

2. RECIPROCAL SPACE IN CRYSTAL-STRUCTURE DETERMINATION

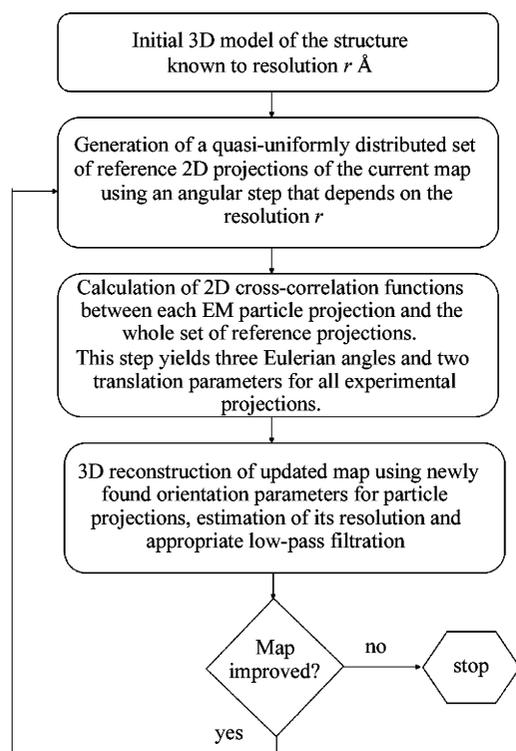


Fig. 2.5.7.4. Schematic of the 3D projection-alignment procedure.

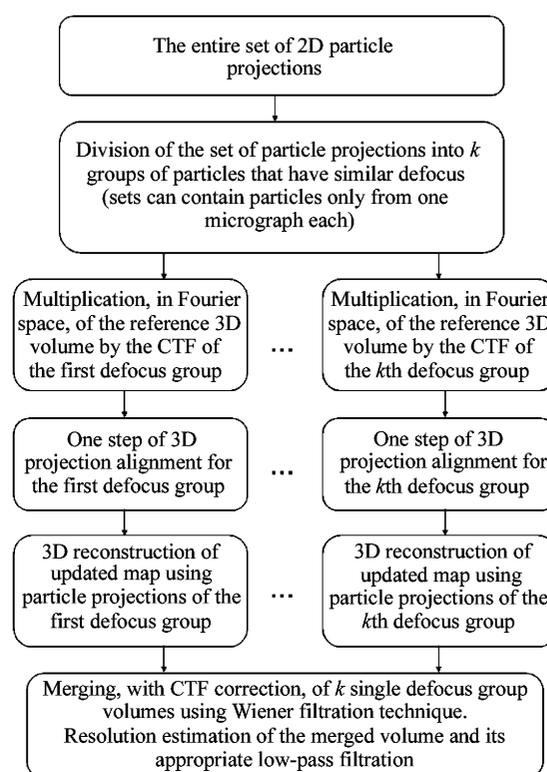


Fig. 2.5.7.5. Schematic of 3D projection alignment with CTF correction performed on the level of 3D maps reconstructed from projection images sorted into groups that share similar defocus settings.

sphere (or, in the case of a symmetric structure, over an asymmetric subunit) by taking fixed steps along the altitude or tilt angle θ and a number of samples azimuthally in proportion to $\sin \theta$ (Penczek *et al.*, 1994). So, for a chosen constant increment $\delta\theta$ and given θ angle the increment of the φ angle varies according to

$$\Delta\varphi = \delta\theta / |\sin \theta|. \quad (2.5.7.17)$$

If all three Eulerian angles are to be sampled, as is necessary in some applications, then ψ is sampled uniformly in steps of $\delta\theta$.

In order to find the orientation parameters of projection images, one step of projection matching is performed. The reference structure is projected in all directions given by (2.5.7.17), yielding a set of reference images. Next, for each projection image, 2D cross-correlation functions with all reference images are calculated using one of the methods described in Section 2.5.7.6 and the overall maximum yields the translation, the in-plane rotation angle, the number of the most similar reference image (thus the remaining two Eulerian angles) and information about whether the image should be mirrored. Given this, a new 3D structure can be calculated using a 3D reconstruction algorithm (see Section 2.5.6). This simple protocol constitutes the core of 3D projection alignment (Fig. 2.5.7.4).

In a simple implementation of the 3D projection-matching procedure, all projection data are assembled into *defocus groups*, *i.e.*, groups of projection images that have similar defocus settings (Frank *et al.*, 2000). During refinement, for each defocus group the reference volume is multiplied by the CTF with the appropriate defocus value, one step of projection matching is performed and a refined structure is reconstructed for this group (Fig. 2.5.7.5). In addition, the within-group resolution is estimated using the Fourier shell correlation (FSC) approach (2.5.7.19) applied to two volumes calculated from two subsets of projection images randomly split into halves. After all defocus groups have been processed, the individual refined volumes are merged in Fourier space with a CTF correction using Wiener-filter methodology (Penczek *et al.*, 1997),

$$F_{\text{merged}} = \frac{\sum_k \text{CTF}_k \text{SSNR}_k F_k}{\sum_k \text{CTF}_k^2 \text{SSNR}_k + 1}, \quad (2.5.7.18)$$

where SSNR_k is the spectral signal-to-noise ratio estimated for each defocus group using (2.5.7.22). Subsequently, the resolution of the merged volume is estimated by merging the half-volumes into two half-merged volumes using (2.5.7.18) and comparing them using (2.5.7.19). Next, the merged volume is filtered using (2.5.7.25) and the structure is centred so that its centre of mass is placed at the centre of the volume in which it is embedded.

The 3D projection-matching approach works very well during the initial stages of the refinement as it constitutes a very efficient approach to an exhaustive search for orientation parameters of all projection data. Once the orientation parameters are known to a degree of accuracy, it is straightforward to modify the procedure such that only subsets of reference projections are generated at a time and projection images are compared only with reference projections within a specified angular distance from their angular direction established during previous iteration. This modification speeds up the procedure significantly and makes it possible to refine structures to very high resolution by using a very small angular step $\delta\theta$. Another possible modification is to introduce an additional step of 2D alignment of the projection data that share the same angular direction (Ludtke *et al.*, 1999). The advantage is that this can correct possible errors of alignment to the projection of a limited-resolution reference structure and also, to an extent, reduces the danger of bias from artifacts in the reference structure. Finally, it is also possible to incorporate into the refinement strategy a correction for the envelope function of the microscope (Ludtke *et al.*, 1999). The 3D projection-matching strategy is widely popular and most EM software packages have implementations of various versions of basic strategies, as outlined above (Frank *et al.*, 1996; Ludtke *et al.*, 1999; Hohn *et al.*, 2007).