

## NEW RECONSTRUCTION CONDITIONS GREATLY IMPROVE THE RECONSTRUCTION QUALITY

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Image processing algorithms have had a deep impact in Three-dimensional Electron Microscopy. The resolution achieved by structural studies using single particles is increasing and there is a hope of eventually achieving atomic resolution (50S and 70S subunits of *E. Coli*'s ribosome at 7.5Å<sup>7</sup> and 11.5Å<sup>3</sup> respectively, *Semliki Forest virus*' capsid at 9Å<sup>5</sup>, and GroEL at 11.5Å<sup>4</sup>). However, these high-resolution studies usually need a large number of projections besides a proper correction of the microscope Contrast Transfer Function (CTF). In this work, we show how imposition of constraints other than those determined by the projections together with a CTF correction can boost the resolution of the resulting volumes to a level which can only be obtained by a much larger number of projection images using the currently-standard approaches.

The algorithm ART (Algebraic Reconstruction Technique) with blobs<sup>6</sup> was modified to include a number of restrictions such as symmetry, occupied volume, macromolecular surface, and density nonnegativity. This kind of information is easily expressed in real space and the use of ART facilitates the incorporation of such conditions into the reconstruction process. These restrictions were applied iteratively and simultaneously to the reconstruction algorithm.

This modified ART was also combined with the Iterative Data Refinement technique (IDR)<sup>1,8</sup> for the CTF correction. The CTF was further adjusted based on a theoretical CTF model using a parametric ARMA spectral estimation<sup>10</sup>. Special attention was paid to the optimization of all parameters involved in the three-dimensional reconstruction.

The efficacy of this approach was investigated using 1400 projections of the large T antigen of SV40 (LAT)<sup>9</sup> obtained by cryomicroscopy. Figure 1 compares the results when the CTF correction provided by IDR and all the proposed restrictions were applied, with those when they were not. Figure 2 compares the corresponding resolutions computed by Fourier Shell Correlation<sup>2</sup>. It can be seen that the incorporation of the constraints together with the proposed CTF correction significantly improves the achieved. This is due to the fact that our proposed approach incorporates *a priori* information that imposes nonlinear and adaptative conditions as the reconstruction process progresses. Further improvements in resolution are expected with increasing the number of images to a level similar to that currently used in high-resolution studies.

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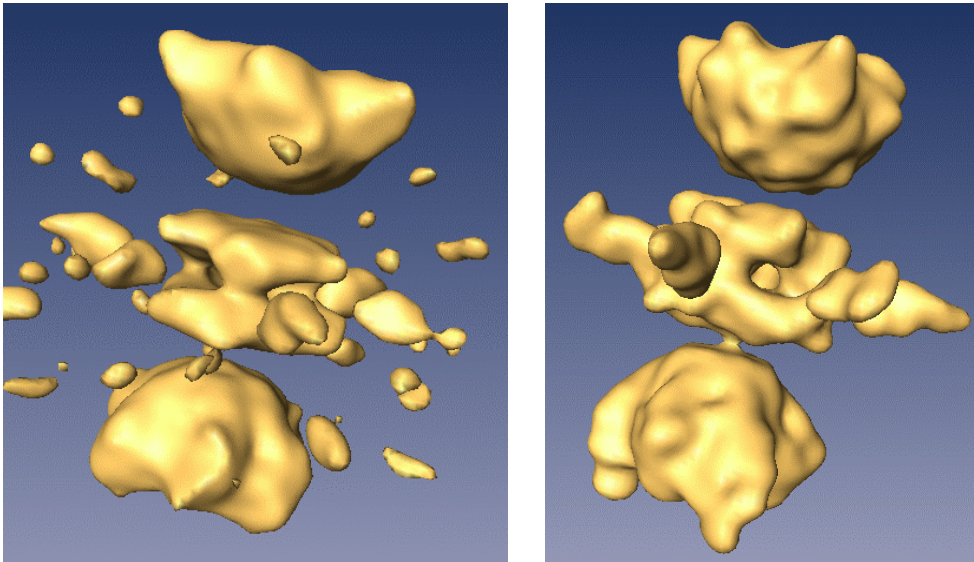


Figure 1. Isosurface representation of the LAT macromolecule when the proposed restrictions are not applied (left) and when they are (right)

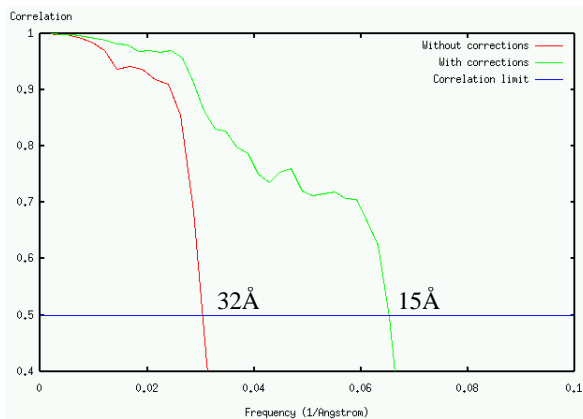


Figure 2. Comparison of the Fourier Shell Correlation for the reconstructed volumes when combining the proposed restrictions (up to 15 Å) and when they are not considered (up to 32 Å).