent. Averaging of the homogeneous portion of the heterogeneous sample has been used when appropriate to increase the resolution of a cryo-ET experiment (Bartesaghi & Subramaniam, 2009).

Cryo-EM methods have been an important addition to the family of structural biology tools. With single-particle cryo-EM, near-atomic-resolution structures can be obtained without the requirement of crystal formation, which is still the rate-limiting step for crystallographic studies. Cryo-ET is particularly useful in studying large molecular complexes and cells that do not possess any symmetry. Further improvements, such as the use of phase plates, further development of automated data-collection software and perfection of reconstruction algorithms, make it likely that cryo-EM will overtake crystallography or NMR as the primary tool for structural biology at low to medium resolution. However, crystallography, NMR and other tools will still be essential to obtain detailed, atomic resolution information.

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