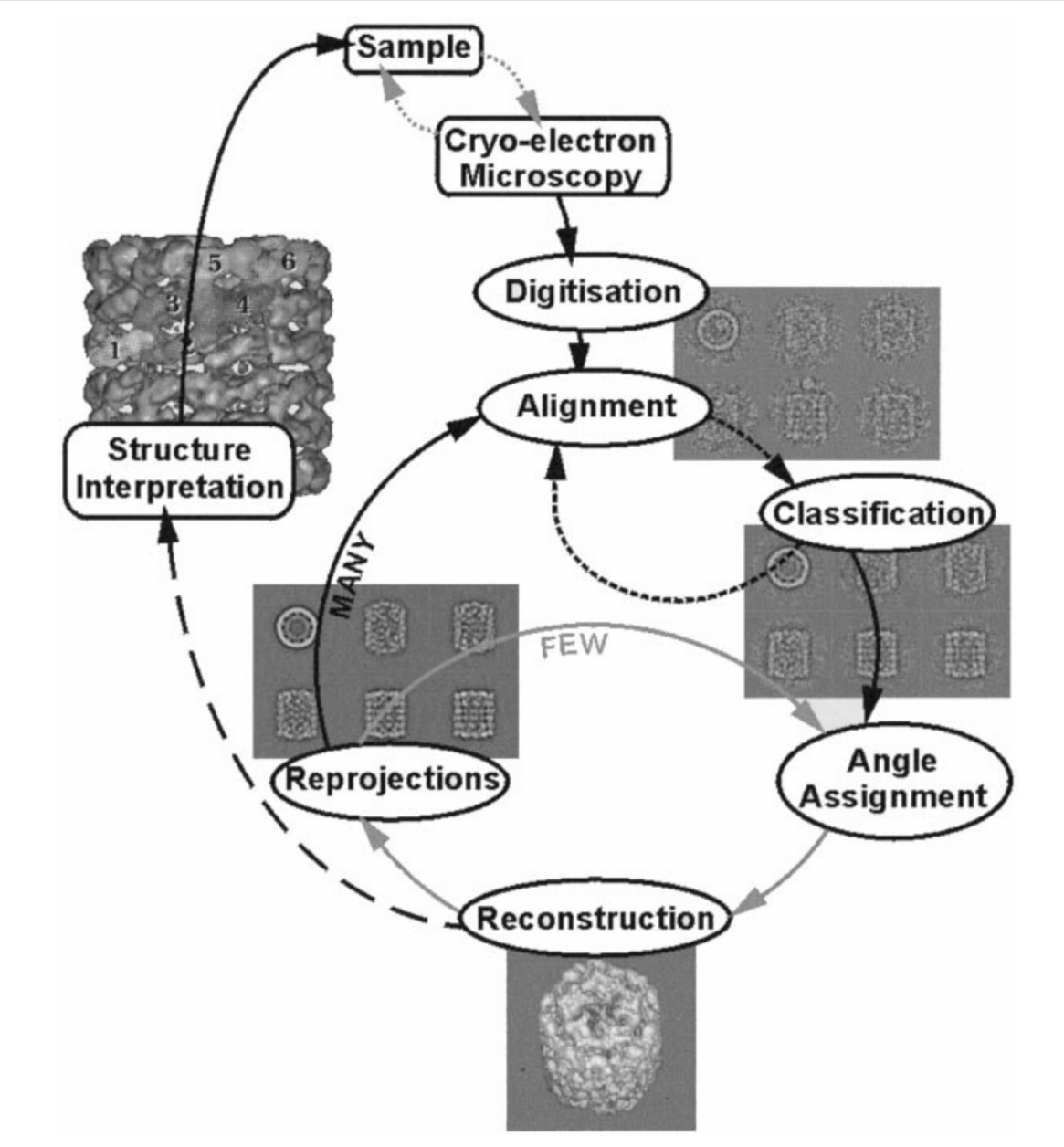
Cryo-electron microscopy

Methods in Molecular Biophysics, Spring 2009

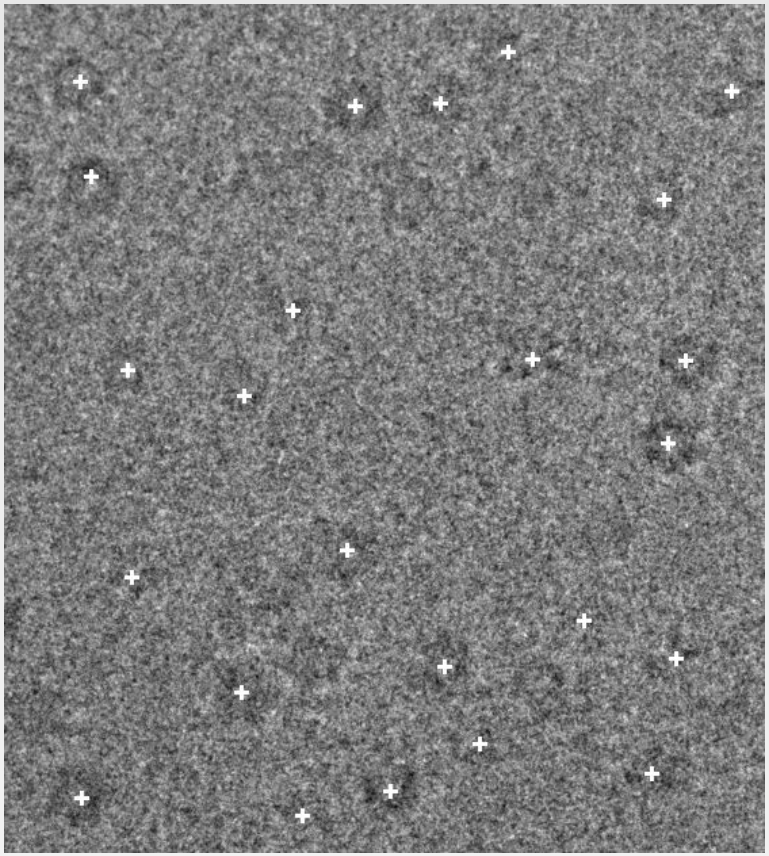
Sample preparation
Single-particle reconstruction
Image manipulation
Examples

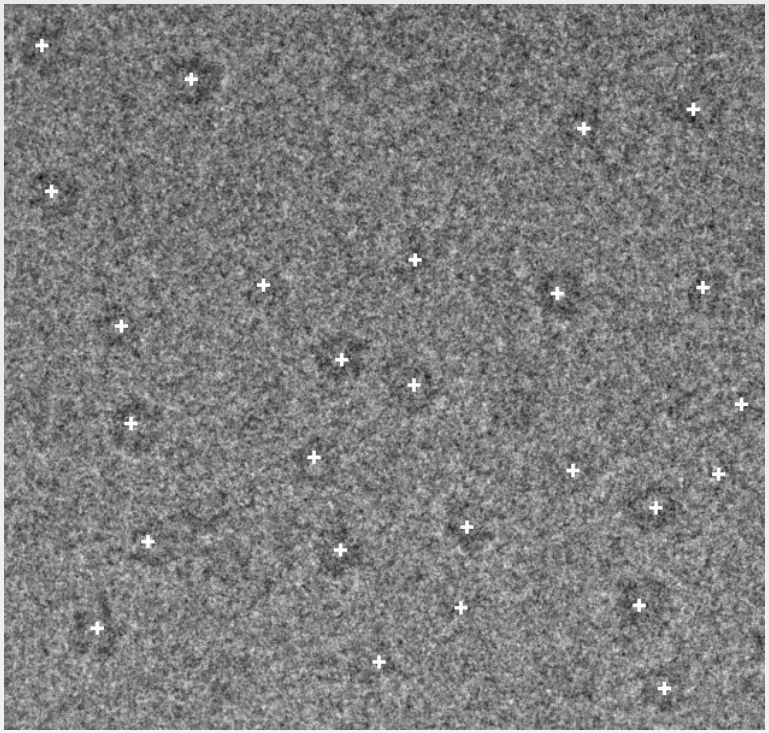
Overview of cyro-EM processing



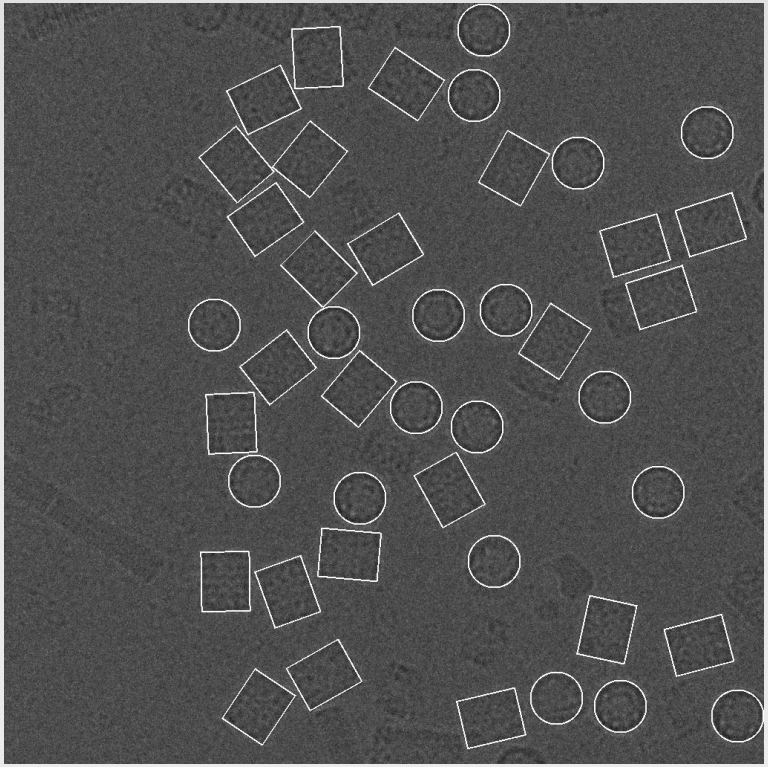
Particle picking

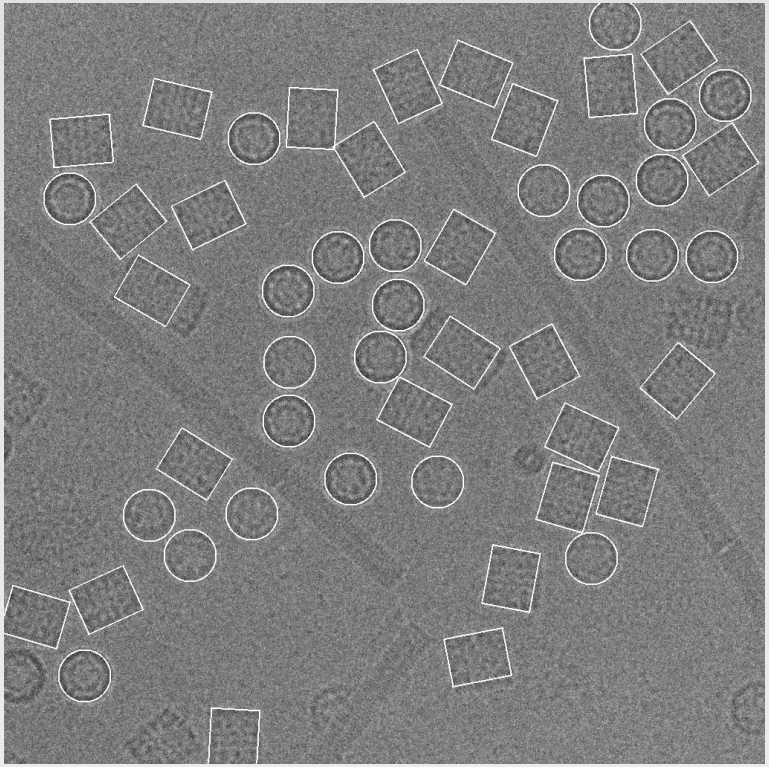
Particle detection is the first thing to do in the single particle reconstruction as soon as the Cryo-EM images are digitized and/or CTF-corrected. The goal of this step is to locate all particles. Since achieving high-resolution reconstruction often requires over hundreds of thousands of particles, it is important to design a fast and automatic algorithm for particle detection. We have developed two methods for this task. One is based on data clustering and the other is based on Voronoi diagram and distance transform. See http://cvcweb.ices.utexas.edu/cvc/projects/angstrom/bm/index.php

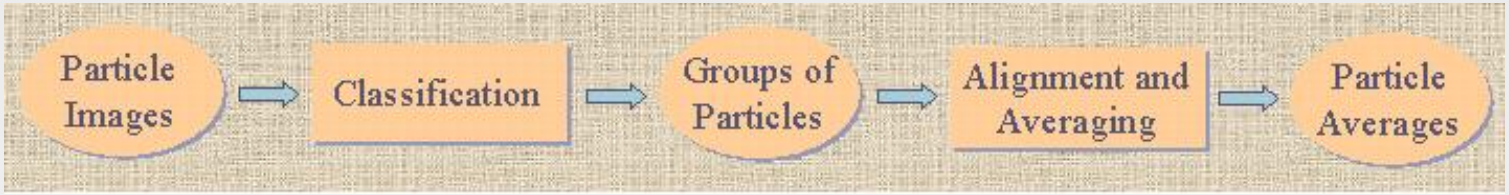




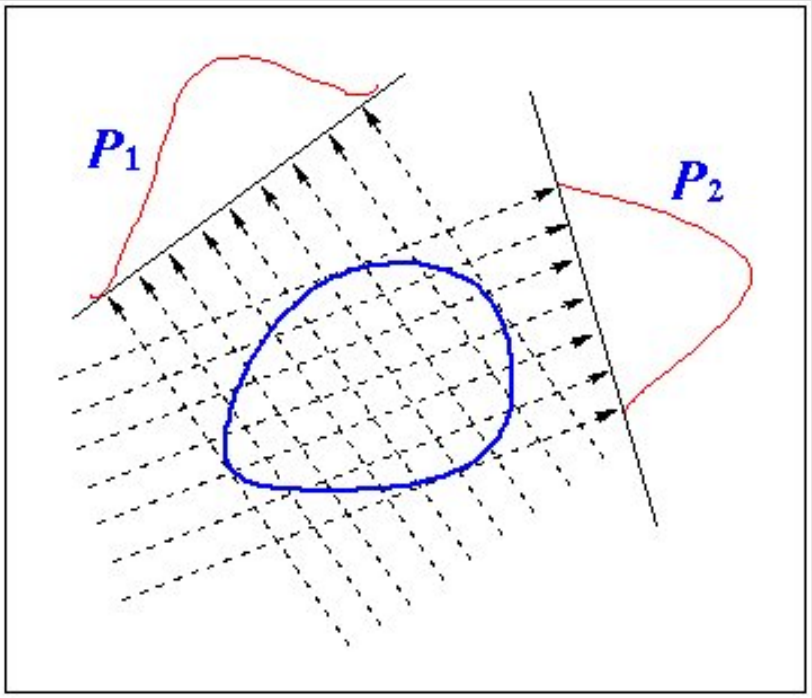
Automated particle picking

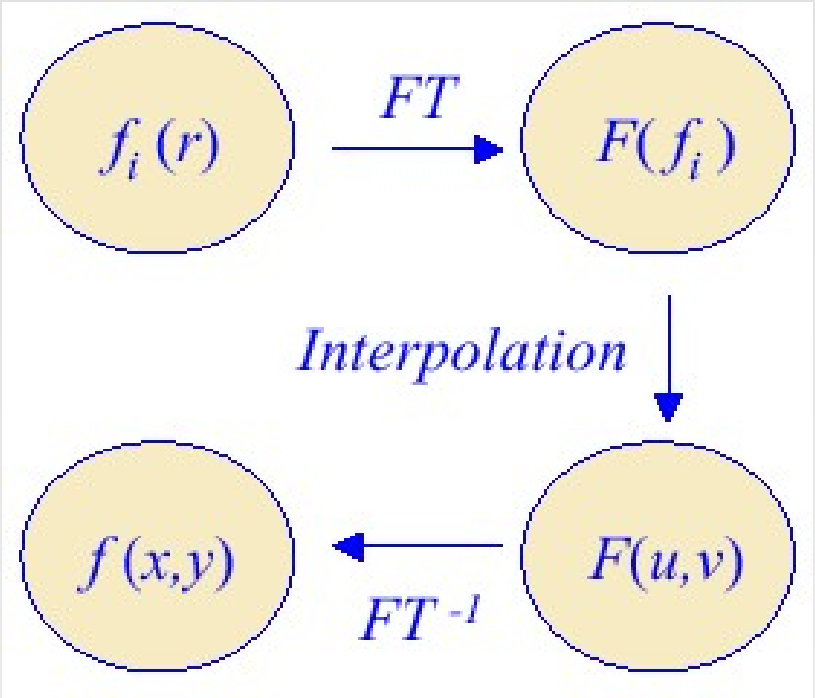






Projecting the image





The CT/MRI reconstruction is based on a theorem called Fourier Slice Theorem, which relates the Fourier transform of any projection of the original structure to the Fourier transform of the original structure. Fig.2(a) shows an example of a 2D structure reconstruction from its 1D projections. With enough number of projections, we can compute their Fourier transforms, each of which is "embedded" in the Fourier space and then an interpolation technique is used to "reconstruct" the Fourier transform of the original structure f(x, y) in the entire Fourier space. After we obtain F(u, v), the inverse Fourier transform of F(u, v) gives the original structure f(x,y). Fig.2 (b) illustrates the overall pipeline of reconstruction. It is straightforward to extend this procedure into 3D structure reconstruction.

More on projections

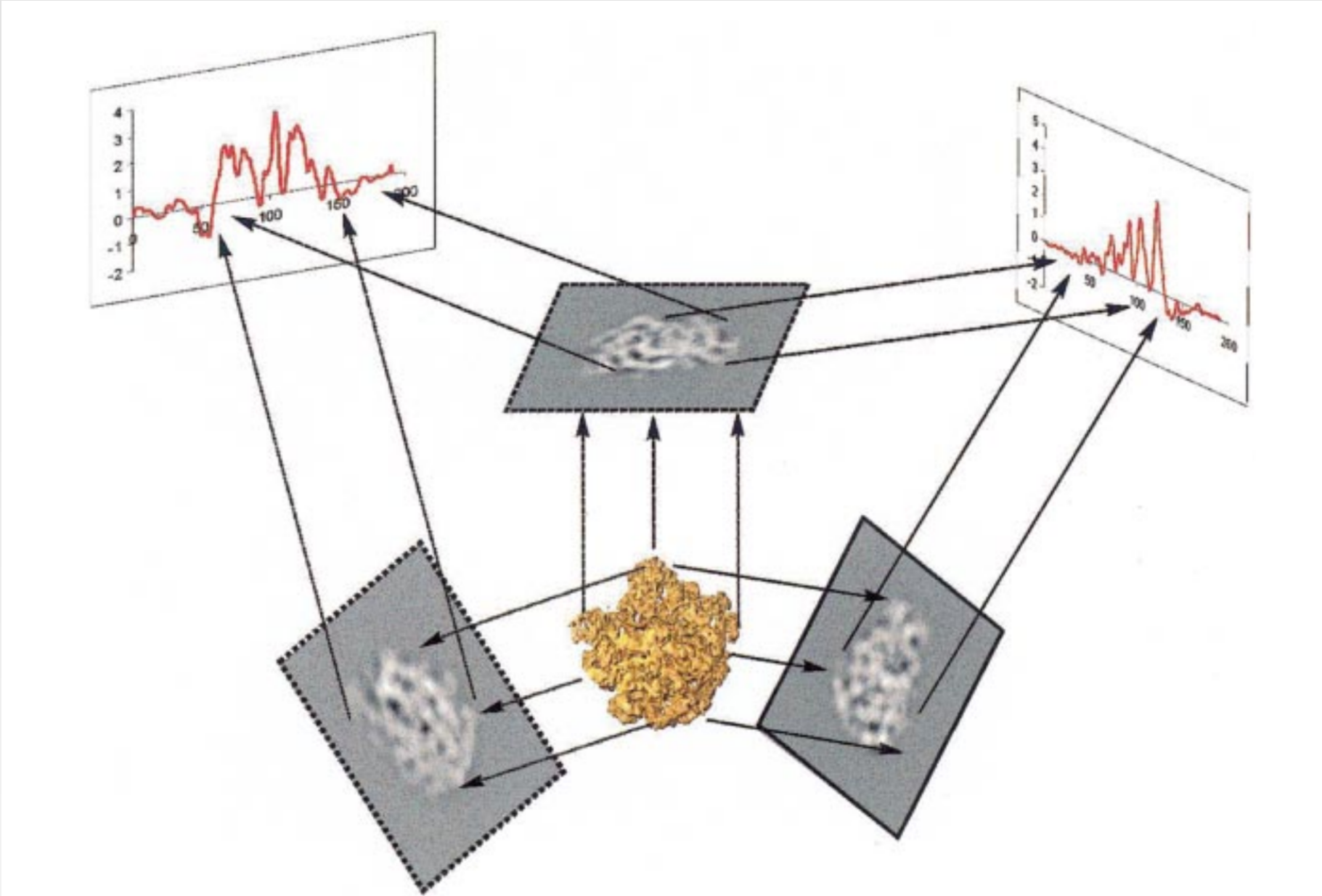


Image enchancement

The images obtained from the cryo-electron microscopy are usually very noisy and have very low contrast. It is necessary to smooth the noise as well as enhance the contrast. This also happens right after the 3D electron density maps are reconstructed. The following pictures show an example of our approaches on the reconstructed electron density map of Rice Dwarf Virus (RDV). The Cryo-EM images or the reconstructed maps may also be improved by Contrast Transfer Function (CTF) correction.

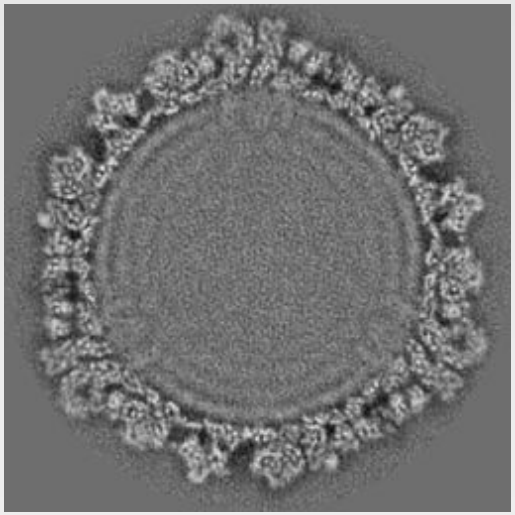
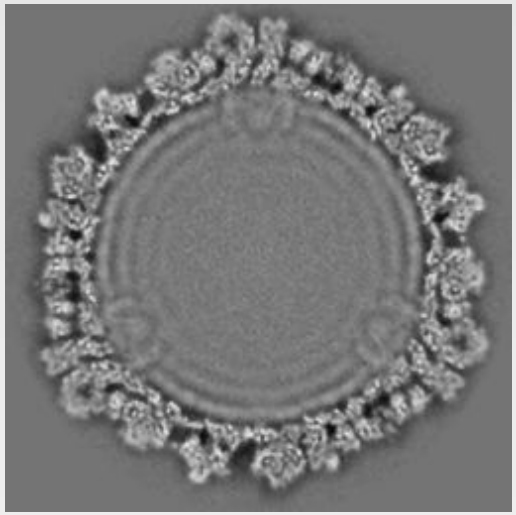
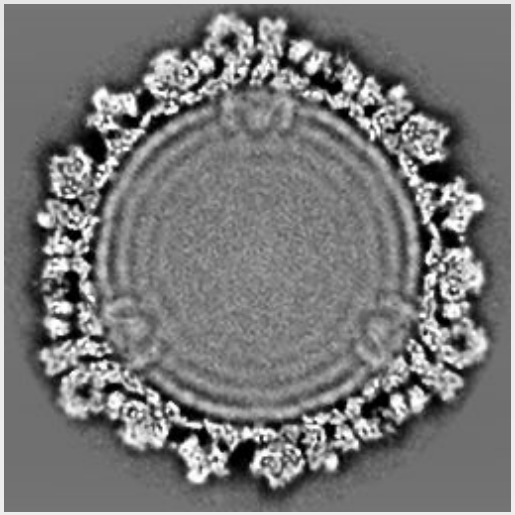
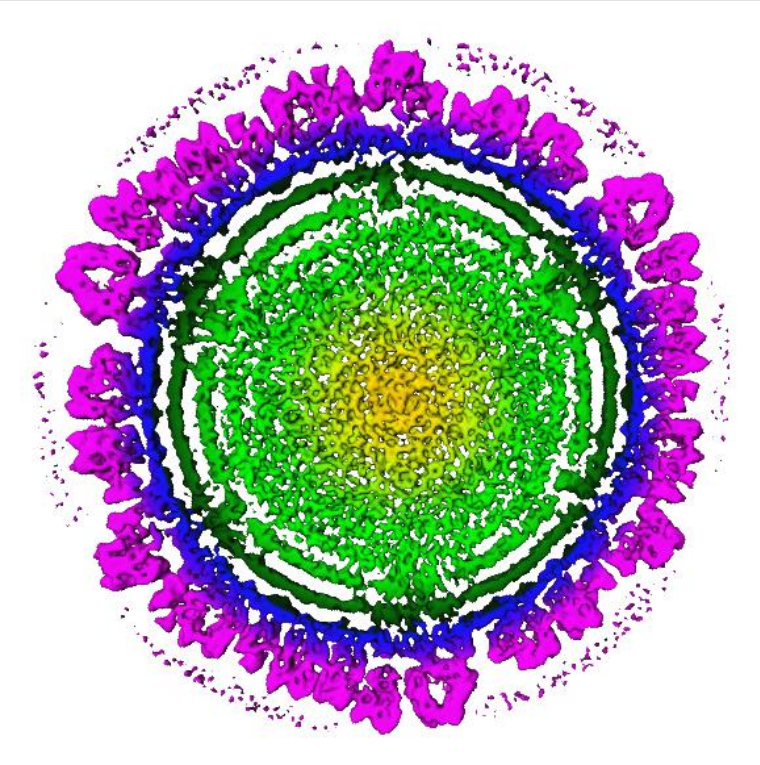
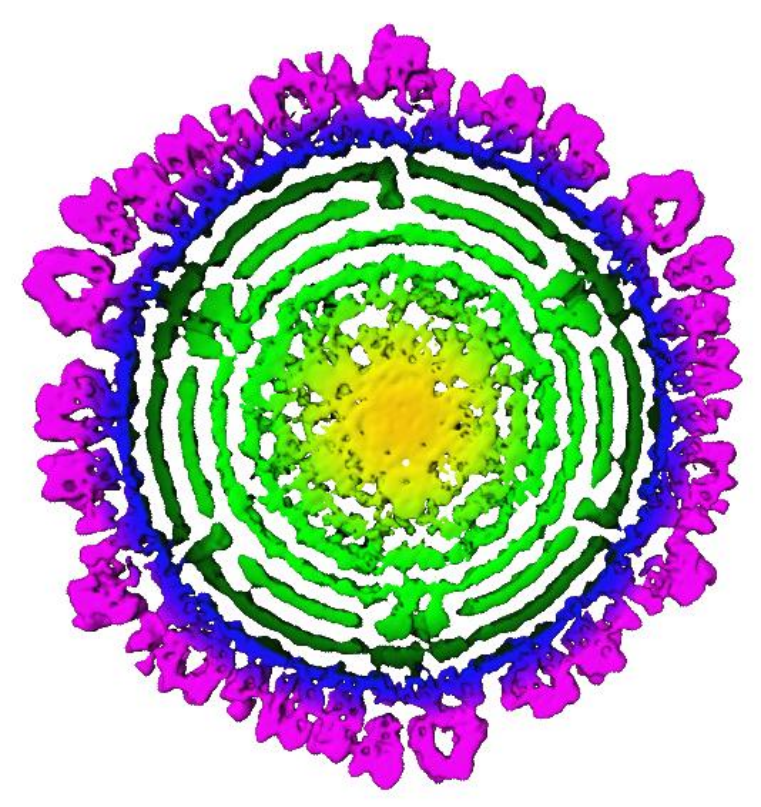




Image enchancement





Moving towards atomic resolution

Although atomic details are not detectable in reconstructed 3D cryo-EM maps, given their low feature resolution, it is sometimes feasible to locate secondary structures (alpha helices and beta sheets). An approach for detecting alpha helices in 3D maps is where the alpha helix is modelled with a cylinder (length and thickness) and the cylinder is correlated with the segmented protein map. Since the best solution is achieved by exhaustively searching in translation space (3D) and orientation space (2D), this method is computationally expensive. A related exhaustive search approach, designed for beta sheet detection uses a disk (planar) model for beta sheets.