Computational Tools for Macromolecular Structure Determination and Visualization
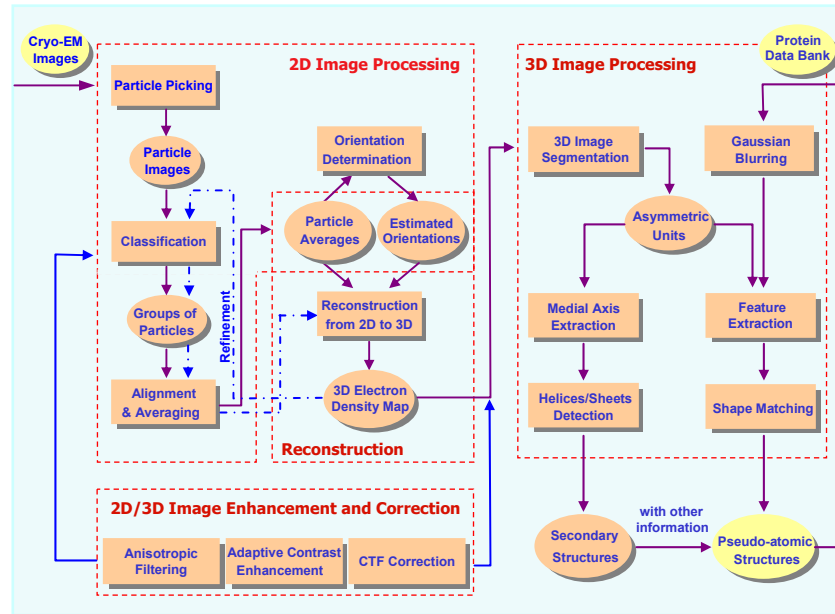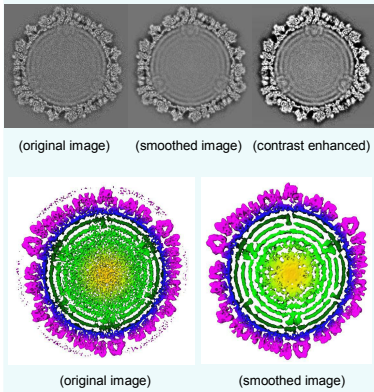
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Introduction

The three-dimensional (3D) structures of molecules are very important for us to study the functions of molecules in many applications. Although the structures of most existing proteins are solved by x-ray crystallography or NMR spectroscopy, they do not give the "full picture" of a functional biological complex. The study of large macromolecular complexes, such as viruses, ion channels, the ribosome and other machines of various types, offers a more complete structural and functional description of the protein machinery. However, it becomes much more difficult for us to crystallize such large macromolecular complexes. Therefore the non-crystallography technique using electron cryomicroscopy (cryoEM), commonly known as *single particle reconstruction,* provides a powerful tool in revealing the structures of large complexes at sub-nanometer resolutions (5 - 10Å). Coupled with this technique are many newly-developed algorithms seen in image processing, which are the main goal of our research in this project. The figure on the right shows the overall procedure of this technique, beginning with the 2D Cryo-EM images and finally yielding pseudo-atomic structures.

Image Enhancement

The images obtained from the electron cryomicroscopy are usually very noisy and have very low contrast. It is quite necessary to smooth the noise as well as enhance the contrast before other tasks can be conducted. This also happens right after the 3D electron density maps are reconstructed. We have developed an adaptive contrast enhancement technique and a PDE-based anisotropic diffusion technique for noise removal.

(original image)   (smoothed image)   (contrast enhanced)

(original image)   (smoothed image)

Cryo-EM Images

2D Image Processing

Particle Picking → Particle Images → Classification → Groups of Particles → Alignment & Averaging

Refinement

Orientation Determination → Particle Averages / Estimated Orientations

Reconstruction

Reconstruction from 2D to 3D → 3D Electron Density Map

2D/3D Image Enhancement and Correction

Anisotropic Filtering   Adaptive Contrast Enhancement   CTF Correction

3D Image Processing

Protein Data Bank

3D Image Segmentation → Asymmetric Units ← Gaussian Blurring

Medial Axis Extraction   Feature Extraction

Helices/Sheets Detection   Shape Matching

Secondary Structures   with other information   Pseudo-atomic Structures

3D Image Segmentation

The goal of image segmentation here is to segment the sub-components from the reconstructed maps such that fast accurate interpretation of the structures could be possible. The following pictures show the segmentation of capsid shells of RDV (left), the segmentation of asymmetric subunits (middle), and the segmentation of P8 monomers (right).
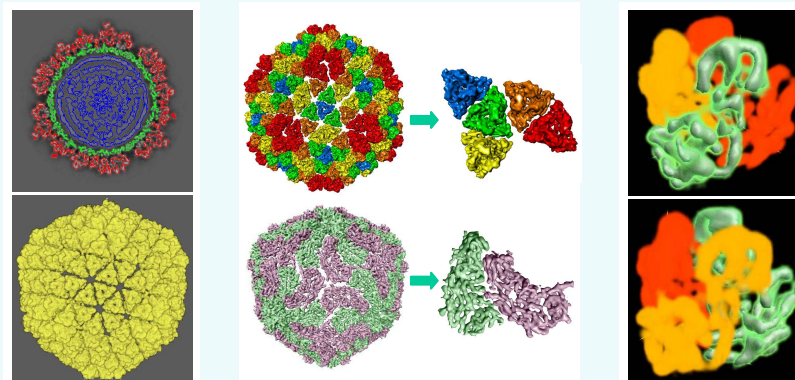

Secondary Structure Analysis

Secondary structure analysis stands for determining alpha-helices and beta-sheets directly from 3D electron density maps. The correspondence between the detected secondary structures and the known atomic structures can then be built and a pseudo-atomic structure could be achieved. Shown below are examples of the secondary structure detection from RDV P8 (left) monomer and P3 (right) monomer, as obtained by W. Jiang *et al.* Currently we are developing a medial axis based technique for fast and accurate secondary structure determination.
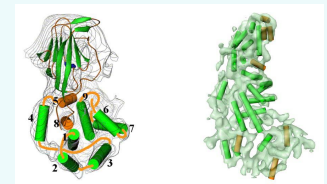
Atomic Structure Fitting

Another way to achieve the pseudo-atomic resolution is by fitting the known atomic structures into the reconstructed electron density maps. Traditional techniques for doing this is by brute-force searching in the six dimensional space (three for translation and three for rotation) for the best fitted position and orientation. Our solution is to calculate the skeletons of both the density maps and the blurred atomic structures and then the fitting is performed in the simplified feature space.
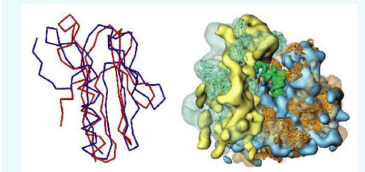
Acknowledgments

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